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The Effect of Omega-3 Fatty Acids on Psychophysiological Assessment for the Secondary Prevention of Posttraumatic Stress Disorder: An Open-Label Pilot Study

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Abstract

Our recent pilot study has shown that the supplementation of omega-3 fatty acids (fish oil) immediately after a traumatic event may be effective toward the secondary prevention of post-traumatic disorder (PTSD). To lay the groundwork for addressing the mechanism by which omega-3 fatty acids can prevent PTSD, we analyzed its psychophysiological data. The psychophysiological data included heart rate, skin conductance, and continuous blood pressure during patient subjection to startling tones and idiographic trauma-related cues. Of the 8 patients, 1 met the diagnostic criteria for PTSD. Compared to the seven patients without PTSD, one patient with PTSD showed relatively large reactivity to the startle tones. In contrast, this patient did not show large reactivity to the trauma-related cue during script-driven imagery. The combination of psychophysiological measurements in our randomized control trial should shed light on the underlying mechanisms by which omega-3 fatty acids can prevent PTSD.

Keywords: Post-traumatic disorder, Fish oil, Fear memory, Script-driven imagery, Startle responses

1. Introduction

We have recently reported that dietary supplementation with omega-3 fatty acids (otherwise known as fish oil) immediately after the occurrence of a traumatic event could be effective in the secondary prevention of post-traumatic disorder (Matsuoka *et al*., 2010). In this open-label pilot study, the scores of the Clinician-Administered PTSD Scale (CAPS) derived from patients who underwent 12-weeks of omega-3 fatty acids supplementation were significantly lower than their historical scores (Matsuoka *et al*., 2009).

Although the exact mechanisms by which omega-3 fatty acids can prevent PTSD are not known, we can posit some reasonable hypotheses. One possibility is that the effect is mediated by the omega-3-related facilitation of the clearance of fear memories from the hippocampus (Matsuoka, 2011). Supporting this hypothesis, emerging evidence suggests that omega-3 fatty acids facilitate neurogenesis in the hippocampus(Beltz, Tlusty, Benton, & Sandeman, 2007; Calderon & Kim, 2004) and that such neurogenesis facilitates the clearance of fear memories(Kitamura *et al*., 2009). Besides this mechanism, a more recent study has revealed that deficits in omega-3 fatty acids can abolish endocannabinoid-mediated neuronal functions (Lafourcade *et al*., 2011) that facilitate the extinction of fear memories (Marsicano *et al*., 2002). It is also possible that omega-3 fatty acids are beneficial because of their ability to mediate a reduction of sympathetic activity. A generally accepted notion is that exaggerated and prolonged sympathetic activity contributes to the development of PTSD (Charney, Deutch, Krystal, Southwick, & Davis, 1993); omega-3 fatty acids have been shown to lower this activity(Delarue *et al*., 2003; Hamazaki *et al*., 2005; Spence, Thornton, Muir, & Westcott, 2003). However, in any of these cases, a single assessment of CAPS is not sufficient to provide new insights into these potential mechanisms.

In order to lay the groundwork for addressing the mechanism of the effects of omega-3 fatty acids on PTSD, we analyzed psychophysiological data in above-mentioned open-label pilot study. Physiological measurements included heart rate, skin conductance, and blood pressure. The measurement paradigms included assaying patients' responses to a startling sound and to a trauma-related cue, otherwise known as script-driven imagery. These paradigms are the same as those used to measure autonomic functions, but they quantify different psychophysiological attributes. The indices derived from the first paradigm reflect a mixture of the hyperarousal symptoms(Griffin, 2008; Shalev *et al*., 2000) and vulnerability(Guthrie & Bryant, 2005; Orr *et al*., 2003) common to PTSD, while those derived from the second paradigm reflect an inner expression of aversive emotions caused by the recollection of a traumatic event—that is, fear memory(Pitman, Orr, Forgue, de Jong, & Claiborn, 1987). In this study, we report the results of these psychophysiological assessments.

2. Methods

2.1 Participants

This study was conducted as a part of the Tachikawa Project for Prevention of Post-traumatic Stress Disorder with Polyunsaturated Fatty Acid (registered as NCT00671489 at http://clinicaltrials.gov). All participants were selected from 122 consecutive Japanese patients who were injured and hospitalized into the intensive care unit of the National Disaster Medical Center, Tokyo between May 2008 and October 2008 (see Figure 1). The inclusion criteria were (a) ≥18 years of age, (b) native Japanese-speaking ability, (c) our ability to contact patients within 240 h of injury, and dosing in oral use, and (d) physical and mental ability to understand the scope of the study and to consent to participating in the trial. The exclusion criteria were (a) clearly irretrievable acute brain parenchyma damage and subdural or subarachnoidal bleeding, as detected by computed tomography and/or magnetic resonance imaging, (b) cognitive impairment: mini-mental state examination score < 24, (c) heavy alcohol use or 100 IU/L ≤ γ GTP in administration, (d) heavy tobacco use (>40 cigarettes per day), (e) history or current diagnostic suspicion of psychosis or bipolar I disorder, (f) diagnostic suspicion of alcoholic or substance-related disorders or eating disorders, (g) existence of serious symptoms, such as suicidal ideation, self-harm behavior, dissociation, or other status necessitating rapid psychiatric treatment, (h) use of anti-epilepsy drugs, lithium, ethyl icosapentate, or anti-coagulant drugs (for example, aspirin, warfarin) at regular intervals within 3 months of injury, (i) regular use of polyunsaturated fatty acids supplements within 3 months of injury, and (j) a habit of eating fish >4 times a week. Of 27 candidates, 15 agreed to participate in the whole study, but eventually only 8 male patients participated in this psychophysiological study. The reasons for nonparticipation were refusal (n = 1), inability to attend due to poor physical condition (n = 2), and non-attendance (n = 4). The mean age of the participants was 36.6 ± 17.6 years; their accident types were motor vehicle accidents (n = 4), fall from a height (n = 2), and work-place accidents (n = 2). The patients who participated in this study did not differ significantly in age or severity of injury from nonparticipants, but participants tended to be male. Written informed consent was obtained from every participant after providing a complete description of the study. This study was approved by the Institutional Review Board of National Disaster Medical Center.

All participants took a dietary supplement of omega-3 fatty acids containing 1470 mg docosahexaenoic acid and 147 mg eicosapentaenoic acid per day for 12 weeks.

A diagnosis of PTSD was made by the Clinician-Administered PTSD Scale (CAPS; Asukai, Hirohata, Kato, & Konishi, 2003; Blake *et al*., 1995) at 12 weeks after participation.

2.2 Stimuli

The startling tones used in this study were essentially the same as those described in previous studies(Orr, Lasko, Shalev, & Pitman, 1995). Stimuli consisted of 15 sudden, 95 dB(A), 1000 Hz, 500 ms pure tones, with 0 ms rise and fall times. Inter-stimulus intervals were randomly ranged from 27 to 52 s.

Idiographic trauma-related cues used in the study were essentially the same as those described in previous studies(Pitman, *et al*., 1987), except for language. On the basis of a prior interview by the clinical psychologist, each patient's experience of their accident was recorded as a traumatic injury script. The script was digitally recorded with a loud, slow, and tense voice by an independent experimenter who had never met the patients before the experiment. The length was approximately 30 s, which corresponds to approximately 160 letters in Japanese.

2.3 Apparatus and Physiological measures

A 3 × 3-m, sound-attenuated, temperature-controlled experimental room was used. Stimuli were presented through binaural headphones (Sony, MDR-CD900ST) plugged into USB audio interface (digidesign Mbox2), controlled by Mac OS X computers (Apple, Mac Pro, MA970J/A, Mac OS X 10.5.5). In developing these applications, we included much of the software code utilized in previous studies(Matsumura, Yamakoshi, & Ida, 2009).

Heart rate (HR) was obtained from electrocardiogram (ECG) readings that were recorded through disposable electrodes placed bilaterally on the arms and connected to a bioamplifier (Monte system, ECG100C). Skin conductance (SC) was obtained from the index and middle finger of the left hand through an Ag/AgCl transducer that was filled with isotonic electrode paste and attached to a bioamplifier (Monte system, GSR100C). Mean blood pressure (MBP) was derived from a blood pressure contour. Blood pressure was measured noninvasively through a finger cuff that was attached to the annular finger of the left hand and connected to a continuous blood pressure monitor (MEDi SENS, MUB101). Analog output was sampled at 16 bit, 1000 Hz by an integrated bioamplifier system (Monte system, BIOPAC MP150 system). Output was then transferred and stored digitally on a Mac Pro computer using AcqKnowledge software (Monte system). Software was run in a virtual Windows XP environment (Parallels, Parallels Desktop 4.0) constructed on a Mac Pro.

2.4 Procedure

This experiment was conducted after the patients' diets were supplemented with omega-3 fatty acids for 12-weeks after injury. After administration of initial instructions and setup of instruments, the participants sat in a reclining chair in the experimental room. A 5-min adaptation period was followed by a 10-min startle period. Participants were told to sit quietly and listen to the tones until the experimenter informs them that they are finished.

After a short break, and a second round of instruction, a 3-min adaptation period was followed by a 2-min script period. The script period consisted of 4 continuous 30 s periods, namely: baseline, read, imagery, and recovery. Participants were asked to sit quietly, listen to the script, imagine it as realistically as possible, and relax. To prevent mistakes, short instruction was given to patients immediately before each period, except pre-baseline.

2.5 Data Reduction

Startle responses of HR, SC, and MBP were calculated by subtracting the mean HR and SC levels 1 s immediately preceding tone onset from the maximum levels 1 to 4 s after tone onset. Initial MPB levels were similarly subtracted from the MBP maxima that occurred 5 to 8 s after tone onset. These values, except for those from the first trial (Lykken, Iacono, Haroian, McGue, & Bouchard, 1988), were averaged to give a mean response score.

HR, SC, and MBP values during script-driven imagery were averaged over each 30 s period. Then, reactivity during script delivery was calculated by subtracting the baseline level from the imagery level.

3. Results

On the basis of CAPS at a 12-weeks-post-injury follow-up examination — that is, immediately after the experiment — 1 patient was diagnosed as having PTSD with a total CAPS score of 76.

3.1 Startling tones

Reactivity values of the patient with PTSD and seven patients without PTSD are presented together in Figure 2. Overall, the reactivity of the patient with PTSD was high.

3.2 Script-driven imagery

In Figure 3, the reactivity values during imagery of idiographic trauma-cues are presented together with their empirical cut-offs for PTSD for both the patient with PTSD and for the 7 patients without PTSD. The sensitivity and specificity of these cut-offs for PTSD are 69% and 89%, respectively.(Orr, Metzger, & Pitman, 2002) No HR responses exceeded the cut-off (1.9 beats per minutes$^{1/2}$). Additional 1-sample *t*-tests indicated that mean HR$^{1/2}$ reactivity (-0.35 ± 1.69) was significantly below the PTSD cut-off (t (7) = 3.77, p = .007, d = 1.33). Overall, the reactivity of the patient with PTSD was not high.

4. Discussion

To begin probing the mechanisms that underlie the prevention of PTSD by omega-3 fatty acids, we conducted a psychophysiological assessment. To our knowledge, no studies have examined the psychophysiology of PTSD in injured Japanese patients. The experimental and measurement procedures were finished without serious difficulty and the raw data obtained had few noticeable artifacts (not shown). It should be noted that, because our laboratory was not barrier-free, patients in wheel chairs or using crutches had some difficulty participating in the experiment.

Reactivity varied according to the measurement paradigm and indices used. The reactivity of the patient with PTSD in the startle tone paradigm (see, Figure 2) was generally large. This tendency is consistent with the previously mentioned notion that startle reactivity reflects a mixture of the hyper-arousal symptoms (Griffin, 2008; Shalev, *et al*., 2000) and vulnerability (Guthrie & Bryant, 2005; Orr, *et al*., 2003) often found in PTSD. In contrast, the reactivity in script-driven imagery in the patient with PTSD was not large at all. Considering that reactivity during script-driven imagery reflects inner expression of aversive emotions caused by the recall of traumatic event (Pitman, *et al*., 1987), this data might suggest that clearance of traumatic event memory—that is, fear memory—from the hippocampus has been facilitated.

We show here the potential usefulness of psychophysiological assessment in examining the underlying mechanisms by which omega-3 fatty acids may prevent PTSD. However, our conclusions are based solely on one patient with PTSD. Therefore, it is currently unknown if such reactivity patterns are representative of PTSD patients in general, or if they are specific to those who have undergone 12 weeks of omega-3 fatty acids supplementation. To examine these questions, we have begun a double-blind, placebo-controlled, randomized trial (registered as NCT00671099 at http://clinicaltrials.gov).

We measured MBP throughout the experiment. To our knowledge, no studies have reported PTSD-related MBP reactivity in a startle paradigm. Because BP increase is observed regardless of patients' responder type (that is, their tendency to have cardiac or vascular responses; Gregg, Matyas, & James, 2002; Julius, 1988) BP measurements are considered to be one of the most robust indices among all psychophysiological measures. In fact, BP has begun to be regarded as a potentially useful index (Pole, 2007; Tucker *et al*., 2007). In future studies, BP will be more closely monitored, in addition to the classical measurements of HR and SC.

We confirmed the feasibility of carrying out psychophysiological assessments in patients who have taken omega-3 fatty acids supplements immediately after traumatic events. Psychophysiological assessment appears to be useful in the examination of the mechanisms by which omega-3 fatty acids prevent PTSD.

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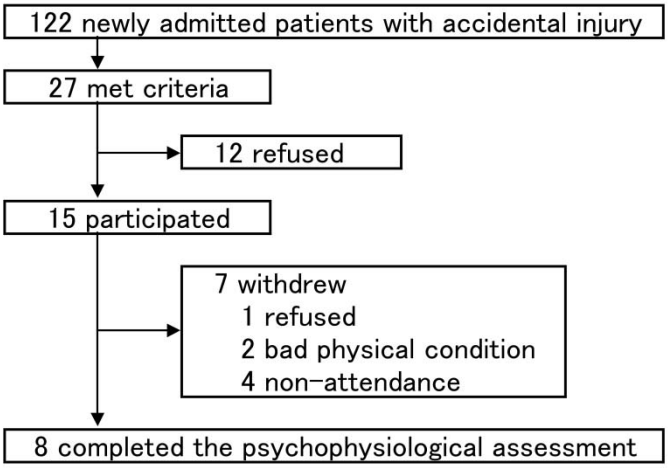

Figure 1. Flowchart of patients

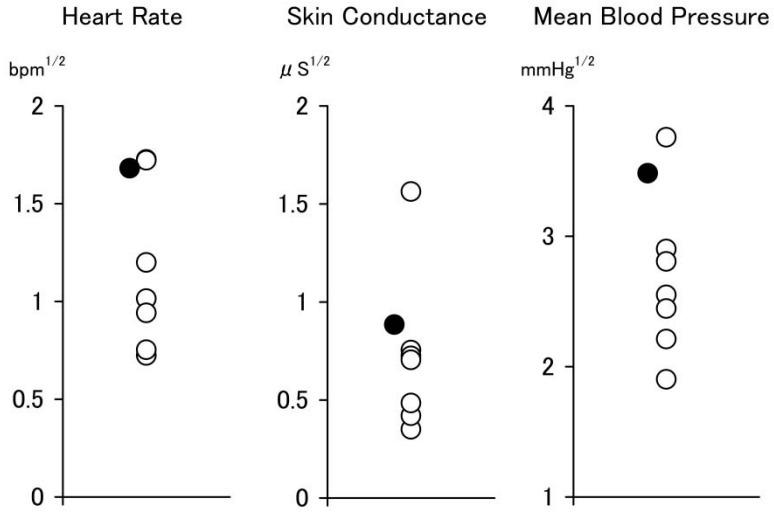

Figure 2. Physiological reactivity to startle sounds in patients with (filled circles) and without (open circles) PTSD after 12-weeks supplementation with omega-3 fatty acids

Note. BPM = beat per minutes; μS=μSiemens.

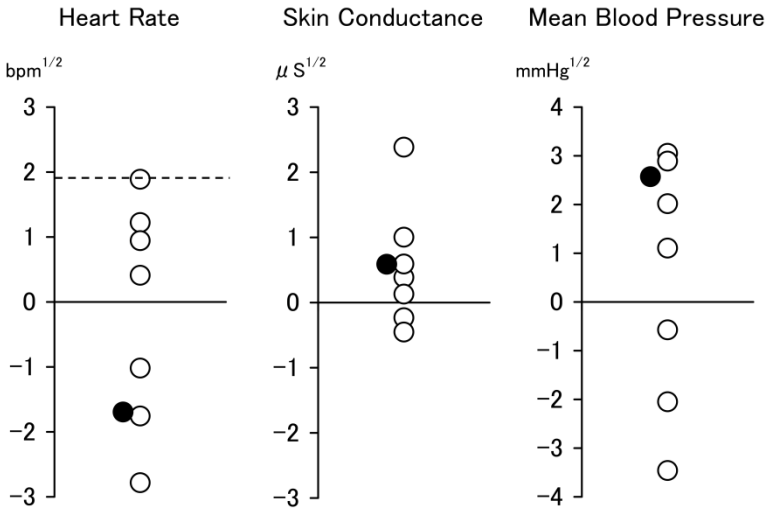

Figure 3. Physiological reactivity during imagery of idiographic trauma-cues in patients with (filled circles) and without (open circles) PTSD after 12-weeks supplementation with omega-3 fatty acids

Note. Dashed lines represent empirical cut-offs for PTSD.

BPM = beat per minutes; μS = μ Siemens.

Understanding the Antecedents of Korean High School Students' Drinking Refusal Self-Efficacy: Parental Influence, Peer Influence, and Behavior

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Abstract

The current study examined the factors that influence Korean adolescents' drinking refusal self-efficacy, which is known to be associated with alcohol use and drinking intentions. Specifically, this study considered parental monitoring, parent-child communication satisfaction, peer influence, and prior alcohol use as possible antecedents of Korean high school students' drinking refusal self-efficacy. High school students (n = 538) in South Korea responded to the current study. The data revealed that parent-child communication satisfaction facilitated parental monitoring, and these factors indirectly predicted adolescents' drinking behavior through peer influence. We also found that prior drinking, parental monitoring, and peer influence were directly associated with drinking refusal self-efficacy, and the self-efficacy, in turn, was associated with drinking intentions. These results not only suggest that drinking refusal self-efficacy are related to drinking behavior and intentions, but they also provide a theoretical explanation for how parental and peer influences are associated with adolescents' drinking refusal self-efficacy.

Keywords: Drinking refusal self-efficacy, Alcohol use, Parental influence, Peer influence, Korean adolescents

1. Introduction

Research suggests that alcohol is the most widely used drug by adolescents regardless of ethnicity, gender, or race (Van Der Vorst, Engels, R., Meeus, & Deković, 2006). Adolescents' drinking behavior has been not only a problem in the U.S. (Perry *et al*., 1996), it is also one of the vital social issues in several countries in Europe and Asia (Shin & Delva, 2004). In Korea, for instance, alcohol use among adolescents is of great concern as the average age of drinking initiation fell from 15.1 years in 1998 to 13.1 years in 2006 (Korean Ministry of Health and Welfare, 2007). This trend is alarming since scholars suggest that adolescents with early exposure to a large amount of alcohol use tend to be at greater risk for later alcohol abuse and dependence, unwanted pregnancy, suicide, domestic violence, accidents, sexually transmitted diseases, and antisocial characteristics, to name a few of the possible consequences (DuRant, Smith, Kreiter, & Krowchuk, 1999; Hingson, Heeren, & Winter, 2006).

Given the problems associated with Korean adolescents' alcohol consumption, studies identifying the ways to prevent teens from abusing alcohol are particularly important.

According to Baldwin, Oei, and Young (1993), educating adolescents on how to refuse drinking alcohol may be one approach that could decrease their alcohol use because individuals' perceived ability to resist drinking alcohol, namely drinking refusal self-efficacy, has a vast impact on their drinking behavior. Adolescents who believe that they could resist alcohol would be more likely to refuse drinking alcohol when compared with those who lack such a perception. In fact, this approach suggests that a lack of such perception is positively associated with alcohol consumption (Oei & Jardim, 2007). Thus, theoretically, identifying the factors that affect adolescents' drinking refusal self-efficacy and fostering the self-efficacy may facilitate decreasing adolescents' drinking intentions and behavior.

Although numerous scholars have demonstrated the inverse association between drinking refusal self-efficacy and drinking behavior (Baldwin *et al*., 1993; Oei & Jardim, 2007), relatively little is known about the attributes that may influence drinking refusal self-efficacy, a type of self-efficacy which ultimately influences individuals' drinking intentions and behavior. What has been identified thus far is that parental and peer influences may be related to adolescents' tendency to use alcohol (Hwang & Akers, 2006; Kunkel, Hummert, & Dennis, 2006; Watkins, Howard-Barr, Moore, & Werch, 2006), and drinking experience may impact individuals' drinking refusal self-efficacy (Aas, Klepp, Laberg, & Aaro, 1995; Oei & Morawska, 2004). In an attempt to identify the attributes that predict Korean adolescents' drinking refusal self-efficacy, a purpose of the current study was to examine how parental influence, peer influence, and drinking experience are related to Korean high school students' drinking refusal self-efficacy.

1.1 Drinking Refusal Self-Efficacy

The notion of self-efficacy affecting people's behavior is originally proposed by Bandura's (1986) social cognitive theory. Bandura states that perceived self-efficacy plays an important role in social cognitive theory because it "supports the type of efficient analytic thinking needed to [discover] predictive knowledge from causally ambiguous environments in which many factors combine to produce effects" (p. 35). He suggests that individuals' beliefs about the ability or capability of performing a behavior are powerful information that can predict people's actual behavior. Self-efficacy has been examined in a variety of behaviors, including exercise (Rimal, 2000), learning (Linnenbrink & Pintrich, 2003), and communication (Afifi, & Weiner, 2004), and a strong relationship between perceptions of self-efficacy on these behaviors and actual behaviors was documented. Research also shows evidence of the connection between drinking refusal self-efficacy and drinking intentions, as well as actual consumption (Baldwin *et al*., 1993; Watkins *et al*., 2006). Adolescents who think that they could resist drinking alcohol would be more likely to refuse to drink when compared with adolescents who perceive a lack of such self-efficacy (Oei & Baldwin, 1994; Watkins *et al*., 2006). In fact, the effects of drinking refusal self-efficacy on drinking behavior have been investigated in diverse samples, and a negative association between drinking refusal self-efficacy and teens' drinking intentions and behavior with both Asian and Caucasian samples were documented (Kim, 2001; Oei & Jardim, 2007). Thus, the following hypothesis was proposed:

H1: Drinking refusal self-efficacy will be negatively associated with Korean adolescents' drinking intentions.

One key predictor of drinking refusal self-efficacy is individuals' past alcohol use. Numerous scholars support the idea that adolescents who are already using alcohol would have relatively low drinking refusal self-efficacy than those without an experience of alcohol (Aas *et al*., 1995; Oei & Morawska, 2004). Research suggests that past behavior strongly impacts intentions and future behavior, particularly relating to habitual behaviors, such as drinking, and this relationship may be mediated by individuals' self-efficacy perception (Aas *et al*., 1995; Oei & Morawska, 2004). Indeed, social cognitive theory (Bandura, 1986) suggests that successful expereince is associated with individuals' self-efficacy, which, in turn, impacts future behavior. Guided by this notion, adolescents who consume alcohol may perceive low drinking refusal self-efficacy because their successful drinking experience may reinforce their perceived ability to drink, and as a consequence, they may not have intentions to turn down alcohol offers and may continue to abuse alcohol. In a similar vein, abstinent adolescents who successfully refused alcohol proposals in the past may have relatively high drinking refusal self-efficacy when compared with alcohol users who lack experience in effectively rejecting drinks from others. Given that past experience may be linked with adolescents' drinking refusal self-efficacy (Bandura, 1986), the following hypothesis was posed to examine the link between Korean adolescents' prior drinking and their drinking refusal self-efficacy:

H2: Prior alcohol use will be negatively associated with Korean adolescents' drinking refusal self-efficacy.

Though past drinking may be a predictor of drinking refusal self-efficacy, in order to fully understand the factors that shape adolescents' drinking refusal self-efficacy, it would be valuable to answer the following inquiry: What is the antecedent of Koeran adolescents' alcohol use? Identifying the factors that impact the adolescents' drinking behavior would be helpful in fully understanding the process in which they perceive drinking refusal self-efficacy.

1.2 Parental and Peer Influences on Drinking

Research demonstrates that parental and peer influences emerge as the two main forces that impact adolescents' drinking. Although some development theorists argue that parental influence tends to give way to peer influence during adolescence (Masten, Faden, Zucker, & Spear, 2008; Wood, Vinson, & Sher, 2001), other scholars continue to suggest that parental influence not only increases during late adolescence (see Duncan *et al*., 1994), it also moderates the effects of peer influence on drinking behavior (Laird, Pettit, Bates, & Dodge, 2003;Wood, Read, Mitchell, & Brand, 2004). Because parenting and family interactions have major influences on adolescent development, behavior, and substance use (Masten *et al*., 2008; Nash, McQueen, & Bray, 2005), the developmental model of Patterson (Patterson, DeBaryshe, & Ramsey, 1989) explains the unique dynamic between parental and peer influence as follows: Children's delinquent behavior is due to peer influences, but children's involvement with deviant peers is a result of poor parenting, suggesting the importance of parenting practices on children's behavior. Similarly, Steinberg (2001) suggests that peer influence plays a role in intensifying adolescents' delinquent behaviors, but "it is unlikely that peer influence leads to the initial emergence of these traits" (p. 12).

1.2.1. Parental Monitoring

Specifically, socialization theories (Grusec & Davidov, 2010) explain that, during adolescence, parents may sense difficulty influencing their children to overcome socialization by peers, indicating the struggle between parents and peers. While some parents may underestimate the power to transmit their values to their children during adolescence, scholars emphasize that parents can protect their children from negative peer influence by monitoring their activities and whereabouts (Bogenschneider, Wu, Raffaelli, & Tsay, 1998). Consistent with the view of this theory, by restricting children's contacts with delinquent peers, parental monitoring can prevent adolescents' involvement in problem behaviors (Dick, Viken, Purcell, Kaprio, Pulkkinen, & Rose, 2007; Westling, Andrews, Hampson, & Peterson, 2008). For instance, Westling *et al*. suggest that little parental monitoring was related to children's poor choice in friends, and as a result, middle and high school students committed deviant behaviors, including drinking. Likewise, Hwang and Akers (2006) demonstrate that peer influence is the mediator between parental supervision and Korean high school students' substance use.

It is important to note that numerous scholars measure parental monitoring by the adolescents' report of monitoring rather than the parents' own account of their monitoring practices (Nash *et al*., 2005; Wood *et al*., 2004). According to Barnes and Farrell (1992), although both mothers' and adolescents' reports of monitoring are negatively associated with the adolescents' alcohol use, because parents often perceive themselves as strict and providing more monitoring than what their children perceive, the use of the adolescents' report of parental monitoring is a relatively more conservative measure when compared with using the parents' report of monitoring. In a related vein, when assessing peer influence, studies typically include the adolescents' own account of influence from the peers rather than asking the respondents' friends' to report on their influence on the respondents (Hwang & Ackers, 2006; Nash *et al*., 2005; Wood *et al*., 2004). For this reason, the current study adopted Korean high school students' own account for peer influence and parental monitoring. Guided by socialization theory, the following hypothesis was posed to examine the mediating role of peer influence between parental monitoring and adolescents' alcohol use:

H3: Peer influence will mediate the association between parental monitoring and Korean adolescents' alcohol use.

1.2.2 Parent-Child Communication Satisfaction

Another perspective, the individuation-connectedness, also emphasizes parental influence on children's peer relationships (Youniss & Smollar, 1985). Youniss and Smollar suggest that, during adolescence, children make steps towards independence from parents while striving to stay connected to them, and this process that occurs in the environment of close relations with parents is optimal. If the parent-child relationship is one of interdependence and has a cooperative climate, adolescents would continue to seek parental support and allow parental influence over peer relationships (Steinberg, 2001; Youniss & Smollar, 1985). Parental responsiveness, which typically occurs during verbal interaction with children, is a correlate of children's social competence and choice of friends (Lamborn *et al*., 1991). In view of that, communication helps children maintain close

relationships with their parents because it promotes children's cognitive and social competence and results in parent-child satisfaction and more competence in adolescents' interactions outside the home environment (Steinberg, 2001).

The individuation and connectedness perspective suggests that parent-child communication, rather than unilateral parental monitoring, is the way in which parents influence adolescent children's peer relationships (Bray, Adams, Getz, & Stovall, 2001; Youniss & Smollar, 1985). Numerous scholars demonstrate that children and parents freely sharing emotional and factual information is indicative of good communication, and it may have a greater impact on children's behavior than parental monitoring alone (Cohen & Rice, 1995; Otto & Atkinson, 1997). In fact, Cernkovich and Giordano (1987) suggest that juvenile delinquents' home environment is short on communication about future plans or children's problems with friends or teachers, and the lack of communication with the parents may result in greater peer influence.

While research focuses on the importance of good parent-child communication on adolescents' peer relationships, it is relatively unclear whether knowledge and skills learned during communication *or* whether the satisfaction children feel from communicating with their parents is the drive behind for adolescents' behavior outside the home. Barbato, Graham, and Perse (2003) suggest that parents' primary motive for communication with their children is affection. In addition, children who communicate about various issues with their parents not only have satisfaction communicating with the parents; they also perceive relational satisfaction (Schrodt, & Afifi, 2007). Therefore, children who frequently communicate with their parents may sense parents' affection and care, and they may perceive relatively more communication satisfaction than those who rarely communicate with their parents. In line with the individuation and connectedness perspective, communication satisfaction, which signifies positive parent-child climate, might negate damaging peer influence, and adolescents may perpetrate behaviors that are in line with values that parents teach (i.e., making good friends and avoiding delinquent behaviors). Hence, we posed a hypothesis to look at the mediating role of peer influence between communication satisfaction and adolescents' alcohol use:

H4: Peer influence will mediate the association between parent-child communication satisfaction and Korean adolescents' alcohol use.

2. Method

2.1 Participants and Procedures

Five-hundred thirty-eight adolescents (363 male, 174 female) from four high schools in the Seoul metropolitan area in Korea participated in the current study. To provide representation from the different districts, four high schools were selected. Male participants were recruited from four high schools (n = 134, 91, 73, 65), and female participants were recruited from one of the high schools (n = 174). Because drinking is problematic among boys in Korea, most of the high school principals authorized male students to be the sample for the current study. As a result, male students were recruited from all four schools and female students were recruited from one school. Respondents' ages ranged from 14 to 17 years, and their mean age was 15.32 years (*SD* = 1.02).

Homeroom teachers announced the current study in their classroom and asked class leaders to administer survey procedures. When the teachers exited the classroom, the class leaders distributed paper survey booklets to the students. Students were informed that completing and returning a survey packet was entirely voluntary. To reduce obtrusiveness, only written directions were provided, and the class leaders did not interact with the students. The survey booklets themselves were anonymous as no personally identifiable information was collected. Researchers obtained institutional approval to collect the data in high schools.

2.2 Measurements

The measures used in the current study were translated into Korean by a researcher. Then, the translations were back-translated into English by a bilingual translator blind to the original English version. Next, the back-translated version was checked for consistency with the original. The back-translated version closely matched the original English version.

Wood *et al*.'s (2004) parental monitoring scale was used for the current study. This scale was based on Steinberg, Lamborn, Dornbusch, and Darling's (1992) strictness-supervision scale. Three items asked respondents what their parents actually know and what their parents attempt to know about their behaviors. Specifically, an example question read "How much do your parents try to know and (really know) about what you do with your free time?" Each item was answered with the following options: 1 = don't try or don't know, 2 = try a little or know a little and 3 = try a lot or know a lot. The *alpha* coefficient of the parental monitoring scale was .88 (*M* = 2.50, *SD* = .45).

Parent-child communication satisfaction was measured with a modified version of Hecht's (1978) interpersonal communication satisfaction questionnaire. Ten items asked how respondents generally describe their communication behavior with their father, and another set of identical questions asked about their communication behavior with their mother. An example questions include "I was very dissatisfied with conversations with him/her". Each item was followed by a 5-point Likert-type scale with 1 representing "strongly disagree" and 5 representing "strongly agree." The *alpha* coefficients of the father-child communication satisfaction scale was .88 (M = 3.29, SD = .76), and the mother-child communication satisfaction scale was .86 (M = 3.65, SD = .68). In the structural model, parent-child communication satisfaction was a latent variable with father-child and mother-child communication satisfactions subscales.

Peer influence was measured with a modified version of Williams *et al*.'s (1995) scale. The items for the scale were adopted from the research of Donovan, Costa, and Jessor (1985), Johnston, O'Malley, and Bachman, (1989), and Oetting, Beauvais, Edwards, Edwards, and Waters (1984). The peer influence scale asked how often the respondents' friends have asked them to (a) smoke cigarettes, (b) drink alcohol, and (c) get drunk. Each item was followed by a 5-point Likert-type scale with 1 representing "never" and 5 representing "many times." Since marijuana, smokeless tobacco, and cocaine are not accessible in Korea, the items concerning these types of drugs were not used in the present study. The *alpha* coefficient of the peer influence scale was .86 (M = 1.84, SD = .98).

Respondents' prior alcohol use was measured by a past alcohol use subscale from Perry and Grant's (1988) alcohol use tendency scale. To assess respondents' past alcohol use, four items asked how many occasions they have had alcoholic beverages to drink (a) during the last 12 months, (b) during the last 30 days, and (c) during the last 7 days. Each item was answered with the following options: 0 = never; 1 = 1 - 2 occasions; 2 = 3 - 5 occasions; 3 = 6 - 10 occasions; 4 = 11 - 20 occasions; 5 = 21 - 39 occasions; 6 = 40 or more occasions. The three-item prior drinking measure had the *alpha* coefficient of .85 (M = .64, SD = .91). The mean score indicates that respondents, on average, had no more than 2 instances of drinking events in the last year.

To assess respondents' drinking refusal self-efficacy, a modified version of Perry and Grant's (1988) drinking refusal self-efficacy scale was selected for the current study. The items asked how sure respondents were that they could say no if they were offered alcohol (a) at a friend's house, (b) by an older brother or sister, (c) by other older persons, and (d) at a party or dance. One question from the original scale that asked if respondents could say no when their boyfriend/ girlfriend offered alcohol was deleted for the current study because dating in high school is atypical in the Korean culture. Each item was followed by a 5-point Likert-type scale with 1 representing "could say no" and 5 representing "could not say no." The items were recoded so that higher scores represented high drinking refusal self-efficacy. The *alpha* coefficient of the scale was .90 (M = 3.24, SD = 1.14).

Drinking intentions was measured with a modified version of an alcohol use prospect scale (Perry & Grant, 1988). The scale asked how likely it would be that they would drink an alcoholic beverage if someone offered it to them (a) in the next 12 months, (b) in the next 30 days, and (c) in the next 7 days. Each item was followed by a 5-point Likert-type scale with 1 representing "likely I would not drink" and 5 representing "likely I would drink." The *alpha* coefficient of the scale was .88 (M = 2.63, SD = 1.06).

3. Results

The hypotheses were tested by Structural Equation Modeling. The model was built with Amos 18. This procedure was appropriate because structural equation modeling was able to clarify the direct and indirect associations in the test of multivariate hypotheses. Our model was developed by constructing the paths predicted by our hypotheses (please see Figure 1). Specifically, parental monitoring and parent-child communication satisfaction were the two exogenous variables predicting peer influence. Peer influence, prior drinking, and drinking refusal self-efficacy were antecedent endogenous variables, with the first predicting prior drinking, the second predicting drinking refusal self-efficacy, and the latter predicting drinking intentions. Finally, drinking intentions was the outcome endogenous variable.

All the variables were operationalized as latent variables, since the latent composite approach could "account for unreliability by extracting measurement error from the latent constructs used in the structural model" (Holbert & Stephenson, 2002, p. 534). Both direct and indirect effects of the related variables were calculated. A bootstrap for each model (number of bootstrap samples is 2000) was performed, and 95% bias-corrected confidence intervals were used to test the significance of the direct and mediation effects. To gauge the fit of the structural equation models, an omnibus model fit was evaluated using the comparative fit index (CFI) and the root mean squared error of approximation (RMSEA). Prior criteria we used were .90 for CFI and .08 for RMSEA. In addition, given the guidelines of Hoyle and Panter (1995), the chi-squared distributed goodness of fit test was

also reported. Results of the analysis revealed that our model did not meet the established priori criteria, χ^2 (df = 14, N = 537) = 359.57, p < .001 CMIN/df = 25.68, CFI = .69, RMSEA = .21.

Subsequently, we made modifications in the model. First we removed paths one by one based on the Lagrange multiplier test, and afterward, we inserted paths one at a time based on the Wald's test (see Knobloch, Solomon, & Cruz, 2001, for an overview of this procedure). We eliminated one path from the proposed model as a result of the Lagrange multiplier test (Fox, 1997): The path from parent-child communication satisfaction to peer influence. Because eliminating the path made parent-child communication satisfaction variable unidentifiable (i.e., there was no path assigned from or to this variable), this model could not be tested. Then, we added paths to the model using the Wald's test (Fox, 1997). When adding paths, we were very careful to follow previous research. Based on the findings of previous work that suggest a positive link between parent-child communication and parental monitoring (Stattin & Kerr, 2000), we examined the association. In view of that, a path from parent-child communication satisfaction to parental monitoring was added.

Two additional paths were added in the model: A path from parental monitoring to drinking refusal self-efficacy and another path from peer influence to drinking refusal self-efficacy. Watkins *et al*. (2006) suggest that parental monitoring is positively associated with adolescents' drinking refusal self-efficacy. Adolescents who believe they receive a lot of parental monitoring also believe that they have high drinking refusal self-efficacy. Nash *et al*. (2005) similarly suggest that family environment, which includes parental monitoring, is positively associated with adolescents' drinking refusal self-efficacy. Based on the findings, a path from parental monitoring to drinking refusal self-efficacy was added. Next, Young, Hasking, Oei, and Loveday (2007) argue that "the role of refusal self-efficacy in the development and maintenance of drinking behavior, including in situations of *peer pressure*, is well established" (p. 863). Given that the drinking refusal self-efficacy measure reflects peer pressure refusal self-efficacy, it is evident that peer pressure may sway individuals' drinking refusal self-efficacy (Young & Oei, 2000). Thus, we added another path from peer pressure to drinking refusal self-efficacy. Finally, based on the previous research that suggest a strong association between prior drinking and drinking intentions (Aas *et al*., 1995), a path from past drinking and drinking intentions was added.

After we made these modifications, the revised model was consistent with the data and established priori criteria, χ^2 (df = 11, N = 537) = 15.40, p = .17, CMIN/df = 1.40, CFI = .996, RMSEA = .027. Please see Figure 2 for the revised model. The direct effect of drinking refusal self-efficacy on drinking intentions (*H1*) (β = - .43, p < .001) and past drinking on drinking refusal self-efficacy (*H2*) (β = - .23, p < .001) were significant. Thus, *H1* and *H2* were supported. The indirect effect of parental monitoring on drinking behavior, mediated by peer influence was also significant (*H3*) (standardized mediation effect = - .09, p < .01). The Sobel mediation test was also conducted to examine the mediating role of peer influence between parental monitoring and drinking behavior. The analysis showed that the indirect effect of parental monitoring on adolescents' drinking was significant (z = - 3.32, p = .001). Accordingly, *H3* was supported by the current data. We were unable to fully examine *H4* since the link between parent-child communication satisfaction and peer influence was removed during the initial stage of the analyses. The revised model, however, suggests that parent-child communication satisfaction is positively associated with parental monitoring (β = .46, p < .001). Thus, *H4* was not supported. Finally, the total indirect effect of parent-child communication satisfaction on adolescents' drinking intentions, mediated by parental monitoring, peer influence, prior drinking, and drinking refusal self-efficacy, was significant (standardized mediation effect = - .03, p < .01).

4. Discussion

The current study examined the function of parental influence, peer influence, and prior drinking on Korean high school students' drinking refusal self-efficacy. The revised model revealed that a number of factors directly influence Korean adolescents' drinking refusal self-efficacy, including prior drinking, parental monitoring, and peer influence. Consistent with social cognitive theory (Bandura, 1986), adolescents' drinking experience was a factor that predicts their self-efficacy regarding drinking refusal. Adolescents who had consumed alcohol in the past reported that they have relatively low drinking refusal self-efficacy compared to abstinent counterparts. In addition, the results of this study extend previous work by indicating that parental factors, including parent-child communication satisfaction and parental monitoring, are uniquely linked to adolescents' alcohol use through peer influence. And, adolescents' drinking refusal self-efficacy was a mediator between prior drinking and their intentions to consume alcohol in the future. The discussion below will highlight the findings with regard to the revised structural model and discuss the present findings in terms of previous research.

4.1 Interpretations of the Findings

In order to fully identify the factors that influence adolescents' drinking refusal self-efficacy, we first sought to understand what motivated those adolescents with prior alcohol experience to initiate drinking. Based on a number of theories (i.e., developmental theory, socialization theory, individuation-connectedness perspective) that suggest a mediating role of peer influence between parental influence and children's drinking (Patterson *et al*., 1989; Steinberg, 2001; Wood *et al*., 2004), we examined the indirect effect of parental influence on adolescents' drinking through peer influence. Indeed, findings of the current study revealed that parental monitoring is associated with peer influence, and peer influence, in turn, is related to adolescents' alcohol use. In line with the notion of socialization theory, parents could protect their children from peer influence by monitoring their activities and whereabouts outside the home (Grusec, & Davidov, 2010). By restricting children's contacts with problem peers, parental monitoring can prevent adolescents' participation in underage drinking (Westling *et al*., 2008). The findings also imply that adolescents who receive relatively little parental monitoring are susceptible to greater peer influence, which directly impacts their alcohol use. The finding also supports previous research that demonstrates the positive association between peer influence and adolescents' drinking (Hwang & Akers, 2006; Wood *et al*., 2004).

However, parent-child communication satisfaction does not have an effect similar to that of parental monitoring on peer influence. Instead, parent-child communication satisfaction facilitates parental monitoring. In agreement with this result is Stattin and Kerr's (2000) findings that highlight the effects of good parent-child communication on parental monitoring practices. Our data indicated that parent-child communication satisfaction was another contributor to Korean adolescents' perceived parental monitoring. Because adolescents are likely to share information about their whereabouts and activities outside the home during communication with the parents, those adolescents who are content communicating with their parents are more likely to believe that their parents know a lot about their daily activities. Consistent with this idea, Cohen and Rice (1995) found that parent monitoring and maintaining communication with parents protected adolescents from substance use.

In addition to the effects of Korean adolescents' prior drinking on their drinking refusal self-efficacy, the present research revealed that parental monitoring and peer influence directly influence their drinking refusal self-efficacy. According to Watkins *et al*. (2006), a high level of parental monitoring may allow adolescents to believe that they can refuse drinking alcohol. Another study that supports the idea is Nash *et al*.'s (2005) study that reports family environment, which includes parental monitoring, is positively associated adolescents' drinking refusal self-efficacy. Nash *et al*. suggest that peer influence mediates the link between family influence and alcohol use; however, they are mute about the link between peer influence and drinking refusal self-efficacy. Although these findings are conclusive, explanations for the effects of parental monitoring and peer influence on drinking refusal self-efficacy are incomplete. The results of the current study are consistent with social cognitive theory (Bandura, 1986), which suggests the importance of environment (i.e., family and friends) on shaping individuals' self-efficacy perceptions. Our respondents who had high level of parental monitoring had relatively little peer pressure, and they also believed they had a relatively high drinking refusal self-efficacy. By contrast, those who reported having little parental monitoring also reported having relatively more peer pressure, and they perceived having a relatively low drinking refusal self-efficacy. Consistent with Steinberg's (2001) argument, our findings show that high level of parental monitoring could not only negate peer influence, it could also boost children's self-efficacy. In a similar vein, peer influence may predict adolescents' drinking refusal self-efficacy. Adolescents who perceive little parental monitoring are in a vulnerable condition for peer influence. Social cognitive theory (Bandura, 1986) suggests that one method to increase self-efficacy is to be with people who are positive about and successful in achieving goals and outcomes. Close friends' alcohol use may influence adolescents' internalization of the behavior and increase confidence in their own ability to drink alcohol themselves. As a result, Korean adolescents who are heavily influenced by their peers may perceive low drinking refusal self-efficacy.

It is also important to note that Korean adolescents' prior drinking not only influences their drinking intentions indirectly through drinking refusal self-efficacy, it also directly affects their drinking intentions. Korean adolescents with drinking experience are more likely to have intentions to use alcohol in the future when compared with the abstinent counterparts. The result is consistent with previous research that suggests past substance use can be a good predictor of future substance use (Patrick, Wray-Lake, Finlay, & Maggs, 2010). Eagly and Chaiken (1993) explain that because behavior over time is the result of people's personal and motivational attributes that are common to the events in which the behavior occurs, they generally act consistently.

4.2 Implications

Taken as a whole, our findings add to the literature that emphasizes the importance of adolescents' drinking refusal self-efficacy in predicting their drinking intentions. Specifically, our data indicated that parental monitoring, peer influence, and prior alcohol use are uniquely associated with adolescents' drinking refusal self-efficacy, and drinking refusal self-efficacy, in turn, predicts their drinking intentions. These findings support Bandura's claim that successful experience predicts self-efficacy, and self-efficacy predicts future behavior. Bandura (1986) also suggest that environment affects people's behavior and attitude. Consistent with this idea, our findings demonstrate that when adolescents are in an environment where the parents closely watch their behaviors and are influenced less by peers, they may perceive relatively high self-efficacy to refuse drinking. The findings considerably add to the literature on antecedents of adolescents' drinking refusal self-efficacy as research on this area is limited.

Another notable contribution of the current study involves our use of a Korean high school sample. As influenced by Confucianism, Asian families or families in a collectivistic culture tend to follow a traditional family structure, characterized by a high degree of cohesiveness and hierarchy (Yum, 1988). In traditional Asian families, parents make the decisions for the family, and children are expected to respect these decisions with compliance (Hofstede, 1991). As a consequence, parental authority is often viewed as customary. Rohner and Pettengill (1985) suggest that Korean high school students' perceived parental control is positively associated with parental warmth and caring. Although adolescents in Western cultures may view parental strictness as aggressive (Rohner & Pettengill, 1985), Korean adolescents may interpret strict parental monitoring as an expression of parental warmth and caring. Therefore, the influence of parents may be more distinct and seen as positive in Eastern cultures than in Western cultures. This may explain the parental influence we found in the current study.

There are a number of practical implications based on this investigation. It is thought that children are heavily influenced by their peers' deviant behaviors (Monahan, Steinberg, & Cauffman, 2009). Although peer influence may be an important predictor of adolescents' drinking behavior, we found evidence of the effects of parental influence on children's drinking. Based on the findings of this research, alcohol prevention interventions need to keep considering parents in the overall strategy. The message is clear: Parents should get actively involved to reduce children's alcohol use by engaging in communication with children to monitor their alcohol-related behaviors. These efforts may influence their adolescent children's drinking refusal self-efficacy perception, and may ultimately decrease children's drinking. We learned that parent-child communication satisfaction is an effective way to show parental monitoring. Children who are satisfied conversing regularly with their parents also believed that they are closely observed by their parents.

4.3 Limitations

A number of limitations of the current research must be recognized. One of these involves the use of retrospective self-reports. Participants may not have accurately recalled or reported their attitudes and behavior (Bernstein, Erdfelder, Meltzoff, Peria, & Loftus, 2011). Since asking adolescents directly about their intentions to drink alcohol is a simple and sensible way to evaluate their intentions, we utilized this method. Another limitation of the present study is the sample. The current sample is the 2 to 1 ratio of males to females. While independent sample t-tests of the study variables suggest that none of the variables exposed sex differences, this sample bias may limit the ability to generalize the results. Further, because the influence of parents may be more marked in Eastern than in Western cultures (Hofstede, 1991), findings of this study may be less generalizable to Western cultures. In addition, we certainly understand that the present investigation is unable to determine the causal directions of the aforementioned effects. More research needs to be done to fully understand the effects of parents and peers on adolescents' drinking refusal self-efficacy and drinking intentions.

5. Conclusion

In conclusion, the main purpose of the present study was to identify the factors that influence Korean adolescents' drinking refusal self-efficacy. Findings revealed that parental monitoring and peer influence directly and indirectly predicted drinking refusal self-efficacy through adolescents' alcohol use. We identified the impact of parents and peers on Korean adolescents' drinking. Furthermore, this study revealed that those factors are antecedents of the adolescents' drinking refusal self-efficacy, which, in turn, predicts their drinking intentions. Given the results of our study, we are convinced that these constructs should be the key attributes researchers should consider when studying Korean adolescents' alcohol use.

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Table 1. *Zero-Order Correlation Matrix of the Study Variables*

	1	2	3	4	5	6	7
1.1. Parental monitoring	--						
2. Father-child comm. satisfaction	.31***	--					
3. Mother-child comm. satisfaction	.35***	.68***	--				
4. Peer influence	-.16***	-.08	-.08	--			
5. Past alcohol use	-.17***	-.09*	-.11**	.57***	--		
6. Drinking refusal self-efficacy	.19***	.14***	.13**	-.46***	-.42***	--	
7. Drinking intentions	-.18***	-.14***	-.14***	.49***	.66***	-.63***	

Note. *$p < .05$, **$p < .01$, ***$p < .001$

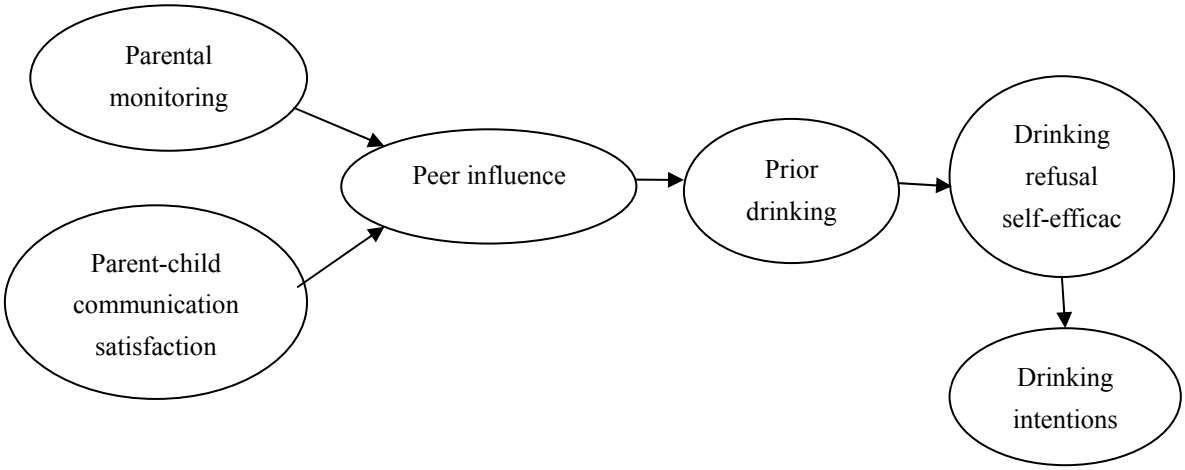

Figure 1: Hypothesized Model

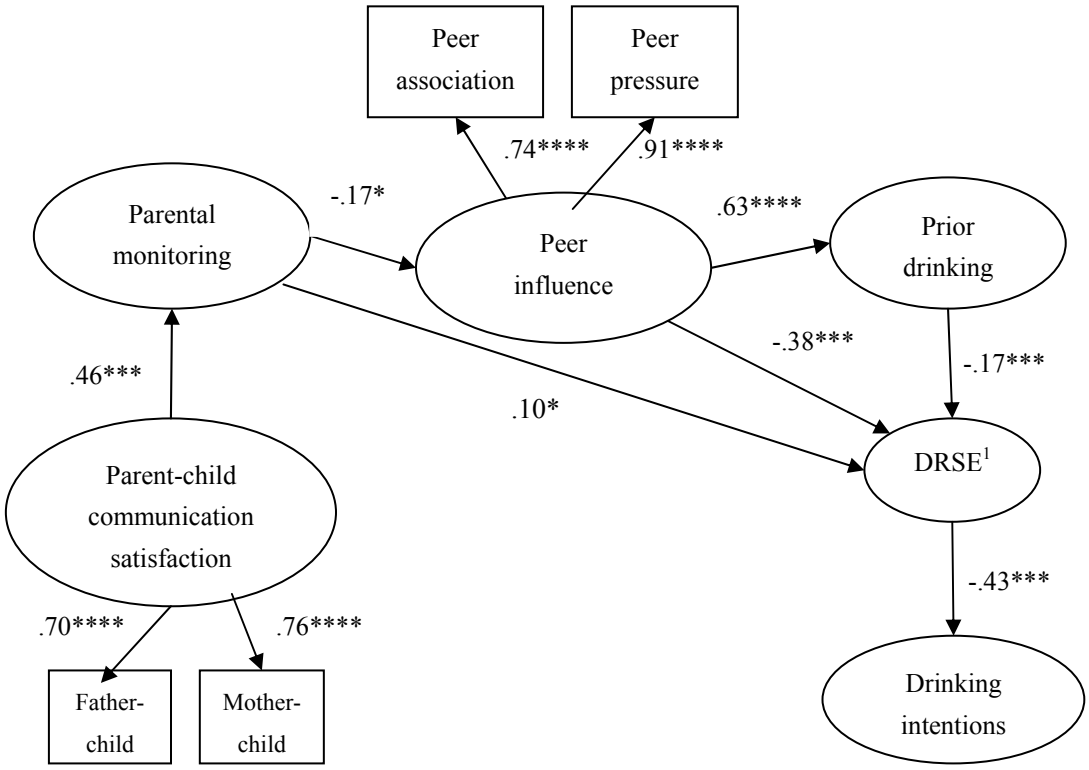

Figure 2: Revised Structural Model

Note: [1] Drinking refusal self-efficacy. All parameter estimates are standardized.

* $p < .05$, *** $p < .001$

Process Evaluation of a Psychosocial Intervention Addressing Women in a Disadvantaged Setting

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Abstract:

Objectives: This paper presents the process evaluation of a community-based randomized psycho-social trial aimed to enhance reproductive and mental health outcomes of disadvantaged women living in the southern suburb of Beirut-Lebanon. Process evaluation of public health interventions involves the monitoring and documentation of interventions' implementation to allow for better understanding of planned outcomes and of intervention effectiveness. **Methods:** A community-based randomized trial was conducted. The intervention consisted of 12 sessions (of combined 30 minutes of relaxation exercises and 75 minutes of structured support groups) delivered twice per week over a period of six-weeks. A process evaluation was conducted during the implementation of the intervention. This process evaluation aimed to ensure that the intervention was delivered and implemented as planned, as well as to monitor women's satisfaction and attendance. The main components of the process evaluation included: dose delivered, dose received, and reach. Closed ended questionnaires were administered before/after/during each intervention session. Data was entered and analyzed using SPSS. Analysis revolved around simple frequency distribution for categorical variables and means (SD) for continuous variables. Limited bivariate analysis (using CHI Square and Anova) was done. **Results:** Results indicated that the delivery, implementation, and reach of the intervention were favorable. Participation was acceptable and satisfaction rates were very high. **Conclusion:** These favorable findings pertaining to intervention satisfaction, reach and participation highlight a number of lessons for future intervention studies in the context of disadvantaged settings. They also support the importance of involving the local community members in intervention planning, implementation and evaluation early on. We believe that the community involvement in this trial directly and significantly contributed to the results of this process evaluation.

Keywords: Process Evaluation, Psycho-social intervention, Disadvantaged Population, Hey el Selloum

1. Introduction

Process evaluation of public health interventions involves the monitoring and documentation of interventions' implementation to allow for better understanding of planned outcomes and intervention effectiveness. Possible challenges to intervention implementation could include low participation and attendance, as well as discrepancy in delivery of what was planned as an intervention (dose intended), and what was delivered or received by participants (actual dose received) (Johnson *et al*., 2009). Process evaluation findings could provide confirmation that the program activities are associated with the outcomes observed (McDonald, 2009). The literature provides numerous frameworks for process evaluation, one of which: is Linnan and Steckler's, 2002. This framework outlines the basic elements of a comprehensive process evaluation plan, which include: a measure of fidelity, dose delivered, dose received, reach, recruitment, and context. *Fidelity* is "the extent to which the intervention was delivered as planned" (Oude Hengel *et al*., 2010, p.5). *Dose delivered* is how much of the intended intervention was delivered as planned. *Dose received* is satisfaction or the "extent to which participants actively engage with, interact with, are receptive to, and/or use materials or recommended resources" (Linnan & Steckler, 2002, p.12). *Reach* is defined as "the degree to which the intended audience participates in an intervention" (Linnan & Steckler, 2002, p.12). *Context* includes characteristics of the environment that may affect implementation development including the physical, social, and political environment (Linnan & Steckler, 2002 p. 12).

This paper will provide a review of the findings of the process evaluation of a community-based randomized psycho-social trial, aimed to enhance reproductive and mental health outcomes of disadvantaged women living in the southern suburb of Beirut-Lebanon. This process evaluation will report on dose delivered, dose received, and reach (the measured process evaluation components as agreed by the research team). Although, there is a multiplicity of public health programs conducted among women by various groups in Lebanon, to our knowledge, this is the first peer reviewed article on process evaluation of an intervention among disadvantaged women in a low income setting in Beirut, Lebanon. This paper will also provide a description of the intervention context, content, evaluation data collection tools, and finally report on process evaluation findings.

2. Methods

2.1 Description of the Context

The intervention setting was in Hay el Sellom (HES), in the southern suburbs of Beirut, the capital of Lebanon. HES is an informal settlement, stretched over an area of less than 1 Km^2 and housing approximately 100,000 to 150,000 inhabitants. During the Lebanese Civil war period, people flocked into the area, which was originally a farming land and built concrete houses with no consideration to urban planning, building laws or basic infrastructure. Due to the prolongation of the Lebanese civil strife, the people forcedly stayed in this area, seeking security and employment. As a result of this unplanned movement, the sporadic and illegal construction resulted in insufficient basic physical infrastructure- thus creating a relatively crowded neighborhood. Despite the end of the

civil war, the government still does not recognize the area in legal terms, and continues to pay little or no attention to providing it with public services (Kobeissi *et al*., 2011; Makhoul, 2003).

2.2 Description of the intervention

The trial aimed to test the impact of a psychosocial intervention package (of combined 30 minutes of relaxation exercises and 75 minutes of structured support groups delivered twice per week over six weeks) on the reporting of medically unexplained vaginal discharge (MUVD)- and common mental disorders (known as CMDs: namely anxiety and/or depression). The package was conducted among 196 currently married women, 18-49 years of age with reported symptoms of MUVD and low to moderate levels of CMDs. Ninety nine women were randomized into the intervention group and 97 were randomized into the control group (Kobeissi *et al*., 2011).

The components and the duration of this intervention package were guided by evidence-based literature supporting the positive impacts of structured social support on disease (Targ & Levine, 2002; Telch & Telch, 1986; Fung & Chien, 2002, Manandhar *et al*., 2004; Bryce, Stanley & Graner, 1991; Panzarella *et al*., 2006; Lumley *et al*., 2006; Dennis, 2005; Constantino *et al*., 2005; Scheidlinger & Kahn, 2005; Wiggins, 2004) and mental health outcomes (Magliano *et al*., 2002; Araya *et al*., 2003; Sumathipala *et al*., 2000; Chen *et al*., 2001; Ning *et al*., 2001; Li, 2001; Krawczynski & Olszewski, 2000; Constantino *et al*., 2005; Scheidlinger & Kahn, 2005; Wiggins, 2004). Also a review of the literature supports the positive impacts of progressive muscle relaxation and/or guided imagery on mental health and persistent unexplained physical symptoms (Kominars, 1997; Baird & Sands, 2004; Crook *et al*., 1998; Baider *et al*., 2001; Carlson & Bultz, 2004). The main aim behind this trial was to develop a simple low cost community-based intervention that has the potential to be later delivered, and easily sustained, at the level of the local community centres (if results deemed favourable).

2.3 The intervention Content

The intervention package consisted of 12 sessions implemented over a six week period using the facilities of the Lebanese Ministry of Social Affairs (MOSA) local center in HES). The 75 minutes semi-structured social support sessions (SSS) were run by masters' level psychologists who moderated the sessions and were assisted by social workers as co-moderators. The SSS sessions were divided into directed and semi-structured social support discussion sessions, incorporating problem solving skills building training coupled with venting (Table 1). To ensure coherence among group members, the group was closed; meaning that once a group was formed its members remained in the same group throughout the intervention period. A manual was developed by a senior doctorate level clinical psychologist highlighting the content of each of the 12 sessions and was pilot-tested with women of similar characteristics. This manual was delivered to the moderators and co-moderators during training sessions, where it was modified and finalized accordingly with the team.

The 30 minutes **relaxation exercise (RE)** sessions were run by physical trainers. The relaxation exercises revolved around the delivery of four main components: progressive resistance training**,** progressive muscle resistance, stretching and breathing, and guided imagery (Table 1). These components were introduced into the sessions gradually, starting at 15 minutes with easy complexity and reaching up to 30 minutes with a relatively harder complexity (by the fourth session). These exercises also included teaching women how to engage in visual guided imagery exercises on their own at home. The sessions were intentionally progressive in nature in order to properly build up resistance. Each new session of the RE package would start with a wrap up about the techniques conducted in the previous sessions, touch at what is being practiced at home and proceed by adding an additional new technique. A manual was also developed by a physical fitness specialist who supervised and trained the physical trainers during a two-day training workshop. The manual was provided for the physical trainers as a guide on each of the sessions. In addition, a brief pamphlet was given to women participants to describe the components of each session.

The implementation and evaluation team included: five clinical psychologists, five social workers, five physical trainers, five process evaluators recruited from the local women's committee (LWC), a field assistant and two supervisors.

<Table 1>

2.4 Data Collection Tools

Process evaluation analysis revolved around: Dose delivered, dose received and reach. Process evaluation data was collected from each session using developed data collection tools to capture the desired components (Table 2). These tools were mainly close-ended questionnaires delivered either before the start of the session to monitor attendance, during the session to assess dose received and reach and at the end of the session to assess satisfaction. All women in the sessions were administered the evaluation tools (questionnaires) by the members of the LWC

and trainers who were trained on data collection. The LWC and the trainers were responsible for this assessment in the RE component of the intervention; while, the co-moderators were responsible for this assessment in the SSS. The co-moderator was assigned for this task in the SSS, in order to maintain the privacy and confidentiality of issues discussed within a group. Hence, no outside evaluator was assigned. These different data collection tools (questionnaires) were pretested during the pilot phase and adjustments were made, as suggested by the LWC and the trainers, accordingly. For example, in the RE component, when rating the extent to which activities were implemented as planned, the initial questionnaire had listed in detail every muscle that the exercise suggested to cover in each session. The LWC thought it was not feasible to evaluate to that level of detail. The evaluation questionnaire was adjusted accordingly and required only noting overall if suggested exercises were implemented or not. One member of the implementation team was assigned to follow-up on the process evaluation, to ensure all forms were filled in due time, and to collect completed forms at the end of each session.

<Table 2>

This trial gained ethical approval by the institutional review board of the American University of Beirut. Two different consent forms were used in the intervention trial: recruitment/baseline assessments and post randomization/post assessments. The first consent form was used during the study recruitment phase to check whether or not the woman conforms with the study inclusion criteria, particularly with respect to the reporting of the reproductive health and common mental distress outcomes. This consent form was read in private to the women orally by an interviewer and in front of a witness prior to examination.

The second consent form was administered to women who were found eligible to take part in the trial. The purpose of this consent form is to make sure that the women understand the randomization process and the reasons behind their selection in the trial. It also made sure that the women agree to comply throughout the trial's period, to being randomized into either the intervention or the control group, and do to having repeated post assessments, clinical exams and laboratory tests. The interviewer explained the consent form to the women privately, which marked the initiation of the intervention trial.

2.5 Analysis

The data was entered by trained personnel using the Statisitical Package for Social Sciences (SPSS) software. Data entry error checks were made by randomly selecting 10% of the questionnaires. An independent research assistant was responsible for undertaking the error checks. Analysis revolved around simple frequency distribution for categorical variables and means (Standard Deviation (SD)) for continuous variables. Limited bivariate analysis (using CHI Square test and Anova) was also done.

3. Results

With regards to ***dose received***, in the evaluation of the RE component, trainers and LWC reported 98% and 94% of the sessions as positive and active, respectively. In the SSS components, the co-moderators reported 88% of the sessions as positive and active. With regards to women's participation rate in the RE component, the trainers and LWC reported 100% and 97% respectively. In the SSS component, the co-moderators reported 100% participation (Table 3).

<Table 3>

For the Relaxation exercise sessions and the SSS sessions, 76% of women had had an over all satisfaction score of 10. (Figure 1)

<Figure 1>

With regards to ***dose delivered***, in the evaluation of the RE component, trainers reported 97% and LWC 99% of the sessions were completed as per the manual. In the SSS component the co-moderator rated 93% and moderator 99% sessions completed as per manual (Table 4).

<Table 4>

In terms of reach, in the RE component 32.2% of women attended 90% of the sessions, 20.9% of the women attended 70-90% of the sessions, 11.3% attended 50-69% , 20.9 % attended less than 50%, and 14.8% none of the sessions (Figure 2). A similar trend of attendance was observed in the SSS sessions. This is expected since the sessions were consecutive to one another, thus, if one woman attended one it was likely that she would also stay for the following one.

<Figure 2>

4. Discussion

The objective of this process evaluation was to ensure that the intervention was delivered and implemented as planned as well as to monitor women's satisfaction and attendance. Overall, process evaluation indicated favorable results pertaining to the delivery and implementation of the intervention as intended and planned. Over 90% of the intervention dose was delivered as outlined in the manual. Sessions were rated as positive and active, and the women were evaluated to have actively participated in the delivered sessions. Thus, participation and satisfaction was very high. Similarly, satisfaction rates were high for each of the two components of the intervention package. Intervention reach, on the other hand, was acceptable. Up to 33% of the women actually attended all 12 sessions. This is contingent with results of studies on process evaluation reported elsewhere on other public health interventions (Anderson, 1985; Dynes *et al*., 2011).

A review of the literature did not result in papers reporting on process evaluations of women of similar age group and intervention. The published literature reports on process evaluations of a variety of public health interventions and varies in the measures used for process evaluation. Anderson (1985), in an evaluation of a psychological intervention among 60 year old or older women (in an aim to decrease loneliness in its social and emotional dimensions), reported 28.6% of the women (total study sample was 64 women) attending all for intervention meetings. This article, however, does not include data on dose received or delivered. A process evaluation of a maternal child health programme that involved a home-based life saving skills program (in an attempt to reduce mortality rates among mothers and children) in Bangladesh reported that over 51% of pregnant women attended all meetings (total study sample was 4500 women) (Dynes *et al*., 2011). The largest percent (85.5%) received 2 sessions, followed by around 70% who received three sessions (Dynes *et al*., 2011).

With regards to dose delivered, a paper reporting on evaluating nurses' implementation of an infant-feeding counseling protocol for HIV-infected mothers: The Ban Study in Lilongwe, Malawi, reported that all 6 nurses implemented the protocol at an acceptable level of 90% implementation adherence or above. This study reported only on implementation but did not include measures of satisfaction, participation, or reach (Ferguson *et al*., 2009).

Our process evaluation findings reveal acceptable attendance rates (> 50% of the women attended 70% of the sessions or more). Reasons given for non-attendance by the women were mostly in line with what the literature cites. In terms of reasons, women mainly responded: sickness in the family death of family member, family commitment (relatives visiting for leisure/vacation), husbands' refusal, work, the intervention locale was far from their houses, or refusal to participate because of the lack of financial benefits. The literature provides a review of challenges to compliance in interventions, which could include: lack of follow-up and contact with subjects, women's employment (Yancey *et al*., 2006), family obligations, lack of proper telephone for communication (Beckie *et al*., 2009). This is consistent with the reasons we found out during intervention delivery due to lack of attendance.

However, despite these barriers, attendance was considered acceptable, particularly given the uniqueness of the trial's location: a disadvantaged setting in the suburbs of the capital. We believe that the implemented community based-participatory approach played a significant role in actively mobilizing the different members of the community. This has strengthened peoples' trust and rapport with the research team, and in turn rendered high commitment to attendance. Working from within was the guiding principle behind implementing this work. Employing a women's committee, whose members were local women, through out the three years of the trial (and since the inception of the project proposal), also equally and significantly rendered the delivery of this intervention successful, and well received at the community level. Further to this, the timing of the delivery of the intervention took into account women's time. The sessions were intentionally delivered during the mornings (between 8am-12:00 pm) allowing time for the children to go to school as well as giving a leeway to go back home for house chores before the children came back from school. Child care at the site of the intervention was also provided in order to accommodate for women with new born babies as well as with little children. In order to further entice women, little incentives were also provided such as training suits (in order to make the less economically privileged women feel at ease about their attire) and incorporating music during the relaxation exercises, which was very well received by participants.

Similar adopted strategies to increase attendance are noted in the literature. For example, Sears *et al*. (2003) suggested the importance of providing transportation, childcare, flexible appointment times as a way to avoid attendance associated problems. Also, distance between home and intervention locations (Oleson *et al*., 2008) as well as timings (Fredman et. al., 2009) were likely cited barriers to attendance.

A number of lessons for future intervention studies in disadvantaged settings could be learnt from our experience. Our findings support the importance of involving the local community in intervention planning and

implementation early on- as we believe this was critical and significantly contributed to the high satisfaction rates as well as our acceptable attendance rate. Involvement of local women in process evaluation gave the study credibility and full trust by the participating women. It also allowed for better communication of objectives and plans. The proximity of the intervention locale to women's homes also meant that they did not have to worry about transportation. The location was both convenient and familiar to participating women. Findings that showed high levels of participation and satisfaction provide a good evaluation of the trainers implementing the intervention. The process evaluation documented the appropriate delivery of the intervention in terms of dose received and dose reached. Findings will help interpret the outcome data.

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Competing Interests: Nothing to Declare

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Table 1. Overview of intervention package

Social Support Component	**General outline of Content**
First two sessions	Introductions and agreement on group rules, ice breaking
Sessions 3-23	Welcoming and attendance taking, getting feedback on the previous session, deciding on the topic of the day, facilitating discussion, teaching problem solving skills, and wrap up of session's conclusions
Session 24	Concluding with a debriefing of gained skills and group experiences. It was accompanied with a farewell ceremony.
Relaxation exercise component	
Progressive muscle relaxation (PMR)	The program focuses on 16 muscle groups which are relaxed in a specified order and lasts for not more than 5 to six minutes to avoid over cooling of the body. Since it is a progressive technique, the relaxation is achieved over a long period of time.
Guided Imagery (GI)	People are coached to create calming, peaceful images to induce relaxation. A pleasant imagery from the past is imagined whereby the details of this image is recalled and relaxation during this imagination process is experienced.
Stretching and Breathing	Promotes relaxation and reduces stress Breathing techniques usually accompany stretching exercises to achieve the desired effect.
Progressive Resistance training (PR)	Progressive resistance is a training that improves muscular fitness. PR is characterized by gradual increases in resistance (weight) over a certain period of time leading in muscular strength and or endurance exp. Weight training is the type of progressive resistance that is highly suggested for improving muscular fitness where using elastic bands can be a part of it.

Table 2. Process evaluation component and description of corresponding data collection tools

PE component	**Form**	**Description**
Reach	A- Attendance	Attendance was taken at the beginning of each session, noting late arrivals and women who left early, if any. Each woman was provided with an ID number to accompany her throughout the intervention period.
Dose received	B- Women Satisfaction	Following each session, the evaluators (the LWC in RE and the co-moderator in SSS) asked the women to indicate on a sheet on a scale of one to ten the extent of satisfaction with the session; with one being the *least* satisfied and ten the *most* satisfied.
	C- Participation	Following each RE session, based on their observation, the trainer and LWC noted the extent of participation and involvement of the women. Following each of the SSS sessions, the moderator filled in a similar evaluation.
Dose delivered	D-Intervention delivery	Following each session, for the relaxation exercise, the trainer and LWC and for the SSS, the moderator and co-moderator evaluated the extent to which the activities were implemented as planned in the manual.

Table 3. Dose received. Participation

	% women rating the session as positive and active		**% of active participation**	
Data collected by :				
Relaxation exercise	Trainer	LWC	Trainer	LWC
All	97.5	94.2	100	96.7
Some	2.5	2.5		
Data collected by :				
SSS	Co-moderator	Moderator	Co-Moderator	Moderator
All	88.3	--	100	-
Some	11.7			

* *n= 120 sessions*

Table 4. Dose delivered. Intervention delivery

% Session completed as per manual		
Data collected by		
Relaxation exercise	Trainer	LWC
Fully	96.7	99.2
Partially	0.8	0.8
Data collected by		
SSS	Co-moderator	Moderator
Fully	93.3	99.2
Partially	6.7	0.8

* *n= 120 sessions*

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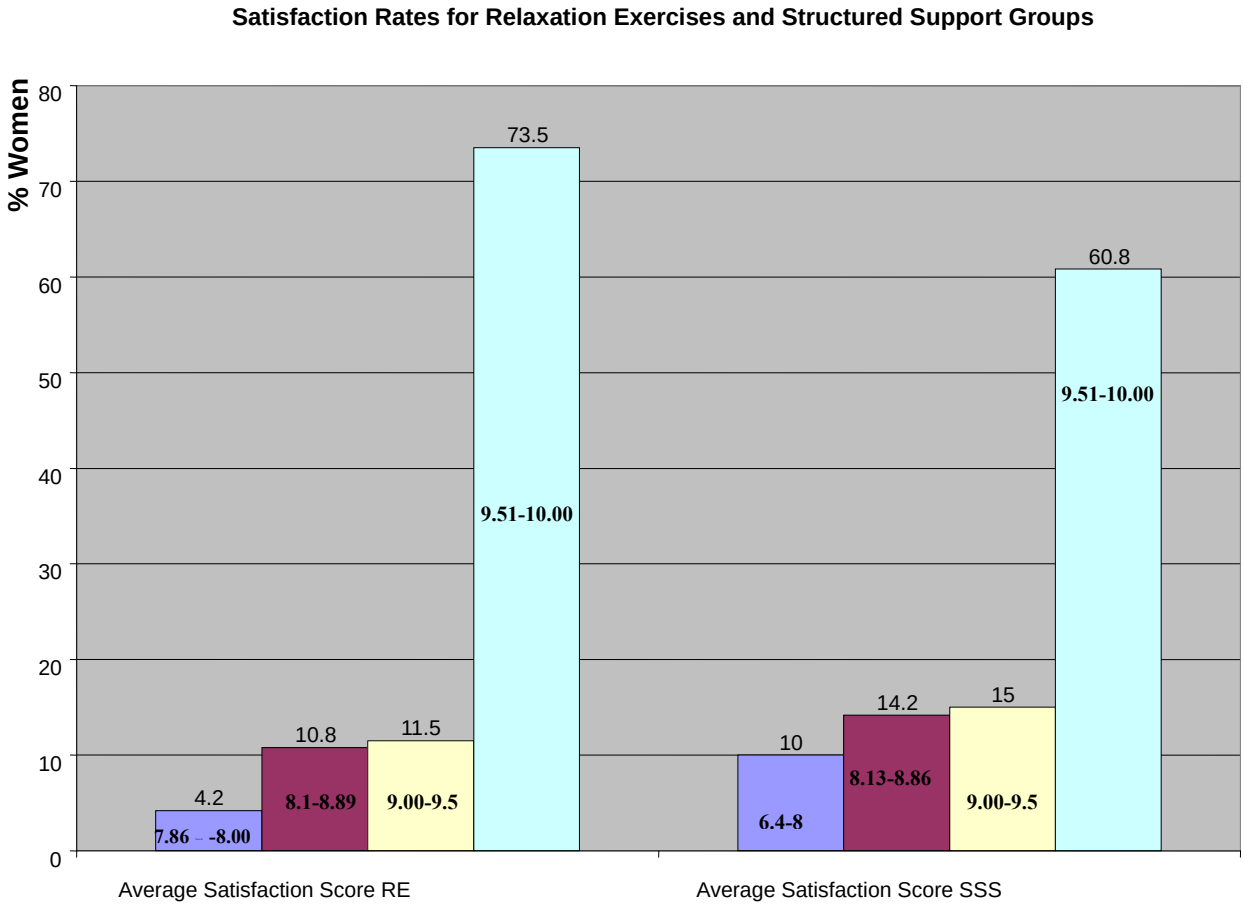

Figure 1: Satisfaction

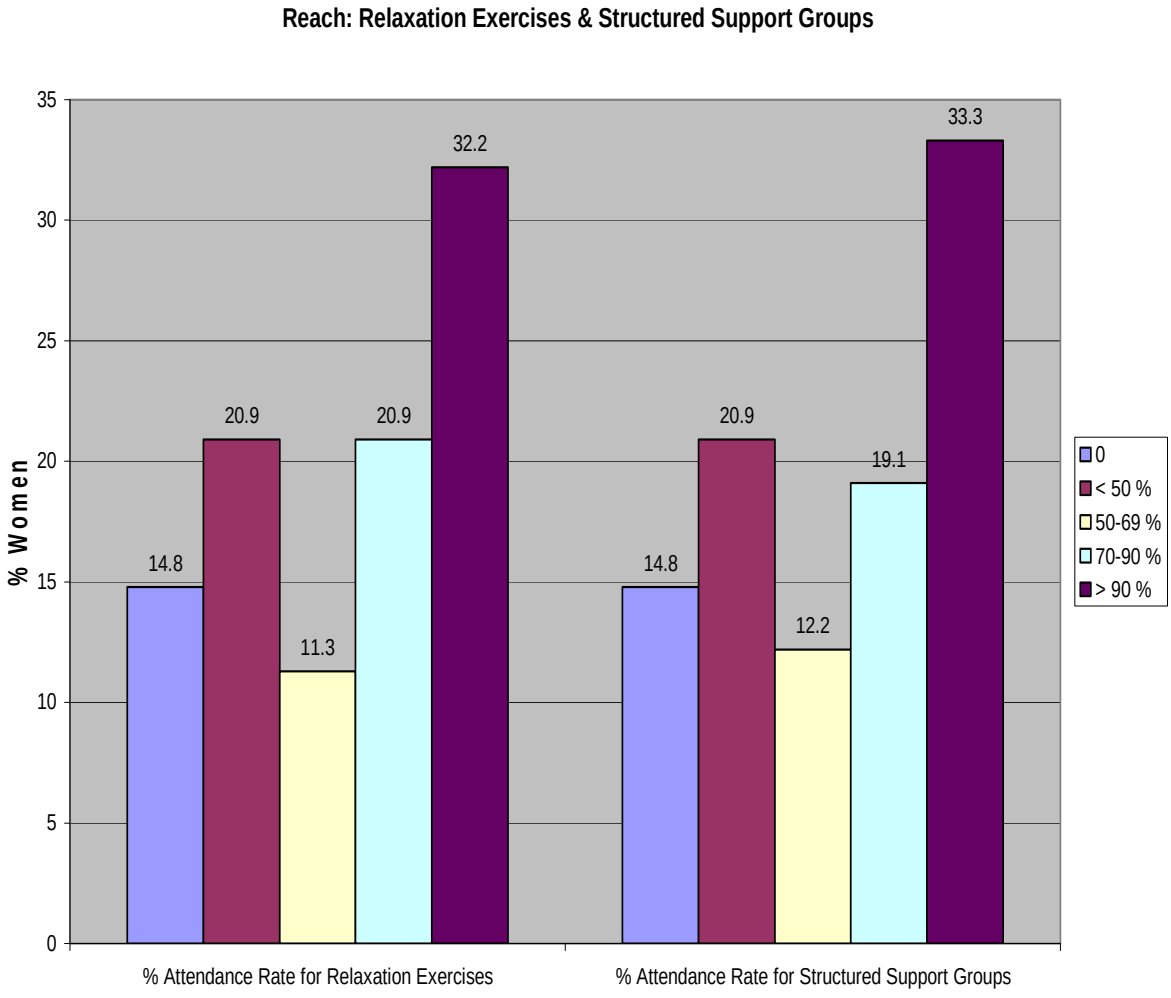

Figure 2. REACH: Attendance Rate (% Sessions Attended)

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Effect of an Education Program on Improving Help-Seeking among Parents of Junior and Senior High School Students in Japan

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Abstract

Early intervention in schizophrenia is important for patient prognosis and quality of life. At the time of the first episode, quality of life is influenced by identification of symptoms and by medical help-seeking behavior. In this prospective cohort study, we investigated help-seeking among 2690 parents of junior and senior high school students before and after the parents viewed a newly developed web-based education program aimed at improving knowledge of schizophrenia. Our web-based education program aimed to improve understanding of schizophrenia, including promotion of help-seeking. Many parents (33.1%-50.0%) consulted a physician in a department of psychosomatic medicine when their child experienced symptoms. Characteristics that predicted a decision not to seek psychiatric medical help were having child with all symptoms, younger parent age, and lower family income ($p<0.05$). After the education program, the rate of parents who sought medical help within

1 week was significantly higher for all symptom categories except sleeplessness (p=0.001). These findings suggest that the present web-based education program was useful in promoting medical help-seeking behavior among parents of junior and senior high school students in Japan.

Keywords: Help-seeking, Parents, Education program, Schizophrenia

1. Introduction

Early intervention in schizophrenia is important for patient prognosis and quality of life (QOL). Another reason for rapidly beginning treatment is that longer duration of untreated psychosis (DUP) is associated with lower long-term treatment effectiveness (Van *et al*., 2005). Thus, early intervention might improve response to antipsychotic treatment and long-term outcome (Perkins *et al*., 2005). Loebel *et al*. (1992) reported that duration of symptoms before treatment was significantly associated with time to remission and level of remission, i.e., longer duration predicted longer time to remission and lesser extent of remission. Longer DUP is associated with mental anguish, declines in QOL and social functioning, and poor clinical outcomes (Bechard-Evans *et al*., 2007). The average DUP was reported to be between 1 and 2 years (Larsen *et al*., 1998). Reducing DUP is an important challenge for mental health professionals, as it influences patient prognosis (Chong *et al*., 2004). In a review by Kessler *et al*. (2005), the authors noted that one-half of cases of mental illness begin by age 14 years and that three-fourths begin by age 24 years. In addition, they found evidence of delays in help-seeking among young people with emerging psychosis (Lincoln *et al*., 1995; Bechard- Evans *et al*., 2007). Bechard-Evans *et al*. (2007) showed that adolescents show poor social and academic adjustment and are socially withdrawn. Furthermore, their changes in behavior are more likely to go undetected when psychosis begins. Therefore, they are less likely to be brought to a mental health professional for help. These findings show that rapid detection of the initial onset of psychosis is important in young people because it would permit treatment to start earlier. However, young patients who are severely mentally ill have few mental health consultations (Nishida *et al*., 2008), and young people who need treatment frequently do not seek help (Boydell *et al*., 2006). Therefore, psychiatric outcomes among young patients might depend on whether their parents can understand the symptoms of mental illness and seek appropriate medical care at an early stage (Helgason, 1990; Perkins *et al*., 2005).

In recent years, there have been a number of studies of help-seeking (Platz *et al*., 2006; Unal *et al*., 2007; Compton *et al*., 2008; O'Callaghan *et al*., 2010). However, there has been no such study among the parents of junior and senior high school students. In this prospective cohort study, we (1) assessed help-seeking among parents when junior and senior high school students have schizophrenia symptoms or prodromal and nonspecific symptoms of schizophrenia, (2) identified factors associated with failure to seek medical help, and (3) investigated the effectiveness of a newly developed web-based education program that aimed to improve understanding of schizophrenia, including the promotion of help-seeking.

2. Methods

2.1 Participants

The participants were 2690 parents of junior and senior high school students. They were extracted from candidates in a large database administered by a private Japanese company that specializes in questionnaire research. Gender and region were used as variables for stratified random sampling. Consent was obtained from all participants by the same company that administered the database. All participants completed a questionnaire on an internet website administered by the survey company. The details have been previously described (Yoshii *et al*., 2011). This study was approved by the Ethics Committee of the Niigata University School of Medicine.

2.2 Questionnaire

The questionnaire used in the present study consisted of 3 sections. Section 1 collected demographic information on respondents. Section 2 asked about consultations their child had for a symptom of schizophrenia, a prodromal symptom, and nonspecific symptoms of schizophrenia. The participants were then asked to indicate all types of consultations they had sought from among 15 choices (e.g., family circle, homeroom teacher, psychiatric clinic, health center) for a child with sleeplessness (nonspecific symptom of schizophrenia), social withdrawal (prodromal symptom), strange behavior (symptom of schizophrenia), or all 3 symptoms. In section 3, the participants were requested to select from 5 items regarding the timing of the consultation with regard to onset of symptoms (within 1 week, about 1 month later, about half a year later, more than 1 year later, treatment not needed) when their child had the above symptoms (the first questionnaire). All participants then viewed the education program. One week later, the questionnaire was answered again (the second questionnaire), and the effectiveness of the education program was evaluated among the participants.

2.3 Web-based education program

After completing the first questionnaire survey, all respondents were invited to view a web-based education program that aimed to improve understanding of schizophrenia, including promotion of help-seeking. This program was developed by the authors (Yoshii *et al*., 2011). The content included help-seeking, i.e. how to prevent progression and exacerbation of the disorder, signs of progression, and consultation alternatives. The education program comprised 12 slides with narration and required 13 minutes to complete. The education program was delivered via the same internet website that was used for the questionnaire survey.

2.4 Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0. McNemar's test was used to compare paired data, i.e., the results of the first and second questionnaires for each respondent. The chi-square test was used to compare both the characteristics of those seeking non-medical help and several demographic characteristics. Differences in rates between groups were assessed with the Bonferroni multiple comparison procedure. All statistical tests were 2-tailed and statistical significance was defined as a P value less than 0.05.

3. Results

3.1 Characteristics of participants

The participants where 2690 parents of junior and senior high school students in Japan, 2465 of whom finished both questionnaires. Mean age ±SD was 45.9 ±4.7 years. A total of 2552 (94.9%) respondents reported being married. Most (51.0%) respondents were employed full-time. The detailed characteristics of the respondents have been previously described (Yoshii *et al*., 2011).

3.2 Medical help-seeking behavior among parents of junior and senior high school students

Table 1 shows the rate of help-seeking behavior, by type of consultation, reported on the questionnaires given before and after the education program. The most frequent (33.1%-50.0%) type of consultation selected by participants was one at a department of psychosomatic medicine. Only 6.5% to 17.3% of participants with children who had the 4 investigated symptoms chose to have a consultation in a mental hospital. The rate of parents seeking help was similar among those with children who showed all 3 symptoms and those with children who showed strange behavior. Thus, strange behavior was the conclusive factor in seeking medical help.

The same questionnaire was administered to the participants 1 week after they had viewed the education program on the website. The rates of those who reported seeking a consultation at a psychiatric clinic for children with social withdrawal or all 3 symptoms were significantly higher as compared with the first questionnaire ($p< 0.05$ for all comparisons).

3.3 Timing of medical help-seeking behavior

For almost all symptom categories, approximately half (43.7%-55.5%) of participants sought help approximately 1 month after symptom onset (Table 1). About 80% of participants sought medical help within approximately 1 month for children with any symptom. Only 1.4% to 2.2% of participants waited longer than 1 year to seek help for a child with any symptom.

After the education program, the rate of participants who sought help within 1 week was significantly higher ($p=0.001$), as compared with the first questionnaire, for all symptom categories except sleeplessness. Those who reported waiting approximately half a year to seek help decreased for all symptom categories ($p<0.05$).

3.4 Factors that predicted a decision not to seek psychiatric medical help

Characteristics that predicted a decision not to seek psychiatric medical help (excepting consultation at a department of internal medicine) were having children with all 3 symptoms, age, and family income ($p<0.05$) (Table 2). Younger parents were less likely to seek psychiatric medical help. Among parents aged 30 to 39 years, 43.9% did not seek psychiatric medical help. The Bonferroni multiple comparison procedure showed significant differences in the rate between parents aged 30 to 39 years and both those aged 40 to 49 years ($p=0.003$) and those aged 50 to 59 years ($p=0.001$). In addition, 51.2% of respondents with a family income less than 11 000 US dollars not seek psychiatric medical help. A lower family income was associated with not seeking psychiatric medical help. The Bonferroni multiple comparison procedure showed significant differences in the rate between parents with a family income of 32 000 to 53 000 US dollars and those with an income greater than 110 000 US dollars ($P<0.05$).

3.5 School help-seeking behavior among parents of junior and senior high school students

Consultation with a homeroom teacher was the most frequent (13.9%-41.4%) school-based help-seeking

behavior (Table 1), and consultation with the school nurse was the least frequent (8.5%-12.5%) school-based help-seeking behavior. After the education program, all school-based consultations were significantly more frequent as compared with responses to the first questionnaire ($p<0.05$ for all comparisons).

4. Discussion

Singh *et al*. (2006) reported that demographic factors associated with longer delays in help-seeking were being single, being unemployed, living alone, living in public housing, and ethnic minority status. Another study reported that patients with schizophrenia might not be fully aware that their condition is deteriorating. In addition, they noted that patients living alone tended to be slower to seek a mental health consultation (Koichi *et al*., 2009). These findings suggest that parents can play an important role in identifying symptoms of schizophrenia in their children, in whom they are well equipped to notice subtle changes. By identifying schizophrenia at an early stage, parents can reduce the time from onset of symptoms to start of treatment, which is important in improving QOL after treatment (Chong *et al*., 2004). However, parents of junior and senior high school students sometimes might do not seek help when a child has signs of schizophrenia.

Help-seeking among parents has been studied in many countries. One study investigated 34 parents with children aged 2-15 years in London (Sayal *et al*., 2010), another study enrolled African American mothers (mean age±SD of children: 14±0.8) in rural Georgia (n = 163) (Murry *et al*., 2011), and a Canadian report studied 506 parents of children aged 4-17 years (Reid *et al*., 2011). However, our study differed from those earlier investigations because it targeted parents of junior and senior high school students, because the gender and regional distributions of our sample were almost identical to those of the Japanese general population, and because the present study had a reliable, large sample size (n=2690). In addition, to our knowledge, no other study has investigated the effectiveness of a web-based education program that aimed to improve help-seeking behavior among Japanese parents of adolescents. Studies of help-seeking have not yielded consistent results with regard to sex-based, socioeconomic, and ethnic determinants of behavior or the impact of such behavior on treatment delays (Anderson *et al*., 2010). Help-seeking may differ due to the nature of available medical care, the economy, and/or culture. Our study is therefore important.

We hypothesized that most parents of junior and senior high school students would consult departments of internal medicine. To test this hypothesis, we investigated help-seeking by inquiring about a nonspecific symptom of schizophrenia (sleeplessness), a prodromal symptom (social withdrawal), and a symptom of schizophrenia (strange behavior). We found that the most common (33.1%-50.0%) form of consultation for all symptom categories was at a department of psychosomatic medicine, a field that is concerned with the diagnosis and treatment of medical diseases and their related psychosocial factors, e.g., essential hypertension and arrhythmia, gastric and duodenal ulcer, bronchial asthma, diabetes mellitus, and migraine. Individuals with mental illnesses can be successfully treated in such departments in Japan. These results disagree with those of Jorm *et al*. (2007), who showed that Australian parents (n=2005) of children aged 12-25 years did not universally recognize the potential value of seeking help from mental health professionals.

In the Australian study, parents frequently mentioned general practitioners (GPs) as an intended source of help for their children when asked questions after vignettes portraying either depression, depression with alcohol misuse, social phobia, or psychosis (Jorm *et al*., 2007). Our results disagree with those findings. In the present study, 21.6% of parents with a child who had a nonspecific symptom of schizophrenia (sleeplessness) and 2.7% of those with a child who had a prodromal symptom (social withdrawal) sought help at a department of internal medicine, which is similar to seeking treatment at a GP. The Japanese medical system permits easy access to specialists. Therefore, patients and their family do not usually have a stable family doctor and can freely seek specialist medical area care.

Our study showed that about 80% of parents of children with symptoms consult a doctor within 1 month of onset. This result differs from a logistic study in Canada (Czuchta *et al*., 2001), which showed that a mean of 7.33 months elapsed before parents (n=20) sought psychiatric help (including help from either a family doctor, a psychiatrist, or a psychologist). Delays in seeking help can negatively affect the course and treatment of schizophrenia (Waddington *et al*., 1995; Wyatt , *et al*. 1997; Marshall *et al*., 2005). Patients may experience such delays in treatment if their parents do not initially consult a medical doctor.

Provision of psychiatric treatment-seeking behavior has been assessed throughout the world (Joa *et al*., 2008; Tanaka *et al*., 2003). For example, a Norwegian study showed that an early intervention program reduced DUP in first-episode schizophrenia from 16 to 5 weeks in a health care setting. The program used a combination of easy-access detection teams (DTs) and a massive information campaign (IC) on the signs and symptoms of psychosis (Joa *et al*., 2008). A previous study in Japan found that an education program significantly improved psychiatric treatment-seeking behavior among workers ($p<0.05$) (Tanaka *et al*., 2003). However, the time

required in that study was much longer than in our program. In addition, that study did not target parents of junior and senior high school students.

Nicola *et al*. found that accessing information on the internet was associated with increased use of any mental health service, GPs, and mental health professionals (MHPs) (Reavley *et al*., 2010). Thus, there is evidence that internet-based therapy programs are an effective means of mental health service delivery (Griffiths *et al*., 2007). Our education program can be viewed over the internet in 13 minutes, which is likely to be more attractive to busy parents in Japan.

5. Conclusions

Many parents consulted a physician in a department of psychosomatic medicine when their child experienced symptoms of mental illness. Our web-based education program was useful in promoting medical help-seeking behavior among parents of junior and senior high school students in Japan.

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Table 1. Rate (%) of help-seeking among parents of junior and senior high school students

	Sleeplessness (A)			Strange behavior (B)			Social withdrawal (C)			A, B, and C		
Type of consultation	**First**	**Second**	**p** *	**First**	**Second**	**p** *	**First**	**Second**	**p** *	**First**	**Second**	**p** *
Medical												
Mental hospital	6.5	6.8	0.822	14.2	14.2	0.717	8.7	8.9	1.000	17.3	17.8	0.941
Psychiatric clinic	13	13.8	0.592	24.1	26.8	0.051	15.9	18.6	0.017	27.4	31.4	0.003
Department of psychosomatic medicine	33.1	29.9	0.024	45.2	43.3	0.319	35.1	36.0	0.532	50.0	51.0	0.313
Department of internal medicine	21.6	17.3	0.001	5.9	5.7	0.952	2.7	2.8	0.931	5.5	5.1	0.609
School												
Homeroom teacher	13.9	19.8	0.001	23.6	31.2	0.001	41.4	45.4	0.001	29.0	35.0	0.001
School nurse	8.5	13.4	0.001	10.8	16.4	0.001	12.5	19.3	0.001	11.5	18.7	0.001
School counselor	10.5	13.8	0.001	16.5	21.7	0.001	27.5	30.3	0.011	20.9	26.1	0.001
Community												
Health center	1.9	2.7	0.055	2.8	4.8	0.001	2.1	4.1	0.001	3.1	5.6	0.001
Mental health center	2.7	4.3	0.004	6.2	9.7	0.001	5.2	8.9	0.001	7.8	12.1	0.001
Other												
Family circle	53.6	61.2	0.001	49.9	56.8	0.001	51.8	57.5	0.001	48.7	54.9	0.001
Neighbor	1.6	1.9	0.445	1.0	0.6	0.511	1.0	0.9	1.000	0.8	0.7	0.868
Classmate's parents	6.6	7.6	0.058	4.6	4.6	0.632	6.7	6.5	0.813	4.5	4.2	0.944
Telephone consultation	5.5	4.7	0.323	8.3	7.0	0.227	8.9	7.5	0.232	9.0	7.8	0.314
Internet consultation	10.8	8.5	0.022	13.3	11.0	0.034	14.1	11.1	0.004	13.8	11.6	0.057
Needless treatment	6.7	6.8	0.824	4.4	4.7	0.737	4.8	4.9	1.000	4.2	4.5	0.730
Timing of medical help-seeking												
Within one week	31.9	34	0.150	40.3	46.0	0.001	24.4	30.8	0.001	44.9	51.4	0.001
About 1 month later	53.3	54.3	0.340	46.8	44.9	0.242	55.5	54.8	0.688	43.7	39.9	0.012
About half a year later	7.5	5.8	0.011	8.3	5.5	0.001	13.2	9.4	0.001	7.1	4.8	0.001
More than 1 year later	1.8	1.1	0.042	1.4	0.9	0.149	2.2	1.7	0.358	1.5	1.4	1.000
Non-medical help sought	5.5	4.9	0.228	3.2	2.6	0.132	4.7	3.2	0.005	2.9	2.5	0.294
*McNemar's test												

Table 2. Associations between parental characteristic and a decision not to seek medical help for children with sleeplessness, strange behavior, and social withdrawal

	Total	Mental hospital: A			Psychiatric clinic: B			Department of psychosomatic medicine: C			A, B, and C			Department of internal medicine		
	n	n	%	p*	n	%	p*	n	%	p*	n	%	p*	n	%	p*
Age (years)				0.528			0.012			0.521			0.006			0.188
30-39	221	187	84.7		179	81.0		119	53.8		97	43.9		203	91.9	
40-49	1904	1564	82.1		1382	72.6		940	49.4		654	34.3		1799	94.5	
50-59	548	461	84.1		381	69.5		278	50.7		170	31.0		525	95.8	
60-69	17	13	76.5		11	64.7		7	41.2		4	23.5		16	94.1	
Gender				0.001			0.001			0.001			0.655			0.396
Male	1381	1093	79.1		953	69.0		748	54.2		469	34.0		1311	94.9	
Female	1309	1132	86.5		1000	76.4		596	45.5		456	34.8		1232	94.1	
Education				0.715			0.012			0.042			0.072			0.673
Junior high school	25	19	76.0		21	84.0		14	56.0		11	44.0		25	1.0	
High school	766	641	83.7		585	76.4		391	51.0		286	37.3		727	28.6	
Vocational school/ junior college	734	610	83.1		540	73.6		333	45.4		241	32.8		696	27.4	
University	1063	875	82.3		736	69.2		559	52.6		361	34.0		1000	39.3	
Graduate school	96	75	78.1		66	68.8		43	44.8		23	24.0		89	3.5	
Other	6	5	83.3		5	83.3		4	66.7		3	50.0		6	0.2	
Domicile				0.003			0.196			0.264			0.323			0.980
Hokkaido/ Tohoku	304	233	76.6		220	72.4		157	51.6		99	32.6		287	94.4	
Kanto/Sin-Etsu/ Hokuriku	1186	980	82.6		848	71.5		593	50.0		407	34.3		1123	94.7	
Tokai/Kinki	822	705	85.8		619	75.3		422	51.3		300	36.5		775	94.3	
Chugoku/Shikoku/ Kyusyu/Okinawa	378	307	81.2		266	70.4		172	45.5		119	31.5		358	94.7	
Marriage status				0.211			0.415			0.842			0.973			0.799
Unmarried	3	2	66.7		2	66.7		2	66.7		1	33.3		3	100	
Married	2552	2117	83.0		1850	72.5		1278	50.1		879	34.4		2412	94.5	
Bereaved	14	9	64.3		8	57.1		6	42.9		4	28.6		14	100	
Divorced	12	97	80.2		93	76.9		58	47.9		41	33.9		114	94.2	

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Family structure				0.635			0.837			0.661			0.759			0.982
2 parents	2092	1738	83.1		1523	72.8		1053	50.3		723	34.6		1976	94.5	
1 parent	89	70	78.7		65	73.0		40	44.9		26	29.2		84	94.4	
3 generations	466	383	82.2		332	71.2		232	49.8		162	34.8		442	94.8	
Other	43	34	79.1		33	76.7		19	44.2		14	32.6		41	95.3	
Employment				0.001			0.006			0.002			0.283			0.777
Full-time	1373	1091	79.5		960	69.9		726	52.9		458	33.4		1298	94.5	
Part-time	471	409	86.8		360	76.4		205	43.5		152	32.3		449	95.3	
Self-employed	259	211	81.5		186	71.8		137	52.9		99	38.2		241	93.1	
Full-time housewife	542	480	88.6		417	76.9		251	46.3		201	37.1		512	94.5	
Unemployed	45	34	75.6		30	66.7		25	55.6		15	33.3		43	95.6	
Occupation				0.535			0.877			0.003			0.154			0.608
Agriculture and forestry	11	10	90.9		8	72.7		7	63.6		5	45.5		11	100	
Production labor service and transportation and communication	772	624	80.8		556	72.0		424	54.9		286	37.0		729	94.4	
Sales and marketing and service industry	160	134	83.8		114	71.3		65	40.6		44	27.5		148	92.5	
Professionals	689	574	83.3		511	74.0		340	49.3		233	33.8		649	94.2	
Other	1058	883	83.5		764	72.2		508	48.0		357	33.7		1006	95.1	
Family income, (US dollars)				0.828			0.103			0.020			0.002			0.199
< 11000	41	36	87.8		33	80.5		22	53.7		21	51.2		36	87.8	
11000–32000	196	160	81.6		146	74.5		98	50.0		74	37.8		189	96.4	
32000–53000	502	421	83.9		377	75.1		284	56.6		198	39.4		476	94.8	
53000–110000	1465	1208	82.5		1065	72.7		710	48.5		485	33.1		1379	94.1	
> 110000	486	400	82.3		332	68.3		230	47.3		147	30.2		463	95.3	

* The chi-square test

The Influence of Psycho-social Factors on Participation Levels in Community-based Breast Cancer Prevention Programs in Tehran, Iran

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Abstract

Background: Although significant consideration has been devoted to women participation in breast cancer prevention programs, our understanding about the psychosocial factors which influence participation remains incomplete. **Method:** The study applied a quantitative approach based on the cross-sectional survey design and multistage cluster random sampling**.** A total of 400 women aged 35-69 years, were surveyed at 4 obstetric and gynecologic clinics affiliated to Tehran University of Medical Sciences in Tehran: the participation levels of 86 women who have had a mammogram were analyzed based on their self-efficacy, belief, social influence, and barriers concerning mammography utilization. **Results:** Consistent with the study framework, in bivariate analysis, the higher level of women's participation in breast cancer prevention programs was significantly related to more positive belief about mammography ($p < .05$), greater social influence on mammography ($p < .01$) and fewer barriers to mammography ($p < .01$). Self efficacy ($p = .114$) was not significantly related to the higher level of participation. **Conclusion:** Results suggest that women's participation levels in breast cancer prevention programs might be associated with the specific psychosocial factors on breast cancer preventive behavior such as mammography screening.

Keywords: Psycho-social factors, Community participation, Participation levels, Community–based programs

1. Introduction

Health promotion encourages individuals and communities to take greater responsibility for their health (WHO, 1978). Women's involvement in health services or programs such as breast cancer prevention programs is another means of community participation in health. A host of individual and psycho-social factors may constrain community participation and its levels.

Previous literatures have documented the influence of individual and structural factors on community participation process. For example, numbers and types of community participants are influenced by geography (Cohen & Syme, 1985), socioeconomic status (Sills, 1968; Widmer, 1987), gender (Wells, *et al*., 1990), and group heterogeneity (Litwin, 1986). It was documented that a rise of participation happened across a number of basic socio-demographic (Boyce, 2001). Women in traditional societies have low social status within their families and communities; hence, their ability to make their own decisions is severely limited (Raju and Leonard, 2000). Community members with low level of incomes and educational levels had minimal levels of participation usually as clients and volunteers, and no interest in taking responsibility at project management positions (Boyce, 2001).

In brief, the relations between communities and the different age, gender, ethnicity, and socioeconomic characteristics affect community participation. Different cultures and various socio-demographic factors make community participation faced many obstacles in higher levels of participation.

To date very few studies have been carried out based on theoretical models regarding women's participation in breast cancer prevention groups (Cameron *et al*., 2005; Gilbar & Neuman 2002; Guidry *et al*., 1997). However, most of previous researchers used the health belief model to explain the psycho-social factors that influence the participation in breast cancer prevention groups such as breast cancer support group in the United States (Sherman *et al*., 2008). Higher levels of women participation in support group were associated with potential benefits of participation in a breast cancer support group, fewer perceived barriers to participation, social influence is caused by friends, family, relatives, oncologist, and medical caregivers, perceived illness severity and consistent support over time (Stvense, 1998; Sherman *et al*., 2008).

Similarly, at the structural level, social, economic and cultural barriers, and at the individual level, motivation can affect local community participation in health in Uganda (Kapiriri, 2003). Boyce (2001) also found these barriers in the study related to community participation of disadvantaged groups such as poor women, street youth, and disabled persons in health promotion projects in Canada.

Community involvement in the diagnosis and solution of health problems is an old opinion of public health. But listening to the concerns and problems of the community residents or starting where the people stand, are more important to participation levels (Minkler, 1990). Volunteer or community activity in other investigations has been linked to use of support groups and may reflect a broader readiness for social engagement and support (Bauman *et al*., 1992; Taylor *et al*., 1986). Of course, volunteer activity is also influenced by functional limitations; individuals with severe illness are less able to participate.

As there are few published reports on influencing factors on women's community participation and its levels in health programs, it is not considerably possible to compare findings of this study with other researches. Literatures regarding socio-psychological attributes on community participation levels are relatively limited in theoretical depth and much of this effort is atheoretical. Additional theory-based study would promote the field forward, particularly given the complex selection of factors that might promote or restrain community participation in health programs such as breast cancer prevention programs.

This study provided a closer look at combining three theories such as the Theory of Reasoned Action, Health Belief Model and Social Cognitive Theory to identify psychosocial factors which affect women's participation in any available community-based breast cancer prevention program or activity in Iran. It intends to identify Iranian women's problems and implementation of systematic interventions involving individual and communities to improve sustainable breast cancer prevention program development.

This paper attempts to understand psychosocial factors such self efficacy, social influence, belief and barriers which affect women's participation in a community-based breast cancer prevention program or activity in the subgroup of women who were adherent to mammography in last two years. It is the only feasible way to understand effective factors on the higher level of women's participation in breast cancer prevention programs. Specifically, we anticipated that greater self efficacy, more positive belief, greater social influence and fewer barriers concerning mammography utilization which could enhance higher level of participation in any available breast cancer prevention program or activity.

1.1 Community Participation Levels in Health

To explain women's participation in community-based breast cancer prevention program or activity, the study argues Rifkin's view on participation levels in community-based program to assess the actual level of participation in the study population. Rifkin (1991) clarified people can participate in a first level to get the benefits of a health project by receiving health services or education which is most passively. At a second level, local people may participate in program activities. People contribute land, labour or money for a health facility or play an important role as health workers. A third level takes place in implementation, where local people are responsible in a program and decide how to conduct certain activities. A fourth level concerns program monitoring and evaluation. But in all these levels so far, local people are still not involved in program planning or in transferring their own needs and interests into a true grassroots development. Only in fifth or final level, people are instructed to decide about the health programs which should be undertaken and ask health staff, agencies and/or the government to provide the necessary expert knowledge and/or resources (Rifkin, 1991). The five levels of participation are as follows:

(1) Health benefits whereby communities are only health or education services users;

(2) Program activities where local communities contribute labour, land or money;

(3) Implementation that focuses on local people's managerial responsibilities to carry out the program;

(4) Monitor and evaluation of program activities;

(5) Deciding on selecting of proper programs to be carried out.

A comprehensive behavioral evaluation of Iranian women on community participation in cancer prevention program has not been undertaken, yet. It is noteworthy to state that community-based breast cancer prevention programs in Iran are related to some informal educational programs which have progressed at the district, and province levels in health centres, work places, voluntary organizations, schools, and hospitals to provide awareness and education on the breast cancer prevention and screening methods at the community level. In other words, these are community- focused programs prepared for health promotion, especially breast cancer prevention along with family planning, maternal health, children vaccination and prenatal care, and other community health programs in Iran. More details related to women's participation in breast cancer prevention programs in Iran have been recently published elsewhere (Ahmadian *et al*., 2010).

2. Method

2.1 Population of the Study and Location

The data for this study consisted of 400 women aged 35-69 years and were selected using multistage cluster random sampling procedure from four obstetric and gynecologic clinics affiliated to Tehran University of Medical Sciences in Tehran, Iran.

Women were classified depending on the mammography participation or non-participation in the past two years into a participant group and a non-participant group, respectively. Participation levels and determinants of community participation in breast cancer prevention program or activity were determined among participant group.

2.2 Measures

Most of the questions for instrument were gained and modified from previous literatures which had illustrated high reliability. Besides, a number of questions were developed only for the purpose of this research to direct important concepts which were not addressed in previous studies, such as questions related to community participation levels in health programs.

The health belief model component, the theory of reasoned action components, and the social cognitive theory component were measured on a 5 point Likert scale from 1 to 5 (1="Strongly disagree" 2="Disagree" 3="Moderate" 4="Agree" 5="Strongly agree"). In this study 5 self efficacy items included the social cognitive theory and it is women's confidence in the ability to participate in a mammography. The scores ranged from 5 to 25 with higher scores indicating higher or greater confidence in doing mammography. Barriers are related to the obstacles which cease women from participating in mammography such as attitudinal and logistic barriers. The scores ranged from 15 to 75 with lower scores indicating fewer barriers in doing mammography and 15 barrier items included in the health belief model components.

Belief is related to women's belief about results of mammography use which is opinionated by the women. This belief can evaluate how positive or negative the attribute of mammography is. The scores ranged from 10 to 50 with higher scores indicating higher or greater belief which has a positive meaning regarding mammography use. 10 belief items and 5 social influence items included in the theory of reasoned action components. Social influence is related to influence from referent individuals such as doctor, nurse, family members, and friends' opinion, media and others in the medical community which approve or disapprove doing mammography in women. The scores ranged from 5 to 25 with higher scores indicating higher influence from referent individuals in doing mammography.

The community participation levels were measured by dichotomous scale that examined women participation levels whether they participated in any program for breast cancer prevention or not (Yes=1, No=0**).** Twenty items of the community participation levels included in Rifkin's perspective on community participation in health programs. Based on some interviews with health care professionals about current women's participation situation in health programs, most of the questions were specified as level 1 (benefits) and level 2 (activity), four items and eight items, respectively, and the late eight items specified to level 3-5 related to women participation at implementing, monitoring and planning levels.

The instrument for the study revised for content validity by an expert panel, which comprised three social scientist with specialty in community development, two specialized doctors in surgery, an oncologist, a radiologist with specialty in breast cancer diagnosis, two family medicine physicians, two epidemiologist, a professor with specialty in public health. Content validity was also evaluated with a review of the literature. Face validity of the research instrument was done by committee members and some experts in this field.

A modified and developed questionnaire was translated by three health care professionals fluent in both English and Persian. Back-translation of the instrument preserved the content validity of the items. The researcher evaluated the linguistic and cultural accuracy of translation by using an expert panel, particularly an expert translator. The two versions (Persian and English) of the questionnaire were reviewed by a group of experts in breast cancer screening and arrangement was achieved over the translation. More information of the psychometric assets of the scale and the instrument have been lately published elsewhere (Ahmadian *et al*., 2010).

2.3 Data analysis

Data analysis was carried out using Statistical Package for Social Science (SPSS 13). Descriptive statistics such as ranges, frequency distribution, percentages, means and standard deviations were calculated to explain data preliminarily. Bivariate analyses were performed via a series of independent t-tests, analysis of variance (ANOVA), and chi-square tests. Preliminary exploratory data analysis was conducted to appraise for missing values, detect outliers and check for normality. The data went through consistency tests and variable frequency analysis and entered into program. Pilot testing evaluated other attributes such as precision (reliability) and accuracy (validity).

Reliability testing was conducted on a convenience sample of 31 women aged 35 or above. The Cronbach Alpha was tested on each dimension of self-efficacy, belief, social influence and barriers, as well as participation levels. Based on the reliability alpha, the instruments have shown the reliability of Cronbach's alpha values (from0.72 to 0.96) in this pilot study. The research program attempted to balance increased model fit and content validity. Correlations between the items of each construct examined and high correlations were desirable to establish convergent validity.

As mentioned above, scales were also pretested and evaluated during the pilot test. The lack of standardized tests to measure a number of constructs related to community participation and psycho-social factors influencing breast cancer prevention required the development of scales using Principal Components Analysis (PCA) and Common Factor Analysis (FA).

Exploratory Factor Analysis was used to determine the fundamental influences on the set of observed variables about the nature of four variables (psycho-social factors) which were counted by examining the extent of each observed variable association with a factor (Tabachnick & Fidell, 2001).The factors explained the data through a reduced number of concepts that returned the original set of variables and were used for further statistical analyses (Hair *et al*., 1995). Principal Components Analysis was applied to describe the psychometric evaluation of instrument for measuring self-efficacy, beliefs, social influence and barriers. Principle axis factoring analysis generated four factors which relate 72% of the variance to the psychosocial items. Barlett Sphericity test was statistically significant, $x^2(595) = 19502.704$, $p = .000$, and the variables were highly correlated to one another. Thus, these data were appropriate to conduct factor analysis. Kaiser-Meyer-Olkin sampling adequacy measure was 0.949. Statistical significance was determined at the level 0.05.

The assessment of frequency distribution for each variable, confirmed that the data set had no problems with skewness and kurtosis. Internal consistency reliability analysis was also performed. Finally, a factor analysis using principle axis factoring with varimax rotation was set to further the evaluation regarding psychosocial aspects of the research framework.

3. Results

3.1 Women's Community Participation Levels in Breast Cancer Prevention Program

A total of 400 women aged 35-69 years, were randomly chosen using random cluster sampling: 86(21.5%) were assessed as the participant group and 314(78.5%) as the non-participant group. Table 1 shows the levels of participation in community-based breast cancer prevention programs or activities which were achieved by the participant group (n=86). Table 1 also shows that the women's participation in breast cancer prevention programs were divided into two levels (benefit, activities). By means of χ^2 test there is a significant difference in frequency between women who were in only level 1(benefits) and those in level 2(activities) [χ^2 (1) 26.79, $p< .01$]. Higher levels (implementing, monitoring, evaluation and planning) were yet controlled by health care professionals in Iran

3.2 Demographic Differences between Women's Level 1 and Level 2

In this study, selected demographic variables such as age, education, occupation, marital status, and income between the level 1 (benefits) and level 2 (activities) were compared using chi-square (Table 2). The study results showed that the largest proportion of participants in level 2 (activities) were those in the 41 to 45 year age

bracket (43.3%), where as the largest rate of the women in level 1(benefits) were older than 51 years old (31.6%). Generally, women in level 2 (activities) tended to be younger. The difference in age between the two levels was significant (χ^2=14.65, p= .002).

In general, a greater percentage of participating women at both levels were graduates. The table 2 indicates 79.1% for level 2 (activities) and 42% for level 1 (benefits) are women graduates. Furthermore, the difference in education was significant between the two levels (χ^2=30.82, p=.001). The study showed that greater percentage of women in both levels are married and the difference in marital status was not statistically significant (χ^2=1.45, p= .482).

The largest proportion of the women in the level 2 (activities) were full-time employees (76%), whereas the largest proportion of women in the level 1(benefits) were unemployed or housewives (52.6%). The statistics shows that participating women in both levels were significantly different in occupation (χ^2=23.66, p= .001). Table 2 presents a greater percentage of participating women in level 2 (activities) related to the middle class (83.6%). It also indicates that more participants in level 1(benefits), were in the middle class (73.7 %). The difference in income was not significant between 2 levels (χ^2=3.64, p= .161). Furthermore, insurance status has not showed a significant difference between the two levels (χ^2=2.85, p= .091).

Notably, however, all participant (n=86) have participated in some programs related to breast cancer prevention, suggesting that most women acknowledged that they seldom participated as an audience in the selected programs. To get better understanding of community participation in breast cancer prevention program among Iranian women, one explanation is that the community participation is referred to some of their activities which embedded into a comprehensive health program on women's health like family planning at the districts level.

3.3 Psychosocial Factors and Its Comparison with the Levels of Community Participation

Independent-sample t-test was conducted to distinguish the influencing factors between the two levels of participation regarding community-based breast cancer prevention program or activity. It was conducted to provide more information regarding self-efficacy among participant women. There was not a significant diversity in self-efficacy between level1 and level 2[t (19.84) =2.39, (p=.114)]. However, the mean self- efficacy score was higher for level 2(M =23.01, SD=2.09) comparing level 1(M=20.21, SD=4.98). It reveals that participant women who were in level 1 (benefit) and level 2 (activity) had no significant difference in self-efficacy in relation to mammography (Table 3).

3.3.1 Belief and Levels of Community Participation

Independent sample t-test also was employed to provide more information regarding beliefs among the participant women on the subject of participation levels in programs (Table 4). There was a significant difference in beliefs regarding mammography utilization between women in level1 and level2; [t (84) =2.590, p= .011]. The mean beliefs score was higher for level 2(M =40.38, SD=3.02) comparing level 1(M=38.36, SD=2.90). It states that the women who have participated in the level 2 (activity) had greater belief in doing mammography than those who participated in the level 1 (benefit).

3.3.2 Social Influence and Levels of Community Participation

Table 5 shows that the mean score for social influence in level 2 was higher than level 1 with a significant variation; [t (20.371) =3.814, p= .001]. The mean social influence score was higher for level 2(M =20.22, SD=1.36) comparing level 1(M=17.63, SD=2.87). It demonstrates that the women who have participated in the level 2 (activity) had greater social influence regarding mammography utilization than those who participated in the level 1(benefits).

3.3.3 Barriers and Levels of Community Participation

Table 6 illustrates that higher mean for the barrier in level 1 (mean =40.52, SD=7.49) than the mean barrier in level 2(mean =33.95, SD=2.85); [t (19.50) =-3.74, p= .001]. It means that women should overcome their barriers to mammography for higher level of participation in any community-based breast cancer prevention program. It presents that the women who have participated in the level 2 (activity) had lower barriers in doing mammography than those who have participated in the level 1 (benefit).

4. Discussion

This study was conducted on 86 Iranian women (participant group) who have had a mammogram within the last two years, attending at 4 obstetric and gynecologic clinics in Tehran to identify significant psycho-social factors influencing their participation levels in breast cancer prevention programs.

The levels of participation which were achieved by the participant group (n=86) were divided into two levels; level 1 (benefit) and level 2 (activities). Based on the findings of this study it can be concluded that, the levels of women's participation are limited.

4.1 Demographic Factors and Women Participation Levels

There is little published information regarding the relationship between socio-demographic characteristics and community participation levels in health programs, particularly in breast cancer prevention. In this study, the chi-square (χ^2) test exposed a significant relationship between age, education, occupation and higher level (activities) of participation ($P < .01$). Furthermore, the study showed marital status ($p= .482$), insurance status ($p= .91$) and income ($p= .161$) have no statistical difference between level1 (benefits) and level 2(activities) among women who participated in any community–based breast cancer prevention program. With respect to demographic variables, one study discovered that socio-demographic characteristics of mothers are associated with the choice to participate in community activities regarding their children health in Indonesia (Nobles, 2006). Another study also demonstrated that low rates of participation of disadvantaged persons in health programs have been characteristic of low socioeconomic status resulting in poor motivational levels Boyce (2001). It is noteworthy to state that numbers and types of community participation are influenced by geography (Cohen & Syme, 1985), socioeconomic status (Sills, 1968; Widmer, 1987), and gender (Wells *et al*., 1990).

The findings from this study showed that older women have participated abundantly in breast cancer prevention program in level one (benefits) than level 2 (activity). Contrary to the result, association between age and participation in cancer support group was not significant in previous study (Sherman, 2008). However, in general, younger women tend to participate in community-based cancer prevention or family planning program in Iran. This again brings to light the better education and employment of these women in this study.

The findings showed that educated women have participated in breast cancer prevention program or activity in higher level (activity) in comparison to non-educated ones. The number of women with high academic qualification has considerably increased in Iran, so it can be a good evident to support the result. Consistent with the result, relationship between education and participation in cancer support group was significant in previous study and participants had marginally higher education than nonparticipants (Sherman, 2008). Similarly, 'established' participants were mostly educated with regard to the participation in a breast cancer support group in the United of States (Stvense, 1998). Several prior studies noted that there were trends for greater participation in cancer support group among those with greater education (Bauman, *et al*., 1992; Eakin & Strycker, 2001; Meyer & Mark, 1995; Stevens & Duttlinger, 1998).

The result of this study also showed that occupation enables women to decide their own breast cancer prevention program in Iran. It can be concluded that women with full-time jobs have less socioeconomic dependency. Thus, occupation encourages their participation in health promotion programs such as breast cancer prevention programs. Having a job can improve women participation at a higher level (activity). These women can be informed more easily of the relevant medical programs such as those many available work-place programs in Iran. Besides, they are less conservative than the housewives and unemployed women. The employed women can conceive this reality that their own health is equal to the whole family health. In contrast, association between occupation and participation in cancer support group was not significant in previous study (Sherman, 2008).

It is believed that socio-demographic factors are important to community participation levels in breast cancer prevention program or activity but it might not be robust predictors. The time of doing mammography and women's involvement in the treatment and diagnosis process also affect women's participation in the breast cancer activities. In this study, the subsample of individuals who have participated in breast cancer prevention program or activity was modest, which may restrict conclusions.

4.2. Psychosocial Factors and Women Participation Levels

Results showed that the higher level of participation (level 2) was significantly related more positive belief ($p< .05$), greater social influence ($p< .01$) and fewer barriers ($p< .01$) towards mammography. Self efficacy ($p= .114$) was not significantly associated to the higher level of participation (level 2).

The study revealed that self-efficacy is not a salient factor for the levels of participation among participant group. In contrast, another study indicated that individuals with low self-efficacy regarding their health behavior restrict their participation in rural NGOs in India (Handy & Kassam, 2004).

It seems self-efficacy did not influence women's participation in community-based breast cancer prevention programs due to the lower levels of their participation in the limited expected benefits and some activities in

Iran. It means that personal factors such as self efficacy cannot influence individual's willingness to participate in community activities, particularly on health matters. Based on our personal observation, Iranian people are more successful in individual activities than working in a community even in the case of health matters. On another note, participating women have not experienced a strong sense of community in the study, since there is no actual formal program regarding breast cancer prevention in Iran.

Self-efficacy of participating women in breast cancer prevention programs may be slowed down because of women's limited positions in those programs in Iran. Thus, it causes difficulty in behavior changes such as voluntary participation in breast cancer prevention program. Health care professionals should have knowledge on behaviors modification in order to enhance women's self efficacy towards voluntary participation in formal or informal breast cancer prevention program.

Results of bivariate analysis showed women who have participated in the level 2 (activity) had greater belief in doing mammography than those who participated in the level 1 (benefit). Consistent with this finding, previous studies showed that positive belief causes people to control the disease individually and to increase their voluntary participation in malaria control activities in Iran (Zaim, 1997; Grantham 2009).

Iranian women specially the traditional and non-educated ones resist against new health behavior and to some extent they refuse to listen to the health care professional's advice. Similarly, previous study showed that specific cultural belief had association with voluntary participation in health program (Boyd-Franklin, 1991; Guidry, *et al*., 1997; Mathews, Lannin, & Mitchell, 1994).

The results of this study demonstrated that participant women in the level 2 (activity) had greater social influence regarding mammography utilization than those who participated in the level 1(benefits). Consistent with this result, higher levels of women's participation in support group were associated with social influence (Stvense, 1998). Similarly, social influence is important to facilitate community involvement at the community level in community-based HIV prevention in Uganda (Leonard *et al*., 2001). The findings from this study showed that social influence mobilized women to reach higher level of participation in any formal or informal breast cancer prevention programs among Iranian women.

The finding also indicated that women who have participated in the level 2 (activity) had lower barriers to mammography than those who have participated in the level 1 (benefit). Consistent with the results of this study concerning individual barriers and participation in community-based program, previous research showed that some individual barriers such as lack of motivation, time, language, economics, social support, family or household responsibilities, socio-cultural (fear) and environmental (traffic-related) barriers which reduced people attendance in community-based programs (Martinez *et al*., 2001). Likewise, participation in cancer support groups was associated with practical barriers, access issues and lack of social support and these items are lower in active participant (Sherman, 2008).

To the best of our knowledge, this study is the first quantitative study that shows how the levels of women's participation in breast cancer prevention program or activity were influenced by psycho-social factors. Earlier researches on community participation levels were mainly qualitative, as a result of which the extent to which participant were influenced by the psycho-social factors could not be determined.

Community-based prevention program is an approach to health promotion and disease prevention that needs high level of motivation for the people to participate. Such motivation comes from a preventive health seeking behaviour of the target population. Women would be active and participate in higher levels of participation in breast cancer prevention programs in an encouraging condition.

As shown in this study, people's socio-psychological aspects influence participation behaviour. As such, using socio-psychological theories can adjust behavioural aspects of health among individuals, and communities. It had been quite well documented that psychosocial factors made individual and community involve more actively in health programs such as HIV programs, chronic disease, child and maternity and family planning. This proves the importance of a sustained breast cancer prevention program using theoretical-based interventions.

It is obvious that engaging the community in different activities in health programs have many benefits but different cultures, and psycho-social characteristics influence different levels of community participation. Carrying out this study, we have demonstrated that more positive belief, greater social influence, and lower barriers toward preventive health behavior (e.g. mammography use) can motivate women to be involved in health activities.

4.3 Limitations of the Study

The current study is a cross sectional survey developed within the positivist approach to social science. Thus, the conclusions are only descriptive in characteristics and do not confirm causal relationships between the variables. Researcher needs to do multiple methods to strengthen the study design, but survey is a basic approach in this study. In addition, Iranian people are very interactive. Thus, more qualitative research with focusing on in-depth interview and focus groups with respondents of study is needed to support this study. Furthermore, the lack of participation of potential key informants is obvious in present research.

Literatures regarding socio-psychological attributes on community participation levels are relatively limited in theoretical depth and much of this effort is atheoretical. The present study cannot be addressed directly to previous literature review. However, the factors influencing active participation in health program can be concluded by highlighting the importance of community participation in health in literature review. Besides, the subsample of individuals who have participated in mammography was reasonable in present study which may limit the conclusions.

These data may be overestimated due to social desirability response bias. Moreover, the present study intends to carry out on a small sample of women and thus our findings may not be generalized to all Iranian women. Specific researches are needed to study medical problems related to women's adherence to mammography and its regularity in the broader construct of medical study and its impact on community participation levels in breast cancer prevention programs and activities.

Previous literature showed that traditionally community participation has been assessed in quantitative forms, for example, by asking how many people have come to a meeting or how many people have joined in a community activity. The problem is that presence does not mean participation. People may be present, but have no commitment or understanding of what is going on (Rifkin, 2001). Therefore, it is another limitation for the studies related to community participation purpose and factors affect its levels.

This research tries to study women who are involved in any community-based breast cancer program. However, some of those organizations or programs are not specific to breast cancer prevention and women are involved in various programs which associated to their health issues like family planning. Another challenging issue is the difference between participation and membership. Women might participate as a member in those programs.

In addition, it must be added that there is a general limitation that affects the interpretation of literatures presented here. The studies identified and included were based on a review of the published literature. Studies with negative findings might be less likely to be published and thus less likely to be included in this review. This would result in an overstatement of the effectiveness of the study incorporated selected theories as it is hard to tackle them all in any single study. Despite the limitations, this study is the first attempt to study psycho-social factors influencing community participation levels in breast cancer prevention program or activities among Iranian women.

5. Conclusion

According to the findings, this study discovered that social influence, belief and barriers to mammography affect women participation levels in breast cancer prevention programs in Iran. In this study, women who had more positive belief, greater social influence and fewer barriers with regard to mammography were more likely to attend in the breast cancer prevention programs which are available at the district level. Thus, attention to social norms and beliefs towards breast cancer prevention in future interventions can increase women's voluntary participation in those programs. Contrary to earlier expectations, self efficacy was not significantly related to the higher level of participation.

Additional studies on the relationship between the psycho-social factors on breast cancer and its prevention, and women's participation in public health programs are needed to improve community participation, and effective health promotion programs. This research could be used as a guide for community participation in participatory research which is now an integral part of Iranian health. The findings of this study also contribute to the existing literature on individual's health-related behavior theories on understanding community participation in future health programs.

Health care depends on the joining of individual and community health care at the local community level (Van Weel *et al*., 2008). In this case, psycho-social characteristics of individuals on preventive health behavior may encourage them to participate in community-based health programs which it can increase their decision-making ability about medical issues such as breast cancer.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. Levels of Women Participation in Community-Based Programs (n=86)

Levels	N	(%)		χ^2	df	P
Level 2	67	77.9		26.79	1	0.01*
Level 1	19	22.1				

Note: * $p<0.01$.

Table 2. Demographic Characteristics of participant Group (Levels of women's Participation in any Community-Based Breast Cancer Prevention Program)

		Only level1 n=19(22.1%)		**level2 n=67(77.9)**		**X^2**	**sig**
		%	**n**	**%**	**n**		
Age	<40	21.1%	4	23.9%	16	14.65	.002*
	41-45	31.6%	6	43.3%	29		
	46-50	15.7%	3	29.9%	20		
	>51	31.6%	6	3.0%	2		
Education	Primary &secondary school	21.1%	4	-	-	30.82	.001*
	diploma	36.8%	7	6.0%	4		
	Graduate	42.1%	8	79.1%	53		
	postgraduate	-	-	14.9%	10		
Marital	Married	78.9%	15	65.7%	44	1.459	.482
	Widow/divorced	10.5%	2	11.9%	8		
	Single	10.6%	2	22.4%	15		
Occupation	Full time Employee	36.8%	7	76.1%	51	23.66	.001*
	Part Time Employee	10.5%	2	17.9%	12		
	Unemployed or Housewife	52.7%	10	6.0%	4		
Income	low	10.5%	2	1.5%	1	3.648	.161
	middle	73.7%	14	83.6%	56		
	high	15.8%	3	14.9%	10		
Insurance	public	100.0%	19	86.6%	58	2.851	.091
	private	-	-	13.4%	9		

Note: * p<0.001.

Table 3. Comparing Self efficacy Scores between Two Levels by Using Independent -Sample t-test (n=86)

Self Efficacy	N	Mean	SD	t	df	P
Level 2	67	23.01	2.09	2.39	19.84	.114
Level 1	19	20.21	4.98			

Note: * p <0.05.

Table 4. Comparing Beliefs Scores between Two Levels by Using Independent -Sample t-test (n=86)

Beliefs	N	Mean	SD	t	df	P
Level 2	67	40.38	3.02	2.590	84	0.011*
Level 1	19	38.36	2.90			

Note: * p<0.01.

Table 5. Comparing Social Influence Scores between Two Levels by Using Independent -Sample t-test (n=86)

Social Influence	N	Mean	SD	t	df	P
Level 2	67	20.22	1.36	3.814	20.371	.001
Level 1	19	17.63	2.87			

Note: * p<0.001.

Table 6: Comparing Barrier Scores between Two Levels by Using Independent -Sample t-test (n=86)

Barrier	N	Mean	SD	t	df	P
Level 2	67	33.95	2.85	-3.74	19.50	0.001*
Level 1	19	40.52	7.49			

Note: * p<0.001.

Appendixes

Following are some questions that are related to your knowledge about mammography. If you do not know, please choose "I do not know".				
		Yes	**No**	**I do not know**
1	**Breast cancer can be cured if it is detected early by screening methods such as mammography.**	**( )**	**( )**	**( )**
2	**Women aged 40 and older should have annual mammogram or every two years.**	**( )**	**( )**	**( )**
3	**Mammogram can find lumps that cannot necessarily be felt by doctor or by yourself when doing breast self exam.**	**( )**	**( )**	**( )**
4	**Although no symptoms exist, mammogram is necessary.**	**( )**	**( )**	**( )**
5	**Women younger than 40 years should have a mammogram if they have family history of breast cancer.**	**( )**	**( )**	**( )**

Following are some questions that are related to your attitude towards mammography. It includes factors such as self efficacy, belief and social influence in connection with doing mammography. **You must choose only one answer for each question.** **1. Strongly disagree 2. Disagree 3. Moderate 4. Agree 5. Strongly agree**						
		Strongly Disagree	Disagree	Moderate	Agree	Strongly Agree
1	**I am confident I will participate in regular mammograms.**	**( )**	**( )**	**( )**	**( )**	**( )**
2	**I am confident I will participate in regular mammograms irresponsible of painful procedure.**	**( )**	**( )**	**( )**	**( )**	**( )**
3	**I am confident I will participate in regular mammograms without recommendation from a doctor.**	**( )**	**( )**	**( )**	**( )**	**( )**
4	**I am confident I will participate in regular mammograms irrespective of time constraints.**	**( )**	**( )**	**( )**	**( )**	**( )**
5	**I am confident I will participate in regular mammograms even if it is expensive.**	**( )**	**( )**	**( )**	**( )**	**( )**

These questions are related to your beliefs about doing mammography. You must choose only one answer for each question.

1. Strongly disagree 2. Disagree 3. Moderate 4. Agree 5. Strongly agree

	If I have mammography in the future it would:	Strongly Disagree	Disagree	Moderate	Agree	Strongly Agree
1	**Aware me whether I have cancer.**	( )	( )	( )	( )	( )
2	**Allow me to live longer.**	( )	( )	( )	( )	( )
3	**Be important to my family.**	( )	( )	( )	( )	( )
4	**Mean making time for my health is important.**	( )	( )	( )	( )	( )
5	**Mean having a mammogram is a part of the good overall health care.**	( )	( )	( )	( )	( )
6	**Expose me too many of X- rays.**	( )	( )	( )	( )	( )
7	**Cause pain.**	( )	( )	( )	( )	( )
8	**Help me feel in charge of my health.**	( )	( )	( )	( )	( )
9	**Give me a sense of control over my health.**	( )	( )	( )	( )	( )
10	**Feel uncomfortable.**	( )	( )	( )	( )	( )

These questions are related to social influence regarding doing mammography. You must choose only one answer for each question.

1. Strongly disagree 2. Disagree 3. Moderate 4. Agree 5. Strongly agree

		Strongly Disagree	Disagree	Moderate	Agree	Strongly Agree
1	**I would seek advice from my doctor or health staff about mammography rather making decision by my own.**	( )	( )	( )	( )	( )
2	**I would follow my family's advice about my mammography even if I prefer doing something different.**	( )	( )	( )	( )	( )
3	**I would follow my friend's advice about my mammography even if I prefer doing something different.**	( )	( )	( )	( )	( )
4	**I would follow mass media and people in the news about doing mammography.**	( )	( )	( )	( )	( )
5	**I would follow awareness program from public health center NGOs or work place health promotion program about doing mammography.**	( )	( )	( )	( )	( )

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Following are some questions related to the barriers which influence your participation in mammography. You must choose only one answer for each question.

1. Strongly disagree 2. Disagree 3. Moderate 4. Agree 5. Strongly agree.

	You DO NOT participate in mammography for many reasons.	Strongly Disagree	Disagree	Moderate	Agree	Strongly Agree
1	**Cost of mammogram is too much.**	( )	( )	( )	( )	( )
2	**Too hard to figure out where to go for mammogram.**	( )	( )	( )	( )	( )
3	**Lack of transportation to get to a mammography center.**	( )	( )	( )	( )	( )
4	**No one to stay with children or grand children**	( )	( )	( )	( )	( )
5	**Worry the breast X-ray might find cancer.**	( )	( )	( )	( )	( )
6	**Doctor /health provider has not advised to do it.**	( )	( )	( )	( )	( )
7	**Do not think mammography can save our life**	( )	( )	( )	( )	( )
8	**People who perform mammography do not treat patients with respect.**	( )	( )	( )	( )	( )
9	**Too many other things are going on in our lives.**	( )	( )	( )	( )	( )
10	**Worry that mammography might give us cancer.**	( )	( )	( )	( )	( )
11	**Do not think we need mammography.**	( )	( )	( )	( )	( )
12	**No one we know talks about getting breast cancer.**	( )	( )	( )	( )	( )
13	**Media and promotional resources about mammograms do not exist in our neighborhood.**	( )	( )	( )	( )	( )
14	**Breast X-ray cannot change our destiny.**	( )	( )	( )	( )	( )
15	**It makes me embarrassed.**	( )	( )	( )	( )	( )

Following are some questions that are related to your participation in mammography, please read carefully and answer them. If you have participated in mammography in last two years, answer the questions.

		Yes	No
1	**Have you ever participated in mammography in last two years? If yes, when.**	( )	( )
		Regular	**Occasional basis**
2	**Do you participate in mammography on a regular or occasional basis?**	( )	( )

3 What was the main reason for your participation in mammography? Just tick one appropriate reason.

( )	**a**	**My own decision making about participation.**
( )	**b**	**Symptoms or breast problems.**
( )	**c**	**Recommended to have yearly mammogram.**
( )	**d**	**Doctor ordered mammogram.**
( )	**e**	**Health care sector ordered mammogram.**
( )	**f**	**Media advice.**
( )	**g**	**Friend or acquaintance developed cancer.**
( )	**h**	**Recommendation received from public health centers or, work place health promotion programs, or non- governmental organization regarding breast cancer prevention**

Following are some questions that are related to your participation levels in any available breast cancer prevention program in any where such as public health center, work place health promotion programs, NGOs, cancer association.

You must choose only one answer for each question.

1.Yes 2.No

		Yes	No
Level 1			
1	**I have participated as an audience in some of the community-based awareness programs about breast cancer prevention held in one of the places such as health center, work place or NGOs.**	**( )**	**( )**
2	**I have followed health care professional's information which was mentioned in community -based awareness programs towards breast cancer prevention.**	**( )**	**( )**
3	**I have consulted with my doctor / health staff regarding breast cancer prevention.**	**( )**	**( )**
4	**I have been informed about breast cancer screening methods by health care staff.**	**( )**	**( )**
Level 2			
5	**I have participated as a member in a breast cancer prevention program.**	**( )**	**( )**
6	**I have participated as a speaker in some of the programs about breast cancer prevention which were held in one of the places such as health center, work place or NGOs.**	**( )**	**( )**
7	**I have participated as a volunteer in some breast cancer prevention programs.**	**( )**	**( )**
8	**I have given consultation, comment or information to others about breast cancer prevention.**	**( )**	**( )**
9	**I have met other members outside of program to cooperate with them about breast cancer issue.**	**( )**	**( )**
10	**I have contacted other members of my current group in community meetings about breast cancer prevention.**	**( )**	**( )**
11	**I have advocated community-based program in my neighborhood or my work place regarding breast cancer prevention program.**	**( )**	**( )**
12	**I have donated money or any resources to help breast cancer prevention program in anywhere such as health center, work place or NGOs.**	**( )**	**( )**
Level 3 (13&14), Level 4 (15,16,17&18) and Level 5(19&20)			
13	**I have taken an active part in organized group activities to carrying out breast cancer prevention programs.**	**( )**	**( )**
14	**I have joined organized committees for voluntary work about how breast cancer prevention program should be run.**	**( )**	**( )**
15	**I have evaluated and organized the community activities about breast cancer prevention program voluntarily.**	**( )**	**( )**
16	**I have encouraged others to join in a breast cancer prevention program group.**	**( )**	**( )**
17	**I have asked health staff agencies or government organization to provide the resources or materials which can help breast cancer prevention program.**	**( )**	**( )**
18	**I have organized individuals or groups to take greater control over breast cancer prevention program.**	**( )**	**( )**
19	**I have participated in planning program to identify the solution about breast cancer prevention.**	**( )**	**( )**
20	**I have made decisions about strategies or addressing the problems that women are faced to in getting breast cancer prevention.**	**( )**	**( )**

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Alcoholic Beverages Drinking among Female Students in a Tourist Province, Thailand

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Abstract

This study aimed to investigate alcoholic beverages drinking and predictive factors among female students. The participants were 377 subjects from 3 high schools in a tourist province, of Thailand. Data collection was done through self-administered questionnaire. Scales of the questionnaire had reliability coefficients ranging from 0.84 – 0.88. The data were analyzed by using descriptive and inferential statistics. The findings revealed as follows. About half (51%) of them have ever drunk and 10.5% of drinkers have drunk once a week. In addition, 15.6% of drinkers began their first drink when they were under 10 years old. Risk factors for alcohol consumption of female student were age, GPA, drinker in family, peer pressure, advertisement and accessibility to alcoholic beverages while protective factors were perception of drinking impacts on family and moral values. Students who have a drinking family member were 4.6 times more likely to drink than those who do not have.

Key words: Female student, Drinking, Alcoholic beverage, Tourist province

1. Introduction

Alcoholic beverage drinking is one of the causes of health problems, crimes, economics, and social problems. Both government and private sectors emphasize politically on alcoholic beverages drinking and participate in activities designed to prevent drinking (Social surveillance and Warning Center, online). Alcohol industry is actively participated in social and school activities, including sport activities, through corporate social responsibility (CSR) program in order to reach out to the target group, who are youth and teenagers. The Thai National Statistical Office in Thailand reported that in 2007, Thai population older than 15 years old consumed

alcoholic beverages about 14.9 million people (29.3%), while 15 – 24 years old age group who were drinkers about 2.3 million people (21.9%) (Sornpaisarn, *et al*, 2008) Prevalence rate of lifetime drinking among students aged 10 – 22 years old were 30.5% and 18.2% for boys and girls, respectively (Assnangkornchai, Mukthong, & Intanont, 2009). Between 1999– 2007, it was found that prevalence rate of lifetime drinking among aged 15 – 19 years old increased from 4.7% to 8.0% or 70%. Moreover, girls tend to drink more than boys when comparing increased percentage of drinkers. In addition, parental attitudes toward alcohol and having drinkers in family, contribute to the risk that youth will likely become a drinker. It is estimated that there will be 67% of drinkers in year 2047 (Sornpaisarn, Kaewmungkun, & Wattanaporn, 2010). Consequences of drinking for young women include academic problems, poor health, mental health problems, accidents, as well as the development of alcohol or other drugs dependence (CASA, 2003).

2. Objectives of research

The objective of this study was to investigate alcoholic beverages drinking and predictive factors among female students in high schools.

3. Methods

3.1 Research design and sample

This study was conducted as a cross-sectional survey among female students from 3 high schools in Chonburi province, in eastern Thailand. Chonburi is well-known as an important tourism area on the Gulf of Thailand. Three hundred and thirty-seven female students from grades 1 – 6 were recruited. Data collection was conducted between August and September 2010.

3.2 Research instruments

The research instrument was the self-administered questionnaire, consisted of 6 parts: 1) alcoholic beverage drinking, 2) family factors: marital status of parents, drinker in family 3) peer pressure, 4) perception of alcoholic beverage advertisement, 5) accessibility to alcoholic beverages and 6) perception of drinking impacts on health, family, social and moral values. Content validity has been reviewed and approved from 3 experts, one in behavioral science, one in family medicine, and one in health policy. Data were verified with internal consistency reliability coefficients between 0.84 and 0.88. This study was approved by the Ethics Committee for Research in Human Subjects of the Faculty of Public Health, Mahidol University (Ref.No. MUPH 2010-131).

3.3 Statistical Analysis

The SPSS for windows was used in both descriptive and inferential statistical analysis of the collected data. The descriptive statistics were frequency, percent, and standard deviation. In addition, Chi-square, t-test, and Multiple Logistic Regression were applied in finding the factors correlating and predicting the alcoholic beverages drinking among female students. Significant level was set to be .05.

4. Results

4.1 descriptive statistics

The samples were between the age of 12 and 17 years old, with the average age was 15.2. Majority of samples (91%) lived with parents or relatives. Out of 377 female students, 74.5% lived with cohabited parent. More than 9 in 10 (92%) had at least one drinker in the family. Approximately 51% of female students have ever drunk. Female drinkers reported having their first drink between the age of 3 and 17 years old (Mean+S.D.= 12.50+2.32). Approximately 35% of female drinkers had their first alcoholic beverages drinking before high schools, and 15.6% began to drink alcoholic beverages when they were under 10 years old. Regarding the reasons that may encourage their first drinking, more than 56% of female student drinkers said they just wanted to try. Secondly, they started to drink in the party or because of peer pressure (38.1%). Wine or Spy (50.5%) is majority of alcoholic beverages which were chosen, followed by beer (25%) and alcoholic smoothies (15.1%) respectively. Approximately one-third (32.8%) of drinkers drank at home. This study reported that 37.3% of female drinkers drank with their parents or relatives, and more than half (56.2%) drank with their friends. More than 4 in 10 (46%) drank once in a few months, followed by few times a month (44%). One in ten (10%) of female drinkers drank once a week (Table 1).

4.2 Inferential statistics

In focusing on the family predictors, namely marital status of parents and drinker in family, the study found that drinking associated with drinker in family with statistical significance at .01. Nonetheless there was no correlation with marital status of parents (Table 2). Additionally, the personal predictors in terms of age and GPA, indicated

that drinkers had older age than non-drinkers, whereas the non-drinkers had higher GPA than drinkers with statistical significance at .001 (Table 3).

With regard to the social predictors such as peer pressure, perception of alcoholic beverages advertisement, and accessibility to alcoholic beverages, comparing the perception scores by alcoholic beverages drinking indicated that drinkers had a higher mean score than non-drinkers, with statistical significance at .001. Referring to perception of drinking impact on health, family, social, and moral values, the difference were statistically significant at .001, with drinkers had less mean scores of drinking impacts than non-drinkers (Table 3).

The findings of association between predictors comprised of personal factors, family factors, social factors, and perception of drinking impacts and alcoholic beverages drinking among female students in high schools revealed that there were 10 predictors which were significantly associated with drinking. The significant predictors were age, GPA, drinker in family, peer pressure, advertisement, accessibility of alcoholic beverages, perceptions of drinking impacts on: health, family, social, and moral values. These variables were analyzed to see how it influences alcoholic beverages drinking, by using Multiple Logistic Regression.

The results of analysis (Table 4) revealed that age, drinker in family, peer pressure, advertisement, accessibility to alcoholic beverages, perception of drinking impact on family and moral values, were predictive of alcoholic beverages drinking with statistical significant at .05. Besides the equation was 77.3% correct in predictive of drinkers and 75.5% correct in predictive of non-drinkers, or 76.4% capable of classifying drinkers and non-drinkers. This finding can make clear as follow.

1) Students live with drinker in family are 4.6 times more likely to drink than those who out drinker in family.
2) For each additional year the students was 1.98 time more likely to be a drinker or 98% [(1.98-1)*100] more likely to be a drinker.
3) Each additional score for accessibility to alcoholic beverages means 1.274 times more likely to be drinker or 27.4% [(1.274-1)*100] more likely to be a drinker.
4) Each additional score for peer pressure means 1.179 times more likely to be drinker or 18% [(1.179-1)*100] more likely to be a drinker.
5) Each additional score for perceived alcohol advertisement means 1.065 times more likely to be drinker or 6.5% [(1.065-1)*100] more likely to be a drinker.
6) Each additional score for perception of drinking impact on moral values means 0.906 times less likely to be drinker or 18% [(1-0.906)*100] less likely to be a drinker.
7) Each additional score for perception of drinking impact on family means 0.820 times less likely to be drinker or 9.4% [(1-0.820)*100] less likely to be a drinker.

Then, alcohol drinking of female students had risk factors such as age, drinker in family, peer pressure, perceived alcohol advertisement, and accessibility to alcoholic beverages, and protective factors, namely perception of drinking impact on family and moral values.

5. Discussion

The study indicated 50.9% of female students in high schools had ever drunk alcoholic beverages. This information indicated a higher rate than previous study of Pichainarong & Chaveepojnkamjorn (2010) that showed only 8.8% in central Thailand. Moreover, this study reported a higher percentage of female students drinkers than the study of Assnangkornchai, Mukthong & Intanont (2009), which was a surveyed with high school students in Thailand, between December 2007 and February 2008. It was reported that 18.2% of female students in high schools had ever drunk alcoholic beverages. The high percentage of female student drinkers in current study may be affected by the context of this study. Chonburi is well-known by foreigners as tourist city. Therefore, there are a lot of accessibility to alcoholic beverages such as cocktail lounges, discotheques, nightclubs, convenient stores, and groceries in many areas. These may be encouraging factors for female students to try drinking alcoholic beverages. Environmental factors in social entertainment may induce a person to touch alcohol (Lunsay, 1997). That is, the percent of female students who had experience with drinking in Chonburi is higher than other provinces which are not tourist destinations. It is consistent with a study of Paupongsakorn in 2005, which stated that one of all causes which had effect on alcoholic beverages drinking was easy access to alcoholic beverages, or having stores near home and school.

The results of this study indicated that about 35% of female students had their first drink of alcoholic beverage at the age under 13. It is consistent with the results of school girls surveyed in the United State of America, which

indicated that nearly 1 in 4 started to drink for the first time before age 13. It was a higher percentage than the survey in 1960 which indicated only 7% of all girls (CASA, 2006). This is particularly disturbing since those who initiate alcohol drinking in early life are at increased risk of becoming problem drinkers (Grant & Dawson, 1997). It would be more troubling issues for Thai female students which tend to start drinking early in their life. With regard to drinking prevention, previous study found that family supervision and support were important factors to prevent substances used for both girls and boys effectively, especially important for girls (Amaro, Blake, Schwartz, & Flinchbbaugh, 2001). Interestingly, this study stated that 37.3% of female students generally drank alcoholic beverages with their parent or relatives at home. The results are consistent with the previous study of Valentine, Jayne & Gould (2010) who found that parents actually introduce their own children to alcohol at home at an early age. This is especially crucial issue when considering why the parents allow female students who are under their responsibility to drink alcoholic beverages. Drinking is accepted by social as a normal activity or familiarity with alcohol may enhance self-protection for female students. The results in the current research indicate the difference from the norm of Thai traditional society, which parents must be the role model for their child and should not allow the child to drink.

There are many factors that control drinking behavior such as perception of drinking impacts on family and moral values. However, the social problems that may influence the drinking behavior such as easy access to buy alcoholic beverages and peer pressure still exist. Other important factor that may increase the risk for alcohol abuse is parental acceptance of female students' drinking together with high percent of having family members who drink alcohol (92%). Moreover, the alcoholic beverages advertisement has the indirect effect to the alcohol consumption of female students. The advertised issues have enhanced drinking by making their images as someone with modern life style, attractive person, or hero for the target groups, especially the young. Young people were stimulated to alcohol consumption by advertisement. This study found that 50% of female drinkers have the first drink as self-trial, and 38.1% of them from peer pressure. It is consistent with the study of Boonleu (2003) who studied Thai youth at the age of 13 – 21 which found that alcohol drinking of these participants was influenced by persuasion of friends and self-trial. It is similar with a study of CASA reported in 2003 that alcohol advertising appeals to girls by making drinking as fun and sexy. Experts agreed that advertisement exerts an influence on youth drinking (Grube, 1993). It is consistent with other studies in Thailand such as Kajorndhum, Kajorndhum, & Sornpaisarn (2004) and Sornpaisarn, *et al*. (2008) which reported the related findings.

In conclusion, the significant predictors toward alcoholic beverages drinking of female students would be more of challenging problems to families and policy regulators. This is especially troubling for each family in Thai society, to be concerned about drinking impacts on social, moral values, economics, and well-being both short and long terms, while family structure seems to be fragile. In reality, these families would be responsible for each problem in the future, which can be destroyed or may not rectify chronic social problems.

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Table 1. Alcohol beverages drinking of female students

Item	Frequency	Percent
Current drinking (n=377)		
Drink	192	50.9
Not drink	185	41.1
Age at first drink (n=192)		
Under 10 years old	30	15.6
11 – 12 years old	37	19.3
13 – 14 years old	97	50.5
15 – 17 years old	28	14.6
Reasons for first drink (n=192)		
Try to drink	108	56.1
Peer pressure	73	38.1
Feel stressed or anxious	9	4.7
Drink followed family members	2	1.1
Alcoholic beverages at first drink (n=192)		
Wines or Spy	97	50.5
Beer	48	25
Alcoholic smoothies	29	15.1
Whisky or Vodka or Rice whisky	18	9.4
Places of drinking (n=192)		
Home	63	32.8
Dormitory	42	21.9
In party or Restaurant	87	45.3
Persons who always drinking (n=192)		
Peers	119	56.2
Parent or Relative	62	37.3
Alone	11	6.5
Favorite alcoholic beverages (n=192)		
Wines or Spy or alcohol mixed with fruit-juice	97	50.5
Beer	37	19.3
Alcoholic smoothies	32	16.7
Whisky or Vodka or Rum	26	13.5
Drinking frequency (n=192)		
Everyday	1	0.5
Always (3 – 4 times a week)	5	2.6
Sometimes (1 – 2 times a week)	14	7.3
Once a month	84	43.8
Once a few months or less	88	45.8

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Table 2. Families predictors associated with alcoholic beverages drinking

Family predictors	**Drinker**	**Non-drinker**	χ^2	**Odd ratio**
Marital status of parent				
Cohabitation	135 (48.0)	146 (52.0)	3.677	0.63
Divorce/Separate/Father or mother dead	57 (59.4)	39 (40.6)		
Drinker in family				
Yes	186 (53.6)	161 (46.4)	12.641**	4.43
No	6 (20.7)	23 (79.3)		

** $p < 0.01$

Table 3. Means comparison of the scale predictors by alcoholic beverages drinking

Predictors	**group**	**n**	**Mean**	**S.D.**	**t-value**
Personal					
Age	Drinker	192	15.16	1.076	5.462***
	Non-drinker	185	14.61	0.853	
GPA	Drinker	159	3.01	0.543	3.658***
	Non-drinker	185	14.61	0.853	
Social					
Peer pressure	Drinker	189	21.91	7.303	10.945***
	Non-drinker	182	15.40	3.623	
Perceived alcoholic advertisement	Drinker	192	16.12	7.432	8.881***
	Non-drinker	185	10.72	3.881	
Accessibility to alcoholic beverages	Drinker	190	2.75	1.504	5.099***
	Non-drinker	184	1.98	1.433	
Perception of drinking impact on					
Health	Drinker	191	23.30	41.69	5.986***
	Non-drinker	185	25.72	3.665	
Family	Drinker	192	19.85	5.682	5.026***
	Non-drinker	185	22.36	3.868	
Social value	Drinker	191	16.84	3.891	3.343***
	Non-drinker	184	18.03	2.952	
Moral value	Drinker	191	11.50	2.294	4.643***
	Non-drinker	185	12.56	2.100	

*** $p < 0.001$

Table 4. Multiple logistic regression between predictors and alcoholic beverages drinking

Predictors	**B**	**Odds Ratio**	**95% CI**
Age	0.683***	1.980	1.463 - 2.680
Grade Point Average (GPA)	-0.435	0.647	0.359 - 1.168
Drinker in family[1]	1.529**	4.614	1.438 - 14.802
Peer pressure	0.164***	1.179	1.100 - 1.263
Alcoholic beverages advertisement	0.063*	1.065	1.000 - 1.133
Accessibility to alcoholic beverages	0.242**	1.274	1.066 - 1.523
Perception of drinking impact on			
- Health	-0.007	0.993	0.910 - 1.085
- Family	-0.098*	0.906	0.825 - 0.995
- Social	0.105	1.111	0.970 - 1.272
- Moral values	-0.198**	0.820	0.707 - 0.952
Constant	-10.183		

[1] Reference group: no

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Risks and Complications of Coronary Angiography: A Comprehensive Review

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Abstract

Coronary angiography and heart catheterization are invaluable tests for the detection and quantification of coronary artery disease, identification of valvular and other structural abnormalities, and measurement of hemodynamic parameters. The risks and complications associated with these procedures relate to the patient's concomitant conditions and to the skill and judgment of the operator. In this review, we examine in detail the major complications associated with invasive cardiac procedures and provide the reader with a comprehensive bibliography for advanced reading.

Keywords: Cardiac catheterization, Angiography, Contrast material, Acute kidney injury, Complications

1. Introduction

Coronary angiography is the gold standard test for identifying the presence and extent of atherosclerotic coronary artery disease (CAD). As with any invasive procedure, there are specific patient-dependent and procedure-related complications that are inherent to the test. Complications range widely from minor problems with short term sequelae to life threatening situations that may cause irreversible damage, if urgent care is not provided. Fortunately, the associated risks have decreased significantly since the inception of coronary arteriography due to advanced equipment design, improved peri-procedural management, and increased experience of diagnostic centers and operators.

Although there are no absolute contraindications to performing coronary arteriography, the associated risks can be attributed to cardiac and non-cardiac complications. Specific disease states pertaining to the general medical profile of the patient (older age, renal insufficiency, uncontrolled diabetes mellitus, and morbid obesity) can increase the risk for complications. The underlying cardiovascular status of the patient can further predispose to adverse events. The extent of CAD, congestive heart failure (CHF) with low ejection fraction, recent stroke or myocardial infarction (MI), and bleeding propensity are just a few of the cardiovascular characteristics that can increase cardiac and vascular complications. Furthermore, the type of procedure being performed, be it diagnostic coronary angiography or additional percutaneous coronary intervention, modulates the risk.

Given the above considerations, however, major complications are uncommon. Because major complications from cardiac catheterization occur in less than 2% of the population, with mortality of less than 0.08%, there are relatively few patients who cannot be studied safely in an experienced laboratory. The use of iso-osmolar contrast media, lower profile diagnostic catheters, measures to reduce the incidence of bleeding and extensive operator experience can all serve to reduce the already low incidence of such complications even further. Therefore, the procedure can be successfully performed even in the most critically ill patient, when clinically indicated, with relatively low risk. However, the risk-to-benefit ratio of cardiac catheterization and familiarity with potential benefits and risks must be assessed on an individual basis in order to minimize any potential problems. In this chapter, we aim to identify the risks associated with coronary angiography and coronary interventions in the modern catheterization laboratory, and describe advances in equipment design and management protocols that have been promoted to reduce potential complications.

2. Allergic and Adverse Reactions

2.1 Local Anesthesia

Allergic local and systemic reactions to local anesthesia are extremely rare. Methemglobinemia, asthma-like reactions, vasodepressor reaction and anesthesia toxicity have been reported (Finder & Moore, 2002). Most reports are with the older agents and have been infrequently reported with amide agents, such as lidocaine. Reactions are generally dermatologic or vagal, and are rarely anaphylactic. The reactions that do occur are generally secondary to the preservatives used in drug preparations. Use of preservative-free agents, such as bupivacaine, and skin testing would be warranted in patients with history of reactions to local anesthetics (T. Feldman, Moss, Teplinsky, & Carroll, 1990).

2.2 General Anesthesia

General anesthesia is not routinely required in the catheterization laboratory, and the vast majority of procedures occur without the presence of an anesthesiologist. Conscious sedation and analgesia with short acting agents such as midazolam or fentanyl at low doses are, however, commonly used to increase patient comfort and relieve anxiety during the procedure. In such cases, care must be taken to avoid over-sedation of the patient. Close monitoring of blood pressure, heart rate, respiratory rate and oxygenation should be performed in all patients. When hemodynamic compromise or oversedation occurs, the use of reversal agents for benzodiazepines (flumazenil) and opiates (naloxone) should be promptly administered. Anaphylactoid reactions happen infrequently with conscious sedation agents and are much more likely to occur following administration of contrast media. Treatment of any adverse reaction depends on its severity, and includes the potential use of oxygen, bronchodilators, epinephrine, histamine blockers, corticosteroids and intravenous fluids (Dewachter, Mouton-Faivre, & Emala, 2009). In cases of severe anaphylaxis not responsive to conservative management, endotracheal intubation and urgent consultation with anesthesia team must be performed. Proper history and review of allergies can help avoid unnecessary exposure to patients with previous allergy or adverse reactions to local or systemic anesthesia. Particular attention should be paid to allergy to sea food, as there is possible cross-reactivity with iodine-containing contrast media.

2.3 Contrast Media

Adverse reactions from contrast media may be classified as chemotoxic or anaphylactoid. Contrast media stimulate an anaphylactoid response through histamine release. It differs from an anaphylactic reaction, in that it is not immune-mediated and does not require prior sensitization to the offending agent to initiate a reaction. Chemotoxic effects are primarily related to the hyperosmolarity, ionic content, viscosity, and calcium binding properties of these agents (Goss, Chambers, & Heupler, 1995). All contrast agents are based exclusively on iodine, commonly combined to a benzoic acid ring in a mixture of meglumine or sodium salt of diatrizoid acid with calcium EDTA. The concentrations of sodium and EDTA are kept roughly equal to that of blood, as higher or lower concentrations have been associated with tachyarrhythmia and myocardial depression. In order to achieve the iodine concentration that is needed for optimal visualization during angiography, solutions of conventional contrast agents were extremely hypertonic. The resulting solutions of these agents (Hypaque (Nycomed) and Angiovist (Berlex)) have an osmolality about 5.8 times (1690 mOsm/kg) that of plasma (Barrett *et al*., 1992). Adverse reactions are common in the ionic, high osmolality contrast agents, reported in > 50% of patients in some studies (Matthai *et al*., 1994). Milder constitutional symptoms are frequently reported (warmth, pain, chest tightness, nausea and vomiting) and are self-limited in the majority of cases. Adverse reactions requiring intervention (hypotension, bradyarrhythmias, pulmonary congestion) have been reported in nearly 30% of patients in one randomized trial (Barrett *et al*., 1992).

The introduction of lower osmolar, ionic agents (ioxaglate (Hexabrix)), and water soluble low-osmolar, non-ionic (iohexol (Omnipaque), ioxilan (Oxilan)) have significantly reduced the incidence of hypersensitivity and adverse reactions. In randomized clinical trials, the use of high osmolar contrast material was associated with a 3.1% increase in risk for need to treat patients for adverse reactions and 3.6% increase in life threatening reactions in comparison to use of lower osmolar non-ionic agents. These reactions were largely confined to patients with severe coronary artery disease or unstable angina (Barrett *et al*., 1992). These results have been duplicated in two other randomized trials that were able to further risk stratify patients at highest risk for developing adverse contrast reactions (Matthai *et al*., 1994; Steinberg *et al*., 1992). Patients with advanced age, higher New York Heart Association CHF class, history of prior contrast reaction, and elevated left ventricular diastolic pressure have been identified as being up to six times more likely to develop adverse reactions with high osmolar ionic agents (Matthai *et al*., 1994). The need for risk stratification initially arose from the high cost of the newer low osmolar agents, which was at one point 10-20 times that of conventional high osmolar agents

(Barrett *et al*., 1992). Selective use of these agents in appropriate populations has been shown to decrease overall cost by 66% with improvement in safety and cost-effectiveness (Matthai *et al*., 1994). The cost of these agents, however, has decreased significantly over the past 10 years allowing for more widespread use of the low osmolar agents to prevent adverse reactions at only a small incremental difference in price.

Most recently, a non-ionic, iso-osmolar compound (iodixanol (Visipaque) has been developed that has an osmolality similar to that of blood (290 mOsm/kg). Hypersensitivity reactions occurred in only 0.7% of the population studied in a large randomized trial comparing iodixanol to the ionic, low osmolar agent ioxaglate, without a significant difference in major cardiovascular events (Bertrand, Esplugas, Piessens, & Rasch, 2000). The introduction of the non-ionic agents was initially met with some concern due to evidence that ionic contrast material exhibited a more pronounced antiplatelet and antithrombotic activity, especially in *in-vitro* studies. These properties may be beneficial during a procedure that may damage the vascular endothelium and cause thrombosis. Fortunately, no increase in the risk of thrombotic complications or major cardiovascular events has been demonstrated in large randomized multicenter trials of angioplasty in which the two classes of contrast agents were compared (Bertrand *et al*., 2000; Schrader *et al*., 1999)

2.4 Prophylaxis and Treatment

Prevention of allergic reactions to contrast material can be successfully achieved. There are two categories of patients at risk for developing anaphylaxis that should be considered for pre-treatment. Patients with previous anaphylactic reactions are at highest risk for developing recurrent reactions. The second category consists of patients with history of atopy, asthma or those who take beta adrenergic blockers, in whom a twofold risk in anaphylaxis has been reported (Lang, Alpern, Visintainer, & Smith, 1991). Despite general concerns, no consistent cross-reactivity has been demonstrated in patients with allergies to food containing iodine (seafood) and contrast anaphylaxis risk (Goss *et al*., 1995; Hildreth, 1987). When encountering patients with history of allergy to shellfish or seafood, further questioning should be addressed toward history of atopy or asthma, as this would identify the patients at highest risk for developing anaphylaxis. In addition to the type of contrast agent, pre-treatment with prophylactic medications is a critical part of preventing recurrent reactions in the population at highest risk. Corticosteroids and histamine blockers are the cornerstone of pretreatment. Prednisone 50 mg administered 13, 7, and 1 hour before the procedure together with diphenhydramine 50 mg orally 1 hour before the procedure are effective in reducing recurrent reactions (Bush & Swanson, 1991; Goss *et al*., 1995; Greenberger, Halwig, Patterson, & Wallemark, 1986; Nayak, White, Cavendish, Barker, & Kandzari, 2009; Wittbrodt & Spinler, 1994). For urgent procedures, intravenous hydrocortisone 200 mg with 50 mg of diphenhydramine should be used prior to the procedure (Table 1) (Greenberger *et al*., 1986).

<Table 1>

It has been hypothesized that the addition of Histamine-2 blockers (cimetidine or ranitidine) to the above regimen may provide greater antihistamine effect on the vascular system in addition to diphenhydramine, a conventional Histamine-1 blocker. The low cost and high safety profile of Histamine-2 blockers have made them a common component of treatment in many catheterization laboratories. Its effectiveness, however, is controversial, and consistent results have not been shown in prospective trials (Goss *et al*., 1995; Greenberger *et al*., 1986; Myers & Bloom, 1981; Wittbrodt & Spinler, 1994). Monteleukast has also been advocated as therapeutic addition. The use of Histamine-2 blockers and Monteleukast has not been advocated by the American College of Radiology (American College of Radiology, 2010).

Despite adequate pre-medication, breakthrough reactions have been shown to occur (Freed, Leder, Alexander, DeLong, & Kliewer, 2001), stressing the role of awareness and careful monitoring in this group of patients. In the case of anaphylactic reactions with laryngeal edema and vascular compromise, 0.3 ml epinephrine at a dilution of 1:1000 subcutaneously or 3 ml at dilution of 1:10,000 intravenously or subcutaneously should be administered immediately. Corticosteroids, diphenhydramine and large volume intravenous fluids should also be given to decrease the severity of the reaction. The use of Histamine-2 blockers remains controversial but should be considered in treatment of refractory cases (Bush & Swanson, 1991; Goss *et al*., 1995).

2.5 Heparin Induced Thrombocytopenia

Heparin Induced Thrombocytopenia (HIT) is a serious immune-mediated complication of heparin administration from flush heparinized saline or during percutaneous coronary interventions. Although the risk will not be manifest during the procedure, the clinical symptoms that develop in the days after the procedure can have potentially devastating thromboembolic complications in patients with prior exposure to heparin. Roughly 1-3% of patients who receive unfractionated heparin will develop a serious form of immune mediated thrombocytopenia with associated venous and arterial thrombosis (HIT-2) (Brieger, Mak, Kottke-Marchant, &

Topol, 1998; Jang & Hursting, 2005). This reaction is caused by antibodies binding to the heparin platelet factor-4 complex, which lead to a cascade of reactions causing platelet activation and the release of procoaggulant and inflammatory factors that consume platelets and incite thrombosis. Patients that develop HIT-2 usually experience a platelet count drop of at least 50%, typically 5-15 days after initiation of heparin, or more suddenly following previous heparin sensitization (Jang & Hursting, 2005). Patients with underlying coronary artery disease and patients with cardiac transplantation have a higher incidence of HIT (2-8% and 11%, respectively) (Hourigan, Walters, Keck, & Dec, 2002; Kappers-Klunne *et al*., 1997), and several patients have been described in whom an acute coronary syndrome (manifesting as acute thrombosis) occurred during coronary angioplasty in association with the onset of HIT (Gupta, Savage, & Brest, 1995). The diagnosis is based on the clinical picture of platelet decrease with or without associated thrombosis. HIT-antibody assays are routinely available for confirming the diagnosis, but treatment should not be delayed when there is a strong clinical suspicion due to severity of comorbidities. Among the patients with HIT and thrombosis, 9-11% require limb amputation and mortality is reported in 17-30% (Jang & Hursting, 2005). Treatment includes immediate and complete discontinuation of heparin and initiation of treatment with direct thrombin inhibitors, such as argatroban, bivlaurdin, or lepirudin. In patients with, or at risk for HIT, who present to the catheterization laboratory, prospective trials of bivalirudin and argatroban have demonstrated safety and efficacy (Campbell *et al*., 2000; Lewis *et al*., 2002; Mahaffey *et al*., 2003). Bivalirudin dose needs adjustment in patients with severe renal impairment, while argatroban is contraindicated in patients with hepatic dysfunction.

3. Infections

3.1 Incidence

Infections are rare after invasive cardiovascular procedures. The reported incidence of catheter-related infections (not involving cut-down techniques) is much less than < 1% based on retrospective studies (Munoz *et al*., 2001; Ramsdale, Aziz, Newall, Palmer, & Jackson, 2004). This may be an underestimation of the true incidence of infections acquired during catheterization, as most signs and symptoms are unlikely to develop immediately following the procedure. In a prospective study of 147 consecutive blood cultures obtained after complex cardiac catheterization procedures, positive blood cultures were found in 18% and 12% of the subjects immediately following and 12 hours after the procedure, respectively. The most common organism was coagulase negative staphylococcus and none of the patients developed clinical signs of infection (Ramsdale *et al*., 2004).

Fever is a relative contraindication for elective procedures. Patients with ongoing infections should be appropriately treated before an elective cardiac catheterization (Chambers *et al*., 2006). Certain catheterization techniques have been shown in case studies to increase the risk of infectious complications. Local infections after angioplasty have been related to early re-puncture of ipsilateral femoral artery (Wiener & Ong, 1989), use of arterial grafts for access (McCready *et al*., 1991), and retention of catheters for prolonged periods (Polanczyk *et al*., 2001). Local hematomas can be a nidus of infection and should be treated urgently upon occurrence. Infection of the suture or collagen anchor in vascular closure devices are infrequent (0.5%), but can lead to limb-threatening arteritis when they occur (Baddour *et al*., 2004; Cooper & Miller, 1999). Insertion of a Foley catheter prior to the procedure should be noted as a potential cause of complicated urinary tract infection. Their use should be avoided when possible and, when inserted, removed when urine output monitoring is not further warranted.

3.2 Infectious Precautions

The American College of Cardiology does not recommend the use of strict operating room sterile techniques for most catheterization procedures (Bashore *et al*., 2001; Chambers *et al*., 2006).

Hair removal should be considered if there is interference with obtaining access site. When removal is necessary, electric clippers should be used and the use of razors should be avoided (Chambers *et al*., 2006; Ko, Lazenby, Zelano, Isom, & Krieger, 1992; O'Grady *et al*., 2002). Skin cleansing with a 2% chlorhexadine based preparation such as Chloraprep should be used prior to local anesthesia. A recent study of ~500 patients did not detect any difference in the rate of infections when caps and masks were worn (Laslett & Sabin, 1989). However, studies looking at the effectiveness of sterile techniques in the catheterization laboratory will require a large patient population in order to be sufficiently powered, given the low incidence of infection. Furthermore, the use of masks and eye shields may provide more protection to the operator to avoid blood splattering during the procedure. Following the procedure, the use of occlusive dressings and topical antimicrobials should be avoided as they can increase the risk of bacterial and fungal infections (Chambers *et al*., 2006). Antibiotic prophylaxis is not routinely indicated during cardiac catheterization (O'Grady *et al*., 2002).

4. Nephropathy

Contrast Induced Nephropathy (CIN) is a potentially serious complication of coronary angiography with significant short and long term sequelae. CIN, however, can be minimized with proper risk stratification, selection of contrast agent and staging of procedures, along with preventive management strategies. CIN has been defined as rise in serum creatinine of $\geq$ 0.5 mg/dl or 25% above the baseline value, based on data that associated such increases with clinically relevant outcomes, such as permanent renal impairment requiring hemodialysis, and death (Gami & Garovic, 2004). Varying definitions of CIN applied in studies with differences in patient co-morbidity have led to difficulty in assessing the true incidence of CIN, with reported rates ranging between 3.3-16.5% (Murphy, Barrett, & Parfrey, 2000). A large observational study in 1,826 consecutive patients uncovered an incidence of 14.4% in a community based population (McCullough, Wolyn, Rocher, Levin, & O'Neill, 1997). Smaller prospective studies in patients with fewer risk factors have shown a much smaller risk, roughly 3% (Rudnick, Berns, Cohen, & Goldfarb, 1997). Most patients, fortunately, experience a mild, transient increase in serum creatinine that is typically not associated with oliguria, peaks within two to four days, and generally resolves by 7 days.

The pathogenesis of CIN appears to be multifactorial. Multidirectional changes in renal hemodynamics due to the effects of contrast media on a number of vasoactive substances (adenosine, nitric oxide, endothelin) along with direct cytotoxicity through the action of free radicals have been implicated as potential causes (Barrett & Carlisle, 1993; R. Solomon, 2005). Preexisting renal insufficiency, diabetes, age, along with osmolality and volume of contrast used are the most significant risk factors to developing CIN. In retrospective studies of patients undergoing angiography, the incidence of CIN in patients with baseline creatinine < 2.0 mg/dl was higher among diabetic than nondiabetic patients. Amongst those with a baseline creatinine $\geq$ 2.0, all had a significantly higher risk of acute renal failure. Of the 7,856 patients studied, the risk of CIN was only 2.5% when creatinine was < 2 mg/dl, but rose to 30.6% when creatinine was > 3.0 mg/dl (Rihal *et al*., 2002). In patients who develop acute renal failure, the two largest studies have reported a nearly identical risk of 7.1% for suffering permanent kidney damage requiring hemodialysis (McCullough, Bertrand, Brinker, & Stacul, 2006; Rihal *et al*., 2002). In addition, multiple studies have shown a correlation between CIN and poor long-term survival (Bartholomew *et al*., 2004; Freeman *et al*., 2002; Rihal *et al*., 2002) with the risk of renal injury requiring dialysis, recurrent hospitalization, and death increasing proportionally to the severity of acute kidney injury (James *et al*.). In large registries, 22% of patients with acute renal failure die during the index hospitalization, compared with only 1.4% of patients without acute renal failure. Among hospital survivors with acute renal failure, 1 and 5 year estimated mortality rates were 12.1% and 44.6%, respectively; much greater than the 3.7% and 14.5% mortality rates in patients without acute renal failure (Rihal *et al*., 2002).

4.1 Prevention and Prophylaxis

Multiple individual risk factors have been reported for the development of CIN. Using multivariable regression models, risk scores have been developed that can assess the risk of CIN (Figure 1) (James *et al*.; Mehran *et al*., 2004). Of the modifiable variables, minimizing the volume of contrast medium administered is a primary defense against CIN. Radiocontrast dose was the most powerful independent predictor of nephropathy requiring dialysis (Cigarroa, Lange, Williams, & Hillis, 1989; Marenzi *et al*., 2009; McCullough *et al*., 1997; Rudnick *et al*., 1997). The overall volume appears to be more relevant in patients with baseline chronic kidney disease, who have a 5-10 fold increase in CIN when more than 125-140 ml of contrast is administered, irrespective of other preventive measures (McCullough *et al*., 1997; Taliercio *et al*., 1991). Therefore, most experts recommend limitation of contrast volume to 3 ml/kg.

<Figure 1>

As previously discussed, the osmolality and ionic content of the selected contrast media have been closely related to a number of adverse reactions, including CIN (Barrett & Carlisle, 1993; Jo *et al*., 2006; Lautin *et al*., 1991; McCullough *et al*., 2006; Rudnick *et al*., 1995). Aspelin *et al*. showed that the iso-osmolar nonionic Iodixanol (Visipaque) reduced the relative risk of CIN by 23% when compared to low-osmolar nonionic agent iohexol (Omnipaque) (Aspelin *et al*., 2003). The concept of osmolality being the sole contributor to CIN has been recently disputed by a randomized, double-blinded trial. The results of the CARE study failed to show any difference in CIN defined by multiple definitions following administration of non-ionic, low-osmolar Iopamidol in comparison to Iodixanol in high risk patients with or without diabetes mellitus (R. J. Solomon *et al*., 2007). A meta-analysis, conducted by the same investigator, also has shown little difference between agents whose osmolality is < 800 mOsm/kg. Such data suggest that factors such as viscosity and ionic content, in addition to the osmolality of the agent chosen, contribute to the overall risk of developing CIN (R. Solomon, 2005).

Volume expansion is the cornerstone for prevention of CIN. The effectiveness of saline administration is well documented by a series of small observational and randomized trials. The first controlled study to explore this relationship was performed in 1994 and showed that administration of 0.45% saline alone over 24 hours was more effective than the combination of volume supplementation and diuresis with furosemide or mannitol (R. Solomon, Werner, Mann, D'Elia, & Silva, 1994). Mueller *et al*. addressed the tonicity of fluids in 1,383 patients, comparing 0.45% saline with 0.9% saline. The rate of CIN was greater in patients receiving 0.45% saline (2.0 vs 0.7%, p = 0.04), without any difference in outcomes for dialysis or length of stay (Mueller *et al*., 2002). Subsequent to this study, a series of underpowered randomized trials have shown a moderate, but consistent benefit of isotonic saline administration at a rate of 1 ml/kg over a 24 hour period, beginning 12 hours prior to the procedure (Bader *et al*., 2004; Krasuski, Beard, Geoghagan, Thompson, & Guidera, 2003; Weisbord & Palevsky, 2008). The success of peri-procedural hydration can be extended to patients with chronic renal failure through continuous veno-venous hemofiltration. Hemofiltration allows for administration of large volumes of fluid without the associated risk of fluid overload. In randomized trials, hemofiltration vs. standard therapy in patients with moderate to severe renal insufficiency (baseline creatinine 3.0 mg/dl) lowered the absolute need for hemodialysis by 18%, with additional reductions in in-hospital events and one-year mortality (10% vs. 30% for controls) (Marenzi *et al*., 2003). Prophylactic hemodialysis, however, has not shown the same benefits (Vogt *et al*., 2001).

The antioxidant agent acetylcysteine, 600-1200 mg orally before and 600 mg twice daily after the procedure for 24-48 hours, has shown inconsistent benefit in prevention of CIN (Briguori *et al*., 2007; Coyle *et al*., 2006; Diaz-Sandoval, Kosowsky, & Losordo, 2002; Fung *et al*., 2004; Marenzi *et al*., 2006; Tepel *et al*., 2000; Webb *et al*., 2004). Meta-analysis of the available data up to 2003 showed that the addition of acetylcysteine to intravenous hydration led to a 56% relative reduction in CIN in comparison to hydration alone (Birck *et al*., 2003). Due to the cost effectiveness, feasibility of use, and benign side effect profile, many experts and institutions have advocated its routine use. A recent randomized international study in 2,303 patients from 46 hospitals across Brazil (ACT trial), however, has failed to show any benefit. In both groups, 12.7% of patients experienced CIN with similar elevations in serum creatinine and need for dialysis. This is the largest study conducted on the topic and may have answered the question on the potential benefit of acetylcysteine. ("Acetylcysteine for prevention of renal outcomes in patients undergoing coronary and peripheral vascular angiography: main results from the randomized Acetylcysteine for Contrast-induced nephropathy Trial (ACT)," 2011) Alkalinizing the urine with sodium bicarbonate infusion has been studied as an attractive mechanism to prevent CIN through attenuation of free radical formation(Brar *et al*., 2008). The results, however, have varied and failed to show a consistent benefit among trials.(From *et al*., 2008; Maioli *et al*., 2008; Merten *et al*., 2004; Recio-Mayoral *et al*., 2007) Most of the benefit appears to have been derived from smaller studies that assessed outcomes soon after radiocontrast administration (Zoungas *et al*., 2009). In some series, significant volume overload and heart failure have been reported after large volumes of sodium bicarbonate. Administration of ascorbic acid as an antioxidant, or the selective dopamine-1 agonist fenoldapam for promotion of renal plasma flow, has also failed to produce a consistent benefit.(Spargias *et al*., 2004; Stone *et al*., 2003)

5. Cholesterol Emboli

Cholesterol emboli are released as cholesterol crystals from friable vascular plaques. Distal embolization of cholesterol crystals after angiography, major vessel surgery, or thrombolysis causes a systemic syndrome.(Bashore & Gehrig, 2003; Kronzon & Saric) The diagnosis is suggested clinically by the appearance of discoloration of the extremities in a mottled purple pattern of livedo reticularis, or when there is digital cyanosis or gangrene, or neurological or renal involvement. Renal involvement is characteristically slowly progressing over a two to four week period following angiography. The diagnosis is confirmed through biopsy of affected tissues showing deposition of cholesterol crystals. Accompanying eosinophilia and elevated C-reactive protein are common laboratory features. The incidence reported in prospective studies is generally less than 2%.(Fukumoto, Tsutsui, Tsuchihashi, Masumoto, & Takeshita, 2003; Saklayen, Gupta, Suryaprasad, & Azmeh, 1997) Interestingly, autopsy studies have reported a much higher incidence, in range of 25-30%, indicating that many of these events are asymptomatic.(Fukumoto *et al*., 2003; Ramirez, O'Neill, Lambert, & Bloomer, 1978) This is further supported by the discovery of plaque debris from > 50% of all guiding catheters in a prospective study of 1,000 patients (Keeley & Grines, 1998). No significant difference in the risk of atheroembolism between brachial and femoral approaches exists, suggesting that the ascending aorta is the predominant source. Major risk factors include advanced age, repeat procedures, diffuse atherosclerotic disease, and elevated pre-procedure C-reactive protein. Treatment is mostly supportive but one retrospective study reported decreased incidence of cholesterol emboli with pre-procedural use of simvastatin.(Woolfson & Lachmann, 1998) Besides

statins, management with steroids and prostaglandins has not resulted in significant benefit.(Elinav, Chajek-Shaul, & Stern, 2002; Graziani, Santostasi, Angelini, & Badalamenti, 2001)

6. Local Vascular Injury

Vascular access site complications are among the most common and dreaded complications of coronary angiography, and are the most significant contributor to morbidity and mortality of the procedure. In the earlier days of cardiac catheterization, the incidence of vascular complications was reported to be between 0.7%-11.7% (Babu, Piccorelli, Shah, Stein, & Clauss, 1989; Omoigui *et al*., 1995; Oweida, Roubin, Smith, & Salam, 1990; Samal & White, 2002; Wyman *et al*., 1988). Over the past decade there have been significant advances in anticoagulant and antiplatelet therapies that have decreased the incidence of major cardiovascular events at a risk of increased bleeding. Major post-procedural bleeding and blood transfusions are associated with increased length of stay and decreased long-term survival. (Doyle *et al*., 2008) Fortunately, increasing experience and strategies aimed at decreasing the risk of access site complications has paralleled improvements in pharmacotherapy. Increasing awareness of the significance of peri-procedural bleeding to overall morbidity and mortality has resulted in the development and validation of scores aimed at identifying patients that are at the highest risk of bleeding. (Applegate *et al*., 2006; Kinnaird *et al*., 2003; Mandak *et al*., 1998; Nikolsky *et al*., 2007) Analysis from the IMPACT II trial has identified modifiable risk factors, such as early sheath removal, avoiding placement of venous sheaths, and careful monitoring of heparin doses as potential ways of decreasing bleeding risk and complications.(Mandak *et al*., 1998) Increasing experience with these complications has allowed for heightened awareness along with earlier detection and treatment techniques. Attempts at optimizing vascular access via fluoroscopic delineation of anatomical landmarks and identifying potential complications via peripheral angiography have come into routine practice (Turi, 2005). Advances in equipment design allow use of lower profile catheters via smaller sheaths, decreasing vascular trauma, and causing fewer complications.(Applegate *et al*., 2008; Metz *et al*., 1997; Talley, Mauldin, & Becker, 1995) The development of vascular closure devices has improved patient comfort following the procedure and, as development progresses further, could reduce the incidence of bleeding complications. Accordingly, these advances have resulted in significant decrease in vascular complications from 1998 to 2007. (diagnostic catheterization from 1.7% to 0.2%, percutaneous coronary intervention from 3.1% to 1.0%, respectively) (c).(Applegate *et al*., 2008)

< Figure 2>

Most of the local vascular complications can be avoided with optimal placement of the sheath in the common femoral artery (Figure 3). The common femoral artery courses over the femoral head in 92% of cases, and 99% of the time the bifurcation of the common femoral artery was below the middle of the femoral head (Garrett, Eckart, Bauch, Thompson, & Stajduhar, 2005), (Jacobi, Schussler, & Johnson, 2009; Sherev, Shaw, & Brent, 2005)(Kim *et al*., 1992)

< Figure 3>

6.1 Hematoma and Retroperitoneal Hemorrhage

Poorly controlled hemostasis following femoral sheath removal can result in a self-limited collection of blood in the anterior compartment of the thigh forming a hematoma. Most hematomas are benign, tender masses without connection to the accessed vessel. Larger hematomas, however, have been associated with formation of deep vein thrombosis and nerve compression resulting in sensory loss.(Butler & Webster, 2002; Shammas, Reeves, & Mehta, 1993) Large hematomas requiring blood transfusion occurred in 2.8% of the population in a large single center registry from 2000-2005 (Table 2).(Doyle *et al*., 2008) Manual compression of the proximal femoral artery and hematoma immediately following discovery and examination should be initiated. From our experience, 20-30 minutes of manual compression results in resolution of the hematoma when no further bleeding or false aneurysm are present. Prompt removal of access sheaths with 2-4 hours of bed rest following removal can help to decrease the incidence of femoral hematomas.

Larger or rapidly expanding hematomas can lead to hemodynamic compromise and multiple blood transfusions. In this setting, free femoral bleeding secondary to laceration of the femoral artery should be suspected. In such cases, a crossover sheath should be inserted into the contralateral femoral artery and bleeding site localized via angiography. In the case of uncontained bleeding, blood loss can be controlled by inflating a peripheral angioplasty balloon or deploying a graft stent at the site of vessel trauma (Samal & White, 2002).

<Table 2>

Retroperitoneal bleeding is a potentially life threatening complication of arterial access, more frequent when the artery is punctured above the inguinal ligament. Such bleeding is typically not evident from the surface, but

should be suspected when patient develops abdominal or flank pain along with hypotension and decreasing hemoglobin level. CT scans can be used to confirm clinical suspicion, but early recognition is essential in order to expedite application of manual compression and administration of fluids (Figure 4). Older age, female gender, low body surface area, and higher femoral artery puncture are significant risk factors for retroperitoneal hematomas. (Farouque *et al*., 2005; Sherev *et al*., 2005) Although there have been some concerns with increased risk with PCI in the glycoprotein IIb/IIIa inhibition era, no associations have been described in large retrospective studies. (Farouque *et al*., 2005) Most patients can be managed with reversal of anticoagulation, compression of the access site, observation, and volume expansion with or without blood products. When conservative management has failed, tamponading the puncture site via balloon angioplasty from the ipsilateral or contralateral femoral artery can be successfully performed (Samal & White, 2002).

< Figure 4>

6.2 Pseudoaneurysm

False aneurysms develop when a hematoma maintains continuity with the arterial lumen, resulting in blood flow into and out of the hematoma cavity during systole and diastole. The origin of the nomenclature stems from lack of normal arterial wall structures (media and adventitia) despite the typical aneurysmal appearance. The incidence is between 0.5-2.0% after diagnostic angiography and has been reported in as many as 7.7% of patients when coronary or peripheral interventions are performed. (Hessel, Adams, & Abrams, 1981; Katzenschlager *et al*., 1995) The principal risk factors for its development are similar to those for hematoma. False aneurysms occur more commonly following low access, where the superficial femoral artery is more likely to be accessed instead of the common femoral artery. This artery is smaller than the common femoral artery, making sheath insertion more traumatic. Furthermore, the lack of underlying bone provides less support for manual compression; too brief a period of manual compression is also a risk factor for its development.(Katzenschlager *et al*., 1995) Clinically, it is detected as a pulsatile mass with bruit adjacent to the site of femoral access. The diagnosis can be made radiographically demonstrating an aneurysmal structure with a thin neck or sinus tract that connects to the femoral artery. Although angiography and contrast CT can be used to make the diagnosis, Doppler color flow imaging has been shown to be the most effective technique for identification of vascular complications (Figure 4a) (Sheikh *et al*., 1989). Prompt diagnosis can avoid the catastrophic risk of rupture, which is likely to occur in larger aneurysms (> 3 cm), in the presence of symptoms, large hematoma, or continued growth of the sac.(Kent *et al*., 1993; Kresowik *et al*., 1991; Webber, Jang, Gustavson, & Olin, 2007)

The treatment depends on the size of the false aneurysm and the rate of growth. False aneurysms less than 2-3 cm in greatest diameter can be managed expectantly and followed on an outpatient basis with serial ultrasound examinations (Johns, Pupa, & Bailey, 1991; Kent *et al*., 1993; Kresowik *et al*., 1991). However, aneurysmal size is not an absolute predictor of thrombosis(Kent *et al*., 1993); therefore, patients with false aneurysms of any size should be followed closely until thrombosis occurs. Most experts advocate for intervention in symptomatic patients who have a false aneurysm > 2.0 cm (Webber *et al*., 2007). Larger aneurysms have traditionally been repaired surgically or through ultrasound-guided compression of the femoral neck without compromising femoral artery flow (Samal & White, 2002). More recently, percutaneous injection of thrombin (1000 US U/mL) has been demonstrated as an effective method of thrombosis, with up to 96% primary success rate (Krueger *et al*., 2005; Webber *et al*., 2007). Ultrasound guided injection of thrombin can be completed in several minutes, has the advantage of avoiding surgical intervention or the pain associated with ultrasound-guided compression, and can be performed effectively in patients who have received anticoagulation (Figure 5a-d) (Lennox *et al*., 1999; Pezzullo, Dupuy, & Cronan, 2000; Taylor *et al*., 1999). Surgical repair of false aneurysms is reserved for cases which exhibit rapid expansion, infection, or failure of closure via thrombin injection. (Samal, White, Collins, Ramee, & Jenkins, 2001; Webber *et al*., 2007)

< Figure 5>

6.3 Arteriovenous Fistula

Arteriovenous Fistulas (AVF) arise when a needle tract crosses both the artery and vein, with subsequent dilation during sheath insertion (Figure 6). They can also arise from on-going bleeding from the puncture site that compresses into an adjacent femoral vein. As such, they are typically caused by low arterial access into the superficial femoral artery because of the anterior-to-posterior relationship of the artery to the superficial femoral vein, as opposed to the side-by-side relationship of the common femoral artery and vein (Kim *et al*., 1992). Diagnosis is made by the auscultation of a thrill or continuous bruit over the puncture site, and confirmed by contrast CT or Doppler sonography. Prospective follow-up studies in over 10,000 patients undergoing

transfemoral cardiac catheterization have shown an incidence of almost 1%. Management is usually conservative with close follow-up, as nearly one-third of AVF closed spontaneously within one year(Kelm *et al*., 2002) Surgical management is reserved for symptomatic patients, high output heart failure, or fistulas that do not close spontaneously within one year(Samal & White, 2002)

< Figure 6>

6.4 Dissection

Dissection of the femoral and iliac arteries occurs very infrequently, (0.42% of the most current cohorts). (Prasad *et al*., 2008) It occurs more commonly in iliac arteries with increased atherosclerotic burden, tortuosity, or traumatic sheath placement. Occlusive dissection is a potentially limb and life-threatening complications that can be identified and treated safely upon diagnosis. Cine images of the femoral access site prior to completion of the study are helpful in localizing potential dissections, and should be performed in patients with difficult access or traumatic sheath placement. When detected, removal of the wires and catheters can allow for spontaneous resolution(Samal & White, 2002) When large, flow limiting dissections occur, angioplasty and stenting have been shown to be a safe and effective method of treatment and surgery can usually be avoided(Scheinert *et al*., 2000)

6.5 Thrombosis and Embolism

Thrombosis typically occurs in female patients with small vessel lumen, peripheral arterial disease, diabetes mellitus, placement of a large diameter catheter or sheath (intraaortic balloon pump), or long catheter dwell-time (Noto *et al*., 1991; Popma *et al*., 1993). Patients typically complain of a painful leg with impaired sensation and motor function in the distal extremity. Loss of peripheral pulses and the appearance of a white painful foot can often be found on physical examination. Thrombotic and embolic complications can be avoided with careful, frequent flushing of the arterial sheaths (prevents thrombus formation) and use of anticoagulation during prolonged procedures and intraaortic balloon pump use. Treatment involves percutaneous thrombectomy or thrombolytic therapy in conjunction with vascular surgery consultation (Samal & White, 2002).

6.6 Vascular Closure Devices

Various methods for percutaneous closure of the femoral artery have been explored over the years in order to avoid manual compression and shorten bed rest, two of the biggest sources of dissatisfaction to patients. Although these devices allow for greater comfort and earlier ambulation, their safety and cost effectiveness in comparison to manual compression has not been unequivocally confirmed. Their benefit has been marginal, at best, and many studies have reported increased incidence of vascular complications following PCI (Koreny, Riedmuller, Nikfardjam, Siostrzonek, & Mullner, 2004; Nikolsky *et al*., 2004). These reviews, however, were of trials of vascular closure devices in their early years of use. Technological improvements to devices and increased operator experience are likely to contribute to improved efficacy and safety. This was demonstrated in the analysis of the ACUITY trial by Sanborn *et al*., where interventional or surgical correction or hematoma ≥ 5 cm occurred significantly less often in patients undergoing PCI when a vascular closure device was used (0.4% versus 0.8%, $P<0.05$ and 1.9% versus 2.7%, $P<0.03$, respectively) (Sanborn *et al*.). The magnitude of these reductions is very similar to those recently reported from the large Northern New England PCI Registry in which the relative risk reduction in bleeding and vascular complications with bivalirudin and vascular closure device use during PCI was 52% and 25%, respectively.(Ahmed *et al*., 2009) Therefore, marked reductions in femoral access complications may be realized with advancement in vascular closure device technology and increased operator experience, along with adjunctive medications that decrease the risk of bleeding.

6.7 Transradial Approach

Transradial approach has gained progressive acceptance since its introduction over 20 years ago, largely due to decrease in vascular complications and patient satisfaction with the procedure. The approach has several advantages, in that, the radial artery is not immediately associated with nearby nerves or veins and is easily compressible, allowing for improved hemostasis. Furthermore, the hand receives dual blood supply through the ulnar and radial arteries via the palmar arch. Therefore, any radial artery occlusion (reported in 5-19%) (Greenwood *et al*., 2005) is not clinically important in most patients because the hand is perfused by extensive collateral flow between the two arteries. When performed properly, the Allen test is an easy and effective method to assess the adequacy of collateral blood flow into the hand. In patients in whom the Allen test fails, there is an increased incidence of complications and occlusion of flow to the hand.

In a meta-analysis by Agostini *et al*. in 2004, similar rates of major adverse cardiovascular events with both access routes were observed, with significantly lower rate of entry site complications in the radial access group.

The advantages are, however, balanced by the higher proportion of procedural failures, 7.2% versus 2.4% for femoral access (Agostoni *et al*., 2004). Data from the National Cardiovascular Data Registry from 2004-2007 has shown that the proportion of radial artery interventions has been increasing steadily. However, they still only constitute 1.3% of all total procedures performed during that time. Compared with the femoral approach, use of radial PCI in recent data is associated with similar rate of procedural success but lower risk of procedural bleeding(Rao *et al*., 2008) Increased operator experience, further development of low profile catheters and stents, along with greater patient satisfaction and comfort, fuel the interest in this field.

7. Conduction Disturbances

7.1 Bradyarrhythmia

Transient bradycardia is a common occurrence in the catheterization laboratory. They were far more frequent in the era of high osmolar ionic contrast agents, but have declined recently due to the widespread use of iso-osmolar, non-ionic contrast material. Prolonged episodes of bradycardia can lead to vagal response with associated hypotension, nausea, sweating, and yawning. This was observed in nearly 3.5% of patients in one study, 80% of which developed during access and 16% during sheath removal (Landau, Lange, Glamann, Willard, & Hillis, 1994). Treatment of anxiety and pain, along with adequate hydration can help avoid prolonged vagal reactions. Furthermore, hypotension and bradycardia can be one of the first signs of perforation and tamponade, as a vagal response is induced through irritation of the pericardium. Coughing forcefully can help to increase coronary perfusion and restore normal cardiac rhythm. When coughing is unsuccessful, rapid intravenous fluid administration, treatment of underlying pain or anxiety, and 0.5-1 mg of atropine intravenously can help reverse the bradycardia. In cases of complete heart block, temporary pacing through a transvenous pacemaker should be rapidly initiated.

Conduction disturbances also occur, but at a much lower frequency than vagal episodes. Passing of the catheter across the aortic valve will usually cause some ectopy. However, in a patient with pre-existing right bundle branch block, the development of left bundle branch block because of septal scraping can lead to complete heart block and cardiovascular collapse. Conversely, in a patient with a pre-existing left bundle branch block, right heart catheterization and right bundle branch block can cause a similar scenario. As such, the electrocardiogram of every patient should be reviewed prior to procedure by the operator. Minimizing the period of ectopy can help in avoiding these complications.

7.2 Tachyarrhythmia

Atrial arrhythmias may occur following irritation of the right atrium by the catheter during right heart catheterization. These arrhythmias usually do not require immediate treatment unless they produce ischemia or hemodynamic instability. The occurrence of ventricular tachycardia and ventricular fibrillation in the current era is related to irritation of the myocardium by the catheter. Identification of ventricular ectopy by trained technicians and engaged operators can help reduce the incidence of these arrhythmias. When a run of ventricular tachycardia is noted, the offending catheter must be pulled back immediately to allow restoration of normal sinus rhythm. Ventricular arrhythmias were more prominent in the era of high osmolar, ionic contrast when intracoronary injection into the right coronary artery caused ventricular dysrhythmia in 1.3% of patients.(Adams, Fraser, & Abrams, 1973; Zukerman *et al*., 1987) The most recent reports, however, place this complication rate at 0.1% (Chen, Gao, & Yao, 2008). In patients with acute myocardial infarction, ventricular tachycardia occurred in 4.3% of the patients with ST- elevation MI during cardiac catheterization in the PAMI trial.(Mehta *et al*., 2004) Pre-treatment of high risk patients with beta-blockers, or initiation of anitarrhythmic therapy with lidocaine or amiodarone during recurrent episodes should be considered as treatment options. Hemodynamically unstable atrial rhythms or any sustained ventricular tachyarrhythmia should be treated with direct current cardioversion.

8. Death

During the last few decades, the incidence of death has progressively declined during left heart catheterization. In the early 1960s, 1% mortality was observed with diagnostic catheterization, which has decreased to 0.08% in the 1990s(Braunwald & Gorlin, 1968; Chandrasekar *et al*., 2001; Johnson *et al*., 1989; Kennedy, 1982; Noto *et al*., 1991) There are a number of baseline variables which contribute to mortality during coronary angiography: presence of multivessel disease, left main coronary artery disease (LMCA), CHF, renal insufficiency and advanced age are the most important of them (Laskey, Boyle, & Johnson, 1993). In recent years, cardiac catheterization and percutaneous coronary intervention have witnessed new developments, such as stents and potent antiplatelet agents that could affect the overall complication rate.(Chandrasekar *et al*., 2001)

Significant LMCA disease increases the risk of dissection during catheter engagement and injection of contrast, which is reported to be around 0.07% and almost twice higher with percutaneous intervention(Cheng *et al*., 2008; Eshtehardi *et al*.) Mortality associated with iatrogenic LMCA dissection is reported around 3% (Eshtehardi *et al*.), particularly if undetected. Emergency therapeutic interventions with either coronary artery bypass surgery or percutaneous coronary intervention with stents are required.

Patient with depressed chronic left ventricular function and those with acute MI who are in shock are at the highest risk for mortality.(Anderson *et al*., 2007; Johnson *et al*., 1989; Shaw *et al*., 2002) If frank cardiogenic shock is present or develops during cardiac catheterization, intra-aortic balloon pump and inotropic support may be required.

If PCI is performed in addition to coronary angiography, the incidence of mortality is higher.(Dorros *et al*., 1983; Shaw *et al*., 2002) Recent data from the American College of Cardiology-National Cardiovascular Data Registry (ACC-NCDR) published in 2010 have shown that factors which are associated with increased risk of mortality during PCI are cardiogenic shock, increasing age, salvage, urgent or emergency PCI, decreased left ventricular ejection fraction, acute MI, diabetes, chronic renal failure, multivessel disease, prior coronary artery bypass grafts (CABG) and chronic occlusion. Overall, PCI in-hospital mortality was 1.27%, ranging from 0.65% in elective PCI to 4.81% in ST- elevation MI patients. (Anderson *et al*., 2007; Peterson *et al*.; Shaw *et al*., 2002)

Patients with aortic stenosis have higher mortality; the VA Cooperative study on Valvular Heart Disease has shown a 0.2% mortality among 1559 preoperative catheterizations. (Folland *et al*., 1989) Bartsch *et al*. have shown a mortality rate 1.1% in patients with aortic value stenosis requiring left heart catheterization to determine the transvalvular gradient.(Bartsch, Haase, Voelker, Schobel, & Karsch, 1999)

Patients with history of CABG who required diagnostic and therapeutic cardiac catheterization are typically older and have generalized atherosclerosis, worse left ventricular function, and require a more lengthy and complex procedure. Varghese *et al*. have shown that patients with coronary artery bypass undergoing graft PCI have no difference in terms of mortality as compared to patients with CABG undergoing native vessel PCI (Varghese, Samuel, Banerjee, & Brilakis, 2009)(Garcia-Tejada *et al*., 2009).

9. Myocardial Infarction

Myocardial damage can occur in different clinical settings: spontaneous, during diagnostic cardiac catheterizations, during percutaneous intervention and during CABG surgery. There have been different thresholds for identifying an infarct in clinical trials: CK-MB > 2 times the upper limit of normal (ULN) for spontaneous MI; CK-MB > 3 times the ULN with coronary interventions; and CK-MB > 5-10 times the ULN for bypass surgery (Alpert, Thygesen, Antman, & Bassand, 2000). In the late 1970s, the data from the Coronary Artery Surgery Study (CASS) showed a MI rate of 0.25% for coronary angiography (Davis *et al*., 1979). In the first, second, and third registries conducted by the Society for Cardiac Angiography, the risk of MI fell progressively, from 0.07%, to 0.06%, to 0.05% (Johnson *et al*., 1989; Kennedy, 1982; Noto *et al*., 1991). The risk of MI during diagnostic catheterization is clearly influenced by patient-related factors that include the extent of CAD (0.06% for single vessel disease, 0.08% for triple-vessel disease, and 0.17% for left main disease) (Johnson *et al*., 1989). With improvement in the equipment and operator skill, the use of more potent antithrombotic and antiplatelet agents, better patient preparation with the use of beta blockers and statins and adoption of low osmolar contrast agents, the incidence of myocardial MI during cardiac catheterization has been reduced considerably (Judkin & Gander, 1974; Pasceri *et al*., 2004).

Approximately 1.5 million patients undergo PCI in the United States every year (Roger *et al*.). Depending on local practices and diagnostic criteria used, 5 to 30% of these patients have evidence of peri-procedural MI.

At the higher estimate, the incidence of these events is similar to the annual rate of major spontaneous MI. The predictors of peri-procedural MI can be broadly categorized as patient-, lesion-, and procedure- related risk factors (Herrmann, 2005). The major risk factors in terms of both frequency and extent, are complex lesions (e.g., the presence of thrombus, stenosis of a saphenous-vein graft, or a type C lesion), complex procedures (e.g., treatment of multiple lesions or use of rotational atherectomy), and procedural complications (e.g., abrupt vessel closure, side-branch occlusion, distal embolization, or no reflow). (Herrmann, 2005; Mandadi *et al*., 2004; van Gaal *et al*., 2009) The occurrence of peri-procedural ischemic symptoms, particularly chest pain at the end of the procedure, or electrocardiographic evidence of ischemia defines the sub-group of patients most likely to have peri-procedural MI (Cai *et al*., 2007).

Large peri-procedural myocardial MIs are usually due to angiographically visible complications; however, this is generally not the case in the vast majority of patients with elevated biomarker levels after PCI. Cardiac magnetic

resonance imaging has confirmed two distinct locations for peri-procedural myonecrosis: adjacent to the site of the intervention, where the injury is most likely due to epicardial side-branch occlusion and downstream from the intervention site, where it is most likely due to compromise of the microvascular circulation.

Studies evaluating the relationship between the post-procedural cardiac troponin level and long term mortality, in general, have not excluded patients with acute coronary syndromes (ACS), many of whom had abnormal cardiac biomarker levels at baseline. (Cantor *et al*., 2002; Cavallini *et al*., 2005; D. N. Feldman, Minutello, Bergman, Moussa, & Wong, 2009; Kini *et al*., 2004; Kizer *et al*., 2003; Nallamothu *et al*., 2003; Natarajan *et al*., 2004; Nienhuis, Ottervanger, Bilo, Dikkeschei, & Zijlstra, 2008; Testa *et al*., 2009) Thus, the reported frequency of post-procedural elevations in cardiac troponin has been highly variable, and although some studies showed that the serum concentration of cardiac troponin was an independent predictor of survival, others did not.

It remains uncertain whether a similar amount of damage in different settings has the same prognostic implications. Mahaffey *et al*. have studied the outcome of peri-procedural MI versus spontaneous MI in a large pool of 16,173 patients from PURSUIT and PARAGON B trials of non-ST- elevation MI. It was clearly evident that patients with peri-procedural or spontaneous MI had significantly higher one and six month mortality. (Mahaffey *et al*., 2005) A recent analysis from the ACUITY trial was conducted among 7,773 patients with moderate to high risk, non-ST elevation MI who underwent PCI. (Prasad *et al*., 2009) Peri-procedural and spontaneous MIs during follow-up developed in 6.0% and 2.6% of the cohort, respectively. After adjustment for differences in baseline and procedural characteristics, spontaneous MI was a powerful independent predictor of an increased risk of death, whereas peri-procedural MI was not significantly associated with an increased risk of death. Similar observation was made among patients with diabetes and stable CAD in Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial. (Chaitman *et al*., 2009)

Taken together, contemporary studies indicate that spontaneous MI is a powerful predictor of mortality. Peri-procedural MI, although frequent, is a marker of atherosclerosis burden and procedural complexity, but in most cases, it does not have important independent prognostic significance in stable CAD or in non-ST-elevation MI. Although large peri-procedural infarcts may affect prognosis, they rarely occur in the absence of procedural complications or in patients with normal baseline cardiac troponin levels.

10. Cerebrovascular Complications

Although the overall incidence of stroke after left heart catheterization or percutaneous intervention is low, it is the most debilitating complication and is associated with a high rate of morbidity and mortality (Table 3, Figure 7) (Akkerhuis *et al*., 2001; Fuchs *et al*., 2002; Lazar *et al*., 1995; Wong, Minutello, & Hong, 2005). Early experience showed an incidence as high as 0.23% in the 1973 study of Adams and others (Adams *et al*., 1973), compared with the 0.07% incidence for the more recent diagnostic catheterization data included in the Society for Cardiac Angiography-registries (Johnson *et al*., 1989; Kennedy, 1982).

< Figure 7>

< Table 3>

The risk of stroke, as expected, is somewhat higher with coronary intervention, because of use of guiding catheters, multiple equipment exchanges in the aortic root, aggressive anticoagulation and longer procedure times. In 20,697 patients who underwent PCI in a large-volume center, stroke occurred in 0.44%(Dukkipati *et al*., 2004). Multivariable analysis has shown that occurrence of stroke was associated with diabetes, hypertension, prior stroke and renal failure and was an independent predictor of in-hospital death (Hamon *et al*., 2008). Patients who suffered a stroke had undergone longer cardiac catheterization procedures, received more contrast, were more likely to have had the procedure for urgent reasons, and to have intra-aortic balloon counter pulsation.(Stone *et al*., 1997) Possible explanations for this latter characteristic include the greater propensity for hemodynamic compromise in these patients, which may increase the risk of ischemic stroke. Indeed, scraping of aortic plaque occurs in > 50% of PCI cases and more frequently with larger catheters (Keeley & Grines, 1998).

Cerebral micro-embolism is thought to be the main mechanism of peri-procedural ischemic stroke occurring with PCI. This finding is supported by transcranial doppler studies performed during cardiac catheterization, which show the systematic occurrence of cerebral micro-emboli.(Bladin *et al*., 1998; Hamon *et al*., 2006; Leclercq *et al*., 2001) Air embolism, thrombus formation in the catheter or its surface, or dislocation of aortic atheroma during manipulation and passage of catheters within the aorta are the main sources of embolic material causing ischemic stroke during cardiac catheterization or PCI. As expected, patients with CAD more frequently have severe atheroma in the descending aorta and aortic arch than patients without CAD (Khoury, Gottlieb, Stern, & Keren, 1997).

In addition to the aortic root, embolic material may also originate in the cardiac chambers, thrombotic coronary arteries, or the surface of cardiac valves. One should avoid placing the pigtail catheter in the left ventricle in patients with suspected aneurysm or recent MI, since either condition may be associated with potentially dislodgeable mural thrombus.

Peri-procedural strokes associated with invasive procedures are predominantly (80%) attributable to embolic material that lodges in cerebral arteries. However, given the increasingly aggressive antithrombotic environment used in PCI, especially in ACS, cerebral hemorrhages are also encountered. This means that ischemic or hemorrhagic mechanisms have to be documented before any treatment can be initiated.

11. Dissection and Perforation of Great Vessels

Perforation of the cardiac chambers, coronary arteries or intrathoracic great vessels is fortunately a rare event in diagnostic catheterization. The incidence of catheter-induced ascending aorta dissection is reported around 0.04% of cases (Gomez-Moreno *et al*., 2006). The incidence of coronary artery dissection is reported around 30% with balloon angioplasty (Figures 8-10).(Cowley, Dorros, Kelsey, Van Raden, & Detre, 1984; Huber, Mooney, Madison, & Mooney, 1991)

<Figure 8, 9, 10>

In recent registries the incidence of coronary artery perforation has been reported to occur in 0.3-0.6% of patients undergoing PCI. (Cowley *et al*., 1984; Dippel *et al*., 2001; Ellis *et al*., 1994; Gruberg *et al*., 2000) With the use of hydrophilic guidewires, platelet IIb/IIIa receptor blockers, and more aggressive atherectomy technologies, the incidence of coronary perforation may be higher. Some perforations, particularly those limited to deep injury to the vessel wall with localized perivascular contrast staining, can simply be observed. But such patients are at risk for delayed tamponade during the several hours following the procedure and must be monitored carefully. In contrast, free perforation may lead to development of frank tamponade within seconds to minutes, particularly when the patient is fully anticoagulated. Immediate measures should be used to seal the perforation with inflation of balloon proximal to the perforation. If after 10-15 minutes or development of ischemia, the extravasation of contrast persists, graft stents need to be utilized to seal the arterial rupture. In parallel, pericardiocentesis should be considered to provide the time necessary to seal the perforation. Overall, the reported incidence of required emergency surgery following diagnostic angiography is 0.05%, and 0.3% following therapeutic procedures (Chandrasekar *et al*., 2001; Loubeyre *et al*., 1999). However, once coronary artery perforation is diagnosed, the reported incidence of emergency surgery requiring pericardial window, bypass surgery or coronary artery ligation is as high as 24-40% (Table 4).

<Table 4>

Perforation of the great vessels (aorta or pulmonary artery) is extremely rare. Ascending aortic dissection can also result from vigorous use of a guiding catheter or extension from proximal coronary dissection.

Right heart catheterization can cause cardiac perforation; it is usually accompanied by bradycardia and hypotension owing to vasovagal stimulation. As blood accumulates in the pericardium, the cardiac silhouette may enlarge and the normal pulsation of the heart borders on fluoroscopy will become blunted. If the patient is hemodynamically compromised, immediate pericardiocentesis is should be performed via the subxiphoid approach. Once pericardiocentesis has stabilized the situation, the operator must decide whether or not emergency surgery will be needed to over sew the site of perforation. Most perforations, in fact, will seal spontaneously, so that surgery is unnecessary.

12. Other Complications

12.1 Hypotension

Reduction in arterial blood pressure is one of the most common problems seen during catheterization. This reduction is the final common manifestation of a variety of conditions including the following: (a) hypovolemia, owing to inadequate hydration before procedure, or excessive contrast-induced diuresis; (b) reduction in cardiac output, tamponade, arrhythmia or valvular regurgitation; or (c) inappropriate systemic arteriolar vasodilatation, due to vasodepressor response to contrast or (d) potential bleeding from retroperitoneal hemorrhage.

Low filling pressures mandate rapid volume administration, whereas low filling pressure combined with inappropriate bradycardia indicates a vasovagal reaction and atropine should be given in addition to fluid resuscitation. High filling pressures, however, suggest primary cardiac dysfunction and should prompt consideration of ischemia, tamponade, or sudden onset of valvular regurgitation. Such patients should be supported empirically by inotropic agents, vasopressors or circulatory support devices.

Patients with hypotension and normal or high cardiac output measured through saturation Swan-Ganz catheters are more likely to have an allergic reaction to contrast and may require vasopressor support, steroids and histamine blockers.

12.2 Hypoglycemia

Diabetic patients who are required to fast before procedure may develop hypoglycemia; special attention should be given to these patients and finger-stick blood glucose should be monitored closely before and during the procedure. If any signs of hypoglycemia, including anxiety, or lethargy develop, prompt action should be taken to administer intravenous glucose.

12.3 Respiratory insufficiency

Respiratory insufficiency can occur from a variety of reasons, including CHF with pulmonary edema, pre-existing lung disease, and allergic reaction or over-sedation. Immediate assessment of patient's condition is required and therapeutic measures should be taken based on the presumed etiology.

13. Conclusion

Cardiac catheterization is a relatively safe procedure with few complications. Although advances in medical management and equipment design have added further significant reductions to the already low incidence of complications, operator awareness and appropriateness of response remain as the most important predictors to adverse outcomes. With each coronary angiography the potential benefit of the procedure should be weighed against the established risk factors with the well-defined morbidity and mortality. The widespread use and availability of angiography will likely fuel further advances in percutaneous modalities that may increase patient comfort while simultaneously reducing complications further.

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Table 1. Specific recommendation for pre-medication regimens. Adapted from the American College of Radiology guidelines (Amreican College of Radiology, 2010). Note that use of H2 blockers is not supported by the current guidelines.

Elective Pre-Medication	1. Prednisone 50 mg by mouth at 13 hours, 7 hours, and 1 hour before contrast media injection 2. Diphenhydramine 50 mg intravenous, intramuscular, or by mouth 1 hour before contrast medium injection
Emergency Pre-Medication (Decreasing order of desirability)	1. Methylprednisolone 40 mg or hydrocortisone sodium succinate 200 mg intravenously every 4 hours until contrast study required plus diphenhydramine 50 mg intravenous 1 hour prior to contrast injection 2. Dexamethasone sodium sulfate 7.5 mg or betamethasone 6.0 mg every 4 hours until contrast study. Must be done in patients with known allergy to methylprednisolone, aspirin, or nonsteroidal anti-inflammatory drugs, especially if asthmatic. Also diphenhydramine 50 mg intravenous 1 hour prior to contrast injection. 3. Omit steroids entirely and give diphenhydramine 50 mg intravenous.

Table 2. Changing incidence of major femoral bleeding and blood transfusions after PCI. (*p < 0.005 versus 2000-2005)

	1994-1995 (n = 2,441)	**1996-1999 (n = 6,207)**	**2000-2005 (n = 9,253)**
Femoral Hematoma	172 (7.0%)*	236 (3.8%)*	257 (2.8%)
Femoral Bleed	60 (2.5%)*	76 (1.2%)*	54 (0.6%)
Retroperitoneal Bleed	20 (0.8%)*	19 (0.3%)	26 (0.3%)
Blood Transfusion	207 (8.5%)*	482 (7.8%)*	516 (5.6%)
1 to 2 Units	98 (4.0%)	288 (4.6%)*	347 (3.8%)
3 + Units	109 (4.5%)*	194 (3.1%)*	169 (1.8%)

Table 3. Incidence of peri-procedural stroke in PCI registries(Hamon, Baron, & Viader, 2008)

Reference	No. Patients	No.	Percentage	95% CI
Lazar *et al*., 1995				
Total	6465	27	0.42	0.27-0.60
Ischemic		NA	NA	NA
Hemorrhagic		NA	NA	NA
Uncertain		NA	NA	NA
Akkerhuis *et al*., 2001				
Total	8555	31	0.37	0.24-0.51
Ischemic		19	0.22	0.13-0.34
Hemorrhagic		12	1.4	0.07-0.24
Uncertain		1	0.01	0.00-0.06
Fuchs *et al*., 2002				
Total	9662	43	0.44	0.32-0.6
Ischemic		21	0.22	0.13-0.33
Hemorrhagic		20	0.21	0.13-0.32
Uncertain		2	0.01	0.00-0.07
Dukkipati *et al*., 2004				
Total	20679	92	0.44	0.36-0.54
Ischemic		43	0.21	0.15-0.28
Hemorrhagic		13	0.06	0.03-0.10
Uncertain		36	0.17	0.12-0.24
Wong *et al*., 2005				
Total	76903	140	0.18	0.15-0.21
Ischemic		NA	NA	NA
Hemorrhagic		NA	NA	NA
Uncertain		NA	NA	NA

Table 4. Incidence of coronary artery perforation with in-hospital complications (Nair & Roguin, 2006)

Reference	Patients	Incidence	CABG	MI	Death
Bittl *et al*., 1993	764	3%	34.7	4.3	9
Ajluni *et al*., 1994	8932	0.40%	37	26	5.6
Holmes *et al*., 1994	2759	1.30%	36.1	16.7	4.8
Ellis., 1994	12900	0.50%	24	19	0
Cohen *et al*., 1996	2953	0.70%	41	45.5	9
Gruberg *et al*., 2000	30746	0.29%	39	34	10
Dippel., 2001	6214	0.58%	22	NA	11
Gunning *et al*., 2002	6245	0.80%	39	29	42
Fejka *et al*., 2002	25697	0.12%	39	29	42
Stankovic *et al*., 2004	5728	1.47%	13	27	8
Witzke *et al*., 2004	12658	0.30%	5	18	2.5
Ramana *et al*., 2005	4886	0.50%	0	20	8

Figure legend

Figure 1 Multivariable CIN risk score (Mehran *et al*., 2004)

Figure 2 Any vascular complications by procedure and closure method. CATH - diagnostic cardiac catheterization; MC - manual compression; PCI - percutaneous coronary intervention; VCD - vascular closure device (Applegate *et al*., 2008)

Figure 3 (a) Fluoroscopy of the femoral head utilizing forceps to note the position of the inferior border of the femoral head on the patient's skin. (b) Correct placement of the sheath in the common femoral artery. (c) Correct placement of the sheath in relation to the femoral head, with the arterial access incorrectly placed in the superficial femoral artery due to the anatomic variant of a high bifurcation. (d) Correct placement of the sheath in relation to the femoral head with a low hypogastric artery causing incorrect arterial placement in the external iliac artery. (e) Low sheath placement in the profunda femoris artery. (f) High sheath placement in the external iliac artery (Jacobi *et al*., 2009).

Figure 4 Retroperitoneal bleeding following cardiac catheterization via right femoral access

Figure 5 Duplex ultrasound image of pseudoaneurysm, demonstrating arterial flow through a long, narrow neck arising from defect in femoral artery and turbulent color flow into cavity (a). With color flow removed, exact position of needle tip can be identified at all times during procedure, because a small amount of echogenic thrombus forms at needle tip when thrombin comes into contact with blood, helping to guide needle placement (b). With needle in position, color flow during injection of thrombin confirms acute development of thrombus within sac (c). Power Doppler image of patent native fem- oral vessels (CFA indicates common femoral artery; SFA, superficial femoral artery; and PFA, profunda femoris artery) and absence of flow after successful thrombin injection into pseudoaneurysm cavity (d) (Lennox *et al*., 1999).

Figure 6 AVF result when needle tract crossing both artery and vein is dilated and catheterized. V = vein, A = artery(Kim *et al*., 1992)

Figure 7 Pooled relative risk (random effects) of mortality after stroke in PCI or in patients with non ST-elevation MI

Figure 8 Angiogram of right coronary artery before (a) and after perforation (b).

Figure 9 Angiogram of right coronary artery prior to intervention (a), after balloon angioplasty (b) and dissection (c).

Figure 10 Angiogram of the left coronary system (a). Dissection of the left circumflex artery with guidewire catheter (b) with subsequent extension in to the left anterior descending artery (c).

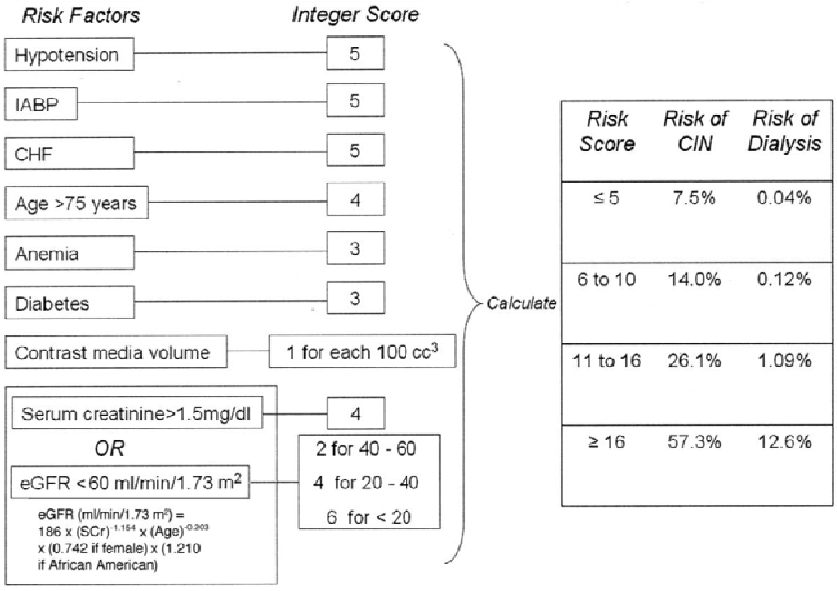

Figure 1. Multivariable CIN risk score (Mehran *et al*., 2004)

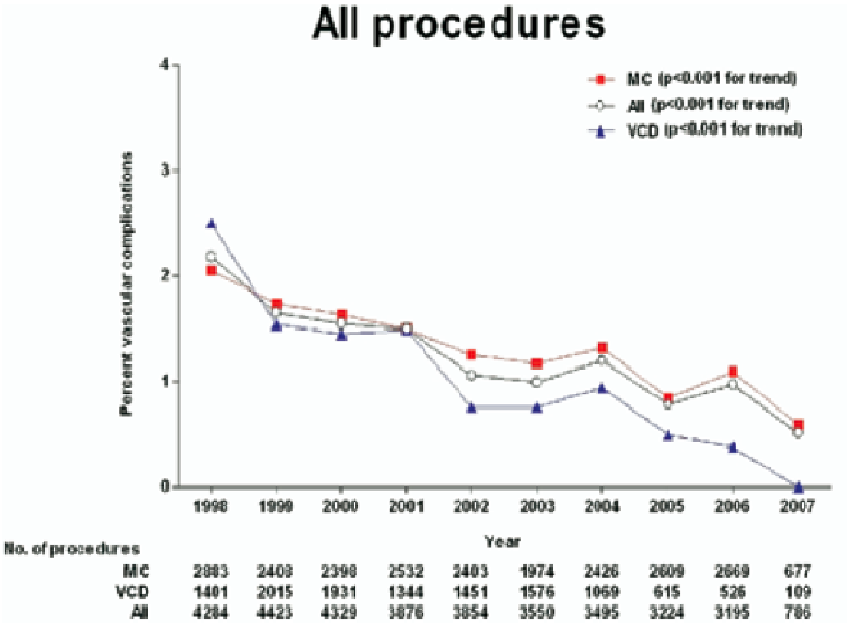

Figure 2. Any vascular complications by procedure and closure method. CATH - diagnostic cardiac catheterization; MC - manual compression; PCI - percutaneous coronary intervention; VCD - vascular closure device.(Applegate *et al*., 2008)

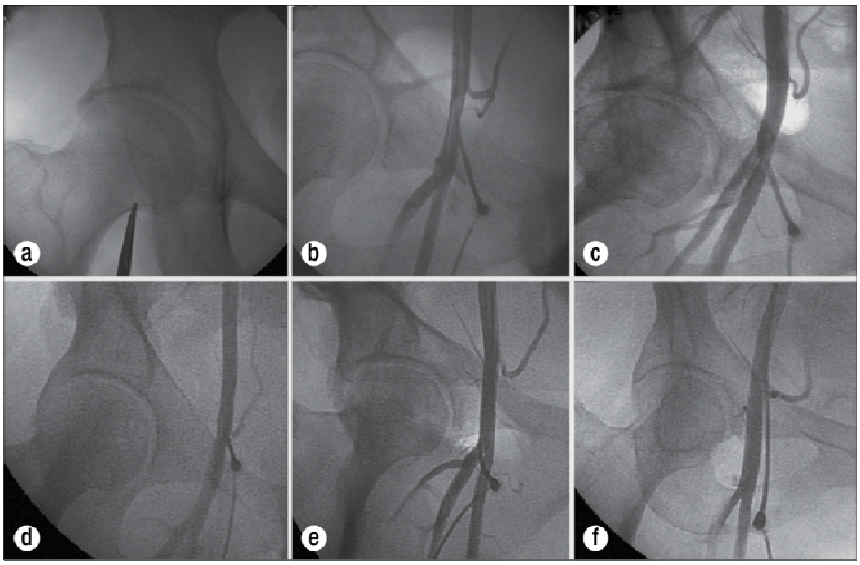

Figure 3. (a) Fluoroscopy of the femoral head utilizing forceps to note the position of the inferior border of the femoral head on the patient's skin. (b) Correct placement of the sheath in the common femoral artery. (c) Correct placement of the sheath in relation to the femoral head, with the arterial access incorrectly placed in the superficial femoral artery due to the anatomic variant of a high bifurcation. (d) Correct placement of the sheath in relation to the femoral head with a low hypogastric artery causing incorrect arterial placement in the external iliac artery. (e) Low sheath placement in the profunda femoris artery. (f) High sheath placement in the external iliac artery (Jacobi *et al*., 2009).

Figure 4. Retroperitoneal bleeding following cardiac catheterization via right femoral access.

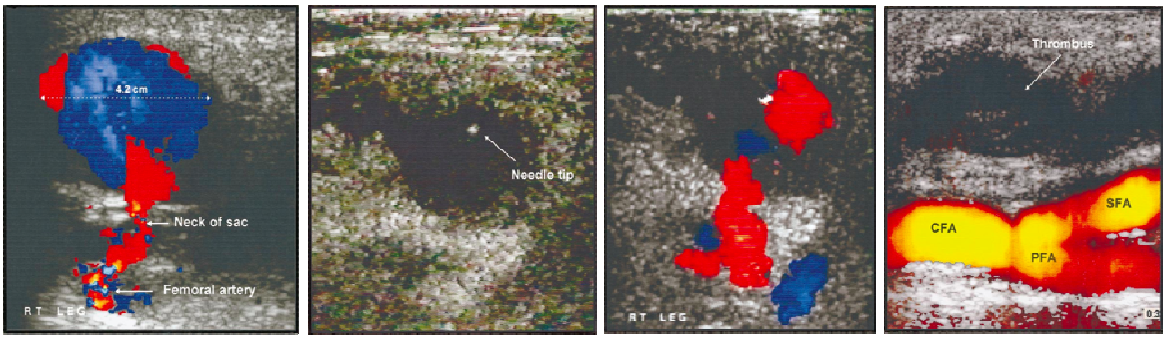

Figure 5. Duplex ultrasound image of pseudoaneurysm, demonstrating arterial flow through a long, narrow neck arising from defect in femoral artery and turbulent color flow into cavity (a). With color flow removed, exact position of needle tip can be identified at all times during procedure, because a small amount of echogenic thrombus forms at needle tip when thrombin comes into contact with blood, helping to guide needle placement (b). With needle in position, color flow during injection of thrombin confirms acute development of thrombus within sac (c). Power Doppler image of patent native fem- oral vessels (CFA indicates common femoral artery; SFA, superficial femoral artery; and PFA, profunda femoris artery) and absence of flow after successful thrombin injection into pseudoaneurysm cavity (d) (Lennox *et al*., 1999).

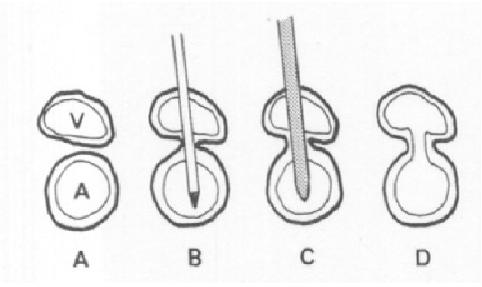

Figure 6. AVF result when needle tract crossing both artery and vein is dilated and catheterized. V = vein, A = artery(Kim *et al*., 1992)

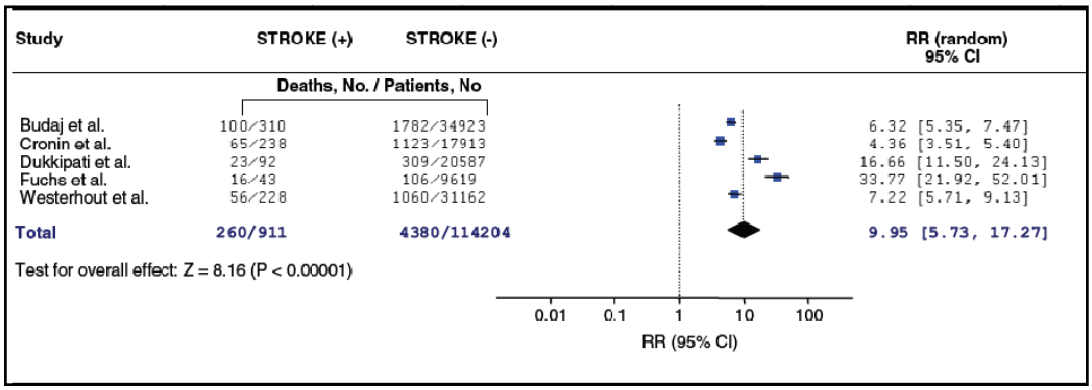

Figure 7. Pooled relative risk (random effects) of mortality after stroke in PCI or in patients with non ST-elevation MI

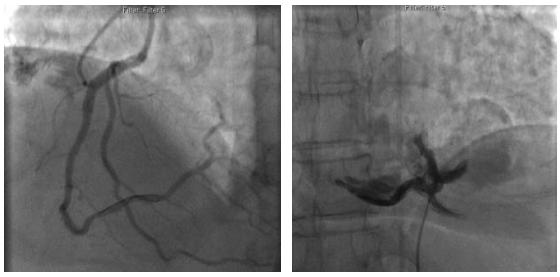

Figure 8. Angiogram of right coronary artery before (a) and after perforation (b)

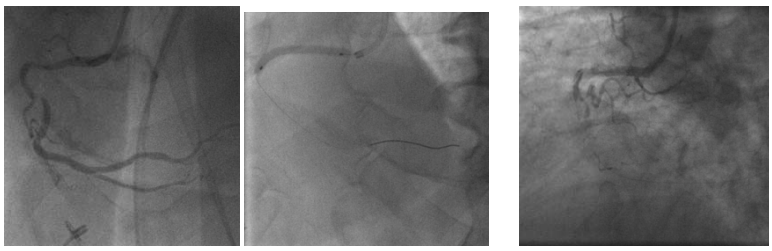

Figure 9. Angiogram of right coronary artery prior to intervention (a), after balloon angioplasty (b) and dissection (c)

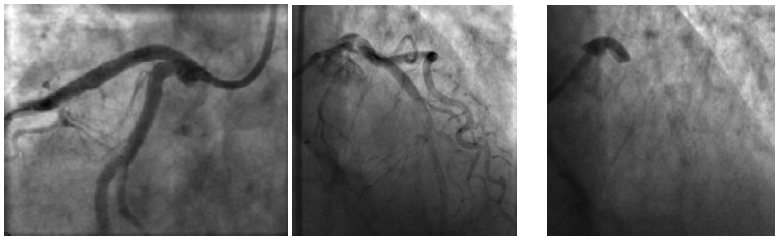

Figure 10. Angiogram of the left coronary system (a). Dissection of the left circumflex artery with guidewire catheter (b) with subsequent extension in to the left anterior descending artery (c)

Prevalence of Neck Pain and Associated Factors with Personal Characteristics, Physical Workloads and Psychosocial among Male Rubber Workers in FELDA Settlement Malaysia

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Abstract

Rubber tapping processes posed potential risk of various health problems among rubber workers. It ranges from simple musculoskeletal aches to more serious and complicated structural damage to bone, muscles, tendons and nerves of musculoskeletal system. These health problems might be linked directly to the arduous demands of farm labor. **Objectives:** A cross-sectional study was conducted to determine the prevalence of neck pain (NP) and musculoskeletal symptoms (MSS) and its association with personal characteristics, physical workloads and psychosocial factors among rubber workers. **Methods:** Stratified random sampling method was adopted and a total of 419 rubber workers in FELDA's scheme Malaysia participated in this study. Data was collected through face to face interview using modified Standardized Nordic Questionnaire (SNQ) and Job Content Questionnaire (JCQ). **Results:** The results revealed the prevalence of NP was 59.9% and weak correlation with age (ρ= -0.184, p= 0.001) and a positive weak correlation with working hours per day (ρ= 0.099, p= 0.043) significantly. All physical workloads (neck flexion or rotation, awkward postures, repetitive motion and static postures) had significant weak to moderate positive correlation with NP (p<0.05). Job insecurity was found to have weak and positive correlation with NP (p<0.05). Binary logistic regression analysis showed risk factors for NP were decreased with age (OR= 3.92, 95% CI 1.61 – 9.58, p=0.003), increase in neck flexion or rotation (OR= 9.52, 95% CI 5.55 – 16.32, p= 0.001), awkward postures (OR=2.23, 95% CI 1.29 – 3.86, p= 0.004) and static postures (OR= 1.86, 95% CI 1.10 – 3.14, p= 0.021). **Conclusion:** This study showed that high prevalence of NP was associated with neck flexion or rotation, awkward and static postures.

Keywords: Neck pain, Musculoskeletal symptoms, Physical workloads, Job insecurity, Rubber workers

1. Introduction

In Malaysia, agriculture is being promoted as the third engine of growth and modernization for poverty reduction as stated in its latest ninth- 5-year development plan (Ninth Malaysian Plan, 2006). Rubber industry is the second largest agriculture industries in Malaysia with the land uses of 1250000 hectare which was 19.58% of the overall land uses for agriculture (MOA and MPIC, 2005). However, farming and agriculture tasks are highly physical demanding, arduous and require extremely large of energy in performing their tasks. Hence, this poses farm workers at potential risk of health problems caused by physical hazards, chemical hazards, biological hazards, psychosocial hazards and ergonomic hazards. Ergonomic hazards poses variety of musculoskeletal symptoms (MSS) encompasses the neck, low back pain, osteoarthritis of the hip and knee, upper limb complaints and hand-arm vibration syndrome (MTUC, 1989; Walker-bone *et al*., 2002).

Neck pain is among the commonest MSS faced by general population and more so among the rubber workers. FELDA (Federal Land Development Authority) is the world's largest plantation operator with 811,140 hectares agriculture area. 722,946 hectares or 84.7 % of agricultural area are planted with oil palm, 84,496 hectares or 9.9 % are planted with rubber, 879 hectares or 0.1 % are planted with sugar cane and 2,819 hectares or 0.3 % are plantation such as timber trees, fruits and also used for research (FELDA Annual Report, 2008). FELDA has implemented replanting program in 42,910 hectares of its rubber plantation because most of rubber trees in FELDA are old as height of tapping trees increase, the tapping tasks prone to focus on upper limb mainly on neck region (FELDA Annual Report, 2008). A preliminary study of prevalence of MSS among Malaysian rubber tappers found that the prevalence of MSS within the last 12 months showed the highest for low back (74.4%) follow by shoulder (53.3%), neck (48.8%) and wrist/hand (48.8%), upper back (41.9%), elbow (39.5%), knee (39.5%) and ankle/foot (9.3%) (Asyraf *et al.*, 2007). In a study by Rosnah *et al.*(2007) on different occupation work posture risk analysis among oil palm plantation mechanical loader operators found that the prevalence of NP was highest with 84 respondents reported NP followed by knee pain (35 respondents), upper back pain (27 respondents) and ankle/feet pain, 24 respondents (Rosnah *et al*., 2007).

Overall tasks of rubber tapping would expose rubber tappers to ergonomics risk factors such as repetitiveness, awkward postures, static muscle loading and forceful exertion (Asyraf *et al.*, 2007). Among the ergonomic factors present in rubber tapping process include age of the trees, height of tapping areas, number of area being tapped, uneven ground and technique of performing the tapping. However, the main risk factors for NP in rubber field are neck extension, twisted head, flexion of neck, awkward postures and repetitive moving of the head. In addition, psychosocial hazards such as low job dissatisfaction, supervisor rating, psychological demands, decision latitudes and social support were the factors to cause sick leaves or disability due to Musculoskeletal Disorders (MSDs) (Hartman *et al*., 2006). These hazards were overlooked as the main cause of MSDs and neck pain among rubber tappers.

Ergonomic risk factors cause wide range of occupational related diseases among agriculture workers especially rubber plantation population and need extensive exploration in Malaysia. In view of limited study on prevalence

of NP and MSDs among rubber workers in Malaysia, this study was attempted to determine the prevalence of NP and its association with personal characteristics, physical workloads and psychosocial factors.

2. Material and Methods

2.1 Study Design and Study Population

A cross-sectional study was conducted among rubber workers from October 2008 until May 2009 in six FELDA in Sembilan State, Malaysia using stratified random sampling in which sample was stratified and drawn from the overall total population in six's FELDA with proportional. The inclusion criteria in this study was Malaysian rubber workers age from 39 to 66 years old without medical diseases or trauma affecting musculoskeletal system and accident in any part of the body as majority of the active rubber workers in FELDA are in this age of range in Malaysia.

Minimum calculated sample size based on Daniel (1999) with 49% (Asyraf *et al*., 2007) prevalence of NP was 384 respondents with $\alpha = 5\%$, and margin error at 0.05. An additional of 10% of calculated sample size brought the total respondent to 423 workers. However, in this study 419 respondents out of 554 male rubber workers were eligible for further analysis.

2.2 Data Collection Material

A modified Standardized Nordic Questionnaire (SNQ) consists of four parts (i) socio-demographic characteristics, (ii) general questionnaire, (iii) detailed body part-specific symptoms and (iv) physical workload rating was used to determine prevalence of NP.

Socio-demographic part contain question on age, education level, height, weight, working hours per day, length of working, smoking habit and trees tapped per day. General questionnaire showed a body map of nine-anatomical body regions and asking about ache, pain, and discomfort for the last 12 months in each of the body regions and past 7 days discomfort. Rubber workers who faced 3 months continuous ache, pain and discomfort in last 12 months was included in the analysis instead of 7 days discomfort as 7 days may be an acute pain which may resolve itself after a while. All answers are in the form of dichotomous yes or no responses. The third part of the questionnaire is a more detailed body part-specific questionnaire (neck, low back and shoulder) embedded with some occupational risk factors such as change of jobs or duties, sick leave or prevented from work and hospitalized because of the ache, pain or discomfort as well as duration of symptom over pastime. The fourth section contains four different physical workload factors (neck flexion or rotation, awkward postures, repetitive motion and static postures) to assess the perception of the risk factors causes pain and the potential contribution to NP. Respondents were ask to indicate on a likert scale of 0-4 (0 = no pain, 4 = severe/very severe pain) on how severity of the risk factors contributed to the NP.

The assessment of psychosocial factors was done using the Malay version of Karasek's Job Content Questionnaire (JCQ) which contains 4 main psychosocial factors (decision latitudes, psychological job demand, social support and job insecurity). A likert-scale of 1 to 4 was used (1=strongly disagree, 2= disagree, 3= agree and 4= strongly agree) to measured all items (decision latitudes, psychological job demand, social support and job insecurity) related to psychosocial risk factors. All psychosocial factors stated above were determined by using median scores as cut off point in which those above median was considered high and those who below median was low. Self-administered SNQ and JCQ were conducted using face to face interview in this study.

Modified SNQ and JCQ were pre-tested the reliability of the questionnaire on 50 rubber tappers with Cronbach's alpha of 0.689 for SNQ and 0.628 for Malay version of JCQ. The reason of Cronbach's alpha for Malay version were low is because of low education level among the rubber tappers and face to face explanation is needed in order they understand the question been asked. However, the content in modified SNQ and JCQ questionnaire were still maintain the original content.

2.3 Statistical Analysis

Statistical Package for Social Science (SPSS) version 16 was used to calculate the means and standard deviations for continuous data with normal distribution, median and IQR for not normally distributed data. For categorical data, frequency and percentage was used. Chi-square test was applied to determine the risk factors contributing to NP. Simple logistic regression was performed with "enter" method to assess the most affected risk factors for NP. Multicolinearity was adopted to check the interaction between the independent variables. Multiple logistic regressions was performed to assess the independents and interaction effect of all the personal characteristics, physical workloads and psychosocial factors and control for potential confounding variables (i.e. age, amount of trees tapped per day, working experience etc) in which p values < 0.05 were included in the model.

2.4 Study Ethic

This study was approved by Ethic Committee of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia on 2nd July 2008 and FELDA Headquarter on 31st March 2008. The data was collected with written consent from respondents.

3. Results

As shown in Table 1, mean age of respondents was 53.01 ± 4.90 years and the age ranges from 39 to 66 years old. Approximate 67.3% (282) of them aged between 49 to 58 years old. Majority of the respondents (95.9%) were Malay and married (99.35%). In terms of educational level, 47.3% of the respondents achieved education up to Standard 6 level (Primary school) with only 9.3% obtained SPM (Certificate of Secondary Education) / STPM (Certificate of Higher Education) level. The mean BMI was 24.96 ± 3.85 kg/m² and 51.1% of the respondents were within ideal BMI whereas 37.9% were overweight and 7.9% were obese. 54.2% of the respondents were smokers. The mean length of working was 23.56 ± 7.16 yrs and 51.1% of them work between 21 to 30 yrs whereas 3.1% of them work less than 10 years. There are 342 respondents work less than 5 hrs per day. The median of trees tapped per day was 200 ± 150 trees and 27.4% of them were able to tap between 200 to 299 trees per day. For psychosocial factors, majority of respondents in this study had low level of social support (71.4%), psychological demand (65.6%), decision latitude (56.3%) and job insecurity (52.5%) (Table 2).

Figure 1 showed the prevalence of MSDs in different body region during last 12 months. The most common affected body region among respondents was NP (59.9%) followed by low back pain (56.3%), shoulders pain (54.9%), knee pain (45.8%), ankles/feet pain (34.4%), elbow pain (33.2%), upper back pain (30.8%), wrists pain (30.1%) and hip/thighs pain (15.3%). However, out of 419 respondents, 37.7% of them sort medical advice due to low back pain followed by neck pain and shoulder pain, 33.7% and 33.4% respectively.

Bivariate analysis showed that neck region had significant association with age (χ^2= 14.732, p= 0.001), working hrs per day (χ^2= 4.107, p= 0.043), neck flexion or rotation (χ^2 = 150.043, p = 0.001), awkward postures (χ^2 =81.147, p = 0.001), repetitive motion (χ^2 =52.569, p = 0.001), static postures (χ^2 =40.954, p = 0.001) and job insecurity (χ^2= 5.539, p= 0.019). Other personal characteristic factors (ethnic, marital status, education level, BMI, smoking habit, amount trees tapped per day and working yrs) and psychosocial factors (decision latitudes, psychological demands and social support) showed no significant association with NP.

Simple logistic regression found NP had significant associations with only 2 selected risk factors: age (39-48 yrs) (OR = 3.19, 95% CI 1.29 – 7.90) and neck flexion or rotation (OR = 9.08, 95% CI 5.09 – 16.17). Binary logistic regression with both stepwise forward and backward elimination showed the risk factors which were significant to the model were age (OR= 3.92, 95% CI 1.61 – 9.58), neck flexion or rotation (OR= 9.52, 95% CI 5.55 – 16.32), awkward postures (OR=2.23, 95% CI 1.29 – 3.86) and static postures (OR= 1.86, 95% CI 1.10 – 3.14). The standard error and the correlation were relatively small for these 4 independents risk factors with no interaction effect among them in the model. Other selected factors such as working hours per day, repetitive motion and job insecurity were not significant risk factors for NP (Table 3). Hosmer and Lemeshow Goodness-of-Fit test showed not significant (p=0.944), which indicated that the model fitted well and 44.3% of the variation in the outcome variable is explained by this logistic model (Nagelkerke R^2 = 0.443).

Model and equation for Prevalence of Neck Pain and associated factors:

Log Y = 0.401 + 1.367* Age (39 - 48) + 2.253 * Neck flexion or rotation + 0.802* Awkward Postures + 0.619 * Static Postures

4. Discussion

4.1 Respondent Background Data

The study population was all male and aged between 39 to 66 years old with more than 20 years working experience. This is in line with a study conducted by Abu Hassan and Abdullah (2003) among male rubber tappers in FELDA Pahang with age range of 23 to 64 years old. The distribution of age and length of working were mainly influenced by FELDA management in year of 1990 where they decide to stop the intake of settlers for all land schemes throughout all the country. Female workers were not included in this study because they form a small portion (20%) in FELDA helping their husband in conducting the tapping work. In addition, work description was different among female and male in FELDA as female mostly keen to be a housewife rather than helping in tapping. Majority of the respondents have less than 5 working hrs per day because some of the rubber trees were old with less yield of the latex and some of them were die off.

51.8% of the respondents tapped the tress in the range of less than 200 trees per day (total of trees for each rubber workers approximately 1000 trees in 10 acres) with the median±IQR of 200±150 trees. However, the number of trees tapped per day was low as compared with a study conducted by Asyraf *et al*. (2007) in which the mean of rubber trees tapped was 395 trees per day. Based on our observation, the reasons for this is due to most of the rubber trees are old and have many roughly plan as compared with young trees; the older the trees, time requirement was more as they need to conduct the rubber tapping process above their head level. On the other hand, physical condition of the respondents will also affect the number of trees that can be tapped per day; respondents that have a good healthy condition are more likely to tap more trees as compared with the weak ones.

4.2 Prevalence of NP and MSDs

The results of this study found overall prevalence of MSDs was high (85.7%) among rubber workers. This finding is in line with a study conducted by Holmberg *et al.* (2002) found 918 (90.6%) reported MSDs and indicating that the odds of reporting musculoskeletal problems were 51% higher among farmers than non-farmers. The highest prevalence of MSDs found in this study was NP with prevalence of 59.9%, followed by LBP (56.3%) and SP (54.9%). These findings was consistent with a study conducted by Kuorinka *et al.* (1987) in which NP, LBP and SP were the most common and predominantly occurring prevalence of MSDs. In the year of 2007, a study conducted by Asyraf *et al.* reported the prevalence of NP was 48.8%. In contrast, Rosecrance *et al*. (2006) reported the prevalence was low among Kansas farmers and Ismail (2007) reported the prevalence as 36.3% among palm oil plantation workers in Sabah. The contrary findings in this study were difficult to conclude as there has been very limited documentation and literature on MSDs and neck MSDs in the agriculture industry (Kermit and Susan, 2007).

Based on observation in rubber field on the tapping process, high prevalence of NP among the rubber workers could be due to the rubber trees condition. Most of the rubber trees are old and need to be tapped above their head level and hence extreme force was required to extend their postures mainly on neck, shoulders and forearm area. This will induce a high risk to the neck pain.

4.3 Association between NP and Personal Characteristics

This study found a significant inverse association between age and NP. Binary logistic regression showed those with age between 39-48 years was 4 times more likely to have neck pain as compared with age group of 48 – 58 and more than 59 years old rubber workers. The finding was in contrast with case control study by Hartman *et al.* (2006) on risk factors for sick leave due to MSDs among self-employed Dutch farmers showed that risk factors for musculoskeletal pain (neck, back, shoulder or upper extremity pain) was increased with age and also with sick leaves due to this disorder.

In this study, young workers was at greater risk to develop neck pain as compared with older age workers because older workers employed foreign workers in helping them to tap the rubber trees in FELDA. Based on observation, most of the rubber tapping process was only conducted in less than 5 hours per day; normally from 7am to 11am. Young rubber workers also involved in helping their parents in cow and sheep farming in the afternoon. The explanation was not uncommon as other study conducted by Park *et al.* (2001) showed older farmers in Iowa performs less physical labor leaving the difficult high force tasks to younger worker. In addition, older rubber workers may have known and learned the right technique of rubber tapping process to avoid pain and discomfort as compared with the young workers who do not have experience in tapping process. “Survivor bias” occurs which means only healthy workers able to continue the current work after the others have dropped out.

There were no significant association found between NP and amount of trees tapped per day. It may due to other physical workload factors such as geographical terrain and neck flexion or different awkward postures were the dominant risk factors for NP. NP had no significant association with working hrs per day, BMI, ethnic, marital status, education level, smoking habit, length of working and trees tapped per day. There was limited literature on association between NP and personal characteristics factors. Nevertheless, a study conducted by Abu Hassan and Hasbullah (2003) on low back pain among rubber tappers show no significant association with socio-demographic factors (educational level, length of working, working experience and pain on low back in previous years).

4.4 Association between NP and Physical Workloads

This study found that all the physical workload factors (repetitive motion, static posture, awkward posture and neck flexion or rotation) have significant association with NP. The findings of our study were in lieu with several

studies that conclude repetitive and monotonous work tasks, awkward posture and neck extension were main risk factors for NP (Meyers *et al*., 2000; MOA and MPIC, 2005; Ohlsson *et al.*, 1995). Multivariate analysis found neck flexion or rotation, awkward and static postures were the main risk factors for NP where repetitive motion showed no significant association.

Binary logistic regression models showed that respondent who fall in the age of 39 to 48 years old had greater risk of NP when conducted rubber tapping tasks with neck flexion, awkward and static postures.

Rubber tapping processes for trees up to 6 meters require the respondents to perform it above their head level. Hence, extreme force required and they need to extend their postures mainly on neck for long duration during tapping task and poses higher risk to develop NP (Figure 2). Similar explanation was elaborated by Meyers *et al*. (2000) which stated neck extension occurs when the farm workers do tasks above their heads in tasks such as fruit harvesting and wine grape pruning.

Awkward postures for example reaching above, twisting, forward or backward bending were significant risk factors for NP. Respondents were forced to flex and rotate their head in order to tap the rubber trees that were above their head level, it causes significant deviation from their neutral position. During tapping process it lead to further over extension of neck muscle. Muscle in this position would not work effectively, required more energy and fatigue faster (Figure 2).

Static postures found significant factors for NP as the rubber workers force to work and tapped the trees above head level for long duration. Neck region required to stay in static postures for average 1 to 2 minutes based on observation to ensure the latex was flow in the right position into the cup. These might increases the load and forces lead to muscle fatigue for neck pain.

4.5 Association between NP and Psychosocial Factors

Job insecurity was associated with a decrease in both physical and mental health status among wide range of occupation, organizations and positions among 729 Norwegian employees (Storseth, 2006). Study among female flight attendants by Hyeonkyeong *et al*. (2008) found that higher feelings of job insecurity (OR = 1.35, 95% CI = 1.09 to 1.68) were associated with a higher incidence of lower-back. Contrary to our finding, we found that job insecurity was associated with NP but not significant with LBP. Different population study may report different sensation of pain in their body parts. A study conducted by Lundberg *et al*. (1994), Melin and Lundberg (1997) found that job insecurity may lead to "physiological vulnerability" of muscles and to the sensation of pain. Nevertheless, further multivariate analysis in this study showed there was no significant association of job insecurity and NP. This shows that other risk factors such as physical workload were dominant associated risk factors for NP rather than psychosocial factors. The specific route or routes by which psychological stress/strain might influence MSS is a matter of debate (Zara, 2008).

5. Conclusion

The high prevalence of MSS shows that MSS is a significant problem among rubber workers. NP showed the highest prevalence as compared with other body regions. This study found that age had inverse relationship with NP. Among ergonomic factors neck flexion, awkward postures, repetitive motion and static postures were found significant relationship with NP. Binary logistic regression revealed age, neck flexion or rotation, awkward and static posture was the most affected risk factors for NP. This study recommended that detail study should be carry out on each ergonomic factors with objective measurement tools which include anthropometry measurement and biomechanic in order to comprehensively understand the mechanism of MSD. In addition, management should instituted health promotion activities and guidelines to empower workers to minimize the risk and better quality of life especially for young workers.

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Table 1. Personal details of respondents (n = 419)

Socio-demography	n	%	Mean ± s.d.	Median ± IQR
Age Group (yrs)			53.01 ± 4.90	
39 – 48	78	18.6		
49 – 58	282	67.3		
≥ 59	59	14.1		
Ethnic				
Malay	402	95.9		
Non- Malay	17	4.1		
Marital status				
Married	416	99.3		
Single/Widow	3	0.7		
Education level				
< Standard 6	92	22.0		
Standard 6	198	47.3		
PMR/SRP	90	21.5		
SPM/STPM	39	9.3		
BMI (kg/m²)			24.96 ± 3.85	
< 18.50	13	3.1		
18.50 - 24.99	214	51.1		
25.00 - 29.99	159	37.9		
> 30.00	33	7.9		
Smoking habit				
Yes	227	54.2		
No	192	45.8		
Length of working (yrs)			23.56 ± 7.16	
< 10	13	3.1		
11 to 20	143	34.1		
21 to 30	214	51.1		
31 to 40	35	8.4		
41 to 50	14	3.3		
Working hours a day (h)			4.49 ± 1.47	
< 5	342	81.6		
> 5	77	18.4		
Trees tapped per day			200 ± 150	
< 200	217	51.8		
200 – 399	114	27.2		
400 – 599	67	16.0		
≥ 500	21	5.0		

Table 2. Frequency of psychosocial factors

Psychosocial Factors	**n (%)**	**Median (IQR)**
Social Support		24 (3)
Low	299 (71.4)	
High	120 (28.6)	
Psychological Job Demands		30 (5)
Low	275 (65.5)	
High	144 (34.4)	
Job Dissatisfaction		0.2 (0.23)
Low	269 (64.2)	
High	150 (35.8)	
Decision Latitude		62 (8)
Low	236 (56.3)	
High	183 (43.7)	
Depression		0.15(0.19)
Low	225 (53.7)	
High	194 (46.3)	
Job Insecurity		6 (3)
Low	220 (52.5)	
High	199 (47.5)	

Table 3: Neck Pain and Associated Factors by Simple Logistic Regression and Multiple Logistic Regression

Variables	**Simple Logistic Regression**			**Multiple Logistic Regression**		
	b	**Crude OR (95%CI)**	**p**	**b**	**Adjusted OR (95%CI)**	**p**
Age (Years)						
39 – 48	1.160	3.19 (1.29 - 7.90)	**0.012***	1.367	3.92 (1.61 – 9.58)	**0.003***
49 – 58	0.522	1.69 (0.81 – 3.51)	0.162	0.589	1.80 (0.88 – 3.70)	0.108
≥59**						
Static Postures						
Yes	0.413	1.51 (0.86 – 2.67)	0.155	0.619	1.86 (1.1 – 3.14)	**0.021***
No pain**						
Awkward Postures						
Yes	0.590	1.80 (0.99 – 3.28)	0.053	0.802	2.23 (1.29 – 3.86)	**0.004***
No pain**						
Neck flexion or rotation						
Yes	2.206	9.08 (5.09 – 16.17)	**0.001***	2.253	9.52 (5.55 – 16.32)	**0.001***
No pain**						

** Reference category, *p is significant when p<0.05

Backward LR Multiple Logistic Regression model was applied

Multicollinearity and interaction term were checked and not found

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Homers-Lemeshow test, (p= 0.944) and Nagelkerke R Square 0.443 were applied to check the model fitness

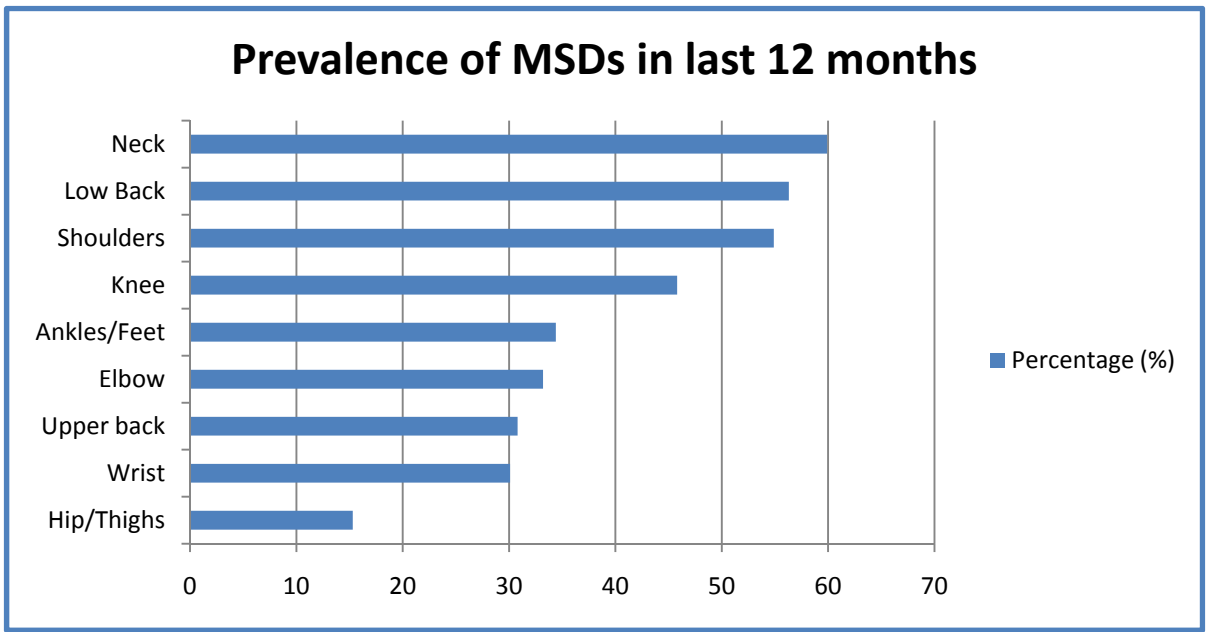

Figure 1. Prevalence of MSDs in last 12 months

Figure 2. Neck flexion or rotation, awkward and static postures while conducting tapping process

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Age at Menarche and Menstrual Cycle Pattern among School Adolescent Girls in Central India

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Abstract:

Background: The onset of menstruation is part of the maturation process. However, variability in menstrual cycle characteristics and menstrual disorders are common. The purpose of this study was to determine the age at menarche and patterns of menstruation among school adolescent girls and explore its variation across socio-economic and demographic factors.

Methodology: This is a cross-sectional descriptive study carried out on 1100 school adolescent girls in district Wardha, Central India. Data were collected using a self-administered structured questionnaire on menstruation. Data was entered and analyzed by using Epi Info 6.04 software package. Chi- square value was used for testing statistical significance.

Results: Mean ages of menarche were 13.51 + 1.04 years and 13.67 + 0.8 years for urban and rural areas respectively. Abnormal cycle length was common and affected 30.48%. The majority 56.15 experienced dysmenorrhoea and 56.16 percent had premenstrual syndrome. Self medication was practiced by 7.13% of the adolescent girls. The most common premenstrual symptom was headache 26.74%. Absenteeism from the school 13.9% was the effect of menstruation related problems on their daily routine. Dysmenorrhea and premenstrual symptoms were perceived as most distressing symptoms leading to school absenteeism. Majority of the girls 75.58% had discussed menstrual problems with someone, most commonly with their mothers 38.15%. There was a general lack of information about menstrual issues especially with regards to cycle length, duration of menses and age at menarche. Girls from families of high socio-economic class have significantly lower mean menarcheal age in both urban and rural area. The mean age of menarche was significantly higher in girls involved in vigorous sporting activity in urban area compared to their non-sporting counterparts.

Conclusion: Age at menarche was delayed. The menstrual disorders among female adolescents are common. A school health education on menstrual problems targeting adolescent girls and their parents and routine screening for menstrual problems by healthcare providers can help to prevent the absenteeism in the school.

Keywords: Age at menarche, Menstrual abnormalities, Socio-economic class

1. Introduction

Adolescence in girls has been recognized as a special period which signifies the transition from girlhood to womanhood. This transitional period is marked with the onset of menarche, an important milestone. Menstruation is a normal physiological process that begins during adolescence and may be associated with various symptoms occurring before or during the menstrual flow. Adolescent girls constitute a vulnerable group, particularly in India where female child is neglected one. Menarche is a part of the complex process of growing up. The age of onset of menstruation varies from 9 to 18 years with the average age in United States being about 12 years and 8 months, whereas in India it is slightly lower and has been reported to be around 12 years (Khadilkar VV *et al* 2006, Chumlea WC *et al* 2003). The age at menarche shows many socioeconomic, environmental, nutritional and geographical differences in the societies (Attallah NL 1978, Ekele BA *et al* 1996).

These problems include psychological adjustment with menstruation, premenstrual and menstrual symptoms and disorders of menstruation. Female experience premenstrual symptoms 7 to 10 days before the onset of bleeding. These include irritability, malaise, headache, acne, abdominal pain etc. the main importance of the premenstrual tension is psychosomatic. The menstruation in majority of female is asymptomatic apart from per vaginal bleeding, however some may have pain in abdomen with or without gastroinstestinal upsets like anorexia and vomiting (Padubidri VN *et al* 1997). Complaints like leg pain, backache may also be associated with normal menstrual cycle (Banerjee D *et al* 1961). The medical and social consequences of premenstrual, menstrual symptoms and disorders of menstruation influence not only the individual but also her family and society. In respect to adolescent girls it may manifest as loss of school days leading to poor progress in education. This may lead to problems in continuation of her education (Deo D S *et al* 2007a). However few studies in India have described the lifestyle factors associated with various menstrual cycle patterns. The present study, therefore, aims to determine the age at menarche and patterns of menstruation among school adolescent girls and explore its variation across socio-economic and demographic factors.

2. Material and Methods

A cross sectional study was conducted among school adolescent girls of classes five to twelve in the District Wardha, Maharashtra, Central India between October to December 2009. The girls were selected according to WHO criteria for the adolescence that is 10 – 19 years (World Health Organization 1984). Random selection of 6 schools and 1100 girls from these schools were enrolled, three rural and three urban schools were surveyed. Using the retrospective method, about 1100 questionnaires were distributed, 1080 were filled correctly. Of this, 519 (48.1%) girls did not yet experience their first menstruation at the time of interview and these students were not included in the study, so we had 390 and 171 answered questionnaires from urban and rural schools respectively. Effort was made to examine the students who were absent on a particular day at the next visit. All the girls were interviewed by the team comprising of doctor, nurse, social worker and school teacher through a scheduled visits. Informed consent of the school adolescent girls and head of the institution was taken for conducting the study. Due approval was obtained from institutional ethical committee.

A pre-designed, pre-tested questionnaire was used for data collection. The questions was administered in local language (*Marathi*) and properly explained to avoid any form of misunderstanding and to facilitate accurate response by the subjects. The questionnaires distributed and collected immediately after completion to prevent interpersonal communication and influence of peers on individual responses amongst the girls. The information was gathered on sociodemographic data, menarcheal age, menstrual cycle pattern, premenstrual symptoms, dysmenorrhea and associated symptoms. The age at menarche was determined by questioning the school girl if she had or not had menstruation and at which age she had menstruated. Data thus generated was entered and analyzed using Epi Info 6.04 software package. Means, standard deviations and simple percentage were determined. Chi-square value was used for significance level. $P < 0.05$ was considered significant.

3. Results

Of the 1100 questionnaires distributed 1080 were filled correctly (98.18%). 324 adolescent girls were of rural residence while 756 were urban ones. 519 (48.1%) girls did not yet experience their first menstruation at the time of interview and these students were not included in the analysis of age at menarche, menstrual pattern and disorders.

The age of the school girls interviewed in this study was ranges between 10 to 19 with mean and standard deviation of 15.45 ± 1.75 years. The mean age and standard deviation at menarche was 13.67± 0.8 years. Out of the total study population, 47 (8.38%) adolescents had a menstrual cycle length shorter than 21 days, 390 (69.52%) had a cycle length between 21 and 35 days and 124 (22.1%) longer than 35 days. There was no statistically significant

difference on cycle length among the subjects living in urban or rural residence. The mean duration of flow was 4 ± 0.8 days (Table 1).

The subjects living in urban areas revealed a mean and median age at menarche of 13.51 and 14.0 years respectively. The subjects living in rural areas revealed a mean and median age at menarche of 13.67 and 14.0 years respectively. The mean age at menarche was 0.16 years younger for urban girls compared to rural area which is not statistically significant. The menstrual cycles were regular in 70 % of the urban and 68.42% rural subjects. There was no statistically significant difference of menstrual cycle regularity by urban or rural residence and age of menarche.

The overall prevalence of dysmenorrhoea was 56.15%. Dysmenorrhoea was significantly more frequent among students from urban as compared with rural residence (Table 2). However, dysmenorrhoea was not significantly associated with age at menarche. Dysmenorrhoea resulted in absence from school of 78 (24.76%) girls with dysmenorrhoea. There was a significant association between the severity of dysmenorrhoea and the duration of menstrual flow.

Dysmenorrhea was more frequently observed among adolescents with irregular cycle (57.9%) as compared to those with regular cycle (42.1%) $p < 0.05$. 7.13% of the adolescents girls use analgesics to relieve the pain. Premenstrual symptoms were present in 56.15% girls. The most common symptom was headache which was experienced by 26.74%. There was statistically significant association between presence of premenstrual symptoms and rural residence. Self medication was practiced by 7.13% of the adolescent girls (Table 2).

School girls from high socio-economic class had significantly lower mean menarcheal age compared to girls from low socio-economic class in both urban and rural area (p value <0.05, Table 3).

In urban area, school girls involved in vigorous sport activity had a significantly higher age of menarche as compared to girls in non sport activity (p value <0.05, Table 4).

24.42% of the adolescents had no information prior to the commencement of menstruation. 38.15% study subjects had got information about menarche from the mother, followed by friends (32.26%), teachers (3.03%) and books or magazines 2.14% (Table 5).

4. Discussion

Menstruation though a normal physiological process is many a time associated with premenstrual and menstrual disturbances. These disturbances may sometimes be very severe leading to loss of work days.

In the present study, the mean age of menarche of the adolescent school girls was 13.67 years whereas various studies conducted in Kalamboli the mean age at menarche was found to be 13.32 years, in West Bengal 12.8 years and in Turkey 12.81 years (Nemade D *et al.* 2009, Dasgupta A *et al.* 2008 & Demir SC *et al.* 2000).

The subjects living in urban area revealed 13.51 mean age at menarche as compared with 13.67 from rural area. This is consistent with the studies conducted in China and Nigeria (Lin WS *et al.* 1992, Ikaraoha CI *et al.* 2005).

In the present study, the intermenstrual interval was reported to be 21-35 days by 69.52% girls whereas it was 36-45 days for 13.73% girls and more than 45 days for 8.38% girls. This could be because of changing trends in lifestyle, dietary habit, stress, hormonal imbalance or some medical reasons which requires gynaecological assessment at the earliest. In a study conducted among tribal Gujjar adolescent girls, 9.9 per cent of the subjects had their menstrual cycle between 45-60 days which is similar to the figure in the present study (Dhingra R *et al* 2009).

30.48% adolescent girls reported that their cycles were irregular. Irregular cycles are common in adolescents as the initial cycles are anovulatory resulting in abnormal uterine bleeding that may be associated with varying amount of blood loss including menorrhagia (Lee HK *et al.* 2006).

The mean duration of menstrual flow was 4 days, whereas in the study conducted in Turkey the menstrual flow lasting more than 8 days (Sule ST *et al.* 2007).

56.15% of the girls had dysmenorrhoea, whereas majority 61.27% of the girls reported dysmenorrhoea in the study conducted in Dharan and Turkey and in urban area 65% (Houston AM *et al.* 2006, Sharma M *et al.* 2003).

Dysmenorrhoea was significantly associated with higher age most likely because menstrual cycles are commonly anovulatory for some time after puberty whereas dysmenorrhoea is associated with ovulatory cycles (Odujinrin OM *et al* 1991). A similar association between dysmenorrhoea and older age has been reported previously (El-Gilany AH *et al.* 2005, Jacks TH *et al.* 2005).

56.16% of the girls had premenstrual symptoms. Headache, fatigue, feeling of increased weight, abdominal bloating, backache, breast heaviness and joint pain were the most common pre-menstrual symptoms experienced by the adolescent girls. Premenstrual symptom has been reported to be one of the most distressing problems associated with menstrual cycle. The complaints of the adolescent girls in the present study were well within the range as reported by other studies (Banikarim C *et al.,* 2000, Joseph GA *et al.,* 1997).

Absenteeism 13.9% from the school was the effect of menstruation related problems on their daily routine. Dysmenorrhea and premenstrual symptoms were perceived as most distressing symptoms leading to school absenteeism. Dysmenorrhea and premenstrual symptoms has also been reported to be one of the most frequent causes of absenteeism from school and of days off work (Drife JO *et al.,* 2004). Women with premenstrual symptoms have reported a greater number of days with impairment in routine work, school and household activities (Dean BB *et al.,* 2004, Robinson RL *et al.,* 2000).

The study results show the disturbance of routine activities of the study subjects due to dysmenorrhea and premenstrual symptoms (Banikarim C *et al.,* 2000, Busch CM *et al.,* 1988). The rate of school absenteeism is slightly lower than reported by other studies this can be explained by responses to various gradients of pain.

Self medication was practiced by 7.13% of the adolescent girls. However, the study conducted at Turkey reported that 56.60% of the adolescent girls practiced self medication (Sule S T *et al.,* 2007).

School girls from a high socio-economic class had a statistically significant lower mean age of menarche compared to those in low socioeconomic class in both urban and rural area. This is consistent with the finding reported by other studies (Ikaraoha CI *et al.,* 2005, Ikechebelu JI, 1991).

In urban area, school girls involved in vigorous sport activity had a significantly higher age of menarche as compared to girls in non sport activity. Similar findings were reported from other studies (Ikaraoha CI *et al.,* 2005, Stager JM *et al.,* 1988).

24.42% of the adolescents had no information prior to the commencement of menstruation. There was general lack of accurate information about all menstrual abnormalities among the girls in urban as well as rural girls. Previous researchers have also found that knowledge about menstrual abnormalities was very poor among adolescent school girls (Sharma M *et al.,* 2003).

Though there was no significant difference regarding awareness in urban and rural girls their source of information varied. In the urban study group mother was the main source of information. This might be due to better literacy level of the mother and better relation among mother and daughter. Friends were the most common source of information in rural girls. However, in urban girls the source of information about menstruation was their mother 27.5% while it was teacher 27.01% in the rural counterparts (Deo DS *et al.,* 2005b). It was also observed that adolescent girls lacked conceptual clarity about menstruation. The reason was that they had no prior information about menstruation due to which they faced several problems about menstruation and related reproductive health issues.

5. Conclusion

In conclusion, the age at menarche was delayed. The menstrual disorders among female adolescents were common. A school health education on menstrual problems targeting female adolescents and their parents, and routine screening for menstrual problems by healthcare providers, can help to prevent the absenteeism in the school.

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Table 1. Menstrual characteristics of school girls

Menstrual characteristics	No	Percentage
Duration of flow (days)		
< 2	9	1.6
2 - 4	379	67.56
5 - 7	166	29.59
> 8	7	1.25
Cycle length		
< 20 days	47	8.38
21 - 35 days	390	69.52
36 – 45 days	77	13.73
> 45 days	47	8.38

Table 2. Menstrual cycle pattern of the school girls in urban and rural area

Variables	Urban (N-390)	Percentages	Rural (N-171)	Percentages	P value
Age at menarche	13.51 + 1.04		13.67 + 0.8		>0.05
Regular cycles	273	70.00	117	68.42	>0.05
Dysmenorrhea	237	60.77	78	45.61	<0.05
Premenstrual symptom	203	52.05	113	65.50	<0.05
Use of self medicine	29	7.44	11	6.43	>0.05
School absence	47	12.05	31	18.13	<0.05

Table 3. Age at menarche and socio-economic status of school adolescent girls

Residence	High Mean+SD	Middle Mean+SD	Low Mean+SD	High Vs Low p value	Middle Vs Low p value
Urban area	12.89 + 1.22 (N-27)	13.62 + 0.95 (N-270)	13.48 + 1.35 (N-93)	<0.05	>0.05
Rural area	12.90 + 1.27 (N-27)	13.71 + 0.69 (N-114)	13.63 + 0.68 (N-30)	<0.05	>0.05

(Socio-economic status: High = Rs. 10000 and more family income per month; Middle = Rs. 3000 to 9999 family income per month; Low = less than Rs. 3000 family income per month)

Table 4. Relationship between menarcheal age and sport activity

Residence	Sport Mean+SD	Non- Sport Mean+SD	p value
Urban (N-390)	13.77 + 0.95 (N-51)	13.4 + 1.11 (N-339)	<0.05
Rural (N-171)	13.72 + 0.7 (N-108)	13.57 + 0.96 (N-63)	>0.05

(sport activities five hours per week: swimming, volleyball, tennis)

Table 5. Source of premenarcheal information

Source	Total	Percentages
Mother	214	38.15
Friends	181	32.26
Teachers	17	3.03
Book/Magazines	12	2.14
None	137	24.42

Effect of an Integrated Case-based Nutrition Curriculum on Medical Education at Qazvin University of Medical Sciences, Iran

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Abstract

Introduction: Nutrition education is identified as an important part of medical education by organizations**.** Qazvin University of Medical Sciences, school of medicine (QUMS SOM), has a required basic nutrition course of 36 hr in second year of medical school, but education experts reports show that the course does not provide required therapeutic skills for graduate student**. Method:** We decided to organize an 8-hr diet therapy work shop in order to develop a patient experience clinical based case study teaching to enhance clinical skills at QUMS SOM. **Results:** Students' perception score about adequacy of nutrition instruction increased from 20% (at baseline) to 70% (after intervention). The mean nutrition knowledge score of total students in clinical nutrition were different between before and one month after integration (8.3±2.5, 13.4±3.2, $P < 0.001$). And two groups of participants including staggers and interns had similar nutritional knowledge score at pre-test (7.9±2.6 and 8.9±2.3 respectively). **Conclusion:** Implemented nutrition curriculum at QUMS was appropriate method to enhance student's perception about nutrition integration and to increase and translate the knowledge to clinical practice.

Keywords: Integrated education, Nutrition curriculum, Perception

1. Introduction

Nutrition training for medical education and its effect on health and the prevention of disease is identified as an important part of medical education by organizations such as American Society for Clinical Nutrition (ASCN) (1990), the American Medical Student Association (AMSA) (1996), and the National Academy of Science (NAS) (1985). Challenges to include more nutrition education in the medical schools education program backs to decades ago (Stare, 1949; Shills, 1994; Dutra-de-Oliveira and Marchini, 1995; Rasmann-Nuhlicek *et al*., 1995). These efforts have had variable success, and nutrition education is continually being discussed as a subject in need of medical students (McLaren, 1994; Weinsier, 1995; Gershoff, 1996). Diet has a main role in the pathogenesis of diabetes, cancer, stroke, osteoporosis, heart disease, liver disease, and other obesity related

disease, however most medical students report that they do not get enough nutritional training during medical school (Lo, 2000). In spite of the minimum medical nutrition education, 25 hr, recommended by NAS (1985), many medical school still do not provide the recommended minimum hr of nutrition education. And those who used to offer nutrition course of 27 hr in first or second year of medical education were enable to provide competency of basic therapeutic applications of foods and diets (Lo, 2000; Woods, 2006). Different approaches in integrated nutrition curriculums on medical education have been performed. At Arizona University, School of Medicine, the implementation of nutrition curriculum resulted in doubling of the total hours of required instruction (35 compared with 75 h) in prospect courses throughout the 4 y of undergraduate medical studies. The objective structured clinical examination (OSCE) score in nutrition significantly improved after implementation of curriculum (41.7% compared with 50.6%) and students perception of inadequacy of medical-nutrition training decreased (68.4% compared with 11.5%) (Taren *et al*., 2001). In integration, at Tufs University, School of Medicine, development of standardized patient cases in nutrition counseling for cardiovascular disease and weight loss and its incorporation into the clerkship and residency programs in internal medicine and family medicine was focused. This procedure was along with expansion of nutrition education including slide show in nutrition topics such as clinical cases, dietary analysis, and patients' handouts. Also nutrition sessions and patient experience included in the education program. This expanded nutrition program in internal and family medicine along with the standardized patient experience resulted in an excellent rating from physicians, residents, and medical students (Woods, 2006).

Qazvin University of Medical Sciences, school of medicine (QUMS SOM), with a total of 7 years education program, has a required basic nutrition course of 36 hr in second year of medical school, but education experts reports show that the course does not provide required therapeutic skills for graduate student. The nutrition course currently is running in year 2 of basic science during which medical students are not involved in clinical setting and they do not know how to apply their learned materials in practice. Therefore we decided to organize an 8-hr diet therapy work shop in order to develop a patient experience clinical based case study teaching to enhance clinical skills regarding the role of nutrition in chronic disease, metabolic, heart, renal, intestinal disease, assessment of current dietary intake, and weight management at QUMS SOM. Also we aimed to increase clinical nutrition skill of students to arrange an objective structured clinical examination (OSCE) and to improve its score over time.

2. Subjects and Methodes

2.1 Subjects

The nutrition curriculum described in this report was implemented at the QUMS COM. The project was initiated in May of 2011 and forty six medical students (22-25 years) who were involved in 4-weeks community medicine training, enrolled in the project. There was no conflict of interest for students to participate. The project was approved by QUMS SOM.

2.2 Methods

For the development and implementation of the integrated nutrition curriculum at QUMS SOM we developed a new curriculum. In addition to baseline 36 hr single basic nutrition course which normally is offered in year 2 of medical school education, we organized an 8-hr diet therapy work shop. Then we developed a patient experience clinical based case study teaching, to enhance clinical skills regarding the role of nutrition in chronic disease, metabolic, heart, renal, and intestinal disease; and assessment of current dietary intake and weight management. The curriculum initiates during 1 month of students' community medicine training (year 4-5), and is intended the 30 cases of patients having different diet pattern to be practiced over time throughout year 5, 6,and 7

2.3 Nutrition curriculum

For developing new nutrition curriculum, we conducted a self-developed, 25 multiple choice nutrition therapy questionnaire (total 25 score) to assess medical students' knowledge. At the beginning of the performed pre test, we sought the students' perception about nutrition instruction at baseline. The question was:

Do you believe that the time devoted to your instruction in nutrition was?

a) inadequate
b) appropriate
c) excessive
d) and also any extra explanation

We were aware, most medical students at QUMS were not satisfied with nutrition course. We were interested to observe whether the proposed nutrition curriculum at QUMS would result in a more favorable response. In other word, would nutrition workshop and also exposure to the curriculum result in either reduced percentage of students reporting that inadequate time was devoted to instruction in nutrition or an increased percentage of students reporting that appropriate time was devoted to instruction in nutrition? This change in perception will be comparable with responses to similar question in post-test, one month after conducting nutrition workshop.

For organizing the diet therapy workshop, the content of an 8-hr case-based, problem oriented tutorial curriculum design workshop reviewed by a nutritional professional and an internal medicine specialist. Next, a meeting was held with the 5 faculty members who actively were teaching the content of workshop, to determine the nutrition objectives and the potentials for expanding the nutrition content. Last, case-based, problem oriented clinical approach teaching implemented and the new nutrition content integrated into the clinical course. This integrated approach allows the nutritional knowledge of the curriculum to be taught and practice throughout the medical education.

2.4 Assessment of outcome measures

Assessment of the project included 3 primary outcomes. The first evaluation included the measurement of knowledge of student in the nutrition therapy area at the end of one month community medicine training. The second evaluation technique is supposed to measure applied skill in diet therapy using the OSCE. The third evaluation method will use the post graduation questionnaire for student evaluation of curriculum nutrition content.

2.5 Objective Structured Clinical Examination

The OSCE is a clinical approach in medical education to evaluate clinical skills among medical students. This multi station format evaluation tools uses standardized, trained patients to assess the performance of a wide range of clinical skills including applied nutrition skills.

2.6 Statistical analysis

To evaluate the nutrition curriculum the questions were asked: 1) Do medical student improve their applied nutrition knowledge after conducted nutrition workshop. 2) Do students apply learned nutrition knowledge in a clinical setting? 3) Do performance on the OSCE nutrition items and its score improve in relation to exposure to the nutrition curriculum overtime? All statistical analysis for this research was conducted with use of SPSS 16 statistical software (SPPSS Inc, Chicago). To address the first question, the statistical analysis (paired t-test) included a comparison of the scores of the medical students before (pre-test before nutrition workshop) and after implementation of the nutrition curriculum (post-test after nutrition workshop).

Performance on the OSCE is the second focus for outcome analysis. To address the second question, we will compare the post-test scores with nutrition subscale score of the first conducted OSCE using paired t-test. To determine whether the nutrition OSCE scores are improving over time, statistical analysis will be conducted among the students of different years after implementation of nutritional curriculum.

2.7 The pilot study

A pilot study was completed prior to the main study on a six internship medical student. The student passed all the procedure of the project including attending clinical nutrition workshop and nutrition clinic for training. Comparison of pre and post test scores and clinical skill evaluation showed a significant improvement in nutrition knowledge and applied nutrition in clinical setting. Theses students were not part of the actual data collection

3. Results

3.1 Nutrition content

The nutrition content before and after the integrated nutrition curriculum is demonstrated in table 1. At the start of the integrated nutrition curriculum in May 2011, there were 34h (2 credits unit) of required nutrition instruction during semester 2 of year 2 of medical education. After the integrated nutrition curriculum, 8 hr extra nutrition instruction in relation to diet therapy included in medical education.

3.2 Nutrition knowledge and OSCE score

In pre-test 20% of the students reported that the time devoted to nutrition was adequate. The students also explained in open question that, they suggest the "Clinical Nutrition" to be offered during years 4, 5, and 6 of their clinical practice education. In post-test 70% of students indicated that the nutrition instruction was

adequate. Also, in open answer question, they expressed that they are satisfied with current ongoing program. Paired t-test showed that the mean nutrition knowledge score of total students in applied nutrition were different between before and one month after integration (8.3±2.5, 13.4±3.2, $P < 0.001$) (table 2). And two groups of participants including staggers and interns had similar nutritional knowledge score at pre-test (7.9±2.6 and 8.9±2.3 respectively). Comparing the score of OSCE with nutrition knowledge and also comparing OSCE scores over time in ongoing integration will be reported separately in due time.

4. Discussion

Our primary evaluation of the nutrition education program indicated that the integrated curriculum was an appropriate and time-efficient model for integrating clinical dietetic into medical education. This approach resulted in significant increase in medical students diet therapy knowledge (8.3±2.5, 13.4±3.2, respectively $P < 0.001$) and enhancing student-reported perception of the adequacy of nutrition education in new implemented program. Observing similar nutritional knowledge score at pre-test among two groups of staggers and interns students (7.9±2.6 and 8.9±2.3 respectively) revealed that the traditional approach in medical education does not significantly improve clinical nutrition knowledge during medical education. This ongoing nutrition education program introduces a clinical orientation to basic nutrition and diet therapy and provides repeated exposure to nutrition information that enhances clinical skill over time. Medical students often do not know how to apply learned nutrition knowledge. The case-based, student-organized learning is appropriate solution to this problem. An important aspect of the evaluation is that how students will be able to use their knowledge and skills in a clinical setting as will be assessed by the OSCE. Previous researches have shown the effectiveness of the OSCE in evaluating the using knowledge and skills gained during medical education to future clinical practice (Rutala *et al*., 1992; Dupras and Li, 1995). OSCE score is similar to traditional methods in evaluating general ability and is also able to evaluate clinical ability in standardized manner (Merrick *et al*., 2000). There are other approaches to nutrition curriculum in medical education. In the nutrition integration program at University of Arizona, College of Medicine program, the curriculum does not comprise a single nutrition course but rather integrated nutrition content into required courses throughout the 4 yr of undergraduate medical studies (Taren *et al*., 2001). We believe that integrating cased-based clinical dietetic allows students to identify patients and medical conditions that would benefit from nutrition intervention for disease prevention, diet therapy, and appropriate nutrition prescriptions and referrals. Our integration program provides totally 42 hr nutrition education which is more than minimum 25 hr recommended by NAS in 1985 (1985). This nutrition education prepares medical students to improves and enhance their medical nutrition skill in clinics during patient managements throughout their clinical practice.

There are limitations for conducting the project. Physicians in clinical settings need to cooperate with integration process. For the supporting the project of integrating nutrition in clinical medical education, it is necessary the clinical nutrition topics and nutrition knowledge take a prominent place on students final and national board examination as has been discussed in existing literature (Hark *et al*., 1997). The final internship exams needs to move away from multiple-choice content questions, turning instead toward more case-based clinical nutrition problem-solving questions

5. Conclusion

In conclusion, the case based nutrition curriculum at QUMS is cost-effective and appropriate method to translate the knowledge to clinical practice. The nutrition curriculum implemented at the QUMS which resulted in improvement of knowledge and also more favorable response to the adequacy of time devoted to instruction in nutrition, needs to be replicated in other medical school for improvement of suggested nutrition integration.

Conflict of interest: There is no conflict of interest

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Table 1. Hours of nutrition curriculum content by course at baseline and after the development and implementation of the integrated nutrition curriculum at the Qazvin University of Medical Science School of Medicine

Nutrition instruction at baseline (h)		Extra medical nutrition therapy instruction after implementation of the nutrition curriculum (h)	
Carbohydrates, sources and related disease	2	gastrointestinal tract disorder	1/2
Protein, sources and related malnutrition	2	Liver disorder & Encephalopathy	1/2
Fat, sources	2	Diabetes mellitus	1/2
Energy, measurement of energy	2	Anemia	1/2
Minerals	2	Heart disease	1/2
Vitamins	2	Renal disorder	1/2
Food groups, food habits and meal planning	2	Infection and diarrhea	1/2
Maternal nutrition	2	Weight management program	1/2
Nutrition during childhood, nutrition in aging	2	Weight gain program	1/2
Nutrition assessment	2	Gluten intolerance	1/2
Anemia (iron and folic acid deficiency)	2	Diet in gout	1/2
Food safety and food poisoning	2	Diet during pregnancy, lactation	1/2
Nutrition obesity and heart disease	2	Food groups and exchange list application in diabetes	1/2
Breast feeding and infants	2	Glycemic index of foods and its application	1/2
Nutrition during pregnancy and lactation	2	Complementary medicine	1/2
Nutrition assessment, PEM	2	Nutrition assessment of patients	1/2
Malnutrition in Iran and around world	2	Vitamins and minerals sources	1/2
Total hr	34		8

Table 2. Students' perception and knowledge score in pre and post-test

	Pre-test	Post-test	*P*
Perception about adequacy of nutrition course hours	20%	75%	
Knowledge score			
Stager (n = 26)	7.9±2.6	13.2±3.9	< 0.001
Internship (n = 20)	8.9±2.3	13.7±2.1	< 0.001
Total students (n = 46)	8.3±2.5	13.4±3.2	< 0.001

A Case Study and State of Science Review: Private versus Public Healthcare Financing

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Abstract

Medicare is a popular program in Canada that offers universal access to medically-necessary healthcare services for all Canadians through a public insurance plan in each province. In spite of its popularity, healthcare privatization has been debated, often over concerns about wait times for select healthcare services. A case report focused on the 2005 Supreme Court's response to the "*Chaoulli v. Quebec*" challenge of the Quebec law banning the purchase of private health insurance for publicly-insured services is presented, along with findings from a state of science review to determine if there would be any benefit from adopting the United States model of private health insurance. This review reveals private health insurance would have significant negative implications, especially by creating inequity in healthcare access for low-income groups. Further study is needed to determine whether Canada's publicly-funded healthcare system would benefit in any way from increased private financing.

Keywords: Case study, Systematic review, Healthcare-insurance, Wait times, Public policy, Healthcare equity, Universality, Privatization

1. Introduction

Medicare, a source of pride and national identity for many Canadians, is a prominent social program that has offered public insurance coverage for all Canadian citizens since 1966 to ensure universal access to medically-necessary healthcare services (Thornhill, Law, Clements, & Stipich, 2008; White & Nanan, 2009; Yalnizyan, 2006). In spite of the widespread popularity of Medicare, healthcare privatization has been a contentious recurrent issue (Brunet, 2011; Schraeder, 2006). Private insurance for publicly-insured healthcare services has been proposed as a solution to ameliorate wait times for select healthcare services in what is now an entirely public healthcare system (Skinner, 2009). Canada's healthcare policy approach of prohibiting competitive private insurance for publicly-funded healthcare services has been noted as "extreme" by proponents of healthcare privatization; particularly as most other developed countries have some form of mixed public and private health insurance with varying levels of government regulation (Skinner, 2009). A case report on the 2005 Supreme Court's response to the "*Chaoulli v. Quebec"* challenge of the law banning the purchase of private health insurance for publicly-insured services in the Canadian province of Quebec is presented below, following an outline of the value of case study research for informing policymaking. Following this, findings are presented from a state of science review that was undertaken in 2011 to determine if there would be any benefit from adopting what Canadians perceive to be the "United States' model" of private healthcare insurance. The privatization issue discussed in this paper is not healthcare delivery (i.e. whether a public or private organization delivers healthcare services) but instead healthcare financing (i.e. by public or private sources, or a combination

of both). Public funding is a key component of the Canadian Medicare system, and if this foundation changes, so too could the nature of Medicare.

2. The Value of Case Studies for Informing Health Policy and Policy Making

A case study involves an in-depth analysis of a specific phenomenon in its real-life context (Gagnon, 2010). Case study research is encouraged, as this type of inquiry can focus on single or multiple cases to produce or challenge a theory; and/or explain, explore, or describe a complex real-life situation or quandary (Gerring, 2007; Yin, 2003). Case studies in health services research are particularly valuable as they enable a better understanding of a large topic by focusing on key components (Gerring).

The typical case study method has five steps. The first is to determine whether the case study is the appropriate method to answer the research question (Gagnon, 2010). A case study is appropriate if the purpose of the research project is to answer *how* and *why* questions about a particular case in its' real-life context (Yin, 2009). The second step is to develop a research design, so as to collect the right types of data and have appropriate data analysis strategies (Yin, 2010). The next three steps involve data collection, analysis and interpretation of data, and dissemination of the findings (Gagnon, 2010; Yin, 1999). Data collection should be done in a structured manner, with the evidence collected from multiple sources triangulated to ensure reliability and validity. During the analysis and interpretation of data, the research team should identify possible explanations, such as *why* a particular policy is effective or ineffective. Based on the disseminated case study results, other jurisdictions may introduce the same policy, a variant of the policy, or avoid the policy. Thus, case studies enable policymakers to learn from mistakes and successes, and ultimately make better policy decisions.

2.1 A Case Report on Private Insurance for Medicare

As indicated above, one current major aspect of the healthcare privatization debate in Canada is the ban on private health insurance for medically-necessary healthcare services (Flood & Haugan, 2010). A case study on private health insurance in a province where this ban has been lifted should inform whether or not this policy has been successful in reducing wait times (Merriam, 2009). Private health insurance in Canada has been traditionally limited to services not covered under the public health insurance plan of each province, as expected through the 1984 *Canada Health Act* (Hurley & Guindon, 2008). The purchase of a private insurance for some publicly-insured services, often referred to as duplicate private health insurance, has also been specifically prohibited in six Canadian provinces, including Quebec (Flood, 2006).

The 2005 Supreme Court "*Chaoulli v. Quebec"* case was initiated by Dr. Jacques Chaoulli, a Quebec physician (originally from France), who was frustrated in his ability to practice privately due to governmental limits; and so challenged the law prohibiting the purchase of duplicate private health insurance in Quebec (Flood & Xavier, 2008; Monahan, 2006; Madore, 2006). Dr. Chaoulli had earlier come into conflict with the Quebec Health Insurance Board over his application to operate a private hospital (by opting out of the provincial health insurance plan), with this application refused by the Board (Tiedemann, 2005). The case involved Dr. Chaoulli's patient, a Quebec resident, Mr. George Zeliotis, who had to wait one year for hip replacement surgery. Mr. Zeliotis' attempts to purchase private health insurance to get earlier hip surgery failed, and they blamed the law prohibiting duplicate private insurance for his treatment delay (Tiedemann, 2005). However, some argued at the time that Mr. Zeliotis, who was 73 years-old with hip and heart problems, would not have qualified for private insurance even if it were available (Flood & Lewis, 2005). The Supreme Court ultimately ruled in favor of Dr. Chaoulli and Mr. Zeliotis, declaring that "Quebec laws preventing the purchase of private insurance, in the face of long wait lists for public treatment, violate guarantees within the Quebec Charter of Human Rights and Freedoms" (Flood & Xavier, 2008, p. 617). In a slim majority (four judges in favor and three against), Chief Justice Deschamps dismissed the Quebec government's claim that prohibiting duplicate private health insurance was necessary to protect the quality of the province's publicly-funded healthcare system (Flood, 2006). This Supreme Court's decision only applied to the *Quebec Charter and* not the *Canadian Charter of Rights and Freedoms*; hence the laws preventing the purchase of duplicate private health insurance remained legitimate in other provinces, such as Alberta and Ontario (Flood & Xavier, 2008).

As a consequence of the "*Chaoulli v. Quebec"* decision, the Quebec National Assembly passed Bill 33 in December 2006 to lift the ban on private health insurance for three publicly-insured surgical procedures - total hip replacement, knee replacement, and cataract removal (Flood & Xavier, 2008; Mehra, 2008). By then, Services Quebec (2011a) had already established a wait time guarantee in 2005 such that if a patient had to wait for more than six months for any of the three surgeries then the government would pay for their treatment in a private clinic in that province or elsewhere. This wait time guarantee was designed to reduce equity concerns as not all Quebec residents had the financial means to pay for private healthcare insurance and thus benefit from

earlier surgery. However, as the specified treatments, when delivered in private clinics, were publicly-funded; this guarantee made Bill 33 redundant (Flood & Haugan, 2010). Since then, due to low public demand, the market for duplicate private health insurance did not grow in Quebec or anywhere else in Canada (Hurley & Guindon, 2008). However, the market for duplicate private health insurance could still grow in Quebec if this wait time guarantee does not operate properly (Flood & Haugan, 2010). According to Services Quebec (2011b), 55% of current patients requiring hip replacement surgeries receive their treatment within three months, while 85% of these patients receive treatment within six months. The Canadian Institute for Health Information (2010) similarly found the wait times for hip replacement surgeries in Quebec between the years 2006 to 2009 showed no evidence of change, with 88% of patients treated within six months over this time period. Although a six month guarantee is present for all residents of Quebec, individual wait times are not rigidly fixed anywhere in Canada, as patients are prioritized on the basis of severity of symptoms and not the length of their waits (Gaudet *et al*., 2007; Services Quebec, 2011c). The Canadian healthcare system is designed to operate on a triage system, where the most ill persons are treated first; and with this prioritization contributing to cost-effectiveness and maximal health services utilization.

3. State of Science Review

As part of the "*Chaoulli v. Quebec"* case study, a state of science review was conducted to examine whether the Canadian healthcare system would benefit from adopting the United States' model of allowing more private financing options. The United States was chosen for comparison over other countries because it is similar to Canada in many ways; however, it differs markedly with respect to healthcare insurance and healthcare access (Siddiqi, Zuberi, & Nguyen, 2009). Private healthcare insurance is dominant in the United States.

State of science or systematic reviews are an efficient method of identifying and reviewing literature in a highly structured manner, in order to provide an evidence base for a practice or policy (Whiting, 2009). The electronic library databases searched were MEDLINE and CINAHL, as these are the two most commonly used health library databases (see Figure 1). The keywords used in the search were: private health insurance, public health insurance, healthcare accessibility, Canada, and/or United States. The search was limited to English-language literature and further limited to research articles published in the last five years, as evidence from older studies was thought to be less relevant to the current context for informing health policy decisions. This search found several articles have been published using data from the 2002-2003 Joint Canada/United States Survey of Health (JCUSH). Of the 10 studies that initially m*et al*l eligibility criteria for review, four were excluded because they were from the same data source (i.e. JCUSH) and because these studies focused on different issues (e.g., access to prescription drugs, access to primary and preventative care services, etc.). A study by Blackwell, Martinez, Gentleman, Sanmartin, and Berthelot (2009), that used the "JCUSH" data, was retained as it took into account several measures of socio-economic status, was inclusive of two of the other excluded studies; and was focused on the topic of interest for this case study. In total, six articles (with one of these a systematic review) were reviewed, with the information gained from each summarized in Table 1.

3.1 Health Insurance Evidence: Private versus Public

The evidence gathered through this literature review revealed, as outlined in Table 1, that encouraging private health insurance for medically-necessary healthcare services would create socio-economic equity concerns. Hence, its' adoption as a means to reduce wait times in the public system is not recommended. This conclusion is based on finding that three studies favored Canada's public healthcare insurance (two through evidence of better health outcomes, and one showing better public access to primary care services) and three provided mixed results. None clearly favored the United States' private healthcare insurance or healthcare system in terms of equity, efficiency, or other factors (see Table 2). Guyatt *et al*.'s (2007) systematic review of studies that compared health outcomes in Canada and United States concluded that "Canada's single-payer system, which relies on not-for-profit delivery, achieves health outcomes that are at least equal to those in the United States at two-thirds the cost" (p. E36). Of the 38 studies reviewed by Guyatt *et al*. (2007), 10 studies included extensive statistical adjustment and among which five favored Canada, two favored the United States, and three showed mixed results. Although neither Canada nor the United States had consistent superior healthcare outcomes, the outcomes for Canada were more often superior to those of the United States for patients with similar underlying medical conditions (Guyatt *et al*., 2007). For instance, Canadian outcomes appeared superior in head and neck cancer, and possibly for various types of cancers for low-income groups; while breast cancer survival rates were better in American women when compared to Canadian women. The findings from the five other reviewed studies follow to better illustrate the identified evidence and conclusion reached.

Gorey, Luginaah, Holowaty, Fung, and Hamm's (2009) study found high-income breast cancer patients in the United States with private health insurance had shorter wait times for surgery and radiation treatment than their

Canadian counterparts. However, the same study found remarkable equity in Canadian breast cancer care, in contrast to a stark socio-economic inequity in access to such care in the United States. A study by Li, Lau, McCarthy, Schull, Vermeulen, and Kelen (2007) used a nationally representative sample of 40,253 emergency department (ED) visits to compare the ED visit rate in the United States and Ontario, Canada. They found that the annual ED visit rates in the United States (39.9 visits per 100 population) was almost identical to the rate in Ontario, Canada (39.7 visits per 100 population); and hence concluded that differences in "health insurance coverage may not have a substantial impact on the overall utilization of emergency care" (Li *et al*., p. 582). Similarly, a study by Blackwell *et al*. (2009) examined whether socio-economic status (SES) and healthcare insurance coverage were associated with difference in the utilization of hospital services among adult patients in Canada and the United States. While the study found no difference in hospitalization based on SES, Blackwell *et al*. noted this lack of difference could be due to hospitalizations resulting from emergency situations in many cases and hence these are likely to occur irrespective of the patient's health insurance coverage status. This argument is supported by their finding that adults who lacked insurance coverage in the United States stayed fewer nights in the hospital when compared to insured Americans. Krajewski, Hameed, Smink, and Rogers (2009) used information on patients diagnosed with acute appendicitis from 2001 to 2005 to determine whether Canada and United States differed in terms of access to emergency operative care. They found no difference in the odds of appendiceal perforation at different levels of SES in Canada; however, there was a significant inverse relationship between the odds of appendiceal perforation and income levels in the United States. Based on their results, Krajewski *et al*. (2009) concluded that unlike Canada, the ability to pay and/or the patient's SES determines access to emergency operative care in the United States. In another comparative study, Rowe, Bota, Clark, and Camargo (2007) examined differences in acute asthma presentations to hospital emergency departments across Canada and the United States. As asthma is a chronic disease, patients in the United States more often reported access barriers and they were less likely to be insured (Rowe *et al*., 2007). In short, this state of science review included evidence from six articles, and these clearly showed no immediate or other benefit from private healthcare insurance.

3.2 State of Science Literature Review Discussion

The findings from this literature review suggest that instead of Canada allowing private healthcare insurance or financing measures, the healthcare system in the United States should consider moving to a publicly funded health care insurance system. The rationale for this is that the United States has a major wait time problem of its own (Gorey *et al*., 2009). The wait times in a multi-payer system like that of the United States are much less transparent than the wait times in Canada's single-payer system. For instance, among the 47-50 million people without any healthcare insurance in the United States, many cannot afford to be on any waiting lists (Gorey *et al*., 2009). While no concrete evidence was found to prove that the Canadian healthcare system would react adversely from adopting the United States model, experimenting with private healthcare financing options would be dangerous since Canada's trade treaties make it difficult to reverse commercialization reforms once initiated (Bryant, 2009). The case study of the "*Chaoulli v. Quebec"* challenge in Quebec is another illustration of the lack of value and need for private healthcare insurance in Canada.

4. Conclusion

This case report of a controversial policy permitting private health insurance in the Canadian province of Quebec hopefully convinces readers of the significance of conducting case studies for informing policy. Case studies are robust, with many sources of information of possible interest and relevance to enhanced policy-making. The evidence from a state of science review presented in this paper also clearly suggests the Canadian healthcare system would not benefit from adopting the United States' healthcare funding model, where private health insurance dominates. Instead, it indicates that if Canadian policymakers decide to adopt private financing options as a quick fix for wait times in the public system, this apparent remedy would have long-term negative implications for Canada, especially by creating inequity in healthcare access. Low-income groups would suffer as they are less able to purchase private insurance. As this "*Chaoulli v. Quebec"* case study illustrates, Canadians so far have upheld healthcare equity through public funding instead of embracing private healthcare financing options.

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Table 1. Results of studies reviewed to compare the performance of United States and Canadian healthcare systems, with focus on health insurance and socio-economic status

Reference	Aim of Study	Methods	Findings	Reviewer's Comments
Blackwell, Martinez, Gentleman, Sanmartin, & Berthelot, (2009)	To examine the factors associated with the utilization of physician and hospital services among adults in Canada and the United States (US), with a focus on socio-economic status (SES) and healthcare insurance coverage.	Study used data from the 2002-2003 "Joint Canada/United States Survey of Health". Country-specific multivariate logistic regressions were conducted to predict healthcare utilization after controlling for predisposing factors, enabling resources (e.g., health insurance), and perceived need for healthcare.	Adults in Canada and the US exhibited similar patterns of hospital utilization, and SES (including health insurance coverage) played no explanatory role. Instead, only the individual's predisposing characteristics (e.g. age and sex) and his/her need for healthcare predicted utilization of hospital services in both Canada and the US.	This study was reviewed as it could explain whether the universal access to hospital services in Canada affects the rate of utilization, when compared to that of the US where private health insurance play a role in access to these services. Since, no difference in service utilization based on the type of insurance coverage was found, this study is determined as offering mixed results.
Gorey, Luginaah, Holowaty, Fung, & Hamm, (2009)	To determine whether SES has a differential effect on waits for surgical and adjuvant radiation treatment (RT) of breast cancer in Canada and the US.	Data was obtained from Ontario and California cancer registries between 1998 and 2000. Residence-based SES data were taken from censuses. Median waits were compared within and between countries using Mann-Whitney U-test.	There were significant associations between lower SES and longer surgical waits and lower access to adjuvant RT waits across diverse places in California. None were observed in Ontario. However, relatively high-income women with breast cancer in Ontario typically waited one to two months longer for adjuvant RT than their counterparts in California did.	This study was reviewed as it could explain whether the US system had shorter waits to cancer care than that of Canada. High-income US patients had shorter waits than Canadians, but since greater inequity was found in the US system, the study is determined as offering mixed results (shorter wait times for high-income groups in the US versus greater equity in Canada).
Guyatt *et al*., (2007)	To perform a systematic review of studies comparing health outcomes in the US and Canada among patients treated for similar underlying medical conditions.	Research of multiple bibliographic databases and resources. Study results were masked before determining study eligibility. For all eligible studies, original authors were asked for additional specific information and also to confirm accuracy of the information drawn from their study.	Of the 38 studies that met the study's eligibility criteria, 14 favored Canada, 5 favored US, and 19 showed mixed results. The only condition in which results consistently favored one country was end-stage renal disease, in which Canadian patients fared better. Overall, the authors concluded that patients cared for in Canada have superior health outcomes than the US.	This study was reviewed as it could identify whether the US system, with a large private health insurance sector, is able to achieve better health outcomes than that of the Canadian system. Since the Canadian system was found to be cost-effective, while achieving equal or better health outcomes than that of the US, this study is noted as favoring Canada.

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Krajewski, Hameed, Smink, & Rogers, (2009)	To determine whether or not there is a difference in access to emergency operative care between Canada and the United States based on socio-economic status (SES), given the difference in health insurance coverage among these two countries.	Data obtained from Canadian Institute for Health Information database and the US Nationwide Inpatient Sample, and included all patients diagnosed with acute appendicitis from 2001 to 2005. Univariate and multivariate analyses were performed to determine the odds of appendiceal perforation at different levels of SES in each country.	In Canada, there was no difference in the odds of perforation between income levels. In the US, there was a significant, inverse relationship between income level and the odds of perforation. The authors conclude this difference in access to emergency operative care could result from concern over the ability to pay medical bills or the lack of a stable relationship with a primary care provider that can occur outside of a universal healthcare system.	Treatment delays in the case of appendicitis would increase the risk of perforation. Since the study found the risk of perforation increased with each decreasing income level in the US patients but no such difference existed in Canada, it is evident that the Canadian system is successful in ensuring equitable access to emergency operative care, without financial barriers. Thus, this study is determined as favoring the Canadian healthcare system.
Li, Lau, McCarthy, Schull, Vermeulen, & Kelen, (2007)	To compare emergency department (ED) visit rate in the US and Ontario, Canada, according to demographic and clinical characteristics.	A cross sectional study with a sample of 40,253 ED visits included in the National Hospital Ambulatory Medical Care Survey in the US, and National Ambulatory Care Reporting System in Ontario, Canada.	The study found annual ED visit rate in the US was identical to the rate in Ontario, Canada; and concluded that differences in health insurance coverage may not have a substantial impact on the overall utilization of emergency care.	With no link found to the type of insurance coverage and overall utilization of emergency care, the study's authors ponder that other factors may be contributing to the ED overcrowding in both countries. This study thus provided mixed results.
Rowe, Bota, Clark, & Camargo, (2007).	To compare emergency department (ED) asthma management and outcomes between Canada and the US, since acute asthma is the most common ED presentation in both countries.	A prospective cohort study of 69 American and eight Canadian EDs was conducted. Patients aged two to 54 years who presented with acute asthma underwent a structured ED interview and telephone follow-up two weeks later.	In terms of asthma chronicity and presentation to the ED, the US patients more often reported barriers to access primary care, demonstrated poor asthma control, and presented with suboptimal preventive medical management than their Canadian counterparts.	This study was reviewed as it could identify whether the universal access to primary care services in Canada play a role in health outcomes related to asthma. The study did find poor asthma control in the US patients without health insurance, and thus the study's results favored Canada.

Table 2. Summary of findings

Results favored Canada	3
Results favored United States	0
Mixed or equivocal results	3

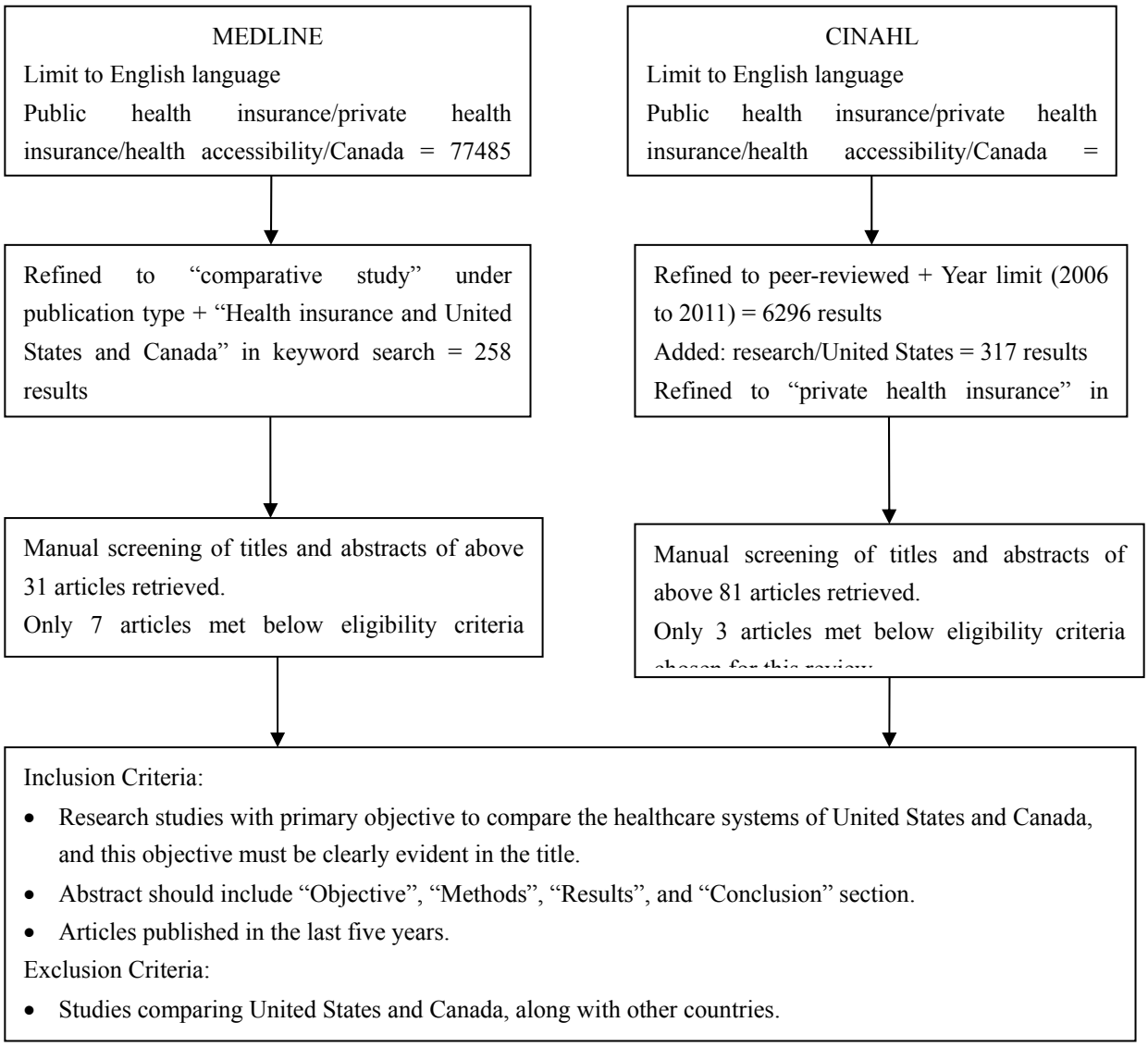

Figure 1. Methodological steps in systematic review

The Impact of Medical Tourism on Thai Private Hospital Management: Informing Hospital Policy

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Abstract

Background: The purpose of this paper is to help consolidate and understand management perceptions and experiences of a targeted group (n=7) of Vice-Presidents of international Private Thai hospitals in Bangkok regarding medical tourism impacts. **Methods:** The method adopted uses a small-scale qualitative inquiry. Examines the on-going development and service management factors which contribute to the establishment and strengthening of relationships between international patients and hospital medical services provision. Develops a qualitative model that attempts to conceptualise the findings from a diverse range of management views into a framework of main (8) - Hospital Management; Hospital Processes; Hospital Technology; Quality Related; Communications; Personnel; Financial; and Patients; and consequent sub-themes (22). **Results:** Outcomes from small-scale qualitative inquiries cannot by design be taken outside of its topical arena. This inevitably indicates that more research of this kind needs to be carried out to understand this field more effectively. The evidence suggests that Private Thai hospital management have established views about what constitutes the impact of medical tourism on hospital policies and practices when hospital staff interact with international patients. **Conclusions:** As the private health service sector in Thailand continues to grow, future research is needed to help hospitals provide appropriate service patterns and appropriate medical products/services that meet international patient needs and aspirations. Highlights the increasing importance of the international consumer in Thailand's health industry. This study provides insights of private health service providers in Bangkok by helping to understand more effectively health service quality environments, subsequent service provision, and the integrated development and impacts of new medical technology.

Keywords: Tourism, Medical, Private hospitals, Hospital policy, Bangkok

1. Introduction

South East Asia and especially Thailand is experiencing an expanding health services sector as hospital management – particularly private hospital management – have realised that offering world-class medical services can result in increased demand from overseas patients (Hazarika, 2010). This has helped private hospitals in Thailand increase their revenues by offering medical services at local premium costs, which are considerably cheaper than in many overseas countries. The Medical News (2009) reported that Thailand becomes Asia's most popular medical hub with approximately 1.2 million medical tourists. However, this was the same total as in 2006 (Med-tourism-thailand, 2011) and in 2008 (Medical2, 2011). Although some forecasts suggest that this has been increased to 1.45 million medical tourists in 2010 (Medical Travel, 2011) with more than half of these going to one hospital (tourismthailand.org, 2011) – although, this hospital had only dealt with 420,000 patients in 2008 (Health-tourism, 2011). Another source recognises that there are perhaps 1.2 million international patients and speculate that 300,000 are medical tourists (Health-tourism, ibid). Consequently, the figures do not allow specific inferences to be made and that rather than a higher figure of 1.4 million, it is perhaps somewhat different and nearer 300,000 medical tourists that arrive in Thailand yearly. Nevertheless, based on these statements, it would seem difficult to know exactly where the medical tourism "dollar" is going in Thailand, as there are no government records published as to this particular purpose or to the revenue each hospital generates from this type of medo-socio-economic activity. As the Thai government does not keep data specifically about whether an individual comes to Thailand as a medical tourist except as a result of a

pre-determined set of options (arrival card) – which does not visually include the term "medical tourist". So the notion of Thailand as the most important medical hub (by numbers) cannot be substantiated, only assumed by NaRanong and NaRanong (2011) and maybe overhyped along with other countries such as Singapore or India. Without independent verification of actual medical tourism numbers then the industry is only speculating. Nonetheless, in some countries in SE Asia, a special medical visa has been developed (Smith, Chanda and Tangcharoensathien, 2009) which will help account for the numbers engaging directly in medical tourism. Given this, as there is a substantial difference in prices between the USA, UK and Canada and what is offered in Thailand, then there is the impression of a trend (Whittaker, Manderson, and Cartwright, 2010) that some tourists do come to Thailand with the express wish to undergo some kind of medical procedure which is the reason and exclusive focus of their visit. This raises the context for the first research question - What is it that patients want from their international health service provider?

For the purpose of this paper, medical tourism has been defined by Carrera and Bridges (2006a) as the organized travel outside one's natural healthcare jurisdiction for the enhancement or restoration of the individual's health through medical intervention. This has resulted in the "growing acceptance" of planned medical tourists (Chee, 2007) – those that have organised their procedures in advance. This is now perhaps a little limited as evidence suggests that there are those individuals who have found out while on holiday that such procedures are available at very affordable prices and quick turnaround times and so this definition must be widened to include ad-hoc patients. Subsequently, some tourists have found that they can combine holidays with medical procedures (Whittaker, 2008) at competitive prices, by providing extensive medical services with no-waiting and on-the-spot demands. In this respect, patients make choices because of their wealth, and are therefore not bound by the state, which often ordinarily provides the medical services for them at no cost. There are others whose circumstances reflect home-situations that are considered expensive, procedures that are often unavailable or have long-queues denoting their country's poorer health service provision (Burkett, 2007). Whilst it can be said that many South East Asian countries offer such services, patients do not always want to take up medical procedures unless they are satisfied that the procedures are carried out in a safe environment and that there is low health risk following the procedure. Therefore, medical tourism suggests patients have choice in where to go for their medical needs and also that the private health insurance or publicly funded health provision in their country of origin does not cover these. In this respect, the patient is balancing the need to be prudent, conservative and safe with lower costs for medical procedures as well as having a choice of when those procedures are carried out and by whom. Often this means selecting where and with whom through a medical services broker or making selective individual decisions. Marketing promotion in home countries create demand for medical services overseas with individuals who have the money and time and where hospitals meet or exceed standards for patient safety and quality of care as they advertise their international accreditations. Whilst this paper indicates why medical tourism occurs, it is firmly targeted at individual medical professional responses at the service use stage, as very little research has been conducted in this area in SE Asia.

It is now common practice for private hospitals in Thailand to advertise and promote their medical services in developed countries as the cost in these countries of providing adequate and specific health increases. Consequently, the direction of medical travel is changing towards developing countries as patients seek faster and cheaper medical solutions (Carrera and Bridges, 2006a).

Private hospitals operating in Bangkok appear therefore to maximise their resultant profits by ignoring the lower to middle income populations that surround them, as no private hospital will treat anyone without the means to pay. Further, medical provision in Bangkok combined with the commercial operating notions of a 5 star hotel has led many first-world patients to select SE Asia as a medical-related destination. However, the impact of medical tourism has raised the question of whether medical tourism can improve the capability for local health services to enhance provision for the poor in SE Asia? (Blouin, 2010). The reverse rational can also suggest that as private hospitals are for-profit, then it is unlikely that in the short-term that health provision for the poor in Thailand will improve as local patients (middle-upper class) also seek medical assistance from such private hospital services Arunanondchai and Fink, 2007a). This raises the context for the second research question - In what ways do hospital services provide for international patient health requirements both in Thailand and overseas?

This paper draws on hospital management views of medical tourism and suggests the difficulty utilising the term for Thai private hospitals as it is perhaps a little too stereotypical just to accept the literature and denote that promotion through brochures, brokers and local/international websites can be used to gauge the numbers taking up such specialist medical activities across borders for cash. In this respect, very little research has been conducted to help condense management opinion regarding the issues involved in medical tourism and how

these can be managed to provide a more effective understanding. Private hospitals by their very nature and focus appear to have major influence on hospital policy in general through their investment and operational orientation and thus appear to be a dominant force in Thai approaches to hospital development. This has helped the Thailand government to propose the development of Thailand - Bangkok - as a medical hub (MPH, 2003). This raises the context for the third research question - What are the reasons for the choices that management make to provide hospital services to medical tourists?

2. Methods

To gain a broader, deeper and more involved understanding of the issues generated within the Thai hospital management context and to consider more implicitly the issues and questions raised, this empirical groundwork utilised an interpretive approach (Walsh, White, and Young, 2008) to understand the perceptions of health service management regarding medical tourism. Hospital management were considered specialist knowledge agents as their opinions and experiences influenced policy application in the hospital. The research used a semi-structured questionnaire, which provided an appropriate element of context and flexibility (Cassell and Symon, 2004) and this further aided by applying an inductive/theory building approach (Glaser and Strauss, 1967). Given the lack of appropriately focused research in this area, this methodology is seen as suitable for creating contextual data for the purpose of forming richer theory development (Cayla and Eckhardt, 2007). The population for this study was managers of seven (7) international private hospitals located in Bangkok, Thailand - chosen through applying the approach of Carman (1990). After discussions with each hospital top management - VP-level managers would take part in the research and this reflected the criteria of theoretical purpose, relevance and appropriateness (Glaser and Strauss, 1967). It also indicated the importance given by the hospital to the research bearing. Using Glaser's (2004) sampling processes, a total of seven VP-level managers were thus determined as the resultant sample frame, which was also considered convenience sampling by Harrel and Fors (1992).

Each interview was audio recorded for future analysis. Interviews were conducted in English and took approximately 50 minutes. All interviews were recorded digitally after gaining explicit permission, and were later transcribed verbatim using NVivo software. The conduct of the interviews follows a similar process used by Gray and Wilcox (1995), with each individual group being asked the same set of questions - modified through ancillary questioning (probes and follow-ups) in the same way as Balshem (1991). To increase the reliability of the data, the actual transcription was returned to each respondent – via e-mail - for correction, addition or deletion and return, which followed the process of validated referral (Reeves and Harper, 1981). Whole-process validity was achieved as the respondents were considered widely knowledgeable of the context and content associated with the research orientation (Tull and Hawkins, 1990).

Each interview was initially manually interrogated and coded initially using the EverNote software according to sub-themes that 'surfaced' from the interview dialogue - using a form of open-coding derived from Glaser (1992a); and Straus and Corbin (1990). This treatment was also reinforced and extended through the use of thematic analysis conducted using the NVivo 9 - qualitative software package (Walsh *et al*., 2008). Each interview was treated and coded independently. In this way, no portion of any interview dialogue was left uncoded and the overall outcome represented the shared respondents views and perspectives through an evolving coding-sequence (Buston, 1999). Various themes were sensed from the use of the software packages, as well as from the initial manual-coding attempts. This dual form of interrogation was an attempt to increase the validity of the choice of both key themes and sub-themes through a triangulation process. NVivo 9 was further used to explore these sub-themes by helping to pull together each of these sub-themes from all the interviews (Harwood and Garry, 2003). In this way, it was possible to capture each respondent's comments across transcripts (Riessman, 1993) on each supported sub-theme and place them together for further consideration and analysis.

The structure of the outcome is greatly influenced by the emergence of the key-themes and sub-themes. The preferred strategy for the analysis of the primary data was to use the stated research questions, which are used as a guide to providing the outcome (based on Yin, 1994).

3. Results/Outcomes

3.1 Theme Outcomes

The various themes developed from the main interviews are presented in Table 1 below, and are essentially broken down into seven (8) key-themes: Hospital Management; Hospital Processes; Hospital Technology; Quality Related; Communications; Personnel; Financial; and Patients distributed across twenty-two (22) sub-themes. The placement of the sub-themes has been influenced by context of the key theme.

The outcomes are stated below where the discussion focuses on the sub-theme elements within each key theme and the subsequent impacts on Hospital Policy and are presented in Table 2, below. The discussion format used in this paper reflects the respondent's voice through a streamlined and articulated approach for reporting. Consequently, the style adopted for reporting and illustrating the data is influenced by Gonzalez, (2008) and also Daniels *et al*. (2007) and is discussed below, focusing on the raised research questions and the resultant main themes.

Table 1, below, also shows the breadth of respondent illustrations/extractions as used in the reporting of this research. Figure 1, above shows a model of the managerial perspective with the main themes centred around the patient.

Q1 - What is it that patients want from their international health service provider?

Main Theme – Hospital Management

Hospital management appeared to recognise that reforms were necessary as changes were perceived to be required not only in the organisation but also in terms of staff development. As one respondent (M3) indicated we had to change. We needed to fundamentally change our whole outlook. Another respondent (M7) suggested we couldn't be the same as everyone else. So, it was a case of focusing all our staff - doctors, nurses, everybody including the staff who carried patient's belongings.

The introduction of newer and very expensive medical technologies had an impact. As one respondent (M1) suggested that it appeared to indicate that every member of staff was expected to learn new things, new ways of doing their job and on top of this was rebranding developments that appeared to expect changes in how staff (from every department) was expected to learn how to meet and greet patients from many countries. Another respondent (M5) supported this and stated with the new technology it was no longer seen as a Thai hospital but an international hospital in a Thai setting. For many hospitals, reforms included, for example as one respondent (M2) indicated internal training for doctors and nurses - who were given training in meeting patient needs, basic-language training - relevant to doctors orientation and more in-depth training for nurses who were expected to meet patients from overseas. Hospital reforms appeared to be more thorough for some as the balance between home-based patients and overseas patients changed, as depicted by another respondent (M6) who suggested that further changes at this hospital included training for receptionist nurses and other frontline medical staff - emergency staff and pharmacists who would explain medication as required. Most hospitals changed their policy on the the Case-Doctor, as one respondent stated each patient was assigned a doctor who would take charge of the medical provision throughout a patients stay together with an assigned international hospital representative who would also speak the patients language fluently.

An issue raised was that regarding core personnel shortages that were seen as inevitable - especially trained doctors in highly specialised disciplines. As one respondent (M3) illustrated this required some creativity, and here the hospital uses a radical form of knowledge sharing through the use of mobile technology - especially in an emergency situation. However, another respondent (M5) suggested that in more normal situations, doctors were shared among hospitals and were driven between appointments and calls by the network of hospitals This appeared to be a change to normal public hospital routines, but as stated by another respondent (M2) was used to ensure that overseas patients were given the attendance as required by their medical conditions.

In terms of hospital capacity, one respondent (M4) indicated that we have never been at full capacity, not even during emergencies. We have enough room to cater for local and international patients including our personnel. Another respondent (M3) suggested that we observe about 50% capacity to 60% - never much more than this. In terms of being private hospitals, the socio-economic profile of the patient would mean a mid to higher income group. Consequently, the expected behaviour of these individuals is that, as one respondent (M6) suggested they would not wait for medical services and expect a personal service delivery and further these two aspects create the focus for our health service delivery.

In terms of Emergency and Disaster preparedness/emergency protocols and teams private hospitals appeared very well prepared. For example, one respondent (M1) stated we have teams of professionals who can move at a moment's notice. This includes the most sophisticated road ambulances and also our helicopter flying doctor service. We can be called to an emergency anywhere within 350 km of Bangkok. However, these are not normal and are used sparingly – and they are fairly expensive, as each trip has to be paid for by the patient or at least guaranteed by the patient through the banking system or through insurance. In many respects, these types of emergency services appear to be a luxury that not many patients can afford. One respondent (M3) indicated that these services are an essential addition to our medical services. We accept that it needs to be paid for, but we see this development as part of a managerial focus that will eventually have an impact on public health services too.

Another respondent suggested that if there is a demand for these emergency services then we have to provide it. It saves lives and helps stabilise our communities.

Main Theme – Hospital Processes

Many of the hospitals "fail" to keep records of whether patients are medical tourists or are say expats. As one respondent (M7) indicated we are here to help patients with their medical problems. It doesn't matter where they come from. Another respondent (M5) suggested that we do not really know whether a patient is a medical tourist or not. However, if a client contacts us before they arrive in the country, we will always do our best to accommodate them – especially if they are on a short-timeframe or if they want multi-procedures carried out at the same time.

Patient support and patient knowledge was raised as a necessary issue. As one respondent (M3) indicated we like our patients to make informed choices. We also want to help the patient professionally and therefore we need as much information as possible to advise them. Another respondent (M4) illustrated this as we help the patient make proper choices – at home, and in the hospital through the use of IT technology. We also can contact the patients' doctor to quickly inform them of medical outcomes. Another respondent (M2) suggested that we try to get as much information from the patient before they leave their home. Otherwise they may have to bring that information with them. We only seek a discussion with the patient's home doctor – if it's necessary and if they give us permission to do so. Sometimes this causes us problems – medically and socially. A comment by another respondent (M1) asked does it really matter if they are tourists? However, from a government point of view this may illustrate an area for development because of the additional service GDP that is created, raising the notion that maybe the level of medical tourism is actually higher than accounted for at present.

Main Theme – Hospital Technology

In terms of medical coverage technology at all private hospitals in this study appears to be contemporary, modern and upto date. As one respondent (M7) indicated oh, yes. We try very hard to ensure we have the latest technology for diagnosing and hopefully confirming prognosis. However, the technology is used as a tool, rather than a singular determinator of evidence. As another respondent (M2) suggested technology is used almost everywhere, but our doctors have a lot of experience, we use the technology to confirm. We don't use the technology to predict. Different points of view were raised regarding the use of technology such as one respondent (M3) who shared. I like the fact that we have technology available, but it isn't always necessary, but it is available - especially if a patient wants it as a record for the future or to take home with them. Another respondent (M1) suggested that it's important we have this backup. We can't always be sure, so it's important for our own development as well as the patient. Further, another respondent (M6) stated that sometimes patients seem a little more reassured when we use the technology, say MRI Scan, which often only confirms cheaper alternative of X-ray pictures.

The notion of cost related to technology was raised and this gave rise to a myriad number of responses. As one respondent (M4) indicated we have to bear the cost of very expensive technologies. Another respondent suggested that yes, very costly. Sometimes the technology just sits there not being used. So, it is very expensive. This aspect was confirmed by another respondent (M1) who stated unfortunately, we either invest or patients don't come here again. We see technology as a way to differentiate this hospital from other hospitals. We either get the technology or our patients become dissatisfied and go elsewhere. On this point another respondent (M5) suggested that we see technology as a means to help our patients very quickly. This is one of the reasons why they come to us.

Private hospitals appear to be utilised at the capacity rate of 60% or less. However, ordinary everyday costs, as one respondent (M7) suggested gives us some problems. We have to manage our costs. There are always unexpected changes and these can affect our general and specific cost structures. However, this does not affect the patient, as another respondent (M2) indicated but when it directly affects our patients we try to communicate with them and show how the costs are built up. If a patient has a fixed cost package, then we will absorb the extra costs – its part of doing business. This is further explained by another respondent (M5) who suggested that when the patient undergoes more than one defined procedure then it can be difficult to precisely cost the outcome before we conduct the work. We always try to give patients an idea, but that isn't enough sometimes – especially if they have come from overseas.

Respondents also showed that there were other costs issues there would Hidden costs – Unexpected charges; Unexpected Complications; Longer hospital stays; additional medicines and/or treatments/procedures; additional medicines after hospital treatment; inaccurate pricing estimates – before treatment; changes in medical

conditions or new information about medical condition; preoperative tests/examination costs; and treatment delay

Main Theme – Quality Related

In terms of hospital accreditation, there would appear to an almost unanimous outcome. As one respondent (M1) stated it provides patients with trust in the hospital management - JCI accreditation, experienced American management, and the use of advanced technology. Another respondent (M6) suggested that our clients require that we are accredited. They can be secure in their mind that our professional judgement will have the client at the centre of what we do. This is further supported by another respondent (M4) who emphasised that accreditation is necessary for us to show both our domestic and international patients that we have the required quality in our systems and processes. It signifies an investment in the structure, processes and people in the hospital.

In terms of quality auditing the response was familiar, as one respondent (M3) stated each patient is given the opportunity to make comments and feedback about the medical services we give in terms of how we treat them etc. These are specifically to add to our processes and procedures that have been adopted for JCI and ISO 9000 accreditations. Another respondent (M1) indicated that we always carry out a service assessment before the patient leaves. It helps us manage for the future services we provide. In order to show how serious hospital management was, one respondent (M4) advised we are assessed on how well we adhere to the procedures and protocols we develop in order to get accredited. We train to make sure that nothing is left to chance. We have to get it right first time, every time.

Main Theme – Communications

Language was raised as a marketing tool. For example, one respondent (M5) indicated that 90% of our doctors speak English and about 45% of our nurses. We have a programme designed to help staff from all departments learn English, which is managed by a teaching group of native English speakers. It is an important part of the communication plan.

Co-ordination appeared to be an important facet of the patient communication strategy. This was seen as a simple measure but one that for overseas patients was a prominent requirement. As one respondent (M1) suggested we have a very good and well-tested system of communication that ensures that we can communicate with patients and their medical advisors while they are at home and supported by another respondent (M3) who stated each patient is allocated a member of the service staff who co-ordinates with the patient directly whether in Thailand or overseas, picks them up at the airport and ensures that everything is to their satisfaction. If they can't solve any problem that's raised then management can help solve any issue.

When the issue of marketing approaches was raised – Domestic and International - one respondent (M7) stated that we have to use many approaches today since there is a higher competition between hospitals – we know this. We have the philosophy that whatever medical procedures a patient requires, we make sure that they know exactly what it is that we will do and when and of course what the cost will be. Another respondent (M2) stated that we will talk directly with the patient in their home via phone, e-mail on videophone etc. This can mean that if surgery is required then a surgeon will talk directly to the patient – even if the patient is thousands of miles away.

Main Theme – Personnel

In terms of personnel, one respondent (M7) indicated that we try to employ the best doctors that we can. It's not easy, as doctors can move from one hospital to another to get better working conditions. Another respondent (M1) stated that we do have a core of very qualified full-time staff, which meets with most needs internally. Sometimes we have to engage a doctor from a specialist field and they cost more – but our patients expect the best – and we always give our best at all times.

In terms of doctor's experience and qualifications, one respondent (M2) stated that most of our doctors are trained in Thailand also internationally as this appears to help the reduction of the doctors leaving for overseas, as they remain better-paid professionals (Ramirez de Arellano, 2007). However, unlike public hospitals, one respondent (M4) stated quite clearly since all the doctors are trained, we do not have groups of young interns following a doctor while on his/her rounds. As such private hospitals are not considered training hospitals as this is reserved for public hospitals. This way we can give more personal attention, and also the patient sees the doctor for longer – and he's always on call, if necessary.

Another respondent (M6) stated many doctors give their personal phone numbers to patients. This further creates a sense of bonding and trust between the doctor and the patient.

Main Theme – Financial

There would appear to be some financial challenges that private hospitals have to meet. For example, managing bad debts. As one respondent (M4) indicated as a doctor I will treat anyone who needs our help. However, as an administrator, we have to be very careful with costs and payments. Another respondent (M2) reiterated that although our priority is always to provide the highest standards in medical care, we have to be cautious with financial issues as some patients lack the funds to support their medical treatments. Given this, we have to behave just like a 5 star hotel, when registering the patient first, we will always take a copy of a credit-card/passport as additional insurance - even if they have validated insurance.

In terms of the revenue cycle, one respondent (M6) indicated that some patients have stays that last several months, but mostly it is less than 5 days. Consequently, there is often a delay of payment to the hospital - especially insurance companies. We recognise that this is the norm. Another respondent (M3) stated that it does create issues when on-going medical costs build-up and we have no cash remittance or revenue. In many cases we ask for partial payment – especially when the costs are likely to be in the hundreds of thousands and more than one procedure carried out. Thus it would seem that the revenue cycle isn't as smooth as say in a manufacturing environment, but it is recognised by management that cycles have to be managed.

Main Theme – Patients

Under the theme patient safety, one respondent (M5) indicated that we are very careful in ensuring that patient safety is a top priority for all staff. For example, before a room is made available for a patient, it is serviced to a meticulous degree to reduce the risk of spreading any hospital-acquired infections. This is one of our major concerns. Another respondent (M1) suggested that at this hospital we have protocols in place that ensure staff, patients and visitors are only allowed in certain areas once they have been screened. On this important point, another respondent (M6) stated that we make sure that patient and staff safety are focused on even before we are fully aware of a patients medical needs.

When patient legal issues were raised, it became apparent that Thai hospitals had a strong standing as complaints were being made, but they were more of process related, rather than medical. For example, one respondent (M4) indicated that sometimes we have complaints, but these are very quickly sorted. These are often to do with being in strange surroundings. We learn how to deal with each patient, as each patient reacts differently. When an patient issue is raised that cannot be solved at the patient level, a wider response ensues. For example, one respondent (M2) indicated that we have protocols that are used to ensure that each patient is looked after very well. We also have protocols when the patient has an issue and we try to ensure that everyone involved in the patients well-being discusses the issues raised. This is what we call targeted patient action. Another respondent (M6) suggested that we don't get many complaints, but sometimes the problems are not with the hospital at all. For example, it may involve special travel arrangements that we weren't told about or an aircraft cancellation. However, we aim to ensure that complaints are reduced to a minimum and we do this by anticipating issues before they become complaints.

Hospital management appeared to be aware that patients were all different, and that unknown medical issues can arise during initial assessment or even during surgery. For example, one respondent (M1) indicated that sometimes, a client is weak or that the client wants a particular procedure and when sometimes we are doing this we find something else also. Another respondent (M7) stated it is difficult to manage because surgery is not like McDonald's – we can't always deliver what is ordered. This is because, each patient is different and we try to ensure that they get what they come here for and also that means getting value for money.

Finding new medical complications may also create new complaints and greater financial issues for the patient as there may be additional costs and changed timeframes associated with a given procedure. One respondent (M3) raised this issue, and stated that it is a difficult task to determine whether to carry out additional unintended surgery or leave it until the patient can make an informed choice. On this point another respondent (M4) suggested we can only do our best as professional doctors. We always make such decisions as a unified team in the best interests of the patient.

An issue was raised by many respondents in terms of emergency medical cover. This appeared to give doctors and management a challenge. For example, one respondent (M4) indicated that we are proud to provide emergency medical cover to anyone. But this is a business, and most patients pay straightaway. Sometimes though, it can be that we have to absorb some costs. That's the medical industry – it happens everywhere. However, in most circumstances such emergencies are managed through the normal patient process. For example, one respondent (M2) suggested that for any patient, if there is an emergency or a patient wants to talk

to his or her own doctor we often arrange that. Whether it's the doctor assigned here or an overseas doctor, we try to accommodate that. We help our patients become as relaxed as possible so that they can recover quicker.

4. Discussion

Figure 2, below illustrates the derived relationships between the major themes informing hospital policy. The model further shows the conceptual development and relationships perceived to correspond to the features informing hospital policy which allows hospital management to focus on how these influence their strategic perceptions and intentions.

Hospital management appeared to make changes associated with the drive to offer foreign patients more facilities, often in better surroundings, guided by staff - nurses and doctors - and using contemporary medical technology that enhanced the working relationship between patients and their health service provider.

Private hospital policy appears to be driven by a contemporary management approach and strategy and this integrates with the patient through links in communications, personnel development and costs/financial arrangements. However, total integration of technology is seen as a barrier because of cost (technologyreview.com, 2009). This is explored further by taking each research question and teasing out the impacts of the related major themes on the main characteristics underpinning hospital policy. These characteristics include: Hospital Management, Quality Related, Hospital Technology, Finance, Hospital Processes, Patients, Communications, and Personnel.

Q1. What is it that patients want from their international health service provider?

The marketing practices of hospital management appeared to engage with patient requirements from first contact, to the conclusion and follow-up of patient outcomes. In this respect, much of the hospital running processes appeared to be developed and focused on providing a diversity of medical provision enhanced by the application of certified quality related processes that underpin the strategies for matching hospital technology and processes with the patient-doctor needs (Weinbroum, Ekstein and Tiberiu, 2003). Good leadership skills (Kizer, 2001) and business skills (Jennings et. al, 2007) therefore appeared to be an essential attribute of this type of hospital strategic engagement. Experienced core doctors and nurses with enhanced working relationships (Hughes, 2008) appear to be a characteristic that observable research outcomes could support resulting in a partnership in care situation (Overton-Brown and Anthony, 1998). In this respect, it would also appear that greater hospital experience would reveal more open communication tendencies and a more informative outlook that underpins the educational interactive-counselling approach (Prideaux *et al*., 2001) used by doctors and nurses (Maguire, 1988). Hospital management also appeared to support the need for extending the range of medical service provision (Friedman *et al*. 2002), which in many circumstances created a larger hospital facility with medical technology utilisation at low levels consistent with developing support information systems required for managing elements of risk and moderating care (Kohn 2000). This appeared to be a fundamental management decision to show that the hospital could accommodate whatever medical plans patients would need and to act as a one-stop medical facility (Kohn 2000) which also impacted on hospital competitiveness (Robinson, 2001) with possible unavoidable duplication (Trinh, Begun and Luke, 2008) of medical services. This may also lead to the creation of additional cost (Lee and Stein, 1980) and reduce overall hospital industry efficiency. Some hospital management do not see this as costly duplication (people and resources), but as complimentary services (O'Malley, 2010) and thus support their overall business and collaborative strategy. All the private hospitals were certificated to appropriate standards in Thailand and some (4) were even certificated to external overseas organisations such as JCI. Consequently, this illustrated the overarching managerial/marketing approach to connect with patients at home and abroad and to provide medical services that were appealing to doctors, nurses and patients (Lake *et al*. 2003). For all private hospitals, it would appear that upto date technology in many areas of expertise were utilised, providing opportunities for staff development (Lake, ibid) marketing communications, more differentiated patient medical needs and greater supplementary facilities utilisation (Lesser and Brewster, 2001). However, the impacts of this hospital strategy may result in lower patient volumes (Chassin and Galvin, 1998) and does little to help doctors and nursing training requirements.

Q2. In what ways do hospital services provide for international patient health requirements both in Thailand and overseas?

Most of the Thai private hospitals sampled in this research have a wide and what appears to be an appropriate range of medical service provision. However, arrangements appear to be in place to meet the needs of most, but not all, of those potentially using the service. Nevertheless, this provision also varies between the hospitals - but hospital management appear to maintain competitive levels of provision and also management targets specific medical and clinical centres/units such as Children's centres or Teenage Psychology units (over 30 such

specialised centres/units available at most private hospitals in the sample) - e.g. www.bumrungrad.com. Hospital processes appeared to be developed very quickly and routinised to ensure appropriate linkages to new medical service provision that included the management of facilities, medication, staff and ancillary personnel; as well as ensuring patient accessibility and availability at the first point of contact. This may be better attenuated with the use of BPR (Gabel *et al*., 1999) and e-health developments (Bliemel and Hassanein, 2004) which is now becoming more common overseas. As hospital technology diversity and scope becomes more technical, hospital management appear to understand that training becomes an issue, not only for hospital technicians, but also for doctors and nurses. However, the future provision of dealing with nuclear or other hazardous medical materials waste may need to be assessed.

It would also appear that patients can expect to receive almost 5* hotel-like ancillary services which includes food prepared by leading Chef's and shopping services. Thus illustrating changes to managed care provision (Lesser and Brewster, 2000). Management further appear to understand the implications and issues involved in communicating more effectively with patients (Hargraves and Trude, 2002) and utilise all available media channels, using appropriate language and personal support mechanisms.

Q3. What are the reasons for the choices that private hospital management make to provide hospital services to medical tourists?

One of the main outcomes of the discussions with hospital management was that initially the private hospital targeted home-based patients and the development of marketing approaches overseas sought more involved assets management because private health expenditure had increased (Chee, 2007). This raised the issues of providing for a range of financial offerings - related to home credit-cards and banks was perceived as needing to be developed - especially for overseas patients as most payments were expected to be in cash. The capability, training and availability of experienced staff has made management focus attention on helping patients through an enhanced service-ethic encapsulating appropriate medical treatments where individuals become certificated following training and hospital protocols. This was especially for protocols requiring meticulous medical attention such as viral/bacterial infections, older long-term patients and children.

Investments in more enhanced personnel training and developments and the integration of technology and hospital processes appears to impact on hospital management as a pragmatic solution to patient requirements. This follows on from Hart, Shleifer and Vishny (1997), who indicated that since private health service providers have well-defined control rights, they have [a] strong incentive to invest in innovations. Such technical innovations and their positive impact on clinical outcomes have been reported elsewhere (Sox *et al*., 1989). Technology is therefore a way for hospital management to drive such innovativeness and together with a enhanced service-ethic and quality to lure new patients (Businessweek.com, 2005) it appears to be a successful and actively encouraging health business model for Thailand.

Finally, the major outcomes of this research that inform hospital policy can be seen in Table 2, below as characterised through the research questions and major themes.

5. Conclusions

Medical tourism has been identified by some researchers as potentially beneficial to the Thai economy and in line with this rhetoric has been an increase in private hospitals whose main focus is to accommodate middle-upper class Thai's and foreigners. However, medical tourism has not been clinically defined in terms of the economic impact as there is little data that directly links patients with medical tourist notions. Nevertheless, it would appear that a number of private hospitals have a management structure, facilities, personnel and the marketing acumen to adapt to changing medical demands located overseas.

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Table 1. Sub-theme observations

Major Theme	Sub-Themes						Total
Hospital Management	Reforms	11	Capacity	8	Emergency	4	23
Hospital Processes	Patient Data	14	Patient Support	12	Legal Issues	5	31
Hospital Technology	Medical Coverage	6	Costs	8	Tool	4	18
Quality Related	Accreditation	16	Auditing	11	QA	9	36
Communications	Co-ordination	7	Marketing Approaches	5			12
Personnel	Doctors	11	Nurses	9	Administration Staff	3	27
Financial	Costs	14	Revenue Cycle	7			21
Patients	Safety	8	Legal Issues	5	Medical Complication	3	16
Total		91		65		28	184

Table 2. Summary of Research Questions and Major Themes that Inform Hospital Policy

Research Question	Major Themes	Informing Hospital Policy
Q1. What is it that patients want from their international health service provider?	Hospital Management	Experienced core doctors and nurses
		Appropriate Range of Medical Service Provision
	Quality Related	Certificated to appropriate standards
	Hospital Technology	Upto date technology
	Finance	Range of financial offerings - related to home credit-cards and banks
Q2. In what ways do hospital services provide for international health requirements both in Thailand and overseas?	Hospital Processes	Appropriate Range of Medical Service Provision
	Patients	Patients receive comparative 5 * hotel services
	Communications	Utilise all available media channels, using appropriate language and personal support mechanisms
	Hospital Technology	Technology diversity and scope
Q3. What are the reasons for the choices that management make to provide hospital services to medical tourists?	Finance	Assets management
		Range of financial offerings - related to home credit-cards and banks
	Personnel	Capability, training and availability of experienced staff

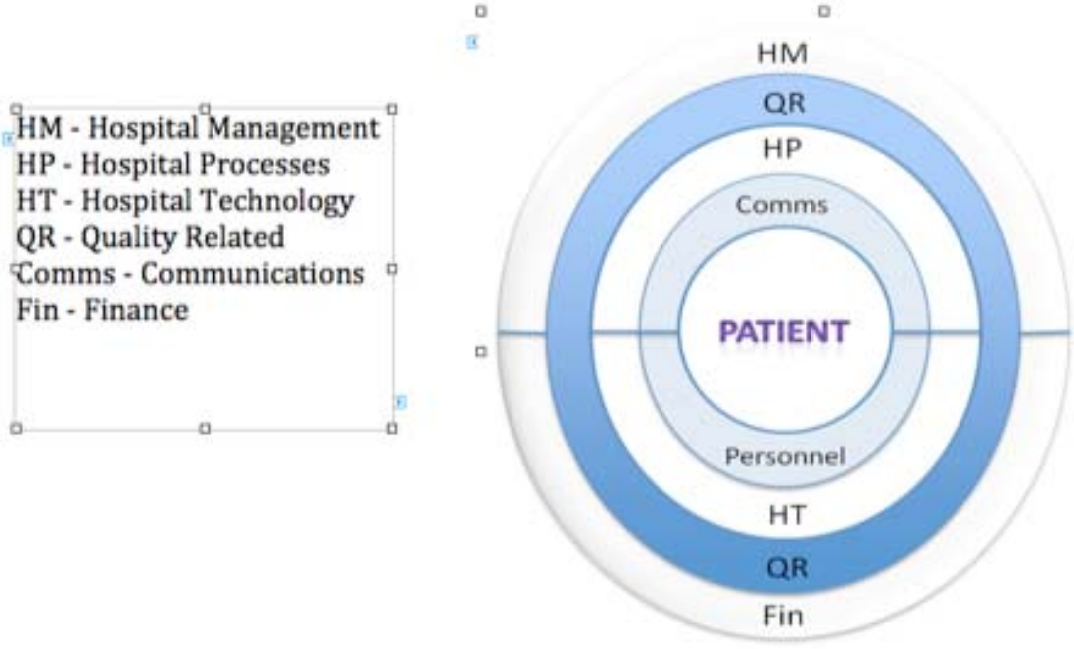

Figure 1. Model of Main Themes

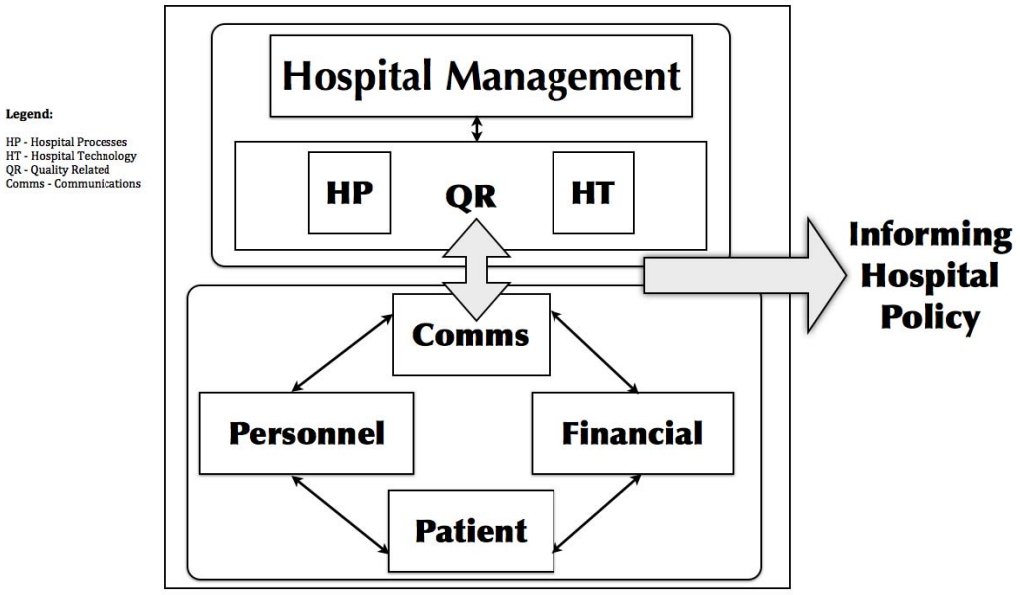

Figure 2. Model of Outcomes

Adhesive Capabilities of *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* Isolated from Tears of HIV/AIDS Patients to Soft Contact Lenses

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Abstract

Fifty conjunctival swab samples collected from ELISA confirmed HIV/AIDS seropositive patients who were referred to the HIV/AIDS laboratories of the University of Benin Teaching Hospital and Central Hospital both based in Benin City, Nigeria were aseptically cultured on appropriate media by standard methods. The resulting isolates/strains, after identification by standard methods, were tested for their ability to adhere to two hydrophobic non-ionic daily wear silicone hydrogel soft contact lenses (i.e. lotrafilcon B, WC 33% and polymacon, WC 38%) as well as to two hydrophilic ionic conventional extended wear silicone hydrogel soft contact lenses (i.e. methafilcon A, WC 55% and omafilcon A, WC 60%) by the adhesiveness/slime production modified vortex/Robin device method. Evidence of adhesiveness/slime production was indicated by presence of a visible stained film lining the surface of the contact lens which was measured and recorded as strong or weak according to the density of the adhered bacterial film. Fourteen (28.0%) *Staphylococcus aureus* strains and 10 (20.0%) *Pseudomonas aeruginosa* strains were obtained among other organisms. *Staphylococcus aureus* strains adhered in decreasing order to lotrafilcon B (55.4 ± 4.7), polymacon (46.4 ± 8.4), methfilcon A (46.4 ± 8.4) and omafilcon A (25.0 ± 6.4) with no significant difference in adhesive strengths of individual strains ($P > 0.05$). *Pseudomonas aeruginosa* strains also recorded decreasing adhesive strengths to lotrafilcon B (37.5 ± 8.2), polymacon (28.6 ± 6.3), methafilcon A (26.8 ± 5.5) and omafilcon A (23.2 ± 5.5) also with no significant difference in adhesive strengths of individual strains ($P > 0.05$). Attachment strengths of *Staph. aureus* strains to all four contact lenses were higher than those of *Pseudomonas aeruginosa* strains. Both organisms adhered most to hydrophobic lotrafilcon B and least to hydrophilic omafilcon A. This invitro adhesion studies revealed that daily wear silicone hydrogel low water content, non-ionic contact lenses are more prone to bacterial adhesion than the conventional extended wear hydrogel high water content, ionic contact lenses and hence, there is more risk of microbial adhesion to the former compared to the latter. Other implications are highlighted.

Keywords: HIV, Contact lenses, Adhesiveness*, Staph. aureus*, *Pseudo. aeruginosa,* Tears

1. Introduction

Contact lenses are the smallest, the least visible devices for correcting refractive error of the eyes. It is a shell-like, bowl shaped glass or plastic that rests on the eye (Mandel, 1981). Contact lens uses range from cosmetic to functional e.g. sports (Poster, 1972). Contact lens fitting is indicated in the management of severe ocular pathology, keratoconus and monocular aphakia although fitting in the presence of active pathology should never be undertaken. Studies have shown that 96% of patients fitted with contact lenses find them a complete success in terms of improved vision (Anon, 1990).

There are two categories of soft contact lenses and they are hydrophilic and hydrophobic types. Hydrophilic lenses allow the passage of water molecules, gas molecules being transported with the water molecules. Soft hydrophilic lenses are divided into those with low water content and those with high water content. Silicon

hydrophilic soft contact lenses are a new generation of supra permeable contact lenses that can transmit unprecedented amounts of oxygen to the cornea. It represents a breakthrough over traditional hydrogel soft contact lenses because silicon allows so much oxygen through the lens. Silicon hydrogel soft contact lenses are made from hydrogel polymers.

The conventional soft lenses are based on polyhydroxyl-ethylmetacrylate (P-HEMA). The lens materials are co-polymers of HEMA and other hydrophilic monomers such as N-vinyl pyrrolidine (NVP) and metacrylates that possess a wide range of water content. The water content is usually above 38% which contributes to the softness and comfort of these lenses. Less than 50% water content is considered to be low water content lenses and greater than 50% water are high water content lenses.

Material surfaces can be considered hydrophobic if the water contact angle is higher than 50^0. Lotrafilcon B (WC 33%) and polymacon (38%) are low water content hydrophobic silicon hydrogel contact lenses while methafilcon A (WC 55%) and omafilcon A (WC 60%) are high water content hydrophilic hydrogel lenses.

Studies have suggested that hydrophobic surfaces are more prone to pathogenic adhesion than hydrophilic ones. Silicon hydrogel contact lens is more prone to bacterial adhesion and this is attributed to the fact that silicon hydrogel lenses have a surface hydrophobicity higher than that of the conventional hydrogel lenses. Holden (2002) disproved this by stating that the adhesion of bacteria to silicon hydrogel contact lenses are as a result of the inherent property of the polymer or the unoxidized surface after treatment.

Cellular adhesion is the binding of a cell to another cell or to a surface or matrix. Bacterial adhesion is the process by which bacteria stick to the surface of host cells. Cellular adhesion is regulated by specific cell adhesion molecules that interact with other molecules. Pilli or fimbriae of gram negative bacteria such as *Pseudomonas aeruginosa* play an important role in adhesion to cell surface. Microorganisms are considered to play a role in the aetiology of certain corneal infiltrative events observed during soft contact lens wear (Padmaja *et al*., 2000). Buehler *et al*. (1992) reported that adhesion of bacteria notably *Staphylococcus* strains and *Pseudomonas aeruginosa* to contact lenses is considered a primary risk factor.

The mechanism used by bacteria to attach to the contact lens surface is poorly understood. Bacteria are thought to attach to a contact lens by interaction of the outer lipoprotein layer with the lens. Once placed in the eye, the contact lens undergoes a profound change in its surface properties. Species of bacteria are however, believed to bind specifically to the carbohydrate residues of this protein including *P. aeruginosa*, *Escherichia coli* etc. *P. aeruginosa* is known to secrete an anionic polysaccharide biofilm matrix on the lens surface in which the organisms are known to metabolize and reproduce. Borazjani *et al.* (2004) however, found no marked differences in the adhesion of *P. aeruginosa* to worn and unworn silicon hydrogel lenses thus suggesting that these lens surface properties were not affected by 6-7days extended wear and thus by the presence of tear film molecules.

Microbial contamination of the lens surface is the main problem associated with contact lenses wear. Although the estimated risk of the incidence of silicon hydrogel lens associated keratitis is one in 15,800 patients yearly, it is 30 times lower than for conventional hydrogel types (Lam *et al*., 2002; Lee *et al*., 2003). This was further elaborated in a study where the frequency of negative cultures was reported to be significantly greater during asymptomatic lens wear in comparison to symptomatic corneal infiltration. Conversely, the frequency of isolation of gram positive bacteria, gram negative bacteria and fungi was significantly greater in symptomatic corneal infiltration than asymptomatic lens wear suggesting that the use of contact lens can pose a threat in terms of bacterial invasion of the ocular tissue.

Factors that play important roles in the adhesion process of bacteria to contact lenses include: surface hydrophobicity/net surface charge, host receptor interaction and binding molecules present on the bacterial cells. Bacterial adherence to epithelial surface occurs due to molecular interactions between bacterial surface proteins and protein receptors on the cell surfaces. Surface hydrophobicity of the contact lens has been found to enhance bacterial adhesion. Bacterial host cells usually have net negative surface charge and therefore repulsive electrostatic forces.

Fujikawa *et al*. (1985) showed that when patients contract HIV, the virus can infect nearly every ocular tissue as well as the tears. The tear gland surface, like every other ocular surface is colonized by microbial agents which are mainly commensals such as *Staphylococcus epidermidis, Staphylococcus aureus, Corynebacterium* spp and *Propionibacterium acnes*. Bacteria commonly isolated from ocular infections include gram positive cocci, *Pseudomonas aeruginosa* among others (Shivitz, 1987). The ability of the bacteria to attach to the lens may depend on the type of lens material, immediate environmental conditions or the bacteria themselves.

Human T-cell viruses (HTLV-III) have been found in tears thus indicating the presence of free virus in tears (Fujikawa *et al*., 1986). Retroviral patients in their immune compromised state would have a wider range of bacterial organisms present in their eyes. These organisms include *Staphylococcus aureus* and *Pseudomonas aeruginosa* among others (Yasuyuki and Ben, 2005). *Staphylococcal* and *Pseudomonas* organisms are opportunistic pathogens in humans which infect the eyes through contaminated fingers/contact lenses.

Silicone-hydrogel soft contact lenses have been found to be more prone to bacterial adhesion than conventional hydrogel soft contact lenses and this is attributable to the hydrophobic nature of the lens. Laurent *et al*. (2002) reported that the extent of bacterial binding was found to range in increasing order from hydrogel to fluorine polyhydroxyl-ethylmetacrylate (PMMA), to hydrophilic acrylic, to heparinized PMMA, to silicone polymer contact lens types.

Mowrey-Mckee *et al*. (1992) carried out a study to determine the relative adhesion of bacteria to HEMA-type contact lenses and an extended wear silicon hydrogel contact lens of high oxygen permeability. They reported that adhesion of *P. aeruginosa* to a hydrogel contact lens does not appear to differ appreciably between the HEMA-type etafilcon and the high silicon hydrogel balafilcon A lens. The ability of *Staphylococcus. epidermidis* 9112 to adhere to the hydrophobic silicone hydrogel lotrafilcon A and balafilcon A was also greater than adhesion to the hydrophilic etafilcon A type thus establishing that hydrophobic silicone lenses are more prone to bacterial adhesion.

Pseudomonas aeruginosa has been shown to adhere strongly to contact lens of low water content than those of high water content. Hart *et al*. (1993) carried out an invitro quantitative study of the adhesion of a *Staphylococcus aureus* strain to two types of disposable contact lenses of ionic and non-ionic water content and reported that the ionic water content lenses were more prone to *Staphylococcus aureus* adhesion than the non-ionic water content lenses. It has been reported that daily wear of soft contact lenses significantly increased the binding of *Pseudomonas aeruginosa* to exfoliated epithelial cells and this binding is inversely proportional to the oxygen transmissibility of the contact lens (Butrus *et al*., 1997).

The effect of continuous wear on physico-chemical surface properties of silicone hydrogel lenses and their susceptibility to bacterial adhesion was studied of which volunteers were made to wear two pairs of either lotrafilcon A or balafilcon A contact lenses. The first pair was worn continuously for a week and the second pair for 4 weeks. One lens of each pair was used for surface characterization and the other one for bacterial adhesion experiments. Lens surfaces were characterized by examination of their wettability, roughness, elemental composition and proteins attached to their surfaces. Results showed that bacteria adhered in lower numbers and less tenaciously to worn lenses except *Staph. aureus* which adhered in higher numbers to worn balafilcon A lenses (Bos *et al*., 1999).

Robert *et al*. (2002) also carried out a study to determine if *Pseudomonas aeruginosa* has the ability to adhere preferentially to unused contact lenses made from different group polymers. They reported that the polymer material (used to construct the contact lenses) may influence subsequent bacterial adhesion and hence, concluded that contact lenses made from non-ionic polymers with low water content may carry higher risks of bacterial contamination.

HIV/AIDS is a disease condition that can affect every ocular tissue as the tear gland and hence, the tears. Contact lens wear by sufferers of this condition therefore may increase the ability of microbial cells to adhere to the cornea leading to keratitis especially if not properly handled during wearing and storage. Extended wear of contact lenses will therefore save sufferers the trouble of constantly removing and storing and this is why extended wear lenses are better than daily wear contact lenses. The risks associated however, with silicone hydrogel lenses on HIV/AIDS patients in terms of microbial contamination have not been fully investigated. This study is therefore aimed at determining the relative adhesion capacities of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from tears of HIV/AIDS patients to the recently manufactured commercially available hydrogel soft contact lenses with the following objectives: 1. Determine the measure of adhesiveness of *Staphylococcus aureus* strains to the selected hydrogel contact lenses. 2. Determine the measure of adhesiveness of *Pseudomonas aeruginosa* strains to the selected hydrogel contact lenses.

2. Materials and Methods

Eye (conjunctival) swabs were carefully collected from 50 ELISA test – confirmed seropositive HIV/AIDS patients who visited the University of Benin Teaching Hospital (UBTH) and Central Hospitals both based in Benin City, Nigeria. To obtain conjunctival swabs, the lower lids of the eye were lowered down gently and the palpebral conjunctiva/culdesac was swabbed with sterile swab sticks. Specimens were collected with informed

consent from the ethical committees of the hospitals involved through correspondences between the Optometry department, University of Benin and the hospitals.

Conjunctival swabs were aseptically cultured on sterile MacConKey agar, Blood agar and Mannitol Salt agar plates and incubated aerobically at 37^0C for 24hours. Pure isolates/strains were obtained and stocked on nutrient agar slants. Pure isolates were then identified culturally, morphologically, biochemically and by sugar fermentation tests according to schemes provided by Cowan and Steel (1993) and Cullimoore (2000).

All circular, white – yellowish, raised entire colonies, gram positive, coagulase positive, glucose positive and mannitol positive colonies/strains (characteristic of *Staphylococcus aureus*) and short gram negative rods in singles, citrate positive, oxidase positive, gray – greenish pigmented colonies/strains (characteristic of *Pseudomonas aeruginosa*) were used for further studies. The *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates and strains were then subjected to the adhesive capability and slime production assay.

3. Adhesive Capability/Slime Production Assay

The hydrogel lenses used in this study were daily wear silicone hydrogel, non – ionic lotrafilcon B (WC 33%), extended wear hydrophilic, ionic omafilcon A (WC 60%), daily wear hydrophobic silicone hydrogel, non-ionic polymacon (WC 38%) and hydrophilic daily wear ionic methafilcon A (WC 55%).

The adhesiveness/slime production assay method used was the modified Vortex/Robin device described by Bertoluzza *et al*. (2004). Each isolate/strain was subcultured aseptically (from their slant stock cultures) into sterile nutrient broth and incubated aerobically at 37^0C for 24 hours. Three milliliters (3ml) of turbid broth culture of each organism was inoculated into a set of 5ml tryptone soya broth in sterile test tubes already containing the different contact lenses listed. The mouths of all inoculated test tubes were bunsen flamed, sealed properly and incubated at 37^0C for 24 hours.

The content of each tube was carefully aspirated leaving the contact lens inside each tube. The contact lens (inside each tube) was then stained with safranin solution for 30 minutes. The contact lens was then taken out of each tube, placed with the convex side up on a blotting paper and then viewed under X 10 objective of a compound microscope.

Evidence of adhesiveness/slime production was indicated by presence of a visible stained film lining the surface of the contact lens and this was measured and recorded as weak or strong according to the density of the adhered bacterial film.

4. Data Analysis

Data obtained were analyzed using the statistical package for social scientists (SPSS) versions 16.0 and 17.0. One way analysis of variance and Duncan's multi-sample test was used to compare the adhesiveness of each contact lens type and for each strain. All analyses were performed at 95% confidence level.

5. Results

Out of the 50 samples processed, 14 (28.0%) *Staphylococcus aureus* strains and 10 (20.0%) *Pseudomonas aeruginosa* strains were obtained among other organisms. *Staph. aureus* and *Pseudomonas aeruginosa* were selected for the study due to their high occurrence in ocular infections and their apparently high resistance to most commonly used eye drops and drugs. *Pseudomonas aeruginosa* for instance, has been severally reported to thrive in commonly used disinfectants. Contact lens solutions also have disinfecting effects.

Staphylococcus aureus strains adhered in decreasing order to lotrafilcon B (55.36 ± 4.7), polymacon (46.4 ± 8.4), methafilcon A (46.4 ± 8.4) and omafilcon A (25.0 ± 6.4). There was no significant difference in the individual adhesion strength values for each strain to all four contact lenses sampled ($P > 0.05$) Table 1. Hence, by implication, *Staph.aureus* strains adhered most to lotrafilcon B contact lens and least to omafilcon A (Table 1). Whereas the attachment of the strains to lotrafilcon B was strong, that to omafilcon A was weak. Attachment strengths to polymacon and methafilcon however, were either weak or strong.

As in the case of *Staphylococcus aureus*, *Pseudomonas aeruginosa* strains recorded decreasing attachment strengths from lotrafilcon B (37.5 ± 8.2), polymacon (28.6 ± 6.3), methafilcon A (26.8 ± 5.5) and omafilcon A (23.2 ± 5.5). There was also no statistical significant difference in the individual strain attachment strengths to the four sampled lenses ($P > 0.05$) Table 2. The attachment strengths of *Pseudomonas aeruginosa* strains however to the sampled lenses were obviously much lower compared to those of *Staph. aureus* strains to the same lenses.

Whereas *Pseudomonas aeruginosa* strains attached most to lotrafilcon B, they attached least to omafilcon A. Attachment strength of *P. aeruginosa* strains to all sampled lenses was weak (Table 2). Both *Staph. aureus* and *P. aeruginosa* strains attached highest to lotrafilcon B and lowest to omafilcon A.

6. Discussion

Staphylococcus aureus and *Pseudomonas aeruginosa* were selected and used for this study because they are the most occurring isolates present in most ocular infections (Henriques *et al*., 2005). In a study carried out by Reichert and Stern (1984), *Staph. aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were found to adhere to corneal epithelium significantly.

Lotrafilcon B (non-ionic) lens having water content of 33% and polymacon (non-ionic) having water content of 38% represent hydrophobic daily wear silicone hydrogel soft contact lenses while omafilcon (ionic) and methafilcon A having water content of 60% and 55% respectively, represent hydrophilic conventional extended wear silicone hydrogel soft contact lenses. The incorporation of silicone into a hydrogel polymer gives the advantage of high oxygen transmissibility, but the disadvantage of decreased hydrophilicity (Tighe, 1999). To render the surface hydrophilic, techniques incorporating plasma into the surface of the lens have been developed.

The two daily wear silicone hydrogel lenses have a surface hydrophobicity higher than that of conventional extended wear silicone hydrogel soft contact lenses. These differences in surface hydrophobicity may explain the differences found in bacterial adhesion. Many studies have suggested that hydrophobic surfaces are more prone to pathogens adhesion than hydrophilic ones (Gomez-Suarez *et al*., 1999; Doyle, 2000). Beattie *et al*. (2003) studied *Acanthamoeba* attachment to a silicone hydrogel lens (balafilcon A) and conventional hydrogel contact lenses and concluded that balafilcon A is more prone to bacterial adhesion. They suggested that the high levels of attachment found in silicone hydrogel lenses may be the result of the inherent property of the polymer.

Our results showed that *Staph. aureus* strains recorded strong adhesion to both hydrophobic daily wear soft contact lenses (i.e. lotrafilcon B and polymacon) of 55.4 ± 4.7 and 46.4 ± 8.4 respectively. Conversely, adhesion strengths to the two hydrophilic conventional extended wear contact lenses (methafilcon A and omafilcon A) by *Staph. aureus* strains were 46.4 ± 8.4 and 25.0 ± 6.4 respectively and this was near weak on average.

The adhesiveness of individual strains to all four lenses was not significantly different from each other ($P > 0.05$). This report is similar to the finding of Grosvenor (2002) which stated that hydrophobic lenses cause higher adhesion because of the formation of biofilms with inherent properties of the polymer of the lens by a biofilm positive strain.

Lotrafilcon B has the lowest water content compared to the others. Omafilcon A has the highest water content. This suggests that contact lenses of low water content are more prone to bacterial adhesion. This explains the finding in this study in which *Staph. aureus* strains adhered greatest to lotrafilcon B followed by polymacon (both of which have low water content and are hydrophobic). The effect of low water content on bacterial adhesiveness to contact lenses was demonstrated by an invitro study carried out by Butrus *et al.* (1997) to determine increased *Pseudomonas aeruginosa* adhesion following five minutes air drying of etafilcon A soft contact lens and reported an increased bacterial adhesion afterwards. They concluded that soft contact lens recorded raised drying results in increased bacterial adhesion.

Pseudomonas aeruginosa strains, like *Staph. aureus* strains reported the highest adhesion strengths to lotrafilcon B followed by polymacon, methafilcon A and omafilcon A. Unlike *Staph. aureus*, the strains of *P. aeruginosa* recorded reduced adhesion strengths of 37.5 ± 8.2, 28.6 ± 6.3, 26.8 ± 5.5 and 23.2 ± 5.5 to lotrafilcon B, polymacon, methafilcon A and omafilcon A respectively. *Staph. aureus* strains and *P. aeruginosa* strains recorded low adhesion to the high water containing (hydrophilic) conventional extended wear lenses of methafilcon A and omafilcon A. This is supported by Grosvenor (2002) who stated that the gas permeability of a hydrogel lens increases exponentially with the water content thereby suggesting that hydrophilic contact lenses will provide better oxygen supply to the cornea and hence possess lower risk of bacterial adhesion This, somewhat differs from the finding of Willcox *et al.* (2001) who reported an increased capability of *P. aeruginosa* to adhere to silicone-hydrogel balafilcon A when compared with the adhesion to conventional hydrogels. Conversely, Borazjani *et al.* (2004) found no significant differences between the adhesion of *P. aeruginosa* to silicon-hydrogel balafilcon A and etafilcon A. These contradictory results may be the result of the different bacterial strains used as well as varying growth conditions employed.

Several authors have reported that the extent of *P. aeruginosa* adherence is strain-dependent and influenced by growth stage and media (Willcox *et al*., 2001; Thuruthyil *et al*., 2001; Bruinsma *et al*., 2002; Cowell *et al*., 1999).

However, studies have shown that deposits accumulation can increase with the length of wear of high water content disposable lenses (Maissa and Franklin, 1998).

In a study carried out to compare the adhesion patterns of three strains of *P. aeruginosa*, it was found that the number of adhered cells of *P. aeruginosa* to etafilcon A was significantly higher than that of *Staphylococcus epidermidis* thus re-enforcing the idea that the hydrophobic silicone lenses are more prone to bacterial adhesion.

Means of adhesion strengths of both organisms to all four contact lenses also showed decreasing adhesion levels from lotrafilcon B, polymacon, methafilcon A to omafilcon B although *Staph. aureus* strains clearly showed much greater adhesion strengths. Based on data obtained, it could be speculated that there are obvious risks associated with hydrophobic silicone hydrogel daily wear contact lenses as compared with the conventional extended wear types in terms of higher microbial adhesion.

This study may provide an indication of the likely transference of bacterial organisms from the wearer's fingers to the contact lenses surfaces. Borazjani *et al*. (2004) however, found no marked differences in the adhesion of *P. aeruginosa* to worn and unworn silicon-hydrogel lenses. Our study is however limited by a small sample size among others. A much larger sample size of contact lens users would show a clearer picture of the extend of bacterial adherence to contact lenses and its far reaching health implications.

7. Conclusion

Staphylococcus aureus strains adhered in decreasing order to lotrafilcon B (55.4 ± 4.7), polymacon (46.4 ± 8.4), methafilcon A (46.4 ± 8.4) and omafilcon (25.0 ± 6.4). Whereas the first two contact lens types are hydrophobic, the last two are hydrophilic. *Staph. aureus* strains therefore adhered most to the hydrophobic lenses. *Pseudomonas aeruginosa* strains also adhered (but with much lower adhesive strengths) in decreasing order to lotrafilcon B (37.5 ± 8.2), polymacon (28.6 ± 6.3), methafilcon A (26.8 ± 5.5) and omafilcon (23.2 ± 5.5).

Both *Staph. aureus* and *P. aeruginosa* strains attached highest to hydrophobic lotrafilcon B and lowest to hydrophilic omafilcon lens. Hence, *Staph. aureus* strains exhibited greater adhesion to daily wear hydrophobic, non-ionic silicone hydrogel lotrafilcon B with water content of 33% while the least adherence was to extended wear hydrophilic ionic silicone hydrogel omafilcon lens. *Pseudomonas aeruginosa* strains exhibited the same but with reduced adhesive strengths.

This invitro adhesion studies revealed that daily wear hydrogel low water content, non-ionic contact lenses are more prone to bacterial adhesion than the conventional extended wear hydrogel high water content, ionic contact lenses and hence, there is more risk of microbial adhesion with the former compared to the latter.

Patients presenting with ophthalmic manifestation of HIV/AIDS opportunistic infections should be discouraged from using hydrophobic ionic daily wear soft contact lenses as it may supply an inoculum of organism on prolonged contact with the cornea thus increasing the risk of cornea infiltration.

The use and advantages of conventional extended wear silicone hydrogel lenses over the daily wear types should be stressed and encouraged by practitioners in the field.

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Table 1. Measure of Adhesiveness of 14 strains of *Staphylococcus aureus* on the four sampled contact lenses

Strain No	LotrafilconB	Polymacon	Methafilcon A	Omafilcon A
	(WC33%)	(WC 38%)	(WC 55%)	(WC 60%)
	Daily wear	Daily wear	Conventional	Conventional
SA_1	50	50	25	50
SA_2	75	50	25	0
SA_3	50	50	50	25
SA_4	50	75	75	0
SA_5	75	25	50	25
SA_6	50	75	25	50
SA_7	75	25	50	0
SA_8	25	75	50	0
SA_9	25	50	50	25
SA_{10}	50	75	75	75
SA_{11}	75	75	50	50
SA_{12}	50	50	25	25
SA_{13}	50	25	50	25
SA_{14}	75	25	50	0
Mean ± S.E	55.4 ± 4.7	46.4 ± 8.4	46.4 ± 8.4	25.0 ± 6.4
P-value	P > 0.05	P > 0.05	P > 0.05	P > 0.05

SA = *Staphylococcus aureus,* 0 = No attachment, 25 = Weak attachment, 50 = Strong attachment, 75 = Very strong attachment (Bertoluzza *et al*., 2004)

Table 2. Measure of Adhesiveness of 10 strains of *Pseudomonas aeruginosa* on the four sampled contact lenses

Strain No	LotrafilconB	Polymacon	Methafilcon A	Omafilcon A
	(WC33%)	(WC 38%)	(WC 55%)	(WC 60%)
	Daily wear	Daily wear	Conventional	Conventional
PA_1	25	50	50	25
PA_2	75	25	50	50
PA_3	50	25	50	25
PA_4	75	25	25	50
PA_5	50	50	25	50
PA_6	25	75	25	25
PA_7	25	50	50	0
PA_8	75	25	50	25
PA_9	50	50	25	50
PA_{10}	75	25	25	25
Mean ± S.E	37.5 ± 8.2	28.6 ± 6.3	26.8 ± 5.5	23.2 ± 5.5
P-value	P > 0.05	P > 0.05	P > 0.05	P > 0.05

PA = *Pseudomonas aeruginosa,* 0 = No Attachment, 25 = Weak Attachment, 50 = Strong Attachment

75 = Very strong attachment (Bertoluzza *et al*., 2004)

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Bio-Activity of Natural Polymers from the Genus *Pistacia:* A Validated Model for Their Antimicrobial Action

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Abstract

The polymers from mastic gum of *Pistacia lentiscose* and subspecies of *Pistacia atlantica*, (sp. *kurdica, mutica* and *cabolica*) have been isolated and characterised by gel permeation chromatography (GPC) and ^{13}C NMR spectroscopy as *cis*-1,4-poly-β-myrcenes. They were screened against *Helicobacter pylori* and other Gram-negative and Gram-positive bacteria to evaluate their antimicrobial action. In order to further test their hypothesised mode of action, two polymer types were synthesized: one from myrcene, and four from polyvinyl alcohols of different molecular weights, derivatised with p-hydroxybenzoate. The anti-microbial activity of these polymers, evaluated through their 'kill' kinetics, was found to be related to their functional groups, their molecular weight and their solubility.

Keywords: *Pistacia Lentiscose*, Atlantica, *Kurdica*, *Mutica*, *Cabolica*, *Helicobacter Pylori*, Anti-Microbial, GEMANOVA

1. Introduction

The use of natural and synthetic organic polymers has become the mainstay of many familiar products due to their excellent electrical, mechanical, optical and thermal properties. Natural and synthetic polymers dominate important industries such as rubber, plastics, packaging, specialist coatings, adhesives, medical devices and pharmaceuticals. In pharmaceuticals, polymers are of interest as a means of drug delivery or as a drug itself, e.g., antimicrobials (Batz, Ringsdorf, Ritter, 1974).

The emergence of antibiotic resistance, where bacteria exhibit reduced susceptibility to antimicrobials by mechanisms such as altered drug uptake, drug targ*et al*ternation and/or drug inactivation, has become a major problem, particularly in hospitals (Cloete, 2003 & Smith, 2005). These mechanisms are undoubtedly significant factors to antibiotic failure in clinical medicine.

One particular problem is where medical devices are contaminated with bacteria growing as adherent biofilms, that is, device-related infections associated with artificial joints or venous catheters. Biofilms are also associated

with some other chronic infections, such as those occurring in the respiratory tract (Smith, 2005). To overcome these difficulties, the process of biofilm formation has been studied with a view to preventing or treating infections. (Kerr, Smith, Cowling, Hodgkiess, 2001). Biofilms are targets for "anti-fouling" systems and novel drug delivery technologies (Kerr, *et al.,* 2001). These include surface modification of devices to reduce bacterial attachment and incorporation of antimicrobials (anti-microbial co-polymers) to prevent bacterial colonization (Kalyon & Olgun, 2001). Electronic instruments have also been used either to release antimicrobials from device surfaces or to drive antimicrobials through the biofilm (Smith, 2005). Other technologies include delivery of antibiotics as aerosols to the lungs and formulations as liposomes and polymer-based vehicles (Smith, 2005). Many biodegradable polymer-based carrier systems have also been used, such as poly (lactide-co-glycolide) and thermoreversible hydrogels (Smith, 2005).

Mastic gum, a product of the *Pistachia* genus containing an unusually high molecular weight polymer, *cis*-1,4-poly-β-myrcene (Van den Berg, Van der Horst, Boon, Sudmeijer, 1998), Figure 1, has a long-standing reputation as a healing agent that has only recently been systematically examined for its antimicrobial activity. For example, it has been found to exhibit anti-plaque formation in dental studies, as well as to inhibit *Helicobacter pyrlori*, and other antimicrobial activity (Al-Habbal, Al-Habbal, Huwez, 1984; Ali-Shtayeh & Abu Ghdeib, 1999; Al-Said, Ageel, Parmar. Tariq, 1986; Ebrahimi, Sharifi, Hazell, Hibbert, 2008; Huwez & Al-Habbal, 1986, Maron, Bono, Leone, Bona, Carretto, Perversi, 2001; Paraschos, *et al.* 2007; Sakagami *et al.,* 2009; Sharifi & Hazell, 2009, 2011; Sharifi, Vagg, Hazell, 2001; Zhou *et al.,* 2009).

As a part of our investigation (Ebrahimi, *et al.,* 2008 & Sharifi *et al*., 2001, 2009, 2011), into the antimicrobial activity of whole gums and their fractions related to mastic, some high molecular weight polymers have been isolated from these naturally occurring gums. In particular, we describe their characterisation by Gel Permeation Chromatography (GPC), and by ^{13}C NMR spectroscopy, and relate their high molecular weight and functional group characteristics to their antimicrobial activity. By comparison of the 'kill' kinetics of the natural polymers, and with those of some high molecular weight synthetic polymers based on polyvinyl alcohol, we propose and validate a mode of action for this antimicrobial behaviour.

2. Experimental

Mastic gum was purchased from Sigma Aldrich (Sydney Australia), composite kurdica gum was collected from Kurdistan, Iran; composite mutica from Shiraz, Iran and composite cabolica from Cabool, Afghanistan. The polymeric fractions of the gums were isolated by dissolving the gums in dichloromethane and precipitating with methanol as described elsewhere (Van den Berg, K., 1998, Ebrahimi, *et al.,* 2008, Sharifi & Hazell, 2009). The collected polymer was then analysed by ^{13}C nuclear magnetic resonance (NMR) to confirm the structure. NMR spectra were obtained at UNSW using a Bruker DMX-500 operating at 500.13 and 125.75 MHz for ^{1}H and ^{13}C respectively, with a 5 mm triple inverse probe, from samples of the polymers dissolved in deuteriochloroform, $CDCl_3$, with the exception of cabolica, for which N,N-dimethylformamide-d_7, was used because of the poor solubility in $CDCl_3$. ^{13}C spectra were acquired with ^{1}H decoupling, and referenced to the central peak in $CDCl_3$, 77 ppm.

Molecular weights were determined using GPC comprised of a NSI-33R Milton Roy Minipump (Riviera Beach FL), a Rheodyne, Model 7010 injector with a 100-µl sample loop, a Gilson (Middleton, WI) 112 UV/Vis detector, and a Waters Associates–Millipore (Milton, MA) R401 Differential Refractometer that was coupled to a Kipp and Zonen recorder. A Viscotek T-Column 300mm L×7.8mm ID from Malvern Innovative Solution was connected between the injector and the detectors and the packing material was a porous styrene divinylbenzene copolymer. Flow rate was 2.0 mL/min and the chart recorder speed was 1 cm/min. Tetrahydrofuran, THF, was used as used as the eluent and all measurements were done at room temperature. UV spectra were measured at room temperature using a Varian Cary 50 and processed with the Cary WinUV software.

2.1 Synthesis of Polymyrcene

β-myrcene (4.74 g), cyclohexane (7.7 g), and sec-butyl lithium (400 µL) were incubated at 60 ºC for 3.5 h. Sharifi & Hazell, Newmark & Majumdar to yield *cis*-1,4-poly-β-myrcene (Figure. 1).

The poly-β-myrcene was collected for molecular weight determination by GPC, NMR analysis (Table 1) and antimicrobial activity as described below.

2.2 Synthesis of co-poly (vinyl-p-benzoate) (CPVPB) - A Novel Anti-microbial Polymer

Four polyvinyl alcohols, PVA's (see 2, Figure 2) with molecular weights ranging from 13,000-146,000 were chosen to synthesise novel soluble antimicrobial polymers, in order to test the hypothesis of the relationship between molecular weight of the polymer and anti-microbial activity. Methyl p-hydroxybenzoate (see 3, Figure 2)

was chosen to be incorporated into the structure of the polymer (see 4, Figure 2) on the basis of its use as a food preservative.

Two methods were used to incorporate the p-hydroxybenzoate through the ester linkage into the PVA's: either direct esterification, or transesterification with the methyl benzoate, 3. Both methods, together with the level of incorporation, influenced the structure of the polymer product, which in turn may have influence on the antimicrobial activity against a range of Gram-positive and Gram-negative bacteria. As the incorporation of p-hydroxybenzoate using transesterification was greater than from direct esterification, the product of transesterification was chosen for detailed studies.

2.2.1 Transesterification

12 g of polyvinyl alcohol was suspended in a solution of 400 mL dry dimethylformamide (DMF), 100 mL of dry benzene (used to remove small quantities of water through its azeotrope), 15 mL triethanolamine (catalyst) and 2 g of methyl p-hydroxybenzoate. The mixture was heated under reflux using a Dean and Stark apparatus, removing 125 mL of distillate, during which time the polymer was had dissolved. A further 10 g of methyl p-hydroxybenzoate was dissolved in 25 mL of dry DMF and added through the condenser and the heating at reflux continued. Approximately 150 mL of distillate was removed slowly over a period of 6 h and then the heating was continued for a further 15 h. The resulting solution was allowed to cool to room temperature, and a semi-transparent gel was produced.

The gel was broken up and suspended in 1.25 L of absolute alcohol. The mixture was stirred for 2 h until the DMF, triethanolamine and un-reacted methyl p-hydroxybenzoate had been leached from the product. The resulting polymer was filtered through Whatman grade 40/8μm (Sigma-Aldrich) and dissolved in 300 mL of milli-Q water, then was precipitated into 1.5 L of acetone and stirred to remove remaining contaminants. Any large polymer "lumps" were broken up with a glass rod, resulting in a white and stringy product. After filtration through Whatman grade 40/8 μm filter paper, the solid product was then re-suspended in 400 mL of absolute ethanol and stirred for 1h. This solid polymer was again filtered off through Whatman grade 40/8μm and then dissolved in 300 mL of distilled water.

The process of dissolution (water), precipitation (acetone), suspension (ethanol) with stirring, was repeated until no detectable Ultra Violet (UV) absorption characteristic of the p-hydroxybenzoate was detected in the supernatant ethanol.

The solid white polymer product CPVPB, 4, Figure 2, was then filtered off through Whatman grade 40/8μm and air-dried. These trans-esterification conditions were expected to result in a low level of incorporation of p-hydroxybenzoate into the polymer and thus the process returned approximately the same mass as the original polymer (12 g).

2.2.2 Purification of the CPVPB polymer

Any remaining low molecular weight impurities under MW 124,000 (including those from the solvents) were removed from the polymer by dialysis against water using high molecular weight cut-off dialysis tubing consisting of a cellulose membrane (DTCM), prior to antimicrobial trials. This was to ensure that any positive result is indeed due to activity of the polymer and not the product of any low molecular weight contaminants.

Some DTCM was softened in distilled water over night. One end of the tube was well sealed, product added and the other end sealed. The tube containing the product was then placed in a large measuring cylinder filled with distilled water, which was slowly stirred by a magnetic stirrer. Every half hour, small aliquots of the water were taken from the cylinder and UV absorption was measured.

No rapid increase in the UV absorbance was observed in the water soon after placing the tube into the cylinder. This was indicative of both the integrity of the tubing and the absence of a high level of contamination from low molecular weight compounds.

The dialysis water was changed several times until no UV absorption could be detected. The solution within the dialysis tube was removed and then dried for determining the level of incorporation of p-hydroxybenzoate by UV followed by antimicrobial trials. The above procedure was repeated for all four different molecular weight compounds.

2.2.3 Mole fraction of incorporated p-hydroxybenzoate

Methyl-p-hydroxybenzoate absorbs at 255 nm at neutral pH and shifts to 285 nm in basic conditions. The polymer-ester exhibited maximum absorbance at 257 nm in neutral aqueous conditions and 299.9 nm under basic conditions; slightly different from the methyl ester in neutral condition and markedly different under basic

condition.

As the molar absorption coefficient of this ester was not known, the mole fraction of p-hydroxybenzoate incorporation was determined by hydrolysing the p-hydroxybenzoate from the polymer in an alkaline medium and measuring the mole fraction of p-hydroxybenzoic acid (di-salt) in the hydrolysate (maximum absorption at 249 nm in neutral conditions and 280 nm under alkaline conditions).

A solution of the polymer in water (310 mg/L) had a maximum UV absorbance at 257 nm. When diluted with 0.1 M NaOH, the maximum shifted to 299.9 nm (theoretical absorbance maximum is at 300.5 nm). To liberate p-hydroxybenzoic acid (as its di-sodium salt), the alkaline solution was warmed to 70^{o}C for 5 min and then cooled to 5^{o}C. The hydrolysis was quantitative and the maximum absorbance at 280 nm was measured.

The hydrolysate (alkaline solution) was acidified with sufficient acid solution (0.2 M HCl) and its UV spectrum was taken at 249 nm to measure the concentration of p-hydroxybenzoic acid present in its protonated form.

Using the calculated molar extinction coefficient, the concentration of the p-hyroxybenzoic acid incorporated into polymer was calculated (Table 2).

2.3 Antimicrobial activity of the natural and synthetic polymers

2.3.1 MIC and MBC

The Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) values were determined against nine strains of *H. pylori*. All other Gram-positive and Gram-negative bacteria tested are listed in result section, for polymer fractions of the gums, for synthetic poly-myrcene and CPVPB, using a broth micro-dilution method Ebrahimi *et al* (2008), and Sharifi *et al* (2001, 2009, 2011).

2.3.2 *H. pylori*, strain 26695

The strain 26695 *H. pylori* was chosen for time-kill kinetic experiments as described previously Ebrahimi *et al* (2008), and Sharifi *et al* (2001, 2009, 2011). The experiments were performed with static liquid cultures containing Isosensitest broth (Oxoid) supplemented with 5% horse serum (Oxoid). The inoculum was harvested with Isosensitest broth from 36 h cultures grown on *Campylobacter* Selective Agar (CSA).

2.3.3 Gram-negative and Gram-positive bacteria

A 100 mL Iso-sensitest broth (Oxoid) culture was inoculated with a 10% inoculum from an 18 h overnight Gram-positive or Gram-negative bacterial culture. The culture was allowed to grow to stationary phase and that was determined by taking the Optical Density 600 (OD600) of the culture. The inoculums were then adjusted with the culture medium to give a starting bacteria concentration of 1.00 x 1.00 E + 08 Ebrahimi *et al* (2008), and Sharifi *et al* (2001, 2009, 2011).

Each culture was incubated for 2 h to allow recovery of the bacteria before the polymers, *cis*-1,4-β-polymyrcene and their partially oxidised forms, 4-hydroxybenzoic acid and CPVPB were added at their respective MIC and 5 × MIC concentrations. Control cultures at MIC and 5 × MIC containing appropriate solvent were also performed Ebrahimi *et al* (2008), and Sharifi *et al* (2001, 2009, 2011).

3. Results and Discussion

Isolation of the polymeric/high molecular weight fractions returned mass fractions of 13.8 % for the kurdica gum, 19.4% for mutica gum, 20.0% for cabolica gum and 35.2% for mastic gum. The number average molecular weight, weight average molecular weight and the distribution of molecular weight of these fractions as well as synthetic *cis*-1,4-poly-β-myrcene are shown in Table 1, which was obtained by GPC (Figures 3-7).

3.1 ^{13}C NMR Analysis

The ^{13}C NMR spectra of polymer fractions are displayed in the Figures 8-12, and show very close similarity in constituents between *P. lentiscus* (Figure 8), *P. a. kurdica* (Figure 9), *P. a. mutica* (Figure 10) and the synthetic *cis*-1,4-poly-β-myrcene (Figure 11). However, *P. a. cabolica*'s NMR spectrum (Figure 12) is totally different, not to mention its vastly different solubility, and is clearly indicative of different structure(s). Also, it has a much lower anti-microbial activity than polymeric fractions of other gums. The NMR spectrum of mastic gum is consistent with literature Van den Berg *et al* (1998) and has been proven to be that of *cis*-1,4-poly-β-myrcene (Figure. 1). The chemical shifts of the relevant major peaks in the spectra of polymer fractions of the gums and synthetic polymyrcene listed in Table 2, and excluding cabolica gum, are assigned to *cis*-1,4-poly-β-myrcene. These chemical shifts (Table 3) are entirely consistent with the literature values Van den Berg *et al* (1998).

3.2 Aerial Oxidation and Mastication

The susceptibility of polymyrcene to aerial oxidation was established by testing with 2,4-dinitrophenylhydrazine (2,4-DNP), to detect the presence of aldehydes and ketones, as would be expected from oxidation of the double bonds in the polymer. Thus, while no colour change was observed when the un-aerated polymer was tested with 2,4-DNP, a yellow precipitate formed with the aerated polymer sample. Likewise, a yellow precipitate was also formed when a sample of masticated gum was treated with 2,4-DNP, but no change was observed when un-masticated gums were treated with 2,4-DNP, indicating that the mastication process resulted in some level of oxidation. Lastly, the behaviour of the synthetic *cis*-1,4-poly-β-myrcene under aerobic conditions also tested positive to 2,4-DNP, forming a yellow precipitate, while the un-aerated one did not, indicating oxidation of the double bonds in the polymer.

The MIC of polymer ranged from 250 to 500 μg/mL. Upon oxidation of this fraction, its MICs shifted to a range of 125 to 250 μg/mL.

3.3 UV Spectroscopy

The incorporation of p-hydroxybenzoic acid into co-poly (vinyl-p-benzoate) for MW of 13000-23000, 31000-50000, 50000-85000 and 85000-146000 were measured are shown in Table 1.

3.4 MIC and Kill kinetics

The MIC results have been tabulated in Tables 4, 5 and 6 for polymers obtained from the gums, synthetic *cis*-1,4-poly-β-myrcene, the four co-poly(vinyl-p-benzoate) and 4-hydroxbenzoic acid against the strains of *H. pylori* listed in Table 4. Activities against Gram-positive and Gram-negative bacteria are listed in Table 5 and 6.

3.5 Kill kinetics

We have reported a Generalized Multiplicative Analysis Of Variance (GEMANOVA) approach to model the complex kill kinetics data Ebrahimi *et al* (2008). The variables studied were: antibacterial agent (*β-myrcene, mastic, kurdica*, *mutica* and *cabolica,* that were labelled by Bm, S, R, T and B due to a pending patent), structure (monomer, polymer), oxidation form (oxidized, non-oxidized), concentration (1 and 5 × MIC) and bacteria (*Escherichia coli*, *Helicobacter Pylori* and *Staphylococcus aureus*). The model obtained from the aforementioned data (shown in results section) revealed the followings:

(a) The polymer fraction of *cabolica*, *kurdica* and *mutica* kill all three genera of bacteria at a concentration of 5 × MIC. Their oxidised form does not have an effect on their activity.

(b) Bacteria *E. coli* and *H. pylori* are more susceptible to the polymer form and at the higher concentration than the monomer and low concentration. *Kurdica* and *β-myrcene* are the most and least effective substances respectively for these two bacteria. Sensitivity is not affected by the oxidised form of the antibacterial substances.

(c) The monomers exhibit the same activity on the three bacteria studied. The oxidised form does not impact on the effectiveness of monomer form of antibacterial substances and greater activity is observed in higher concentrations (5xMIC).

It was demonstrated that the mass fractions of the polymers within the gums and their average molecular weight (Mn)s showed values ranging from 13.8-35.2% and 682-221823 respectively (Table 2). NMR Spectra (Figure 3-6) are indicative of very similar chemical structures with respect to the natural occurring and synthesised polymers, and, apart from cabolica gum, which requires further investigations, the polymeric fractions of the gums could be readily assigned to *cis*-1,4-poly-β-myrcene.

The MIC values and the 'kill' kinetics data of the polymers (naturally occurring *cis*-1,4-poly-β-myrcene, β-myrcene, and synthetic *cis*-1,4-β-poly myrcene, 4-hydrobenzoic acid and CPVPB and their antimicrobial relationship were statistically investigate (Ebrahimi, *et al.,* 2008).

The antimicrobial activities of the polymeric fractions of the gum suggest that β-myrcene, the monomer of these polymers, may also have antimicrobial activity (Ebrahimi, *et al.,* 2008). However, while knowing the structure, the question arises as to what are the active sites of these polymeric fractions and why they are more active against Gram-negative than Gram-positive bacteria, despite of the fact that the monomer has the same MIC values for Gram-positive and Gram-negative bacteria.

One of the activating factors is oxidation or mastication, as a result of which, aldehydes and ketones may be formed. Strong supporting evidence for this comes from testing the partially oxidised polymyrcene with 2,4-DNP to give a yellow precipitate i.e. a positive test. Thus oxidative activation leads to the formation of

aldehydes and ketones as shown schematically in Figure 13, by oxidative cleavage of the double bond between carbons 7 and 8. The polymeric backbone will then carry the reactive aldehyde groups able to interfere with the bacterial surface, possibly augmented by the released acetone.

The antimicrobial activities of aldehydes and ketones are well known (Melrose & Keleppe, 1998). The broad-based antimicrobial properties of a polymer having the repeating polymeric unit of an aldehyde (Figure 14) forms the basis of a recent patent (Melrose & Keleppe, 1998), active against *H. pylori* (Melrose & Keleppe, 1998).

4. Conclusions

It has been shown that poly-myrcene as part of mastic, kurdica and mutica gum and it is masticated in use, a practice consistent with its physical form and properties which suggests a possible *in situ* oxidation of the gums, taking place in therapeutic use. The concept of polymer being "activated" through a process of oxidation generating reactive aldehyde groups, is consistent with our understanding of the mechanism of action of synthetic poly-myrcene (before and after oxidation), a model which was designed to validate the suggested mode of the action. These results are consistent with the concept that the antibacterial activity of the components of gums increase as their polarity increases, and in particular as a consequence of the presence of aldehyde, ketone and/or carboxylic acid groups within their structures, the level of these groups potentially increasing as a consequence of oxidation *in situ*. They are also consistent with higher activity being demonstrated by higher molecular weight polymer, in which CPVPB was synthesized to validate this hypothesis.

The MIC data of these polymeric compounds were not consistent with contemporary antibiotics but were rather more consistent with topical disinfectant-like compounds. This work has provided insights into natural mechanisms for activating polymeric components found in the gums of *Pistacia* sp., for increasing antimicrobial activity as well as processes for incorporating active functional groups into polymers.

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Table 1. Polymeric fractions of the *Pistacia* gums and synthetic polymyrcene analysed by GPC

Source of Gum	High Molecular Weight Fraction(Polymer) %	Whole Gum (Total weight) %	Number average molecular weight (Mn)	Weight average molecular weight (Mw)	Distribution of molecular weight
P. a. kurdica (kurdica gum)	13.8	100	1023	11876	250-55000
P. a. mutica (mutica gum)	19.4	100	906	3584	250-50000
P. a. cabolica (cabolica gum)	20.0	100	682	3611	250-30000
P. lentiscus (mastic gum)	35.2	100	20000	80000	50000-130000
cis-1,4-poly-β-myrcene	N/A	N/A	221823	405703	50000-500000

Table 2. Mole fractions of p-hydroxybenzoic acid incorporated into the polymer

Molecular weight	**Mole fraction p-hydroxybenzoate (%)**
13000-23000	0.15
31000-50000	0.14
50000-85000	0.13
85000-146000	0.12

Table 3. Chemical shifts of the relevant peaks in the 13C NMR spectra of the polymer fractions of the gums and synthetic polymyrcene in CDCl3

	Synthetic Polymyrcene		**Mastic gum**		**Kurdica gum**	**Mutica gum**
Assignment	Chemical Shift		Chemical Shift		Chemical Shift	Chemical Shift
C1	30.73	30.56[a]	30.90	31.50[a]	30.90	30.93
C2	138.91	138.86[a]	38.92	139.50[a]	38.90	39.00
C3	124.62	124.52[a]	124.81	125.00[a]	124.78	124.70
C4	27.00	26.74[a]	27.96	28.10[a]	27.76	27.93
C5	37.73	36.85[a]	37.85	38.10[a]	37.63	37.90
C6	27.04	26.92[a]	27.63	27.80[a]	27.60	27.80
C7	124.37	124.36[a]	125.00	125.00[a]	125.05	125.10
C8	131.23	131.01[a]	130.93	131.80[a]	130.80	131.00
C9	17.57	17.64[a]	18.78	18.80[a]	18.70	19.01
C10	26.59	25.62[a]	26.50	26.70[a]	26.50	27.03

a Literature values from reference (6, 20).

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Table 4. MIC values of partially oxidized polymers from cis-1,4-β-polymyrcene, from Pistacia gums and polymers of CPVPB activity against strains of H. pylori (μg/mL)

Bacterial Strains	β-myrcene/ Oxidised	Mastic Polymer/ Oxidised Polymer	Kurdica Polymer/ Oxidised Polymer	Mutica Polymer/ Oxidised Polymer	Cabolica Polymer/ Oxidised Polymer	Polymyrcene / Oxidised Polymyrcene	4-hydroxy-benzoic acid	CPVPB MW 13000-23000	CPVPB MW 23000-50000	CPVPB MW 50000-85000	CPVPB MW 85000-146000
26695	1000/ 1000	200/100	500/250	500/250	1000/ 1000	100/50	2000	1000	750	500	500
J99	1000/ 1000	250/100	250/125	250/125	1000/ 1000	100/50	2000	1000	750	500	500
RSB6	1000/ 1000	250/100	500/250	1000/500	1000/ 1000	100/50	2000	1000	750	500	500
P10	1000/ 1000	200/100	500/250	500/250	1000/ 1000	100/50	2000	1000	750	500	500
SS1	1000/ 1000	250/100	250/ 200	1000/500	1000/ 1000	100/50	2000	1000	750	500	500
SS2000	1000/ 1000	250/100	500/200	1000/500	1000/ 1000	100/50	2000	1000	750	500	500
N6	1000/ 1000	250/100	500/250	250/125	1000/ 1000	100/50	2000	1000	750	500	500
NCTC 11637	1000/ 1000	250/100	500/250	250/125	1000/ 1000	100/50	2000	1000	750	500	500
RU1	1000/ 1000	250/100	500/250	250/125	1000/ 1000	100/50	2000	1000	750	500	500

Table 5. The MIC values of partially oxidized polymers from cis-1, 4-poly-β-myrcene, from Pistacia gums and polymers of CPVPB activity against gram negative bacteria (µg/mL)

Gram negative bacteria	β-myrcene/ Oxidised	Mastic Polymer/ Oxidised Polymer	Kurdica Polymer/ Oxidised Polymer	Mutica Polymer/ Oxidised Polymer	cabolica Polymer/ Oxidised Polymer	Polymyrcene/ Oxidised Polymyrcene	4-hydroxy-benzoic acid	CPVP B MW 13000-23000	CPVP B MW 23000-50000	CPVP B MW 50000-85000	CPVP B MW 85000-146000
Escherichia coli type 1	1000/ 1000	250/ 100	400/250	500/ 250	1000/ 1000	100/75	2000	1000	750	500	500
Salmonella typhimurium	1000/ 1000	250/ 100	500/200	250/ 125	1000/ 1000	100/75	2000	1000	750	500	500
Serratia marscens	1000/ 1000	250/ 100	500/400	1000/ 500	1000/ 1000	100/75	2000	1000	750	500	500
Pseudomonas aeruginosa	1000/ 1000	250/ 100	500/250	500/ 250	1000/ 1000	100/75	2000	1000	750	500	500
Alcaligenes faecalis	1000/ 1000	250/ 100	500/300	1000/ 500	1000/ 1000	100/75	2000	1000	750	500	500
Enterobacter aerogenes	1000/ 1000	250/ 100	500/200	1000/ 500	1000/ 1000	100/75	2000	1000	750	500	500
Pseudomonas fluorescens	1000/ 1000	250/ 100	250150	250/ 125	1000/ 1000	100/75	2000	1000	750	500	500
Proteus vulgaris	1000/ 1000	250/ 100	250125	250/ 125	1000/ 1000	100/75	2000	1000	750	500	500
Porphyromonas gingivalis	1000/ 1000	200/ 100	200/125	250/ 125	1000/ 1000	100/75	2000	1000	750	500	500

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Table 6. The MIC values of partially oxidized polymers from cis-1,4-poly-β-myrcene, from Pistacia gums and polymers of CPVPB activity against gram positive bacteria (µg/mL)

Gram positive bacteria	β-myrcene/ Oxidised	Mastic Polymer/ Oxidise Polymer	Kurdica Polymer/ Oxidise Polymer	Mutica Polymer/ Oxidise Polymer	Cabolica Polymer/ Oxidise Polymer	Polymyrcene/ Oxidised Polymyrcene	4-hydroxy-benzoic acid	CPVPB MW 13000-23000	CPVPB MW 23000-50000	CPVPB MW 50000-85000	CPVPB MW 85000-146000
Bacillus cereus	1000/ 1000	1000/ 1000	500/ 250	500/250	1000/ 1000	1000/ 1000	2000	1000	750	500	500
Staphylococcus aureus	1000/ 1000	1000/ 1000	500/ 250	500/250	1000/ 1000	1000/ 1000	2000	1000	750	500	500
Streptococcus faecalis	1000/ 1000	1000/ 1000	500/ 200	500/200	1000/ 1000	1000/ 1000	2000	1000	750	500	500
Staphylococcus epidermidis	1000/ 1000	1000/ 1000	500/ 200	500/200	1000/ 1000	1000/ 1000	2000	1000	750	500	500
Bacillus subtilis	1000/ 1000	1000/ 1000	500/ 200	500/200	1000/ 1000	1000/ 1000	2000	1000	750	500	500
Corynebacterium sp.	1000/ 1000	1000/ 1000	500/ 200	500/200	1000/ 1000	1000/ 1000	2000	1000	750	500	500

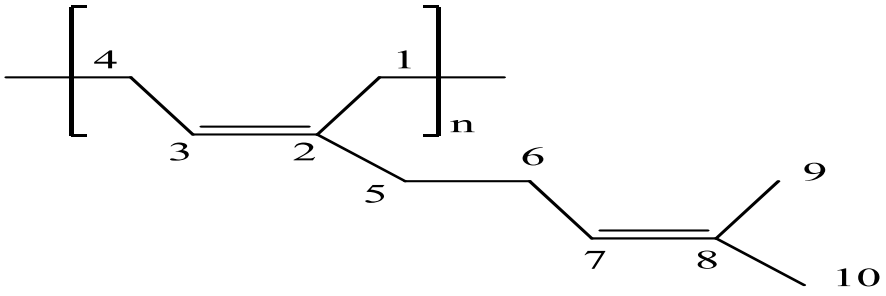

Figure 1. *Cis*-1,4-poly-β-myrcene (polymyrcene)

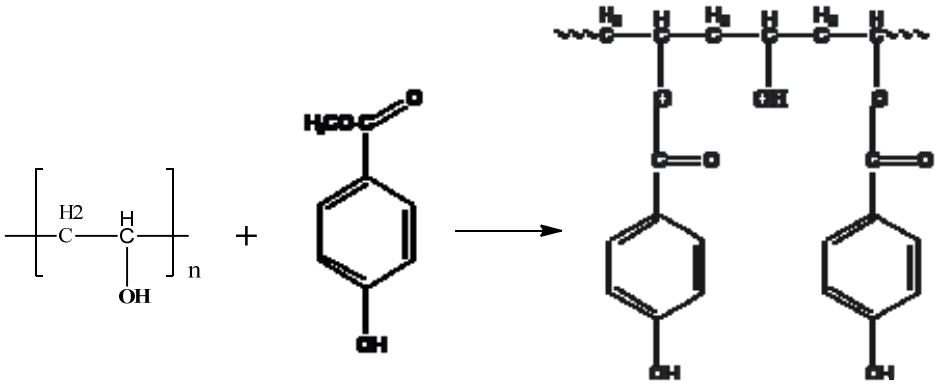

Figure 2. Reaction of Polyvinyl Alcohol, 2, with Methyl p-hydroxybenzoate, 3, to give Co-poly (vinyl-p-benzoate) (CPVPB), 4

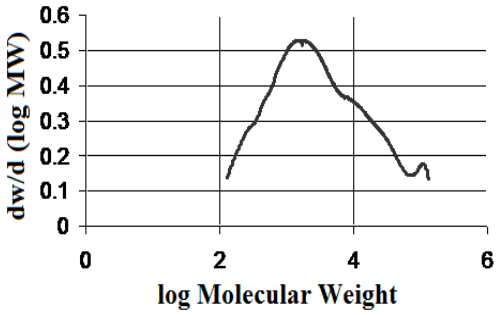

Figure 3. Molecular weight distribution of polymer from "kurdica gum"(Mn 1023, Mw 11876)

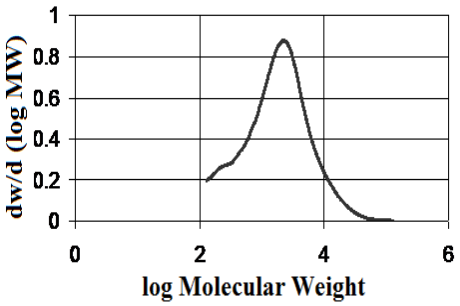

Figure 4. Molecular weight distribution of the polymer from "mutica gum"(Mn 906, Mw 3584)

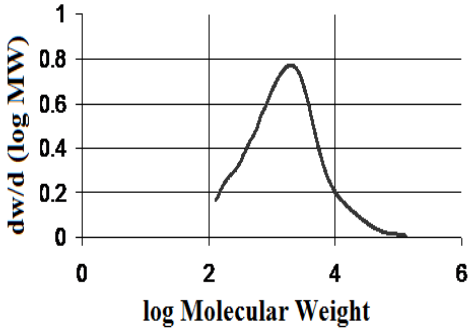

Figure 5. Molecular weight distribution of the POLYMER from "cabolica gum"(Mn 682, Mw 3611)

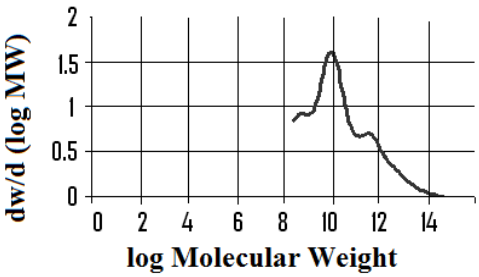

Figure 6. Molecular weight distribution of the polymer fraction from "mastic gum" (Mn 80,000, Mw above 120,000)

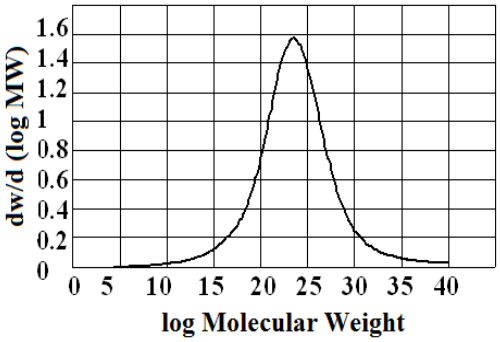

Figure 7. Molecular weight distribution of synthetic polymyrcene (Mn 221823, Mw 405703)

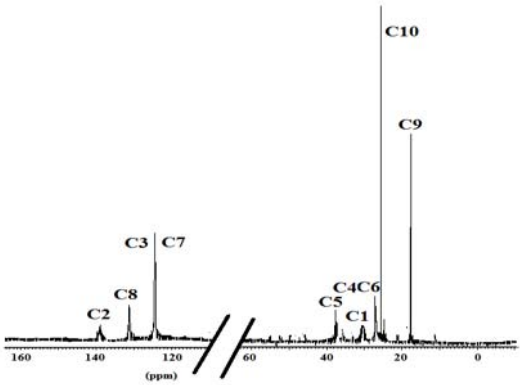

Figure 8. 13C NMR spectrum (CDCl3 at 298 K, DMX 500) of the polymer from Mastic gum

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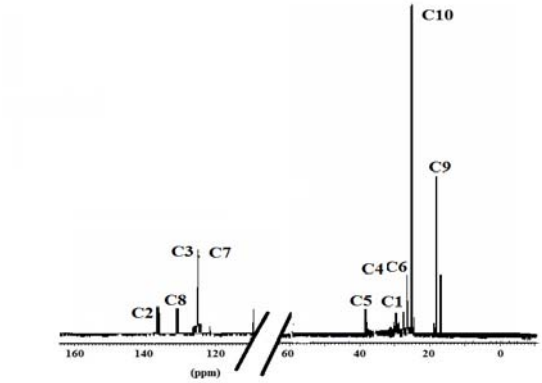

Figure 9. 13C NMR spectrum (CDCl3 at 298 K, DMX 500) of the polymer from kurdica gum

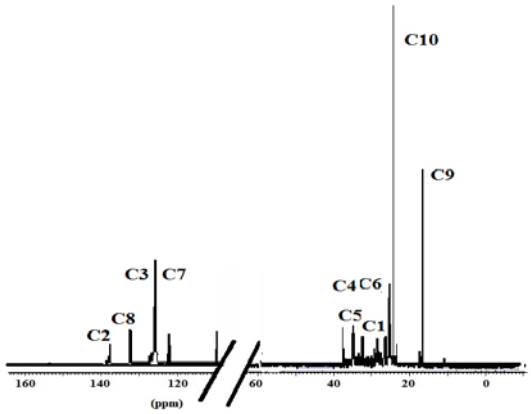

Figure 10. 13C NMR spectrum (CDCl3 at 298 K, DMX 500) of the polymer from mutica gum

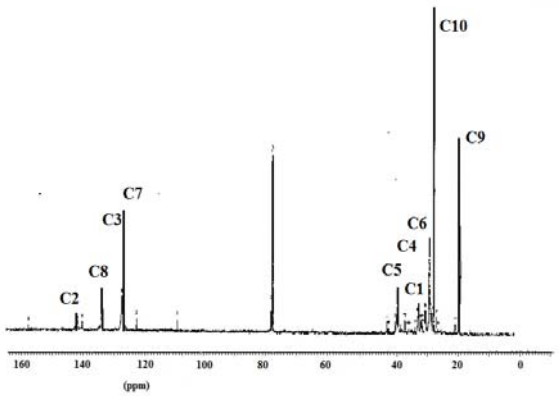

Figure 11. 13C NMR spectrum (CDCl3 at 298 K, DMX 500) of the synthetic cis-1,4-β-polymyrcene

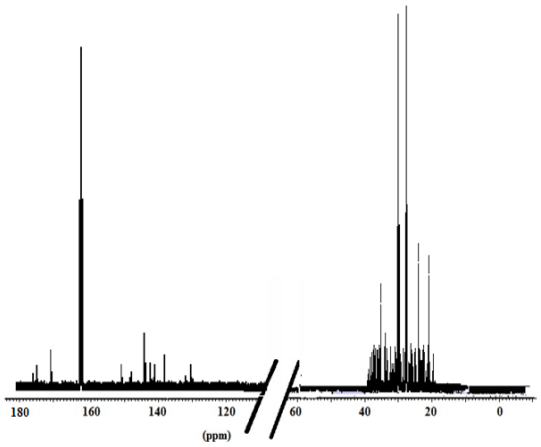

Figure 12. 13C NMR spectrum (DMF-d7 at 298 K, DMX 500) of the cabolica gum

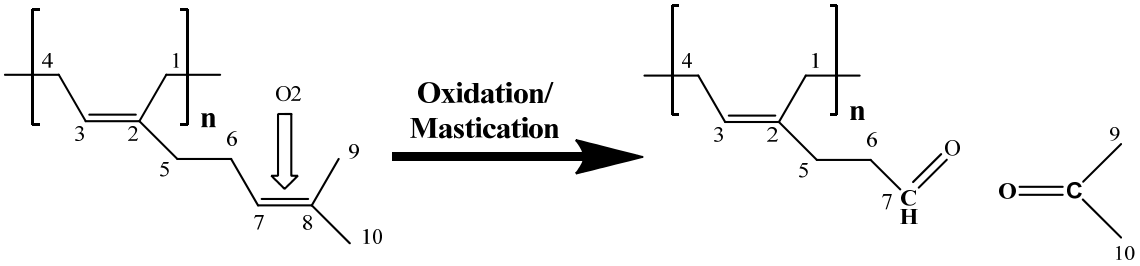

Figure 13. Oxidation and mastication of polymeric fraction

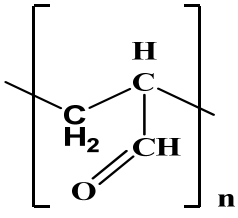

Figure 14. Bioactive polymer with the aldehydic functional group active against H. pylori

Uses of 2-Ethoxy-4(3*H*) quinazolinone in Synthesis of Quinazoline and Quinazolinone Derivatives of Antimicrobial Activity: The Solvent Effect

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Abstract: 2- Ethoxy-4(3*H*) quinazolinone 1 was synthesized and allowed to react with various halides, namely: alkyl, benzyl, allyl, acyl, haloacetyl, crotonyl, benzoyl, 2-furoyl and 1-naphthalenesulphonyl halides affording quinazoline and quinazolinone derivatives. The reactions of compound 1 with phosphorus oxychloride, phosphorus pentasulfide, ethyl chloroformate, ethyl chloroacetate, α-bromoglucose tetraacetate, *p*-acylaminobenzenesulfonyl chloride, acrylonitrile, chalcone and chalcone oxide were also investigated. Depending on the reaction condition and reactant halide, the type of substituent (alkyl, acyl, aroyl, etc.) that will reside on either of the expected positions (3 or 4) on the quinazoline moiety can control the reaction pathway for synthesis of the promising products. The significant role of solvent responsible for determining both the reaction pathway and type of products synthesized was also discussed. Some derivatives were chosen for biological screening test against Gram (-ive) and Gram (+ive) bacteria and two strains of fungi.

Keywords: Quinazolinone, Quinazoline, Tautomerisation, Nucleosides, Chalcone

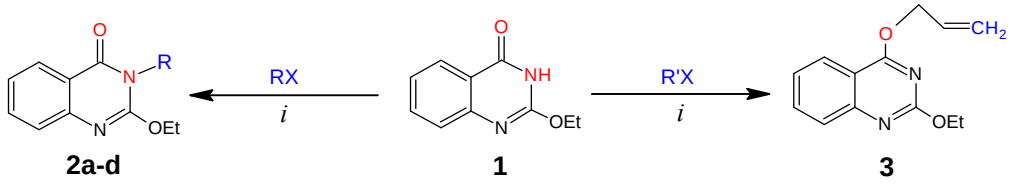
1. Introduction

In recent years there has been an increasing interest in the chemistry of 4(3*H*)-quinazolinones because of their biological importance. Many of them show antifungal, antibacterial, anticancer, anti-inflammatory, anticonvulsant, immunotropic, hypolipidemic, antitumor, antiulcer, analgesic, antiproliferative activities and inhibitory effects for thymidylate synthase and poly (ADP-ribose) polymerase (PARP) [1-13]. The 4(3*H*)-quinazolinones can act as semicyclic amides or iminols, due to the tautomeric phenomenon they have. Their reactions in either form with alkyl or acyl halides are perhaps the most interesting due to the large number of heterocycles that are obtained either directly or through further transformations of the initially formed products.

2. Results and Disscussion

By the solvent free reaction of 2-ethoxy (4*H*)-3, 1-benzoxazin-4-one with ammonium acetate 2-ethoxy (4*H*)-3,1-quinazolin-4-one 1 was synthesized to be used as the starting material. In this paper we report its behavior towards various types of organic halides, with the aim of obtaining more precise information about the course of reaction and bearing into consideration the effects of changing the reaction conditions (dry solvent, solvent free, base, etc.) on the reaction pathway to obtain the promising products. Therefore the interaction of compound 1 with alkyl halides such as methyl iodide, ethyl iodide, benzyl chloride and ethyl chloroacetate, carried out in dry acetone and anhydrous potassium carbonate gave the corresponding *N*-substituted quinazolinones 2a-d (Scheme 1), respectively. The IR spectra of products 2a-d showed absorption bands in the range 1672-1791 attributable to υ_{max} of C=O groups. But when the reaction was carried out using allyl bromide the *O*-substituted quinazoline 3 was obtained, confirming the allylic effect caused by the unsaturated moiety adjacent to the carbon atom bearing the halide. In addition, the IR spectrum of product 3 showed an absorption band at 1264 attributable to υ_{max} of (C-O-C) and no any band for the C=O group.

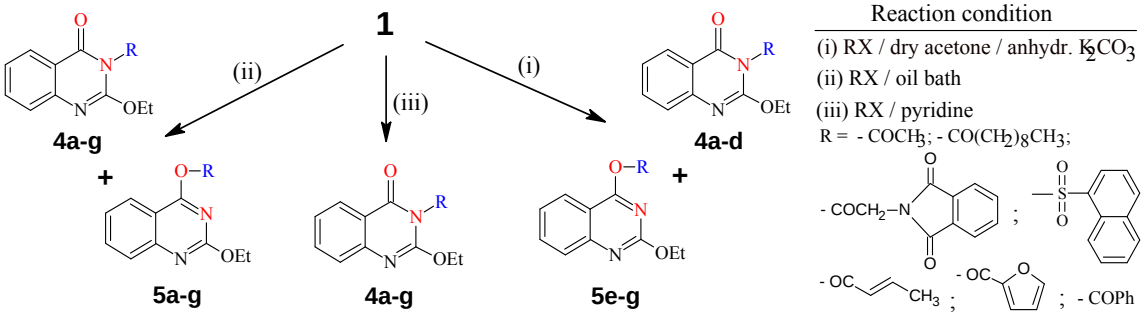
Scheme 1 : synthetic pathway for compounds **2** and **3**



(a) RX : CH_3; CH_3CH_2I; $PhCH_2I$; $ClCH_2COOEt$ (b) R'X : CH_2=CH - CH_2Br (c) i : dry acetone / anhydrous K_2CO_3

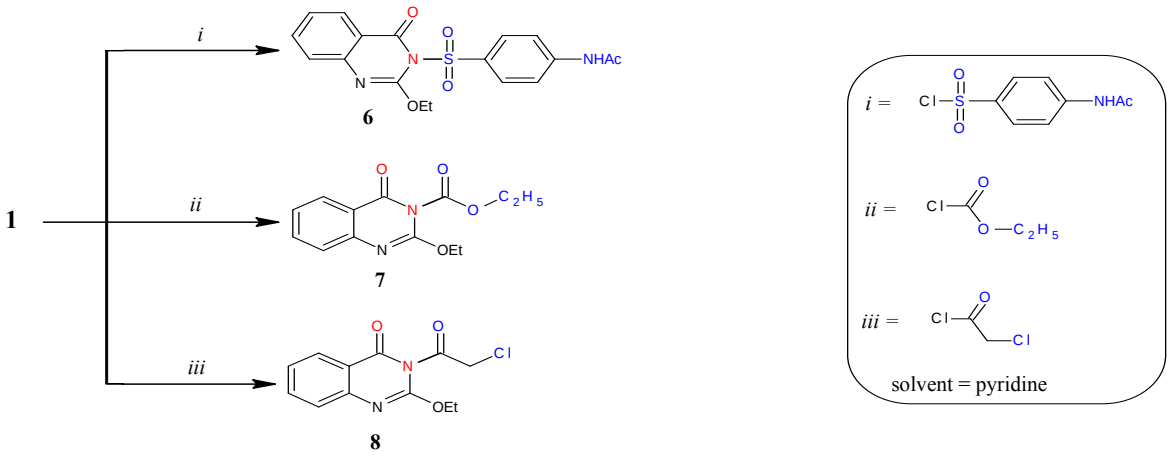
Compound 1 was reacted with acid chlorides, namely: acetyl, *n*-decanoyl, phthalimidoacetyl and 1-naphthalene sulfonyl chlorides in dry acetone / anhydrous K_2CO_3 affording the 3-substituted 4a-d products, respectively, and with *α,β*-unsaturated or aromatic halides namely: crotonyl, furoyl and benzoyl chlorides affording the *O*-substituted derivatives 5e-g respectively. But when the reactions were done using oil bath, both the *O*-substituted 5a-g and *N*-substituted 4a-g, with lower yields of the latter. The IR spectra for 4a-d showed two groups of absorption bands in the ranges 1671-1691 and 1714-1738, which are attributable to the endocyclic and exocyclic carbonyl groups of quinazolinone derivatives, respectively, whereas the IR spectra of 5e-g showed absorption bands in the range 1763-1769 attributable to C=O groups of the ester. This can be a good proof in explaining why compound 1 when reacted, in dry acetone, with acid chlorides having the carbonyl groups directly linked to an aliphatic moiety (e. g. acetyl, decanoyl, phthalimidoacetyl) the amide form of quinazolinone enhanced the formation of the *N*-substituted quinazolinones, whereas its reactions with the *α, β*-unsaturated or aromatic acid chlorides (e. g. crotonyl, furoyl and benzoyl chlorides) allowed the imidol form to predominate enhancing the formation of *O*- substituted quinazolines. Moreover, this behavior was opposed under solvent free condition. However, in dry pyridine only products 4a-g were obtained with the formation of the more favorable pyridinium chloride. This confirmed that the more basic pyridine, compared with the only polar acetone, preferred to interact with the slightly more acidic amide form affording the pyridinium salt according to the expected reaction pathway, and therefore enhancing the formation of the 3-substituted quinazolinones, independent of the type of acid chloride used (Scheme 2). The structures of 4a-g and 5a-g were based on the microanalytical and spectral data.

Scheme 2 : synthetic pathway for compounds **4** and **5**



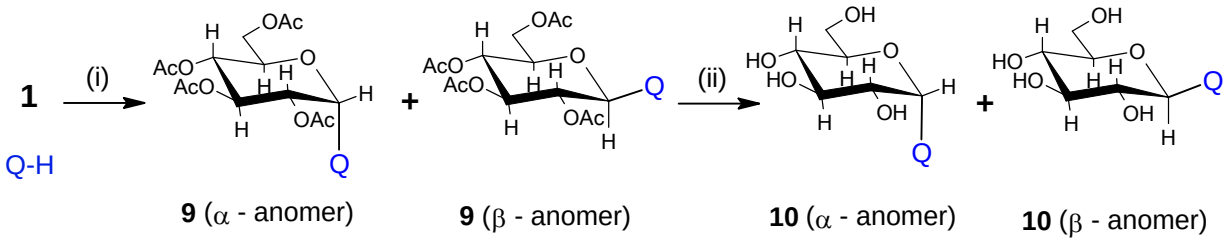
Compound 1 was reacted with *p*-acetylaminobenzenesulfonyl chloride, ethyl chloroformate and chloro-acetyl chloride in dry pyridine affording derivatives 6, 7 and 8 respectively (Scheme 3). The IR spectra of products 6-8 showed two types absorption bands in the ranges 1666-1672 and 1712-1762 attributable to υ_{max} of the endocyclic C=O group of quinazolinone and exocyclic C=O groups of acetyl- aminophenyl, ethoxycarbonyl and chloroacetyl moieties, respectively.

Scheme 3 : synthetic pathway for compounds **6 - 8**



It is well known that the *N*-glycosylated heterocycles are of important significance because of the cytotoxic activitiy of their acetylated derivatives, which increases dramatically after hydrolysis [14]. Herein we discuss the interaction of derivative 1 with α-bromoglucose tetraacetate in 1,4-dioxane to afford α and β anomers of the *N*-substituted acetylated quinazolinone derivative 9 followed by the deacetylation using potassium carbonate /methanol to give α and β anomers of the deacetylated product 10 (Scheme 4). The IR spectra for 9 showed two absorption bands at 1662 and 1738 attributable to υ_{max} of the endocyclic C=O group of quinazolinone and the acetyl groups of per-*O*-acetylated glucose moiety respectively. The H NMR showed no indication for the N-H of quinazolinone and elemental analysis showed no indication for the bromide atom which was originally attached to the glucose moiety. This confirmed its removal during the reaction of glucosamine with quinazolinone.

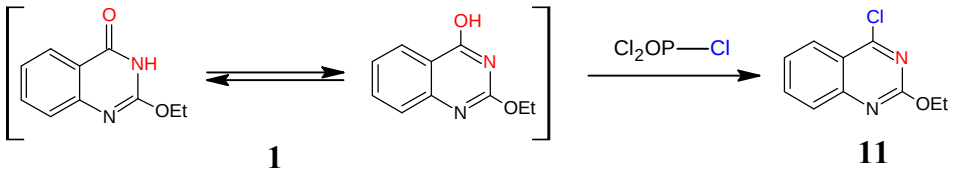
Scheme 4: synthetic pathway for compounds **9** and **10**



(i) a-bromoglucose tetraacetate; 1,4-dioxane; overnight (ii) Deacetylation, Na_2CO_3 / MeOH; r. t. / 4 h.

Moreover, compound 1, in its imidolic form, was reacted with $POCl_3$/2 h affording quinazoline 11 (Scheme 5) [15]. The IR data showed no indication for the NH of quinazolinone.

Scheme 5: synthetic pathway for compound **11**



Compound 1 interacted with P_2S_5/dry xylene / sand bath / 6 h affording quinazolin-4-thione 12 (Scheme 6) [16]. The IR spectrum, in addition to the elemental analysis, showed an absorption band at 1319 attributable to υ_{max} of C=S with no indication for the C=O group.

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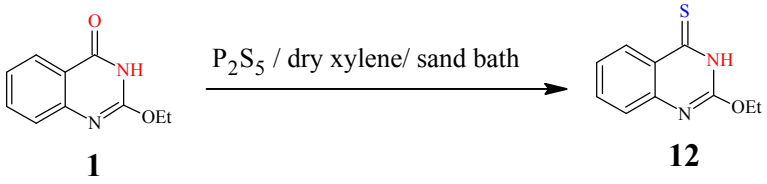
Scheme 6: synthetic pathway for compound **12**

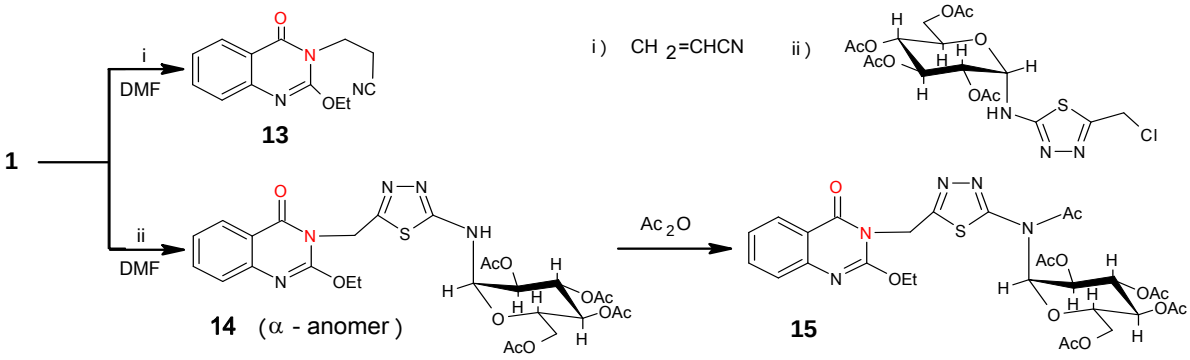


1 **12**

During our attempts, it was noted that most of quinazolinones resemble secondary amines in their Michael-type addition to acrylnitrile [17]. Thus, compound 1 was treated with acrylonitrile in DMF for 5 h giving product 13 (Scheme 7). The IR spectrum showed an absorption band at 2246 attributable to υ_{max} of the CN group and the H NMR spectrum showed a triplet at δ 4.15 for the N-CH_2 bonding.

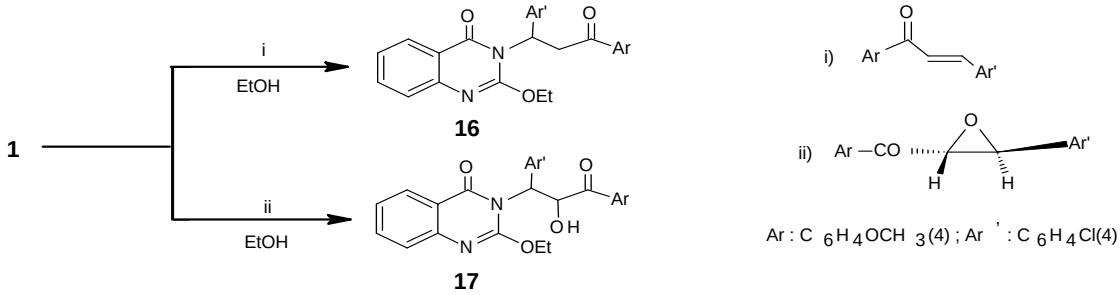
On the other hand, compound 1 was reacted with an alkyl halide having an *N*-glucosidated thiadiazole moiety, the 2-chloromethyl-5-glucopyranosylaminothiadiazole, in DMF for 8 h affording compounds 14. The H NMR spectrum of 14 showed a singlet at δ 5.29 for the N-CH_2 bonding. Acetylation of 14 afforded derivative 15. The IR spectrum was devoid any NH group band. Derivatives like 14 and 15 are believed to have biological activities similar to those of cyclic and acyclic nucleosides [18].

Scheme 7 : synthetic pathway for compounds **13-15**



Similarly, compound 1 was submitted to react with a selected chalcone and its epoxide in absolute ethanol for 5-6 h to give derivatives 16 and 17 respectively (Scheme 8). The IR spectra for 16 and 17 showed two absorption bands at 1671 and 1713-1719 characteristic for the endocyclic and exocyclic C=O groups. The H NMR spectra of 16 and 17 showed no band for the NH group of quinazolinone. Derivatives 16 and 17 are also expected to have biological activity [19].

Scheme 8 : synthetic pathway for compounds **16** and **17**



3. Antimicrobial Evaluation

Compounds 4b, 4c, 4d, 4f, 4g, 6, 7, 8, 9, 10 and 15 were tested for antimicrobial activity against *Escherichia coli* (Gram negative bacterium), *Staphylococcus aureus* (Gram positive bacterium), *Aspergillus flavus* and *Candida albicans* (fungi) using the disc diffusion method. The antimicrobial evaluation was done in the Microanalytical Center at Cairo University.

3.1 General disc diffusion (agar-based) method

Standard discs of tetracycline (antibacterial agent) and amphotericin B (antifungal agent) served as positive controls and references for antimicrobial activities respectively, but filter discs impregnated with 10µL of solvent (chloroform, ethanol, DMF) were used as a negative control. The agar used is Mueller - Hinton agar that is rigorously tested for composition and pH. The depth of the agar in the plate is a factor to be considered in this method. Blank paper discs (Schleicher and Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10 µL of the tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area susceptible to the chemical around the disc. This area of no growth around the disc is the "zone of inhibition" or "clear zone". For disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS) [20]. Agar-based method is a good alternative method being simpler and faster than broth–based methods [21, 22].

3.2 Antibacterial Activity

Concentration of 1 mg/mL of test compounds were prepared by dissolving the compounds in its proper solvent. For each concentration, 0.2 mL of synthesized compounds (1 mg/mL) was added to each hole. The plates were allowed to stand at room temperature for two hours and then incubated. The organisms were grown in nutrient agar at 37^{o}C for 24 hours. After incubation period, the growth inhibition zones diameters were carefully measured in mm. The clear zone around the wells was measured as inhibition zones. The absence of a clear zone around the well was taken as inactivity.

Results of antibacterial activity tested against *E. Coli* (G-) and *S. Aureus* (G+) showed that all of the selected compounds were antibacterially active and comparatively efficient.

3.3 Antifungal Activity

The samples were dissolved, each in its proper solvent, then 0.5 mL sample of each compound (1 mg/mL) plus 0.1 mL of the tested fungal suspension were mixed thoroughly with 20 mL of agar medium, which was maintained at 45^{o}C. The inoculated medium was poured into sterile Petri-dishes, allowed to solidify, and incubated at 25^{o}C for seven days. Results of antifungal activity tested showed that compounds 4c, 4d, 6, 9 and 10 were active against both fungi, none was active with *A. flavus*, 4f, 4g, 8 and 15 were active only with *C. albicans*, whereas the rest of compounds were totally inactive.

All the results for the antimicrobial evaluation are given in (Table 1) showing the inhibition zone diameter in mm/mg sample. Both compounds 9 and 10 showed the highest inhibition with *S. aureus* whereas compounds 6 and 9 showed the highest inhibition towards *C. albicans*.

In conclusion all the products 4b-d,f,g, 6-10 and 15 were antibacterially active and comparatively efficient. In addition, compounds 4c, 4d, 6, 9 and 10 were active against both fungi, 4f, 4g, 8 and 15 were active only with *C. albicans*, and the rest were inactive.

The antimicrobial activity of the products compared to those of tetracycline and amphotericin B are given in Fig 1.

4. Experimental

All melting points recorded are uncorrected. The IR spectra were recorded on a Pye Unicam SP1200 spectrophotometer using KBr wafer technique. The ^{1}H-NMR spectra were determined on a Varian FT-200, Brucker AC-200 MHz instrument using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm. The mass spectra were determined by EI technique using MP model NS-5988 and Shimadzu single focusing mass spectrometer (70 eV).

2-Ethoxy-4(3H)quinazolinone (1): 2-ethoxy(4*H*)-3,1-benzoxazin-4-one (0.01 mol) and ammonium acetate (0.01 mol) were fused using an oil bath for 2 h. The mixture was poured into an ice/water mixture and stirred. The off-white precipitate that separated out was filtered, washed, air-dried and crystallized from ethanol to give off-white crystals of compound 1. M.p. 155-156ºC; yield 85%; Anal. for $C_{10}H_{10}N_2O_2$ (m.w. 190); Found: C, 63.16; H, 5.26; N, 14.74; Calcd: C, 63.22; H, 5.18; N, 14.72; IR υ (cm^{-1}) 1671 (C=O), 3229 (NH); MS: *m/z* (int. %) [M+] 190 (58%); H NMR (DMSO-d_6) δ 1.19 (t, 3H; OCH_2CH_3, *J* = 7.4 Hz), 4.29 (q, 2H; -OCH_2CH_3, *J* = 7.4 Hz), 7.31-8.17 (m, 4H; ArH), 12.30 (br s, 1H, NH).

General procedure for the synthesis of compounds 2a-d and 3.

A mixture of quinazolinone 1 (0.01 mol) and any of the alkyl halides methyl iodide, ethyl iodide, benzyl chloride, ethyl chloroacetate or allyl bromide (0.01 mol) in dry acetone and anhydrous K_2CO_3 (50 mL/2 g) was heated under reflux for about 24 h. The excess acetone was distilled off and the residue was poured into cold water with stirring. The solid that separated out was filtered by suction, washed with water, dried and crystallized from suitable solvent affording derivatives 2–3, respectively.

2-Ethoxy-3-methyl-4(3H)quinazolinone (2a):

Light brown crystals from ethanol; m.p. 203-204 ºC; yield 70 %. Anal. for $C_{11}H_{12}N_2O_2$ (m.w. 204); Found: C, 64.82; H, 5.78; N, 13.68; Calcd: C, 64.70; H, 5.88; N, 13.72; IR υ (cm^{-1}) 1686 (C=O), 2982 (CH); MS: *m/z* (int. %) [M+•] 204 (38.3); H NMR (DMSO-d_6) δ 1.15 (t, 3H; -OCH_2CH_3, *J* = 7.4 Hz), 3.44 (s, 3H, NCH_3), 4.29 (q, 2H; -OCH_2CH_3, *J* = 7.4 Hz), 7.36 - 7.97 (m, 4H; ArH).

2-Ethoxy-3-ethyl-4(3H)quinazolinone (2b):

Light brown crystals from ethanol; m.p. 217-218 ºC; yield 75 %. Anal. for $C_{12}H_{14}N_2O_2$ (m.w. 218); Found: C, 66.12; H, 6.38; N, 12.88; Calcd: C, 66.06; H, 6.42; N, 12.84; IR υ (cm^{-1}) 1691 (C=O), 2982 (CH); MS: *m/z* (int. %) [M+•] 218 (47.3); H NMR (DMSO-d_6) δ 1.19 (t, 3H; OCH_2CH_3, *J* = 7.4 Hz), 1.22 (t, 3H, NCH_2CH_3), 4.01 (q, 2H, NCH_2CH_3), 4.32 (q, 2H; -OCH_2CH_3, *J* = 7.4 Hz), 7.42 - 8.16 (4 d, 4H; ArH).

3-Benzyl-2-ethoxy-4(3H)quinazolinone (2c):

Light brown crystals from ethanol; m.p. 279-280 ºC; yield 85 %. Anal. for $C_{17}H_{16}N_2O_2$ (m.w. 280); Found: C, 72.82; H, 5.66; N, 10.14; Calcd: C, 72.86; H, 5.71; N, 10.00; IR υ (cm^{-1}) 1689 (C=O), 2984 (CH); MS: *m/z* (int. %) [M+•] 280 (38.2); H NMR (DMSO- d_6) δ 1.19 (t, 3H; OCH_2CH_3, *J* = 7.4 Hz), 4.35 (q, 2H; -OCH_2CH_3, *J* = 7.4Hz), 5.09 (s, 2H, CH_2Ph), 7.25-7.33 (m, 5H, PhH), 7.38-8.17 (m, 4H; quinazolinone).

2-Ethoxy-3-ethoxycarbonylmethyl-4(3H)quinazolinone (2d):

Off-white crystals from ethanol; m.p. 275-276 ºC; yield 65 %. Anal. for $C_{14}H_{16}N_2O_4$ (m.w. 276); Found: C, 60.82; H, 5.83; N, 10.22; Calcd: C, 60.87; H, 5.80; N, 10.14; IR υ (cm^{-1}) 1672, 1734 (2C=O), 2904 (CH); MS: *m/z* (int. %) [M+•] 276 (55.2); H NMR (DMSO-d_6) δ 1.19 (t, 3H; OCH_2CH_3, *J* =7.4 Hz), 1.15 (t, 3H, $COOCH_2CH_3$), 4.96 (s, 2H, CH_2CO), 4.24(q, 2H, COOCH_2CH_3), 4.34(q, 2H; OCH_2CH_3, *J* = 7.4Hz), 7.43-8.17(m, 4H; ArH).

3-Allyl-2-ethoxy-4(3H)quinazolinone (3):

White crystals from ethanol; m.p. 231-232 ºC; yield 75 %. Anal. for $C_{13}H_{14}N_2O_2$ (m.w. 230); Found: C, 67.80; H, 6.02; N, 12.22; Calcd: C, 67.83; H, 6.08; N, 12.17; IR υ (cm^{-1}) 1619 (C=N), 2846, 2885 (CH), 1264 (C-O-C); MS: *m/z* (int. %) [M+] 230 (33.8); H NMR (DMSO-d_6) δ 1.20 (t, 3H; -OCH_2CH_3, *J* = 7.4Hz), 2.46 (t, 2H, *J* = 6.4), 4.16 (q,2H; -OCH_2CH_3, *J* = 7.4 Hz), 4.73 (d, 2H, -CH_2 - CH=CH_2), 5.01, 5.05 (2 dd, 2H, -CH_2-CH=CH_2), 6.01 (m, 1H, -CH_2 - CH=CH_2), 7.43 - 8.82 (m, 4H; ArH).

General procedure for the synthesis of compounds 4a-d and 5a-c.

To a solution of compound 1 (0.01 mol) in 50 mL of dry acetone was added the acid chloride, acetyl, *n*-decanoyl, phthalimidoacetyl, naphthalene sulphonyl, crotonyl, furoyl or benzoyl chlorides (0.04 mol) and anhydrous K_2CO_3 (0.04 mol). The reaction mixture was refluxed for 24 h. The excess acetone was evaporated and the residue was poured into cold water with stirring. The separated solid was filtered off, washed with cold water, dried and crystallized from the proper solvent to give 4 and 5, respectively.

General procedure for the synthesis of compounds 4a-g and 5a-g.

The quinazolinone 1 (0.01 mol) and the same acid chloride (0.04 mol) were fused at150 ºC in an oil bath for 2 h, and poured into an ice/water mixture. The solid that separated out was filtered, washed, dried, and then crystallized from the proper solvent affording 4a-g and 5a-g.

Another procedure for the synthesis of derivatives 4a-g.

A mixture of quinazolinone 1 (0.01 mol) and the acid chloride (0.01 mol) was refluxed in 50 mL of dry pyridine for 4 h. The excess solvent was distilled off and the reaction solution was cooled then poured into crushed ice with stirring leaving a crude product which was filtered off, washed with cold water, dried and crystallized from the proper solvent to afford 4a-g.

3-Acetyl-2- ethoxy-4(3H)quinazolinone (4a):

Brown crystals from ethanol; m.p. 221-222 ºC; yield 65%. Anal. for $C_{12}H_{12}N_2O_3$ (m.w. 232); Found: C, 62.09; H, 7.72; N, 12.05; Calcd: C, 62.07; H, 7.76; N, 12.07; IR υ (cm^{-1}) 1671, 1734 (2xC=O), 2982 (CH); MS: *m/z*

(int.%) [M+•] 232 (34.6); H NMR (DMSO-d_6) δ 1.19 (t, 3H; -OCH$_2$*CH$_3$*, *J* = 7.4 Hz), 2.21 (s, 3H, -CO*CH$_3$*), 4.36 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 7.44 - 8.20 (m, 4H, ArH).

3-n-Decanoyl-2-ethoxy-4(3H)quinazolinone (4b):

Yellow crystals from light petroleum (80-100 ºC); m.p. 145-146ºC; yield 75%. Anal. for $C_{20}H_{28}N_2O_3$ (m.w. 344); Found: C, 69.74; H, 8.18; N, 8.12; Calcd: C, 69.77; H, 8.14; N, 8.14; IR υ (cm^{-1}) 1691, 1738 (C=O), 2925 (CH); MS: *m/z* (int. %) [M+•] 344 (56.7); H NMR (DMSO-d_6) δ 0.87 (t, 3H, *CH$_3$*), 1.23- 1.56 (m, 14H, *CH$_2$* grps), 1.2 (t, 3H; OCH$_2$*CH$_3$*, *J* = 7.4Hz), 2.63(t, 2H, *CH$_2$*CO), 4.36 (q, 2H; O*CH$_2$*CH$_3$, *J* = 7.4), 7.11-8.30 (m, 4H, ArH).

2-Ethoxy-3-phthalamidoacetyl-4(3H)quinazolinone (4c):

Yellow crystals from DMF; m.p. 297-298 ºC; yield 85%. Anal. for $C_{20}H_{15}N_3O_5$ (m.w. 377); Found: C, 63.62; H, 3.92; N, 11.18; Calcd: C, 63.66; H, 3.98; N, 11.14; IR υ (cm^{-1}) 1667, 1714, 1774 (4xC=O), 2993 (CH); MS: *m/z* (int. %) [M+•] 377 (41.6); H NMR (DMSO-d_6) δ 1.20 (t, 3H; -OCH$_2$*CH$_3$*, *J* = 7.1 Hz), 4.63 (m, 2H, *CH$_2$*), 4.46 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.1 Hz), 7.31-8.21 (m, 8H, ArH).

2-Ethoxy-3-(1-naphthalenesulphonyl)-4(3H)quinazolinone (4d):

Light brown crystals from ethanol; m.p. 182-183 ºC; yield 75 %. Anal. for $C_{20}H_{16}N_2O_4S$ (m.w. 380); Found: C, 63.12; H, 4.19; N, 7.38; S, 8.42; Calcd: C, 63.16; H, 4.21; N, 7.38; S, 8.42; IR υ (cm^{-1}) 1157 (S=O), 1671 (C=O); MS: *m/z* (int. %) [M+•] 380 (56.2); H NMR (DMSO-d_6) δ 1.23 (t, 3H; -OCH$_2$*CH$_3$*, *J* = 6.8 Hz), 4.44 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 6.8 Hz), 7.40-8.22 (m, 11H, ArH).

3-Crotonyl-2-ethoxyquinazolin-4-one (4e):

Off-white crystals from ethanol; m.p. 227-228 ºC; yield 75%. Anal. for $C_{14}H_{14}N_2O_3$ (m.w. 258); found: C, 65.18; H, 5.48; N, 10.81; Calcd: C, 65.11; H, 5.43; N, 10.85; IR υ (cm^{-1}) 1671, 1728 (2 C=O), 2938 (CH); MS: *m/z* (int. %) [M+•] 258 (55.4); H NMR (DMSO-d_6) δ 1.2 (t, 3H; -OCH$_2$*CH$_3$*, *J* = 7.4Hz), 1.86 (t, 3H, *CH$_3$*), 4.36 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 6.35 (d, H, CH$_{trans}$), 7.05 (d, H, CH$_{trans}$), 7.39-8.2 (m, 4H, ArH).

2-Ethoxy- 3-furan-2-oyl-quinazolin-4-one (4f):

White crystals from ethanol; m.p.177-178 ºC; yield 80 %. Anal. for $C_{15}H_{12}N_2O_4$ (m.w. 284); found:C, 63.18; H, 5.48; N, 10.81; Calcd: C, 63.38; H, 4.23; N, 9.86; IR υ (cm^{-1}) 1671, 1734 (2C=O); MS: *m/z* (int. %) [M+] 284 (56.1); H NMR (DMSO-d_6) δ1.19 (t, 3H, -OCH$_2$*CH$_3$*, *J* = 7.4 Hz), 4.37 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 6.75 (dd, 1H, Furan-H), 7.34 (d, 1H, Furan-H), 7.91 (d, 1H, Furan-H), 7.46-8.20 (m, 4H, ArH).

3-Benzoyl-2-ethoxyquinazolin-4-one (4g):

White crystals from ethanol; m.p. 117-118 ºC; yield 65%. Anal. for $C_{17}H_{14}N_2O_3$ (m.w. 294); Found: C, 69.35; H, 4.73; N, 9.54; Calcd: C, 69.39; H, 4.76; N, 9.52; IR υ (cm^{-1}) 1671, 1734 (2xC=O); MS: *m/z* (int. %) [M+] 294 (58.4); H NMR (DMSO-d_6) δ 1.2 (t, 3H, -OCH$_2$*CH$_3$*, *J* = 7.4 Hz), 4.37 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 7.46-8.2 (m, 9H, ArH).

[2-Ethoxyquinazolin-4-yl] acetate (5a):

Off-white crystals from ethanol; m.p. 167-168 ºC; yield 65%. Anal. for $C_{12}H_{12}N_2O_3$ (m.w. 232); Found: C, 62.13; H, 7.70; N, 12.10; Calcd: C, 62.07; H, 7.76; N, 12.07; IR υ (cm^{-1}) 1605 (C=N), 1780 (C=O); MS: *m/z* (int. %) [M+] 232 (67.5); H NMR (DMSO-d_6) δ 1.2 (t, 3H, -OCH$_2$*CH$_3$*, *J* = 7.4 Hz), 2.14 (s, 3H, -CO*CH$_3$*), 4.19 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 7.50-8.85 (m, 4H, ArH).

[2-Ethoxyquinazolin-4-yl]-n-decanoate (5b):

Light brown crystals from benzene; m.p. 167-168ºC; yield 70%. Anal. for $C_{20}H_{28}N_2O_3$ (m.w. 344); Found: C, 69.79; H, 8.18; N, 8.14; Calcd: C, 69.77; H, 8.14; N, 8.14; IR υ (cm^{-1}) 1619 (C=N), 1769 (C=O); MS: *m/z* (int. %) [M+] 344 (44.8); H NMR (DMSO-d_6) δ 0.87 (t, 3H, *CH$_3$*), 1.23-1.56 (m, 14H, *CH$_2$* grps), 1.2 (t, 3H, OCH$_2$*CH$_3$*, *J* = 7.4 Hz), 2.4 (t, 2H, *CH$_2$*CO), 4.19 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 7.51-8.85 (m, 4H, ArH).

[2-Ethoxyquinazolin-4-yl]phthaloylglycinate (5c):

Off-white crystals from ethanol; m.p. 272-273ºC; yield 65%. Anal. for $C_{20}H_{15}N_3O_5$ (m.w. 377); Found: C, 63.68; H, 3.94; N, 11.16; Calcd: C, 63.66; H, 3.98; N, 11.14; IR υ (cm^{-1}) 1590 (C=N), 1730, 1774 (3 x C=O), 2990 (CH); MS: *m/z* (int. %) [M+] 377 (66.5); H NMR (DMSO-d_6) δ 1.21 (t, 3H, -OCH$_2$*CH$_3$*, *J* = 7.4 Hz), 4.7 (m, 2H, -*CH$_2$*), 4.17 (q, 2H; -O*CH$_2$*CH$_3$, *J* = 7.4 Hz), 7.69-8.85 (m, 8H, Ar-H).

[2-Ethoxyquinazolin-4-yl]naphthalene-1-sulphonate (5d):

Brown crystals from light petroleum (80-100 ºC); m.p. 152-153 ºC; yield 70 %. Anal. for $C_{20}H_{16}N_2O_4S$ (m.w. 380); Found: C, 63.18; H, 4.16; N, 7.40; S, 8.44; Calcd: C, 63.16; H, 4.21; N,7.38; S, 8.42; IR υ (cm^{-1}) 1163

(S=O), 1620 (C=N); MS: *m/z* (int. %) [M+] 380 (72.3); H NMR (DMSO-d_6) δ 1.21 (t, 3H, OCH_2CH_3, *J* = 6.8 Hz), 4.15 (q, 2H; $-OCH_2CH_3$, *J* = 6.8 Hz), 7.68-8.86 (m, 11H, ArH).

[2-Ethoxyquinazolin-4-yl] crotonate (5e):

Light brown crystals from benzene; m.p. 197-198 ºC; yield 65 %. Anal. for $C_{14}H_{14}N_2O_3$ (m.w. 258); Found: C, 65.15; H, 5.42; N, 10.79; Calcd: C, 65.11; H, 5.43; N, 10.85; IR υ (cm^{-1}) 1264 (C-O-C), 1617 (C=N), 1767 (C=O), 2881, 2939 (CH); MS: *m/z* (int. %) [M+] 258 (68.4); H NMR (DMSO-d_6) δ 1.2 (t, 3H, $-OCH_2CH_3$, *J* = 4.7 Hz), 1.89 (t, 3H, $-CH_3$), 4.19 (q, 2H; OCH_2CH_3, *J* = 4.7 Hz), 6.19 (d, H, CH_{trans}), 7.24 (d, H, CH_{trans}), 7.50-8.85 (m, 4H, ArH).

[2-Ethoxyquinazolin-4-yl] furan-2-carboxylate (5f):

Off-white crystals from benzene; m.p. 153-154ºC; yield 75%. Anal. for $C_{15}H_{12}N_2O_4$ (m.w. 284); Found: C, 65.18; H, 5.48; N, 10.81; Calcd: C, 63.38; H, 4.23; N, 9.86; IR υ (cm^{-1}) 1588 (C=N), 1763 (C=O); MS: *m/z* (int. %) [M+] 284 (44.3); H NMR (DMSO-d_6) δ 1.2 (t, 3H, $-OCH_2CH_3$, *J* = 7.4 Hz), 4.17 (q, 2H; $-OCH_2CH_3$, *J* = 7.4 Hz), 6.80 (dd, 1H, Furan-H), 7.32 (d, 1H, Furan-H), 7.98 (d, 1H, Furan-H), 7.61-8.85 (m, 4H, ArH).

[2-Ethoxyquinazolin-4-yl] benzoate (5g):

Off-white crystals from benzene; m.p. 101-102 ºC; yield 70 %. Anal. for $C_{17}H_{14}N_2O_3$ (m.w. 294); Found: C, 69.35; H, 4.73; N, 9.54; Calcd: C, 69.39; H, 4.76; N, 9.52; IR υ (cm^{-1}) 1611 (C=N), 1769 (C=O); MS: *m/z* (int. %) [$M^{+\bullet}$] 294 (55.8); H NMR (DMSO-d_6) δ 1.2 (t, 3H, OCH_2CH_3, *J* = 7.4 Hz), 4.17 (q, 2H; OCH_2CH_3, *J* = 7.4 Hz), 7.55-8.85 (m, 9H, ArH).

General procedure for the synthesis of compounds 6, 7, and 8.

Compound 1 (0.01 mol) was refluxed with reagents such as p-acetylaminophenylsulphonyl chloride, ethyl chloroformate and chloroacetyl chloride (0.01 mol) in 50 mL of dry pyridine for 4 h. The excess solvent was distilled off and the solution was left to cool and then poured onto ice with stirring to obtain the crude product which was filtered off, thoroughly washed with cold water, dried and crystallized from the proper solvent affording products 6, 7 and 8 respectively.

2-Ethoxy-3-(p-acetylaminophenylsulphonyl) quinazolin-4-one (6):

White crystals from ethanol; m.p. 261-262ºC; yield 88 %. Anal. for $C_{18}H_{14}N_3O_5S$ (m.w. 387); Found: C, 55.79; H, 4.41; N, 10.89; S, 8.31; Calcd: C, 55.81; H, 4.39; N, 10.85; S, 8.27; IR υ (cm^{-1}) 1160 (S=O), 1666, 1712 (2 C=O), 2992 (CH), 3271 (NH); MS: *m/z* (int. %) [M+] 387 (68.2); H NMR (DMSO-d_6) δ 1.23 (t, 3H, $-OCH_2CH_3$, *J* = 7.4 Hz), 2.13 (s, 3H, $COCH_3$), 4.44 (q, 2H; $-OCH_2CH_3$, *J* = 7.4 Hz), 7.72 (d, 1H, NH, *J* = 7.1 Hz), 7.45-8.18 (m, 8H; quinazolinone-H and ArH). ^{13}C-NMR: 15.0, 64.6, 154.0, 146.9, 120.8, 161.9, 126.7, 133.4, 127.3, 126.6, 129.4, 132.2, 129.4, 118.0, 144.8, 118.0, 168.9, 24.0.

3-Ethoxycarbonyl-2-ethoxyquinazolin-4-one (7):

White crystals from benzene; m.p. 177-178ºC; yield 92%. Anal. for $C_{13}H_{14}N_2O_4$ (m.w. 262); Found: C, 59.65; H, 5.39; N, 10.68; Calcd: C, 59.54; H, 5.34; N, 10.69; IR υ (cm^{-1}) 1672, 1762 (2 C=O); MS: *m/z* (int. %) [M+] 262 (88.5); H NMR (DMSO-d_6) δ 1.19 (t, 3H, $-OCH_2CH_3$, *J* = 7.4 Hz), 1.15 (t, 3H, CH_3, $COOCH_2CH_3$), 4.24 (q, 2H, CH_2, $COOCH_2CH_3$), 4.34 (q, 2H; $-OCH_2CH_3$, *J* = 7.4 Hz), 7.43-8.17 (m, 4H, ArH).

3-Chloroacetyl-2-ethoxyquinazolin-4-one (8):

Brownish white crystals from ethanol; m.p. 152-153ºC; yield 85%. Anal. for C12H11N2O3Cl (m.w. 266.5); Found: C, 54.08; H, 4.15; N, 10.56; Cl, 13.32; Calcd: C, 54.03; H, 4.13; N, 10.51; Cl, 13.28; IR υ (cm-1)1668, 1717 (2 x C=O), 2823 (CH); MS: m/z (int. %) [M+] 266.5 (77.3); H NMR (DMSO-d6) δ 1.2 (t, 3H, -OCH2CH3, J = 7.4 Hz), 4.36 (q, 2H; -OCH2CH3, J = 7.4 Hz), 4.29 (d, 2H, CH2Cl), 7.45-8.20 (m, 4H, ArH).

Synthesis of Compounds 9 *(acetylated α and β anomers) and* 10 *(deacetylated α and β anomers)*

A crude mixture of compound 1 (0.05 mol) and α-bromoglucose tetraacetate (0.01 mol) in 1,4-dioxane (100 mL) was heated with stirring under reflux for 4 h. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was then dissolved in ethyl acetate, washed sequentially with saturated NaHCO3, dried over MgSO4. Purification and separation was achieved using column chromatography (3:1 EtOAc: Hexane) affording the acetylated derivative 9 as a white solid which was later on crystallized using dichloromethane-diethyl ether -hexane solvents. A solution of product 9 in MeOH (0.004 mol/ 65mL) was treated with sodium carbonate solution (0.002 mol). A white syrupy solid began to precipitate. The residue was purified using column chromatography (3:1, EtOAc:Hexane) to give the deacetylated product as a white solid, which was later on crystallized using dichloromethane-diethyl ether-hexane solvents to give the crystalline product 10 (α and β anomers).

N-(2, 3, 4, 6-Tetra-0-acetylglucopyranosyl)quinazolin-4-one (α + β) (9):

α-Anomer: Compound was obtained as amorphous white crystalline solid from methanol; yield 14 %; m.p. 182-183°C. Anal. for $C_{24}H_{28}N_2O_{11}$ (m.w. 520); Found: C, 55.22; H, 5.41; N, 5.48; Calcd: C, 55.38; H, 5.38; N, 5.38; IR υ (cm^{-1}) 1662, 1738 (2 C=O); MS: *m/z* (int. %) [M+] 520 (33.6); H NMR ($CDC1_3$) δ 1.2 (t, 3H, -OCH_2CH_3, *J* = 7.4 Hz), 2.05-2.06 (s, 12H, 4 Ac-H), 3.74-5.12 (m, 6H, H-2, H-3, H-4, H-5, H-6_a, H-6_b), 4.45 (q, 2H; -OCH_2CH_3, *J* = 7.4 Hz), 6.17 (d, 1H, H_{anom}), 7.40-7.85 (m, 4H, ArH).

β-Anomer: Position of the spot on TLC plate was lower to the α-anomer, flaky amorphous solid; yield 17 %; m.p. 169-172 °C. Anal. for C24H28N2O11 (m.w. 520); Found: C, 55.48; H, 5.31; N, 5.24;Calcd: C, 55.38; H, 5.38; N, 5.38; IR υ (cm-1) 1666, 1738 (2 x C=O); MS: m/z (int. %) [M+] 520 (38.3); H NMR (CDC13) δ 1.20 (t, 3H, -OCH2CH3, J = 7.4 Hz), 2.05-2.06 (s, 12H, Ac-H), 3.74-5.11 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 4.45 (q, 2H; -OCH2CH3, J = 7.4 Hz), 5.81 (d, 1H, Hanom), 7.28-7.85 (m, 4H, ArH).

2-Ethoxy-3-(glucopyranosyl-2-yl)quinazolin-4-one (10) (α-anomer):

White syrupy crystals of α-anomer from methanol; m.p. 197-198 ºC; yield 24%. Anal. for $C_{16}H_{20}N_2O_7$ (m.w. 352); Found: C, 54.52; H, 5.69; N, 7.92; Calcd: C, 54.54; H, 5.68; N, 7.95; IR υ (cm^{-1}) 1662 (C=O), 3268 (OH bonded), 3384 (OH non-bonded); MS: *m/z* (int. %) [M+] 352 (33.8); H NMR (D_2O) δ 1.2 (t, 3H, OCH_2CH_3, *J* = 6.8 Hz), 3.17-3.87 (m, 5H, H-2, H-3, H-4, H-5, H-6_a, H-6_b), 3.58 (m, 2'-OH, 3'-OH, 4'-OH), 3.65 (s, 6'-OH), 6.09 (d, 1H, H-1), 4.34 (q, 2H; -OCH_2CH_3, *J* = 6.8 Hz), 7.29-8.18 (m, 4H, ArH).

2-Ethoxy-3-(glucopyranosyl-2-yl)quinazolin-4-one (10) (β-anomer):

Amorphous flaky solid of β-anomer from 1,4-dioxane; m.p. 189-191ºC; yield 14 %. Anal. for C16H20N2O7 (m.w. 352); Found: C, 54.52; H, 5.69; N, 7.92; Calcd: C, 54.54; H, 5.68; N, 7.95; IR υ (cm-1) 1668 (C=O), 3247 (OH bonded), 3362 (OH non-bonded); MS: m/z (int. %) [M+•] 352 (36.2); H NMR (D2O) δ 1.1 (t, 3H, OCH2CH3, J = 6.8 Hz), 3.54-4.79 (m, 5H, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.58 (m, 2'-OH, 3'-OH, 4'-OH), 3.65 (s, 6'-OH), 5.78 (d, 1H, H-1), 3.58 (q, 2H; -OCH2CH3, J = 6.8 Hz), 7.63-8.03 (m, 4H, ArH).

4-Chloro-2-ethoxyquinazoline (11):

A solution of 2-ethoxy-4(3H)quinazolinone 1 (0.01 mol) with phosphorus oxychloride (20 mL) was heated on a water bath at 70°C for 2 h. The reaction mixture was cooled and diluted with ice-water and the resulting precipitate was collected by filtration and then crystallized from chloroform affording product 11. Light brown crystals from ethanol; m.p. 180-182 ºC; yield 85 %. Anal. for C10H9N2OCl (m.w. 208.5); Found: C, 57.45; H, 4.31; N, 13.42; Cl, 17.00; Calcd: C, 57.55; H, 4.30; N, 13.43; Cl, 17.02; IR υ (cm-1) 1622 (C=N); MS: m/z (int. %) [M+] 208.5 (57.9); H NMR (DMSO-d6) δ 1.19 (t, 3H, -OCH2CH3, J = 7.4 Hz), 4.19 (q, 2H; -OCH2CH3, J = 7.4 Hz), 7.49-8.86 (m, 4H, ArH).

2-Ethoxy-4(3H)quinazolin-4-thione (12):

A solution of quinazolinone 1 and P2S5 (0.03 mol each) in dry xylene (50 mL) was boiled for 6 h. The reaction mixture was filtered while hot and then concentrated. The solid separated on cooling was crystallized from the suitable solvent to give product 12 as brown crystals from ethanol; m.p. 137-138 ºC; yield 65 %. Anal. for C10H10N2OS (m.w. 206); Found: C, 58.15; H, 4.81; N, 13.52; S, 15.53; Calcd: C, 58.25; H, 4.85; N, 13.59; S, 15,53; IR υ (cm-1) 1319 (C=S), 1597 (C=N), 3137 (NH); MS: m/z (int. %) [M+•] 206 (55.7); H NMR (DMSO-d6) δ 1.19 (t, 3H, OCH2CH3, J = 7.4Hz), 4.39 (q, 2H; OCH2CH3, J = 7.4), 7.29-7.67 (m, 4H, ArH), 12.3 (br s, 1H, NH).

3-[2-Ethoxyquinazolin-3-yl]propionitrile (13):

A mixture of quinazolinone 1 and acrylonitrile (0.01 mol each) was heated under reflux in DMF (30 mL) for 5h. The reaction mixture was then poured into crushed ice. The solid that precipitated out was filtered, washed dried and crystallized from ethanol to give white crystals of product 13; m.p. 190-192 ºC; yield 85 %. Anal. for C13H13 N3O2 (m.w. 243); Found: C, 64.14; H, 5.29; N, 17.32; Calcd: C, 64.20; H, 5.35; N, 17.28; IR υ (cm-1) 1675 (C=O), 2246 (CN), 2989 (CH); MS: m/z (int. %) [M+•] 243 (69.3); H NMR (DMSO-d6) δ 1.19 (t, 3H, -OCH2CH3, J = 7.4Hz), 3.14 (t, 2H, CH2CN), 4.15 (t, 2H, NCH2), 4.2 (q, 2H;-OCH2CH3, 7.42-8.16 (m, 4H, ArH).

2-(2-Ethoxyquinazolin-4-one-3-ylmethyl)-5-(2,3,4,6-tetraacetyl-β-glucopyranosyl-2-ylamino)thiadiazole (14):

Preparation of the reagent for compound (14):

A mixture of thiosemicarbazide (0.01 mol) and chloroacetyl chloride (0.015 mol) was heated under reflux for 2h. The reaction mixture was then poured into crushed ice. The precipitated solid was filtered, washed, dried and

crystallized from the proper solvent to afford 2-chloromethyl-5-aminothiadiazole. A mixture of this product and α-bromoglucose tetraacetate (0.01mol each) in anhydrous acetonitrile (300 mL) was left for 24 h at room temperature with occasional stirring. The suspension was filtered through Celite and the filtrate was taken to dryness. The resulting residue was purified by flash chromatography using a gradient of hexane/ethyl acetate (2:1 to 1:1) affording the acetylated α-anomer of the reagent, the 2-chloromethyl-5-N- (2,3,4,6-tetra-O-acetyl-β-glucopyranosyl) aminothiazole. A mixture of this reagent (0.01 mol) and quinazolinone 1 (5 eqv.) was heated under reflux in 1,4- dioxane (50 mL) for 8 h. The excess solvent was then evaporated and the residue was poured into cold water with stirring. The solid that separated out was filtered, washed with cold water, dried and crystallized from DMF to give light brown crystals of the acetylated product of derivative 14; m.p. above 300ºC. Deacetylation was achieved by adding CH3ONa solution (0.01mol) to this derivative (0.02mol) in methanol (65 mL). White crystals began to precipitate within 5 minutes by quenching with acetic acid (0.003 mmol). The solvent was removed in vacuo and the residue was purified; m.p. 133-135 ºC; yield 65 %. Anal. for C29H33N5O12S (m.w. 633); Found: C, 54.99; H, 5.18; N, 11.05; S, 5.04; Calcd: C, 54.97; H, 5.21; N, 11.06; S, 5.06; IR υ (cm-1) 1669 (C=O), 1768 (OAc), 2992 (CH), 3123 (NH); MS: m/z (int. %) [M+] 465 (33.8); H NMR (DMSO-d6) δ 1.44 (t, 3H, OCH2CH3, J = 6.8 Hz), 2.05-2.06 (4 s, 12H, 4OAc), 2.6-2.7 (br. s,1H, NH), 5.29 (s, 2H, NCH2), 3.88 (dt, 1H, H-3'), 4.49 (d, 1H, H-4'), 4.52 (q, 2H; -OCH2CH3, J = 6.8 Hz), 4.91 (2d, 2H, H-6a' and H-6b'), 4.95 (t, 1H, H-5'), 5.02 (dd, 1H, H-2'), 5.47 (d, 1H, H-1'), 7.32-7.93 (m, 4H, ArH).

N-Acetyl-2-(2-ethoxyquinazolin-4-one-3-ylmethyl)-5-(2,3,4,6-tetraacetyl-β-glucopyranosyl-2-ylamino)thiadiazole (15):

A cold solution of product 14 (0.01 mol) in dry pyridine (25 mL) was reacted with Ac2O (25 mL). The mixture was kept overnight at room temperature, with occasional shaking, and then poured onto crushed ice, and the residue was collected by filtration, washed repeatedly with water, air-dried and crystallized from ethanol affording product 15; m.p. 123-125 ºC; yield 65 %. Anal. for C27H31N5O11S (m.w. 675); Found:C, 48.08; H, 4.69; N,10.54; S, 4.84; Calcd: C, 48.00; H, 4.59; N,10.37; S, 4.74; IR υ (cm-1) 1669, 1772 (2C=O), quinazolinone and OAc), 2992 (CH); MS: m/z (int. %) [M+•] 465 (66.6); H NMR (DMSO-d6) δ 1.44 (t, 3H, -OCH2CH3, J = 6.8 Hz), 2.06-2.15 (5 s, 15 H, 5 OAc), 5.27 (s, 2H, NCH2), 4.89 (dt, 1H, H-3'), 5.01 (d, 1H, H-4'), 4.53 (q, 2H; OCH2CH3, J = 6.8Hz), 4.49 (2d, 2H, H-6a' and H-6b'), 3.77 (dt, 1H, H-5'), 5.18 (dd, 1H, H-2'), 5.93 (d, 1H, H-1'), 7.47-7.96 (m, 4H, ArH). 13C-NMR: 15.0, 64.9, 150.1, 126.7, 133.4, 127.3, 126.6, 146.9, 120.8, 161.6, 40.7, 168.0, 163.4, 21.0, 169.9, 23.3, 170.2, 95.7, 68.2, 81.9, 28.7, 51.3, 170.2, 20.4, 65.6, 62.4, 170.2, 21.0, 170.2, 20.7.

1-(p-Methoxyphenyl)-3-(p-chlorophenyl)-3-(2-ethoxyquinazolin-4-one-3-yl) propan-1-one (16):

A mixture of compound 1 and 4'-methoxyphenyl-4-chlorophenyl chalcone (0.01mol each) in ethanol (50 mL) was heated under reflux for 6 h. The solid that separated out was filtered, washed and air-dried. The residue was purified by chromatography using a gradient of hexane and ethyl acetate (2:1 to 1:1) to afford product 16; pale yellow crystals from methanol; m.p. 154-156 ºC; yield 75 %. Anal. for C26H23N2O4Cl (m.w. 462.5); Found: C, 67.48; H, 5.01; N, 6.01; Cl, 7.70; Calcd: C, 67.46; H, 4.97; N, 6.05; Cl, 7.68; IR υ (cm-1)1671, 1713 (2 C=O), 2990 (CH); MS: m/z (int. %) [M+] 462.5 (56.8); H NMR (DMSO-d6) δ 1.22 (t, 3H, OCH2CH3, J = 7.4 Hz), 2.72 (d, 2H, CH2CO), 3.8 (s, 3H, OCH3), 5.61 (t, 1H, -CH), 4.51 (q, 2H; -OCH2CH3, J = 7.4 Hz), 6.99-7.98 (m, 4H, -C6H4OCH3); 7.45-7.63 (m, 4H, -C6H4Cl); 7.45-8.17 (m, 4H, quinazolinone).

2-Ethoxy-3-{2-hydroxy-3-oxo-1-(4-chlorophenyl)-3-(4-methoxyphenyl) quinazolin-4(3H)-one (17):

Compound 1 (0.01mol) and trans-1-(4-methoxyphenyl)-3-(chlorophenyl)- 2-oxiranylpropanone (0.01 mol) in absolute ethanol (50mL) were heated together under reflux for 5 h. The solid that separated out was filtered, washed, dried and crystallized from methanol to afford pale yellow crystals of product 17. The purity of 17 was checked by chromatography and change in the melting point, 149-151 ºC, compared with that of reactant; yield 75%. Anal. for C26H23N2O5Cl (m.w. 478.5); Found: C, 65.23; H, 4.86; N, 5.82; Cl, 7.37; Calcd: C, 65.20; H, 4.80; N, 5.85; Cl, 7.41; IR υ (cm-1) 1671, 1719 (2C=O), 2966 (CH), 3223 (OH); MS: m/z (int. %) [M+] 478.5 (58.3); H NMR (DMSO-d6) δ 1.22 (t, 3H, -OCH2CH3, J = 7.4 Hz), 2.80 (1H, br s, OH), 4.82 (d, 1H, CHCO), 3.8 (s, 3H, OCH3), 5.52 (m, 1H, CH-C6H4 Cl), 4.44 (q, 2H; -OCH2CH3, J = 7.4 Hz), 7.06-8.01 (m, 4H, -C6H4OCH3); 7.46-7.53 (m, 4H, -C6H4Cl); 7.23-8.17 (m, 4H, quinazolinone). 13C-NMR: 15.0, 64.9, 150.1, 126.7, 133.4, 127.3, 126.6, 120.8, 146.9, 161.3, 52.5, 141.6, 127.2, 128.6, 132.3, 128.6, 127.2, 88.2, 197.0, 126.5, 129.8, 129.8, 114.2, 114.2, 165.0, 55.8.

CompetingInterests

The authors declare no conflict of interest.

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Table 1. *In vivo* antimicrobial activity by agar diffusion method of tested compounds

Compound	**Inhibition zone diameter (mm / mg sample)**				
	E. coli	*S. aureus*	*A. flavus*	*C. albicans*	Control solvent
Tetracycline	33	31	00	00	----
Amphotericin B	00	00	17	21	----
4b	10	10	00	00	Chloroform
4c	09	10	09	10	DMF
4d	16	16	12	15	Ethanol
4f	14	16	00	12	Ethanol
4g	14	15	00	12	Ethanol
6	18	18	17	16	Ethanol
7	08	08	00	00	Chloroform
8	12	12	00	12	Ethanol
9	08	21	08	18	Ethanol
10	08	21	08	12	Ethanol
15	14	16	00	16	Ethanol

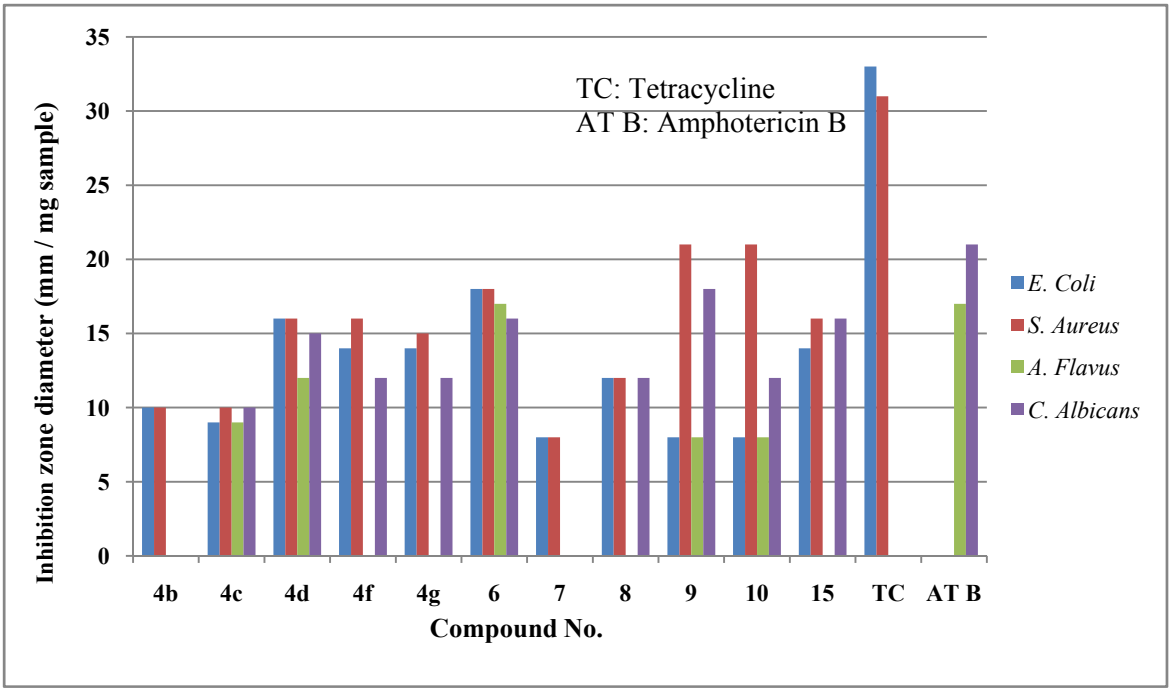

Figure 1. Graphical representation for the antimicrobial activity of the tested compounds

Reactivity of 2-Ethoxyquinazolin- 4-yl hydrazine and its Use in Synthesis of Novel Quinazoline Derivatives of Antimicrobial Activity

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Abstract: The reactions of 2-ethoxy-4-hydrazinoquinazoline 2 with diethyl oxalate and ethyl chloroacetate gave 6-ethoxy-2*H*-[1,2,4] triazino [4,3-*c*] quinazoline-3,4-dione 3 and 6-ethoxy-2,3-dihydro-4*H*-[1,2,4] triazino [4, 3-*c*] quinazolin-4-one 4 respectively. A series of 5-ethoxy-2-X-[1, 2, 4] triazolo [1, 5-c] quinazolines 5a-d was also produced by reacting 2 with the acid chlorides namely: benzoyl, crotonyl, cinnamyl and 2-furoyl chlorides via Dimroth rearrangement. Also, 2 reacted with ethyl chloroformate giving 6. Condensation of 2 with acetone gave Schiff base 7, and with monosaccharides gave the sugar hydrazones 8a-e which was thereafter acetylated giving the corresponding 9a-e. Cyclization of 8a-e by iron(III) chloride gave triazoloquinazolines 10a-e acyclic *C*-nucleosides which, by acetylation, afforded 11a-e. All products were confirmed by elemental, IR, MS, and ^{1}H-NMR analysis. Products 8-11 were chosen for biological screening test against gram (+ ive) and gram (- ive) bacteria.

Keywords: Iminamine, Triazoloquinazoline, Sugar Hydrazones, *C*-Nucleosides

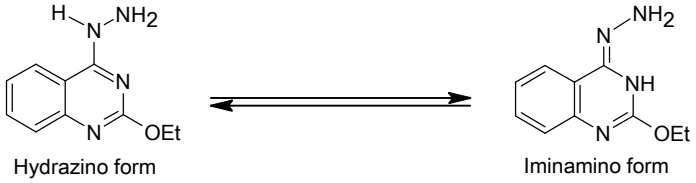
1. Introduction

Quinazolines are a big family of heterocyclic compounds, which have shown broad variety of biological activity profiles, e. g. analgesic, antiinflammatory, antipyretic [1, 2], antimicrobial [3], anticonvulsant [4], anticancer [5], antitumoral [6], antihypertensive [7], antimalarial [8], diuretic [9], antidiabetic [10], antihistamine/sedative [11], antibiotic [12] and many others. Heterocycle-bearing N-glycosides are well known to play a significant role as inhibitors. An example is the tetrazole-bearing N-glycosides used as SGLT2 inhibitors [13], where their hypoglycemic activity is tested *in vivo* by mice oral glucose tolerance test (OGTT). Moreover, sugar hydrazones exhibit remarkable biological activity [14]. Herein we report the synthesis of hydrazones of D-exoses and D-pentoses with 4-hydrazinoquinazoline and the screening of their antimicrobial potentials.

2. Result and Discussion

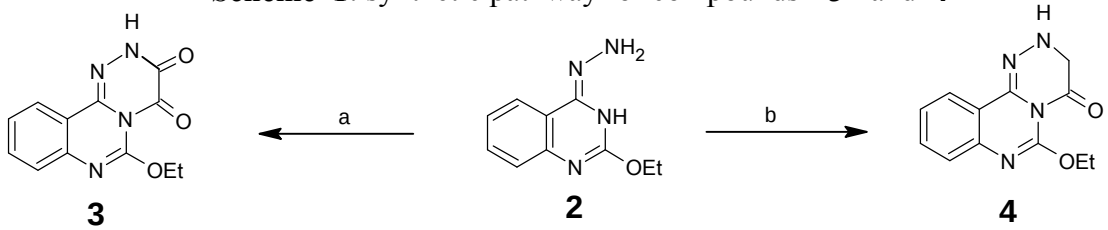
Recently, it was reported that 4-substituted-aminoquinazolines are exploited as potent antitumor compounds [15]. The 4-hydrazinoquinazolines resemble primary amines in being good substrates for aldehydes, ketones, alkyl and acid halides, anhydrides, etc. Therefore, they play a significant role in the synthesis of biologically active products [16]. The tautomeric behavior of hydrazinoquinazoline is used whenever necessary. For example, any necessary cyclization prior to product formation requires the presence of iminamine rather than hydrazine - form (Chart 1).

Chart 1: Tautomeric phenomenon of compound **2**



Compound 2 reacted with diethyl oxalate and with ethyl chloroacetate in boiling ethanol to giving products 3 and 4 respectively (Scheme 1). The reaction possibly started with a nucleophilic attack of NH2 of hydrazine moiety on C=O of the ester group through a tetrahedral mechanism intermediate to yield a fleeting acyl derivative followed by 1,3-tautomerism and ring closure via SN2 mechanism.

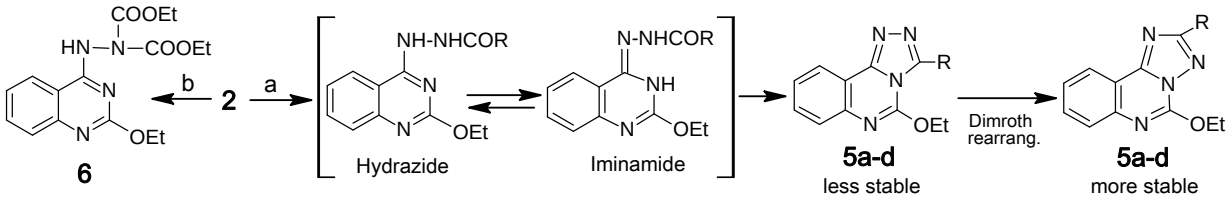
Scheme 1: synthetic pathway for compounds **3** and **4**



(a) EtOOC-COOEt / EtOH; (b) Cl-CH_2COOEt / EtOH.

Similarly, compound 2 was reacted with acid halides namely: benzoyl, crotonyl, cinnamyl and furoyl chlorides in dry $CHCl_3$ and K_2CO_3 giving the 2-acyl-1-(2-ethoxyquinazolin-4-yl)hydrazine derivatives, which tautomerized into the iminamide form upon heating and then underwent cyclization and Dimroth rearrangement affording the more stable derivatives 5a-d respectively (Scheme 2). Compound 2 was also reacted with ethyl chloroformate in dry pyridine affording derivative 6.

Scheme 2: synthetic pathway for compounds **5a-d**

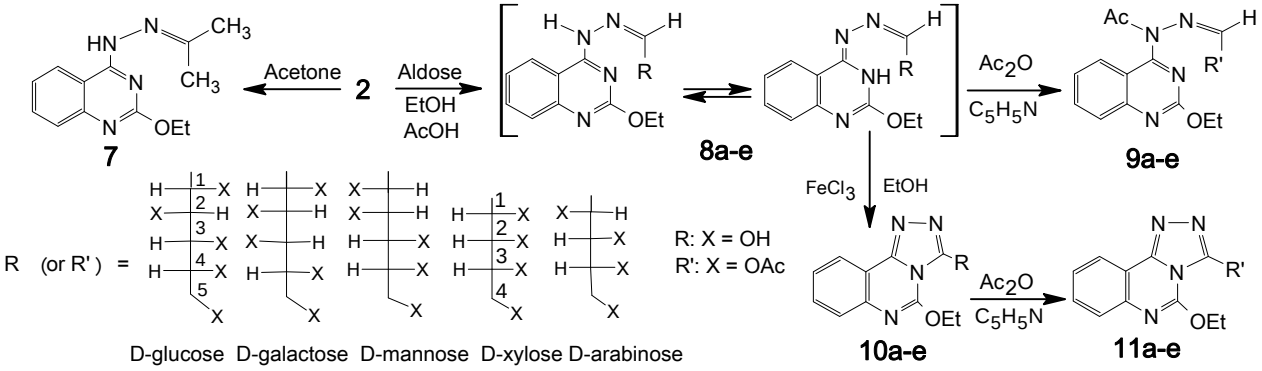


(a) RCOCl / $CHCl_3$ /K_2CO_3; R: - Ph; - CH=CH-CH_3; - CH=CH-Ph; furan-2-yl; (b) ClCOOEt / pyridine.

Compound 2 was reacted with acetone affording derivative 7, whose mass spectrum showed a molecular ion peak at *m/z* 244,246 whereas the ^{1}NMR spectrum showed a singlet at δ 2.40 ppm characteristic for CH_3 groups of the hydrazone. A number of sugar hydrazones 8a-e were prepared by condensation of compound 2 with equimolar amounts of D-aldohexoses and D-aldopentoses namely: glucose, galactose, mannose, xylose and arabinose, respectively in boiling ethanol and drops of acetic acid as a catalyst (Scheme 3). Their IR spectra revealed characteristic absorption bands at 3459-3135 cm^{-1} attributed to OH and NH groups. Acetylation of these hydrazones 8a-e by acetic anhydride in pyridine at room temperature afforded the corresponding per-acetyl products 9a-e, whose IR spectra revealed disappearance of the bands of OH groups and appearance of absorption bands in the carbonyl group frequency region at 1711-1725 cm^{-1} and 1673 - 1692 cm^{-1} due to the OAc and NAc groups, respectively. The ^{1}H-NMR spectra showed signals corresponding to *O*-acetyl groups in addition to NAc groups; whereas no signals could be found for NH groups confirming that per-*O*- and *N*-acetylation had taken place. The

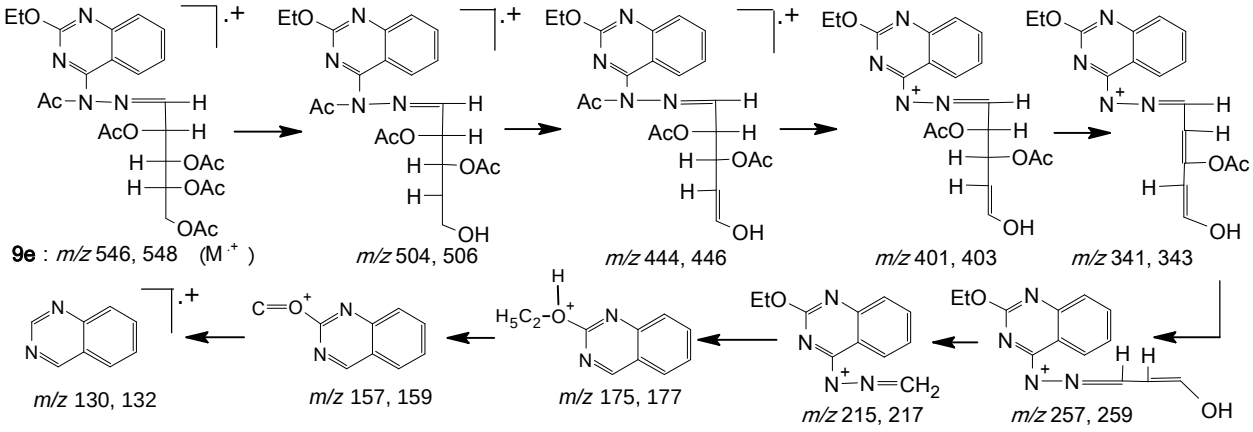
spectra also confirmed the presence of the HC=N proton as a doublet at δ 6.55-6.74 ppm low field in addition to the rest of alditol-1-yl side chain.

Scheme 3: synthetic pathway for compounds **7 - 11**



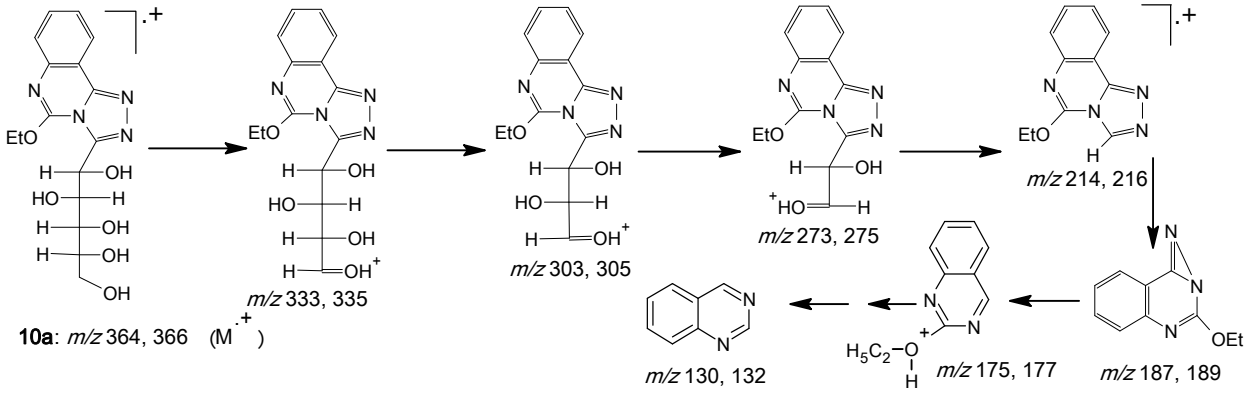
The mass spectral data of 9e showed a molecular ion peak at *m/z* 546, 548 which agreed with the molecular formula $C_{25}H_{30}N_4O_{10}$. The ion at *m/z* 215, 217 confirmed a loss of sugar residue from the molecular ion. The fragment at *m/z* 175, 177 referred to quinazoline ring (Scheme 4).

Scheme 4: MS data interpretation of compound **9a**



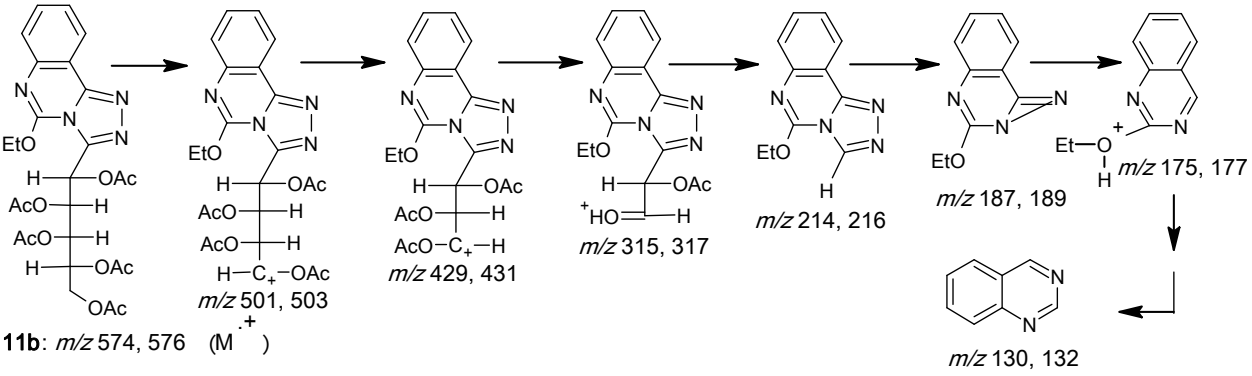
The oxidative cyclization of the hydrazones 8a-e with ethanolic iron (III) chloride afforded the triazolo[4, 3-a]quinazolines 10a-e. The oxidation must have taken place by an electrophilic attack of the hard acid site of ferric chloride on the hardest basic site of sugar hydrazones 8a-e followed by an elimination of hydrogen chloride and formation of possibly a nitrilimine that undergoes 1,5-electrocyclization to give 10a-e. The IR spectra showed bands at 3240-3488 cm^{-1} (OH) and the mass spectral data of 10a showed a molecular ion peak at *m/z* 364 and 366 and an ion peak at *m/z* 214 and 216 presumably attributable to the triazoloquinazoline ring (Scheme 5).

Scheme 5: MS data interpretation of compound **10a**



The ^{1}H-NMR spectrum of compound 10c showed a doublet at low field at δ 5.22 ppm assigned to H-1, followed by the rest of the alditol-1-yl chain at higher field. The spectrum of 10e is similar, showing a doublet at low field at δ 5.03 ppm for H-1. Acetylation of 10a-e by acetic anhydride in pyridine at room temperature gave polyacetoxyalkyl derivatives 11a-e, whose IR spectra showed only one absorption band in the C=O frequency region (OA). The OAc groups were confirmed by the ^{1}H-NMR spectra showing singlets at δ 2.03-2.19. The doublets at δ 5.74-6.02 were attributed to H-1. The mass spectra of products 11b and 11d showed molecular ion peaks at *m/z* 574, 576 and 501, 503 (Scheme 6) which, on combination with the elemental analysis, led to the assignment of their molecular formulas $C_{26}H_{30}N_4O_{11}$ and $C_{23}H_{26}N_4O_9$ respectively. In addition, the characteristic fragment at *m/z* 214,216 was shown attributable to the triazoloquinazoline ring.

Scheme 6: MS data interpretation of compound **11b**



2.1 Antimicrobial Activity

All compounds were screened for their antimicrobial activity. Compounds 8-11 were tested against gram-positive bacteria *Staphylococcus aureus, Streptobacillus moniliformis* and *Bacillus subtillis* and gram-negative bacteria *E. coli, Streptobacillus moniliformis* and *Pseudomonas acru- ginosa* species applying the agar plate diffusion method. The screening results (Table 1) indicated that all the tested products exhibited antimicrobial activities against one or more type of bacteria. Almost all triazoloquinazoline products 10a-e and 11a-e showed more inhibition against the gram positive bacteria specially *Streptobacillus* than the gram negative one.

<Table 1>

2.2 Experimental

All melting points recorded are uncorrected. The IR spectra were recorded on a Pye Unicam SP1200 spectrophotometer using KBr wafer technique. The ^{1}H-NMR spectra were determined on a Varian FT-200 or Brucker AC-200 MHz instrument using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm. The mass spectra were determined using MP model NS-5988 and Shimadzu single focusing mass spectrometer (70 eV). All the solvents used were of HPLC/AnalaR grade. All reagents were used as received from Alfa Aesar.

Synthesis of 2-Ethoxyquinazolin-4-ylhydrazine 1.

An emulsion of 4-chloro-2-ethoxyquinazoline 1 (0.01mol) and hydrazine hydrate (0.05 mol) in benzene (15 mL) was stirred for 2h.The benzene-insoluble gum obtained was treated and washed with water, dried and crystallized from ethanol affording reddish brown crystals of product 2. Evaporation of solvent from the benzene-soluble fraction afforded a residue which was rinsed with water and air dried. Crystallization of the residue from absolute ethanol afforded product 2.

2-Ethoxyquinazolin-4-ylhydrazine 2.

Yield 68%; m. p. 156-158°C. *Anal*. for $C_{10}H_{12}N_4O$ (M. wt. 204); Found: C, 58.86; H, 5.78; N, 27.45; Calcd: C, 58.82; H, 5.88; N, 27.45; IR υ (cm^{-1}) 1620 (C=N), 3160 (NH), 3250, 3300 (NH_2); MS: *m/z* $[M+H]^+$ 204; ^{1}H -NMR (DMSO-d_6) δ 1.18 (t, 3H, CH3 of ethoxy *J* = 7.4 Hz), 4.19 (q, 2H, CH_2 of ethoxy *J* = 7.4), 4.95 (br. s, 3H, NH and NH_2), 7.43 - 8.08 (m, 4H, ArH).

6-ethoxy-2H-[1,2,4]triazino[4,3-c]quinazoline-3,4-dione 3.

A mixture of 2 (2.04 g, 0.01 mol) and diethyl oxalate (1.46 g, 0.01 mol) in boiling ethanol (30 mL) was heated under reflux for 10 h. After cooling the separated solid was collected and recrystallized from THF to give white crystals of 3; m. p. 237-239 ºC; yield 58 %. *Anal.* for $C_{12}H_{10}N_4O_3$ (M. wt. 258); Found: C, 54.21; H, 3.56; N, 21.89; Calcd: C, 55.81; H, 3.88; N, 21.71; IR υ (cm^{-1}) 1680 -1690 (C=O), 3275 (sec NH); MS: *m/z* $[M+H]^+$ 258 (77%). ^{1}H-NMR (DMSO-d_6) δ 1.21 (t, 3H, CH_3 of ethoxy *J* = 7.4 Hz), 4.38 (q, 2H, CH_2 of ethoxy *J* = 7.4), 7.21 -8.13(m, 5H, ArH nd NH), 10.20(s, 1H, NH, exchangeable).

6-ethoxy-2H-[1,2,4]triazino[4,3-c]quinazolin-3(4H)-one 4.

A mixture of 2 (2.04 g, 0.01 mol) and ethyl chloroacetate (1.22 g, 0.01 mol) in boiling ethanol (35 mL) was heated under reflux for 10 h. The solid that separated after cooling was recrystallized from dioxane affording light brown crystals of 4; m. p. 213-216 ºC; yield 58 %. *Anal.* for $C_{12}H_{12}N_4O_2$ (M. wt. 244); Found: C, 58.85; H, 4.53; N, 23.02; Calcd: C, 59.02; H, 4.92; N, 22.95; IR υ (cm^{-1}) 1675 (C=O), 2993 (CH), 3312 (sec NH); MS: *m/z* $[M+H]^+$ 244; ^{1}H-NMR (DMSO-d_6) δ 1.19 (t, 3H, CH_3 of ethoxy *J* = 7.4 Hz), 3.94, 4.30(m, 2H, CH_2CO), 4.30 (q, 2H, CH_2 of ethoxy *J* = 7.4), 7.01- 8.31 (m, 5H, ArH and NH of triazine), 9.96 (s, 1H, NH, exchangeable).

Synthesis of 5-ethoxy-2-substituted[1,2,4]triazolo[1,5-c]quinazoline 5a-d.

To a solution of derivative 2 (0.01mol) in dry chloroform (100 mL) containing anhydrous K_2CO_3 (1g) the acid chloride namely: benzoyl, crotonyl, cinnamyl and furoyl chlorides (0.015 mol) was added slowly. After the addition was complete, the mixture was stirred at room temperature for 30 min and then heated on a steam bath for1h. The mixture was filtered, evaporated and the crude product was collected and crystallized from the proper solvent affording product 5a-d.

5-Ethoxy-2-phenyl[1,2,4]triazolo[1,5-c]quinazoline 5a.

Colorless needles from ethanol; m. p. 168 -170ºC; yield 68%. *Anal.* for $C_{17}H_{14}N_4O$ (M. wt. 290); Found: C, 70.17; H, 4.91; N, 19.38; Calcd: C, 70.34; H, 4.83; N, 19.31; IR υ (cm^{-1}) 1622 (C=N), 3050 (CH); MS: *m/z* $[M+H]^+$ 290 (32.2), 292 (12.3), 214 (100), 216 (23.5), 174 (43.8), 176 (8.2), 78 (13.2), 80 (0.3); ^{1}H-NMR (DMSO-d_6) δ 1.19 (t, 3H, CH_3 of ethoxy *J* = 7.4), 4.33 (q, 2H, CH_2 of ethoxy *J* = 7.4), 7.57-8.10 (m, 5H, phenyl), 7.62 - 8.65 (m, 4H, ArH).

5-Ethoxy-2-[(1E)-prop-1-en-1-yl][1,2,4]triazolo[1,5-c]quinazoline 5b.

Brown white crystals from ethanol; m. p. 223-225 ºC; yield 71%. *Anal.* for $C_{14}H_{14}N_4O$ (M. wt. 254); Found: C, 66.28; H, 5.31; N, 22.23; Calcd: C, 66.14; H, 5.51; N, 22.05; IR υ (cm^{-1}) 1635 (C=N), 3050 (CH); MS: *m/z* $[M+H]^+$ 254 (48.2), 256 (14.2), 174 (100), 176 (38.1); ^{1}H-NMR(DMSO-d_6) δ 1.21(t, 3H, CH_3 of ethoxy *J* = 7.4), 1.67 (t, 3H, CH_3), 4.31(q, 2H, CH_2 of ethoxy *J* = 7.4 Hz), 6.13 (d, H, CH_{trans}), 6.70 (d, H, CH_{trans}), 7.53 - 8.21 (m, 4H, ArH).

5-Ethoxy-2-[(E)-2-phenylethenyl][1,2,4]triazolo[1,5-c]quinazoline 5c.

Off-white crystals from ethanol; 153 - 155 ºC; yield 62%. *Anal.* for $C_{19}H_{16}N_4O$ (M. wt. 316); Found: C, 72.84; H, 5.19; N, 17.76; Calcd: C, 72.15; H, 5.06; N, 17.72; IR υ (cm^{-1}) 1633(C=N), MS: m/□ $[M+H]^+$ 316 (29.3), 318 (12.8), 174 (100), 176 (41.1), 103 (12.7), 105 (0.8); ^{1}H-NMR(DMSO-d_6) δ 1.2(t, 3H, CH_3 of ethoxy *J* = 7.4 Hz), 4.38 (q, 2H, CH_2 of ethoxy *J* = 7.4), 7.09, 7.48 (2d, 2H, of two olefin protons), 7.4-7.6 (m, 5H, PhH), 7.67-8.71 (m, 4H, quinazoline).

5-Ethoxy-2-(furan-2-yl)[1,2,4]triazolo[1,5-c]quinazoline 5d.

White crystals from benzene; 163-164ºC; yield 68%. *Anal.* for $C_{15}H_{12}N_4O_2$ (M. wt. 280); Found: C, 64.38; H, 4.31; N, 20.07; Calcd: C, 64.29; H, 4.29; N, 20.00; IR υ (cm^{-1}) 1619 (C=N), MS: *m/z* $[M+H]^+$ 280 (33.2), 282

(12.4), 174 (100), 176 (31.3), 60 (0.8), 61 (0.1); ^{1}H-NMR(DMSO-d_6) δ1.2 (t, 3H, CH_3 of ethoxy J = 7.4Hz), 4.2 (q, 2H, CH_2 of ethoxy J = 7.4), 6.79(dd, 1H, J = 3.6Hz, J = 1.6, Furan-H), 7.29(d, 1H, J = 4.4Hz, Furan-H), 7.76(d, 1H, J = 1.6Hz, Furan-H), 7.5 - 8.2 (m, 4H, ArH).

1-(2-Ethoxyquinazolin-4-yl)-2-bis(ethoxycarbonyl) hydrazine 6.

A mixture of 2 (0.01 mol) and ethyl chloroformate (0.02 mol) in dry pyridine (20 mL) was heated at boiling water bath for 4 h. The solvent was evaporated under vacuum, the residue was cooled and crystallized from ethanol giving colorless needles of 6; m.p. 123-125 ºC; yield 52 %. *Anal.* $C_{16}H_{20}N_4O_5$ (M.wt. 348); Found: C, 55.27; H, 5.83; N, 16.08; Calcd: C, 55.17; H, 5.75; N, 16.09; IR υ (cm^{-1}) 1250 (C-O), 1622 (C=N), 1731 (C=O), 2986 (C-H); MS: *m/z* $[M+H]^+$ 348 (31.5), 350 (14.1), 275 (2.8), 277 (13.2), 187 (0.8), 189 (0.2), 174 (100), 176 (39.4), 74 (0.7), 75(0.1); ^{1}H-NMR(DMSO-d_6) δ 1.11-1.25 (t, 9H, 3 CH_3 of ethoxy), 4.15-4.25(q, 6H, 3 CH_2 of ethoxy), 8.5-8.8(m, 4H, ArH), 10.05(s, 1H,NH).

2-ethoxy-4-hydrazinoquinazoline Acetone hydrazone *7.*

A solution of crude 2 (0.01 mol) in acetone was left to stand for several days when the solvent had evaporated to give a solid from which the hydrazone 7 (80%) was isolated by chromatography on silica gel (30 g, 2.5% absolute ethanol-chloroform). Crystallization from hexane gave product 7 as colorless solid that turned deep yellow on exposure to light and air; m. p. 115 -116 ºC; yield 85 %. *Anal.* for $C_{13}H_{16}N_4O$ (M. wt. 244); Found: C, 63.98; H, 6.61; N, 22.95; Calcd: C, 63.93; H, 6.56; N, 22.95; IR υ (cm^{-1}) 1634 (C=N), 2993 (CH), 3243 (sec NH); MS: *m/z* $[M+H]^+$ 244 (33.1), 246 (12.6), 215 (100), 217 (19.6), 174 (55.8), 176 (1.2); ^{1}H-NMR(DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4), 2.4 (3H, s, -N=C-CH_3), 4.23(q, 2H, CH_2 of ethoxy J = 7.4), 7.1-8.3(m, 4H, ArH), 8.3(br. s,1H, NH).

General procedure for the synthesis of sugar (2-ethoxyquinazolin-4-yl) hydrazones 8a-e.

To a suspension of 2-Ethoxy-4-hydrazinoquinazoline 2 (0.01 mol) in ethanol (30 ml), was added a solution of selected sugar (D-glucose, D-galactose, D-mannose, D-xylose and D-arabinose (0.01 mol)) in water (10 ml) and few drops of glacial acetic acid. The mixture was heated under reflux until reaction was judged complete by TLC (2-6 h). The solid product formed upon cooling was filtered off, washed with the minimum amount of ethanol, dried and finally crystallized from ethanol to afford the corresponding hydrazones 8a-e.

2-ethoxy-4-hydrazinoquinazoline-D-glucose hydrazone 8a.

Yield 62 % (from ethanol); m. p. 212 - 214 ºC; *Anal.* for $C_{16}H_{22}N_4O_6$ (M. wt. 366); Found: C, 52.66; H, 6.16; N, 15.41; Calcd: C, 52.46; H, 6.01; N, 15.30; IR υ (cm^{-1}) 1615 (C=N), 3225 - 3417 (OH, NH); MS: *m/z* $[M+H]^+$ 366; ^{1}H-NMR (DMSO-d_6) δ 1.17 (t, 3H, CH_3 of ethoxy J = 7.4), 4.17 (q, 2H, CH_2 of ethoxy J = 7.4), 7.1- 8.3 (m, 4H, ArH), 8.33 (br. s, 1H, NH).

2-ethoxy-4-hydrazinoquinazoline-D-galactose hydrazone 8b.

Yield 88% (from DMF/ethanol); m. p. 193-195 ºC; *Anal.* for $C_{16}H_{22}N_4O_6$ (M. wt. 366); Found: C, 52.56; H, 6.23; N, 15.48; Calcd: C, 52.46; H, 6.01; N, 15.30; IR υ (cm^{-1}) 1615 (C=N), 3135-3391 (OH and NH); MS: *m/z* $[M+H]^+$ 366; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4 Hz), 4.21 (q, 2H, CH_2 of ethoxy J = 7.4), 7.3 - 8.2 (m, 4H, ArH), 8.37 (br. s, 1H, NH).

2-ethoxy-4-hydrazinoquinazoline-D-mannose hydrazone 8c.

Yield 77% (from DMF/ethanol); m. p. 222-224ºC; *Anal.* for $C_{16}H_{22}N_4O_6$ (M. wt. 366); Found: C, 52.42; H, 6.04; N, 15.37; Calcd: C, 52.46; H, 6.01; N, 15.30; IR υ (cm^{-1}) 1618 (C=N), 3232-3459 (OH and NH); MS: *m/z* $[M+H]^+$ 366; ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, CH_3 of ethoxy J = 7.4 Hz), 4.23 (q, 2H, CH_2 of ethoxy J = 7.4), 7.0 - 8.1 (m, 4H, ArH), 8.29 (br. s, 1H, NH).

2-ethoxy-4-hydrazinoquinazoline-D-ribose hydrazone 8d.

Yield 61% (from DMF/ethanol); m. p. 217-219 ºC; *Anal.* for $C_{15}H_{20}N_4O_5$ (M. wt. 336); Found: C, 53.68; H, 6.04; N, 16.57; Calcd: C, 53.57; H, 5.95; N, 16.67; IR υ (cm^{-1}) 1616 (C=N), 3210-3439 (OH and NH); MS: *m/z* $[M+H]^+$ 336; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH3 of ethoxy J = 7.4 Hz), 4.23 (q, 2H, CH_2 of ethoxy J = 7.4), 7.1- 8.2 (m, 4H, ArH), 8.52 (br. s, 1H, NH).

2-ethoxy-4-hydrazinoquinazoline-D-arabinose hydrazone 8e.

Yield 63% (from ethanol); m. p. 197-198 ºC; *Anal.* for $C_{15}H_{20}N_4O_5$ (M. wt. 336); Found: C, 53.62; H, 5.98; N, 16.63; Calcd: C, 53.57; H, 5.95; N, 16.67; IR υ (cm^{-1}) 1613 (C=N), 3230-3414 (OH and NH); MS: *m/z* $[M+H]^+$ 336; ^{1}H-NMR (DMSO-d_6) δ 1.11 (t, 3H, CH_3 of ethoxy J = 7.4 Hz), 4.19 (q, 2H, CH_2 of ethoxy J = 7.4), 7.2- 8.3 (m, 4H, ArH), 8.33 (br. s, 1H, NH).

Synthesis of per-O-acetylsugar [1-acetyl-1-(2-ethoxyquinazolin-4-yl)] hydrazones 9a-e.

A cold solution of 8a-e (0.02 mol) in dry pyridine (50 mL) was treated with Ac_2O (50 mL). The mixture was kept overnight at room temperature, with occasional shaking, and then poured onto crushed ice, and the residue was collected by filtration, washed repeatedly with water, dried and recrystallized from ethanol affording product 9a-e .

2,3,4,5,6-Penta-O-acetyl-D-glucose[1-acetyl-1-(2-ethoxyquinazolin-4-yl)] hydrazones 9a.

Yield 58 %; m. p. 63 - 64 ºC; *Anal.* for $C_{28}H_{34}N_4O_{12}$ (M. wt. 618); Found: C, 54.41; H, 5.56; N, 9.01; Calcd: C, 54.37; H, 5.50; N, 9.06; IR υ (cm^{-1}) 1608 (C=N), 1673 (NAc), 1718 (OAc); MS: *m/z* $[M+H]^+$ 618; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4 Hz), 2.02, 2.04, 2.10 (3s, 15H, 5 OAc), 2.50 (s, 3H, NAc), 4.1(q, 2H, CH_2 of ethoxy J = 7.4),4.15 (dd, 1H, H-6'), 4.26 (dd, 1H, H-6), 5.02-5.10 (m, 1H, H-5), 5.44-5.55 (m, 2H, H-4, H-3), 5.62 (dd, 1H, H-2), 6.74 (d, 1H, H-1), 7.11-8.23 (m, 4H, ArH).

2,3,4,5,6-Penta-O-acetyl-D-galactose[1-acetyl-1-(2-ethoxyquinazolin-4-yl)] hydrazones 9b.

Yield 80 %; m. p. 158 -160 ºC; *Anal.* for $C_{28}H_{34}N_4O_{12}$ (M. wt. 618); Found: C, 54.43; H, 5.53; N, 9.03; Calcd: C, 54.37; H, 5.50; N, 9.06; IR υ (cm^{-1}) 1633(C=N), 1692(NAc), 1722(OAc); MS: *m/z* $[M+H]^+$ 618; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4 Hz), 1.96, 1.99, 2.02, 2.03, 2.08, 2.09 (5s, 15H, 5OAc), 2.47(s, 3H, NAc), 3.88(dd, 1H, H-6'), 4.1(q, 2H, CH_2 of ethoxy J = 7.4), 4.28 (dd, 1H, H-6), 5.38-5.88 (m, 4H, H-5, H-4, H-3, H-2), 6.55 (d, 1H, H-1), 7.15- 8.33 (m, 4H, ArH).

2,3,4,5,6-Penta-O-acetyl-D-mannose[1-acetyl-1-(2-ethoxyquinazolin-4-yl)] hydrazones 9c.

Yield 63 %; m. p. 58 - 60 ºC; *Anal.* for $C_{28}H_{34}N_4O_{12}$ (M. wt. 618); Found: C, 54.40; H, 5.51; N, 9.02; Calcd: C, 54.37; H, 5.50; N, 9.06; IR υ (cm^{-1}) 1615(C=N), 1682(NAc), 1711(OAc); MS: *m/z* $[M+H]^+$ 618; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4 Hz), 2.05, 2.06, 2.10 (3s, 15H,5OAc), 2.52 (s, 3H, NAc), 4.11(q, 2H, CH_2 of ethoxy J = 7.4), 4.14 (dd, 1H, H-6'), 4.28 (dd, 1H, H-6), 5.22-5.40 (m, 1H, H-5), 5.42 (d, 1H, H-4), 5.54 (dd, 1H, H-3), 5.66 (dd, 1H, H-2), 6.68 (d, 1H, H-1), 7.00 - 8.13 (m, 4H, ArH).

2,3,4,5-Tetra-O-acetyl-D-ribose[1-acetyl-1-(2-ethoxyquinazolin-4-yl)] hydrazones 9d.

Yield 52 %; m. p. 92 - 93 ºC; *Anal.* for $C_{25}H_{30}N_4O_{10}$ (M. wt. 546); Found: C, 54.97; H, 5.54; N, 10.29; Calcd: C, 54.94; H, 5.49; N, 10.26; IR υ (cm^{-1}) 1619 (C=N), 1682 (NAc), 1725 (OAc); MS: *m/z* $[M+H]^+$ 546; ^{1}H-NMR (DMSO-d_6) δ 1.91, 1.94, 2.00, 2.19 (4s, 12H, 4OAc), 1.13 (t,3H, CH_3 of ethoxy J = 7.4 Hz), 2.51 (s, 3H, NAc), 4.15 (q, 2H, CH_2 of ethoxy J = 7.4), 4.22 (dd, 1H, H-5'), 4.39 (dd, 1H, H-5), 5.38 - 5.40 (m, 1H, H-4), 5.78 (dd, 1H, H-3), 6.01 (dd, 1H, H-2), 6.59 (d, 1H, H-1), 7.10- 8.32 (m, 4H, ArH).

2,3,4,5-Tetra-O-acetyl-D-arabinose[1-acetyl-1-(2-ethoxyquinazolin-4-yl)] hydrazones 9e.

Yield 67 %; m. p. 108-111 ºC; *Anal.* for $C_{25}H_{30}N_4O_{10}$ (M. wt. 546); Found: C, 54.95; H, 5.52; N, 10.27; Calcd: C, 54.94; H, 5.49; N, 10.26; IR υ (cm^{-1}) 1610 (C=N), 1682 (NAc), 1715 (OAc); MS: *m/z* $[M+H]^+$ 546 (42.9), 548 (14.3), 504 (10.5), 506 (3.4), 444 (25.0), 446 (8.2), 401 (13.3), 403 (4.4), 341 (17.6), 343 (5.8), 257 (40.5), 259 (13.4), 215 (100), 217 (33.3), 175 (16.2), 177 (5.3), 157 (2.8), 159 (0.9), 130 (58.3), 132 (0.1).

General method for preparing 1-(alditol-1-yl) - 5-ethoxy[1,2,4]triazolo[1,5-c] quinazoline 10a-e.

A 2 M solution of iron (III) chloride in EtOH (2 mL) was added dropwise to a boiling solution of 8a-e (0.01 mol) in ethanol (50 mL). Heating was continued for 10 min and the mixture was then kept overnight at room temperature. The product was filtered, washed repeatedly with water, air dried and recrystallized from EtOH affording product 10a-e.

1-(D-gluco-pentitol-1-yl)-5-ethoxy[1,2,4]triazolo[1,5-c] quinazoline 10a.

Yield 90 %; m. p. 85-87 ºC; *Anal.* for $C_{16}H_{20}N_4O_6$ (M. wt. 364); Found: C, 52.79; H, 5.52; N,15.43; Calcd: C, 52.75; H, 5.49; N, 15.38; IR υ (cm^{-1}) 1613 (C=N), 3240-3454 (OH); MS: *m/z* $[M+H]^+$ 364 (14.4), 366 (4.8), 333 (0.3), 335 (0.1), 303 (0.6), 305 (0.2), 273 (0.6), 275 (0.2), 214 (100), 216 (33.4), 187 (59.8), 189 (19.6), 175(6.2), 177 (2.1), 157 (2.5), 159 (0.5), 130(63.4), 132 (0.1).

1-(D-galacto-pentitol-1-yl)-5-ethoxy[1,2,4]triazolo[1,5-c] quinazoline 10b.

Yield 85 %; m. p. 80 ºC; *Anal.* for $C_{16}H_{20}N_4O_6$ (M. wt. 364); Found: C, 52.77; H, 5.56;N, 15.48; Calcd: C, 52.74; H, 5.49; N, 15.38; IR υ (cm^{-1}) 1619 (C=N), 3340-3450 (OH);

1-(D-manno-pentitol-1-yl)-5-ethoxy[1,2,4]triazolo[1,5-c] quinazoline 10c.

Yield 75 %; m. p. 101-102ºC; *Anal.* for $C_{16}H_{20}N_4O_6$ (M. wt. 364); Found: C, 52.82; H, 5.59; N, 15.53; Calcd: C, 52.74; H, 5.49; N, 15.38; IR υ (cm^{-1}) 1615 (C=N), 3290-3466 (OH); ^{1}H-NMR (DMSO-d_6) δ 3.97-4.20 (m, 2H, H-5', H-5), 4.40 (dd, 1H, H-4), 4.62 (dd, 1H, H-3), 5.03 (t, 1H, H-2), 5.22 (d, 1H, H-1), 7.10- 8.32 (m, 4H, ArH).

1-(D-ribo-pentitol-1-yl)-5-ethoxy[1,2,4]triazolo[1,5-c] quinazoline 10d.

Yield 68 %; m. p. 128 ºC; *Anal.* for $C_{15}H_{18}N_4O_5$ (M. wt. 334); Found: C, 53.92; H, 5.43; N, 16.81; Calcd: C, 53.89; H, 5.39; N, 16.77; IR υ (cm^{-1}) 1623 (C=N), 3310 - 3444 (OH); MS: *m/z* $[M+H]^+$ 334 (21.1), 336 (7.0), 303 (3.5), 305 (1.1), 273 (0.6), 275 (0.2), 214 (9.9), 216 (3.2), 187 (100), 189 (33.2), 175 (5.4), 177 (1.1), 157 (1.8), 159 (0.3), 130(56.4), 132 (0.1).

1-(D-arabino-pentitol-1-yl)- 5-ethoxy[1,2,4]triazolo[1,5-c] quinazoline 10e.

Yield 63 %; m. p. 108 ºC; *Anal.* for $C_{15}H_{18}N_4O_5$ (M. wt. 334); Found: C, 53.97; H, 5.46; N, 16.88; Calcd: C, 53.89; H, 5.39; N, 16.77; IR υ (cm^{-1}) 1619 (C=N), 3320-3480 (OH); ^{1}H-NMR (DMSO-d_6) δ 3.49-3.75 (m, 2H, H-4', H-4), 4.18-4.39 (m, 2H, H-3, H-2), 5.03 (d, 1H, H-1),7.1-8.3 (m, 4H, ArH).

Preparation of 1-(penta-O-acetylsugar-1-yl)-5-ethoxy-1,2,4-triazolo[1,5-c] quinazoline 11a-e

A cold solution of 10a-e (0.002 mol) in dry pyridine (10 mL) was treated with Ac_2O (6 mL), and the mixture was kept overnight at room temperature, with occasional shaking, and then poured onto crushed ice, and the residue was collected by filtration, washed repeatedly with water, dried and recrystallized from ethanol affording product 11a-e.

1-(1,2,3,4,5-penta-O-acetyl-D-glucopentitol-1-yl)-5-ethoxy-1,2,4-triazolo[1,5-c] quinazoline 11a.

Yield 78 %; m. p. 71 ºC; *Anal.* for $C_{26}H_{30}N_4O_{11}$ (M. wt. 574); Found: C, 54.42; H, 5.26; N, 9.83; Calcd: C, 54.36; H, 5.23; N, 9.76; IR υ (cm^{-1}) 1650 (C=N), 1725 (OAc); ^{1}H-NMR (DMSO-d_6) δ 1.99, 2.01, 2.03 (3s, 12H, 4OAc), 3.98 (dd, 1H, H-5'), 4.30 (dd, 1H, H-5), 5.30-5.52 (m, 3H, H-4, H-3, H-2), 5.86 (d, 1H, H-1), 7.26-8.30 (m, 4H, ArH).

1-(1,2,3,4,5-penta-O-acetyl-D-galactopentitol-1-yl)-5-ethoxy-1,2,4-triazolo[1,5-c] quinazoline 11b.

Yield 81 %; m. p. 66 ºC; *Anal.* for $C_{26}H_{30}N_4O_{11}$ (M. wt. 574); Found: C, 54.39; H, 5.24; N, 9.79; Calcd: C, 54.36; H, 5.23; N, 9.76; IR υ (cm^{-1}) 1619(C=N), 1719(OAc); MS:*m/z* $[M+H]^+$ 574 (19.3), 576 (6.3), 501 (27.4), 503(9.1), 429(31.7), 431 (10.5), 315 (9.5), 317 (3.2), 214 (100), 216 (33.3), 187 (59.8), 189 (19.9), 175(5.4), 177 (2.1), 157 (1.3), 159 (0.2), 130 (48.4), 132 (0.1).

1-(1,2,3,4,5-penta-O-acetyl-D-mannopentitol-1-yl)-5-ethoxy-1,2,4-triazolo[1,5-c]quinazoline 11c.

Yield 63 %; m. p. 76 ºC; *Anal.* for $C_{26}H_{30}N_4O_{11}$ (M. wt. 574); Found: C, 54.41; H, 5.27;N, 9.81; Calcd: C, 54.36; H, 5.23; N, 9.76; IR υ (cm^{-1}) 1639 (C=N), 1745 (OAc); ^{1}H-NMR (DMSO-d_6) δ1.96, 1.99, 2.03, 2.08(4s, 12H, 4OAc), 3.90(dd, 1H, H-5'), 4.32 (dd, 1H, H-5), 5.30-5.53 (m, 3H, H-4, H-3, H-2), 5.76 (d, 1H, H-1), 7.15-8.31(m, 4H, ArH).

1-(1,2,3,4-tetra-O-acetyl-D-ribopentitol-1-yl)-5-ethoxy-1,2,4-triazolo[1,5-c] quinazoline 11d.

Yield 58 %; m. p. 71 ºC; *Anal.* for $C_{23}H_{26}N_4O_9$ (M. wt. 502); Found: C, 54.94; H, 5.22; N, 11.14; Calcd: C, 54.98; H, 5.18; N, 11.16; IR υ (cm^{-1}) 1629 (C=N), 1731 (C=O); MS: *m/z* $[M+H]^+$ 502 (21.3), 504 (7.1), 430 (17.6), 432 (5.8), 358 (21.4), 360 (7.1), 286(16.9), 288(5.6), 214 (100), 216 (33.2), 187(8.4), 189(2.8), 175(5.8), 177(1.9), 157(1.6), 159 (0.1), 130 (59.3), 132 (0.1). ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4), 2.03, 2.06, 2.08 (3s, 12H, 4OAc), 4.05 (dd, 2H, H-4', H-4), 4.35-4.55 (m, 1H, H-3), 5.56(dd, 1H, H-2), 5.74 (d, 1H, H-1), 7.1 - 8.3 (m, 4H, ArH).

1-(1,2,3,4-tetra-O-acetyl-D-arabinopentitol-1-yl)-5-ethoxy-1,2,4-triazolo[1,5-c] quinazoline 11e. Yield 67%; m. p. 98ºC; *Anal.* for $C_{23}H_{26}N_4O_9$ (M. wt. 502); Found: C, 54.92; H, 5.24; N, 11.19; Calcd: C, 54.98; H, 5.18; N, 11.16; IR υ (cm^{-1}) 1618 (C=N); ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, CH_3 of ethoxy J = 7.4), 1.81, 1.86, 1.91, 2.19 (4s, 12H, 4 OAc), 4.22 (dd, 2H, H-4', H-4), 5.35 - 5.41 (m, 1H,H-3), 5.76 (dd, 1H, H-2), 6.02 (d, 1H, H-1), 7.17 - 8.32 (m, 4H, ArH).

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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Table 1. Antimicrobial activity

Compd No	Gram-positive Bacteria			Gram-negative Bacteria		
	Staphy-lococcussp	Strepto-bacillussp	Bacillus-subtillissp	Esch-colisp	Strepto-bacillusussp	Pseudo-monassp
8a	-	+	-	-	-	-
8b	-	+	-	-	-	-
8c	-	+	+	-	-	-
8d	-	+	-	-	-	-
8e	-	+	-	-	-	-
9a	-	+	+	-	+	-
9b	-	+	+	+	-	-
9c	-	+	+	-	-	+
9d	-	+	-	+	-	-
9e	-	+	+	+	-	+
10a	-	+	+	+	-	-
10b	-	+	+	+	+	+
10c	-	++	++	+	+	+
10d	+	+++	++	+	-	-
10e	+	+	+	+	-	-
11a	-	+++	++	+	+	+
11b	-	++	+	+	-	-
11c	-	++	+	+	-	-
11d	+	+	+	+	-	-
11e	-	++	+	+	-	-

Computed Tomographic Pattern of Physiological Intracranial Calcifications in a City in Central Africa

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Abstract

Objective: Intracranial calcifications underlie certain brain diseases which may be de novo or systemic. But calclfications un-connected to pathologies are classified physiological. **Aim:** To evaluate physiological intracranial calcifications in Douala with establishment of earliest age range of detection. **Materials and Methods:** Prospective study of brain computed tomograms was done from April to October 2009 using Schumadzu CT Scan machine. Axial, reconstructed and bone window images as well Hounsfield unit measurements were used for final evaluations. Results were analysed with SSPS 3. **Results:** 132 patients with 75 males and 57 females were studied and 163 separate calcifications were identified due to co-existent calcifications. The highest calcification was in choroid plexi, constituiting 56.82% of the studied population. This was followed by pineal gland. Both were commonly co-existent with advancing age. These calcifications were first seen at 10-19years. No type of physiological intracranial calcification was seen below age 10. The least calcification of 0.76% of population was in dentate nucleus. **Conclusion:** No intra-cranial physiological calcifications started earlier than 9years in Douala, a city in Cameroon, Central Africa.

Keywords: Intracranial calcifications, Pineal, Choroid plexus, Basal ganglia, Computed tomography

1. Introduction

Intracranial physiological calcifications are unaccompanied by any evidence of disease and have no demonstrable pathological cause (Daghighi *et al*, 2007, pp115-9).They are due to calcium and sometimes iron depositions in the blood vessels of the different structures of the brain (Daghighi *et al*, 2007, pp115-9). CT remains the imaging modality of choice in the detection of two processes: acute intracranial haemorrhage and calcifications (Glo & Zee, 1998, pp542-58). Computed tomography is a veritable imaging modality for best identification and characterizations of any intracranial calcifications (Sarmiento de La Iglesia *et al*, 2006, pp19-26, Rozylo-Kalinowaska *et al*, 2003, pp602-5). It is also the most sensitive means of detections of these calcifications (Daghighi *et al*, 2007, pp115-9). A number of factors including slice thickness, window width, and level may affect the detectability of calcification on CT (Glo & Zee, 1998, pp542-58). It is superior to conventional radiography in this respect as the specific regional localisation of the calcification can be ascribed. Besides, most calcifications are not visualized on plain radiographs especially if the CT attenuation values are less than 200 Hounsfield units (HU) (Patel, 1987, pp177-80). CT is superior to MRI in the detection of

calcifications as .MRI cannot reliably rule out or determine the presence of calcifications (Glo & Zee, 1998, pp542-58 (Sarmiento de La Iglesia *et al*, 2006, pp19-26). Also, the magnetic resonance imaging findings for intracranial calcifications previously demonstrated at CT are variable and unspecific (Glo & Zee, 1998, pp542-58 (Sarmiento de La Iglesia *et al*, 2006, pp19-26). The most frequent appearance of intracranial calcifications on T1W sequence is an area isointense with the cerebral cortex while most frequent appearance on T2W sequence is focus of hypointensit (Sarmiento de La Iglesia *et al*, 2006, pp19-26).

Depositions of calcium salts in intracranial structures forms calcifications (Rozylo-Kalinowaska, *et al.,* 2003, pp602-5). Intra-cranial calcifications can be sub-divided into physiological or pathological (Chapman& Nakiel, 2003, p428). Physiological intracranial calcifications are seen in sites like pineal gland, choroid plexus, basal ganglia, lens, cerebellum, ligaments, dura, vessels,arachnoid granulations and habenula.(Daghighi *et al*., 2007, pp115-9, Dahnert,2003, p237).

Physiological intracranial calcifications are asymptomatic and detected by neuro-imaging (Basak, 2009, pp 220-224). They may have no clinical importance but may be critical findings in diagnosing underlying pathology (The Intracranial calcifications, The Free-online Librarary),. Most importantly, these statistics can be used in comparing physiological and pathological intracranical calcifications (Daghighi *et al*, 2007, pp115-9). To the best of our knowledge, the publications on this topic is scant in this African region, encouraging us in pursuit of this study.

To evaluate the pattern of physiological intracranial calcifications in Douala and observation of earliest age range of each calcification

2. Materials and Methods

A prospective study of cranio-cerebral CT done from 8/4/09 to 18/10/2009 was undertaken in Department of Radiology, Polyclinic Bonanjo, Douala, Cameroon, (a tertiary health care provider). This was done using Schumadzu CT scan machine with continuous rotational system. Axial sections were done using slice tissue thickness of 2mm from the base of the skull to the sella turcica, thence 5mm from the sella to the vertex. IV Iopamidol at 1mm/kg was given when indicated. Images were reconstructed to achieve sagital and coronal images. Hounsfield unit (HU) measurement and bone window were employed in some cases of doubt so as to differentiate calcifications from acute haemorrhage. The HU of calcifications is above 100HU while HU of acute haemorrhage is in the range of 60-90HU taking into account the effect of partial volume averaging. The structures evaluated consisted of (a) the pineal gland, (b) the choroid plexus, (c) the habenula, (d) the basal ganglia, (e) the tentorium cerebelli, sagittal sinus and falx cerebri, (f) vessels and (g) lens and other structures which could be calcified. Patients consent was obtained. The ethical board review committee's approval was obtained. All patients with any pathology linked or associated with intra-cranial calcifications and those with improper data documentation were excluded. Results were analysed using SSPS 3.

3. Results

132 of 174 brain computed tomograms were considered optimal for our study and analysed. There were 75 males (56.82%) and 57 females (43.18%) with age range of 0.4-81years and mean age of 45.5years. Largest population studied was 38 in 40-49 year age range with 24 males and 14 females. This age range also had the highest number of intra-cranial calcifications of 58 (35.58%) of the total number of 163 intracranial calcifications detected in this study. In this 40-49years, 40 (68.96%) calcifications were seen in males and 18 (31.04%) females with male: female ratio 2.22:1. This is followed by 26 calcifications (14 in males, 12 in females with ratio of male to female 1.6:1) in the 50-59 age range. 8 calcifications (2 in males and 6 in females with male to female ratio 1:3) were seen in 80-89 year age range. No calcification was seen below 10 years of age in both sexes. In older age of 70 years and above, females had more calcifications whereas from 69years and below, males had more calcifications. The highest number of calcifications of 75, constituting 46.01% of total number of detected calcifications and 56.82% of total studied populations was seen in the choroid plexus (atria), with 44 calcifications in males and 31 in females (male: female ratio is 1.42:1). Highest number of choroid calcification 24 (32%) was in the 40-49 year age range with male predominance followed by 15 in the 50-59years with female predominance.

Pineal gland calcifications were 61 (37.42%) of all calcifications and 46.21% of studied population. It was the second highest with 36 cases in males and 25 in females (male: female 1.44:1). Peak age is also 40-49years followed by 50-59 years. Least calcifications of 1 case were in the dentate nucleus in a female. The earliest age of calcification of choroid plexus/ pineal gland in males was in 10-19 year age range whereas earliest calcifications of choroid plexus/pineal gland in females were 20-29 years. Both show equal predominance of choroid plexus/pineal calcifications with increasing age, females tend to have increasing pineal gland calcifications than males.

Anterior falx calcifications (21) is more than posterior falx calcification 3 (anterior to posterior 7:1). Both show male predominance (anterior falx male: female ratio 1.6:1) and (posterior falx male: female ratio 2:1). Earliest calcification of anterior falx started at 20-29 year age range. Highest incidence is also at 40-49 years, with 6 cases in males and 3 cases in females (male to female ratio 2:1). Posterior falx calcifications started at 40-49 years.

4. Discussion

Intra-cranial calcifications can be physiological or pathological. When physiological, it is asymptomatic and detected incidentally in neuro-imaging (Verulashirili *et al*, 2006, pp 39-43). Intracranial calcifications seen on computed tomography (CT) are the most common finding in the everyday practice of neuroradiology, because noncontrast-enhanced CT of the head is the preferred imaging modality worldwide for the initial evaluation of patients with acute or chronic neurological problems (The Intracranial calcifications,The Free-online Librarary). Possible sites of physiological calcifications are pineal gland, choroid plexi, habenula, dura (falx cerebri, tentorium, vault), ligaments (petro-clinoid, interclinoid), dura, pacchonian bodies, basal ganglia, cerebellum, pituitary gland, and lens (The Intracranial calcifications, The Free-online Librarary, Sutton, 1995, p746, Medical definitions-online). This physiological calcifications is thought to be an adaptive metabolic processes which depend on many factors, among which include the individual constitutional ground and aging (Guja *et al*, 2005, pp1-8).

In this study, understandably no intracranial calcifications of any type was seen in the young age range of 0-9 years as in other studies (Guja *et al*, 2005, pp1-8). Presence of pineal calcifications in a child less than 6 years suggests neoplasm (Dahnert, 2003, p237, Medical definition, online). Doyle and Anderson, 2006, pp822-6) observed 1% of pineal calcifications in those less than 6years (Menon & Harinarayan, 2009, pp55-60). Males started choroid plexus calcification earlier than females in this study, 10-19years and 20-29years respectively. Evidence of choroid plexus calcifications has been recorded in 9.5% of children from 9 to 15 years of age in some reports. (The Intracranial calcifications,The Free-online Librarary).

Individual calcifications were 163 from 132 patients. This is because a single patient can have multiple calcifications. Such co-existence was commonly between pineal gland and choroid plexus and between anterior and posterior falx cerebri. Choroid plexus calcification is known to be associated with pineal gland calcification Doyle and Anderson, 2006, pp822-6)

In this study, the commonest calcifications noted were choroid plexi and pineal glands 46.01% (56.82% of total population) and 37.42% (46.21% of total population) respectively. This choroidal plexus calcification predominance has been reported by other authors (Menon & Harinarayan, 2009, pp134-135). However a reversal of this pattern was noted by other studies (Daghighi *et al*, 2007, pp115-9; Admassie & Mekonne, 2009, pp55-60). Admassie & Mekonne, 2009, pp55-60Admassie and Mekonne reported an overall incidence of normal pineal gland calcifications of 72.0% and that of choroid plexus 43.3%. Similarly, Daghighi *et al* observed 71% of their studied population had pineal gland calcifications while 66.2% had choroid plexus calcifications (Daghighi *et al*, 2007, pp115-9). Both choroid and pineal calcifications in this study peaked at 40-49 years with male predominance. The physiologic calcifications of the choroid plexus are very common after the age of 40 years (The Intracranial calcifications, The Free-online Librarary).

The pattern of choroid plexi calcification in this study was symmetrical and bilateral in 100% of positive cases of intracranial calcifications. Such calcification increased with age with maximum of 80% in 80-89 years. Choroid plexi calcifications are known to occur in all ventricles, most commonly in the glomus within the atrium of lateral ventricles near foramen of Monro. Other sites are tela choroidea of third ventricles, roof of fourth ventricle along foramen of Luschaka (Dahnert, 2003, p237). In this study, all the calcifications were in the atria of lateral ventricles.

Physiologic pineal calcification is more common in children than previously reported, mostly because of improving computed tomography technology. The pathogenesis of pineal gland calcifications is that the pineal organ (pineal gland, epiphysis cerebri) contains several calcified concretions called "brain sand" or acervuli corpora arenacea. (Vigh *et al*, 1998, pp851-70) Predominantly composed of calcium and magnesium salts, corpora arenacea are numerous in old patients. In smaller number they can be present in children as well. (Vigh *et al*, 1998, pp851-70). Corpora arenacea occur not only in the actual pineal tissue but also in the leptomeninges, habenular commissure and in the choroid plexus (Vigh *et al*, 1998, pp851-70). The size of physiological pineal calcification is usually 3-5 mm, if greater than 1 cm, raise concerns for underlying tumor,like pinealoma, teratoma, AV malformation (Medical definition, online). Usually, pineal gland calcifications are in the form of cluster of amorphous, irregular densities or it may be solitary (Medical definition, online). But the incidence of pineal calcification noted in this study across all ages was 46.21% of the population compared to $2/3_{rd}$ of the population in some literature (Dahnert, 2003, p237; Admassie & Mekonne, 2009, pp55-60). Some literatures

recorded 40% of pineal calcification at 20 years, but only 15.79% of our studied population below 20years and 30% below 30years had physiological pineal gland calcifications (Dahnert, 2003, p237, The Free-online Library). The pattern of pineal calcification across ages in this study is that females showed more calcifications in older age group of 70 years and above whereas males had more calcifications below 69 years. The plausible explanation is the complete removal of the effect of the female sex hormonal control. The incidence of pineal gland and choroid plexus calcifications show male bias in this study as in other studies. The incidence of normal pineal gland and choroid plexus calclfications were higher in males than females by 13.1% and 6.0% respectively Admassie & Mekonne, 2009, pp55-60). The frequency of pineal gland and choroid plexus calcifications show a steady increase in both sex groups Admassie & Mekonne, 2009, pp55-60).

The only dura calcifications in this study were in the falx cerebri. The anterior to posterior falx calcification ratio is 7:1. Males show predominance in the above mentioned two dural calcifications. 15.91% of this studied population had falx calcification compared to 10% of populations in some studies (Dahnert, 2003, p237. Anterior falx calcification was first noted at above 19years (compare to >3years in some literatures) while posterior falx started at a later age of 40-49 (Dahnert, 2003, p237). Displacement of falx calcifications has been a good indicator of raised intra-cranial pressure or intracranial mass lesion in adults in earlier days of only conventional radiography. This is because physiologic calcifications of the dura are very common in older age groups and are usually located in the falx or the tentorium (The intracranial calcifications,The Free-onlineLibrary).. Presence of dural calcifications in children should raise the suspicion of underlying pathology, mainly basal-cell nevus syndrome. (The intracranial calcifications, The Free-onlineLibrary)

Brain calcinosis syndrome (BCS) is usually defined as bilateral calcium accumulation in the brain parenchyma, most often within the basal ganglia. Various terms have been used to describe basal ganglia calcification including calcification(s) of the basal ganglia, basal ganglia calcification(s), Fahr syndrome, intracranial calcification, pallidal calcification, and striopallidodentate calcinosis (Basak, 2009, pp220-224). More than 50 reported clinical conditions have been associated with BCS, including sporadic entities and the heredofamilial conditions (Basak, 2009, pp220-224). Basal ganglia are supplied by perforating arteries which are prone to small vessel ischaemia with increasing age (Rossi *et al*, 1993, 192-8). The review of literature shows, now, that there is no definite pathogenesis of basal ganglia calcifications (Rossi *et al*, 1993, 192-8). The calcifications in the basal ganglia are usually punctate and are located within the globus pallidus, the head of the caudate nucleus, and the putamen and are very common in middle-aged individuals and the elderly. The intracranial calcifications, The Free-online Library), Computed tomography is superior to conventional skull radiographs in detecting basal ganglia (Rossi *et al*, 1993, 192-8). Basal ganglia calcifications have been associated with different conditions, abnormality with calcium-phosphorus metabolism being the most of these associations (Rossi *et al*, 1993, 192-8). Basal ganglia calcifications incidence of 1.52% in our studied population is within the known range of 0.3-1.5% (Basak, 2009, pp220-224, Verulashvili *et al*, 2006, pp39-43). Though lower values of 0.8% in other studies has been recorded (Daghighi *et al*, 2007, pp115-9). The earliest basal ganglia calcification was first noted at middle age of life (40-49years) in this study which agrees with other assertions (The intracranial calcifications, The Free-online Library). The presence of basal-ganglia calcifications in patients <30 years of age should prompt careful clinical evaluation to rule out another etiology like hyperparathyroidism, hypoparathyroidism,congenital disorders such as Fahr syndromp (Intracranial calcifications, The Free-online Library). In general, pathological basal ganglia calcification is due to various causes such as congenital, metabolic disorders, idiopathic, aging neuro-degenerative(Fahr syndrome,Cockayne syndrome), infectious (cytomegalovirus, toxoplasmosis) and genetic disorders, birth anoxia, radiation, lead and carbon monoxide poisoning and others (Basak, 2009, pp220-224; The Free-online Library; Menon & Harinarayam, 2009, pp134-135; Rossi *et al*, 1993, 192-8; Erdem *et al*, 1994 pp111-22). Hypoparathyroidism and pseudohypoparathyroidism are the most common causes of pathological basal ganglia calcification (Basak, 2009, pp220-224; Verulashvili *et al*, 2006, pp39-43). Before CT, 70% to 80% of brain calcification detected on plain skull radiography was associated with hypoparathyroidism (Medical definition, online).

All types of intracranial calcifications increased at old age except for lens and other non-defined calcifications (Daghighi *et al*, 2007, pp115-9). The calcification of the intra-cavernous segment of internal carotid artery (ICA) or para-sellar ICA is thought to be due to intima carotid siphon (double –bent shape of the para-sellar ICA) (Weninger *et al*, 1999, pp85-97). Physiologic calcifications can be seen in the cerebellum, with the dentate nucleus being the most common site as noted in this study (The intracranial calcifications, The Free-online Library).

5. Conclusion

No any type of intracranial calcification was seen below 10 years of age in Douala. The commonest physiological intracranial calcification is choroid plexi, followed by pineal gland. Both calcifications started at 10-19 year age range and both also show male predominance, Choroid plexus calcifications were all bilateral and

symmetrical. Choroid plexus and pineal gland calcifications were co-existent with advancing age. The least calcification is in the dentate nucleus of cerebellum.

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Table 1. Showing the numbers of intracranial calcifications at different locations in age groups and sex

Age range	Ant	falx	Post	falx	Choroid	plex	Pineal	gland	Basal	gland	Dentate	nucl	Habenu	Liga	Arach	Lens	Total
	M	F	M	F	M	F	M	F	M	F	M	F				F	
0-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10-19	-	-	-	-	3	-	3	-	-	-	-	-	-	-	-	-	6
20-29	2	1	-	-	6	1	5	1	-	-	-	-	-	-	-	-	16
30-39	1	2	-	-	5	4	3	4	-	-	-	-	-	-	-	-	19
40-49	6	3	1	-	16	8	16	7	1	-	-	-	-	-	-	-	58
50-59	2	-	-	-	7	8	5	4	-	-	-	-	-	-	-	-	26
60-69	1	-	-	-	3	2	1	2	-	-	-	-	-	-	-	-	9
70-79	1	1	1	1	3	5	2	4	-	1	1	-	-	-	-	-	21
80-89				-	1	3	1	3	-	-	-	-	-	-	-	-	8
Total	13	8	2	1	44	31	36	35	1	1	1	-	-	-	-	-	163

Distribution of Studied Population			
Age Ran	Males	Females	Total
0-9	8	2	10
10,-19	5	4	9
20-29	8	3	11
30-39	4	13	17
40-49	24	14	38
50-59	14	8	22
60-69	6	2	8
70-79	4	8	12
80-89	2	3	5
90-99	0	0	0
Total	75	57	132

Table 2. Showing total number calcifications pattern across age ranges

Age Range	Males	Females	
0-9	0	0	0
10--19	6	0	6
20-29	13	3	16
30-39	9	10	19
40-49	40	18	58
50-59	14	12	26
60-69	5	4	9
70-79	7	14	21
80-89	2	6	8
90-99	0	0	0
Total	96	67	163

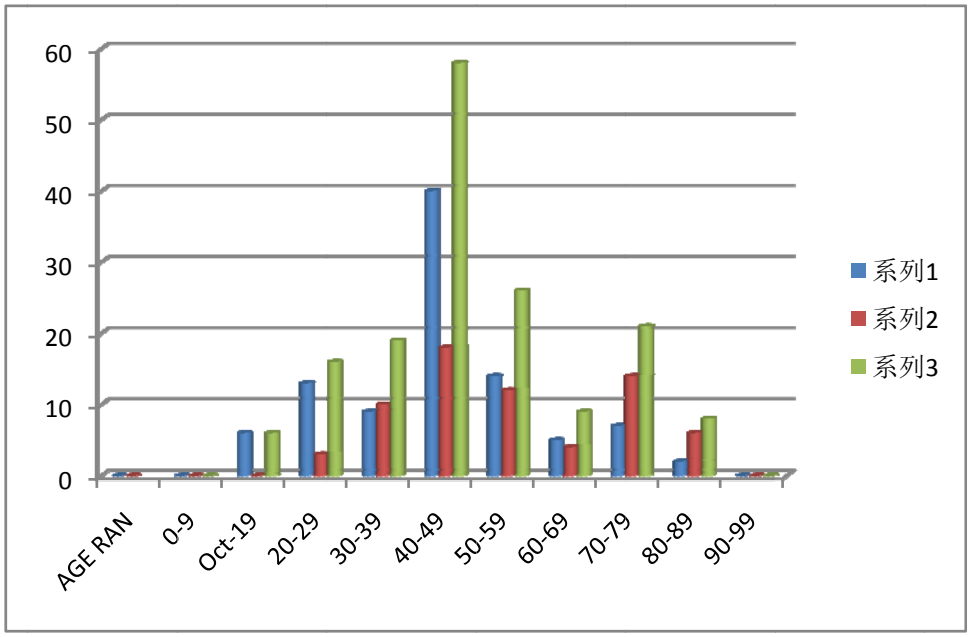

Figure 1. Calcification distributions across age ranges

Series 1=Males, Series 2=Females, Series 3=Total

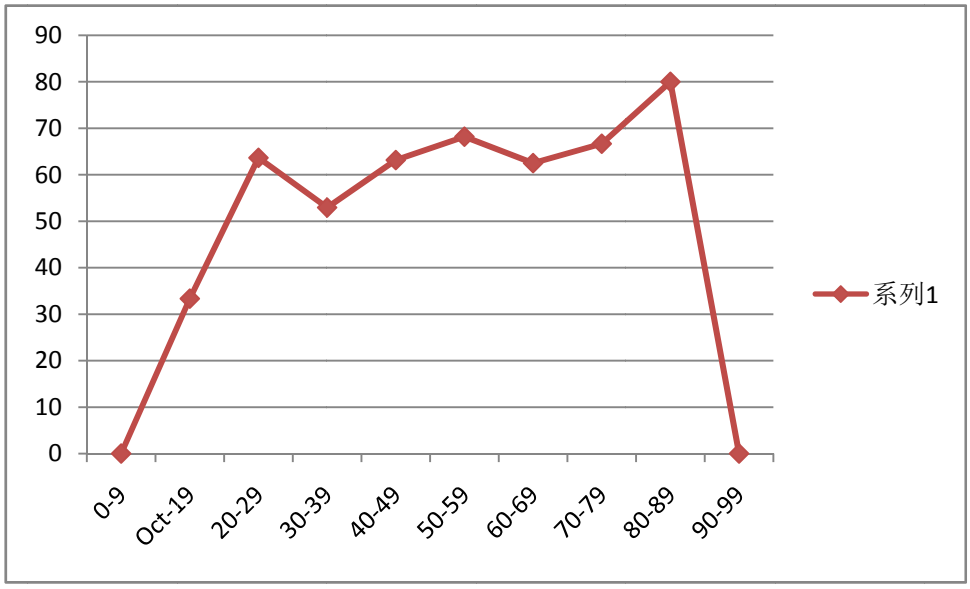

Figure 2. Choroid plexus graphical representations of %calcification pattern across age ranges

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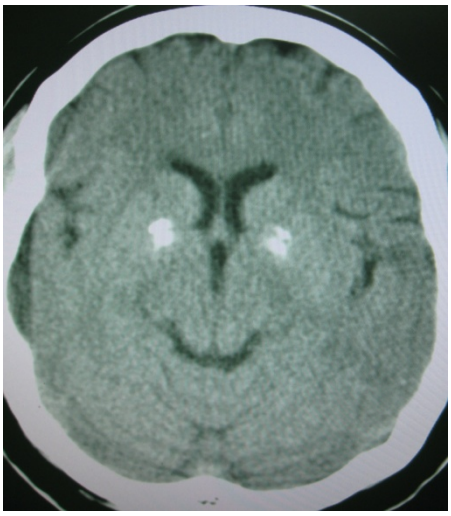

Figure 3. Axial brain ct showing bilateral symmetrical basal ganglial calcification

Figure 4. Axial brain ct showing anterior falx calification

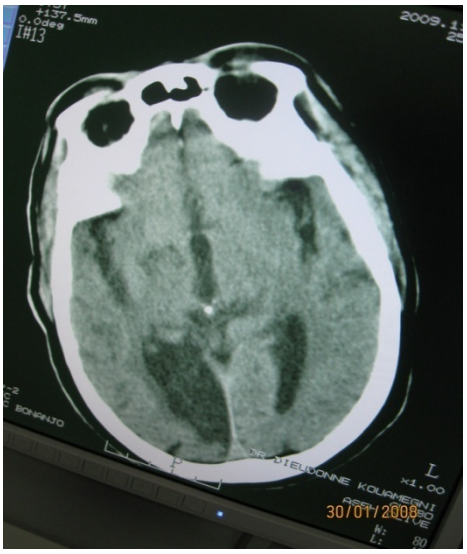

Figure 5. Axial brain ct showing pineal gland calcification

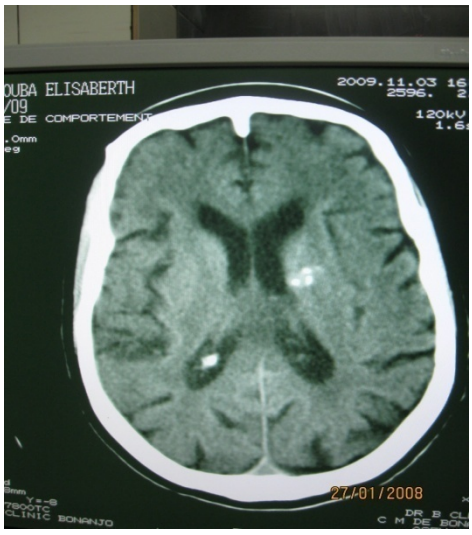

Figure 6. Axial brain ct showing choroid plexus and left basal ganglial calcifications

The Relationship between Spatial Patterns of Illnesses and Unemployment in Iraq-2007

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Abstract:

Studies of the relationship between spatial patterns of chronic illnesses (CI) and unemployment rate (UR) characteristics were not well documented. However, when analyzing the data that were collected on geographic areas, the spatial effects were seldom considered. This study addresses this concern by applying the mapping and spatial analysis techniques in studying how UR pattern is related to the CI pattern in Iraq. The aim is to assess the existence of spatial pattern in CI across geographical areas, and find whether this pattern was influenced by the pattern of socioeconomic indicators such as UR. The study design was cross-sectional census data obtained in 2007. Governorates were used as the respective units of the analysis. Two statistics of spatial autocorrelation based on sharing boundary neighbours known as global and local Moran measures were used to investigate the global and local clustering respectively. To investigate the bivariate spatial relationship between CI and UR, Wartenberg's (1985) measure was used. It was found that UR varied significantly across different governorates, while CI didn't. Significant local clusters in UR, in northern and southern parts of the country were found, while no significant local clusters were found in CI. No significant spatial association was found between CI and UR based on bivariate spatial correlation coefficient.

Keywords: Spatial autocorrelation, Sharing boundary neighbors, Mapping, Global and local moran, Monte carlo simulation, Unemployment, Chronic illnesses

1. Introduction

Unemployment is one of the main socioeconomic issues that negatively affect both economic activity and social life. In recent years, there has been a growing interest in examining the existence of spatial autocorrelation of UR and its spatial relationship with many indicators such as poverty, education, health, etc. Low wage flexibility and limited labour mobility involve persistent unemployment differentials across governorates in Iraq. The present paper focuses on the spatial structure of UR disparities across governorates and its relationship to CI disparities.

Regions, independent of their geographic level of aggregation, are known to be interrelated partly due to their relative locations.Governorates are tightly linked by migration, commuting, and enter-governorate trade. These types of spatial interactions are exposed to the frictional effects of distance, which could cause the spatial dependence of governorate labour market conditions. Similar economic performance among governorates can be attributed to proximity. Benach *et al*. (2009) stated that the employment conditions and associatedwork organization of most migrant workers are dangerous to their health inequalities.For example, theimpact of Korean employment conditions on workers'mental health is worse for immigrants, minorities and unionmembers because of their labor market vulnerability and thehostility of management to union organizers and members. The study of Wahl *et al*. (2009) in Norway supported the assumption of a complex and indirect relationship between chronic pain and global quality of life (GQOL). Wahl *et al*. found negative effect of CI on GQOL. The CI is a major cause of disability (Wehdell, 2001). Smith (2008) in a least-squared regression model found that UR has a moderate effect on infant mortality rates.

The UR was studied in many countries using different statistical measures. Given the large literature documenting the important relationship between employment outcomes and health, Johnston and Lordan(2011) concluded that this is a potentially important pathway through which discrimination affects health.Evidence provided by Eichengreen (1993) indicated that the responsiveness to health, social, and other factors of unemployment differentials was much greater in US than in Europe. Bertola (1999), as well as Balu and Kahn

(1999), analyzed the impact of different institutions and regulations on labour market outcomes. According to their results, wage adjustment and labour mobility wereaffected by minimum-wage provisions unemployment benefits and welfare payments. However, the results of Layard (1997) implied that strict labour market regulations, employment protection and minimum wages were not the main target areas of policies aiming at a significant decline of unemployment. Instead they advised reform of social security systems combined with active labour market policies.

In US, unemployment rates remained near a 25-year high and global unemployment was rising (Roelfs *et al*., 2011). In Europe and Chile, Jeffers, *et al*. (2010) found that unemployed individuals have poorer mental health than the employed and possible subsequent major depression, which could lead to suicide. Being unemployed were considereda major risk factor causing suicide by several authors (Chida, 2006; and Hawton & Herringen, 2009). Jin *et al*., (1995) found a strong positive association between unemployment and health problems, such as morbidity at the population level.Tontsa *et al*. (2011) concluded that socioeconomic wellbeing across 33 small mining towns in Western Australia is highly variable. Eriksson *et al*. (2010) addressed unemployment and related social factorsas risk factors for impaired mental illness in Denmark.They found that experiences of long- and medium-termunemployment are followed by an increased probability of the individualbeing admitted for the first time to a psychiatric hospital.

The relationship between unemploymentand health is expected in the poorest areas of the world, and morespecifically in countries with severe economic and socialcrises, such as war, poverty and mass migrations in Iraq, Afghanistan, and Africa South of the Sahara (Benach *et al*. 2009).In Iraq unemployment remained at very high levels.The most affected are women and young people (Agency for Technical Cooperation and Development, 2010). Epilepsy is a chronic illness that affects all ages andhas long-term complications, includingun-employability (AL-Saad, *et al*. 2001). Their study was conducted in Salahuddin governorate. Unemployment in this governorate was found 33%, and this rate was significantly greater among those stricken by epilepsy especially the young. Youth unemployment is high and increasing: 57% of those aged between 15 and 29 are unemployed and 450000 young men are new entrants of labour market each year (UNIAU Iraq labour force analysis 2003-2008).

A common feature of most of the above mentioned studies is that they investigated the functioning of labour market adjustments and the effects of labour market regulations without considering the spatial dimension of area labour market disparities. Most of these studies have shown that unemployed persons have an increased risk of death. Some studies investigated the wage curve taking spatial effects into account. Manning (1994) and Buettner (1999) analyzed the relationship between earnings and unemployment for British countries and German regions respectively. An analysis by Molho (1995) confirmed that there is significant spatial interaction among regional labour markets in UK. Overman and Puga (2002) analyzed unemployment clusters across European regions. The results of their nonparametric approach indicted that unemployment rates were much more homogeneous across neighbouring areas than across regions in the same EU country. The results found by Filiztekin (2007) indicated that the provincial unemployment rates were quite persistent and the gap across different regions widens further with spatial clusters emerging across Turkey. Lopez-Bazo, Barrio and Artis, (2000) discussed the role of neighbouring effects in explaining the spatial distribution of unemployment, where their results pointed to the emergence of at least two clusters in the regional distribution of UR in Spain.

To understand the linkages between socioeconomic variables, investigations should focus on features of the areas rather than on the compositional characteristics of residents of the area, which cannot fully describe the social environment in which people live (Macintyre, Maciver & Sooman, 1993). So, spatial autocorrelation and geographical pattern of UR and CI were studied using lattice data. Spatial autocorrelation is the term used for the interdependence of the values of a variable over space. However, it was argued that lattice data are spatially correlated, where exploratory spatial data analysis (ESDA) was used using lattice data. The ESDA quantifies the spatial pattern in order to increase the analyst's knowledge of the spatial system. As well as mapping plays an important role in the monitoring of unemployed people and CI. Maps can reveal spatial patterns not previously recognized or suspected from the examination of a table of statistics and reveal high risk communities or problem areas (Lawson & Williams, 2001). However, the purpose of spatial analysis is to identify pattern in geographic data and attempt to explain this pattern. Findings are expected to enhance unemployment and CI monitoring and policing interventions across governorates in Iraq.

Available evidence was shown that employment conditions and associated work organization of most migrant workers are dangerous to their health (Benach *et al*. 2009).Reducing UR and CI inequalities are not a primary objective but emergent prosperity is. The importance of this objective emanates the argument that unemployment is a standard indicator of poverty which means that poor people cannot pay for medical treatment especially

those who suffered from CI. Also the job prospects for the people who suffered from CI may be limited. Cattaneo, (2006) stated that a strong link existed between poverty and unemployment, being the lack of employment one of the main determinants of poverty. According to the study in Jordan by Amerah, (1993), health was affected negatively by unemployment. Elhorst, (2003) proposed several reasons that make studying the spatially uneven distribution of unemployment worthwhile. One of these reasons is that unemployment differentials imply inefficiency in the economy as a whole and reduce growth. To the author's knowledge no studies used the spatial analysis techniques and geographical mapping in studying the inequalities in UR and CI gradient and their relationship to each other in Iraq. Furthermore, the studies that used advanced statistical techniques, such as structural equations modeling, in examining the inequalities of UR and CI were very limited in Iraq.

The importance of mapping was stated by Koch (2005): why make the map if detailed statistical tables carry the same results? Perhaps the most important reason for studying spatial statistics is not only interested in answering the "how much" question, but the "how much is where" question (Schabenberger&Gotway, 2005). In light of these: (1) the existence of spatial global clustering and (2) spatial local clusters of governorates with respect to UR and CI were investigated. Also, the bivariate spatial association between UR and CI was examined. Thisstudy contributes to the literature by examining the geographical distribution of UR and CI, spatial global clustering, and local clusters of UR and CI. The study design was a cross-section analysis in a census survey conducted in Iraq in 2007.

The data analysis follows five steps. In step 1, the levels of UR and CI were visually inspected using the quartiles based on choropleth mapping. Step 2 includes the calculation of global Moran's I for each UR and CI to detect the global clustering and also the significance of I statistic was examined using permutation test. Step 3 involves the calculation of local Moran's I_i for each ithgovernorate to detect the local clusters of UR and CI, and also the p -values of local Moran's I_i values were calculated using Monte Carlo simulation. In step 4 the gradients of quartiles of local Moran values were visually inspected. In Step 5, the bivariate spatial correlation between UR and CI was examined based on Wartenberg's (1985) statistic. In conclusion, spatial global clustering was found for UR but was not found for CI.Local clusters in UR were found but for CI were not found. The major contribution was the demonstration that spatial locations had statistically significant effects on the likelihood and disparity of UR.

2. Materials and Methods

Data: Data were collected from Iraq Household Socioeconomic Survey, based on a census conducted in Iraq in 2007. For ($N = 18$) governorates, transformed UR and CI variables were used. The UR is defined as the percentage of unemployed persons in the total economically active population (the total of unemployed and employed persons).An unemployed person is a person aged (15-65) years who are without work, able to work, available for work,actively looking for work, and willing to accept the market wage. The percentages ofpersons reported to have CI in each governoratewereapplied. The CI included the following: Diabetes, hypertension, heart disease, kidney, Tumors and high cholesterol, psychiatric and nervous sensory,stomach, intestines, ulcers, Thyroid, hepatitis, respiratory and chest, gynecological, hematological, Skin, venereal, parasitic and other, Urinary and genital, and Impotency and other.

Analysis: Data analysis involved five steps. In step 1, the UR and CI were tested for normal distribution.They weren't found to follow normal distribution. Therefore, both variables were transformed to follow normal distribution using LISREL software. The LISREL scales the normal scores so that the transformed variables have the same sample mean and standard deviation as the original variable. Thus, the normal score is a monotonic transformation of the original score with same mean and standard deviation (this characteristic can be considered as an advantage in this transformation) but with the values of skewness and kurtosis much reduced. In step 2, visual inspection based on the quantified gradients for transformed UR and CI using quartiles were conducted. Step 3 included the calculation of global Moran's I for transformed UR and CI to detect the global clustering and also the significance of I -statistic using permutation test for each variable was examined. Step 4 involved the calculation of local Moran's I_i for the ith governorate and it's p -value using Monte Carlo simulation to detect the local clusters fortransformed UR and CI. In step 5, using quartiles, visual inspection of local Moran values for each variable was inspected based on choropleth mapping.

The UR and CI values were categorized into four intervals. These intervals were used for all maps using darker shades of gray to indicate increasing values of UR and CI. Such approach enables qualitative evaluation of spatial pattern. In the neighbourhood researches, neighbours may be defined as governorates which border each other or within a certain distance of each other. In this research neighbouring structure was defined as

governorates which share a boundary. The *second order* method (queen pattern) which included both the first-order neighbours (rook pattern) and those diagonally linked (bishop pattern) was used. A neighbourhood system was given in Fig. 1, where ID neighbour for each governorate was shown. Although maps allow visual assessment of spatial pattern, they have two important limitations: their interpretation varies from person to person, and there is the possibility that a perceived pattern is actually the result of chance, and thus not meaningful. For these reasons, it makes sense to compute a numerical measure of spatial pattern, which can be accomplished using spatial autocorrelation. Therefore, global spatial clustering and local spatial clusters were identified.

Identification of global spatial clustering: The goal of a global index of spatial autocorrelation is to summarize the degree to which similar observations tend to occur near to each other in geographic space. In this exploratory spatial analysis, the spatial autocorrelation using standard normal deviate (z-value) of Moran's I under normal assumption was tested. Moran's *I* is a coefficient used to measure the strength of spatial autocorrelation in regional data. Global clustering test was used to determine whether clustering has existed throughout the study area, without determining statistical significance of local clusters.Moran's I is calculated as follows (Cliff andOrd, 1981):

$$I = \frac{N \sum_{i=1}^{N} \sum_{j=1}^{N} w_{ij} (x_i - \bar{x})(x_j - \bar{x})}{S_0 \sum_{i=1}^{N} (x_i - \bar{x})^2} \quad \text{and} \quad S_0 = \sum_{i=1}^{N} \sum_{j=1}^{N} w_{ij}, i \neq j$$

where, $N = 18$ is the number of governorates, $w_{ij} = 1$ is a weight denoting the strength of the connection between two governorates i and j that shared a boundary, otherwise, $w_{ij} = zero$, x_i and x_j represent the transformed UR or CI in ith and jth governorate respectively.Theautocorrelation coefficient can be used to test the null hypothesis of no spatial autocorrelation or spatially independent versus the alternative of positive spatial autocorrelation:

H_0 : No clustering exists (no spatial autocorrelation)

H_1 : Clustering exists (positive spatial autocorrelation)

A significant positive value of Moran's I indicates positive spatial autocorrelation, showing the overall pattern for the governorates having a high/low level of UR or CI similar to their neighbouring governorates. A significant negative value for Moran's I indicates negative spatial autocorrelation, showing the governorates having a high/low level of UR or CI unlike neighbouring governorates. To test the significance of global Moran's I , z -statistic which follows a standard normal distribution was applied. It was calculated as follows (Weeks, 1992):

$$z = \frac{I - E(I)}{\sqrt{\text{var}(I)}}$$

Permutation test wasapplied. A permutation test tells us that a certain pattern in data was or was not likely to have arisen by chance. The observations of each UR and CI were randomly reallocated 1000 times with 1000 of spatial autocorrelations were calculated in each time to test the null hypothesis of randomness. The hypothesis under investigation suggests that there will be a tendency for a certain type of spatial pattern to appear in data, whereas the null hypothesis says that if this pattern was present then this was a pure chance effect of observations in a random order. The analysis suggested an evidence of clustering if the result of the global test was significant but it didn't identify the locations of any particular clusters. Beside, clustering which represent global characteristic, the existence and location of localized spatial clusters in the study population are of interest in geographic sociology. Accordingly, local spatial statistic was advocated for identifying and assessing potential hot spots or clusters.

Identification of local spatial clusters: A global index can suggest *clustering* but cannot identify individual *clusters* (Waller &Gotway, 2004). Anselin, (1995) proposed the local Moran's I_i statistic to test the local autocorrelation, where local spatial clusters, sometimes referred to as hot spots, may be identified as those locations or sets of contiguous locations for which the local Moran's I_i was significant. Anselin stated that the indication of local patterns of spatial association may be in line with a global indication, although this is not necessarily the case. It is quite possible that the local pattern is an aberration that the global indicator would not

pick up, or it may be that a few local patterns run in the opposite direction. However, Moran's I_i for ith governorate may be defined as (Waller &Gotway, 2004):

$$I_i = \frac{(x_i - \bar{x})}{S} \sum_{j=1}^{N} \left[\left(w_{ij} \Big/ \sum_{j=1}^{N_i} w_{ij} \right) \frac{(x_j - \bar{x})}{S} \right], \; i = 1,2,...,18$$

where, analogous to the global Moran's I, the x_i and x_j represents the UR or CI in ith and jth governorate respectively, N_i = number of neighbours for ith governorate, and S is the standard deviation. It was noteworthy that the numbers of neighbours for ith governorate were taken into account in I_i statistic by the amount:

$$\left(w_{ij} \Big/ \sum_{j=1}^{N_i} w_{ij} \right)$$

where w_{ij} was measured in the same manner as in Moran's *I* statistic. Local Moran statistic was used to test the null hypothesis of *no clusters*. However, local Moran statistic is a decomposition of global Moran's *I* into the contributions of small areas.

Clusters may be due to aggregations of high values, aggregations of low values, or aggregations of moderate values. Thereby, high values of I_i suggested clusters of similar (but not necessarily large) values across several governorates, and low value of I_i suggested an outlying cluster in a single governorate i (being different from most or all of its neighbours). A positive local Moran value indicates local stability, such as governorate that has high/low UR surrounded by governorate that has high/low UR. A negative local Moran value indicates local instability, such as governorate has low UR surrounded by governorate has high UR or vice versa. However, each governorate's I_i value can be mapped to provide insight into the location of governorates with comparatively high or low local association with its neighbouring values. In the statistical analysis, all programs performed in S+8 Software.

Bivariate spatial association: So far, spatial method have only presented that quantified the spatial structure of one variable at a time. There is controversy about the appropriate measure for bivariate spatial association. However, spatial dependence or spatial clustering causes loss in the information that each observation carries. When N observations were made on a variable that was spatially dependent (and that dependence was positive so that nearby values tend to be similar), the amount of information carried by the sample was less than the amount of information that would be carried if the N observations were independent, because a certain amount of the information carried by each observation was duplicated by other observations in the cluster. A general consequence of this was that the sampling variance of statistics was underestimated. As the level of spatial dependence increases the underestimation increases. The problem is when spatial autocorrelation is present, the variance of the sampling distribution of e.g.,Pearson correlation coefficient which is a function of the number of pairs of observations, is underestimated. Spatial autocorrelation coefficients are therefore modified to estimate the spatial correlation between two variables (Wartenberg 1985):

$$I_{xy} = \frac{1}{S_0} \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} w_{ij} (x_i - \bar{x})(y_j - \bar{y})}{\left[\sqrt{\sum_{i=1}^{N} (x_i - \bar{x})^2 \Big/ N} \right] \left[\sqrt{\sum_{j=1}^{N} (y_j - \bar{y})^2 \Big/ N} \right]}$$

Where x and y are the UR and CIvariables respectively. Although the mathematics is quite straightforward, very few software packages allow computing I_{xy} (Fortin & Dale, 2005). Thus, programming was used to find the value of I_{xy}. To test the significance of I_{xy}, z-statistic was applied which follows approximately standard normal distribution:

$$z = I_{xy} \sqrt{N - 1}$$

3. Results

Descriptive analyses were performed to assess the demographic characteristics of the data set. The mean and standard deviation for transformed URwere found 11.71 and 4.83 respectively; skewness and kurtosis were

found 0.00 and -0.10 respectively. The five-number summary of transformed UR data set consists of the minimum, maximum and quartiles written in increasing order: Min=2.14, Q_1=8.29, Q_2=11.71, Q_3=15.14 and Max=21.28. From the five-number summary, the variations of the four quarters of UR data were found 6.15, 9.57, 3.43 and 6.68 respectively, where the second quarter has the greatest variation of all. Also, descriptive statistics were calculated for transformed CI, where the mean and standard deviation were found 9.94 and 7.11 respectively; skewness and kurtosis were found 0.00 and -0.10 respectively. The five-number summary of transformed CI data set was found: Min=-4.13, Q_1=4.91, Q_2=9.70, Q_3=14.08 and Max=24.02. From the five-number summary, the variations of the four quarters of CI data were found 9.04, 4.79, 4.38 and 9.94 respectively, where the fourth quarter has the greatest variation of all.

Thiqar governorate accounted for the highest UR (20.9%). It was followed by the governorates Diala and Missanwhich accounted for (20.4%) and (19.6%), respectively. The lowest rate was in the governorate of Suleimaniya with (2.14%). This can be explained by the persistent growth of economic activity in most fields, which provide more job opportunities. However, the UR in Iraq decreased dramatically from 28.10% in 2003 to 11.71% in 2007. Suleimaniya governorate accounted for the highest CI (29.6%). It was followed by the governorates of ALnajaf and ALqadisiyawhich accounted for (21.5%) and (16.5%), respectively. The lowest CI was in the governorate of Karkuk with (2.2%).Figure 1 shows the study area explaining all governorates with their identification numbers (ID).

Since the local Moran's I_i varied by location, it was easier to interpret visually by color coding of each governorate. Maps displayed geographical inequalities across governorates of Iraq. Figures 2a, b, c, and d show visual insight for transformed UR, and its local Moran values, transformed CI and its local Moran values respectively, with the darkest shade corresponding to the highest quartile. Based on visual inspection, an overall worsening pattern (higher scores) for UR was found in the western-northern, mid, and southern parts of the country.The suggestion of spatial clustering of similar values for UR was confirmed by a positive significant global Moran's I of 0.23 with an associated standard normal z-value of 2.25 and $p-\text{value}=0.024$. Also, based on visual inspection, an overall worsening pattern (higher scores) for CI was found in the northern and southern parts of the country. The suggestion of spatial clustering of similar values for CI was not confirmed by a negative global Moran's I of -0.12 with an associated standard normal z-value of -0.48 and $p-\text{value}=0.632$.The high level of CI in somegovernorates such as 14 and 15 could probably be contributed by the high level of UR in these governorates, in some of their neighbours,and/or by the UR inequality among their neighbours as shown in Fig. 2a and 2c.Seven significant clusters have high level in UR were found as shown from their p-values, where their ID (3, 4, 5, 12, 15, 16, and 17). For CI, no significant clusters were found.

To investigate global clustering, permutation test was done, where the permutation $p-$value=0.018for transformed UR was found significant; while $p-$value=0.636for transformed CI was found not significant. Thus, the null hypothesis of no spatial autocorrelation was rejected for UR but not rejected for CI. The results of local Moran's I_i values for transformed UR and CI,and their p-values are reported in Table 1.

The Pearson correlation coefficient between UR and CI variables was found negative (-.06), which is not significant with ($p=0.809$). Bivariate spatial correlation between the UR and CI was found ($I_{xy}=-0.01$) which is not significant with (z=-0.04 and $p=0.569$). However, although both results were not significant, Pearson coefficient is always over estimated when used in finding the spatial correlation. That's why, in investigating the bivariate spatial correlation, it is recommended to use Waternberg (1985) measure.

4. Discussion

The association between the spatial patterns of UR and CI was examined, allowing for the effects of neighbouring governorates that share the boundary with a particular governorate. Findings allow policy makers to better identify what types of resources are needed and precisely where they should be employed. The above framework is proposed to analyze the spatial pattern of CIand revealed some noteworthy findings. The rationale behind the relationship between CI and UR is that unemployed people usually suffer from financial strain that could be caused by increased health problems.

After rejecting the null hypothesis, it becomes possible to conclude that there is some form of clustering.It is of course of interest to know the exact nature of the clustering process. Is it only global type clustering or are there hot-spot clusters? If the later, how many hot-spots are there and where are they located? Our analysis of the association between CI and UR used exploratory tools such as descriptive tables and small area choropleth maps. Geographical distributions of CI and UR in quantiles were examined visually using maps.

Research on neighbourhoods and health is motivated by the idea that we live in places that represent more than physical locations. They are also the manifestation of the social, cultural, political and geographic cleavages that shape a constellation of risks and resources. The first wave of studies on neighbourhoods and health focused on showing the relevance ofneighbourhoods and the effects beyond individual socioeconomic characteristics. These studies argued that neighbourhoods influence health by behavioralpatterns such as collective socialization, peer-group influence, and institutional capacity. The second wave of the studies evaluated these mechanisms with latent measures of neighbourhood characteristics, such as level of segregation, collective social and economic capacity (Sampson *et al*. 2002).

The URmay be associated with CI reflectingtheexisting of individual income which provides good medical care, high quality of food, and acceptable household conditions. The usual correlation coefficients, such as Pearson, only test whether there is an association between two attributes by comparing values at the same location. Map comparison involves more than pair wise comparisons between data recorded at the same locations as spatial units were arbitrary subdivisions of the study region and people could move around from one area to another and could be affected by UR levels in areas other than the area they live in. i.e., the level of CI in ith governorate was thought to be influenced by the levels of UR not just in ith governorate but also in neighbouring governorates. Neighbourhood residential turnover had been linked to poor child development, problem behavior, and health risks (Jelleymanand Spencer, 2008).

Permutation distribution can be used to test the significance of the global Moran statistic, for this purpose 1000 random permutations were used. The $p = 0.018$ for Morans's*I* of UR and $p = 0.636$ for Morans's *I* of CI were found. Simulated data is useful for validating the results for such analysis. However, using Monte Carlo simulation, 9999 random samples were simulated, 18 values for each sample, for both CI and UR. These samples (9999 matrices, each had two columns) were generated under bivariate normal distribution.

As stated by Charloit *et al*. (2005), CI is one of the indicators that measure children's health in Denmark and Sweden. They found that children in families with one or both parents without paid work had an increased prevalence of recurrent psychosomatic symptoms and CI. The essay of Bambra (2011) showed that the relationship between work, worklessness, and health inequalities was influenced by a broader political and economic context in the form of welfare state regimes. Public health initiatives could target unemployed persons for more aggressive cardiovascular screening and interventions aimed at reducing risk-taking behaviors (Roelfs, *et al*., 2011). When people are unemployed they have less money to spend on medical treatment and paydoctors' bills, purchase healthy food, water, and shelter.All of which leads to a higher risk of CI. Better allocation of resources to healthcare and health awareness can help lead to lower levels of CI. One cannot believe that these problems will ever be fully resolved.However, with more effort, time, and better allocation of resources, an enormous impact can be achieved.

When UR is low, unemployed individuals may have special problems or characteristics that make them more vulnerable to ill health (Martikainen and Valkonen, 1996). The analysis showed that several governorates (4, 6, 8, and 10), confirmedthe findingsof Martikainen and Valkonen. However, one could also argue that being unemployed in times of low levels of unemployment may lead to a social stigma to a greater extent than when unemployment levels are high, which may partly explain the increased risk of ill health.

Although this study could not confirm any causation, there are two possibilities. First, UR could be caused CI or second, vice versa. Epidemiologic evidence suggests that the direction of causation from unemployment to illness has a greater possibility than the converse (illness causes unemployment). Although more research can be done to elucidate mechanisms and mediating factors, the present author found sufficient evidence to recommend that intervention research, to determine ways to reduce the adverse effect of unemployment on health, be a priority. Unemployment may exert detrimental effects on health through many mechanisms: (1) by disrupting community and personal social relationships (Jackson, 1993), (2) by leading to greater risk behavior,such as alcohol consumption and poor diet (Morrell *et al*. 1993), (3) by causing stress (Moser, 1986), and (4) by precipitating reaction, like that caused by other losses (Jackson, 1993). It didn't assess the evidence for any particular mechanism or series of mechanisms since the main purpose was to assess whether, not how, UR pattern is related to the pattern of CI.

5. Conclusion

Clustering of transformed UR and CI are studied, and the spatial association between them. The association was not found significant based on Wartenbergcoefficient. Although, a causal relationship between UR and CI cannot be provided, the results are conclusive in at least five aspects: First, based on mapping the quartiles of UR, high UR was concentrated along the north-south axis, for instance in the governorates (2, 6, 14and 16). High CI

was concentrated along the north-south axis, particularly in the northernareas and multiple governorates in southern areas, for instance, the governorates (2, 3, 5, 13, 14, 16 and 17). Based on *visual* inspection, the patterns formed by those governorates with highest ranking in UR and those with highest ranking in CI were somewhat identical.Second, many governorates were not observed visually as hot spots for both UR and CI, but after considering the information of their neighbours (i.e., calculating local Moran's I_i values), it can obviously see the patterns of hot spots, For example, governorates (3, 4, 5, and 12) for UR, and governorates (6 and 12) for CI. Third, based on global Moran index the clustering tendency showed that UR for each governorate can be spatially correlated with UR in neighbouring governorates, while the clustering tendency in CI was not found significant. Fourth, the significance of bivariate spatial correlation didn't support the hypothesis of the association between the spatial patterns of CI and UR. Fifth, neighbouring governorates with high degree of inequality in UR seem to show higher levels in CI, for instance governorates (12 and 16). This was consistent with what Haining (2003) stated, the levels of such variable in area i was thought to be influenced by the levels of another variable not just in area i but also in its neighbouring areas. This supports the hypothesis that the degree of variations in UR between these governorates and their neighbours could somewhat influence CI.

The analytical approach used here accurately delineates governorates of high unemployment, and permits policy makers to develop strategies to minimize the difference between governorates. Policy which pays attention to area characteristics will reduce unemployment inequalities and consequently improve the health of the overall population. Additional research is needed to characterize more fully this relationship. Although unemployment and economic issues may seem beyond the usual bounds of health care, physicians and other health care professionals have the opportunity to recognize, treat and possibly prevent the adverse consequences of unemployment for their patients. Beyond caring for individuals, however, health care professionals can also pay an important role in collective action against unemployment by advocating better health.

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Table 1. Shows both transformed UR (%) and CI (%), Local Moran's I_i values for UR and CI, and their corresponding p -values

ID	Transformed UR	I_i for UR	p -value	Transformed CI	I_i for CI	p -value
1	10.02	0.09	.344	11.41	0.10	.333
2	15.80	-0.65	.925	12.43	-0.07	.615
3	2.14	0.95	**.011**	24.02	-1.86	.999
4	6.55	1.49	**.004**	-4.13	-0.84	.940
5	5.12	0.93	**.007**	14.66	-0.11	.666
6	18.31	-1.01	.975	2.35	0.01	.431
7	9.30	0.03	.342	7.46	0.04	.312
8	12.04	-0.01	.493	8.97	0.09	.260
9	8.51	-0.01	.480	8.97	-0.01	.500
10	13.05	-0.08	.613	3.92	-0.16	.703
11	10.71	-0.15	.717	6.39	-0.05	.587
12	7.62	0.32	**.058**	0.24	0.11	.201
13	11.38	0.00	.462	19.65	0.06	.301
14	14.13	0.17	.181	17.54	0.28	.112
15	14.91	0.45	**.076**	13.50	0.23	.167
16	21.28	0.91	**.006**	10.43	0.02	.396
17	16.87	0.73	**.046**	15.97	-0.31	.788
18	13.05	0.34	.135	5.23	-0.31	.785

ID	governorate	ID Neighbours
1	Duhouk	2,5
2	Nineveh	1,5,7,12
3	Suleimaniya	4,5,6,12
4	Karkuk	2,3,5,12
5	Erbil	1,2,3,4,12
6	Diala	3,8,11,12
7	Alanbar	2,8,9,10,12,13
8	Baghdad	6,7,9,11,12
9	Babil	7,8,10,11,13,14
10	Kerbala	7,9,13
11	Wasit	6,8,9,14,16,17
12	Salahuddin	2,3,4,5,6,7,8
13	Alnajaf	7,9,10,14,15
14	AlQadisiya	9,11,13,15,16
15	Almuthanna	13,14,16,18
16	Thiqar	11,14,15,17,18
17	Missan	11,16,18
18	Basrah	15,16,17

Figure 1. Study area shows all governorates with their ID and the neighbours of each governorate

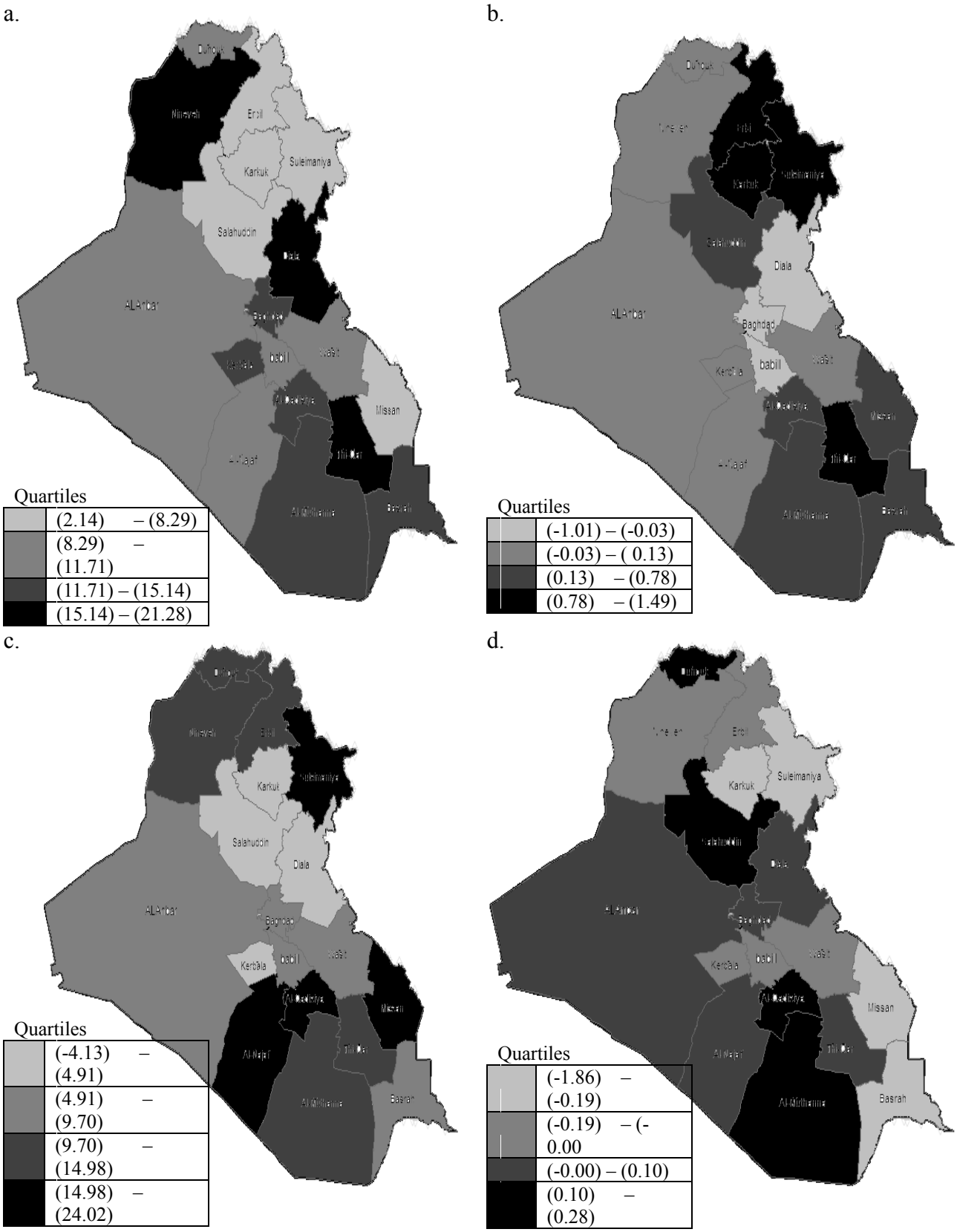

Figure 2. Choropleth maps show: a. transformed UR variable, b. local Moran values of UR variable, c. transformed CI variable, and d. local Moran values of CI variable

3-Self Behavior Modification Programs Base on the PROMISE Model for Clients at Metabolic Risk

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Abstract

The objectives of this mixed methods research were 1) to study effects of the health behavior modification program (HBMP) conducted under the principles of the PROMISE Model and the CIPP Model and 2) to compare the 3-self health behaviors and the biomedical indicators before with after the program completion. During the program, three sample groups including 30 program leaders, 30 commanders and 120 clients were assessed, and there were assessments taken on 4,649 volunteers who were at risk of metabolic syndrome before and after the program conducted in 17 hospitals. The collected data were analyzed by the t-test and the path analysis. The research instruments were questionnaires used for program evaluation, structuralized interview forms, and questionnaires used for 3-self health behavior assessment. The findings were as follows: 1) During the program, the assessment result deriving from comparing the overall opinions toward the program among the three sample groups showed no difference (F=2.219), 2) The program management factors based on the PROMISE Model (positive reinforcement, optimism, context, and process or activity provision) had an overall influence on the product or success of the HBMP ($p< 0.05$) with size effects at 0.37, 0.13, 0.31 and 0.88 respectively. All of the factors could predict the product of the program by 69%. 3) After participating in the program, the clients' 3-self health behaviors (self-efficacy, self-regulation, and self-care) were significantly higher than those appeared before the participation ($p< 0.05$), and their biomedical indicators (BMI, blood pressure, waistline, blood glucose, lipid profiles, cholesterol, and HbA1c) were significantly lower than those measured before the program ($p< 0.05$).

Keywords: CIPP Model, Behavior modification, Metabolic syndrome, Self-regulation, Self-care

1. Introduction

The National Health Security Office (NHSO) has underlined health problems associated with metabolic syndrome (MS) including obesity, high hypertension, diabetes, and stroke as such diseases cause illness to people worldwide: the U.S. (24% of the total population or 44% of adults aged over 50 years); Saudi Arabia (39.3%); Turkey (33%); Tehran, Iran (30%); and South Korea (14.2%) (Shila *et al*., 2010). In Thailand, 36% of the people living in Bangkok suffered from MS. In addition, the mortality rate per 100,000 Thai people due to cardiovascular disease constantly increased from 48.58 persons in 2002 to be 55.29 persons in 2007. During the period, the mortality rate attributed to both cardiovascular disease and diabetes was as high as 85,000 people per year or 236 people per day. It was also found that 58% of people who survived a stroke remained permanently disabled and became a burden on the society due to high medical expenditures of long-term treatment (Health Research Network, 2009). Thus, health behaviors and MS are key indicators of having good quality of life (Wood, 2005). Promotion of health behaviors needs cooperations from all segments of the society in an effort to solve health problems for overall achievement and to yield substantial results to the public (Knezevic *et al*., 2008; Knezevic, 2009; WHO, 2010). At present, the health behavior modification adhering to accurate psychological techniques and strict occupational ethics is approved to be sustainable prevention of health problems and the safest way for people in that it can reduce mortality rate and risk of medications (Innis, 1981; Watanabe *et al*, 1998). Many developed nations lay importance on the health problem prevention with the use of health behavior modification techniques that combine psychological theories with social cognitive learning (Bandura, 1986). The techniques are used to design activities that suit specific features of the at risk group so as to reduce risky health behaviors and reinforce preferred ones which consequently lessen occurrence of chronic diseases (Jones,

Brockway & Atkinson, 1995). Behavior modification includes group activities and community relations which help reduce risk of obesity (Nilsen, 1996); it can also be achieved through sports and exercise (Larsen & Manderson, 2009). People of all ages and occupations can individually and collaboratively participate in behavior modification activities; however, such activities must rely on an understanding in psycho-behavioral science. Hence, the providers of health behavior modification must receive training on accurate modification techniques, methods, and procedures by the experienced, and there must be a regular follow-up in order to achieve the set goals (Elder, 1987; Leventhal & Linda, 1987; Reighard, 2010).

The plan to modify the 3-self health behaviors (self-efficacy, self-regulation, and self-care) of Thai people at risk of MS was developed by the NHSO, in collaboration with Srinakharinwirot University, on the basis of the PROMISE Model: P = Positive Reinforcement, R = Result-based Management, O = Optimism, M = Motivation, I = Individual or Client Center, and SE = Self-esteem (Ungsinun, 2009). In addition, in 2010 the Thai Public Health Ministry has launched the policy of health adjustment village with an aim to decrease the risk of cancer, high blood pressure, and cardiovascular disease by using key behavioral indicators which are an exercise program of at least three to five days a week and daily consumption of half a kilogram of fruits and vegetables (Health Education Division, 2011). This research was conducted with the cooperation of 100 program leaders from 17 hospitals in Bangkok Metropolis, amounted to 30 projects all together. After the participating leaders received a 4-day training in the techniques of 3-self health behavior modification in accordance with the PROMISE Model, they had to carry out ongoing health activities for 4,649 people who were at risk of MS for at least five times during five to seven months. Meanwhile, they were periodically supervised, monitored, and assessed by the researcher team.

2. Objectives of the research

The main purpose of this study were 1) evaluating the HBMP carried out by the participating hospitals, based on the CIPP Model and the 360 Degree Feedback, 2) examining effects of the health programs administered according to the PROMISE Model by focusing on the context, the input, and the process toward the product received from the programs, 3) comparing the 3-self health behaviors of the clients before participating the program with the ones occurred at the end of the program, and 4) making a comparison of the biomedical indicators (BMI, blood pressure, waistline, blood glucose, lipid profiles, cholesterol, and HbA1c) before and after the program.

3. Methods

3.1 Extent of evaluation

The evaluation research was conducted in line with the CIPP Model of Stufflebleam & Shinkfield (2007), as well as the Logic Model of Pankratz (2008).

3.2 Setting

The setting included 30 projects of 17 hospitals in Bangkok Metropolis which were funded by the NHSO in fiscal year 2010.

3.3 Samples

3.3.1 The data used in experimental research were 4,649 Thai clients aged over 15 years, living in Bangkok Metropolis, and proved to be patients or at risk of MS; obesity, diabetes, high hypertension, and stroke. The clients volunteered to participate in the health behavior modification program for 5 to 7 months.

3.3.2 The data used in the evaluation research were collected during the program from 30 program leaders, 30 commanders of the program leaders, and 120 clients.

3.4 Instruments

3.4.1 Structured interview forms were used to investigate the context, the input, the process, and the product of the HBMP.

3.4.2 Four-point rating scale questionnaires consisting of 40 questions were used to evaluate the program based on the CIPP Model. The item discrimination of the questionnaires was found to range between .478 to .733 with the Cronbach's alpha coefficients of reliability at .873.

3.4.3 Four-point rating scale questionnaires containing 20 questions were used to evaluate the program administered under the PROMISE Model. The questionnaires were completed by the clients and the program leaders. The item discrimination of the questionnaires were between .509 to .754 with the reliability at .938.

3.4.4 Four-point rating scale Questionnaires with 17 questions were launched before and after the program inquiring about the 3-self health behaviors of the clients: 1) *Self-efficacy* involves the confidence in one's ability to adjust health behaviors by oneself with patience and endeavor until achieving the set goal, the part's reliability equaled .730, 2) *Self-regulation* is to observe one's behaviors with changes in one's health and to set a goal of having good health and a plan to achieve the goal by recording changes in health behaviors as well as reminding of ongoing action, the part's reliability was at .800; 4.3) *Self-care* referred to one's habit of having regular health check, searching for health knowledge, consuming proper food, exercising, and managing stress with an aim to live in good health. Behaving accordingly relied on a practice and a continuous process. The reliability was at .850.

3.4.5 Biomedical instruments comprising, as examples, measurements of BMI, blood pressure, waistline, blood glucose, lipid profiles, cholesterol, and HbA1c which were taken before and after the program.

3.5 Hypotheses

3.5.1 The result of the CIPP Model-based evaluation of the health program is at the good level and indicates the consistency in opinion among the groups of program leaders, clients, and commanders of the program leaders.

3.5.2 The factors of the program administration based on the PROMISE Model, namely context, input, and process has an impact on the product of the HBMP.

3.5.3 At the completion of the program, the clients' self-efficacy, self-regulation, and self-care are higher than the beginning of the program.

3.5.4 The clients' biomedical indicators (BMI, blood pressure, waistline, blood glucose, lipid profiles, cholesterol, and HbA1c) are better than before the program.

3.6 Statistical Analysis

3.6.1 To test Hypothesis 1, one-way ANOVA was used in comparing the consistency of the CIPP Model-based evaluation scores deriving from the three sample groups.

3.6.2 To test Hypothesis 2, the path analysis was used to analyze the quantitative data about the program administration and the activity provision based on the PROMISE Model during the program so as to study the effect size representing the influence of the factors including positive reinforcement, result-based management, optimism, motivation, individual or client center, self-esteem, context, input, and process that affected the product or success of the program.

3.6.3 To test Hypothesis 3 and 4, there were comparisons of the 3-self health behaviors and the biomedical indicators before and after the program with the use of the dependent t-test.

4. Results

4.1 As for general information concerning *the clients*, most of them were female, totaling 3,241 persons (69.71% of the total); 1,197 of them aged between 50 to 59 (25.75%). 3,308 of the clients held an academic degree lower than a bachelor's degree (71.16%). 1,734 clients were under the medical benefit scheme for civil servants or state enterprise employees, including their family members (37.30%). 1,545 clients faced the risk of obesity (33.23%), and 3,248 clients had the BMI higher than 23 kg/m^2 (69.86%). 3,783 clients (81.37%) were very satisfied with the HBMP.

4.2 The outcome of the CIPP Model-based evaluation of the program revealed that, generally, the evaluation scores of the clients, the program leaders, and the commanders of the program leaders were not diverse and found to be in the good level (the average scores=3.52, 3.47, 3.45 respectively) as show in Table 1.

4.3 The results of the analysis on the impact of the key factors on the product of HBMP carried out by the participating hospitals using the LISREL program showed that the factors concerning positive reinforcement, optimism, motivation, context, and input of program generally affected the product or success of the HBMP with effects at 0.37, 0.13, -0.02, 0.31, and -0.02 respectively, and the process was found to had direct effect on the product of the program with highest effect at 0.88 (χ^2= 29.8; df =19; p-value = 0.15489; RMSEA = 0.051; RMR = 0.0082; CFI = 0.99; AGFI = 0.86; GFI = 0.95; CN = 142.10). The relationship between the casual factors and the product of the program was displayed in Figure 1.

4.4 After finishing the HBMP, the client's self-efficacy, self-regulation, self-care, as well as biomedical indicator scores improved as shown in Table 2.

4.5 Key elements to successful HBMP were that the clients displayed attention and willingness toward the program by attending the health activities every time they were conducted. In addition, the clients were given

positive reinforcement, optimism, motivation by the staff, as well as support by the commanders. Besides, the activities were diversified and mainly focused on the service receivers.

5. Discussion

5.1 Having tested Hypothesis 1, the evaluation scores deriving from the clients, the program leaders, and the commanders of the program leaders were found consistent for many reasons. First, all of them perceived the necessity of the program and wanted to respond to the hospital's policies. Next, all people that at risk were permitted to be part of the health care program; they were allowed to set their own goals and run activities by themselves in order to solve their health problems. Finally, all of the three groups showed hospitality and friendliness to one another; such phenomenon conformed to the concept of Bandura (1986) on behavior modification in that success of behavior modification derives from willingness of the individuals whose behaviors are to be adjusted and the others involved.

5.2 According to Hypothesis 2, the factors including positive reinforcement, optimism, context, and process of HBMP were found to have an impact on the product or success of the program at the 0.05 level of significance with effects at 0.37, 0.13, 0.31, and 0.88 respectively, and they were able to predict the product or success of the program by 69%. This explained that the staff administered the program by relying on 'positive reinforcement' of the PROMISE Model. In such doing, when the clients' behaviors continually changed to the target ones, the clients were convinced that they would receive awards and acceptance for their efforts. This finding was in line with Burnet *et al*. (2002) who synthesized the research on the development of behavior modification programs for diabetes type 2 prevention and found that many research studies succeeded in implementing positive reinforcement into health programs and the effects of an optimistic program on a sense of happiness in life of patients with high blood pressure revealed that the experimental group were happier than the controlled group at the 0.01 level of significance.

5.3 Regarding the tests of Hypothesis 3 and 4, it was found that the clients' 3-self behaviors and biomedical indicators improved. This was because the PROMISE Model-based program administration encouraged the clients to play an important role in designing and doing health behavior modification programs by themselves. In addition, they were allowed to be a program initiator and an activity administer till the end of the program; as a result, the clients developed the feeling of being the program owner. Besides, there was use of positive psychological techniques to build the clients' motivation resulting in the demand for activity attendance. These findings agreed with that of Grey *et al*. (2004) who conducted a pilot study testing the effectiveness of the prevention program conducted for 41 teenagers who were at risk of diabetes type 2 in two middle schools in Connecticut for a period of 12 months. The students of the experimental group were permitted to be part of activities in order to adjust their own behaviors. The activity provider only persuaded the students by talking to them, and invited their parents to take care of the students' nutrition and to be part of their physical activities. The children were given supports and advices via telephone. Grey el al.'s research explained that, in general, the students of the experimental group and their parents gained more health knowledge and developed better health behaviors. Also, the result of this research was consistent with that of Thitima (2006) who investigated the 10-week behavior adjustment regarding weight loss in the overweight people based on Bandura's self-regulation concept, combining with social supports which included knowledge providing, self-observation processing, decision making processing, exercise practicing, stimulating, and morale support and advice giving. The overweight clients in the experimental group were found to have higher frequency in weight loss, lose more weight, and consequently have less weight than the controlled group at the 0.01 level of significance. Achara (2008; cited in Ungsinun, 2009) also suggested that motivation could cause an ongoing behavior which occurred for a particular purpose. The fact that the staff of the participating hospitals brought motivation reinforcement into the health projects resulting in the occurrence of self-care behaviors of the clients. This was considered to conform with the thought of Barofsky (1978; cited in Orasa, 1999) explaining that self-care was a person's intentional action with some reason concerning health; therefore, without motivation or intention regarding self-care, self-care behaviors would not occur. Moreover, the research of Suwannee (2002) that examined effects of self-control skill development of students on avoidance of snack consumption by using the concept of motivation reinforcement found that after the experiment the experimental group developed the behaviors of snack avoidance more than before the experiment and than that of the controlled group at the 0.05 level of significance. In the same way, Mananya (2005) studied effects of individual consultation toward self-control concerning weight loss in a group of 20 volunteers during an eight-week period. There were six stages of self-control which were relationship building, risky behaviors examining, weight estimating, planning and self-promising, plan implementing, and consultation ending. The research results revealed that the experimental

group had significantly more improvements in behaviors of noticing and recording their eating and exercising than the controlled group and than before the experiment at 0.01.

6. Conclusion

The health behavior modification program for people at risk of metabolic syndrome conducted on the PROMISE Model was able to cause increases in the clients' 3-self behaviors and improve their biomedical indicators.

7. Recommendations for future research

7.1 Commanders should encourage the staff by giving them rewards and positive reinforcement.

7.2 Clients with similar ages or health problems should be grouped together so that it will be more convenient for developing motivation strategies.

7.3 Regarding the features of activities, the clients must voluntarily participate in the program provided, and they should be allowed to create and adjust activities that individually suit their behaviors. The activities may be divided into three parts: games of cognitive adjustment and thinking change (30%); knowledge of 3-E (energy diet, exercise, and emotional management) (20%); and skill practices of the 3-self behaviors and 3-E (50%). Group activities should be carried out under the regulations firmly established in order to discipline the clients, and all groups of people should be welcomed to participate in the activities. To be more sustainable, these health programs should be promoted as routine work.

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Table 1. The comparison of the consistency of the evaluation scores among the clients, the program leaders, and the commanders of the program leaders using the ANOVA and the t-test.

Variables (scale 1 – 4)	clients(1) n=120		program leaders (2) n=30		commanders (3) n=30		F-test / (t-test)	Scheffe test		
	Mean	S.D	Mean	S.D	Mean	S.D		1-2	1-3	2-3
1.Context	3.54	.32	3.50	.33	3.48	.30	.478	.04	.06	.02
2.Input	3.45	.35	3.40	.32	3.39	.35	.550	.05	.06	.01
3.Process	3.51	.39	3.52	.34	3.13	.48	10.448**	-.01	.38*	.39*
4.Product	3.56	.54	3.38	.32	3.39	.39	2.415	.18	.17	-.01
5.Total	3.52	.33	3.45	.28	3.35	.32	3.219*	.07	.17*	.10
6.PROMISE	3.53	.37	3.58	.31	-	-	(t=.596)	-	-	-

* P-value < .05, ** P-value <.01

Table 2. Comparison of 3-self behavior and biomedical indicator scores between before and after participating in the program

Health risk indicators	n	Before		After		MD.	t
		$\overline{X}$	S. D.	$\overline{X}$	S.D.		
Self-Efficacy	4,649	13.59	2.89	15.72	2.74	-2.12	52.850*
Self-Regulation	4,649	13.44	3.11	15.75	2.74	-2.32	56.378*
Self-Care	4,649	18.48	3.75	21.28	3.48	2.93	54.991*
BMI	3,832	26.39	4.61	25.95	4.51	.44	19.896*
Systolic BP	2,754	127.73	16.88	124.51	14.60	3.21	8.115*
Diastolic BP	2,753	78.95	10.76	77.15	12.65	1.80	8.115*
Waistline	918	31.57	3.99	31.00	3.91	.57	6.858*
Waist hip ratio	100	.8761	.0642	.8673	.0652	.0088	1.701
Fasting blood sugar	712	105.59	38.95	103.26	39.32	2.33	2.296*
Lipid profiles	100	31.25	8.91	30.24	9.64	1.01	2.335*
Cholesterol	355	236.62	28.57	205.26	36.06	31.36	12.810*
HbA1c	144	9.08	2.28	8.01	1.67	1.06	7.189*

* P-value < .05

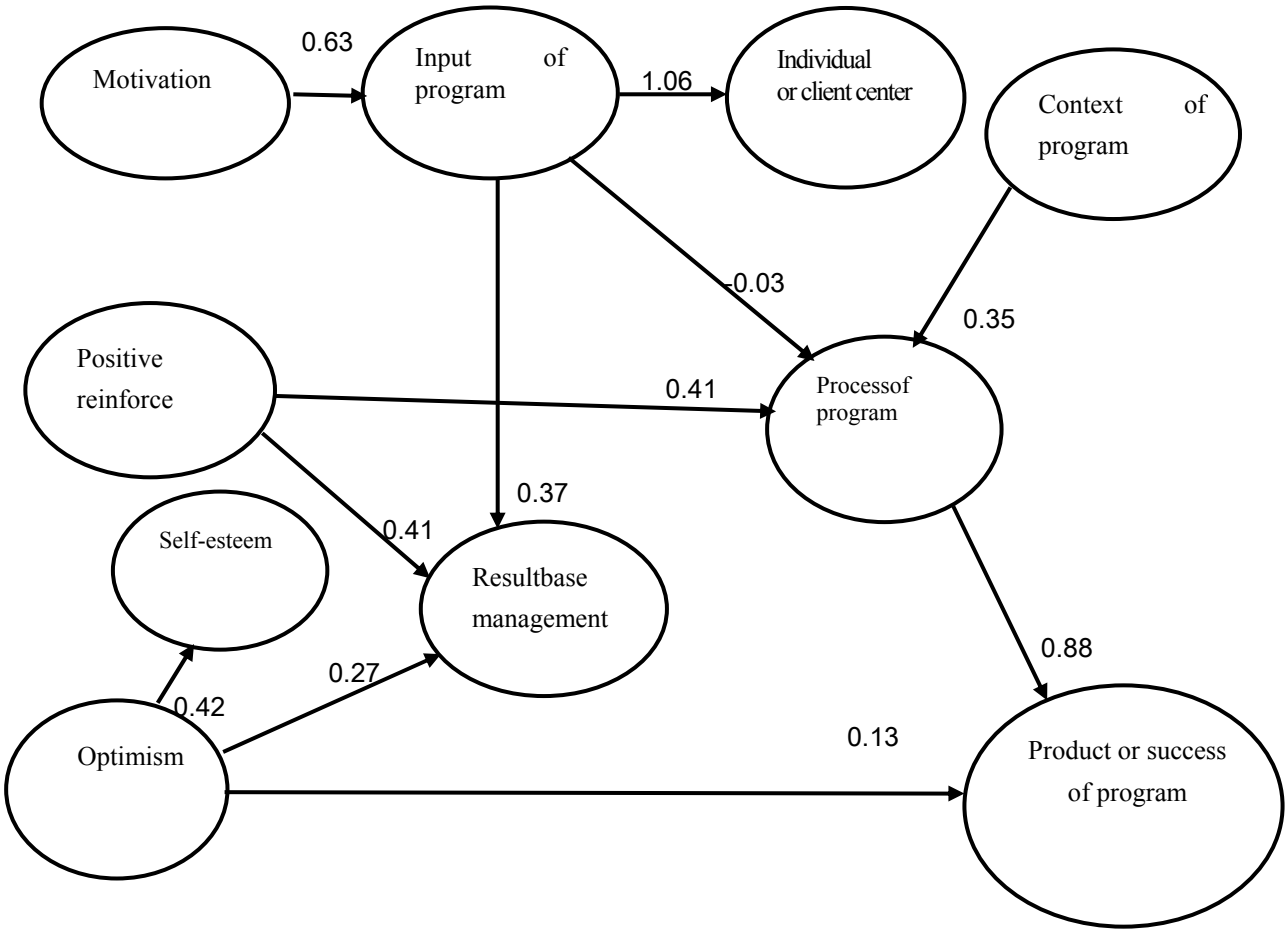

Figure 1. The casual correlation model and effect size of the product or success of the program

Effect of Low Glycemic Load Diet on Glycated Hemoglobin (HbA1c) in Poorly-Controlled Diabetes Patients

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Abstract

Different carbohydrate diets have been administrated to diabetic patients to evaluate the glycemic response, while Poor-controlled diabetes is increasing world wide. To investigate the role of an alternative carbohydrate diet on glycemic control, we explored the effect of a low glycemic load (Low GL)-high fat diet on glycemic response and also glycated hemoglobin (HbA1c) of poor-controlled diabetes patients. Hundred poorly-controlled diabetes patients, HbA1c > 8, age 52.8 ± 4.5 y, were administrated a low GL diet , GL = 67 (Energy 1800 kcal; total fat 36%; fat derived from olive oil and nuts 15%; carbohydrate 42%; protein 22%) for 10 weeks. Patients did their routine life style program during intervention. Fasting blood glucose and HbA1c before and after intervention with significant reduction were: 169 ± 17, 141 ± 12; 8.85% (73 mmol/mol) ± 0.22%, and 7.81% (62 mmol/mol) ± 0.27%; respectively (P < 0.001). Mean fasting blood glucose reduced by 28.1 ± 12.5 and HbA1c by 1.1% (11 mmol/mol) ± 0.3% (P=0.001). There was positive moderate correlation between HbA1c concentration before intervention and FBS reduction after intervention (P < 0.001, at 0.01 level, R =0.52), and strong positive correlation between FBS before intervention and FBS reduction (P < 0.001, at 0.01 level, R = 0.70). This study demonstrated that our alternative low glycemic load diet can be effective in glycemic control.

Keywords: Poorly-controlled diabetes, Glycemic load, Glycated hemoglobin

1. Introduction

Poorly-controlled diabetes that is characterized with increased glycated hemoglobin (HbA1c) > 8% (64 mmol/mol) (Mahan and Escott-Stump, 2007) is increasing world wide, especially in North America and Europe which resulted in an increasing prevalence of disease associated with poor glycemic control (Livesey and Tagami, 2009). Different interventions to lower the glycemic response to carbohydrate foods have been introduced. Theses approaches included: Diets containing 50-60% calories from carbohydrates (Arora and McFarlance, 2005), consumption of soluble fiber, non-soluble fiber, low viscosity fiber (resistant maltodextrin) (Livesey and Tagami, 2009) , and administration of low glycemic load diet (100 g) (glucose equivalents per day) without elevating fat intake (Livesey and Tagami, 2009). High carbohydrate intake recommended in diabetes,

resulting in suboptimal glycemic control and lipoprotein profile, gradually increasing insulin and/or oral hypoglycemic medication requirement and eventually weight gain (Arora and McFarlance, 2005, 2:16). Several studies have demonstrated that viscous soluble fibers suppress the glycemic response to carbohydrate foods, (Garcia *et al*., 2007; Livesey *et al*., 2008), and beneficial effect of insoluble dietary fiber for glycemic control has been reported in different studies (De Munter *et al*., 2007; Schulze *et al*., 2007); however such polysaccharides have limited palatability and insoluble dietary fiber produce flatus and is not suitable for most subjects suffering from gastrointestinal disease. In addition, in prospective cohort studies, it is mainly insoluble cereal dietary fiber (i.e., cellulose and hemicelluloses) and whole grains, not soluble dietary fiber, that associated with reduced diabetes risk (De Munter *et al*., 2007; Schulze *et al*., 2007). In relation to consumption of non-viscous soluble palatable polysaccharides (resistant maltdextrin, RMD) a systematic review of randomized, placebo controlled trials revealed that administration of ≤ 10 g RMD per meal significantly reduces the postprandial glycemic response to a carbohydrate meal in acute studies (Livesey and Tagami, 2009) , however its effect in relation to reducing risk of diabetes in long period consumption is not clear. Also RMD is fermented; it increases the production of flatus and has potential to contribute to abdominal discomfort in higher doses and continues use. (Ohkuma and Takahashi, 1990). Also RMD is more potent in drinks consumed with starch foods than when placed directly into such foods (Livesey and Tagami, 2009).

Therefore the aim of the present study was to investigate the role of low glycemic load diet having lower amount of carbohydrate and higher fat content than traditionally introduced diets as an alternative approach to reduce glycemic response to carbohydrate and also reducing HbA1c concentration of poor-controlled diabetes. We hypothesized that carbohydrate-based low glycemic load diet (GL ≈ 67), with 36% fat, and 42% carbohydrate suppress glycemic response and reduces HbA1c concentration in poor-controlled diabetes.

2. Materials and Methods

One hundred and twenty Poor-controlled (HbA1c > 8%) (Mahan and Escott-Stump, 2007) diabetes patients who were referred to endocrine clinic during 6 months (January 2009 to Jun 2010), and were receiving either insulin or oral medication during study were volunteers for this study. Patients were receiving conventional high carbohydrate low fat diabetes diet. Subjects were excluded if they were unwilling to consume the administrated diet and their medications have not been changed during the study. The procedures were followed in accordance with the ethical standards of the Qazvin University of Medical Science and the study was approved by the Human Research Ethics Committee of the institution. Subjects underwent on low glycemic load diet, GL = 67 (Energy = 1800 kcal, total fat = 36%, fat derived from olive oil and nuts 15%, carbohydrate = 42%, protein = 22% (Table 1) for 10 weeks. Patients were recommended to do their routine daily life style program during intervention. Fasting blood glucose (FBS), HbA1c, weight and BMI were measured before and after intervention. Data were inspected for normality of distribution before use of parametric statistics with SPSS version16 (SPSS Inc, Cary, NC). Data are reported as means ± SDs. Data were analyzed by using paired t-test and Pearson correlation to compare weight, BMI, FBS, and HbA1c of patients before and after intervention.

3. Results

Hundred subjects (55 M, 45 F), aged 52.8 ± 4.5 y, weight 74.0 ± 5 kg, BMI = 27.2 ± 1.9 kg/m^2 who had recruitment criteria took part in this study. Fifteen persons had BMI ≤ 25, while 85 persons had BMI > 25. The mean values for data are shown in Table 2. FBS concentration, HbA1c percentage, weight and BMI was significantly different between two values of before and after intervention (P <0.001). Mean fasting blood glucose reduced by 28.1 ± 12.5 mg/dl (16.6%), HbA1c by 1.1% (11 mmol/mol) ± 0.3%, weight by 3.3 ± 1 kg and BMI by 1.2 ± 0.4 kg/m^2 after diet intervention (P <0.001). There were positive weak correlation between BMI kg/m^2 before intervention and HbA1c level reductions (P = 0.01, at 0.05 level, R = 0.27), between BMI kg/m^2 reduction and HbA1c reduction (P = 0.01, at 0.05 level, R = 0.25), and between HbA1c concentration before intervention and HbA1c reduction (P < 0.001, at 0.01 level, R = 0.36). Also there was positive moderate correlation between HbA1c concentration before intervention and FBS reduction (P < 0.001, at 0.01 level, R = 0.52), and strong positive correlation between FBS before intervention and FBS reduction (P < 0.001, at 0.01 level, R = 0.70), (Table 3, Figure 1). Observed variable changes were significant in both normal and overweight groups.

4. Discussion

This study showed a significant effect of low glycemic load diet on FBS and HbA1c. In our study as we hypothesized, the administrated low glycemic load diet suppressed the HbA1c of poor-controlled diabetes patients to 7.8% (62 mmol/mol) ± 0.3% level which does not considered as poorly-controlled level (Mahan and Escott-Stump, 2007) and was our target in current study.

This study revealed that the more sever the dysglycemia, the greater effect of low GI diet on glycemic control was observed. This finding was parallel with point view of conducted workshop by Howlett and colleagues (Howlett and Ashwell, 2008). Similarly, researchers (Kiens and Richter, 1996) in their study found that both two isoenergetic diets which were composed of 46%, 41%, and 13% as carbohydrate, fat, and protein respectively and the carbohydrate contents were either a high GI (90) or a low GI (66), both didn't have significant effect on normal blood glucose of healthy subjects at the end of 30 days of intervention. In addition it is reported that unavailable carbohydrate reduces fasting blood glucose or HbA1c in persons with diabetes but not in individuals having normal fasting blood glucose (Livesey *et al*., 2008). These studies support our finding in which lower blood glucose levels and also normal blood glucose were less affected by low glycemic load diets.

Diets having composition of 50-60% of total energy as carbohydrates is recommended for diabetics and subjects with metabolic syndrome. Even recommendation of some health organizations is 55-70% carbohydrate, 15-20% proteins and 20-30% fats (Krauss *et al*., 2000; Liu *et al*., 2000; Franz *et al*., 2002). However, epidemiological studies such as the Nurses Health Study and Health Professional Follow-Up Study (Hu and Willett, 2001), and also Framingham Offspring Study (Mckeown *et al*., 2004) have demonstrated the association between glycemic load with type 2 diabetes, CVD and metabolic syndrome. High carbohydrate intake results in suboptimal glycemic control and lipoprotein profile, and subsequently increasing insulin and/or oral hypoglycemic medication requirement and weight gain (Surender and Samy, 2005), while the effect of low carbohydrate diets with 20% of total energy as carbohydrate on glycemic control was greater and independent of weight loss. However in long term compelling with restricted carbohydrate diet is difficult and adherence to such a diet having around 100 g carbohydrates a day which is far away from patients' food habits is weak. In addition physicians are reluctant to advice such a diet to their patients. Considering accumulating evidence for benefits of restricted carbohydrate diets, the American Diabetes Association (ADA) agrees with role of carbohydrate restriction "*in weight management of type 2 diabetes, replacing carbohydrate with monounsaturated fats reduces post prandial glycemia and triglyceridemia*" and recommends that carbohydrates and monounsaturated fat together should provide 60-70% of the energy intake in which their ratio should be individualized. However, alternatively, there is statement from ADA which limits carbohydrate intake to 45-65% of the calories intake (Blades *et al*., 1997). In our study the moderate carbohydrate diet with GL = 67g/day, including 42% carbohydrate as energy intake, and 15% of fat intake from monounsaturated fatty acids sources was almost similar to ADA's recommendation which is more appropriate and compelling for glycemic control in long period. The GL < 80 g/day is considered low GL diet (Brand-Miller, 2005). The higher the GL, the greater the glycemic effect (Afaghi *et al*., 2007) and insulinogenic effect (Foster-Powell *et al*., 2002). The GL of diet in our study was 67 g/d which was even lower than maximum g/day recommendation for low GL diet.

In current study we increased the energy derived from fat up to 36%. Adherence to standard dietary advice to reduce fat intake while increasing carbohydrate intake generally increase the glycemic effect of diet. Both the quantity and quality of a carbohydrate influence postprandial glycemia, and the interaction between the two may be synergistic (Brand-Miller *et al*., 2002). Therefore our meal plan was based on high fat foods that produce a low glycemic response (low- GI foods) and may promote weight control because they increase satiety, minimize postprandial insulin secretion, and maintain insulin sensitivity (Brand-Miller *et al*., 2002).

Fiber consumption has significant effect on glycemic control (Howlett and Ashwell, 2008). However large amounts of fibers ingestion (25 grams per meal) is needed to achieve 10% reduction in 2 hr postprandial blood glucose level (Afaghi *et al*., 2011). In practice due to limited palatability, produced flatus and discomfort by insoluble dietary fiber (DF), consumption of large amount of fiber is not pleasure and diabetic subjects will not compel with such a diet.

Different factors in current study may affected on glycemic control including: moderate energy intake (24 kca/per kg bod weight), low glycemic load diet, and consumption of monounsaturated fatty acids. Moderate energy intake lowers body weight (Freedman *et al*., 2001) and consequently increases insulin sensitivity. Weight loss of 5-10% of initial body weight may significantly improve glycemic and other metabolic abnormalities, and prevents the development of diabetes in high risk populations (Tuomilehto *et al*., 2001; Knowler *et al*., 2002; McFarlane *et al*., 2003). We observed 3.3%, 4.6% and 4.4% weight loss in persons having BMI ≤ 25, BMI > 25, and in total subjects respectively. Due to observed poor correlation between BMI kg/m^2 and HbA1c and lack of any correlation between BMI reduction and FBS reduction, the weight loss in our study, less likely affected on glycemic profile improvement. We believe that the effect of administrated low glycemic load diet was dominant for weight reduction, appetite and also suppress postprandial blood glucose through slow absorption and resulting in reducing HbA1c. We did not have control group which was the limitation of our study.

5. Conclusion

Our provided meal plan for glycemic control of poor-controlled diabetes subjects is appropriate and further investigation for long term effect of low GI diet for glycemic control of poor-controlled diabetes patients is suggested.

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Table 1. Low glycemic load diet administrated to poor-controlled diabetes patients*

Food	Weight (g)	Protein (g)	Fat (g)	Carbohydrate (g)	GI	GL	Energy (kcal)
4 exchange from starch list, (whole-wheat bread, rice, backed beans, sliced fried potato), all low GIs	different	12	---	60	47	28	320
4 exchange from milk list (low fat milk, yogurt)	1000	32	20	48	30	14	480
8 exchange from meat and meat substitutes list (lean meat, low fat cheese, egg whites)	different	49	21	-----	---	----	440
2 exchange from vegetable list (letus, cucumber, tomato)	2 cups raw vegetable	4		10	1	1	50
4 exchange from fruit list (fresh low GI fruits, apple, orange)	480	-----		60	40	24	240
6 exchange from fat list (olive oil, olives, nuts, walnut)	different		30			----	270 (15%)
Total		97 (22%)	71 (36%)	178 (42%)		67	1800

*Source of analysis of ingredients foods: GI, & GL of foods (Taleban and Esmaeili, 1999)

Table 2. Blood glucose profile of diabetic patients before and after diet intervention

patients	no	age	weight	BMI	FBS	HbA1c
at baseline	100	52.8±4.5	74.0±5 CV=6.7%	27.2±1.9, CV=7%	169±17 CV=10%	8.85% (73 mmol/mol) ±0.22% CV=2%
after 10 weeks			70.7±4.6 CV=6.5%	26.0±1.8, CV=7%	141±12 CV=8%	7.81% (62 mmol/mol) ±0.27% CV=3%
P			*P*<0.001	*P*<0.001	*P*<0.001	P<0.001

Table 3. Correlation between different variables of before and after intervention

Variables	FBS reduction 28.1 ± 12.5 mg/dl	HbA1c reduction 1.1 ± 0.3
Weight 74.0±5 kg Before intervention	_____	_____
BMI 27.2±1.9 kg/m^2 Before intervention	_____	*P* = 0.01, at 0.05 level, R= 0.27
FBS 169 ±17 mg/dl Before intervention	*P* < 0.001, at 0.01 level, R = 0.70	_____
HbA1c 8.85 ±0.22 Before intervention	*P* < 0.001, at 0.01 level, R = 0.52	*P* < 0.001, at 0.01 level, R = 0.36
Weight reduction 3.3 ± 1 kg	_____	_____
BMI reduction 1.2 ± 0.4 kg/m^2	_____	*P* = 0.01, at 0.05 level, R = 0.25

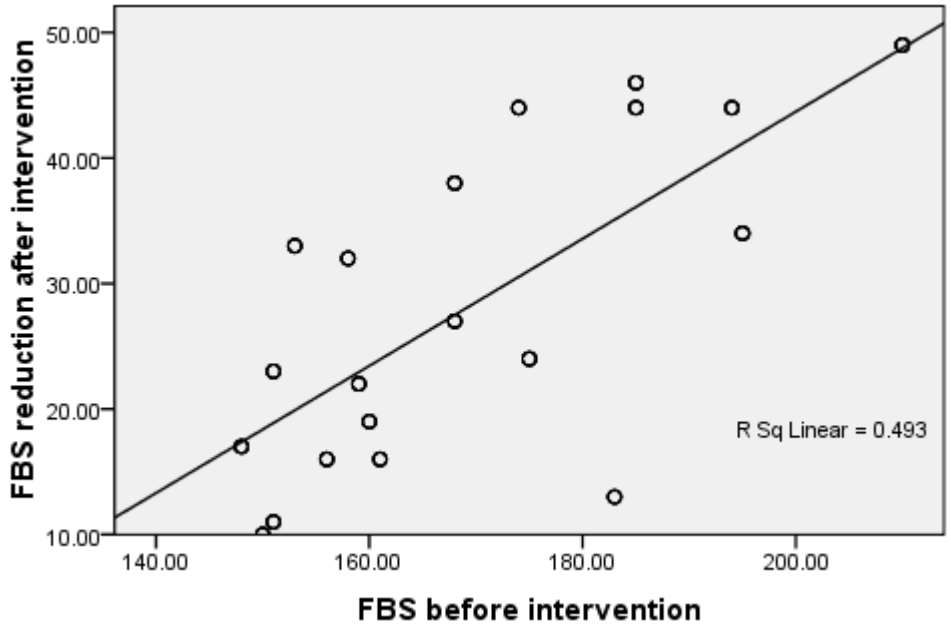

Figure1. Correlation between fasting blood glucose before intervention and fasting blood glucose reduction after intervention in diabetes patients

Isolation, Analysis and Antimicrobial Activity of the Acidic Fractions of Mastic, Kurdica, Mutica and Cabolica Gums from Genus *Pistacia*

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Abstract

The chemical entities of Mastic, Kurdica, Mutica and Cabolica gums from genus *Pistacia* have been isolated and characterised by GC-Mass Spectrometry, High Performance Liquid Chromatography and Column Chromatography. These chemical entities were screened for anti-microbial activities against nine strains of *Helicobacter pylori* and some other Gram-negative and Gram-positive bacteria. The most bioactive components were structurally analysed. These components mimic steroid compounds, in particular, the known antibiotic Fusidic acid. Some of these chemical entities have produced promising data that could lead to the development of a novel class of antimicrobial agents that may have application in the treatment of infectious disease.

Kill kinetics have been also performed, and the produced data were evaluated by Generalized Multiplicative Analysis Of Variance (GEMANOVA) for the bactericidal and bacteriostatic activities which can be clinically significant. The isolated components were all bactericidal.

Keywords: *Pistacia lentiscose*, Atlantica, Kurdica, Mutica, Cabolica, *Helicobacter pylori*, Anti-microbial, GEMANOVA, Fusidic acid, Steroid compounds

1. Introduction

The chemical composition of mastic gum has been studied by a number of researchers. The first attempt to characterise the chemical composition of mastic gum was made in 1904 by Tschirsh and Reutter followed by Casparis and Naef 1934 (Barton & Seaone, 1956). However, they failed to demonstrate any of those components that are known to us today. The first published account detailing elements of the chemical composition of mastic gum was by Barton and Seaone (1956). They isolated and identified three crystalline compounds from the "acidic fraction" (masticadienonic, isomasticadienonic and oleanonic acids) and one compound from the "neutral fraction" of mastic gum (tirucallol) (Barton & Seaone, 1956; Seoane, 1956). In 1973 nine esters were isolated from "acidic fraction" of the galls of *Pistacia lentiscus* (*P. lentiscus*) by chromatography after methylation with diazomethane (Monaco *et al*, 1973). They also isolated eight triterpenes from neutral fraction of the galls produced by *Aploneura lentisci* (Monaco *et al*, 1973). These data were used as a source of authentic measurements of melting point (m. p.) and optical rotations.

Isolated triterpenes from the bled resin of *Pistacia vera* have been documented to show some similarity with those that had been isolated from *P. lentiscus* (Caputo *et al*, 1978). As mastic had been used as a protective layer for artistic works including painting, there was an interest in understanding the characteristics of the gum and so it was subjected to pyrolysis gas chromatography-mass spectrometry (Chiavari *et al*, 1995) for chemical identification. Similar works have been undertaken by other researchers looking for varnishes that had been used for art and paintings (Rene de la Rie, 1989; Van der Doelen, 1998; Van der Doelen & Boon, 2000; Zumbuhl *et al*, 1998).

Eight components were identified by other workers with High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) using atmospheric pressure Chemical Ionisation (APCI) (Van der Doelen *et al*, 1998).

Much of the work that had been undertaken prior to 1987 has been summarized (Mills & White, 1989). Interestingly, they have also identified components of mastic resin together with some other resins from wrecked ships of the late Bronze Age (Mills & White, 1989). While good work has been undertaken, some attempts at characterization have not been reliable and others are perhaps trivial or irrelevant.

Thin Layer Chromatography TLC has also been used for preliminary comparisons (Hairfield & Hairfield, 1990). Some of the previously identified components of mastic identified by less reliable techniques have been confirmed using different methods. For example, Papageorgiou and associates have identified ten triterpenoid acids from acidic fraction of mastic gum by Gas Chromatography-Mass Spectrometry (GC-MS) (Papageorgiou *et al*, 1997). Also, the chemical composition of the resins extracted from insect galls found on the plant species of *Pistacia* has been analysed by a number of researchers (Caputo *et al*, 1978). Apart from a couple of papers that have been published by the same authors (Ebrahimi *et-al,* 2008; Sharifi & Hazell, 2011) on sub-species of *Pistacia atlantica* no other work have been published.

The identified triterpenes and triterpenoids from acidic fractions of mastic gum had structures that mimic those of steroidal compounds. Therefore action was taken to search the literature with respect to possible antimicrobial activity that triterpenes and steroids may exhibit. In China ten triterpenic acids and two steroids had been isolated from the root of *Rubus innominatus* from the *Rosaceae* family in which some of these components were shown to exhibit antibacterial activity (Mingkui *et al*, 2003). Steroid compounds have also been isolated from the sponge *Erylus lendenfeldi* (Geodiidae) collected in the Red Sea with demonstrated antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (Al-Trabeen *et al*, 2004). They have also shown antifungal activity against *Candida albicans* (Al-Trabeen *et al*, 2004). Cholic acid also exhibits a structure similar to some of the acidic fractions of mastic gum. Some novel cationic steroid antibiotics have been synthesized by conjugating tripeptides to a triamino analog of cholic acid. These compounds have demonstrated activity against Gram-negative and Gram-positive bacteria (Bangwei *et al*, 2004).

Preliminary analysis of extracts from *Bryophyllum Pinnatum* (Lam) Oken has shown the presence of steroids, flavanoids, saponins, tannins, glycosides and acids. Such extracts has shown antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Akpuaka *et al*, 2003).

Two novel steroidal phenols have been synthesized and screened against strains of multiresistant *Staphylococcus aureus*, a vancomycin resistant *Enterococcus faecalis* and fast growing mycobacteria (Lange *et al*, 2004). Their antibacterial activity was dependant on the length of alkyl chain (Lange *et al*, 2004). Similarly, stigmasterol and β-stigmasterol glycoside were isolated and identified by 2D NMR; these compounds demonstrated significant antimicrobial activity (Nacef *et al*, 2003).

A tetraoxane derivative of steroid: I [R = H, ethanoyl, propanoyl, benzoyl; R1 = H, Me, Et, isopropyl; R2 = H, Me, Et; R3 = H, Me, Et; R4 = H, Me, Et, tert-Bu, aryl, ester, etc.; X = alkoxy, amino, N-alkylamino, N-arylamino; n = 0-3], and all other possible stereoisomers has demonstrated high antimicrobial activity against the malarial parasite *Plasmodium falciparum* chloroquine-susceptible strain D6, and the chloroquine-resistant strain W2 respectively (Solaja *et al*, 2003) (Figure. 1).

The chemical compositions of gum extracted from the *atlantica* species and its sub-species are not known but are expected to be similar to that of mastic. The acidic fractions of the sub-species of *atlantica*, particularly *kurdica* have been demonstrated to contain compounds exhibiting anti-microbial activity; (Sharifi, 2006) therefore work was undertaken to identify the compositions of extracted fractions and compare the spectra with the published spectra in literature. In this study, the analysis of acidic fractions of mastic gum as a reference and kurdica, mutica and cabolica gums are reported. The identification of the GC-MS peaks was performed by published mass spectra and retention characteristics of mastic gum (Papageorgiou *et al*, 1997). The retention time and characteristics of mastic gum that was obtained by GC-MS was first verified with published data, and then the verified obtained data was used as a criterion for identification of sub-species of *atlantica.*

The acidic fractions of mastic (Sigma Aldridge), kurdica, mutica and cabolica gum (Kurdistan Saghez Sazi and Surij from Iran) were analysed using GC-MS (Sharifi, 2006). Thirteen triterpenoid acids were identified by retention characteristics as their methyl esters (i.e., Moronic acid, Oleanonic acid, Ursonic acid, Oleanolic acid, Isomasticadienonic acid, 3-epi-isomasticadienolic acid, Masticadienonic acid, Dihydromasticadienonic acid, 3-O-acetyl-3epi(iso)masticadienolic acid, Masticadienolic acid, Dihydromasticadienolic acid, 3-acetoxy-3-epiisomasticadienolic acid, 3-acetoxy-3-epimasticadienolic acid) in mastic gum. Their structures were identified by analysis of their spectral data, optical rotation and melting point and also by comparing with

authentic reported data and co-injection with authentic samples (Chiavari *et al*, 1995; Papageorgiou *et al*, 1997; Van der Doelen & Boon, 2000).

This data was used to identify the chemical composition of kurdica, mutica and cabolica gums and to compare the composition by reference to their biological activity. The objectives of the work outlined in this study were as follows:

- To identify the individual components of mastic gum in relation to their antimicrobial activities.
- To use mastic gum data (GC-MS) for identification of kurdica, mutica and cabolica gums components.
- To screen for compounds in gum extracts that have antimicrobial activity seeking to identify and isolate specific compounds with substantial antimicrobial activity.
- To investigate any differences between the active components identified within the fractions.
- To perform kill kinetics on individual chemical isolates seeking to identify bactericidal or bacteriostatic activities.
- To identify any common structure/s of the active component/s.

2. Experimental

2.1 GC-MS Analysis of Acidic Fractions

The acidic fractions of the gums were extracted (Sharifi & Hazell, 2009). These fractions were dissolved in acetonitrile 11mL. This solution was then methylated. The methylated solution was evaporated under vacuum and solids were analysed by GC-MS (Sharifi, 2006). The methylated acidic fractions of kurdica, mutica, cabolica and mastic gum were analysed by GC-MS in split mode (20:1) 0.5 μm injection volume in a Shimadzu QP-5000 GC-MS System with a 30m BP-5 fused silica capillary column of 0.25 mm I.D. and 0.11μm film thickness as described previously (Sharifi, 2006). High Resolution Mass Spectrometry used to determine the molecular formula. The obtained spectrum of mastic gum was used in order to obtain a retention time for the ten previously identified methylated triterpenoid.

2.2 Column Chromatography

A vertical glass column was used to separate and collect the most active fractions of the gum (Sharifi, 2006). In this technique, the mixture to be analysed is placed on the top of the column and flows down through the column (by either gravity or external pressure). This process of fractionation was performed parallel to GC-MS for further validations and also to collect the fractions for antimicrobial assay in the existing form whereas in GC-MS these fractions were methylated. While the intention was to repeat the method reported in the literature (Barton & Seaone, 1956), allowing the comparison with authentic data, some modification in the mobile phase was necessary to optimize the separation. This optimization was obtained by TLC (Sharifi, 2006). Purity of the fractions was tested by Chemical Ionisation (CI) Mass Spectrometry, followed by identification by EI Mass- Spectrometry. These spectra were then correlated with authentic spectra in the literature (Chiavari *et al*, 1995; Monaco *et al*, 1973; Papageorgiou *et al*, 1997; Sharifi, 2006; Van der Doelen & Boon, 2000).

The impure sub-fractions were discarded and the pure components were then crystallized from ether/benzene or methanol where applicable. Rotations were determined in $CHCL_3$, at the concentration of 0.2%, UV absorptions were taken by Varian UV spectrophotometer equipped with Carry 50 software. The molecular weight, m/z fragments, peak intensities and $[\alpha]_D$ are tabulated in Table 1, Table 1.1 and Table 1.2. The pure components were then kept for further analysis and screening for any antimicrobial activities against the strains of *H. pylori* and Gram-positive and Gram-negative.

2.3 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a method of analysis that is not limited by the volatility or stability of the sample compound. HPLC is used to separate, identify, purify and quantify various compounds. Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS) method was used to avoid any changes in chemical structure of triterpenoids components of the acidic fractions (Van der Doelen *et al*, 1998) and also to validate the identification made as a result of data obtained by GC-Mass and collected following column chromatography (Sharifi, 2006). The identified collected fractions were kept for antimicrobial screening against the strains of *H. pylori* and Gram-positive and Gram-negative bacteria, and also to investigate the mode of the action that will be reported in a separate paper.

3. Antimicrobial Activity of the Isolated Components of the Acidic Fractions

3.1 Minimum Inhibitory Concentration (MIC)

The MIC and MBC values were determined for all the sub-fractions of acidic (Sharifi & Hazell, 2009) and all chemical entities that are listed in Table 1, 1.1 and 1.2 against 9 strains of *H. pylori* table 2 and all other Gram-positive and Gram-negative bacteria listed in table 3 and 4 using the broth micro-dilution method (Sharifi & Hazell, 2009).

3.2 Time-kill kinetic

The 26695 strain of *H. pylori, Escherichia coli type 1 and Staphylococcus aureus* were chosen for time-kill kinetic experiments with static liquid cultures. The cultures were allowed to grow to stationary phase and that was determined by taking the Optical Density 600 (OD600) of the cultures (Ebrahimi *et-al*, 2008; Sharifi & Hazell).

The data collected from kill kinetics with MIC and 5X MIC for individual components were recorded on Microsoft Excel and analysed using Microsoft Excel, Sigma plot and MATLAB. As a large number of data was produced from the complex interactions, the classic ANOVA offered limited interpretability. Therefore, Generalized Multiplicative Analysis of Variance (GEMANOVA) method was proposed to tackle this problem in data generated mainly by these complex interactions (Ebrahimi *et-al* 2008). This method was the first application of GEMANOVA to model the data from the field of microbiology and the first GEMANOVA model in which more than two multi-way terms are used and interpreted (Ebrahimi *et-al* 2008).

3.3 Minimum Inhibitory Concentration

The MIC results have been tabulated in Tables 2-4 for all the components of acidic fractions of the mastic, kurdica, mutica and cabolica gums, against the strains of *H. pylori* Table 2 and all other Gram-positive and Gram-negative bacteria listed in Table 3-4

The MIC values for the components listed in Table 1, 1.1 and 1.2 ranged from 0.1-50μg/mL against the strains of *H. pylori* and all other Gram-negative bacteria (Table 2 and 3) and ranged from 2-100μg/mL against Gram-positive bacteria (Table 4).

3.4 Kill Kinetics

The rate of killing for all the components was almost constant. Statistically significant results were determined by a P value of less than or equal to 0.05 (Ebrahimi *et-al* 2008) in their respective MIC and 5MIC.

4. Results and Discussion

The chemical characteristic of the specific components of mastic gum, galls from *P. lentiscus* and *P. vera* are well known. Some of these characteristics were used as authentic data (Monaco *et al*, 1973).

The characteristics of isolated components of acidic fractions of mastic, kurdica, mutica and cabolica gums such as molecular weight, GC-MS data, melting point and optical rotations listed in Table 1, 1.1 and 1.2 correlate with literature (Barton & Seaone, 1956; Papageorgiou *et al*, 1997; Seoane, 1956).

When this study began in January 2000, no published data were available on antimicrobial activity of the mastic gum and other related gums and their fractions. The first abstract from this work was published on June 2001 (Sharifi *et al*, 2001). Soon after that abstract was published, a short paper was published reporting on the antimicrobial activity of whole mastic gum (not defined fractions) against *H. pylori* (Marone *et al*, 2001). In 2003 a patent was published reporting the antimicrobial activity of the mastic as a whole together with a mixture of some of the fractions, that is not pure chemical entities (Fotinos *et al*, 2003).

Important features of the chemical entities that have been identified and isolated in this study are their antimicrobial activity. Most of the chemical entities above have not been tested for antibacterial activity previously, particularly with respect to *H. pylori*. Thus it was important to further characterise these compounds with respect to their capacity to inhibit or kill bacteria.

The antimicrobial screening of these chemical entities led to fundamentally new information that went beyond *H. pylori*, expanding the original parameters of the study. Such was the extent of these findings that a new class of antibiotics may have emerged and their structure have been characterised. The mechanism of their action and structural related activities will be discussed in a separate paper. Furthermore, the potential to enhance the antimicrobial activity of antibiotics has been incremented and as a result the ability to design a new class of antibiotics has become possible.

The MIC values for moronic acid ranged from 5-20 μg/mL. *H. pylori* strain SS1 was more sensitive with MIC 5 μg/mL (Table 2). MIC values ranged for all other Gram-negative bacteria tested from 10-20 μg/mL (Table 3) and

for Gram-positive bacteria ranged from 50-100 μg/mL (Table 4). Antimicrobial activity of moronic acid isolated from *Ozoroa mucronata* has been previously reported in 1979 (Hostettmann-Kaldas & Nakanishi, 1979). However, antiviral activity of this substance is well known and it is reported to be active against Herpes (Kurokawa *et al*, 1999). Purified Moronic acid from *Rhus javanica* has shown significant anti-HSV activity *in vitro* and *in vivo* with therapeutic index of (10.3–16.3). The effective concentrations for 50% plaque reduction of moronic acid for wild type HSV type 1 (HSV-1) was 3.9 mg/mL (Kurokawa *et al*, 1999). Moronic acid has also been isolated from *Myrceugenia euosma* and shown significant anti-HIV activity with therapeutic index of over 186 (Singh *et al*, 2005). This substance and its derivatives were classed "Structure I" for structural analyses (Table 5).

The MIC values for oleanonic acid against the nine strains of *H. pylori* ranged from 5-10 μg/mL. Oleanolic acid and ursonic acid were less active against the 9 strains of *H. pylori* with MIC values ranged from 25-100 μg/mL (Table 2), for all other Gram-negative bacteria the MIC was 50 μg/mL and for all other Gram-positive bacteria the MIC was 100 μg/mL (Table 3 and 4). Antimicrobial activity of oleanonic acid and its derivatives have not been previously reported. Oleanonic and ursonic structures and their derivatives were classed "Structure II and Structure III" respectively (Table 5). Lanosta base skeletons were classed Structure IV, V and VI table 6.

MIC values for masticadienonic acid, isomasticadienonic acid and masticadienolic acid against 9 strains of *H. pylori* was 5μg/mL. Testing masticadienonic acid against the strain P10 of *H. pylori* yielded an MIC of 10 μg/mL (Table 2). Masticadienonic acid and isomasticadienonic acid had MIC values of 5 μg/mL and masticadienolic acid, 2 μg/mL against all other Gram-negative bacteria (Table 3). Their MIC values against Gram-positive bacteria ranged from 5-10μg/mL; (Table 4) these three compounds are designated structures IVb, VIb and IVd respectively (Table 6).

MIC values for dihydromasticadienonic acid against all the strains of *H. pylori* and all other Gram-positive and Gram-negative bacteria ranged from 1 to 5μg/mL. The MIC values of dihydromasticadienolic acid exhibited a tight cluster ranging from 0.5-2μg/mL. This compound is designated structure Va (Table 6).

MIC values for 3-epi-isomasticadienolic acid against *H. pylori* strains ranged 5-10μg/mL. This MIC was higher than that observed for all other Gram-negative bacteria at 1μg/mL and was similar to that observed in Gram-positive bacteria 5μg/mL (Table 2-4). No particular antimicrobial pattern with respect to Gram-positive and Gram-negative bacteria was identified. This compound exhibited broad spectrum activity with an MIC ranged of 1-10 μg/mL and is designated structure VId (Table 6).

MIC values for 3-acetoxy-3-epiisomasticdienolic acid and 3-acetoxy-3-epimasticdienolic acid ranged from 0.1-0.5μg/mL against *H. pylori*, 1μg/mL against all other Gram-negative bacteria tested with the exception of *E. coli* type 1 which had an MIC of 5μg/mL. When tested against Gram-positive bacteria these agents exhibited an MIC of 2μg/mL (Table 2-4).

The most active chemical isolate was 3-O-acetoxy-3-epiisomasticadienolic acid with the MIC values ranged from 0.01 to 5μg/mL against all the strains of *H. pylori* and all other Gram-negative and Gram positive bacteria. This compound is found only in the acidic fraction of the kurdica gum (Table 1, 1.1 and 1.2). This compound is designated structure VIj (Table 6) and constitutes 9.00% of this fraction. Hypothetically, the higher activity of the acidic fraction of kurdica gum in comparison to acidic fractions of the other gums may be attributed to this compound (Sharifi, 2006). Antimicrobial activities of all the Structure IV, V and VI and their derivatives have not previously been reported (Table 6) thus the identification and characterisation of these compounds may represent an important finding that could lead to the development of a novel class of antimicrobial agents that may have application in the treatment of infectious disease.

Antibacterial agents can be bactericidal or bacteriostatic. The difference can be significant clinically. Bactericidal agents may be more effective in the treatment of disease particularly in immuncompromised individuals. The isolated components were all bactericidal (Ebrahimi *et-al,* 2008; Sharifi & Hazell, 2009).

The isolated components were divided into two major groups; Olean base skeleton, with three sub-group of Structure I, II and III (Table 5) and Lanosta Base skeleton with three sub-group of Structure IV, V and VI (Table 6). These components mimic steroid compounds, and the known antibiotic Fusidic acid. As a consequence an investigation of the possible mode of the action/s was undertaken which will be reported in a separate paper.

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Table 1. Chemical composition of acidic fractions of mastic, kurdica, cabolica and mutica gum

No.	I.Time -F.Time	Mastic %	Kurdica %	Cabolica %	Mutica %	Common name/Systematic name	mp	$[\alpha]_D$ 25° CHCl3	M	m/z characteristic ions of methylated compounds.EI (70ev) (int. %)/Under APCI
1	30.017-30.550									
2	34.267-34.6		0.71	0.39	0.53					
3	36.517-36.7		0.85		0.23					
4	37.983-38.350	11.22	11.97	7.38	8.20	Methyl moronate 3-oxo-olean-18-en-28-oic Fig 5-2 methyl ester	218-220º	+60º	468	468(61), 249(55), 189(100)
5	38.583-38.850	7.87	4.93	4.80	5.2	Methyl oleanonate 3-oxo-olean-12-en-28-oic. Fig 5-3 methyl ester	180-182º	+75º	468	468(25), 262(53) 203(100)
6	38.850-38.967		0.76		0.31	Ursonic acid methyl ester 3-oxo-urs-12-en-28-al)	174-176º		468	438(16), 232(21), 203(100)
7	38.967-39.217		2.92		2.51					
8	39.217-39.517	1.38	0.81		1.10				468	468((25), 262(53), 203(100)
9	40.200-40.733		4.46						438	438(16), 232(21), 203(100)
10	40.733-41.100	0.53	3.39	1.02	2.80	Methyloleanolate 3β-hydroxy-olean-12-en-28-oic. Fig 5-5 methyl ester	196-198º	+85º	470	470(16), 410(14), 262(70), 203(100)
11	41.100-41.517	30.74	14.79	13.16	20.50	Methyl isomasticadienonate 3-oxo-13α, 14β, 17βH,20αH-lanosla-8, 24-dien-26-oic. Fig 5-6 methyl ester	110-112º	+37º	468	468(31), 453(100), 421(21)

Table 1.1 Chemical composition of acidic fractions of mastic, kurdica, cabolica and mutica gum

12	41.517-41.900	0.87	1.16	0.43	1.10	Methyl 3-epi-isomasticadienolate3α-hydroxy-13α,14β,17βH, 20αH-lanosta-8,24-dien-26-oic Fig6-7 methyl ester	140-142º	+12º	470	437(100), 121(52) 95(75)
13	42.433-42.650		0.62		1.20					
14	42.233-42.433		0.58							
15	42.433-42.65		0.62							
16	43.050-43.700	40.13	20.06	21.11	32.90	Methyl masticadienonate 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic Fig-6.8methyl ester	123-124º	-71º	468	468(30), 453(100), 421(21)
17	43.700-43.917		0.61							
18	43.917-44.133		0.60			Methyl Dihydromasticadienonate 3-oxo-13α,14β,17βH,20αH-lanosta-7,en-26-oic methyl ester	90-92º	-75°	470	455 (50), 423 (100)
19	44.133-44.667		9.00			Methyl 3-O-acetyl-3epi-iso-masticadienolate (3α-acetoxy-13α, 14β, 17 βH,20αH-Ianosta 8,24-dien-26-oic acid Or 3α-acetoxy-3α,14β17 βH,20αH-Ianosta- 7 ,24--dien-26-oic acid	85-87º	-2º	512	512(26), 497(29), 437(100)

Table 1.2 Chemical composition of acidic fractions of mastic, kurdica, cabolica and mutica gum

20	44.667-44.950	0.79	0.62	3.45	0.71	Methyl masticadienolate 3βhydroxy13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic Fig 6-9 methyl ester	121-122º	-44º	470	455 (60), 437(100), 121(30), 95(50)
21	45.200-45.75		1.24							
22	45.817-46.283		2.29							
23	46.483-46.817		1.06			Methyl Dihydromasticadienolate 3β-H-hydroxyl 3α, 14β,17βH, 20αH-lanosta-7-en-26-oic methyl ester	115-116º	-44º	472	457 (10), 454 (80) 439 (100), 301 (10) 257 (15)
24	46.817-47.283	2.76	10.14	2.74		Methyl 3-acetoxy-3-epiisomasticadienolate 3α-acetoxy13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic methyl ester Fig 6-10	118-122°	+22	512	512(21), 497(26), 437(100)
25	47.550-48.050	2.94	4.03	2.65		Methyl 3-acetoxy-3-epimasticadienolate 3α-acetoxy-13α, 14β, 17βH, 20αH-lanosta-7,24-dien-26-oic. Fig 6-11 methyl ester	100-102º	-45º	512	497(20), 437(100), 189(19), 127(25), 95(34)

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Table 2. The MIC values of the isolated components of the acidic fractions of the gums against the strains of *H. pylori* (μg/mL)

Sub-fractions of acidic fractions of the gums	*H. pylori* 26695	*H. pylori* J99	*H. pylori* RSB6	*H. pylori* P10	*H. pylori* SS1	*H. pylori* SS2000	*H. pylori* N6	*H. pylori* NCTC 11637	*H. pylori* RU1
Moronic acid	10	15	20	10	5	10	10	10	10
Oleanonic acid	10	10	10	10	5	10	10	10	10
Ursonic acid	50	100	50	50	50	100	50	50	50
Oleanolic acid	25	20	20	25	25	25	20	25	25
Isomasticadienonic acid	5	5	5	5	5	5	5	5	5
3-epi-isomasticadienolic acid	10	5	5	5	5	10	5	5	5
Masticadienonic acid	5	5	5	10	5	5	5	5	5
Dihydromasticadienonic acid	1	1	1	1	1	1	1	1	1
3-O-acetyl-3epi(iso)masticadienolic acid	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Masticadienolic acid	5	5	5	5	5	5	5	5	5
Dihydromasticadienolic acid	1	0.5	0.5	1	0.5	1	1	1	1
3-acetoxy-3- epiisomasticadienolic acid	0.1	0.5	0.1	0.1	0.1	0.5	0.5	0.5	0.5
3-acetoxy-3-epimasticadienolic acid	0.5	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 3. The MIC values of the isolated components of the acidic fractions of the gums against the Gram-negative bacteria (μg/mL)

Sub-fractions of acidic fractions 'a' and 'b' of gums	*Escherichia coli*	*Salmonella typhimurium*	*Serratia marscens*	*Pseudomonas aeruginosa*	*Alcaligenes faecalis*	*Enterobacter aerogenes*	*Pseudomonas fluorescens*	*Proteus vulgaris*	*Porphyromonas gingivalis*
Moronic acid	20	20	20	20	20	20	10	10	10
Oleanonic acid	20	20	20	20	20	10	10	10	10
Ursonic acid	50	50	50	50	50	50	50	50	50
Oleanolic acid	25	25	25	20	25	25	25	25	25
Isomasticadienonic acid	5	5	5	5	5	5	5	5	5
3-epi-isomasticadienolic acid	1	1	1	1	1	1	1	1	1
Masticadienonic acid	5	5	5	5	5	5	5	5	5
Dihydromasticadienonic acid	1	1	1	1	1	1	1	1	5
3-O-acetyl-3epi(iso) masticadienolic acid	0.01	0.02	0.01	0.05	0.01	0.01	0.01	0.01	0.01
Masticadienolic acid	2	2	2	2	2	2	2	2	2
Dihydromasticadienolic acid	1	1	1	1	1	1	1	1	1
3-acetoxy-3-epiiso-masticadienolic acid	1	1	1	1	1	1	1	1	1
3-acetoxy-3-epimasticadienolic acid	1	1	1	1	1	1	1	1	1

Table 4. The MIC values of the isolated components of the acidic fractions of the gums against the Gram-positive bacteria (μg/mL)

Sub-fractions of acidic fractions 'a' and 'b' of gums	*Bacillus cereus*	*Staphylococcus aureus*	*Streptococcus faecalis*	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Corynebacterium sp.*
Moronic acid	50	50	75	50	100	50
Oleanonic acid	50	50	50	50	50	50
Ursonic acid	100	100	100	100	100	100
Oleanolic acid	20	20	20	25	20	20
Isomasticadienonic acid	5	5	10	5	5	5
3-epi-isomasticadienolic acid	5	5	5	5	5	5
Masticadienonic acid	5	5	5	5	5	5
Dihydromasticadienonic acid	2	5	2	5	2	2
3-O-acetyl-3epi(iso)masticadienolic acid	0.01	0.05	0.01	0.02	0.02	0.01
Masticadienolic acid	5	10	5	5	10	5
Dihydromasticadienolic acid	2	2	2	2	2	2
3-acetoxy-3-epiisomasticadienolic acid	2	2	5	2	2	2
3-acetoxy-3-epimasticadienolic acid	2	5	2	2	2	2

Table 5. Chemical structure of isolated components of the gums

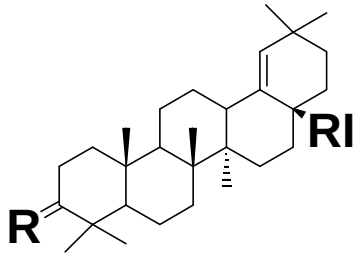
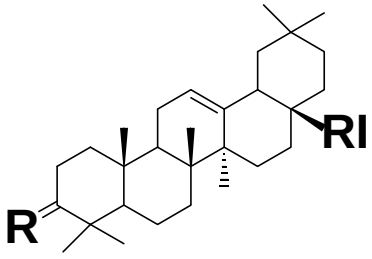
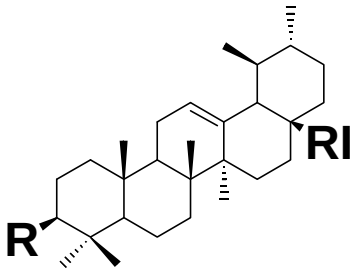
Structure I	**Structure II**	**Structure III**
		
a: R= O RI= COOMe Moronate b: R= O RI= COOH Moronic acid c: R= O RI=CHO Moronic aldehyde	a: R = O, RI = COOMe Oleanonate b: R = O, RI = COOH Oleanonic acid c: R = H, β-OH, RI = COOMe Oleanolate d: R =O RI = CHO Oleanolic aldehyde	a: R = H, α-OH RI = CHO Ursonic aldehyde

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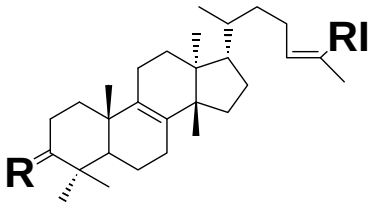
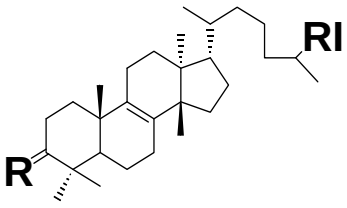
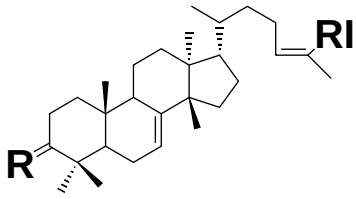
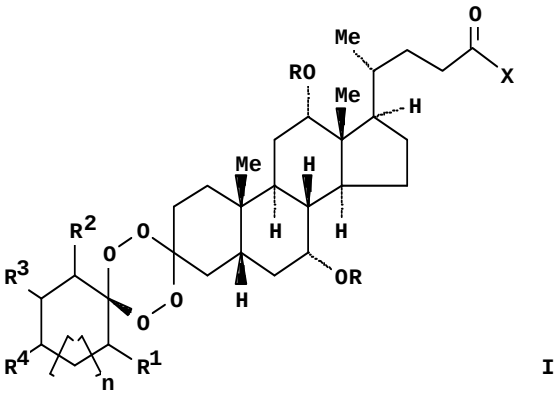
Table 6. Chemical structure of isolated components of the gums

Structure IV	**Structure V**	**Structure VI**
		
a: R = O, RI = COOMe Masticadienonate, b: R = O RI = COOH, Masticadienonic acid c: R =H, β-OH, RI = COOMe Masticadienolate, d: R =H, β-OH RI = COOH, Masticadienolic acid e: R = α-CH3COO, RI = COOMe 3α-acetoxy-3-epimasticadienolate f: R = α-CH3COO β-OH, RI = COOMe 3-α-acetoxy-masticadienonate g: R = α-CH3COO, RI = COOH 3-α-acetoxy-masticadienonic acid h: R = α-CH3COO, RI = COOMe 3-α-acetoxy-3-epimasticadienolate i: R = α-CH3COO β-OH RI = COOH 3-α-acetoxy-3-epimasticadienolic acid	a: R = O RI = COOMe Dihydromasticadienonate b: R = O RI = COOH Dihydromasticadienonic acid c: R =H, β-OH RI = COOMe Dihydromasticadienolate d: R =H, β-OH RI = COOH Dihydromasticadienolic acid	a: R = O, RI = COOMe, Isomasticadienonate b: R = O, RI = COOH Isomasticadienonic acid c: R =H, α-OH, RI=COOMe 3-epi-isomasticadienolate d: R =H, α-OH, RI = COOH 3-epi-isomasticadienolicacid e: R = α-CH3COO, RI = COOMe 3-α-acetoxy-masticadienonate f: R = α-CH3COO, RI = COOH 3-α-acetoxy-isomasticadienonic acid g: R = α-CH3COO β-OH, RI = COOH 3-acetoxy-3-epi- isomasticadienolate h: R = α-CH3COO β-OH, RI = COOH 3-acetoxy-3-epi- isomasticadienolic acid, i: R =H; α-OAc, RI = COOMe 3-O-acetyl-3epi-iso-masticadienolate j: R =H; α-OAc, RI = COOH 3-O-acetyl-3epi-iso-masticadienolic acid



I

Figure 1. Tetraoxane derivative of steroid

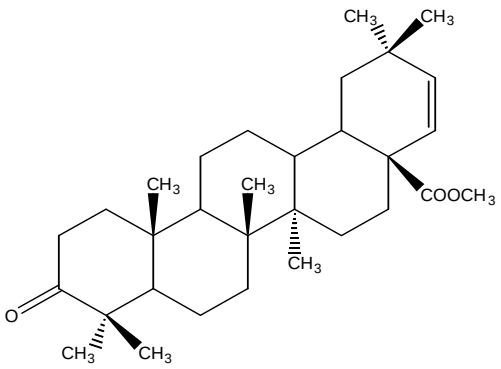


Figure 2. Methyl moronate

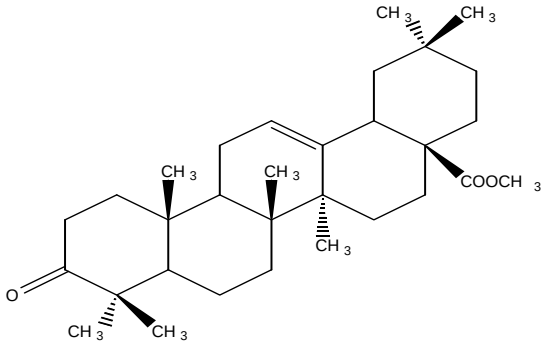


Figure 3. Methyl oleanonate

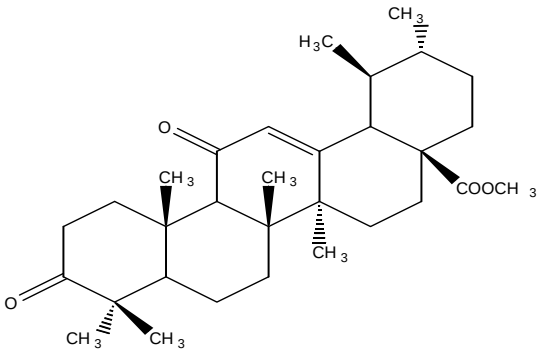


Figure 4. Ursonic acid (Methyl ester)

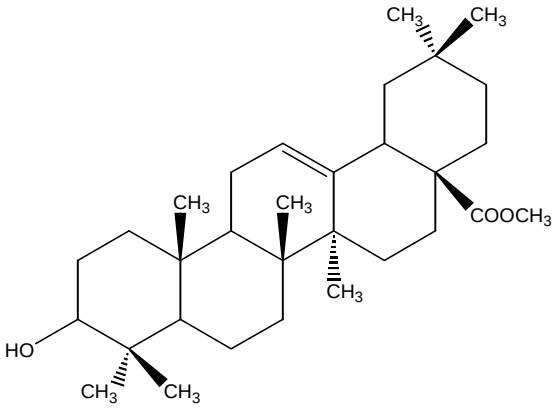


Figure 5. Methyl oleanolate

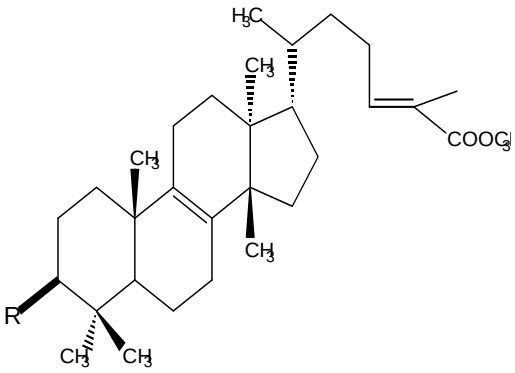


Figure 6. (R=O), Figure 9 (R=β-OH, H) and Figure 11 (α-CH3COO)

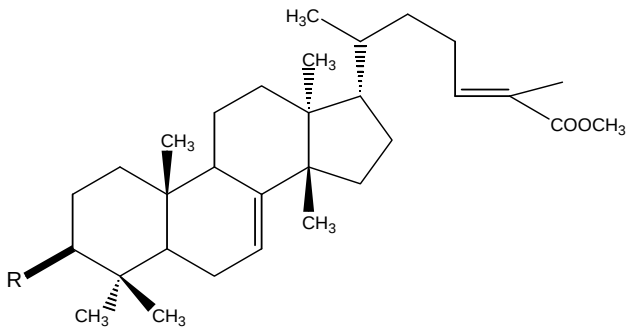


Figure 7. (R=O), Figure 8 (R=α-OH, H) and Figure 10 (α-CH3COO)

Extrachromosomal DNA Length and Antibiograms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolated from Tears of HIV/AIDS Patients after Curing with Sodium Dodecyl Sulphate

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Abstract

Staphylococcus aureus and *Pseudomonas aeruginosa* strains were isolated from eye swab samples randomly obtained from 100 seropositive HIV/AIDS patients who reported to various anti-retroviral treatment clinics at the University of Benin Teaching Hospital and Central Hospital both based in Benin City, Nigeria. Invitro antibiotic sensitivity patterns of strains before curing were determined by the Kirby-Bauer disc diffusion technique. Resistance plasmid DNA of multidrug resistant strains was cured with 0.1% sodium dodecyl sulphate and cured strains were again subjected to invitro antibiotic sensitivity testing. EcoRI and Hind III restriction endonuclease enzymes were used to make cuts on extracted plasmid DNA whose length sizes were then determined. A total of 36 (36.0%) strains made up of 27 (75.0%) *Staphylococcus. aureus* and 9 (25.0%) *Pseudomonas aeruginosa* were isolated of which 7 (19.4%) strains showed multidrug resistance to ciprofloxacin, pefloxacin, ofloxacine, gentamycin, tetracycline, ampicillin, chloramphenicol, nitrofurantoin and erythromycin. All seven multidrug resistant strains before curing, recorded 85.7%, 42.9%, 14.3% and 14.3% sensitivity in that decreasing order to ciprofloxacin, pefloxacin, ofloxacin and gentamycin respectively. There was 0.0% sensitivity each to tetracycline and ampicillin. After curing, there was enhanced sensitivity of 100.0%, 85.7%, 28.6% and 71.4% respectively. There was also 28.6% and 57.1% improved sensitivity to tetracycline and ampicillin after curing. Before curing, there was 76.2% average resistance to all used antibiotics and this reduced to 47.6% after curing *Staph. aureus* plasmid DNA. In the case of *Pseudomonas aeruginosa*, there was an average resistance of 76.3% before curing which fell to 42.5% after curing. EcoRI restriction enzyme gave the plasmid DNA length of *Staphylococcus aureus* strain 04 as 4.0Kb and this size depended upon the distance between recognition sites. Isolation of 36 (36.0%) strains of both isolates from 100 eye swabs shows the danger these organisms portend to all categories of opticians. The cheapness and high sensitivity of gentamycin justifies its use as eye drops for treatment of some eye infections. Curing of plasmid DNA is an indication that if SDS is administered to the organisms in sublethal doses, it can lead to the elimination of plasmid DNA without adverse effect on the genomic DNA of the bacterial strains.

Keywords: Extrachromosomal, DNA, Antibiogram, *Staph aureus, P. aeruginosa*, Sodium dodecyl sulphate

1. Introduction

AIDS nearly always affects the eyes and ophthalmic signs (symptoms) were the initial signs (among others), that led to the diagnosis of HIV infection in its terminal stage (Cheesborough, 1990). Fujikawa *et al*. (1985) reported that when patients contact HIV, the virus can infect nearly every ocular tissue as well as the tear (lachrymal) gland. They found human T-cell lymphotrophic virus (HTLV-III) in tears.

Staphylococcus aureus and *Psuedomonas aeruginosa* are opportunistic pathogens in humans and animals and are one of the frequent sources of hospital and community acquired infections. They can infect the eyes through contaminated fingers and contact lens. Contact lens also causes abrasion of ocular structure thereby increasing the chances of the "wearers" developing ocular infection. Disinfecting systems are important part of infection control practices and aid in the prevention of infection.

Pseudomonas aeruginosa, among other diseases, causes eye infections such as keratitis and neonatal ophthalmia. *Pseudomonas aeruginosa* can colonize the ocular epithelium by means of a fimbrial attachment to sialic acid receptors. If the defense of the environment is compromised in any way, the bacterium can proliferate rapidly and through the production of enzymes such as elastase, alkaline protease as well as exotoxins which can cause rapidly destructive infections which may eventually lead to loss of the entire eye *(*Alejandro *et al.,* 2002*).*

Capriotti *et al.* (2008) in their study of normal flora in 276 conjunctival swabs of healthy eyes of a rural population in Sierra Leone, isolated coagulase negative *Staph aureus* (28.6%), coagulase positive *Staph aureus* (19.9%), *Haemophilus* spp (9.8%) and *Norcadia* spp (6.5%). Grasbon *et al.* (1995) examined 100 conjunctival swabs for the prevalence of coagulase negative *Staph aureus* and isolated 151 different strains of coagulase positive *Staph aureus* in 86 samples while coagulase negative *Staphylococci* accounted for the remainder.

Maria *et al.* (2000) worked on 40 conjunctival swabs of health professionals to know the conjunctival microflora of clinically healthy persons who work in the hospital environment. They isolated *Staph. epidermidis* (45.0%), *Bacillus* spp (29.0%), *Proteus* spp (61.0%), *Citrobacter* spp (2.1%), *Moraxella* spp (2.1%) and *Proteus mirabilis* (2.1%). Yasuyuki *et al*. (2005) in a study to compare the conjunctival flora of HIV seropositive and seronegative patients found no difference between the conjunctival flora of HIV positive and negative patients

Most bacterial strains are lysogenic and many of the toxins and products of these strains are mediated by plasmids which play a major role chemically as mediators of antimicrobial resistance (Alejandro *et al.,* 2002). Griffith (1986) reported that antibiotic resistance plasmids were molecules of circular DNA containing an aONA segment called the replication region which allows the plasmid to propagate itself independently of the machinery that reproduces the chromosomal DNA.

Aminocyclotol resistance plasmid was isolated and characterized in *Staphylococcus aureus* by Gray (1999). According to him, the plasmid was divided into two interrelated groups due to loss or gain of defined DNA sequences. Also, Stiffer *et al*. (1974) isolated and characterized Kanamycin resistance plasmid form *Staph. aureus*. They reported that the plasmid had a molecular weight of 9.2 x 104 Daltons.

A restriction endonuclease functions by scanning the length of a DNA molecule. Once it encounters its particular specific recognition sequence, it binds to the DNA molecules and makes one cut in each of the two sugar-phosphate backbones of the double helix. The positions of these two cuts both in relation to each other and to the recognition sequence itself are determined by the identity of the restriction endonuclease used to cleave the molecule in the first place.

Lofdahi *et al.* (1978) characterized small molecular weight plasmids from *Staph. aureus* with respect to size, restriction endonuclease pattern and transforming capacity. The plasmids p5194 and pCM4 which encode streptomycin and chloramphenicol resistance respectively contained 3.0 and 2.0 megadalton of DNA. Both plasmids transformed *Staph. aureus* with high efficacy. Whereas pC 194 contained only one average size for endonuclease Hind II, pS194 contained single cleavage for endonuclease Hind III and EcoRI.

The multidrug resistance nature of some organisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (among others), found in seropositive HIV/AIDS patients have posed a serious problem in the effective management of eye infections suffered by these patients from time to time. This study therefore, is aimed at determining the "extrachrosomal DNA length and antibiograms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from tears of HIV/AIDS patients after curing with Sodium Dodecyl Sulphate" with the following objectives:

1) Isolate *Staph. aureus* and *Pseudomonas aeruginosa* from eye swabs of HIV/AIDS patients.

2) Determine the sensitivity patterns (antibiograms) of these isolates to some selected antibiotics.

3) Cure the isolates of their plasmid DNA using sodium dodecyl sulphate (SDS).

4) Use restriction endonucleases (EcoRI and Hind III) to cut and determine the length of the isolates plasmid DNA.

2. Materials and Methods

2.1 Sampling

With informed consent and approval obtained from the ethical committee of the hospital managements, eye swabs of 100 seropositive HIV/AIDS patients receiving treatment at the medical wards of the University of Benin Teaching Hospital (UBTH) and the Central Hospital both based in Benin City, Nigeria were randomly selected and obtained for the study. Collection of samples was done without consideration for age, sex, profession and family background. The seropositive HIV/AIDS status of patients used was as confirmed by the Enzyme Linked Immunosorbent Assay (ELISA) technique.

2.2 Processing of Samples

Obtained eye swabs were taken to the laboratory immediately for processing. Swabs were collected in duplicates per patient. While one was used for gram staining, the other was used for culture. Swabs were aseptically cultured on sterile MacConkey, Blood and Mannitol salt agar plates and incubated aerobically at 37^0C for 24hours.

Isolates were identified culturally, morphologically, biochemically and by sugar fermentation according to schemes provided by Cowan and Steel (1993) and Cullimoore (2000). All catalase positive, coagulase positive colonies, gram positive cocci in clusters, glucose positive, mannitol positive (characteristic of *Staphylococcus aureus)* and all citrate positive, oxidase positive colonies with gray-greenish pigmentation, short gram negative rods in singles (characteristic of *Pseudomonas aeruginosa*) were stocked on agar slants for further use. Before curing, the strains were subjected to invitro antibiotic testing.

2.3 Invitro Antibiotic Testing

This was done according to the modified Bauer and Kirby (1997) disc diffusion technique. Sterile Nutrient agar plates and peptone water were prepared and dispensed into bijou bottles in accordance with manufacturer's instructions. An inoculum of the stock culture of each strain was subcultured into sterile peptone water bijou bottles and incubated on the bench for 2-3hours. A sterile peptone water bottle (which was not subcultured) was used as control. All steps above were carried out with proper labellings. Sterile Nutrient agar plates were arranged and labeled for the strains of each isolate and one as control. The plates were flooded with the liquid culture in the bijou bottles and the control bijou bottle was equally used to flood the control plate. The excess liquid culture was drained off the plates. Using well sterilized pair of forceps, commercially obtained gram positive and gram negative multidrug discs was impregnated on flooded agar surface. While gram positive discs was used for *Staph. aureus* strains, gram negative discs were used for *Pseudomonas aeruginosa* strains. The control plate was divided into two halves. On one half, gram positive discs were impregnated and gram negative discs on the other half. All plates were then incubated aerobically at 37^0C for 24hours. Results were interpreted according to the National Committee for Clinical Laboratory Standards (1997). Strains that were resistant to the drugs used invitro were subjected to curing with sodium dodecyl sulphate (SDS).

2.4 Plasmid Curing

Plasmid curing of *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains that were resistant (did not show any zone of inhibition after incubation) was done according to the method described by Tomoeda *et al*. (1968). To 90ml of Nutrient broth, 10g of sodium dodecyl sulphate (SDS) was added. The resulting suspension was autoclaved, steamed for 1 hour, pH adjusted to 7.6 and then used as stock solution. Overnight broth culture of the multidrug resistant strains of *Staph. aureus* and *Pseudomonas aeruginosa* was each diluted 100 fold (0.1ml of broth + 9.9ml sterile water). To fresh 30ml of Nutrient broth, 0.5ml of diluted broth was added and mixture maintained at pH 7.6. This was incubated for 2 hours and 1% SDS stock solution was added and then incubated for up to 72hours (3days).

2.5 Invitro Antibiotic sensitivity testing of cured strains

Plasmid cured strains were again subjected to antibiotic sensitivity testing according to the method of Kirby-Bauer already described above.

2.6 Isolation of Plasmid DNA

Cured plasmid DNA of multidrug resistant strains of *Staph. aureus* and *Pseudomonas aeruginosa* was isolated by the alkaline phosphate method of Birmborn and Doly (1979).

2.7 Restriction Endonuclease Activity

Commercially available restriction endonucleases - EcoRI and Hind III were obtained and used to determine the

sizes of short cuts on the isolated Plasmid DNA. With these sizes known, the entire plasmid DNA length was then determined. According to the method described by Esiobu (2008), 0.51 microlitre (0.51µl) of restriction endonucleases of EcoRI and Hind III each, was added to 1 microlitre of 1µg/ml of extracted DNA in a tube and mixed. The mixture was centrifuged for few seconds in a microcentrifuge and incubated at 37^0C for 2hours. Ten microlitres of the mixture was taken and put in another tube and this was loaded into 1% Agarose gel stained with ethidium bromide. The electrophoretic tank was filled with 1XTBE. Agarose gel was also loaded with 5µl of extracted (uncut) genomic DNA. Gel was then run at 120 volts for 45mins and was visualized on a trans-illuminator at long UV range. The gel results were then analyzed to determine length of extracted (cured) DNA.

3. Results

Out of the 100 samples processed, 36(36.0%) strains made up of 27(75.0%) *Staphylococcus aureus* and 9(25.0%) *Pseudomonas aeruginosa* strains were obtained of which 3(11.1%) *Staph. aureus* and 4(44.4%) *Pseudomonas aeruginosa* strains showed multidrug resistance (Table 1).

Before curing, 3(11.1%) *Staph.aureus* strains showed resistance to three or more antibiotics invitro while 4(44.4%) *Pseudomonas aeruginosa* strains showed resistance to three or more (multidrug resistance). After curing, 2(7.4%) *Staph. aureus* strains showed resistance to more than three antibiotics invitro while 2 (22.2%) *Pseudomonas aeruginosa* strains showed resistance to three or more antibiotics (Table 2).

After curing, *Staphylococcus aureus* didn't show any sensitivity change to ciprocin as all strains remained sensitive before and after treatment with 0.1% sodium dodecyl sulphate. There was 33.3% reduction in resistance to pefloxacin after treatment. Similarly, there were 66.7%, 66.7% and 33.3% changes (or reduction) in resistance to gentamycin, ampicillin and erythromycin respectively after treatment or curing with SDS. However, all strains remained resistant to ofloxacin and tetracycline before and after curing (Tables 3 and 4). On average, there was 28.6% reduction in resistance to all the antibiotics after curing.

As for *Pseudomonas aeruginosa*, there was no change in resistance to nitrofurantoin after curing as all strains remained resistant. There was 25% change in resistance to ciprocin after curing. There were 20%, 50%, 50%, 50% and 75% reduction in resistance to tetracycline, pefloxacin, ofloxacin, gentamycin and ampicillin respectively after curing. There were however no resistance reduction (changes) for nitrofurantoin and chloramphenicol (Table 4). On the average, there was 33.8% resistance reduction of *Pseudomonas aeruginosa* strains to all the antibiotics used.

The fragmented (cut) DNA sizes as mediated by EcoRI and Hind III restriction enzymes are shown in Table 5. All the three multidrug resistant strains of *Staph. aureus* showed one plasmid DNA each (after plasmid DNA isolation). While two *Pseudomonas aeruginosa* strains showed two plasmid DNA each, the other two showed one each (Table 5). EcoRI and Hind III restriction enzymes made one cut (one fragment) each on the three strains of *Staph. aureus* separately. The same restriction enzymes made three and two DNA cuts respectively on *Pseudomonas aeruginosa* strain 11. Similarly, the enzymes made four and two cuts respectively on *Pseudomonas aeruginosa* strain 12 and one cut apiece on strains 13 and 14. The plasmid DNA length of 4.0kb for *Staphylococcus aureus* strain 04 using the fragment size as mediated by restriction enzyme EcoRI for example was obtained by adding the antilog of the log of the DNA molecular marker weight (MMW) i.e

$$\text{Plasmid size} = \text{Sum of Restricted fragments}$$
$$= 3122.0 + 398.1 + 239.9$$
$$= 3760.0\text{bp}$$
$$= 4.0\text{kb approximately}$$

4. Discussion

HIV has been recovered from ocular tissues, tears and soft contact lenses of patients with AIDS (Dennehy *et al.,* 1989). All ophthalmic offices especially those with contact lens practice must be aware of any potential risk of transmission of HIV to both the office staff and other non-HIV patients through the use of trial contact lenses and tonometry (ocular examination involving contact with tears and ocular tissues).

The isolation of 36 strains made up of 27 (75.0%) *Staphylococcus aureus* and 9(25.0%) *Pseudomonas aeruginosa* from the 100 eye swab samples processed (Table 1) shows the danger these organisms may portend to all categories of opticians due to continuous exposure to them.

Pseudomonas aeruginosa can cause a rapidly destructive infection that can lead to loss of the entire eye (Slonim and Tampa, 1987). The organism has been found to grow better on the cornea than any other part of the eye and

has also been reported to be a contaminant of many commonly used eye solutions and disinfectants (Morrison *et al.,* 1984 and Gilgardi, 1972). *Staphylococcus aureus* is the leading cause of soft tissue infections and has been implicated in eye infections (Slonim and Tampa, 1987).

All seven multidrug resistant strains before curing, recorded 85.7%, 452.9%, 14.3% and 14.3% sensitivity in that decreasing order to ciprofloxacin, pefloxacin, ofloxacin and gentamycin respectively (Table 2).

There was 0.0% sensitivity each to tetracycline and ampicillin. After curing, there was improvement in sensitivity due to removal of resistance plasmid DNA. Consequently, there was 100.0%, 85.7%, 28.6% and 71.4% improved sensitivity to the same drugs respectively. Of note, is 28.6% and 57.1% improved sensitivity to tetracycline and ampicillin after curing (Table 2). This shows or suggests that ciprofloxacin, pefloxacin, ofloxacin and gentamycin will prove to be effective in inhibiting or killing most of the strains. However after curing, ampicillin recorded 57.1% improved sensitivity as against 0.0% sensitivity before curing. However, apart from gentamycin, the others are expensive and sometimes scarce. This finding did not agree with the report of Anyanwu (1983) which stated that there is high resistance to most of the commonly used antibiotics except gentamycin. Drugs used in this study are not commonly used apart from gentamycin, tetracycline ampicillin, chloramphenicol and erythromycin. Resistance to the others apart from gentamycin and ampicillin (after curing) was high. This finding somewhat justifies the continued use of gentamycin eye drops to treat most eye infections. A combination of ampicillin and gentamycin may provide a synergistic effect to combat very debilitating eye problems.

The average resistance to all the antibiotics used on *Staphylococcus aureus* before curing was 76.2% and this reduced to 47.6% after curing suggesting a 28.6% change or reduction in resistance (Table 3). The curing of the plasmid DNA which brought about improved sensitivity is an indication that if sodium dodecyl sulphate (curing agent) is administered to the organisms in a sub lethal dose, it can lead to the elimination of plasmid DNA without adverse effect on the genomic DNA of the bacterial strains under study (Singleton and Sainsbury, 2001). A higher improvement in sensitivity to the antibiotics including nitrofurantoin and chloramphenicol was recorded for *Pseudomonas aeruginosa*. After curing, there was 33.8% reduction in resistance to the drugs on the average (Table 4).

Gel electrophoresis analysis of plasmid DNA of multidrug resistant strains after restriction enzyme digestion showed various plasmid numbers and DNA fragment sizes. The fragment sizes were used to determine overall length of plasmid DNA. For example EcoRI enzyme gave the plasmid DNA length of *Staphylococcus aureus* strain 04 as 4.0kb. The size of the DNA fragments generated by restriction enzyme cleavage depends on the distance between recognition sites (Esiobu, 2008).

5. Conclusion

Thirty six strains of isolates made up of 27 (75.0%) *Staphylococcus aureus* and 9 (25.0%) *Pseudomonas aeruginosa* isolated from 100 eye swabs shows the danger these organisms portend to all categories of opticians due to continuous exposure to them.

All seven multidrug resistant strains before curing, recorded 85.7%, 42.9%, 14.3% and 14.3% sensitivity in that decreasing order to ciprocin, peflacin, ofloxacin and gentamycin respectively. There was 0.0% sensitivity each to tetracycline and ampicillin. After curing, there was improvement in sensitivity as there was 100.0%, 85.7%, 28.6% and 71.4% enhanced sensitivity respectively of note, is 28.6% and 57.1% improved sensitivity to tetracycline and ampicillin after curing.

Average resistance to all antibiotics used on *Staphylococcus aureus* before curing was 76.2% which reduced to 47.6% after curing suggesting a 28.6% change or reduction in resistance. The curing of the plasmid DNA which brought about improved sensitivity is an indication that if sodium dodecyl sulphate (curing agent) is administered to the organisms in a sublethal dose, it can lead to the elimination of plasmid DNA without adverse effect on the genomic DNA of the bacterial strains under study.

Gel electrophoresis analysis of plasmid DNA of multidrug resistant strains after restriction enzyme digestion showed various plasmid Nos and DNA fragment sizes. EcoRI enzyme gave the plasmid DNA length of *Staphylococcus aureus* strain 04 as 4.0kb and this size depended upon the distance between recognition sites.

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Table 1. Occurrence of multidrug resistant strains among samples processed.

Isolates	No of strains isolated	No of strains showing multidrug resistance
Staphylococcus aureus	27 (75.0%)	3 (11.1%)
Pseudomonas aeruginosa	9 (25.0%)	4 (44.4%)
Total	**36 (100.0%)**	7

Table 2. Invitro Antibiotic Susceptibility Patterns of Isolates strains before and after curing

Code of strains	Organisms	Antibiotics									
			CIP	PEF	OF	GN	TE	AMP	C	N	E
04	*Staph. aureus*	Before	S	R	R	R	R	R	NA	NA	R
		After	S	S	R	R	R	R	NA	NA	S
06	*Staph. aureus*	Before	S	S	R	R	R	R	NA	NA	S
		After	S	S	R	S	R	S	NA	NA	S
10	*Staph. aureus*	Before	S	R	R	R	R	R	NA	NA	R
		After	S	R	R	S	R	R	NA	NA	R
11	*Pseud. aeruginosa*	Before	R	R	S	S	R	R	R	R	NA
		After	S	S	S	R	S	R	R	R	
12	*Pseud. aeruginosa*	Before	S	S	R	R	R	R	S	S	NA
		After	S	S	S	S	R	S	R	S	
13	*Pseud. aeruginosa*	Before	S	R	R	R	R	R	S	S	NA
		After	S	S	R	S	S	S	R	S	
14	*Pseud. aeruginosa*	Before	S	S	R	R	R	R	R	S	NA
		After	S	S	R	S	R	S	R	S	
Total % sensitivity		Before	**85.7%**	**42.9%**	**14.3%**	**14.3%**	**0.0%**	**0.0%**			
		After	**100%**	**85.7%**	**28.6%**	**71.4%**	**28.6%**	**57.1%**			

Table 3. Summary of Resistance pattern of Staph. aureus after curing with 0.1% Sodium Dodecyl Sulphate

Antibiotic	Before	After	% Change (Reduction) in Resistance
Ciprofloxacin	0%	0%	0%
Pefloxacine	66.7%	33.3%	33.3%
Ofloxacine	100.0%	100.0%	0.0%
Gentamycin	100%	33.3%	66.7%
Tetracycline	100.0%	100.0%	0.0%
Ampicillin	100%	33.3%	66.7%
Erythromycin	66.7%	33.3%	33.3%
Average	**76.2%**	**47.6%**	**28.6%**

Table 4. Summary of Resistance Pattern of *Pseudomonas aeruginosa* after curing with 0.1% SDS

Antibiotic	Before	After	% change (Reduction) in Resistance
Ciprofloxacin	75.0%	50.0%	25.0%
Pefloxacine	50.0%	0.0%	50.0%
Ofloxacine	75.0%	25.0%	50.0%
Gentamycin	75.0%	25.0%	50.0%
Ampicillin	100.0%	25.0%	75.0%
Nitrofurantoin	75.0%	75.0%	0.0%
Chloramphenicol	100.0%	100.0%	0.0%
Tetracycline	60.0%	40.0%	20.0%
Average	**76.3%**	**42.5%**	**33.8%**

Table 5. Plasmid DNA fragment sizes after digestion with EcoRI and Hind III Endonucleases

Code	Organisms	Plasmid No	EcoRI fragment size/No	Hind fragment Size/No
04	*Staph. aureus*	1	4.0kb(1)	4.0kb (1)
06	*Staph. aureus*	1	4.2kb(1)	4.2kb (1)
10	*Staph. aureus*	1	3.9kb(1)	3.9kb
11	*Pseud. aeruginosa*	2	4.0kb 2.1kb 1.5kb (3)	4.0kb 4.4kb (2)
12	*Pseud. aeruginosa*	2	3.2kb, 3.0kb 1.3kb, 0.9kb (4)	4.0kb, 4.6kb (2)
13	*Pseud. aeruginosa*	1	3.9kb (1)	3.9kb (1)
14	*Pseud. aeruginosa*	1	3.8kb (1)	3.8kb (1)

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Effects of Dao De Xin Xi Exercise on Balance and Quality of Life in Thai Elderly Women

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Abstract

The objective of this study was to evaluate the effects of a 12-week Dao De Xin Xi exercise, modified short forms of Tai Chi, on balance and quality of life in Thai elderly population. Quasi-Experimental research, pretest-posttest one group design was done at Physical Medicine and Rehabilitation Department, Phramongkutklao Hospital. Thai healthy elderly women over the age of 60, requiring regular Dao De Xin Xi exercise were recruited from either patients or workers in the hospital. A 60-minute Dao De Xin Xi exercise class was set as 3 times per week for 12 weeks. At baseline and 12 weeks, participants were tested in their static balance (Single-Leg Stance Timed Test with eyes open and close), dynamic balance (Expanded Timed Up and Go (ETUG) Test). Quality of life was measured by the abbreviated Thai version of the World Health Organization Quality of Life (WHOQOL-BREF) questionnaire. Fourteen females were studied with mean age of 62.8±4.3 years old. The Single-Leg Stance Timed Test with eyes open and close, Expanded Timed Up and Go (ETUG) Test improved significantly (before versus after exercises $p < 0.001$). Significant improvement in quality of life were also found in each 4 domains, including physical health, psychological, social relationship, and environment (before versus after exercises $p = 0.001$, 0.001, 0.004 and 0.005 respectively). No significant improvement was found only in the right Single-Leg Stance Timed Test with eyes close ($p = 0.091$). A three times per week for 12-week Dao De Xin Xi exercise may help Thai elderly women improve both static, dynamic balance and quality of life.

Keywords: Dao De Xin Xi exercise, Balance, Quality of life, Elderly

1. Introduction

Evolutions in medical technology lead to long-lived population, which further result in an increased number of elderly person. Thus, the elderly have to face health problems contributed from physical decline. Falls are recognized as the most common and major health problem among the elderly. It is also shown that falls are the leading cause of major injuries; for example fractures, head trauma, which account for 5-15% of falls (George, 2000) with disability and death. Falls are responsible for 70% of accidental death in persons 75 years of age and older and have psychological impacts as loss of self confidence-efficacy and then fear to fall (Scheffer *et al*, 2008) These contribute to functional decline of daily activity and low quality of life in the elderly population.

A number of studies have been performed to determine causes, risk factors, and intervention to prevent falls among elderly population (Gillespie *et al* 2003; Tinetti *et al* 1995; Thaiamwong *et al* 2008; Assantachai *et al* 2003). Exercises and other forms of physical activity; such as swimming, yoga, walking, jogging, aerobic and Tai Chi Chaun, are recommended to prevent falls(Howe *et al* 2007).

Tai Chi Chaun or Tai Chi (TCC, TC), originally developed in China is a slow and graceful Chinese exercise that includes a form of mindful meditation. TCC consists of series of individual movements linked together in a continuous manner that flow smoothly from one movement to another. TCC is now worldwide used for health benefit. A number of studies have demonstrated the beneficial effects of Tai Chi Chuan on health conditions. Wayne *et al* (2004) reviewed the studies about the improvement of vestibulopathic postural control by Tai Chi. The 24 studies supported evidences that Tai Chi may have beneficial effects for balance and postural impairments in those associated with aging. Eight of ten randomized controlled trials(RCTs) showed that Tai Chi alone, or combined with other therapies, can reduce risk of falls, improve balance and dynamic stability, increase musculoskeletal strength and flexibility, improved performance of activities of daily living (ADLs), reduced fear of falling and general improvement in psychologic well-being. Audette *et al* (2006) conducted a 12-week RCT comparing the effects of a short style of Tai Chi that was 40-45 min of the 10-movement form versus brisk walking training program in twenty six community-dwelling, sedentary elderly women on many aspects of fitness, balance and quality of life in elderly women. The participants were randomly assigned to Tai Chi Chuan (TCC; n = 11) or brisk walking group (BWG; n = 8). A separate group of elderly women was recruited from the same population to act as a sedentary comparison group (SCG; n = 8). **S**ignificant improvement was found in estimated VO_2max in the Tai Chi Chuan group. Significant gains were also seen in the non-dominant knee extensor strength and single-leg stance time that measured balance. Kuramoto (2006) made research review about benefit of Tai Chi. There were 9 randomized controlled trials, 23 non randomized controlled trials and 15 observation studies. The author concluded that Tai Chi may lead to improved balance, reduced fear of falling, increased strength, increased functional mobility, greater flexibility, and increased psychological well-being, sleep enhancement for sleep disturbed elderly individuals, and increased cardiac functioning. Tsang *et al* (2007) conducted an RCT with thirty-eight older adults with stable type 2 diabetes comparing the benefits of a 16-week "Tai Chi for Diabetes" group with a sham-exercise-control group. Static and dynamic balance index and maximal gait speed improved significantly over time, with no significant group effects. Hackney *et al* (2008) conducted a 10-13 week RCT in thirty-three people with parkinson disease comparing a Tai Chi intervention with a non-exercise control group. The Tai Chi group participated in 20 one-hour long training sessions whereas, the control group had two testing sessions between 10 and 13 weeks apart without interposed training. The Tai Chi group improved more than the control group on the Berg Balance Scale, Unified Parkinson's Disease Rating Scale (UPDRS), timed up and go, tandem stance test, 6-minute walk, and backward walking. Improvement in Berg Balance scores was significantly greater for the Tai Chi than the Control group. All Tai Chi participants were satisfied with the program and improvements in well-being. Almost all of the mentioned studies supported the exact evidences of balance improvement, fall prevention and also the improvement of quality of life.

Now in western, TCC has been modified in different forms (Audette *et al* 2002). A lot of studies use long form of TCC (Yang style-108 movements) which seems to be more difficult and harder to learn than short forms (8–13 movements). Short form of TCC is much easier to learn than original form and becomes better known and more favorite in urban area and city.

Dao De Xin Xi exercise was created in Bangkok, Thailand in 1998. Dao De Xin Xi exercise is a modified short form of TCC, includes 9 selected movements believed to have health benefits for 9 physiological systems in the body such as cardiovascular system, digestive system, and respiratory system etc. Dao De Xin Xi exercise is easier to perform than original TCC, softly, gently, smartly and flow slowly in a continuous repetitive left to right circular manner. Deep breathing and mental concentration are also required during exercise. In addition, each movement of Dao De Xin Xi exercise was done simultaneously with 9 easy-listening Chinese songs of Dao Xin melody arranged in Thai version, playing during exercise. The meaning and theme of all 9 songs are about mercy of mankind according to philosophical aspect, mentioned to improve mental functions by meditative effect. Today, Dao De Xin Xi exercise is well-known in almost provinces in Thailand and many participants of Dao De Xin Xi exercise got a lot of better health benefits and well being after exercise. By the way, Department of Physical Medicine and Rehabilitation, Phramongkutklao Hospital has initially adopted Dao De Xin Xi exercise for patients and interested people since the year 2006.

2. Objective

In Thailand, many studies of original or complete long form of Tai Chi have been done .No studies of short form of Tai Chi have been reported especially the potential effects of Dao De Xin Xi exercise. This quasi-experimental research, therefore, was the first study of Dao De Xin Xi exercise aiming to determine the effects of a 12-week exercise on balance and quality of life in Thai elderly women.

3. Methods

3.1 Population

The target population of this study was volunteers who are either patients or workers in the hospital, over the age of 60 who expressed their willingness to keep on Dao De Xin Xi exercise regularly.

3.2 Inclusion criteria

1) The volunteers had experience of 2 months or less Dao De Xin Xi exercise and were healthy enough for the completion of standard balance tests. 2) They could consent to participate in the study.

3.3 Exclusion criteria

1) The volunteers were unable to complete the standard balance tests, as specified in the study. 2) They withdrawn from the study.

3.4 Setting

The study setting was the activity field, Chalermprakiat building, 5th floor, Phramongkutklao Hospital. A 60-minute Dao De Xin Xi exercise class was set as 3 times per week for 12 weeks, with a total of 36 times.

3.5 Study procedure

1) Participants were informed by the author about what Dao De Xin Xi exercise is, what to do during the study and safety of exercise. Any questions regarding the study were then answered and the written informed consent was obtained from all participants before the study.

2) The participants were asked to complete a questionnaire on demographic data including age, sex, history of illness, medications, experience of falls in the last 6 months, visual problem, and information on other forms of exercise in addition to Dao De Xin Xi.

3) The participants were tested in their balance before and after 12-week Dao De Xin Xi exercise. These tests started from Single-Leg Stance Timed Test with eyes open, followed by Single-Leg Stance Timed Test with eyes close and Expanded Timed Up and Go (ETUG) Test respectively. The author explained how to perform each tests to each participants, showed the example of tests and then let each participants try doing test 1-2 times only.

4) Conducting the test (Figure 1.)

- Single-Leg Stance Timed Test (SLST) with eyes close and open

The participant was asked to stand on a firm surface, place arms with the body, stand on one leg, and look straight ahead with eyes open. The duration of standing without trunk bending was recorded in seconds. Then, duration of single leg standing with eyes close was recorded by the same method.

- Expanded Timed Up and Go (ETUG) Test (Figure 2.)

The participant was asked to sit on a chair, with back against the chair and arms on the lap. When the researcher said the word “go” or gave any signal, the participant stood upright, walked at normal pace on a 10 meter- walkway to the specified mark, turned around, returned to the chair, and sat down. The stopwatch was started on the word “go” or the first signal, and stopped when the participant returned to the starting position.

5) Each participant was tested twice and the scores of both times were averaged. After the first test, each participant was allowed to rest for one minute before undertaking the next test.

6) The duration of each test was recorded in seconds.

7) Quality of life was measured by the abbreviated Thai version of the World Health Organization Quality of Life (WHOQOL-BREF) questionnaire.

8) Data were collected at baseline that was before Dao De Xin Xi exercise(experience of 2 months or less Dao De Xin Xi exercise) , and at the end of the 12-week of Dao De Xin Xi exercise

3.6 Statistical analysis

1) Descriptive statistics including frequency, percentage, and mean were conducted to present demographic data.

2) Within participant comparison, Paired t-test was used to compare the differences in balance and quality of life before and after 12-week Dao De Xin Xi exercise. The significance level was set at 0.05.

4. Results

Demographic data were shown in Table 1. As shown in the table, a total of fourteen females with mean age of 64.1±4.2 years old participated in the study. Most participants had dyslipidemia and hypertension which could be improved or maintained by medications, and no walking aid was reported by all participants. 14.3% of the participants fell in the last 6 month. A half of the participants wear glasses. Prior to the study, mean duration of Dao De Xin Xi exercise was 3.9±1.4 weeks, and 35.7% of the participants performed other forms of exercise such as tennis, jogging, aerobic, and swimming.

The Single-Leg Stance Timed (SLST) Test with eyes open and close and the ETUG Test have been used extensively in order to study static and dynamic balance respectively among the elderly; which reliability of the tests have also been reported.

As presented in Table 2, the SLST-Test with eyes open (right and left), the left SLST-test with eye close, and the ETUG Test improved significantly (p <0.001) after a 12-week of Dao De Xin Xi exercise. Although there was an increase in the right SLST-test with eye close after the exercise, no significant difference was found (p =0.091). During the study, no falls have been reported by the participants.

The data about quality of life obtained from WHOQOL-BREF questionnaire(Table 3) showed that the overall quality of life improved significantly after a 12-week of Dao De Xin Xi exercise (p <0.001). Regarding each domain, significant improvement in quality of life were also found in each 4 domains, including physical health, psychological, social relationship, and environment (p =0.001, 0.001, 0.004 and 0.005 respectively).

5. Discussion

Dao De Xin Xi exercise seems to be most likely a kind of Taoist Tai Chi that is also an exercise form of Tai Chi Chuan. Taoist Tai Chi, developed by Taoist monk in Toronto, Canada has become more popular and favorite in Western since 1970 (http://www.taoist.org/). The main foundations of Dao De Xin Xi exercise resemble those of Taoist Tai Chi such as a basic forearm rotation, a rotation of arms in front of body, a variant of the "Wave Hands like Clouds" move, repetitions of various other movements etc. Dao De Xin Xi exercise consisted of 9 movements selected from 108 movements of the Taoist Tai Chi set.

The risk factors responsible for falls could be both extrinsic, such as environmental hazards, and intrinsic, such as age-related physiologic changes especially nervous system and muscle strength (Rossat *et al*, 2003). A single fall may have multiple causes. Therefore, it is essential to evaluate and determine risk factors in order to prevent further falls. Balance problem is recognized as a major risk factor of falls and it is suggested that balance can be improved by exercise intervention (Liu and Frank, 2010; Logghe *et al*, 2010; Guan and Koceja, 2011). In addition to prevent falls, exercise can promote health status both physical and psychological functions.

5.1 Balance control

Balance was defined as the ability to align body segment against gravity to maintain or move the body(center of mass)within the available base of support without falling; the ability to move the body in equilibrium with gravity via interaction of the sensory and motor system(Kisner and Colby 2002).

Balancing requires concurrent processing of inputs from multiple senses, including equilibrioception (from the vestibular system), vision, and perception of pressure and proprioception (from the somatosensory system), while the motor system simultaneously controls muscle actions. The senses must detect changes of body position with respect to the base, regardless of whether the body moves or the base moves (Shumway-Cook *et al* 1988).

The study results revealed that both static and dynamic balance improved significantly after a 12-week of Dao De Xin Xi exercises. This is consistent with the studies determining the effect of TCC among elderly population. Zhang *etal* (2006) found that the elderly who performed 8-week TCC showed significant improvements in balance, flexibility, and a reduced fear of falling, when compared with the control group. Uhlig *et al* (2010) investigated the benefits of Tai Chi for fifteen patients with rheumatoid arthritis. The results revealed Tai Chi led to improved lower-limb muscle function, confidence in moving, balance and less pain during exercise and in daily life. Chyu *et al* (2010) reported sixty-one postmenopausal women with osteopenia. Subjects in Tai Chi group significantly both increased in stride width and improved in general health, vitality and bodily pain compared with those in the control group. Leung *et al.* (2011) reported systematic and meta-analytical review.The reviewed 13 RCTs showed the effectiveness of Tai Chi in improving balance in older adults and Tai chi could also reduce falls in the nonfrail elderly in the absence of other interventions. Bula *etal* (2011) conducted systematic reviewed and found the exercise including Tai Chi appears as the most promising monofactorial intervention. These are also consistent with other studies such as Schaller *et al* (1996) and Li *et al* (2008). In Thailand, Kumkate *et al* (2007) investigating the effects of original long form TCC exercise on static and dynamic balance among Thai elderly

people demonstrated that the TCC group had significantly better balance control than the control group in both Single-Leg Stance Timed Test with eye open and the Expanded Timed Up and Go (ETUG) Test.

Inconsistent with some aspects of the previous research, our study found that there was an increase in the SLST-test with eyes close after the exercise, with significant difference was found only for the left leg SLST-test. However, this result was supported by Hong *et al* (2000). This may be due to the 9 individual movements of Dao De Xin Xi exercise which improved proprioceptive function. Previous studies also supported this. For example, Li *et al* (2008) examining the benefits of the 16-week TCC intervention among the elderly found that the proprioception of the knee improved significantly after the intervention, when compared to the control group. Tsang and Hui-Chan (2008) demonstrated that there were improvements in the proprioception of the knee, flexibility, and balance with eye close among the elderly who regularly performed TCC. However, because of the limited number of participants and intervention period, future studies may verify these results.

Contrary to the results of foreign research, but consistent with a Thai study conducted by Kumkate *et al* (2007), our study found that Dao De Xin Xi exercise significantly improved dynamic balance. This may be because this study used the same test as the study of Kumkate *et al* (2007), the ETUG Test(Wall *et al*, 2000), which the stopwatch was started on the word "go", stand upright, walk at normal pace on a 10 meter-walkway to the specified mark, turn around, return to the chair, and sit down. For the foreign research, which the Time Up and Go Test was used, the walkway distance was only 10 feet. It is possible that the longer walkway may lead to the more sensitivity in balance test which possibly results in higher performance.

It's not yet clear which mechanism plays role in balance improvement. The combination of improvement of proprioception, increased trunk, leg muscle flexibility and strength is more likely to be than only single specific mechanism. The improvement of vestibular function and the possible linkage of psychological well-being may probable be the adjuvant mechanism of balance improvement (Wayne *et al* 2004).

5.2 Quality of life

The results of quality of life showed that the overall quality of life improved significantly after a 12-week of Dao De Xin Xi exercise. The significant improvements were also found in each 4 domains, including physical health, psychological, social relationship, and environment. This is consistent with Deschamps *et al* (2009) and Taylor-Piliae *et al* (2006). Some explanations are responsible for this. The first may be due to the improvement in balance control after exercise. Second, as Dao De Xin Xi is considered to be an aerobic exercise, is easy to perform with melodious Dao Xin song providing meditation and mental training, these may result in psychological relaxation. The final explanation is social relationship among the group of elderly during the exercise.

Philosophically, the meanings of 9 songs, simultaneously playing with exercise are intended to help the people regain original nature of goodness in human such as taking care of each other etc. This itself may improve psychological being and social relationship included as parts of quality of life.

5.3 Limitation of the study

The first is the small sample size which may be a limitation in terms of statistical significant. Second, the generalization of the findings is limited by characteristics of the participants: female, healthy, have no history of illness, and moderate to high quality of life.

Recommendations for further study are as follows. First, studies in a larger sample size of elderly population and also in elderly population with health problems; such as coronary heart disease, diabetes, hypertension, and at risk of falls, should be conducted in order to further create useful exercise guideline. Second, there should be a study examining the association between the improvement in balance and quality of life using more reliable instrument, i.e. computerized dynamic posturography. Finally, there is a need for additional studies evaluating the potential effects of Dao De Xin Xi exercise on other physical and mental conditions; such as bone density, cognitive function, and sleep quality in elderly.

6. Conclusion

A 60-minute Dao De Xin Xi exercise 3 times per week for 12 weeks may help Thai elderly female participants improve both static, dynamic balance, and quality of life.

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Table 1. Demographic data of the participants

Demographic data	Frequency	Percentage
Sex (male/female)	0/14	0/100
Age (average/year)	64.1±4.2	
Weight (average/kg)	55.2±7.1	
Height (average/cm)	156±5.2	
Having history of illness	12/14	85.7
4 or more of concurrent medicines	2/14	14.3
Walk aid	0/14	0
Experiences of falls in the last 6 months	2/14	14.3
Visual problem(wear glasses)	7/14	50.0
Perform other forms of exercise	5/14	35.7
Mean duration of Dao De Xin Xi exercise before intervention (weeks)	3.9±1.4	

Table 2. Balance test before and after Dao De Xin Xi exercise (mean ± SD)

Balance tests	Before (seconds)	After (seconds)	Mean Difference (95%CI)	P-value
Right SLST-Test * (eye open)	9.72±9.17	23.94±11.65	14.22±8.59	**<0.001**
Left SLST-Test * (eye open)	8.1±6.25	20.67±6.94	12.57±5.85	**<0.001**
Right SLST-Test * (eye close)	2.89±1.10	6.11±7.22	3.23±6.62	0.091
Left SLST-Test * (eye close)	1.59±0.53	3.13±1.43	1.55±1.10	**<0.001**
ETUG Test**	17.36±1.14	15.98±1.07	-1.38±1.10	**<0.001**

* SLST-Test = Single-Leg Stance Timed Test

** ETUG Test = Expanded Timed Up and Go Test

Table 3. Quality of life obtained from WHOQOL-BREF questionnaire before and after Dao De Xin Xi exercise (mean ± SD)

Quality of life score	Before	After	Mean Difference (95%CI)	P-value
1. Physical	27.21±3.96	28.93±3.15	1.71±1.49	**0.001**
2. Psychological	22.00±2.91	23.64±2.27	1.64±1.69	**0.003**
3. Social relationship	11.29±2.13	12.50±1.51	1.21±1.37	**0.006**
4. Environment	31.00±4.91	33.29±3.77	2.29±2.52	**0.005**
Overall	99.07±12.55	106.71±10.21	7.64±6.06	**<0.001**

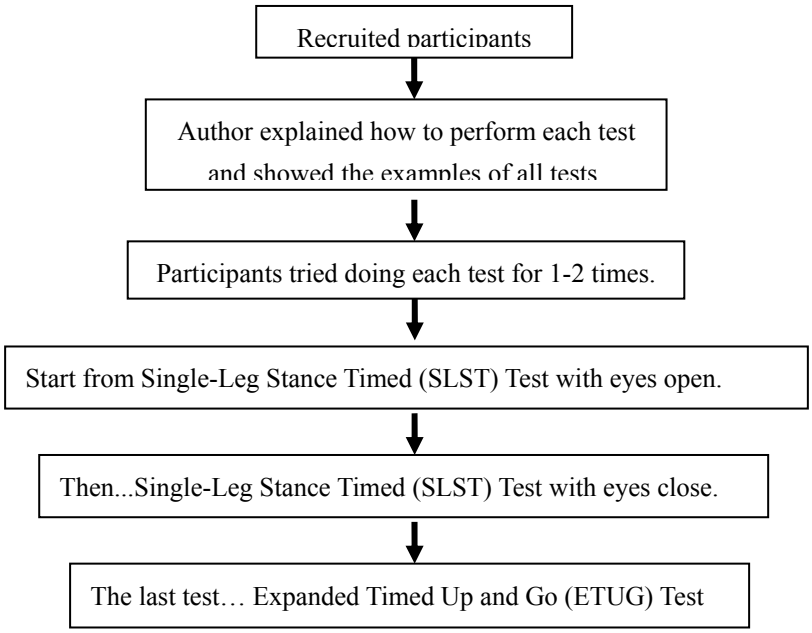

Figure 1. Flowchart of the balance testing procedure

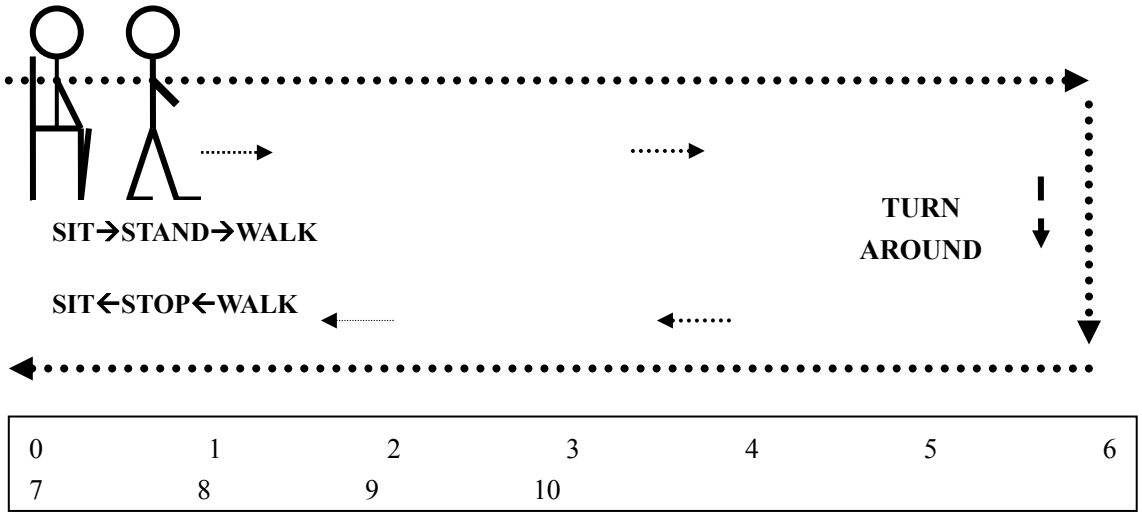

Figure 2. Expanded Timed Up and Go test

The Effects of HIV/AIDS Scourge on Production and Income among Rural Households in Adamawa State of Nigeria

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Abstract

The paper investigates the determinants and the impact of HIV/AIDS on households in Adamawa State. 120 respondents affected with HIV/AIDS were selected for interview using simple random sampling techniques. Both primary and secondary data were used in its analysis to determine the impact of the disease on household's income. The data collected were analyzed using descriptive analytical techniques and a logistic regression model was employed to estimate the likelihood that a household witnessed a fall in income as a result of the disease. The paper revealed that HIV/AIDS had an adverse impact on household's productivity, income, saving and capital formation. The paper, therefore recommends an intensive Aids education programme and Government at all level as well as NGO's should endeavor to provide adequate HIV testing kits, medication and free counseling services to enable the households determine their HIV status.

Keywords: Labour force participation, HIV/AIDS, AIDS scourge, Household productivity

1. Introduction.

The health status of the people in any country is a critical element in its economic growth as well as an important factor in the overall quality of household's life and productivity. AIDS tend to selectively affect women, youths, which are the vulnerable groups; it influences a wide range of social and economic factors. In Adamawa State, agriculture is the dominant industry in the state, thus any serious infection of HIV could lead to low productivity of the industry. Recent studies have revealed that HIV/AIDS prevalence among women and youths is increasingly worrisome. The target is the most productive group of the population (15-49 groups). This is the age bracket involved in social and economic sectors as well as the civil service and education. Thus these sectors could suffer severely from labor supply, loss of time due to illness, funeral bills, orphans, street children, medical expenditure and other vices. The consequences could be disastrous if nothing is done to control the spreads.

National prevalence has been steadily increasing from 1.8 percent in 1990 to 5.5 percent in 1999. The difference between HIV seroprevalence in urban and rural areas is not large, indicating that the AIDS problem in Nigeria is not strictly an urban one. This trend dictates labour participation rates both in the rural Nigeria; where the labour force is engaged in agricultural production, and in Urban Nigeria where most Nigerians migrate, find information to travel abroad for so called better life.

Factors that determine the labour force participation are the size of the family, level of education, job opportunities and migration amongst others. These are the main demographic dynamics in Nigeria over the years. Demographic indicators show that the dynamics in the population are skewed towards low productivity and increased social problems – high population movements to cities (Urban), high HIV/AIDS infections, and porous borders of the study area (Adamawa State) with Cameroun and Niger resulting to increased congestions and the rate infections.

Labour is total human efforts used in the productive process. It can also be defined as the strength expanded on the creation of goods and services. It includes number of hours worked and the physical strength. Labour can be services that have value and exchange. Economics is therefore concerned with efficiency – best use of productive resources and value. This is why policies for enhancing the labour force productivity is vital in achieving growth goals of the Nigerian economy, for instance the Vision 2020, which depended on the quality of the labour force, that will propel this economy to the desired growth by the year 2020.

The main objective of this paper is to examine the effects of HIV/AIDS scourge on labour force productivity of Adamawa state households.

2. Problem Statement

The health status of the people in any country is a critical element in the growth of its economy as well as an important factor in the overall quality of life. AIDS tend to selectively affect women, youths, which are the vulnerable groups; it will influence a wide range of social and economic factors. In Adamawa State, agriculture is the dominant industry; high rate of infection could lead to the stagnation of the industry. Recent studies have revealed that HIV/AIDS prevalence among women and youths is increasingly worrisome. The target is the most productive group of the population (15-49 groups).

3. Literature Review and Conceptual Framework

Until the early 1990's, the empirical economic growth literature focused exclusively on the role of capital and labour (The later often augmented by schooling and technological change, but hardly ever on health as a key element of human capital (Baro, 1991). Even where a relationship has been found between indicators of health and income per capita, it has either been discounted or thought to be an indication of the impact of economic development on health. The standard perspective of this earlier literature appears to have been that of Preston (1976) who noted that key role of economic development in improving life expectancy.

Studies carried out by UNAID and ECA in 2003 revealed that HIV/AIDS appear to be having devastating effects on the economies of East and South African countries. Evidence from these studies shows that: (a). Millions of children are orphaned by AIDS; and the number is growing. (b). Poverty is intensifying and deepening. (c). Productive capacity is being reduced in all sectors such that:-

i. Food crises of 2003 in South Africa could be traced to HIV/AIDS infection.

ii. Depriving those economies scarce skills, and children of their parents. In addition, it is observed that HIV/AIDS could leave the Kenyan economy one-sixth smaller than it would be without a high prevalence by the year 2015. And in Southern Africa, by the year 2020, the level of GDP could be lowered by 17% due to HIV/AIDS while the level of per capita GDP could be lowered by 17%.

An empirical study of this nature is therefore necessary in an attempt to determine the actual effect of HIV/AIDS on the household's productivity, income and standard of living. The objectives of the paper are therefore to assess the impact of HIV/AIDS on household in Adamawa State.

However, there is now significant evidence demonstrating the aggregate impact of health on growth and on level levels of real GDP per capita (Bhargava, 2001).

Bloom, Canning and Sevilla (2004) found that a one year improvement in the population's life expectancy (a standard measure of health status) contributions to a 4% increase in output. In another study, the same authors estimate that a one percentage point increase in adult survival rates boosts labour productivity by about 2.8% formal analysis suggest that a country can, on average, expect to see per capita income grow by an extra 0.3 – 0.5% points a year for every 5 years it adds to its life expectancy. This is a considerable boost, given that between 1965 and 1990 global income per capita grew by an average of 2.0% per year (Bloom, Canning and Malaney, 2000).

Similar studies have been done in Asia by Andrew and Mason (2002) – that the effects of controls for initial income, (developing Asian) countries with infant mortality rates (Mason, 2002). The potential returns to health investment he concludes appear to be substantial in the region.

Moreover, studies that consider full income which assigns economic value to changes in life expectancy-suggest that falling mortality rates have a more substantial positive impact on economic development than is shown by GDP per capita data. For example, in a assessment of the growth of real income per capita on the United States over the 20th century, (Nordhaus, 2003) concluded that over half of the growth in full income up to 1950 was attributable to mortality decline.

Considering the effect of mortality rates on full income suggest that estimates of the impact of AIDS on economic performance are under stated.

Bloom, Canning and Jamison (2004) suggest a new review of the literature on "value of statistical life (VSL) indicators that the adverse economic impact of AIDS in sub-Saharan Africa has already been more significant than GDP per capita data indicate.

Health affects labour productivity. Healthier workers are more energetic, have better attendance records and are likely to have higher mental capacity and morale. In development countries in particular, manual work makes up a large production of output, and physical endurance and strength rely crucially on sound health. A major study by Weil (2001) estimated that health differentials accounted for 17% of the difference in workers productivity between countries giving health roughly the same influence on productivity as physical capital (8%) and education (21%). Several micro-economic studies support this finding Strauss and Thomas (1998). The second channel from health to wealth involves the effect of health on education on education. Healthy children are better able to attend school and learn, and have more to gain by doing so because they can expect to live and work longer; and healthy families impose fewer burdens on children of having to care for sick relatives. An extra year of life expectancy is estimated to increase schooling levels by 0.25 years (Bill and Klenow, 2000).

Health improvements therefore spur the increase in savings which, by enabling greater investments in physical capital, spurs economic growth. East Asia's dramatic savings boom between 1950 to 1990, which contributed greatly to its unprecedented economic growth (Bloom *et al*, 2004) was driven by the region's rapidly improving life expectancy and by the increased production of people in the age groups that save the most (Lee, *et al*. 2000).

The household impact begins as soon as the member of a household starts suffering from AIDS related diseases. In addition to social and psychological consequences, three kinds of economic impacts can be distinguished. The first is the loss of income of the family members, in particular if he or she is the breadwinner. The second impact is the increase in household expenditures to cover the medical costs. The third impact is the indirect cost resulting from the absenteeism of members of the family from work or school to care for Aids patients.

The household impact begins as soon as a member of a household starts suffering from AIV/AIDS related disease. In addition to social and psychological consequences, three kinds of economic impacts can be distinguished. The first is the loss of income of the breadwinner. The second impact is the increase in household expenditures to cover the medical costs. The third impact is the indirect costs resulting from absenteeism of the members of the family from work or school to care for the aids patients.

A study in the same location (Adamawa State) by *Abdulazeez Abubarkar, Alo*

Eammanuel and Naphthali Rebecca (2008) found that, "*In spite of the persistent war against HIV/AIDS world all over, an amazing prevalence rate (14.2%) of the infection was still recorded in this part of the globe comprising of 1%, HIV-1, 0.51% HIV-2 and 1.6%, HIV-1+2 serotypes".* This shows the degree of devastation of the disease in the state and its consequent effects on productivity of the state, showing its impacts on the working population over time. This has been manifested in low agricultural productivity of the state compared to the productivity of the sector in the 1980s and early 1990s.

4. Methodology

Study Area: The study was conducted in Adamawa State. The state is located in the North Eastern region of Nigeria. The study area was chosen in the North-Eastern Geo-political zone and export to other parts of the country, Central Africa, Niger and Federal Republic of Cameroon. The state has a total human population of 3, 194, 781 (NPC, 2006). The state has a total land area of 38, 741. 12 square kilometers (Uyang, 1993 and Tukur and Ardo, 1999). Out of these, the lowland, the hills and mountain ranges and upland plains constitute 32. 58%, 26.56% and 40.58 respectively (Tukur and Ardo, 1999). The state has (21) local government areas. Geographically, Adamawa State lies between latitude 7^0 and 11^0 and longitudes 11^0 and 14^0 (Adebayo, 1999). It share boundary with Taraba State in the South and West, Gombe in the North West and Borno State to the North. The state has an international boundary with Cameroon Republic along its eastern state.

The study was conducted between the periods of January 2010 to June 2010. The study covered six (6) local government areas out of 21 local government areas in the state. Two local governments were randomly selected from each of the three senatorial zones. And from each local government, four (4) villages were randomly selected, making a total of 24 villages sampled from the state. Five (5) respondents were also randomly picked from each village, making a total of one hundred and twenty (120) respondents to be randomly selected for the study. Data are obtained from both primary and secondary sources. While the former includes direct interviews with concerned individuals and families and the use of questionnaires, the latter encompasses hospitals and medical laboratories records from ten (10) major centres in the state. There are, however, series of HIV/AIDS related cases that were/are not recorded, for there is a very high tendency of several non-hospital HIV/AIDS issues, due to reasons peculiar to and inherent in rural communities.

The paper adopts a descriptive analytical technique resulting in evaluating the impact of HIV/AIDS on the households in the state.

5. Model Specification

A logistic regression model was employed to investigate the livelihood that household's income level falls as a result of HIV/AIDS. The logistic regression model is as follows:

$$P(Y=1) = g(X) \quad (1)$$

Where ***P (Y=1)*** is the probability that a household' income level falls as a result of the disease.

X is vector of the determinants of the odds of income level. The regression equation was build up by including variables that were presumed to affect the household poverty status. Such as:

(1) Sex of the household (Female=1 and Male=0),

(2). Household size.

(3). The sales of household assets which takes the value of 1 if the household sold the property and 0 if it did not.

(4). Total amount of money spend on caring for AIDS patients; on funeral and on other mourning expenses.

6. Result, Discussion and Analysis.

Majority of the HIV/AIDS patients interviewed were males (61.7%), while only 38.3% of the respondents were females as shown on Table 1.0.

It could be concluded that most of the HIV/AIDS patients in the study area are males. The study also shows that (58.3%) of the 120 respondents sampled were married, 10.5% were not married. And 14.2% and 16.7% of the respondents were divorcees and widows respectively.

Most of the HIV/AIDS patients are married and within the ages of 30 – 39 years (51%). More so, most of the sampled respondents have 4 – 6 persons per household, 91% were with the disease from 1– 5 years and most of them (58.3%) did not attend formal education.

Table 2.0 reveals that the pulled monthly labour and their values in maintaining and caring for AIDS patience. It shows that from the sampled of 120 respondents 120,500 man-hours were pulled from active labour force, while 555,700 man-hour were also pulled from those caring for them which would have been economically used productively.

On the average, unit price per man-hour in N150.00 and the value pulled man-hours for the respondents per months is N15, 062.05 and for those caring for them, the estimated value stood at N69, 462.5

It can therefore be concluded that the man-hour and income lost to AIDS are tremendously. Hence, the income lost would have been saved or invested that in capable of improving the per capita income and standard of the hiring of the household in the state. The finding is in line with that of Mason (2004) who also confirmed that a lot of income has been lost away because of AIDS infection

This finding also agrees with (Dauda and Shuiabu, 2006) who positioned that HIV/AIDS may result in absence from work, loss of income temporary and if the patient dies the temporary loss income becomes a permanent loss.

The analysis in table 3.0 above reveals that many as 65% of the respondents in the households affected with HIV/AIDS attested experiencing a significant reduction in production and income as well. It was also observed that 82.0% said their tangible portion of their spending go on medication of the members affected with the disease in their households. Most of the respondents attested selling their assets ranging from jewelleries to landed property to coup with the cost of living and medication. About 57.0% of the respondents experienced reduction in quantity and quality of food consumption. Some of the respondents (58.0%) were compelled to adopt orphans within the extended family after the death of the parents. This may exert extra pressure on the affected families and which might be responsible for (56.0%) withdrawal of students from school.

The result table 4.0 reveals that HIV/AIDS contribute significantly to a reduction in household's income. A large household is more prone to poverty than smaller one because HIV/AIDS diminishes a household's ability to produce which more mouths to feed. Even when labor for participating in income generating activities is available, much of it is devoted to caring for the patients and less to production. More so, larger households are more likely to be poor; this may imply that the households of small size spend less on food and other materials than larger household.

Also the result shows that female headed households are more likely to have fall in income due to AIDS infection since men may be have more access to productive resources such as land credits technology, job opportunities etc. than female headed. Another explanation is that widows may themselves be infected and

lacking the physical strength to engage effectively in income generating activities and food production. The result also revealed that household sale of family assets to cope with HIV/AIDS tends to reduce income levels of households.

Conclusively, from the analysis above, HIV/AIDS significantly reduces household income.

7. Conclusion

HIV/AIDS scourge has serious adverse effects on the household productivity, savings and capital accumulation and investments in Adamawa State as confirmed by study. Findings of the study reveal that HIV/AIDS has made significant contributions in reducing household income especially for female headed households. Further, the results revealed that the scourge has been responsible for loss of income, increased household expenditure for medical, funeral related expenses and a greater fall of household savings and assets.

8. Recommendations

An intensive HIV/AIDS education programme among the Nigeria populace is desirable and recommended. Government at all levels as well as NGO's should provide adequate HIV/AIDS testing kits and free counseling services to enable every household to determine their status and consequently take necessary steps to prevent the spread and manage those already affected provision of affordable anti-retroviral drugs to the already affected households can go a long way in prolonging their lives as well as enhance their productivity.

The government design and implement effective, appropriate policies and programmes for poverty alleviation, targeting HIV/AIDs victims.

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Table 1. Socio-economic characteristics of the respondents

CHARACTERISTICS	FREQUENCY	PERCENTAGE (%)
Sex		
Male	74	61.7
Female	46	38.3
Total	120	100
Marital Status		
Single	13	10.8
Married	70	58.3
Divorced/Separation	17	14.
Widow	20	16.7
Total	120	100
Age Range		
20-29	22	18.3
30-39	62	51.7
40-49	37	26.7
50 and above	4	1.3
Total	120	100
Family Size		
1-3	22	18.3
4-6	43	35.5
7-10	34	28.4
11 and a above	21	17.5
Total	120	100
Years of Infection		
1 – 5	110	91.7
6 – 10	6	5
11 – 15	4	3.3
16 and above	0	0.0
Total	120	100
Educational Status		
No formal education	70	58.33
Primary	30	25.00
Secondary	14	15.83
ND/University	1	0.08
Total	120	100

Source: Field Survey, 2010

Table 2. Analysis of value pulled monthly man-hours of labour to Aids

ITEM	**HIV/AIDS patient man-hr.**	**CARING MEMBERS OF HOUSEHOLD: (man-hr)**
Total man-hour lost to AIDS	120500	555.700
Average man-hour lost to AIDS	1004.167	4630.83
Average unit price of man-hour in Naira	150.00	150.00
Gross valued of pulled man-hour in Naira	18,075,000.00	83,355,00.00
Average valued of pulled man-hour in Naira	15,062.05	69,462.5

Source: Analysis of Field Survey Data, 2010

Table 3. Effects of HIV/AIDS scourge o rural household

Effects	Yes (%)	No (%)
Reduction in production income	78(65.0)	42(35.0)
Spending of household saving/income on medical care	99(82.5)	21(17.5)
Selling of household assets to meat cost of living	84 (70.0)	36(30.0)
Reduction in quality and quantity of food consumption	68(56.67)	52(43.33
Adoption of orphans	58(48.33)	

Source: Field survey, 2011

Table 4. Logistic regressed results of income status among households with AIDS

Variables	**Coefficients**	**Std error**	**Wald**	**P-value**	**Exp(B)**	**95% CI**
Constant	-13.629	5.410	6.348	.012	0.00	
Household Size (Hs)	1.049	0.395	7.064	0.008	2.854	1.3176.184
Sex of head of household (Shh)	2.060	0.845	5.949	0.015	7.845	1.49841.073
Sales of household assets (Sha)	1.381	0.806	2.939	0.087	3.979	0.82019.31
Total spending on Medicare (Tsm)	0.785	0.408	3.699	0.054	2.191	0.9854.875

Wald Chi-square= 49.669

Pseudo R-squared=0.484(p-value= 0.000)

Log likelihood=-47.139

Source: Analysis of field survey, 2010

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Intra-orbital Malignant Melanoma: Role of Mr Imaging (a Case Report and Literature Review)

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Abstract

Magnetic resonance imaging is a non-invasive modern imaging tool that can definitely diagnose malignant melanoma despite its anatomic localisations. This is borne out of tumour paramagnetic melanin pigment content. Melanin is known to shorten T1 and T2 relaxation times of protons thereby exhibiting hyperintense T1W and hypointense T2W signals, hence conferring some histiological diagnosis. This is unlike Amelanotic melanoma, other intra-orbital tumours and tumours in general that show usual hypointense T1W and hyperintense T2W signals. However a few mimics of signal characteristics of malignant melanoma like sub-retinal serous collection exist. This therefore needs additional MRI sequences like fat suppression with Gado-pentetate Dimeglumine enhancement for differentiations.

1. Introduction

Malignant Melanoma is malignant tumours of melanocytic origin, commonly seen on skin and various mucous membranes (Pandey *et al*, 2007, p30). This neoplasm results from malignant transformation of normal melanocyte (Garandawa *et al*, 2007, volume 9, number 2). Melanocytes are the cells that produce the pigment melanin, the main substance responsible for pigmentation of the skin and non-cutaneous sites) (Garandawa *et al*, 2007, volume 9, Number 2). Malignant melanoma is the commonest intra-ocular malignant neoplasms in adults. (Pandey *et al*, 2007, p30).

Evaluation of this intra-orbital melanoma ranges from ophalthmoscopy, Duplex Doppler ultrasonography (USS), Computed tomography (CT) to Magnetic resonance imaging (MRI) (3) (Bond *et al*, 1991, pp 459-466). But Malignant melanoma (MM) is uniquely suited for evaluation by MRI due to paramagnetic effect of the melanin pigment content of the tumour (Bond *et al*, 1991, pp 459-466, Uozum *et al*, 1990, pp 143-1469). This melanin shortens both T1 and T2 relaxation times causing hyperintense T1W and hypointense T2W images, thereby eliciting neuro-imaging interest and providing histiological diagnosis of this neoplasm.(Song *et al*, 1990, pp76-9)

Mucosal MM like intra-orbital malignant melanoma belongs to the class of tumours that on light microscopy may be confused with other malignancies unless the intra-cytoplasmic pigment or the melanoma cytoplasmic antigen is sought (Garandawa *et al*, 2007, volume 9, number 2). Such seeking could be by immuno-histiopathology of biopsied tumour sample or pre-operative MRI imaging. Thus, necessitating this report to highlight the need to equip African hospitals with modern imaging tools and immunohistopathological equipments

2. Case Report

MB is an un-married 31year old Cameroonian Lady who was referred by the Ophthalmologist for brain MRI in Polyclinic Bonanjo, Douala. Her history was 7years of intermittent right eye pain with last one year progressive

proptosis though without visual impairment. Brain MRI showed right intra-orbital, extra-ocular 52 X 33.3 X 20mm soft tissue well marginated mass that is hyperintense on T1W (Figure 1) and hypointense to vitreous on T2W (Figure 3) and FLAIR sequences reminiscent of paramagnetic melanin. This mass is markedly enhancing except an anterior-inferior part of the mass which is hypointense relative to the rest of the mass (Figure 2 coronal image). This suggests a recent intra-tumoral haemorrhage. Sagital T1W shows normal optic nerve with no optic foraminal nor intra-cranial extensions (Figure 4). There is proptosis of the right eye by a distance of 17.2mm (Figure 1). T1W intermediate and T2W hyperintense small polypoidal masses measuring 6.7mm, 6.4mm and 5.8mm in diameter are seen in the nasopharynx, right and left maxillary antra respectively. Ocular echography was not contributory, but shows near symmetrical globes with right and left measuring 23 x19.4mm and 21.7 x 18.7mm in dimension. Patient visited the Opthalmologist with the result once, thereafter vanished denying us of any management and immune- histiology.

3. Discussion

Primary orbital melanoma is an exceedingly rare tumour that probably develops from congenital rests of neural crest cells in the orbit (Delaney *et al*, 2004, pp118-121). It represents less than 1% of primary orbital neoplasm and usually occurs in the presence of clinical or histiological evidence of ocular melanosis or blue nevus syndrom (Delaney *et al*, 2004, pp118-121). Naevi (mole) are aggregation of melanocytes that are present from birth, often do not make their appearance until puberty (2) (Gaeandawa *et al*, volume 9, number 2). During embryologic development, precursors melanocytes arise in the neural crest, as the fetus develops, these cells migrate to areas including the skin, meninges, the eye, upper oesophagus and the mucosal surface of the oral cavity, nasal cavity, paranasal sinuses, anorectal and urogenitalia (Garandawa *et al*, 2009,volume 9, number 2). Dark brown melanocytic pigmentation is normal observation in the conjunctiva, a condition referred as facial melanosis is especially evident bilaterally in more heavily pigmented races. (Goldberg *et al*, 1996, pp456-461) This condition is usually in the form of an excess production of melanin or hyperpigmentation by the melanocytes (forming an ephelis) or benign proliferation of melanocytes (forming a benign lentigo). (Goldberg *et al*, 1996, pp456-461)

Several factors are important in producing malignant transformation of the melanocyte, eg age, hormonal status, genetic predispositions, environmental factors, other risk factors eg trauma, dysplastic naevus syndrome, xeroderma pigmentosum, family history of melanoma, exposure to certain carcinogens and sunlight. For instance, Persons born in the Southern US had a relative risk of orbital and intra-orbital MM of 2.7 as compared with those born in the North (Turkar, 1985, pp 789-792). In general MM are more common in Caucasians than in Asia and Blacks population since lightly pigmented individual are at the higher risk of development of melanomas than are darkly pigmented individual (Garandawa *et al*, 2009, volume 9, number 2, Scott *et al*, 1976, pp 489-491, Lohman & Bridges, 1990, p 115). For example, uveal melanoma occurs 6-7 cases per million among Caucasians, 4.3 cases per million in US, most of which occurs in the white population and 0.25 per million in Japan (Kato *et al*, 2006, 404-9). Minutes areas of pigmentation that could predispose to the development of melanoma like small foci of pigmentation, subdermal or orbital melanocytosis are sometimes missed (Delaney *et al*, 2004, 118-121).

Primary orbital melanoma is a rare condition that is histopathologically similar to uveal melanoma). The commonest orbital malignant melanoma (MM) is uveal melanoma. This is because the uvea is the most vascularised portion of the eye, hence a substrate for primary and metastatic neoplasm (Peyman & Mafee, 1987, 471-88). Uveal melanoma is sub-classified into anterior uveal melanomas when the tumour arises from the iris and posterior melanomas when it arises from the choroid or ciliary body. The choroidal sub-type is the commonest MM among adults. WHO and International agency for Research on Cancer (IARC) reported 0.1-2.3 per 100,000 world-wide (Lutz *et al*, 1999, 1190-1193).

Women develop MM slightly more than men (Garandawa *et al*, 2007, volume 9, number 2). It is extremely rare for melanoma to occur before puberty, and the median age for diagnosis is in the late fortie (2) (Garandawa *et al*, 2007, volume 9, number 2). MM of mucosal surfaces eg head and neck are very rare according to American College of Surgeons Commission report (1998), 91% of all MM were cutaneous, only 1.3% were mucosal (Garandawa *et al*, 2007, volume 9, number 2, Chang *et al*, 1998, 1664-1678). Mucosal MM tends to occur in an older age group than the cutaneous counterpart from 5^{th} -8^{th} decade. Men are more affected than women in mucosal melanoma unlike in cutaneous melanoma (Garandawa *et al*, 2007, volume 9, number 2)

The aetiology of the paramagnetic relative enhancement seen in MM on proton MRI has been the subject of many investigations and has previously been ascribed to iron from associated haemorrhage or chelated metal ions, rather than directly due to melanin (Atlas *et al*, 1990, 547-554). Atlas *et al* indicated that T1 shortening

correlates with increasing melanin content and not with increasing iron deposition, electron paramagnetic resonance (EPR) EPR-active metallic cation, necrosis or water content. Atlas *et al*, 1990, 547-554). In fact, they even found a probably unrelated statistical correlation between increased iron and T1 prolongation (Atlas *et al*, 1990, 547-554). Also T2 relaxation times did not correlate with the presence of any single factor other than proton density) Atlas *et al*, 1990, pp 547-554). Although the unique relaxation behaviour of non-haemorrhagic MM in vivo cannot be traced to a single cause, their data suggested that contrary to previous investigations, its strongly influenced by the presence of melanin rather than iron or other naturally occurring paramagnetic ions (Atlas *et al*, 1990, pp 547-554). The intensity of the tumour T1W signal but not the contrast enhancement is statistically associated with the degree of pigmentation of the tumour (Scot *et al*, 1998, pp897-899). Majority of MM with anticipated MR imaging melanotic pattern of high signal intensity relative to that of cortex on T1W and low signal intensity relative to that of cortex on T2W have more than 10% melanin containing cells (Isiakler *et al*, 1995, pp503-512). (Giovanni *et al*, 2006, pp605-8) Giovani *et al* also found a direct correlation between melanin content and T1W hyperintensity but no correlation between T2*W intensity and melanin (Giovanni *et al*, 2006, pp605-8).

Newer diagnostic modalities like MRI have modified the treatment of MM. Currently, Opthalmologists aim to save the eye and preserve any possible useful vision (Shanmugan *et al*, 1997, pp143-161). Supplementary modalities like MRI is warranted since dense vitreous may prevent view of fundus with opthalmoscopy. Also innumerable benign and malignant lesions may mimic the opthalmoscopic features of MM like choroidal haemorrhages (Shanmugan *et al*, 1997, pp143-161). Supplementary modalities are Fundus Fluorescein Angiography (FFA), Colour Doppler ultasonography (USS), Computed tomography (CT), Magnetic resonance imaging (MRI), Single photon emission tomography (SPECT) and Positron emission tomography (PET). Though FFA can differentiate certain Pseudo-melanomas from MM but Small choroid melanomas may not produce any appreciable change in angiogram due to absence of retinal pigment epithelium alteration and associated exudative retinal detachment (Shanmugan *et al*, 1997, pp143-161). ^{123}I-IMP Sctingraphy using planar imaging or SPECT can provide specific localization of melanoma but the sensitivity of ^{18}F-FDG-PET for diagnosis of MM is low due to high incidence of false negative results(13) (Kate *et al*, 2006,1404-9). CT is used when MRI is contraindicated and Non-contrast CT can only diagnosed 34% of uveal melanoma but increased to 75% in contrast CT (Kate *et al*, 2006, 1404-9). But CT might miss out brain metastasis from orbital melanoma < 2cm in diameter and the role of CT has been limited by poor tissue definition (Shanmugan *et al*, 1997, pp143-161, Golleri *et al*, 1991, pp27-34). Nearly all MM are confirmed by immunohistiological analysis using S-100 protein, HMB-45, Vimentin & Cytokeratin regardless of the site (Garandawa *et al*, 2009, volume 9, number 2)

MRI appears to be the most helpful and non-invasive imaging study of choice for evaluation of selected orbital lesions. MRI has been proven to be more sensitive and specific than USS in the detection of extra-ocular extension of uveal melanoma (Scott *et al*, 1998, pp 897-899). Information obtained from MR studies allow the identification of compounds such as melanin, met-haemoglobin, deoxyhaemoglobin and proteinacous fluid (Shanmugan *et al*, 1997, pp143-161). This is important because MM is a very vascular tumour , therefore the above blood degradation products, water and fat gives variations occasionally to the classical MR features of MM.

The MRI characteristics of orbital melanoma have been mainly attributable to paramagnetic properties of melanin. This melanin shortens T1 and T2 relaxation times leading to T1W hyperintense orbital melanoma which is a hypointense on T2W with respect to the hyperintense vitreous (Uozum *et al*, 1994, pp 76-9; Shanmugan *et al*,1997, pp143-161; Peyster *et al*, 1988, pp 773-9; Mafee *et al*,1989, pp 773-80) (Peyster *et al*, 1988, pp 773-9) Peyster *et al* reported these characteristic pattern in 93% of melanoma in their evaluations of intra-ocular tumours (De Potter *et al*, 1994, pp 340-8). De Potter *et al* also reported 95% of same patterns. However the presence of tumour necrosis containing water, presence of blood degradation products and iron content may explain the varying combinations of signal intensities such as decreased signal on T1W and/or increased signal on T2W (Shanmugan *et al*, 1997, pp143-161). He uses of fat suppression techniques help to improve the conspicuousness of the tumour and in differentiations from pseudo-melanomas and assess orbital extension (De Potter *et al*, 1994, pp 340-8). This technique combined with enhanced Gadopentetate Dimeglumine MRI images help to detect small intra-ocular mass with thickness of >1.8mm (22, 27) (Shanmugan *et al*, 1997, pp143-161; De Potter *et al*, 1994, pp 340-8).

Amelanotic melanoma exists, behaving just like any other tumour with hypointense or isointense T1W and hyperintense/isointense T2W (Ogwa, 2003, pp548-551, Escott, 2001, pp 625-639). On T1W Gado-pentetate enhancement, MM show as moderate to marked enhancement and larger tumours show more heterogenous

enhancement than smaller tumours (Shanmugan *et al*, 1997, pp143-161). Preliminary reports based on limited number of cases have proposed that specific MR imaging patterns may permit a distinction between melanotic and amelanotic brain metastastasis in MM patients (Isiklar *et al*, 1995, pp 503-512). Rare orbital metastasis from cutaneous melanoma and contralateral choroidal melanoma have been reported (Sen Hadj Hamidi *et al*, 2009, pp416-420). The imaging features of metastatic melanomas are distinctive due to the presence of melanin and the propensity for haemorrhage. Both haemorrhage and melanin can produce T1W hyperintensity and T2W signal intensity loss (GIOV). T2W images improve detection of metastastic melanoma through T2W signal intensity loss due to susceptibity effect (Giovanni *et al*, 2006, pp 605-8)

The combination of T1W hyperintensity and T2W hypointensity signals have been seen in other intra-ocular lesions like serous retinal detachment, secondary sub-retinal fluid, uveal melanocytoma, choroidal osteoma, sub-acute choroidal/retinal haemorrhagic detachment, Retinoblastoma, Retinal capillary haemangioma, Focal retinal gliosis, Medulloepithelioma, and Inflammatory granuloma. This is due to proteinacious content or blood degradation product (Shanmugan *et al*, 1997, 143-161). Post-contrast T1W with fat suppression may help to differentiate these masses from MM as retinal detachment do not enhance and Choroidal haemangioma with high vascular flow and enhancement exhibit isointensity to slight hyperintensity on T1W and hyperintense T2 weighting which is isointense to vitreous.

4. Conclusion

MRI is pivotal in anatomical localisations and tumour extent of malignant melanoma. Interestingly, unlike in other tumours, it can suggest histiological diagnosis of Malignant melanoma based on unique signal intensities on different MRI sequences.

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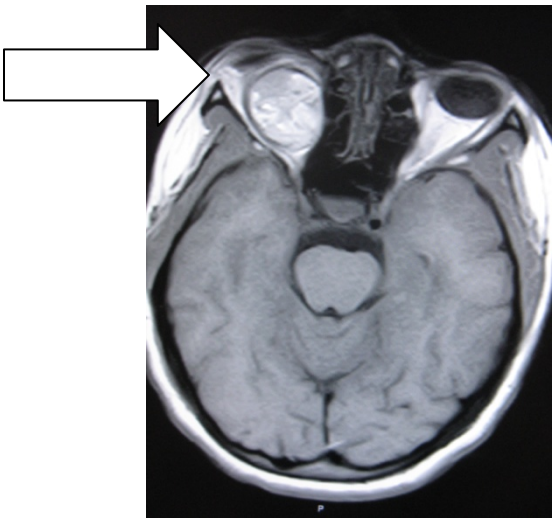


Figure 1

Figure 2

T1W

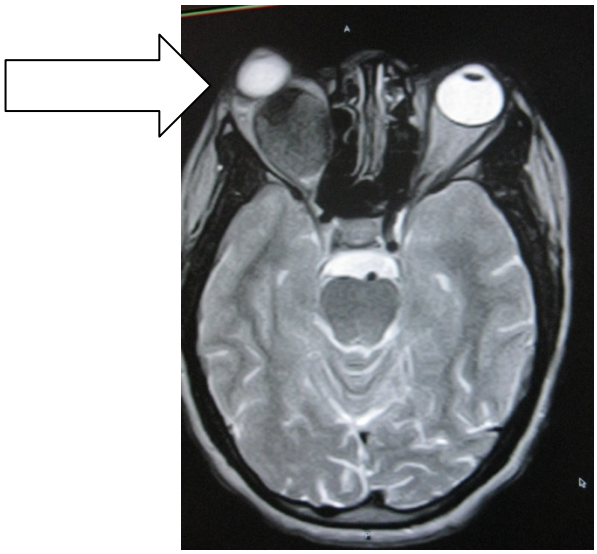
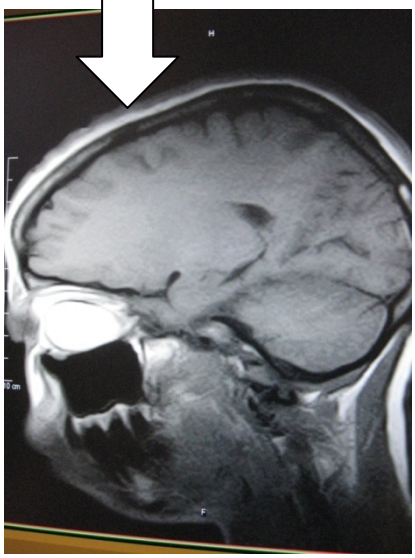


Figure 3



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