Journal of
Molecular Biology Research

Editorial Board

Contents

Examining the Factors Affecting Endodontic Therapy Failure

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Abstract

The purpose of the study was to examine the factors affecting Endodontic Therapy Failure (ETF). The study was descriptive cross-sectional conducted at College of Dental Science & Research Centre.
Dental school in Pune, India. In the present study, 90 patients were analyzed in the treatment ward. According to the results, the highest endodontic failure was observed in 41-50 years age group (41.11%) and the least was in 21-30 (24.44%). According to the tooth type, most endodontic failures were observed in maxillary molars (44.4%), mandibular molars (20%) and maxillary premolars (15.5%). Endodontic treatment by general dental practitioners (GDPs) showed the highest failure rate (78.8%). The factors contributing to the most common endodontic problems were under-filled canals (33.3%) and unfilled and missed canals (17.7%). The study concluded that ETF occurred when the treatment was not according to the accepted standards. The main causes of ETF are microbial infection in the root canal system and these patients' not attending the specialists.

Keywords: Failure, Endodontic Therapy, Dentistry

1. Introduction

Endodontic therapy is a treatment sequence for the infected pulp of a tooth that eliminates infection and protects the infected tooth against the subsequent microbial invasion (Cohen & Hargreaves, 2006). This therapy has been investigated in various studies (Farbod and Bolhari, 2018; Vigneshwar and Ramesh, 2017).The root canals and their paste shield are physical intra-dental holes that naturally exist in living connective tissue and blood vessels and together form the tooth pole (Nanci, 2012). Endodontic therapy includes the elimination of these structures, formation, cleaning cavity contamination with disinfectant solutions and the blockage of contaminated canals. Filling clean and contaminated canals is done using inert filler such as gutta-percha and typically zinc oxide eugenol (Marciano et al., 2011). Endodontics include both primary and secondary endodontic treatment as well as periradicular surgery, generally used for the teeth that can still survive (Setzer & Kim, 2014; Kishen, Peters, Zehnder, Diogenes, & Nair, 2016).

The keys to success in endodontic treatment are complete sealing of the root canal space and complete extraction of the infected root tissues of the pulp and microorganisms. This prevents recurrence and infection of the root canal space. ETF can be diagnosed according to clinical signs and symptoms and root canal radiographic findings. Many factors affect ETF, such as necrotic pulp of periradicular infection, periodontal disease, root fracture, broken instruments, mechanical perforations, root canal underfillings, and missed or unfilled canals (Siqueira, Rôças, & Ricucci, 2019).

Endodontic treatment is not always successful (Feiz, 2017). Zimpolas et al. (2012) reported the most common causes of failure, as prosthetic failure (59.4%), periodontal reasons (32%), and endodontic reasons (8.6%) (Tzimpoulas, Alisafis, Tzanetakis, & Kontakiotis, 2012). Foss et al. (1999) reported that 43.5% of endodontic treatments were not successful and the most common reason for failure was root fracture (21.1%) (Fuss, Lustig, & Tamse, 1999). According to Chen et al. (2008) and Zadik et al. (2008), the most common causes of failure were irreversible caries (46.4% - 61.4%) (Zadik, Sandler, Bechor, & Salehrabi, 2008; Chen, Chueh, Hsiao, Wu, & Chiang, 2008). Toreh et al. (2011) prepared a questionnaire for planning a prospective study to examine the causes of failure, where the main reasons were disease (40.3%), endodontic failure (19.3%), vertical root fractures (13.4%), suspended fractures and irreversible crowns (15.1%), irreversible caries (5.2%), estrogen piercing (4.4%) and prostheses (Touré, Faye, Kane, Lo, Niang, & Boucher, 2011). This study discussed the factors mainly

responsible for ETF, so to improve the quality of dental practice in terms of endodontic treatment, applications had to taken into account. Thus, the purpose of the study was to examine the factors affecting ETF.

2. Methodology

The study was descriptive cross-sectional conducted at College of Dental Science & Research Centre

Dental school in Pune, India

. In the present study, 90 patients were analyzed in the treatment ward. Teeth with root fractures, periodontal and endodontic lesions, fractured crown, and irreversible and fractured teeth were excluded from the study. Faculty members of Endodontics Department of Dentistry participated in the study. Strindberg criteria (Strindberg, 1956) were used to judge the cases of endocrine failure. These criteria were as follows: 1) clinical symptoms such as pain, swelling and discharge of the sinus tract, and so on, 2) treatment of the sinus tract, and 3) increased size or incidence of new periadicular lesion. The proposed criteria of De-Moor et al. have been used to access root canal filling quality (De Moor, Hommez, De Boever, Delmé, & Martens, 2000). Each case was fully radiographically examined with a magnifying glass for observation: untreated or missed canals, any unwanted injury, periapical condition of the involved tooth and root canal filling status. After careful clinical and radiographic evaluation of the involved teeth, the patients were scheduled for re-treatment. The patients were randomly selected from the clinic. They were divided into three age groups: Group 1 (20-30 years), Group 2 (31-40 years), and Group 3 (41-50 years). Informed consent from the study subject and approval of this study were obtained from the College Ethics Committee. The collected data from the subjects to study on ETF were analyzed in SPSS, version 10, software as frequency and percentage of the results. Chi-square test was used to analyze the study parameters.

3. Results

Table 1. The relationship between ETF and dental practitioners and specialists

Therapist	Frequency	Frequency percentage	p-value
Dental practitioners	71	78.88%	< 0.001
Specialists	19	21.1%	< 0.001

Table 2. The relationship between ETF and various ages

	First group (21-30 years)	Second group (31 to 40 years)	Third group (41 to 50 years)	Total
p-value	22 (24.44%)	31 (34.44%)	37 (41.11%)	90 (100%)
	< 0.001	< 0.001	< 0.001	

Table 3. Frequency percentage of ETF according to tooth type

	Max. Anterior tooth	Min. Canine teeth	Max. Premolar	Min. Molar	Max. Anterior tooth	Min. Canine teeth	Max. Premolar	Min. Molar	Total
Tooth type	4	3	14	40	5	1	5	18	90
Frequency percentage	4.4	3.3	15.5	44.4	5.5	1.1	5.5	20	100

Table 4. Frequency percentage of the factors effective in ETF by radiographic evaluation

Endodontic Failure Factors	Frequency	Frequency percentage
Broken instruments	6	6.6
Untreated canals	11	12.2
Unfilled and lost canals	16	17.7
Under-filled canals	30	33.3
Too moist canals	9	10
Holes	5	5.5
Poor crown restorations	13	14.4

Ninety patients aged 21-50 years were enrolled in the study. Endodontic treatment done by general dental practitioners has shown a high rate of failure (78.8%), whereas the specialists have had the lowest rate (21.1%).

The highest endodontic failure was observed in the third age group (41%) and the least was observed in the first age group (24.44%). Concerning the type of tooth, most endodontic failures were in maxillary molars (44.4%) and then mandibular molars (20%) and maxillary premolars (15.5%), whereas mandibular showed the least endodontic fractures (1.1%). The factors that had identified the most endodontic problems were under-filled canals (33.3%), unfilled and missed canals (17.7%) and the factors least responsible for endodontic damage were mechanical holes (5.5%) and broken instruments (6.6%).

4. Discussion

ETF occurs when the treatment is not performed according to the accepted standards (Seltzer, Bender, & Turkenkopf, 1963; Sundqvist, Figdor, Persson, & Sjögren, 1998). The main causes of ETF are microbial infection in the root canal system and periradicular tissue (Nair, Sjögren, Krey, Kahnberg, & Sundqvist, 1990; Lin, Skribner, & Gaengler, 1992). The results in line with these studies showed that root canal filling quality is effective in endodontic treatment (Noor, Maxood, & Kaleem, 2008; Nie & Lin, 1999). Usually, as the result of inadequate preparation, dryness in root canal (more than 2 mm) often leads to the lack of success in treatment. Chagal et al. showed that in case of 1 mm drop in length, there is a 14% chance of failure of endodontic treatment in teeth with apical periodontitis. The cause of pericardial tissue stimulation is the residual necrosis and pulp-infected tissue in inadequate and defective canals (Chugal, Clive, & Spångberg, 2003). Unfilled canals were the second most common cause of unsuccessful endodontic treatment. These unfilled canals may be lost by the physician during root canal filling or may remain hidden during root canal excavation or he may be unable to find these canals. This was in line with the similar studies showing that due to the complexity of the root canal system, there was a risk of loss of root canal anatomy during endodontic therapy (Cantatore, Berutti, & Castellucci, 2006).

Age is a significant factor in the success of endodontic treatment in the individuals. Results showed that most endodontic failures (41.11%) were in the age group of 50-41 years, whereas the least endodontic failures (24.44%) occurred in the age group of 21-30 years and their difference showed that the difference between the first and the third age groups was significant (p = 0.011). The clear reason for failure in the age group of 41-50 was the calcified channels in the higher age groups. The second reason may be poor oral health and lack of proper patient-specialist interaction.

ETF depends on the location of the tooth, as well. Regarding this, most failures occur in the posterior teeth. The results showed that the greatest defects in endodontic treatment occurred in maxillary molars (44.4%), mandibular molars (20%) and maxillary premolars (15.5%). The rate of endodontic failure was high (5.5%) compared to maxillary injectors. The most general explanations on endodontic failure were untreated or unfilled canals after endodontic therapy. In mandibular injectors, the reason for the high ETF rate was the presence of extra canals not been treated during initial endodontic treatment (Cantatore, Berutti, & Castellucci, 2006). Another reason could be the presence of curved and firm canals in multi-canal teeth, making successful endodontics treatment of these teeth difficult for specialists. The test statistics in the current study showed that this difference was significant (P = 0.001). Navar et al. reached similar findings in their study (Noor, Maxood, & Kaleem, 2008). Skill, experience, and specialized training play an important role in the success of endodontic treatment. In this study, 78.8% of injured patients were treated by a GDP. The results of similar studies have indicated that failure rates can be significantly higher for those treated by a GDP and not by a specialist (Sjögren, Hägglund, Sundqvist, & Wing, 1990; Weiger & Axmann-Krcmar, 1998). Studies have indicated that endodontic treatment done by a GDP shows a success rate of 65-75%, whereas endodontic therapy by specialists has a success rate over 90% (Eriksen, 1991). This difference in success rate may show a difference in technical quality of endodontic treatment by a GDP and specialist. By comparing GDPs and dentists, the study showed that test statistics were very significant (P = 0.001). Of the limitations of the study was the lack of specific information about the experience of GDPs and their training.

The study concluded that ETF was mostly related to the lack of awareness and lack of continuing training periods by general dental practitioners. Moreover, ETF was due to the lack of proper specialized tools and the lack of proper training of these tools even when used by a dentist, complex anatomy of the involved teeth and these patients not visiting a specialist. Thus, it is suggested that the teeth with complex anatomy should be evaluated by high quality preoperative radiography and the GDPs should be encouraged to continue dental education, especially in the endodontics section.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Correlation Between mpMRI Staging and Final Surgical Pathology in Prostate Cancer

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Abstract

Purpose: We evaluated the role of multiparametric magnetic resonance imaging (mpMRI) in the diagnosis of prostate cancer and predicting of surgical staging of prostate cancer.

Materials and Methods: The study was done in 110 subjects who got mpMRI before radical prostatectomy in our hospital from 2016 to 2019. Preoperative mpMRI findings of 110 were compared to surgical pathology results following radical Prostatectomy. A comparison was made between pathologic staging of prostate cancer and the mpMRI findings.

Results: pathologic evaluation confirmed prostate cancer foci (237) were recognized in 110 subjects. Generally, mpMRI sensitivity of 46.4% was found for prostate cancer detection (110/237). Pathological tumor volume was a significant predictor of prostate cancer detection using mpMRI. In 33% of the cases, the pathologic staging is precisely similar to mpMRI and in 43%of the cases, there was a slight difference between the pathologic staging and staging by mpMRI but the cancer was confined to the prostate.in 24% of the cases, there was a significant difference between the pathologic staging and staging by mpMRI. The mpMRI was not able to identify the significant cancer in 24% of the cases.

Conclusion: The preoperative mpMRI was useful in detecting prostate cancer and in predicting surgical staging. However, the detection of 24% of clinically significant cancer was missed using mpMRI. As we move toward personalized medicine, use of MRI to biopsy each man's prostate differently rather than based on a pre-defined 12 core seems to be supported in the recent literature.

Keywords: Multiparametric MRI (mpMRI), Prostate Cancer (PCa), Radical Prostatectomy (RP)

1. Introduction

Malignant neoplasms have remained as a leading cause of death worldwide (Abdel-Sattar et al., 2018). Cancer is the uncontrolled proliferation of abnormal cells that leads to a malignant growth, and ultimately severe morbidity and mortality (Alshammari, 2018). The diagnostic pathway for prostate cancer detection is initiated on prostate-specific antigen (PSA) level and digital rectal exam (DRE). Use of PSA as a screening tool followed by systematic transrectal ultrasound-guided (TRUS) biopsy has resulted in increased detection of prostate cancer with stage migration toward low-risk disease (Mottet et al., 2017). For evaluation of the clinical staging, routine diagnostics (i.e., digital rectal examination, serum prostate-specifc antigen (PSA) level, transrectal ultrasound, and Gleason score) are insufficient (Carroll et al., 2016). MRI findings in patients are of importance in detection and management of disorders (Mosarrezaii et al., 2017). Multiparametric MRI has become a valuable tool in the diagnosis of prostate cancer. Although most large, high grade cancers are visible on mpMRI, intermediate grade and low volume cancers are often difficult to identify. Furthermore, the usefulness of MRI for determining the true size and shape of a tumor remains incompletely characterized. (Ukimura et al., 2013)

Multiparametric MRI is increasingly used in prostate cancer assessment for its diagnosis and detection and for staging and risk stratification (Thompson, Lawrentschuk, Frydenberg, Thompson, & Stricker, 2013; Felker, Margolis, Nassiri, & Marks, 2016). A concern regarding mpMRI is the considerable interobserver variability

(Fedorov, Vangel, Tempany, & Fennessy, 2017; Marin et al., 2017). Mp-MRI has been used to assess prostate cancer aggressiveness and to identify anteriorly located tumors before and during active surveillance. With the technological advancement of imaging modalities used in prostate cancer assessment, Introduction of the Prostrate Imaging Reporting and Data System (PI-RADS), constitutes a globally accepted standard for the detection, scoring and reporting of suspicious lesions on mpMRI (Weinreb et al., 2016). In two studies, improved detection of clinically significant cancer and a decrease in the identification of indolent cancer were demonstrated with the use of mpMRI in conjunction with PI-RADS and a subsequent targeted biopsy (Borkowetz et al., 2016; Toner et al., 2017). However, as mpMRI with PI-RADS is rapidly being adopted for prostate cancer detection and surveillance, additional efforts to identify the diagnostic accuracy of mpMRI in prostate cancer detection and its ability to predict tumor aggressiveness are warranted to determine if it should play a decisive role in prostate cancer management.

Thus, the essential aims of present study were to evaluate the diagnostic accuracy of mpMRI in the detection of prostate cancer and prediction of pathological staging, and to directly compare the findings on mpMRI with the histological findings from the radical prostatectomy specimen.

2. Materials and Methods

For this study, we have identified a hundred and ten patients who had experienced mpMRI prior to radical prostatectomy for localized prostate cancer between 2016 and 2019 at our institute. Inclusion criteria were histologically confirmed prostate adenocarcinoma. Finally, 110 patients were selected for inclusion in the study. Data were collected in a database in which patient demographics, clinical results, prostate biopsy results, mpMRI-related information and final histopathological results were documented.

A total of 110 patients underwent mpMRI at the Department of Radiology of our Hospital. In the Department of Radiology, all mpMRI of the prostate is performed on a 3-Tesla MRI system (Siemens Medical Solutions Germany). The mpMRI protocol of the prostate included T2-weighted images in transverse and coronal orientation, T1-weighted images in transverse orientation, diffusion weighted images in transverse orientation, dynamic contrast enhanced imaging in transverse orientation and contrast enhanced T1-weighted images with fat suppression in transverse orientation. The total MRI acquisition time was 30min. Endorectal coil was not used. The mpMRI findings have been scored by using PI-RADS version 2.

The prostate specimens were reviewed by a dedicated uropathologist who was blinded to the mpMRI findings. All prostatic biopsies and radical prostatectomy specimens were investigated at the Pathology unit of our Hospital. The whole prostate was prepared in 3-5mm increments and embedded in paraffin. These sections were cut and stained with haematoxylin and eosin for microscopic examination. Each individual tumor focus in the radical prostatectomy specimen graded according to the Gleason grading system. The highest Gleason score recorded per tumor foci was equated to a corresponding score using the new grading system For example, grade group 1 equated to a Gleason score of 3+3 (the least aggressive), grade group 2 was the equivalent of a Gleason score of 3+4, grade group 3 amounted to a Gleason score of 4+3, grade group 4 equated to a Gleason score of 4+4, 3+5 and 5+3 and a grade group 5 was the equivalent of a Gleason score of 9-10 (the most aggressive).The pathological index tumor was defined as the tumor in the highest-grade group. Based on the calculation of tumor volume, clinically significant cancer was defined as a tumor in grade group 2 with a cancer volume of 0.5 cc. (Weinreb et al., 2016; Wolters et al., 2011; Lee, Ku, Park, Lee, & Ha, 2018).

Data were analysed using SPSS v.23.0. Categorical data are presented as absolute and relative frequencies. Continuous variables are described using mean values, complemented by median and range values. Linear-by-linear association and the chi-square test were used to compare the rate of tumors detected and missed on mpMRI, according to the pathological features. Spear-man's rank-correlation coefficient was used to determine an association between the PI-RADS score and the pathological features of grade and volume. Cut-off values for pathological tumor volume detected by mpMRI were obtained using the Youden index. A P value <0.05 was taken to indicate statistical significance.

3. Results

The demographic characteristics of the study are shown in Table 1. The median age of the 110 men who underwent mpMRI prior to RP was 63 years (50-75 years) and median PSA was 10.7 ng/mL (5.1-30). The median time taken from performing mpMRI to conducting RP was 29 days (7-63 days). Unique pathologically confirmed prostate cancer foci (237) were identified in 110 patients, 39 (36%) of whom had solitary and 68 (64%) of whom had multifocal tumors. mpMRI successfully identified 249 lesions.

Overall sensitivity of 46.4% was achieved using mpMRI with PI-RADS for the detection of prostate cancer

(110/237). In total, 106 (44.7%) tumor lesions on radical prostatectomy specimen were identified as clinically significant cancer. The sensitivity, specificity, negative predictive value (NPV), PPV and accuracy of detection of clinically significant cancer were 75.5%, 77.0%, 79.8%, 72.7% and 76.3%, respectively. A total of 75.7% (81/107) of the pathological index tumors in the RP specimens were detected using mpMRI with PI-RADS. The median pathological index tumor volume was 2.31 cc (0.10-11.21 cc). The sensitivity, specificity, NV, PPV, and accuracy of detection of pathological index tumors were 75.7%, 77.7%, 79.5%, 73.6% and 76.8%.

Table 1. The demographic characteristics for 110 men

Age, years; median (min; max)	63 (50; 75)
PSA, ng/mL; median (min; max)	10.7 (5.1; 30)
Positive findings in DRE	37
Prostate volume, mL; median (min; max)	41 (15; 112)
Time from biopsy to RP, days; median (min; max)	29 (7; 63)
Histological findings	
Tumor multifocality	
Solitary	39(36.4)
Multifocal	68 (63.6)
Number of multifocal tumor	
2 foci	29 (27.1)
3 foci	21 (19.6)
4 foci	14 (13.1)
≥5 foci	4 (3.7)
Pathological stage	
pT2a	21 (19.6)
pT2b	22 (20.6)
pT2c	52 (48.6)
pT3a	3 (2.8)
pT3b	9 (8.4)
Grade group	
Grade group 1 (Gleason score 3+3)	15 (14.0)
Grade group 2 (Gleason score 3+4)	44 (41.1)
Grade group 3 (Gleason score 4+3)	22 (20.6)
Grade group 4 (Gleason score 8)	7 (6.5)
Grade group 5 (Gleason score 9-10)	19 (17.8)
mpMRI findings	
PIRADS score of each tumor focus	
PIRADS ≤2	127 (51.0)
PIRADS 3	31 (12.4)
PIRADS 4	50 (20.1)
PIRADS 5	41 (16.5)

In addition, higher PI-RADS scores were significantly associated with increased tumor volume (P < 0.001). Pathological index tumor volume was the strongest predictor of tumor detection by mpMRI with PI-RADS (P = 0.03). In 33% of the cases, the pathologic staging is precisely similar to mpMRI and in 43%of the cases, there was a slight difference between the pathologic staging and staging by mpMRI but the cancer was confined to the prostate.in 24% of the cases, there was a significant difference between the pathologic staging and staging by mpMRI. The mpMRI was not able to identify the significant cancer in 24% of the cases.

4. Discussion

The aim of our study was to determine the accuracy of MRI for predicting pathological staging of prostate cancer. The main role of mpMRI with PI-RADS in prostate cancer diagnosis is to identify clinically significant cancer. Although there is no general consensus on the definition of clinically significant PCa, clinically significant cancer is defined on pathology as grade group 2 with a cancer volume of 0.5 cc (Weinreb et al., 2016; Wolters et al., 2011). In present study, 3T mpMRI with PI-RADS demonstrated overall sensitivity of 46.4% and specificity of 75.5% in detecting clinically significant cancer. These results are consistent with those of previous studies. Jesse et al. reported overall sensitivity and specificity of 47% and 72%, respectively, for the detection of

clinically significant cancer,12 and Bratan et al. reported 53-59% overall sensitivity (Bratan et al., 2013). Although the individual sequences are useful, T2WI in combination with two functional sequences has been shown to provide better characterization of tumor in the prostate ([42, 43, and 44). In a diagnostic meta-analysis of seven studies, de Rooij et al. revealed a high overall sensitivity and specificity on accuracy of mp-MRI using T2WI, DWI and DCE MRI. Pooled sensitivity and specificity were 0.74 and 0.88, respectively, with negative predictive value (NPV) ranging from 0.65 to 0.94 (de Rooij, Hamoen, Fütterer, Barentsz, & Rovers, 2014).

Although, theoretically, mpMRI with PI-RADS is known to be able to detect intermediate- to high-grade cancers with volumes 0.5cc (Weinreb et al., 2016). Our data showed that 24% of clinically significant cancer and pathological index tumors were missed using this approach.

Wang et al. reported that a decrease in the degree of intensity in the peripheral zone on T2WI correlated with Gleason grade (Wang, Mazaheri, Zhang, Ishill, Kuroiwa, & Hricak, 2008). Similarly, in the case of DWI, the diffusion of water molecules was more restricted in tightly packed high-grade prostate cancer compared to that in low-grade PCa, which is more loosely packed or more normal prostate-like tissue architecture, thus depicting hyperintense focal lesions on high b-value DWI and hypointense focal lesions on ADC mapping. Elsewhere, an inverse correlation between quantitative ADC mapping values and Gleason grade was demonstrated (Hambrock et al., 2011; Nowak et al., 2016). Although our data and those in previous studies suggest that mpMRI with PI-RADS can be used as a tool to predict prostate cancer aggressiveness, attention should be paid to the fact that 36% of lesions classified as PI-RADS 2 were grade group 2. Borkowetz et al. and Truong et al. reported that most tumors that could not be visualized on mpMRI were found to be cribriform ones, which are currently interpreted as Gleason pattern 4 (Borkowetz et al., 2016; Truong, Hollenberg, Weinberg, Messing, Miyamoto, & Frye, 2017). It was suggested that the more open cellular architecture of cribriform tumors, compared to Gleason pattern 4 or 5 tumors, could lead to misinterpretation of high-grade tumors as normal architecture or low-grade tumors on mpMRI.

The value of using staging and treatment planning for prostate cancer has been demonstrated in contemporary studies on mpMRI in prostate cancer (McClure et al., 2012; Somford et al., 2013). In addition, it was shown in a recent prospective trial that when used with targeted biopsy, mpMRI improved the detection of significant prostate cancer and reduced the need for unnecessary invasive tests (Ahmed et al., 2017).

5. Conclusion

We evaluated the ability mpMRI with PI-RADS (version 2) to detect prostate cancer and a direct comparison was made with the pathology findings for RP specimens. Sensitivity of 76% was found for both clinically significant cancer detection and the findings for pathological index tumors. A moderate and significant correlation was observed between a high PI-RADS score and a high pathological grade, tumor volume, index tumor status and clinically significant cancer status. Pathological index tumor volume was the strongest predictor of tumor detection. The mpMRI was not able to identify the significant cancer in 24% of the cases. MRI/US-fusion biopsy was associated with a higher detection rate of clinically significant prostate cancer while taking fewer cores, especially in patients with prior negative biopsy. As we move toward personalized medicine, use of MRI to biopsy each man's prostate differently rather than based on a pre-defined 12 core seems to be supported in the recent literature.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Comparison of Magnesium Sulfate and Nifedipine in Prevention of Preterm Labor

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Abstract

Introduction: Fetal and neonatal complications are more common in premature than full term pregnancy. Treatment of preterm labor and postpone delivery increases neonatal survival and better quality of life and reduces health care costs for premature infants. This study aimed to compare the effects of Nifedipine and Magnesium sulfate in arresting preterm labor and to adverse the effects of these drugs.

Materials and Methods: This randomized and clinical trial study was performed on 100 pregnant women who were hospitalized for preterm labor pain. The participants were pregnant women with the gestational age of 28 to 34 weeks and with a single pregnancy and symptoms of preterm were studied. They were randomly divided into two equal groups. After not suppressing the pain by fluid therapy, in the first group Magnesium sulfate infused injection (N=50) was performed, while in the second group, oral Nifedipine were given. The research uses SPSS software (version 20) statistical software issue 20 to analyze the result of tests with descriptive statistical methods including independent T test and chi square test.

Results: Mean maternal age, gestational age, parity converted Magnesium sulfate and Nifedipine group had no significant difference in statistical analysis. Delivery was delayed more than 48 hours in 48% (24 person) of cases in the Magnesium sulfate group and in 72% (36 person) in Nifedipine group (p=0.03). A statistically significant difference in response to treatment was more in group of Nifedipine.

Conclusion: The results showed that Nifedipine is more effective than Magnesium sulfate in postponing delivery (more than 48 hours), producing minimal side effect, having adequate price and applying an easy use method. Therefore, Nifedipine, as a tocolytic, can be a good substitute for Magnesium sulfate in preterm labor treatment.

Keywords: Preterm Delivery, Nifedipine, Magnesium Sulfate

1. Introduction

Giving birth is one of the most amazing physiologic events in human life and it can be safe and pleasant in many circumstances, but in some situations it might end with known difficulties and complications for mother and her fetus. Pregnancy may be influenced by many factors (Valadbeigi et al., 2017). Preterm labor is one of these complications. (Saadati, Moramezi, Cheraghi, & Sokhray, 2014) The care and treatment of birth complications of a premature baby costs a great deal annually and sometimes mental and psychological strokes can cause irreparable stress for families. In fact, the birth of a healthy and uncomplicated newborn is the primary goal of pregnancy. (Cunningham, Leveno, Bloom, Hauth, Gilstrap III, & Wenstrom, 2005)

Due to the importance of the subject and the increasing prevalence of preterm delivery in recent years, many efforts and studies have been done to identify, prevent and treatment of preterm labor. Nevertheless, no progress in reducing the prevalence of preterm delivery has been achieved in developed countries over the past two decades and the successes have only been limited to the field of treatment. (Petraglia, Strauss, Gabbe, & Wiess, 2007) Pyelonephritis, a history of abdominal and pelvic surgery, diabetes, urinary tract and genital tract infections are all contributing to the increased risk of preterm labor. (Chehre, Karaallahi, Eivazi, Borji, & Safar, 2018)

All pregnant women are taught about the symptoms of premature labor by the antenatal care practice. Should uterine contractions occur, they are to be self-monitored, and if regular painful contractions are detected the women have to go to the hospital for examination. (Songthamwat, Nan, & Songthamwat, 2018)

Preterm delivery cannot be stopped, but can be postponed for a few days. With this delay, it can have a profound impact on the outcome of preterm labor, including mortality and morbidity of premature infant (in terms of Physically, mentally, and evolutionarily, the economic burden and complications that sometimes persist throughout life) (Saadati, Moramezi, Cheraghi, & Sokhray, 2014; Moore, 2003)

Uterine contractions are produced by the effects of prostaglandins. Prostaglandins are a group of paracrine hormones so they act where they produce. Prostaglandin producing by the decidua and fetal membranes might be one of the essential events of parturition that is followed by initiation of the uterine contraction. It seems likely that prevention of producing prostaglandins or suppression of their effects can stop preterm uterine contraction. (Saadati, Moramezi, Cheraghi, & Sokhray, 2014)

Conventional treatment options for postponing preterm labor include bed rest, fluid therapy and sedation, and tocolytic agents, that each of these has its advantages and disadvantages. However, no convincing evidence has been provided as to the effectiveness of bed rest and hydration. (Saadati, Moramezi, Cheraghi, & Sokhray, 2014; Scott, Gibbs, Karlan, & Haney, 2003)

Several tocolytic agents are used for uterine contractions. Therefore, prior counseling, training and screening before and during pregnancy can help identify these factors that affect preterm labor. In preterm labor with cervical dilatation, the efficacy of tocolytics was proven for prolonging pregnancy. (Songthamwat, Nan, & Songthamwat, 2018) The tocolytic that is most commonly used in our country is Magnesium sulfate. Magnesium works by competing with calcium or by reducing calcium entry into the cell through cell membranes during depolarization. According to recent studies, since this drug has been known to cause maternal complications (such as nausea and vomiting, lethargy, dyspnea, headache, hypotension, and dizziness (Kim et al., 2018)) and fetal complications (as respiratory depression, hypotonia, and hyporeflexia (W. H. Kim, Y. H. Kim, An, Moon, Noh, & J. W. Kim, 2018)), and contradictory reports on its efficacy and cost, in most prestigious research centers around the world has been deleted from preterm labor treatment. In fact, Magnesiom sulfate has been replaced by some drug like a calcium channel blocker group called Nifedipine with better efficacy, faster rate of action, less side effects and less costs. (Taherian & Dehdar, 2007)

Nifedipine inhibits myometrial contraction by blocking calcium entry into smooth muscle cells and by blocking voltage-dependent calcium channels. Many studies have now suggested that tocolytics is the preferred treatment for preterm labor. (Cunningham, Leveno, Bloom, Hauth, Gilstrap III, & Wenstrom, 2005)

However the use of Nifedipine is associated with some side effects such as a headache, dizziness, flushing, and peripheral edema, and is contraindicated in women with hypotension, congestive heart failure, and aortic stenosis. (Haghighi, Rashidi, Najmi, Homam, Hashemi, Mobasseri, & Moradi, 2017)

In general, medicine prescribed for pregnant mother has a therapeutic effect (Afiqah Amani et al., 2017). Clinicians are often faced with the problem of managing an established preterm labor with a variety of drugs which may have a lack of uterospecificity, low efficiency or potentially severe side effects for the mother or the unborn baby. (Moramezi, Barati, Saadati, Masihi, & Hemadi, 2011)

Therefore, in this study, by considering the potential of delaying preterm labor and its effects, comparison was made between Magnesium sulfate and Nifedipine.

In case of achieving similar results to those reported in recent reports, based on better efficacy and fewer side effects of Nifedipine, It can be a suitable replacement for Magnesium sulfate in the treatment of preterm labor.

2. Method

This study is a phase 2 randomized clinical trial on 100 pregnant women with gestational age of 28 to 34 weeks who have been admitted to Imam Khomeini Hospital in Ahwaz since 2017 years with the diagnosis of preterm labor. It has been done after obtaining consent and in accordance with the inclusion criteria (At least 3 contractions lasting 30 seconds for 20 minutes with increased dilatation and cervical effacement) and exclusion criteria (All those who continuation of pregnancy to have contraindications or have problems with the administration of Nifedipine or Magnesium sulfate).

In this study, vital signs are checked first for all patients, 500cc Ringer is then administered with a rapid infusion and Then, if the uterine contractions continue, The patient was randomly assigned to either group A or Nifedipine (n = 50) or group B or Magnesium sulfate (n = 50). Then Nifedipine is administered with an initial dose of 20mg orally and then every 6 hours for 24 hours and Magnesium sulfate is administered intravenously at 4 mg and then 2g/h for 24 hours.

In this study, for blinding, the Nifedipine group received ringer serum in addition to the oral drug and

Magnesium sulfate recipient group receive oral placebo in addition to injectable drug. As such, the patient, physician, and treatment staff of the patients under study have no knowledge of how individuals are placed in groups.

Thereafter, a check is made between initiation of treatment to relieve pain or the occurrence of preterm labor. During the study, vital signs, vaginal bleeding, rupture of fetal membrane, heart rate, uterine contractions and maternal blood pressure are monitored.

Midwifery information about the pregnant woman and her examinations and the type of prescription medication, as well as side effects and cases of treatment failure and interval between initiation of treatment and improvement of pain or stillbirth will be recorded in the questionnaire. In the cases in which the induction of labor due to fetal distress or other causes have occurred, the information is excluded.

Variables such as maternal age, gestational age, number of deliveries, history of preterm labor, rate of cervical dilatation and effacement, severity of uterine contractions, duration between onset of preterm labor until initiation of treatment, and duration between initiation of treatment to improvement of pain or delivery measured and examined.

To describe the data, the mean and standard deviation of the quantitative variables and the frequency and percentage of the qualitative variables are used.

For data analysis, t-test (Mann-Whitney if necessary), chi-square test and if necessary, logistic regression and survival analysis method were used.

All analyzes were performed using SPSS software version 20 and $P<0.05$ was considered statistically significant.

3. Results

Based on the results of 100 pregnant women with gestational age of 28-34 weeks who were randomly divided into Nifedipine and Magnesium sulfate groups, there was no statistically significant difference in maternal age, gestational age, number of previous deliveries, dilatation and cervical effacement at the start of treatment and number of uterine contractions. (Table 1).

Table 1. Comparison of clinical and demographic characteristics of Nifidipine group and Magnesium sulfate group

Variables	Nifedipine group Mean+/- SD		Magnesium sulfate group Mean+/- SD	P value
Maternal age (year)	24.4 +/- 5/13	0.88	23.8 +/- 5.43	
Gestational age(week)	31.6+/- 1.8	0.14	30.6+/- 2.7	
Number of previous deliveries	1.5 +/- 0.97	0.10	1.8+/-1.2	
Cervical dilatation(Cm)	1.5 +/-0.5	0.14	1.6+/-0.49	
Cervical effacement(%)	35.5 +/- 10.01	0.96	38+/-9.47	
Number of uterine contractions	4.91 +/- 0.66	0.51	5.2+/- 0.45	

The duration mean (+/- SD) duration between the onset of preterm labor and until treatment begins, was 35.42 +/- 32.52 hours in women receiving Nifedipine and 34.48+/- 34.46 in women receiving Magnesium sulfate, that means the difference in time distribution between the two groups was not statistically significant. The duration mean (+/- standard deviation) between the start of treatment and the recovery of preterm labor was 14.32+/- 15.39 hours in the Nifedipine group and 15.86+/- 14.72 hours in the Magnesium sulfate group. Statistical analysis showed that there was a significant difference between the two groups (p = 0.01). However, there was no statistically significant difference between the two groups in the duration between treatment initiation and preterm delivery. (p = 0.473) (Table 2)

Table 2. Frequency distribution of treatment response in the Nifedipine and Magnesium sulfate groups

Variables	Nifedipine group	Magnesium sulfate group	P value
Duration between the onset of preterm labor and until treatment begins(h)	35.42 +/- 32.52	34.48+/- 34.46	0.668
Time between the start of treatment and the recovery of preterm labor(h)	14.32 +/- 15.39	15.86 +/- 14.72	0.01
Time between treatment initiation and preterm delivery(h)	16.7+/- 13.97	16.3+/- 14.63	0.473

In 36 patients (72%) of Nifedipine group and 24 patients (48%) of Magnesium sulfate group, delivery was delayed more than 48 hours, which was statistically significant (p = 0.03) and Also in delayed delivery for less than 48 hours, there was 14 cases (28%) in the Nifedipine group and 26 cases (52%) in the Magnesium sulfate group (p = 0.04) (Table 3).

Table 3. Frequency distribution of treatment response in the Nifedipine and Magnesium sulfate groups

group	delayed delivery for less than 48 hours person (%)	delayed delivery for less than 48 hours person (%)	Total Person (%)
Magnesium sulfate	**26 (52%)**	**24 (48%)**	**50 (100%)**
Nifedipine	**14 (28%)**	**36 (72%)**	**50 (100%)**
P value	**0.04**	**0.03**	**-**

Chi-square test showed a statistically significant difference in complications in the two groups of Nifedipine and Magnesium sulfate. (p <0.00001) (Table 4)

Table 4. Comparison abundance of complications between patients in the two groups of Nifedipine and Magnesium sulfate

complications	have		Have not		Total	
abundance	number	%	number	%	number	%
Nifedipine group	12	24	38	76	50	50
Magnesium sulfate group	26	52	24	48	50	50
Total	38	38	62	62	100	100

The most common complications in Magnesium sulfate-treated group were hot flashes (32%) and headache (8%) and in the Nifedipine-treated group hypotension (10%) and dizziness (8%). None of the two groups had a serious maternal complication leading to discontinuation of treatment. (Table 5)

Table 5. Frequency of complications according to Nifedipine and Magnesium sulfate treatment groups

group	Magnesium sulfate		Nifedipine		Total	
	number	%	number	%	number	%
Headache	4	8	1	2	5	5
Dizziness	3	6	4	8	7	7
Hypotension	-	-	5	10	5	5
Tachycardia	-	-	1	2	1	1
Hot flashes	16	32	-	-	16	16
Nausea & vomoting	3	6	-	-	3	3
Dyspnea	-	-	-	-	-	-
Palpitation	-	-	1	2	1	1
Total	26	52	12	24	38	38

4. Discussion

Prevention and treatment of preterm labor is essential to reduce undesirable neonatal complications and increase survival and quality of life. In fact, the ultimate goal of preterm birth management is not just to prolong pregnancy, but to improve neonatal outcomes and reduce morbidity and mortality.

For this reason, we should try to prevent preterm labor as much as possible by eliminating the underlying factor or suppressing uterine contractions. Therefore, the aim of this study was to compare the effect of Nifedipine and Magnesium sulfate in preventing preterm labor which findings showed that there was a statistically significant difference in response to treatment of the two groups, such as the Nifedipine group had a higher response to treatment.

None of the patients in the two groups had any complication leading to discontinuation of treatment.

In a study in 2007 by Lille, the efficacy of the two drugs was similar in delaying delivery for 48 hours, and there were fewer maternal complications in the Nifedipine-receiving group (Lyell, Pullen, Campbell, Ching, Druzin, Chitkara, Burrs, Caughey, & El-Sayad, 2007).

Dr. Faraji's study in 2013 in Iran, it was found that Nifedipine was more effective than Magnesium sulfate in delaying labor for more than 48 hours, which is consistent with the results of the current study. (Faraji, Asgharnia, Dalil Heyrati, & Nemati, 2013)

Deirdre, in a study in 2007 at Stanford University, found that stopping contractions within Magnesium sulfate was better than Nifedipine during the first 48 hours (87% compared to 72% at p = 0.01), but delayed labor and gestational age at delivery and neonatal major outcomes were similar in the two groups, the results of which are in contrast to our recent study, and Nifedipine was significantly associated with fewer maternal complications (Deirdre, Pullen, Campbell, Ching, Druzin, Chitkara, Burrs, Caughey, & EL-Sayed, n. d.).

In a 1999 study by Dr. Haghighi at the University of Tehran, Nifedipine and Magnesium sulfate both had similar efficacy and side effects, but Nifedipine had a faster effect on stopping uterine contractions, which is similar to the results of our study (Haghighi, 1981).

In another study by Glock in 2002, aimed to compare the effects of Nifedipine and Magnesium sulfate in patients with preterm labor, oral Nifedipine was as effective as Magnesium sulfate in stopping preterm labor, which is similar to our study's result showing that Nifedipine had a faster effect on preventing preterm labor. (Glock & Morales, 2009)

In a study in 2013 by Dr Nikbakht in Iran, 100 women with documented preterm labor were randomly assigned to receive Magnesium sulfate (n=50) and Nifedipine (n=50) as tocolytic therapy. Both drugs were equally effective in prevention of labor and delaying delivery more than 7 days, 56% vs. 64% in the Nifedipine and Magnesium sulfate groups, and the days gain in utero was no statistically different in two groups. 6% of Nifedipine group and 2% of Magnesium sulfate group required drug discontinuation due to severe symptoms. There were also no significant differences in maternal characteristics between two groups. The total success rate and side effects were similar in two groups. (Nikbakht, Moghadam, & Ghane'ee, 2014)

In the present study, Nifedipine has many benefits including fewer side effects, better tolerance and acceptance by the patient, and oral administration of intravenous infusion.

Side effects of Nifedipine are also dose dependent and rarely lead to intolerance and discontinuation of the drug. The most common complication is hypotension less than 90.50 mmHg reported in the mother and is seen approximately 20 minutes after the second oral dose and is usually transient and not clinically significant, such that with adequate fluid therapy, it is preventable. (Jeyabalan & Caritis, 2002)

In the present study, there were maternal adverse events in 12 patients (24%) of Nifedipine group and 26 patients (56%) of Magnesium sulfate group, which were statistically significant.

5. Conclusion

According to the results, Nifedipine can be a good substitute for Magnesium sulfate in preterm labor due to its good efficacy and low side effects, low cost and easy use as a tocolytic.

Acknowledgments

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Efficacy of Laparoscopy and Hystroscopic Dilation and Curettage Procedure in Managing Cesarean Scar Pregnancy

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Abstract

Objective: To evaluate the success rate of laparoscopy and hysteroscopic Dilation & Curettage in treating cesarean scar pregnancy.

Methods: This cross-sectional study was carried on patients suspicious to CSP referred to Imam Khomeini Hospital, Ahvaz from Jun 2016 to March 2018. Gestational site was carefully evaluated by hysteroscopy and then curettage was carried out under laparoscopic guidance. The primary outcome in the study was time interval from treatment to hCG resolution. Moreover, the secondary outcomes were vaginal bleeding time and residual mass size.

Results: In this study, 30 women with intrauterine CSP were evaluated. Of these, 5 had live embryos and 25 had dead fetuses. The success rate of treatment was 100%. Only one patient needed to transfusion. The mean and median duration of Bhcg resolution were 30.9 and 28 days, respectively. Moreover, The mean and median duration of vaginal bleeding were 30.9 and 28 days, respectively. The mean level of residual mass was 9.8 mm^3 (ranged from 1 to 25 mm^3).

Conclusion: Overall, the findings of this study showed that D&C effectively treated CSP patients (100% success rate), reduced the time of Bhcg resolution and also reduced vaginal bleeding time.

Keywords: Dilation and Curettage, Ectopic Preganacy, Cesarean, Hysterescopy

1. Introduction

Pregnancy may be influenced by many factors (Valadbeigi et al., 2017). Ectopic pregnancy (EP) with an incidence of 1-2% of the total pregnancy is responsible for 3 to 4% of maternal deaths. In the United States, it has been reported that EP causes 31.8 deaths per 100,000 pregnancies. It is the most common cause of maternal death in the first trimester of pregnancy. Although Ep appears in the fallopian tube in more than 98% of cases, it rarely presented in other sites (inside the abdomen, ovaries, and in the cesarean section scar). Cesarean scar pregnancy (CSP) is referred to as replacement of a pregnancy product within the myometrium at the site of the previous cesarean section. In fact, CSP is due to a uterine endometrial defect, which can be caused by other reasons such as rough curettage, myomectomy, metroplasty, Hysteroscopy or manual removal of the placenta (Lin, Yang, Chi, Lian, Wang, Huang, Lu, Liu, & Qiao, 2017).

There are two types of CSP: 1- Implantation of the gestational sac (GS) in the scar and progressing towards the cervical uterus; 2- Progressing the GS to the myometrium and uterine serous. Thirty-two percent of CSP have a history of more than 2 times CS, while it can be seen even in those with a history of one previous CS. It has recently been estimated that the overall incidence of CSP is 1 per 531 pregnancies in women with CS and 4.2% of those with EP. CSP incidence is rising due to an increase in CS. CSP must be diagnosed quickly, because its life-threatening nature. It could be rupture the uterus, severe bleeding, circulatory dysfunction and maternal death (Litwicka & Greco, 2011; Petersen, Hoffmann, Larsen, & Nielsen, 2016). There are several strategies for treating CSP. In patients who are clinically stable, conservative treatments are include uterine artery embolization with methotrexate, D & C, curettage and hysteroscopy. D&C under trans-abdominal ultrasound guidance is the common procedure in treating CSP. Despite the D&C advantages including simple, low-cost, and well-therapeutic effect, it carried series dangers such as uterine perforation, bleeding, and low success rates.

Patients treated with D&C have a longer time to resolve the ectopic mass and B-hCG, and in some cases, such as intense hemorrhage, they require laparotomy and hysterectomy (Jurkovic, Knez, Appiah, Farahani, Mavrelos, & Ross, 2016). According to some studies, hysteroscopic removal of CSP is an effective, safe, and non-invasive process. This method provides direct observation, less surgical operation, less hospitalization after surgery, and faster resolution of B-hCG levels.

Due to the low prevalence of CSP, there is no general agreement to choose the best treatment. Although studies have shown the benefits of hysteroscopy and D&C in the managing of CSP, most of them designed as case studies or case series with little sample size that reduce the reliability of their results (Litwicka & Greco, 2013). In the current study we have aimed to evaluate the success rate of hysteroscopic and D&C in treating CSP.

2. Material and Methods

2.1 Study Design

This cross-sectional study was carried on patients suspicious to CSP referred to Imam Khomeini Hospital, Ahvaz from Jun 2016 to March 2018. Inclusion criteria was confirmed diagnosis of CSP. Patients with acute severe blood loss, hypovolemic shock, renal failure, active pelvic infection and those with coagulation disorders were excluded from the study. Patients demographic informations were extracted from the files. This study was approved by the Ethics Committee of Ahvaz Jundishapour University of Medical Sciences. Prior to entering the study, patients were provided with necessary explanations. The signed informed consent was received from all participants.

2.2 Measurements

EP diagnosis was performed based on history, clinical examinations, and B-HCG level. The B-HCG serum level more than 25 IU is considered positive. CSP diagnosis confirmation was done by abdominal or vaginal ultrasonography by an expert prenatalogist. Hemodynamic factors and B-hCG serum level were checked weekly. The patients were followed-up until B-hCG resolution. Moreover, one week after treatment, residual mass size was evaluated by transvaginal sonography (TVS) (Medison Accuvix V20, Samsung, Korea).

2.3 Interventions

Initially, patients were treated by medical procedures including single or multiple dose of MTX. To cervical ripping, 400 milligrams of misoprostol suppository was administrated. Gestational site was carefully evaluated by hysteroscopy and then curettage was carried out under laparoscopic guidance.

2.4 Outcomes

The primary outcome in the study was time interval from treatment to hCG resolution. Moreover, the secondary outcomes were vaginal bleeding time and residual mass size.

2.5 Statistical Analysis

All variables were subjected to descriptive analysis including mean, standard deviation and frequency. The mean time to event was calculated by Kaplan-Meier plot. Computations were performed using SPSS version 19 software (Statistical Package for Social Service Inc., Chicago, IL, USA). The significant level for analyses assigned 0.05.

3. Results

Table 1. Patients characteristics

Variables	Median	Mean	Std. Deviation	Minimum	Maximum
Age	31.00	30.66	2.581	25.00	35.00
BhCG	2887	8176	11601	224.0	49717
Time to BhCG resolution	28.00	30.97	14.05	7.000	75.00
Time of bleeding	30.00	40.48	20.41	14.00	120.0
Gravidity	3.0	3.4	1.09	2.0	7.0
Parity	2.0	2.24	0.78	1.0	4.0
Abortion	0.0	0.31	0.66	0.0	3.0
Cesarean section	2.0	2.17	0.84	1.0	4.0
Residual Mass(mm^3)	6.0	9.8	7.4	1.0	25.0
Hospitalization	3.0	2.9	0.5	2.0	4.0

In this study, 30 women with intrauterine CSP were evaluated. Of these, 5 had live embryos and 25 had dead fetuses. Of the 5 patients with the live fetus, 3 patients were injected with one dose of MTX into the GS. The mean Gravidity and Parity levels in patients were 3 and 2 (range 2 to 7 and 1 to 4) respectively. The average abortion rate in patients was 0.3 with a maximum of 3 (Table 1). One patient was excluded during the study.

The success rate of treatment was 100%. Only one patient needed to transfusion. We have not detected any procedure related complications in the studied patients. BHcg levels strictly decreased during monitoring time in all studied patients (Figure 1).
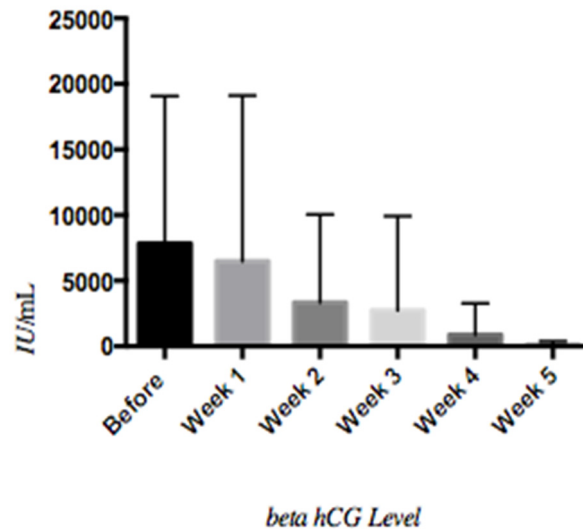
Figure 1. Changes in BHcg level

The mean and median duration of Bhcg resolution were 30.9 and 28 days, respectively. Moreover, The mean and median duration of vaginal bleeding were 30.9 and 28 days, respectively (Table 2, Figure 2). The mean level of residual mass was 9.8 mm^3 (ranged from 1 to 25 mm^3).
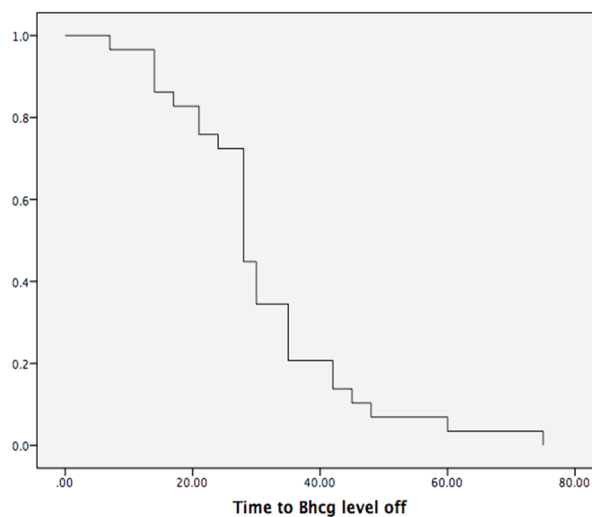
Figure 2. The Kaplan Meier chart of the Bhcg resolution time

Table 2. Outcomes assessment

Variables	Mean			Median		
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval	
		Lower Bound	Upper Bound		Lower Bound	Upper Bound
BhCG resolution time	30.966	25.850	36.081	28.000	25.375	30.625
Vaginal bleeding time	40.483	33.054	47.912	30.000	29.046	30.954

Comparison of live and dead embryos in terms of the studied factors have shown that the initial level of Bhcg, BhCG resolution time and vaginal bleeding time in patients with live fetuses were significantly higher than those with dead embryos (Table 3).

Table 3. Comparison of the studied variables between dead and live embryos

Group Statistics	Live N=5	Dead N=24	P value
Bhcg	23480±18091	4987±6736	p<0.0001
Mass	2.5±3	6.44±7.9	p=0.29
Bhcg resolution Time to	47.6±20.3	27.5±9.9	p=0.002
Bleeding Time	63±34.2	35.79±13.06	p=0.004
Hospitalization	3±0	2.8±0.54	p=0.612
Gravity	3.8±0.44	3.4±1.17	p=0.484
Parity	2.4±0.54	2.2±0.83	p=0.629
Abortion	0.4±0.54	0.2±0.69	p=0.745
Cs	2.4±0.54	2.12±0.89	p=0.719

4. Discussion

Larsen and Solomon reported the first case of CSP in 1978 (Larsen & Solomon, 1978). However, there are still no specific guideline available for the treatment of these patients. Some researchers have suggested that conservative treatment is preferred in patients with cesarean section scar greater than 2 mm, absence abdominal pain and hemodynamically stable (Ghezzi, Lagana, Franchi, Fugazzola, & Bolis, 2002; Khunda & Tay, 2007). However, recent studies have shown that surgical-based therapies, including local lesion resection of the uterus, and D&C have many beneficial effects when compared with uterine embolization. Patients undergoing surgical treatment needs significantly less blood transfusion than patients treated by uterine embolism, shorter hospitalization time, and shorter duration of time for Bhcg resolution. Wang et al. Also showed that the recovery time is much shorter when surgical treatment performed along with systemic or topical methotrexate administration (Wang et al., 2009). In addition, preservative treatments can be associated with various complications such as uterine rupture, bleeding and the need for surgery (Stevens & Ogburn, 2011; Jurkovic, Hillaby, Woelfer, Lawrence, Salim, & Elson, 2003). Surgical treatment not only results in the termination of pregnancy, but also preserves fertility, reduces the amount of bleeding and shortens the duration of hospitalization. Although abdominal surgery is an effective treatment and first choice for CSP patients, it causes severe complications (Litwicka & Greco, 2011; Chang et al., 2011; Deans & Abbott, 2010). On the other hand, hysteroscopic surgery can reduce the incidence of hemorrhage by identifying the location of the sac and the vascular distribution surrounding the gestational implant (Deans & Abbott, 2010; Wang, Yuen, Chao, Lee, Yen, & Soong, 2005; Chao et al., 2005). Laparoscopic hysteroscopy can reduce the need for bladder surgeries and reduce the amount of bleeding during surgery (Li, Guo, Han, Wang, Xiong, Shen, & Zhang, 2011). This surgery requires specialized equipment and specialized surgeons with specialized skills, which makes it difficult to do in small hospitals. Abdominal surgery is preferred in patients with confirmed uterine rupture. This can actually be the best treatment for patients with CSP, which can remove the pregnancy sac and restore uterine in order to reduce the risk of relapse (Hasegawa et al., 2005; Fylstra et al., 2002). Although this procedure causes more trauma, longer recovery time and hospitalization time (Fylstra et al., 2002). Although a general consensus suggests that D&C is not a good treatment for CSP patients, it also has a high risk of extensive bleeding and uterine rupture, but recent studies have shown that ultrasound D&C can treat nearly 100 % of patients (Liu, Sun, Cai, Xi, Yang, & Sun, 2016).

According to the findings, the average time needed to normalization the Bhcg level and stopping vaginal bleeding was 30.9 and 40.4 days, respectively. In a recent study, Liu and colleagues evaluated the success of

D&C in managing patients with CSP and found that the treatment was successful in 97.6%, and their findings showed that after three weeks of treatment Bhcg was unditectable. Also, 40 days after treatment, all patients had normal menstrual cycle (Liu, Sun, Cai, Xi, Yang, & Sun, 2016). Contrary to the current study, Liu et al. Did not prescribe methotrexate in any of the patients. Qian et al. Compared hysteroscopy with curettage in treating CSP. The researchers concluded that both D&C and hysteroscopy along with curettage are successful in treating CSP (the success rate was 95.56%) (Qian, Huang, & Zhu, 2015s).

5. Conclusion

Overall, the findings of this study showed that D&C effectively treated CSP patients (100% success rate), reduced the time of Bhcg resolution and also reduced vaginal bleeding time. The lack of allocation of comparative groups was the main limitations of this study, which is suggested to be addressed in future studies.

Acknowledgements

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the local ethics committee of Tehran University of Medical Sciences and with the 1964 Helsinki declaration. Written informed consent was obtained from all patients and normal individuals.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Frequency of Ectasia among Patients with Coronary Atherosclerosis by Angiography Dual Source & 64 CT SLICE Evaluatin

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Abstract

Introduction: The aim of this study is Frequency of ectasia among patients with coronary atherosclerosis.

Materials and Methods: This is a cross-sectional study, which retrospectively had evaluated a percentage of total frequency ectasia among patients with coronary atherosclerosis by angiography Dual a source & 64 CT SLICE between 2007-20012 in Imag Center of Imam Khomeini Hospital and shahid Rajai Hospital.

Results: Totally 2770 patients were enrolled in the study. The frequency of ectasia among all patients was 42 [1.5%]. The frequency of stenosis in patients with LAD ectasia was 36.8% [7 out of 19] while the frequency of LAD stenosis in other patients was 59.5% [1637 out of 2751] [P=0.045]. Distribution of stenosis in ecstatic LCx and RCA was not statistically different with patients without non ecstatic LCx and RCA. [P=0.47 and 0.45 respectively]. In patients with ectasia, the frequency of stenosis was 71.4 while it was 64.4% in patients without ectasia [P=0.35].

Discussion: Furthermore, detection and investigation of Ectasia in patients with stenosis will lead to more accurate determination of the treatment plan and the purpose of this study is finging a new assess prevalence of morphological changes Ectasia by CT angiography and coronary sclerosing Prevalence this group of patients.

Keywords: Ectasia, Coronary Atherosclerosis, Angiography Dual Source & 64 CT SLICE

1. Introduction

Coronary-related disease is one of the common diseases (Liu-qiang et al., 2017). Many Ectasia coronary means uncommon dilatation in coronary artery, Ectasia coronary incidence is about 1%-5%. Some levels of spasticity, cardiac ischemia, thrombosis and coronary artery dissection and rupture in patients with Ectasia were more reprted. So, it is important to diagnose coronary artery Ectasia for antiplatelet therapy to reduce surgical complications.

Recent advances in CT angiography can be increased diagnosis of coronary artery disease. Today, this common method is used for the detection of coronary artery stenosis in patients who suspected of low or moderate corner disease.

Despite these advances, very few papers had been investigated about Ectasia diagnostic coronary by CT angiography. The aim of this study is Frequency of ectasia among patients with coronary atherosclerosis.

2. Materials and Methods

This is a cross- sectional study by census sampling, which retrospectively had evaluated a percentage of total frequency ectasia among patients with coronary atherosclerosis by angiography Dual source & 64 CT SLICE between 2007-2012 in Imam Khomeini Hospital and shahid Rajai Hospital.

All CT angiography had been done by 64-slice GE Light speed VCT models, and Siemens Dual source FLASH model. Patient's heart rate should not be higher than 70 during CT angiography so, in the absence of contraindications, beta-blockers were used, too. Dualsource be injected for all patients (by a isosorbidnitrate dose).

This dose was adjusted based on each specific time (CT) and BMI Rate of 5 ml per second, followed by a mixture of contrast and saline injection. CT in the Cardio-caudal level of the carina to the diaphragm was performed, too.

ECG was done for all patients and images were taken in all phases of systole and diastole. (0% to 90% Sytsvl and diastole). Images MRP, MIP, VRT done for all patients, so, Ectasia and stenosis in all images had been reviewd and evaluated. Time fore image examination in 64-slice device was 10-12 minutes and for Dual source device was 4-8 minutes.

3. How to Calculate CT Angiography

An observer had measured of maximum diameter for each Ectasia segment. It also differs in size from the diameter of a normal segment was also calculated.Ectasia ratio (diameter segment of Ectasia/segment average according to normal diameter) was calculated. Aneurysm Ectasia was defined by a ratio in two groups with a ratio of 1.5 up to 2 and 2 or more. Descriptive analysis was used to evaluate the frequency Ectasia (percentage of total stereotypes) and frequency separately of each gender and coronary artery disease. 95% confidence intervals were estimated and the results are presented in tables and graphs.

4. Results

Totally 2770 patients were enrplled in the study. The mean age of the patients was 57.1 ± 12.5 [7-93] years. Totally, 1652 were male [59.6%] we assessed the number and frequency of ectasia in terms of each coronary artery and in sub segments of main coronary artery. There was 12 ectatic left main amang the patients [0.43%] The frequency of ectasia in LAD, LCx and RCA were 19, 12 and 24 amang the patients.

Detailed frequency of ectasia in coronary subsegments have been mentioned in Table 1.

Table 1.

Coronary Artery	Number [%]	Coronary subsegment	Number [%]	Gender	
				Male	Female
LM	12[0.43]		12[0.43]	10	2
LAD	19[0.69]	Proximal portion	17[061]	14	3
		Middle portion	5[0.18]	4	1
		Distal portion	2[0.07]	1	1
		Ramus intermedius	1[0.04]	1	0
		D1	3[0.11]	2	1
		D2	1[0.04]	1	0
LCx	12[0.43]	Proximal portion	6[0.22]	4	2
		Middle portion	9[0.32]	5	4
		Distal portion	3[0.11]	2	1
		OM1	4[0.14]	3	1
		OM2	3[0.11]	3	0
RCA	24[0.87]	Proximal portion	15[0.54]	11	4
		Middle portion	12[0.43]	11	1
		Distal portion	12[0.43]	11	1
		RVB	0	0	0
		PDA	3[0.11]	3	0
		PLV	0	0	0

The frequency of ectasia among all patients was 42 [1.5%].

The frequency of stenosis in patients with LAD ectasia was 36.8% [7 out of 19] while the frequency of stenosis in other patients was 59.5% [1637 out of 2751] [P= 0.045].

Distribution of stenosis in ecstatic LCx and RCA was not ststistically different with patients without non ecstatic LCx and RCA. [P=0.47 and 0.45 respectively].
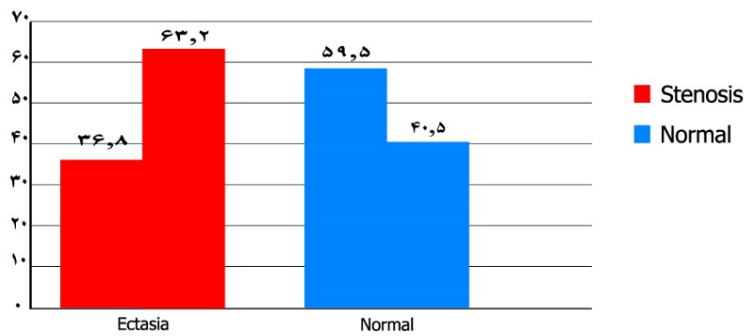
Figure 1. Correlation of Ectasia and Stenosis in LAD
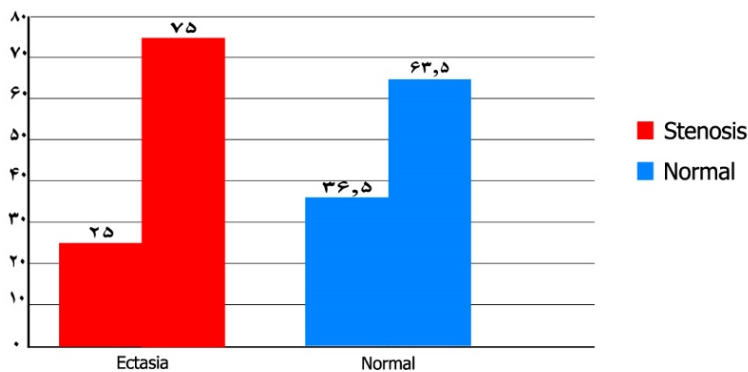
Figure 2. Correlation of Ectasia and Stenosis in LCx
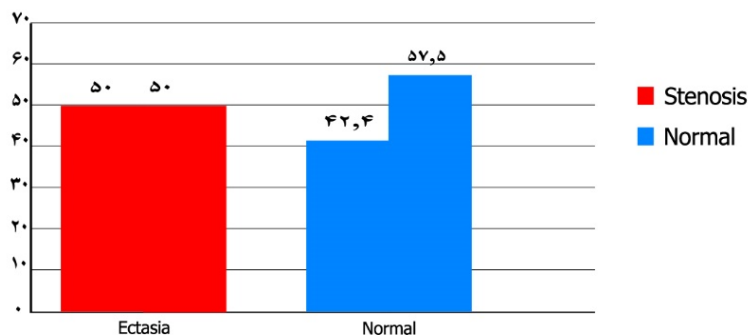
Figure 3. Correlation of Ectasia and Stenosis in RCA

Inpatients with ectasia, the frequency of stenosis was 71.4 while it was 64.4% in patients without ectasia [P=0.33].

5.Discussion

Coronary Ectasia more than two centuries has been observed by pathologists and cardiologists. (Falestti & Carrol, 1976)

First described by Morgagni et Ectasia was conducted in 1761. Deaud and colleagues in patients older than 16 years reported a prevalence of 1.4% for Coronary Ectasia. (Daoud, Pankin, Tulgan, & Floretin, 1963). 1%-5% Of Ectasia have been reported in other articales. In our study, totally, 1652 were male [59.6%] we assessed the number and frequency of ectasia in terms of each coronary artery and in sub segments of main coronary artery. There was 12 ectatic left main amang the patients.

Although these rates may be higher than the incidence in the normal population. In the largest study by Sway and colleagues in CASS Registration, the prevalence of Ectasia in 200,000 cases was 4.9% (Sway et al., 1983) The clinical significance of coronary Ectasia there is conflicting evidence. In some articales, not to mention the

difference in prognosis in this group of patients is normal (Sway et al., 1983) and in some articales, Ectasia had no effect on coronary artery bypass grafts has been mentioned. (Alford Jr, Stoney, Burrus, First, & Thomas Jr, 1976) In the large number of papers, it was mentioned that non-uniformity prognosis of this group. One of these items are increase the amount of spasms (Suzuki et al., 1994), exercise-induced cardiac ischemia (Kruger, Stierle, Herrmann, Simon, & Sheikhazadeh, 1999), thrombosis (Perlman & Ridgewey, 1989), and dissection (Myerburge, 1991) and coronary artery rupture (Satoda, Tatsukawa, & Katoh, 1998). And the detection of coronary artery Ectasia was important and complications. (Ramappa, Kottam, Kuivanemi, & Thatai, 2007) Also have some sources mention that Ectasia coronary is important as coronary stenosis artery size (Herman & Gorlin, 1976). Several studies are shown that CT angiography is a noninvasive approach for of coronary artery disease diagnosis, and also, angiography as a noninvasive method for precise determination of coronary artery variants can be used, too. (Kosar, Ergun, Ozturk, & Kosar, 2009) For example, in a retrospectively study that conducted in Turkey by Koser and colleagues, is shown that CT angiography in 700 patients with suspected coronary artery disease were examined and different variants were investigated, resulting in coronary CT angiography as a noninvasive method for precise determination of coronary artery variants can be used. (Kosar, Ergun, Ozturk, & Kosar, 2009)

Accourding our study, the frequency of ectasia among all patients was 42 [1.5%]. The frequency of stenosis in patients with LAD ectasia was 36.8% [7 out of 19] while the frequency of LAD stenosis in other patients was 59.5% [1637 out of 2751] [P=0.045].

In patients with proximal and middle segments of coronary artery disease, right Corner is most commonly involved segments for ectasia, and then left descending and rotation coronary artery is the second segment for ectasia incidence. (Demopoulos et al., 1997)

Distribution of stenosis in ecstatic LCx and RCA was not statistically different with patients without non ecstatic LCx and RCA. [P=0.47 and 0. 45 respectively]

In Iran, few studies have examined the coronary Ectasia different variants. In the case study reported by Hashmi et al reported a case of aneurysm of the report has been expanded with a septal hypertrophy. (Petrossians, 1993)

In our study, In patients with ectasia, the frequency of stenosis was 71.4 while it was 64.4% in patients without ectasia [P=0.35].

In another case reported by Leach and colleagues conducted a case of the left main coronary artery stenosis that diagnosed with a mass in the right atrium diagnosis by computed tomography (Safi et al., 2009).

In another retrospectively study was conducted by the AKSU and colleagues 12514 coronary angiography were examined and then compared. The prevalence of Ectasia was 1.59%.

Ectasia incidence in the cohort study that conducted in India by Sharma and colleagues was 10% in patients with ischemia. (Sharma et al., 1990) According to the results of this study, it be suggested, Future studies, it is need to examine the relationship Ectasia coronary atherosclerosis in prospective studies with larger sample size.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Investigating Job Stress among Professional Drivers

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Abstract

Purpose: Psychological stress is one of the main occupational hazards. The aim of this study was evaluating psychological stress in terms of role stress and its domains in professional drivers.

Methods: This cross-sectional study was conducted on 300 heavy vehicle drivers and 330 light vehicle drivers. Data were collected using interview and Osipow job stress questionnaire. T-test ، ANOVA ، chi-square test and linear regression were used in analyzing the data.

Results: 33.2% of the participants had mild to moderate stress. Independent psychological stress predictors were vehicle type, shift work, job satisfaction, and income. Stress scores were higher in work overload, role conflict, responsibility, and work environment in heavy vehicle drivers than light vehicle drivers ($p<0.001$), while this difference was not significant in terms of role insufficiency and ambiguity.

Conclusion: Nearly one-third of the drivers had mild to moderate stress level. Overall stress level was higher in heavy vehicle drivers than light vehicle drivers. The highest score in stress domains in all drivers attributed to the role insufficiency.

Keywords: driver, job, psychological stress

1. Introduction

Undoubtedly, stress is an integral part of today's life. On the basis of related literature, it is one of the main occupational hazards with numerous adverse effects on individual's health.

According to definition of the National Institute of Occupational Safety and Health (NIOSH), job stress occurs when there is no congruence between individuals' abilities and needs with their job demands (Hoel, Sparks, & Cooper, n. d.). Job stress has been investigated by many reseracgers (Haider et al., 2018; Hannani et al., 2018) and it not only imposes direct costs on individuals due to physical and emotional diseases and disrupts individual performance (LaDou & Harrison, 2014), but it also wastes millions of dollars of organizational costs via decreasing productivity and increasing absenteeism, accidents and insurance indemnities (Varmazyar, Mortazavi, Hajizadeh, & Arghami, 2013). Ironically, stress costs account for 1 to 3.5% of Gross Domestic Product (GDP) (Tangri, 2003). The cost of stress-related issues reaches 20 billion euros a year in the EU (KIZILOĞLU, 2018).

Numerous variables affect individuals' vulnerability to job stress; however, in order to manage this problem there is a need for higher emphasis on stressors of workplaces than individual susceptibility. Stressors of workplaces are mainly classified into the following five categories: 1- Organizational (such as organizational changes and communication problems), 2- career development (such as lack of promotion opportunity), 3- role of the individual (e.g. role conflict and role ambiguity), 4- job task (such as the level of responsibility and decision-making latitude), and 5-Work environment (such as physical and chemical exposures). (LaDou & Harrison, 2007)

Obviously, each job has its own stress characteristics. According to recent research, driving is among the stressful jobs. Driving requires skill, high concentration and accuracy, careful judgment and confidence in decision-making; therefore, increasing in drivers' stressors will end in accidents (Aminian, 2005). Some factors,

which may cause job stress in driving, are as follows: long working hours; irregular work schedules; even and repetitive work; inappropriate physical conditions; lack of job support; and familial and social constraints. (Aminian, 2005; Siedlecka, 2006)

Job stress increases driver's mental and cognitive fatigue and leads to impaired job quality. According to Simon and Corbett's theory, stress and anxiety do not allow drivers to follow traffic laws and this increases the incidence of fatal accidents and economical loss. (Vrijkotte, Van Doornen, & De Geus, 1999)

Considering the importance of the issue and since there are a few studies on different stress domains in professional drivers, and given different results in various studies, we decided to evaluate job stress in term of role stress and its domains in heavy vehicle and light vehicle drivers.

2. Method

The present study was a cross-sectional study conducted on professional drivers who were referred to an occupational medicine center for periodic examinations. Simple random sampling was used in this study. The required sample size was 627 based on the sample size formula in correlation studies:

$$z_{1-\pi/2} = 1.96$$

$$z_{1-\beta} = 1.28$$

$$r = 0.3)$$

$$n = \left\lceil \frac{1.96 + 1.28}{0.13} \right\rceil^2 + 3 = 627$$

The inclusion criteria were as follows: at least one year of work experience in professional driving and willingness to participate in the study. The exclusion criteria included a diagnosed anxiety disorder or history of consuming psychiatric drugs before initiating professional driving. Based on the inclusion criteria, 658 light and heavy vehicles drivers were selected to participate in the study. After considering the exclusion criteria, a total of 630 subjects were included in the study. 300 subjects were heavy vehicles drivers (bus, chemical tankers, trailer, truck, dump truck, light truck and other heavy vehicles) and 330 subjects were light vehicle drivers (taxi driver, agency driver, school service and company drivers). A two-part questionnaire was used in this study. The first part of the questionnaire included questions about demographic and occupational data of the subjects (age, education, marital status, smoking, regular exercise, type of vehicle, shift work pattern, monthly income level, rest duration (in hour), and job satisfaction). The second part covered Osipow job stress questionnaire (Osipow & Spokan, 1998). This 60-item questionnaire evaluated the individuals' roles in six domains including work overload, role ambiguity, role insufficiency, role conflict, responsibility and work environment. Each sub-group had 10 questions. Responses were based on five-point Likert scale and each one was scored from 1 to 5. Summing the scores, we put the individuals' total stress into one of the following four categories according to the questionnaire guide: mild stress (scores of 60-119), mild to moderate stress (171-120), moderate to severe stress (180-239), and severe stress (240-300). Validity and reliability of the questionnaire had been already investigated in Iran and the Cronbach's alpha coefficient was 83% (Sharifian, Aminian, Kiani, Baruni, & Amiri, 2005). It should be noted that the applied questionnaire of this study was completed by the participants under the supervision of a trained occupational hygiene expert.

Drivers were categorized into 3 groups according to their working shift pattern: 1) day-workers whose working hour was between 6 am to 6 pm, 2) night-workers whose working hour was between 6 pm to 6 am and 3) drivers with varied working shift pattern.

In this study monthly income was defined in 3 groups: less than 188$, 188 to 375$ and more than 375$.

Finally, collected data was statistically analyzed by SPSS-20 software. T-test, ANOVA, chi-square test and regression analysis were used to analyze data. Significance level was considered less than 5% (P-value<0.05) in all calculations.

3. Results

The present study evaluated 630 professional drivers including 300 heavy vehicles drivers (bus, trailer, chemical tankers, truck, light truck, dump truck and other heavy vehicles) and 330 light vehicle drivers (taxi driver, agency driver, school service and company drivers). All of the participants were male. 610 subjects (96.8%) were married. 600 subjects (95.3%) had high school diploma and lower degrees. 370 subjects (58.7%) had monthly income of less than 375$. In terms of shift work, 259 subjects (41.1%) were day-shift workers and 23 subjects (3.6%) were night-shift workers, and 347subjects (3.55%) had varied shifts. 357 subjects (56.6%) were smokers.

The mean age in heavy and light vehicle drivers was 44.33±6.6 and 37.5±5.5 years, respectively. According to the analysis, majority of the heavy vehicle drivers were older than or equal to 40 years of age, while only 37.9% of light vehicle drivers were in this age category (P<0.001.) There were significant statistical differences between two groups of drivers in terms of monthly income, cigarette smoking, and type of shift work, so that monthly income and smoking were higher in heavy vehicle drivers, and 82.3% of heavy vehicle drivers had varied working hours, whereas most of the light vehicle drivers (68.5%) were day-workers (P<0.001).

According to the study on job stress level in all drivers, most of the subjects (33.2%) had mild to moderate stress level. Frequency distribution of job stress level in two groups of drivers is shown in Figure 1.
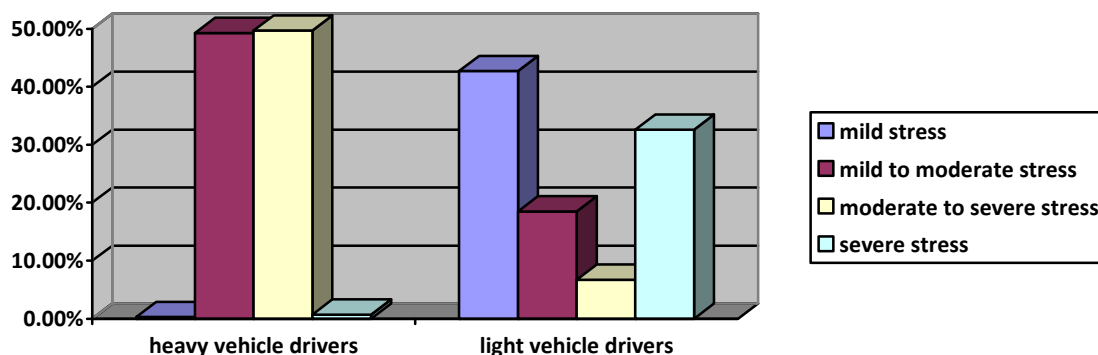
Figure 1. Job stress level in heavy and light vehicle drivers

Linear regression model was used to eliminate the effects of confounding variables on stress. Variables such as age, income, educational level, type of vehicle (heavy and light), rest duration, shift work, and job satisfaction were entered in the model. Based on the analysis (R square= 0.11, p≤0.001), independent stress predictors were as follows: vehicle type, shift work, job satisfaction, and income level. light vehicle driving reduced stress by 33 units compared to heavy vehicle driving (p≤ 0.001, CI 95%:19-41); income level of over 188 dollars increased stress by 33 units (p≤ 0.001, CI 95%:18-49), job dissatisfaction increased stress by 20 units (p≤ 0.001, CI 95%:11-28) and shift work and varied working shift pattern reduced stress 25 units compared to day-working (p≤ 0.001, CI = 15-35).

Given the low number of night-shift drivers (n=23) than day-shift drivers and those with varied working shift pattern, regression analysis was performed to compare day-working with varied working shift pattern. Based on the analysis, working in varied shifts decreases stress score by 25 units.

Table 1. Logistic regression model controlled for type of vehicle (heavy and light), age, education, income, varied shift, rest duration and job satisfaction

Variable	Standard Error	Beta	P-value	CI
Vehicle type	5.64	-0.29	≤ 0.001	-41.50 to -19.32
Age	0.38	-0.02	0.63	-0.94 to 0.57
Education	4.81	0.02	0.61	-7.03 to 11.88
Income level	8.03	0.17	≤ 0.001	18.07 to 49.62
Varied shift	5.18	-0.25	≤ 0.001	-35.81 to -15.46
Rest duration	4.61	0.06	0.12	-1.94 to 16.19
Job satisfaction	4.43	0.17	≤ 0.001	11.39 to 28.83

In assessment of stress categories in all drivers, the highest score was attributed to role insufficiency (29.86±9.29). In comparison between two groups of drivers, the mean stress scores were significantly higher in work load, role conflict, responsibility, and physical work environment as well as overall stress level in heavy vehicle drivers than light vehicle drivers (p<0.001), while this difference was not statistically significant in terms of role insufficiency and ambiguity.

According to the analysis of variance (ANOVA), there was a significant difference between total stress score in light vehicle drivers in terms of type of task (taxi, agency, school service and company drivers) (p<0.001). After performing Dunnett T3 test in Post hoc test, we found that taxi drivers had significantly higher stress than other light vehicle drivers (p<0.001, p<0.002, and p= 0.006, respectively). Post hoc test also indicated that agency drivers significantly had less stress than taxi and company drivers (p<0.001). Total stress score and its domains in light vehicle drivers are presented in Table 2.

Table 2. Comparison of stress score and its domains in light vehicle drivers*

Type of task	Total Stress Score	Workload	Role Ambiguity	Role Insufficiency	Role Conflict	Responsibility	Physical Environment
Taxi Driver	203.42±67.65	23.17±12.17	35.18±11.98	35.12±12.5	31.83±7.24	36.39±13.7	31.6±10.98
Agency Driver	130.63±38.42	19.48±7.34	24.35±6.75	23.68±6.75	24.64±4.87	20.13±8.09	18.45±6.43
School Driver	152.78±64.32	24.21±11.99	28.43±11.72	27.43±11.63	27.71±6.54	22.93±12.74	22.03±10.4
Company Driver	172.36±64.20	28.19±12.35	30.77±10.11	31.16±11.95	28.93±6.87	28.66±13.76	24.55±10.39
P- value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

*mean±SD

According to compared mean score of total stress in heavy vehicle drivers in terms of type of task (bus, chemical tanker, trailer, truck, and other heavy vehicles drivers), the ANOVA indicated that there were significant differences between these subgroups (p<0.001). According to Dunnett T3 in Post hoc test, we found that the chemical tanker drivers (fuel, solvents, pesticides, etc) and bus drivers had significant higher stress than others (p<0.001). Total stress score and its domains in heavy vehicle drivers are presented in Table 3.

Table 3. Comparison of stress score and its domains in heavy vehicle drivers*

Type of task	Total Stress Score	Workload	Role Ambiguity	Role Insufficiency	Role Conflict	Responsibility	Physical Environment
Chemical tanker driver	216.45±22.4	37.56±2.69	32.79±3.86	35.56±3.39	36±3.34	36.49±3.69	41.46±2.34
Bus driver	221.4±10.08	40.82±2.02	33.44±5.15	35.71±3.91	35.37±3.89	42.8±3.53	33.24±2.40
Tanker & trailer driver	157.11±21.54	29.71±5.9	27.22±4.36	27.88±4.39	28.86±4.26	28.87±5.85	32.50±2.53
others	166.35±10.97	27.66±6.73	26.33±2.71	26.46±3.24	28.4±4.46	25.66±2.19	31.66±1.83
P- value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

*mean±SD

4. Discussion

According to the results of this study, the total stress was moderate to severe in heavy- vehicle drivers, while it was mild to moderate in light vehicle drivers. This case is justifiable since job stressors are clearly higher in heavy vehicle drivers than the light vehicles. Higher responsibility for individual's life and capital in heavy-vehicle drivers is amongst these stressors.

44.3% of the whole participants had moderate to severe stress level. However, in a research by Aminian et al, 78% of public transport drivers had moderate to severe stress perhaps due to the differences in the studied population. (Aminian, Farjami, Pouryaghoob, & Sadeghniiat, 2011)

In terms of different domains of stress in all drivers, the highest score belonged to the role insufficiency. According to the definition of role insufficiency, individuals believe that their training, qualifications and experience do not fit their job requirements (Jackson, 2004). In fact, professional drivers are expected to show faster reaction time, regularly control their vehicles and carry out routine maintenance, and have minimum mental competence. For instance, they should remain calm in critical conditions and avoid aggressive behavior, but in Iran professional drivers are deprived such specialized training both before and during their career and they can only get benefit from their pioneers' experience.

In the present study, the mean score of total stress was significantly higher in chemical tanker (fuels, solvents, pesticides, etc) and bus drivers than truck and trailer drivers and the others (e.g light truck, dump truck, etc.) perhaps due to the importance of type of cargos.

Heavy vehicle drivers had higher stress scores in work load, role conflict, responsibility and physical work environment than light vehicle drivers; and the highest scores in role conflict and work environment belonged to chemical tanker drivers. Stressors of work environment include physical hazards (noise, heat, cold, and vibration), ergonomic problems, unpleasant odors and safety hazards. Certainly, the first two stressors are common in heavy-vehicle drivers, but safety hazards and unpleasant odors are considered more serious problems in chemical tanker drivers. According to a research by Krishnan et al on gas and oil tanker drivers, there was significant relationship between psychological factors including job stress and fatigue, which this might result in reducing drivers' efficiency and increasing traffic accidents. (Krishnan, Hizam, Firdhaus, Sarah, & Taufiq, 2017)

According to the previous studies, role conflict occurs when a person is in contradictory situations. Furthermore, it may come about when a person is dissatisfied with existing job expectations (Yongkang, Weixi, Yalin, Yipeng, & Liu, 2014). Those experiencing role conflict may gradually begin to believe that they are unable to successfully carry out their job tasks and expectations (Piko, 2006). Therefore, there is a need to pay special attention to the importance of adverse consequences of this issue especially in heavy vehicle drivers.

According to the analysis, the highest scores of work load, role insufficiency, role ambiguity and responsibility in heavy-vehicle drivers belonged to bus drivers. In other words, bus drivers believed that there was not any consistency between quantity and quality of expected occupational activities with their abilities and required time to do their job roles. Numerous studies have been conducted on job stress in bus drivers (Bergomi, Modenese, Ferretti, Ferrari, Licitra, Vivoli, Gobba, & Aggazzotti, 2017; Bathija, Bant, Itagimath, Lokare, Godbole, Nekar, Mahesh, & Kantesh Reddi, 2014; Roohi & Hayee, 2010; Du, Lin, Lu, & Tai, 2011; Yamada, Mizuno, Sugiura, Tanaka, Mizuno, Yanagiya, & Hirosawa, 2008; Kompier, Aust, Van den Berg, & Siegrist, 2000), despite the fact that these studies have not paid much attention to various subgroups and domains of job stress. According to all of these studies, bus driving is among the jobs with low control and high demand and includes significant psychological expectations due to the following reasons. A bus driver should be adapted to conflicting demands and expectations. Employer companies and people expect drivers to establish good relationships with passengers and provide good service. For instance, bus drivers are expected to provide necessary and sufficient information about departure time, routes and bus stop time for passengers and stop at certain intervals, but fulfillment of such expectations is often in conflict with obligations to keep an accurate schedule in heavy traffic. Driving should be also in accordance with driving rules and regulations. In addition, this group of professional drivers is faced with threats and violence by troublesome passengers. Bus drivers are often forced to work in varied shifts, and thus they have irregular rest days which do not match with family members' programs. Since bus drivers are responsible for passengers' safety and health, higher stress in this group of drivers is inevitable. In a research by Golmohammadi et al., 76% of bus drivers complained of high stress. Quality of individual relationship with supervisors and colleagues, amount of individual interest in his job and the work environment were among important stress-related factors in that study (Golmohamadi, Damyar, Mohamadfam, & Faradmal, 2013). In another research, one third of bus drivers suffered from intense stress. Based on results of this study, there was a significant relationship between stress with older age and varied shift work pattern (Varmazyar, Mortazavi, Hajizadeh, & Arghami, 2013). These results were consistent with the results of the present study.

According to the analysis on light vehicle drivers, total stress score was significantly higher in taxi drivers than others (agency, school service, and company drivers). Moreover, taxi drivers had the highest stress score in all stress domains. Conversely, the minimum stress domains scores were belonged to agency drivers. What has been observed in taxi drivers was probably due to the competition with other coworkers for picking up the passengers, dealing with different social classes during a working day, further use of the vehicle and thus higher depreciation and its imposed excess costs on taxi owners, longer exposure to urban traffic, and lack of specific working schedule and determined monthly income in taxi drivers. (B. Choi, S. Choi, Jeong, Lee, Shu, Yu, Ko, & Zhu,

2016; Wang & Delp, 2014; Apantaku-Onayemi, Baldyga, Amuwo, Adefuye, Mason, Mitchell, & Blumenthal, 2012; Chen, Chang, Chang, & Christiani, 2005; Hattori & Azami, 2001; Nakano et al., 1998)

The relatively high sample size and including different groups of professional light and heavy-vehicle drivers were among the strengths of the present study. Furthermore, a few studies have investigated different domains of stress in professional drivers so far.

This study was a cross-sectional study; thus, existence of a causal relationship could not be verified, though this is an inevitable issue in cross-sectional studies. Self-reported questionnaires might have led to bias in responses; however, there has not been any reliable objective method in this regard so far. It should be noted that our study was only conducted on professional male drivers. Since the majority of drivers in Iran are male, the studied sample probably represents the Iranian professional drivers' society.

5. Conclusion

According to the results of this study, nearly half of the participants had moderate to severe stress; and overall stress level was higher in heavy vehicle drivers than light vehicle drivers. In heavy vehicle drivers, the highest level of stress belonged to chemical tanker and bus drivers. Taxi drivers received the highest scores of stress among light vehicle drivers. Among various domains of stress in all drivers, the highest score belonged to the role insufficiency. Heavy vehicle drivers also received higher scores in work load, role conflict, responsibility, and physical work environment than light vehicle drivers.

According to previous studies and the present research, despite the proven adverse effects of psychological problems on drivers' performance and safety, this issue is still neglected in professional drivers, and unfortunately there is not enough attention to the origins of job stress and its management -especially in heavy vehicle drivers- in Iran. Therefore, health planners should pay greater attention to this issue and put tanker, bus and taxi drivers in the first priority for job stress management programs due to their higher stress levels in the case of limited resources.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Emergency Nurses Job Satisfaction Prediction Model: Personality traits, Resilience, Emotional Expression and Ambiguity Tolerance

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Abstract

Background: Nursing is considered as one of the most stressful jobs due to the emotional nature of the patient's demands, long working hours, professionals and interpersonal conflicts.

Aim: The purpose of present research was the study of job satisfaction predictors consisted personality traits, resilience, emotional expression and ambiguity tolerance of emergency nurses in Tehran hospitals.

Methods: This is a descriptive and correlational study. The population includes all emergency nurses in all hospitals of Tehran in 2017. The sample size consisted of 300 nurses who were selected randomly. For data collection were used NEO Personality Inventory, Berkeley Emotional Expression Questionnaire (BEQ), Job Satisfaction Survey (JSS), Resilience Scale (RS) and Ambiguity Tolerance Scale (ATS).

Results: The results demonstrate that ambiguity tolerance, resilience and emotional expression respectively had respectively the highest impact on job satisfaction, but personality traits had the lowest impact on it.

Implications for Practice: Personality traits can be a predictor of the job satisfaction of emergency nurses in hospital environment.

Keywords: Ambiguity Tolerance, Emotional Expression, Job Satisfaction, Nurses, Personality Resilience, Trait

1. Background

Nursing is considered as one of the most stressful jobs due to the emotional nature of the patient's demands, long working hours, professionals and interpersonal conflicts. Also nursing is under the constant social pressure. In recent decades, nurses' job satisfaction has received the attention of nursing researchers (Asri et al., 2017). Financial demands, psycho- political and human pressure in the caring field and increase workload of personnel all affect the nurses' mentality and professional performance and their job satisfaction. In recent years, the job satisfaction of the nurses has been affected by new healthcare technologies, budget cuts and change in the healthcare environment, increasing the stress in nurses (Khamisa, Peltzer, Ilic, & Oldenburg, 2017). Health systems around the world are facing with increasing challenges, growing health needs and economic constraints. Nursing shortage and the high level of nurses leave this profession is a global problem. Job dissatisfaction is one of the important factors in nurses leave this profession. Researchers report that the low level of job satisfaction of nurses is accompanied by the high rates of desire to change the job (Meeusen, Brown-Mahoney, van Dam, van Zundert, & Knape, 2010). Job satisfaction is positive attitudes and internal emotional reactions of a person toward his/her job that is depending on both the nature of the job and the individual's expectations of what the job should offer them (Nwafor, Immanel, & Obi-Nwosu, 2015). Job Satisfaction is an important predictor to keep the nurses in their careers (Chang, Li, Wu, & Wang, 2010). Nurses who have a high level of job satisfaction, they will likely stay at their job (Chang, Li, Wu, & Wang, 2010). According to the results of a study, job satisfaction of nurses is possible not by job rotation or wage increase but by strengthening friendship and co-operation in working environment (BAŞ et al., 2017).

Job satisfaction of Nurses is one of the most studied topics in occupational and organizational research. Most studies are being implemented on external predictor factors related to job satisfaction of nurses such as organizational perspectives, working conditions, nursing job characteristics, workplace relationships, work planning, rewards, promotion, responsibilities, and job security, also leadership style and Organizational policies.

But relatively little attention has been paid to the investigation of nurses' job satisfaction in terms of individual perspective (Chang, Li, Wu, & Wang, 2010). Study of individual characteristics of nurses such as personality traits, resilience and the method of emotional expression and tolerance of ambiguity can be used in identification of the individual situational factors involved in nursing job satisfaction.

Personality traits are features that generally are stable in individuals and distinguish them from others and effect on their behavior. The five-factor model of the personality traits with the experimental support is one of the most prestigious and prominent personality model (Ahmadi, 2012). Personality factors in the 5-factor model are as follows: Neuroticism involves a general desire to experience negative emotions, including fear, guilt, anxiety, and hatred and disorientation. Extraversion includes features like publicity, daring and decisiveness. Experiences include imagination of inner feelings, mental curiosity, diversity, and independent judgments. Openness to experience includes imagination about inner feelings, mental curiosity, novelty seeking, and independent judgments. Agreeableness includes altruism, a high tendency to help and sympathizing with others, moving toward people and accepting criticism. Conscientiousness, includes the ability to control impulses appropriate to the community demand, and delaying satisfaction of desire and observance the norms of society. (Ahmadi, 2012). Researches have indicated to the strong relationship between personality traits and job satisfaction among nurses (Judge, Heller, & Mount, 2002). It has been observed that people working in a similar stressful working environment report different levels of job satisfaction (Judge, Heller, & Mount, 2002). Specter 1997, quoted by Chang et al. (Judge, Heller, & Mount, 2002), argued that job satisfaction depends not only on the nature of the job but also on the perception and subjective assessment of the individual in which the person is working. In other words, the individual's personality can directly effect on how the work environment is interpreted and evaluated. Another variable in relation to job satisfaction in nurses is resilience (Hudgins, 2016). Resilience is the ability of successful adaptability, facing unpleasant situations (Benadé, du Plessis, & Koen, 2017). It also involves managing daily problems and making constructive changes to events, and positive adaptation in accordance to the problems that was experienced by a person (Fletcher & Sarkar, 2013).

Although this concept was originally developed in developmental psychology by Werner (quoted by Sefay, 2009; translated by Sadeghi et al. (Sefay, 2016)), but gradually entered other areas of psychology such as social and clinical psychology. Resilience includes a set of phenomena that, despite the serious threat to adaptation or development, are matched with good outcomes and results (Masten, 2001). Researches show that nursing students who have high resilience are more powerful in difficult and stressful conditions and show better response (Pines et al., 2014; Stephens, 2013; Taylor & Reyes, 2012). Hudgins (2016) research shows that there is a significant relationship between job satisfaction and nurses resiliency.

Another important variable related to job satisfaction in nurses can be how people respond to confusion or tolerance of ambiguity. The concept of tolerance of ambiguity has been widely discussed since 1940 by social cognitive theorists, but there is no definite agreement on the definition and how to evaluate it. Dominant thinking about tolerance of ambiguity returns to personality traits. The concept of tolerance of ambiguity has been widely studied outside the context of social cognition, including the clinical decision of nurses and their behavior (Knight, Kenny, & Endacott, 2016). Tolerance of ambiguity refers to the systematic and continuous tendency of individuals in respond to perceived ambiguity (more or less intensity) (McLain, Kefallonitis, & Armani, 2015)

Emotion is another variable that is related to the level and quality of job satisfaction of nurses. Emotions at any moment affect attention, decision making, memory, physiological responses, and social interactions, even a wide range of interpersonal and intrapersonal processes are influenced by emotions (Hasani & Bemani Yazdi, 2015). Emotion expressing is referred to as one of the main components of emotion, external emotion, regardless of value (positive or negative). Emotional expression includes behavioral changes with emotion such as facial, sound, gestures, and body movements changes (Shahgholian, Moradi, & Kafee, 2007). People who are emotionally more powerful compare to those who do not have the ability to understand and express emotional states are more successful with negative experiences and show more suitable adaptation in relation to the environment and others (Goleman, 1995). Andonian (2013) study shows that there is a positive correlation between emotional intelligence structures (emotional expression and personality traits) and the efficiency of nurses in the internship period. The researchers did not find a report that looked at the cognitive emotional and personality dimensions as a structural model of nursing job satisfaction through careful studies of the previous research. As Chang et al. (2010) indicate that relatively little attention has been paid to assessing the job satisfaction of nurses in terms of individual and personality perspectives.

2. Materials and Methods

This is a descriptive and correlational research and it's a quantitative study. The population consisted of all

nurses in the emergency department of Tehran hospitals in 2017. The sample was selected randomly from this population. The sample size was 300 nurses.

At first, a list of all public and private hospitals in Tehran was compiled. Next step, letters of request for consent to the research were sent to all hospitals in 2017. Among the hospitals, Firoozgar, Hafte Tir, Firozabad, Hazrat Rasool, Hasheminejad and Mutahari Hospitals of Tehran approved and responded and accepted the reasearch. Then, the approval questionnaires were reviewed at the Ethics Committee of hospitals. Among 500 nurses who were randomly invited to participate in the research, 350 accepted to participate in the research. After performing the research 50 questionnaires were excluded from the research process due to lack of complete response or inappropriate response, in the correction and grading phase. Finally, about 300 people's data were analyzed.

2.1 NEO Personality Inventory

In 1989, Costa and Mc Carey designed the short form of the five-factor Neo-personality questionnaire to measure five main factors (neuroticism, extroversion, experience, consistency, and conscientiousness) (McCrae and Costa, 1989; quoted by Muszadeh and Haji Alizadeh) (Mousazadeh & Haji Alizadeh, 2017). This questionnaire has 60 items and it merely measures the five main factors of the personality, while its long form, in addition to the five factors, also measures its thirty dimensions. Scoring of this questionnaire on a five-point scale Likert from totally disagree to fully agreement. Correlation between short form and long form is 0.75 for conscientiousness to 0.89 for neuroticism. Validity coefficient and re-testing of the scale of this questionnaire were obtained between 75% and 83% within three months (Ahmadi, 2012). In a research, the internal consistency coefficients for each factors of neuroticism, extraversion, experience, consistency and conscientiousness were reported to be 0.86, 0.73, 0.56, 0.86 and 0.87, respectively (Ahmadi, 2015). The re-test validity of this questionnaire is in a range from 0.86 to 0.90. As well; the internal consistency of the subscales has been reported from 0.74 to 0.89 (Mousazadeh & Haji Alizadeh, 2017).

2.2 Berkeley Emotional Expression Questionnaire (BEQ)

This questionnaire is a sixteen item- instrument that was developed by Gross and John (quoted from Narimani, Porsour and Basharpour) (Narimani, Porzour, & Basharpour, 2016) and expresses the amount of emotional expression in a 7-point Likert scale of 1 (completely disagree) to 7 (I fully agreement) Measures. The impulse intensity subscale consists of 6 items and measures the severity of emotional impulses. The negative expression subscale includes 6 items that assess the intensity of the negative emotions and the positive expression subscale includes 4 items that assess the intensity of the positive emotions. The whole questionnaire shows a high internal consistency and has good test- retest reliability. Convergent and divergent validity of each subscale of this questionnaire has also been well reported (Narimani, Porzour, & Basharpour, 2016) The scale of the Persian version of this questionnaire has shown a high correlation with the components of re- cognitive assessment and repression of the emotion regulation questionnaire (quoted by Basharpour et al. (2013)). In the study of Dobbs, Sloan and Karpinski (Dobbs, Sloan, & Karpinski, 2007), the Cronbach's alpha coefficient of each subscale of impulsive intensity, positive and negative expressions were 0.78, 0.63, 0.63 respectively and for the whole questionnaire 0.82 were obtained. Cronbach's alpha coefficient in this study was 0.79.

2.3 Job Satisfaction Survey (JSS)

This survey was developed by Spector in 1985. It is used to evaluate the attitudes of employees towards their jobs. This questionnaire was translated to Persian in 2001 by Chit Chin and was used in a research (Chit Chin, 2001; quoted by Bahadori Khosroshahiet al) (Khosroshahi Bahadori, Hashemi Nosratabadi, & Bayrami, 2013). It has 36 terms. Some of them are scored positively and others scored negatively. A positive term is a term that agreeing to it represents a job satisfaction. A nagative term is a term that agreeing to it represents job dissatisfaction (Khosroshahi Bahadori, Hashemi Nosratabadi, & Bayrami, 2013). In a research in order to ensure the accuracy of the translation carried out by Chit-chin (Khosroshahi Bahadori, Hashemi Nosratabadi, & Bayrami, 2013), translated Persian text into English and compared with the original questionnaire. He reported the Cronbach Alpha coefficient of 0.80 for this questionnaire. In Van Saaneet al research (van Saane, Sluiter, Verbeek, & Frings-Dresen, 2003) with using Cronbach's alpha, the internal consistency coefficient of this test was reported 0.91 and test retest 0.71 in 18 months. And in the research of Bahadori Khosroshahi et al. (Khosroshahi Bahadori, Hashemi Nosratabadi, & Bayrami, 2013), Cronbach's alpha was reported 0.86. Cronbach's alpha coefficient of this questionnaire was 0.83.

2.4 Resilience Scale

This scale was developed by Canner and Robinson (quoted by Zautra et al.) (Zautra, Johnson, & Davis, 2005) It is designed to test resilience (the ability to cope with stress). This scale has 25 items and the subject answers

each item on a 5-point Likert scale (from always false (0) to completely correct (Chang, Li, Wu, & Wang, 2010). High scores on this scale shows high resilience. The Cronbach's alpha coefficient has been reported 0.86 (Zautra, Johnson, & Davis, 2005). This scale was standardized in Iran by Mohammadi. To determine the validity of this scale, first the correlation of each item with the total score was calculated and then the analysis method was used. The calculation of the correlation of each score with the total score excluding 3 items showed coefficients between 0.41 and 0.44. Then, the items of the scale were subjected to factor analysis by principal component method. The KMO value was 0. 87 and the value of the chi square test in the Bartlett test was 5556.28, which showed two indicators of the adequacy of the evidence for the analysis of the factor. To determine the reliability of the Conor's self-resilience scale and Cronbach's alpha coefficient was 0.85 (Ahmadi & Sharifi Daramadi, 2015).

2.5 Ambiguity Tolerance Scale

This scale created by Simons & Gaher (Simons & Gaher, 2005). It is a self-reporting tool that contains 15 items in which subjects are asked to degree the extent of their acceptance or opposition with the sentences on a 5-point Likert scale (Khamisa, Peltzer, Ilic, & Oldenburg, 2017) to totally disagree (Ahmadi, 2012). The options of this scale will measure tolerance of ambiguity based on individual's ability to tolerate emotional disturbances, Mental impairment assessment, the amount of attention to negative emotions in case of occurrence and adjusting actions to relieve confusion. The disturbance tolerance with emotional disturbance ($r = -0.59$) and maladaptation ($r = -0.51$) has a negative relationship and has a significant positive relationship with positive excitement measures ($r = 0.26$). Cronbach's alpha coefficients have been reported for tolerance, evaluation, absorption and adjustment substitutions of 0.73, 0.84, 0.77 and 74 respectively (Simons & Gaher, 2005). In the research of Shams, Azizi and Mirzaie (Shams, Azizi, & Mirzaee, 2010), the Cronbach's alpha coefficient of this questionnaire was 0. 67 and the alpha coefficient of reliability were reported by test re-test method for the whole scale was 0.81. Cronbach's alpha coefficient in this study was 0.81.

3. Procedure

After being coordinated with Roozbeh Psychiatric Hospital and gaining the satisfaction of the participants, as well as identifying men with bipolar disorder, first aim of this study was explained to them and were asked them to read the questions carefully and choose the desired responses according to their own characteristics, and answer to questions as many as possible. Data were collected on an individual basis at related centers. Finally, the collected data were analyzed using Pearson correlation and multiple regression statistical techniques. Also, the ethical issues including ensuring the participants about the confidentiality of their information and freedom of choice for participation in the study project observed in this research.

4. Ethical Considerations

The research objectives were fully described for subjects. The consent was obtained for participation in the research (we agreed that the personal information of the subjects should remain confidential).

5. Results

To describe the research data, some descriptive statistics values are presented below.

Table 1. Results of descriptive statistics (mean and standard deviation) of research variables

	variables	Maximum	Mean	Standard Deviation
	Job Satisfaction	191	159.153	15.85
	Tolerance of ambiguity	75	54.156	5.464
	Resilience	104	73.21	14.41
	Extraversion	78	55.243	8.280
	Openness to experience	34	25.100	3.248
Personality trait	Agreeableness	41	32.456	4.159
	Neuroticism	47	34.893	4.784
	Conscientiousness	39	27.220	3.859
Emotional expression	Expressive	49	35.873	4.944
	Impulsive	66	45.933	10.018

Table 2. One-way variance ANOVA analysis of variables based on gender

	Observed Variables	F	Significancy	result
	Job Satisfaction	2.433	0.120	Meaningless difference
	Tolerance of ambiguity	0.092	0.762	Meaningless difference
	Resilience	2.727	0.100	Meaningless difference
	Extraversion	5.754	0.017	significant difference
	Openness to experience	6.633	0.010	significant difference
Personality trait	Agreeableness	11.991	0.001	significant difference
	Neuroticism	9.105	0.003	significant difference
	Conscientiousness	1.585	0.209	Meaningless difference
Emotional expression	Expressive	2.709	0.101	Meaningless difference
	Impulsive	1.029	0.311	Meaningless difference

According to the results of the above table, the significance levels of the test for all variables are higher than the significance level (0.05). Consequently, in this dimension, the gender is estimated to be the same, and the assumption of the test based on the difference in variables is not accepted by the 95% confidence level. Except extraversion, Openness to experience, agreeableness and Conscientiousness whose significance level is less than (0.05). The result is not the same in terms of gender, and the assumption of the test based on the difference of extraversion, Openness to experience, agreeableness, and Conscientiousness by gender is accepted confidently 95%.

Table 3. One-way variance analysis ANOVA Research variables based on work shift

	Observed Variables	F	Significancy	result
	Job Satisfaction	7.504	0.007	significant difference
	Tolerance of ambiguity	11.055	0.001	Meaningless difference
	Resilience	1.614	0.205	significant difference
	Extraversion	11.680	0.001	significant difference
	Openness to experience	2.271	0.133	Meaningless difference
Personality trait	Agreeableness	0.215	0.643	Meaningless difference
	Neuroticism	17.781	0.000	significant difference
	Conscientiousness	0.435	0.510	significant difference
Emotional expression	Expressive	0.017	0.896	Meaningless difference
	Impulsive	2.849	0.092	Meaningless difference

According to the results of the above table, the significance levels of the test for all variables are higher than the significance level (0.05). Consequently, in this dimension, the work shift is estimated to be the same, and the assumption of the test based on the difference in variables is not accepted in a 95% confidence interval, except job satisfaction, tolerance of ambiguity, extraversion, Conscientiousness a significant level of less than (0.05). As a result, the work shift is not the same, and the assumption of the test based on the difference in job satisfaction, tolerance of ambiguity, extraversion, and Conscientiousness is based on a 95% confidence.

Figure 1 shows the estimated standard coefficients for the variables. Job Satisfaction was considered as dependent variable and tolerance of ambiguity, personality traits, emotional expression and resilience were considered as independent variables. Standardized coefficients show that tolerance of ambiguity, resilience and emotional expression have the highest impact and ultimately personality traits have the least impact on job satisfaction. The value of the determination coefficient for the model is below 0.76. In other words, 76 percent of job satisfaction is expressed by personality factors.
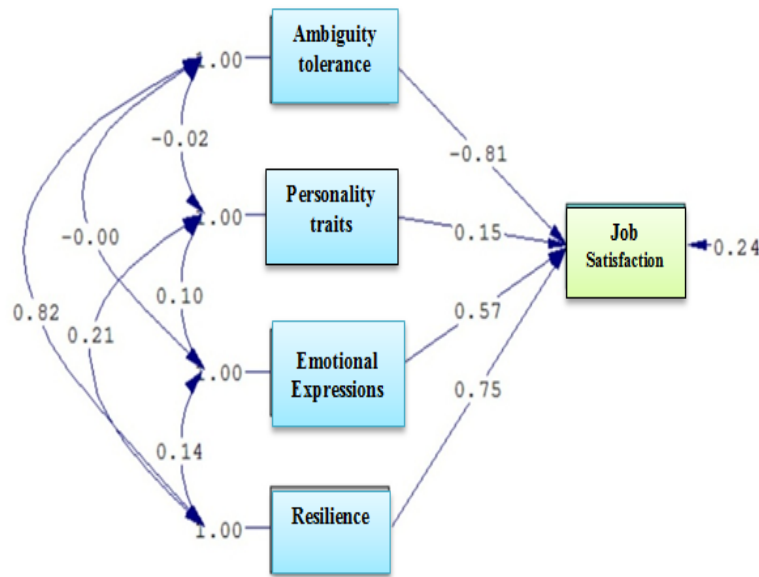
Figure 1. The model for estimating standard coefficients for variables was observed
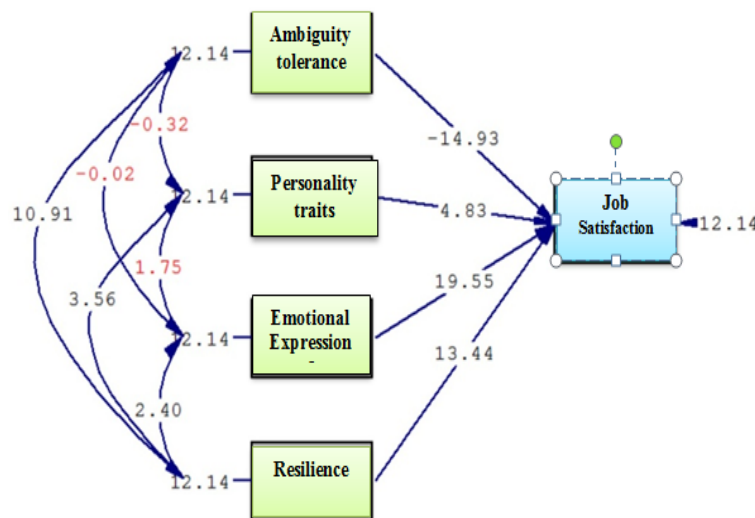
Figure 2. Test model Estimates of standard coefficients for observed variables

Also Pearson correlation coefficients between the observed variables have been tested. Regarding the significance level, linear relationship between resilience, tolerance of ambiguity, personality traits and emotional expression with job satisfaction variable was significant at 99% confidence level. The linear relationship between resilience and emotional expression, tolerance of ambiguity, emotional expression and personality traits, and emotional expression were at the 95% confidence level (meaningful less than 0.05).

6. Discussion

The purpose of this study was to study the predictors of job satisfaction among nurses in the emergency unit. The results of the standard estimation test showed that the tolerance of ambiguity, resilience and emotional expression had the highest impact and personality traits had the least impact on job satisfaction. The value of the determination coefficient for the model is below 0.76. In other words, 76 percent of job satisfaction is expressed by the above factors. This finding is in line with previous studies such as Wittenberg and Norcross (Wittenberg & Norcross, 2001), Hudgins (2016) and Chang et al. (2010).

Researchers have identified and studied many factors as predictors of job satisfaction among nurses, most of

these factors emphasize on nursing work status. Because nursing is associated with physical and emotional aspects of working environments. In other words, the environment and situation in which the nurse is placed needs some of the characteristics and personality traits that predict their job satisfaction. One of the predictors of job satisfaction is the ability of the individual to respond in a systematic and sustainable way to ambiguity in the workplace. In stressful working environments such as the hospital and the emergency department, a nurse can have a good performance when s/he is prepared to deal with and manage critical and unpredictable situations; and on the other hand, show high resilience facing with of different conditions (Benadé, du Plessis, & Koen, 2017). Hudgins (2016) showed that there is a significant relationship between job satisfaction and resilience of nurses. Persons who are emotionally and capable and capable of expressing it will be more successful in coping with the ahead of challenges (Dobewall, Aavik, Konstabel, Schwartz, & Realo, 2014). Individuals who are emotionally empowered recognize their feelings, understand implicit concepts, and more effectively express their emotional states to others. Therefore, emotional expressiveness can affect the individual's job satisfaction. As in the present research, it was found that there was a significant relationship between emotional expressiveness and job satisfaction among nurses. In other words, expressing emotions in time, place and position can lead to positive outcomes (Rahdari, Modarres Gharavi, & Tabibi, 2010).

As well, in the present research model it was found that personality traits (although low impact) can effect on job satisfaction. Judge et al. in their research in 2002 pointed to the relationship between personality traits and job satisfaction among nurses. Person who have more neurotic traits than other personality traits have a lot of emotional and cognitive problems, therefore have many shortcomings and tensions in interpersonal, social and occupational environments. In other words, with the increase in neuroticism, the active interaction with the environment decreases and the individual loses some of its supporting resources (Ahmadi, 2015). Therefore, nurses with higher neurotic symptoms will probably have more negative emotions. As a result, the person's tendency to openness to experience and to get into new situations will be reduced and this creates a situation of tension due to dissatisfaction with the current situation and the lack of a change in the present situation. In other words, the existence of some negative personality traits can lead to negative emotional expressions, which can be attributed to the low level of job satisfaction in the individual. The limitations of the present study were to conduct research in the private hospitals. Because working conditions in government hospitals is different in terms of salaries and benefits, etc. This is due to the lack of cooperation of state hospitals in participating in the research process. Also, the conditions and facilities of Tehran hospitals are very different from the other cities; generalizing these events to the whole society should be considered cautiously. The lack of investigation of the subjects' mental status during the conduct of research was one of the other limitations of this study. Therefore, it is suggested that in future studies governmental hospitals be investigated, and studies should be done in other provinces and cities.

Implications for Practice

Considering the findings of this study, it seems that, the nursing profession is associated with the physical and emotional aspects of working conditions, consideration of the environment, position and personality traits of nurses in accordance with the work situation can be they are important in their job satisfaction. This can be more important, especially for the nurses working in the emergency unit in the hospitals, and experiencing more difficult and critical situations.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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The Effect of One Session Acute Exercise on the Urinary Excretion of Urinary Gamma-Glutamyl Transferase, Protein and Creatinine Levels of Elite Football Players

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Abstract

The aim of the present study was to evaluate the effect of one session acute exercise on the urinary excretion of urinary Gamma-glutamyl transferase, protein and creatinine levels in elite football players. A total of 30 Premier League football players with a mean age (26.1 ± 3.80), a mean height (180.01 ± 7.39), a mean weight (78.6 ± 9.26) and a mean body mass index (24.87 ± 1.13) were voluntarily and purposefully selected as statistical samples. The urinary sample of the players was collected in two stages. In the first stage, the players' urinary samples were taken on the rest day when they had not exercised for 24 hours. In the second stage, it was taken immediately after exercise and transferred to the laboratory. To test the hypotheses, especially to compare urinary protein, creatinine, and gamma-glutamyl transferase levels before and after exercise, and to compare excretion levels of protein, creatinine, and gamma-glutamyl transferase between the positions, ANOVA test was used. There was a significant difference between the levels of gamma-glutamyl transferase, proteinuria and creatinine before and after exercise (P <0.001). There was a significant difference between the results of level of excretion of protein, creatinine and gamma-glutamyl transferase among different positions (P <0.001). In addition, LSD post hoc test was used to compare the pairwise scores of the variables among the positions.

In general, the results of numerous studies suggested that long-term and high-intensity exercise increases the levels of urinary protein and creatinine more than normal level. If it continues for a long time, it can cause irreparable injury to the kidney tissue. In addition, it seems that gamma-glutamyl transferase enzyme can be more affected by exercise intensity and events of exercise and competition.

Keywords: Gamma-Glutamyl Transferase, Protein, Urine, Creatinine

1. Introduction

Physical activity is extensively constructive for every single individual (Ibrahim et al., 2018). Resisted exercise is effective in optimal health (Mady et al., 2018). Physical activities affect different body systems and lead to their adaptation to the specific needs of the organs during physical activity. Muscles, cardiovascular systems, respiratory system, kidneys are the organs whose function changes during physical activity. Components of physical activity arlead to cardiorespiratory fitness, muscle strength, flexibility, agility, and muscle endurance (Zaeimi Fard et al., 2017). The kidneys are vital organs that are important in controlling volume, osmotic pressure, electrolyte content, and the stability of the internal environment of the body. Thus, it is not surprising that kidney dysfunction affects almost the function of other body systems. Hence, recognizing the interactions between exercise and the kidneys is crucial to fully understanding how physical activity works. Recognizing these two effects is also important for recognizing their control mechanisms for designing and setting exercise programs and the type of exercise activity. Exercises can cause dramatic changes in renal hemodynamics and electrolytes.

Various factors are involved in the occurrence of these renal hemodynamic changes, one of which is ischemia, caused by the redistribution of blood during exercise and its abnormal circulation to the active muscles (Poortman, 1984). Based on the results of the Poortman et al. (1988), reduced kidney blood flow is accompanied

by narrowing of the arteries by epinephrine and norepinephrine hormones during exercise, which can have continuous effects on the ratio of filtration. In this regard, lack of using fluids during exercise can reduce the kidney blood flow below the normal level and reduced glomerular filtration rate can cause acute tubular necrosis (Harman et al., 2003). Investigation of urinary proteinuria, creatinine and gamma-glutamyl transferase enzymes play major roles in the prognosis of kidney tissue injury. Studies conducted in recent years have shown that an increase in these three indices after prolonged and high-intensity exercise can be a sign of injury to the parts of the kidney tissue. Ayca et al. (2006) in a study on 12 male and 12 female Volleyball players showed that urinary creatinine and protein increased in male players. However, only urinary protein increased significantly in female players. In this article, the level of excretion of urinary gamma-glutamyl transferase enzyme increased only in male spiker players after exercise and this difference was significant, while there was no significant difference in other positions. Ayca attributes this increase to gamma-glutamyl transferase caused by high-intensity exercise and jumps in spiker players. In 2003, Turgut studied the effects of exercise on protein, insulin-like growth factor IGF1 and insulin-like growth factor-binding factor (IGFB-3) in the urine of volleyball players. Results of the study showed significant increase in urinary protein and creatinine and IGF, while significant increase was not seen in IGFB-3.

Nowadays, one of the most important concerns of professional athletes who get engaged in various sports activities is the possibility of various disorders and failures in various body systems, including immune, respiratory, renal systems, etc. Numerous uncertainties with regard to kidney function during exercise require conducting further research to answer existing questions. Football game requires the ability to withstand over 90 minutes of varied and high-intensity physical effort. Nowadays, professional football players travel an average of 10 kilometers in a match, whose 90% of consumed energy is supplied by aerobic metabolism. Moreover, unlike most sports, football players often have to participate in different club or national competitions 2 or even 3 times a week and repeat exercise sessions, because lack of receiving adequate energy put the player at the risk of reduced performance and kidney injury (Bangsbo, 1991).

Although it has been stated that exercise have beneficial effects on people's health, research findings in recent years have shown that professional exercise and consequently heavy and continuous exercises can cause problems for professional athletes. Repeated high-intensity exercise sessions, reduced kidney blood flow, poor recovery, dehydration, and the blows imposed on the athlete are some of the factors that can cause kidney tissue injury in professional athletes (Poortmans et al., 1988). Kidney tissue injury is associated with physiological symptoms. These physiological indicators change based on the exercise pressures. The kidneys are responsible for clearing some of the waste and toxins caused by metabolism. Kidney function tests evaluate the excretion power of these substances by the kidney. To measure kidney excretion power, the concentration of certain substances in the blood and urine is often measured. In this regard, changes in urinary excretion of creatinine, protein, gamma-glutamyl transferase play a major role in the prognosis of kidney tissue injury. If the urinary excretion of these three indicators increases after the exercise, it can be stated that the kidney tissue of athletes are at risk of injury. One of the most important symptoms that indicate kidney injury or disease is increased protein excretion in the urine after exercise or proteinuria. The level of protein secreted in the urine has clinically high importance (Dilena, 1983).

The term "proteinuria" is an indicator that indicates urinary protein excretion is higher than normal level. The normal level of proteinuria in healthy individuals is 150 mg/day or 10 mg/liter (Bergstein, 1999). Two major mechanisms can cause proteinuria, including increased glomerular secretion with no change in tubular reabsorption and defects in the reabsorption of filtered protein from tubular (Poortmans, 1985). Proteinuria is a term indicating that urinary protein is excreted above normal level. Increased protein excretion can be a result of increased glomerular permeability and defect in tubular reabsorption of plasma proteins (Poortmans, 1985). Factors such as changes in the hormonal system, reduced kidney blood flow, dehydration, and reduced glomerular electrostatic barrier cause proteinuria. Therefore, excessive protein excretion in urine after high-intensity and prolonged activity is a sign of impaired kidney tissue. Acute kidney failure is a syndrome that can be defined as a sudden reduction in kidney function leading into the retention of nitrogenous waste materials (such as nitrogen, blood urea, and creatinine) in the body. Urinary creatinine is a byproduct of the degradation of creatine and phosphocreatine, found more in muscles. It turns into creatinine with loss of water and flows into bloodstream. Kidneys also excrete it through urine. Its excretion is relatively stable under normal conditions. This level of produced creatinine is proportional to the muscle mass of the person. The level of creatinine in the urine for adults is 15 to 25 mg per day. Determining the level of urine creatinine is used to diagnose kidney and muscle diseases, and its estimation can determine the extent of kidney injury (Alissio et al., 1985; Rieh et al., 2004). The gamma-glutamyl transferase is a gamma glutamyl cycle enzyme involved in glutathione and

degradation and restoration (Nemesanzkj et al., 1985). The highest activity of this enzyme is seen in the kidney tissue, which is mainly located on the luminal surface of the epithelial cell membrane. It seems that gamma-glutamyl transferase plays an important role in plasma glutathione excretion (Tate et al., 1985; Zie et al., 1980). Gamma-glutamyl transferase is one of the kidney enzymes located mainly on the luminal surface of the proximal membrane of the tubular epithelial cells. To measure gamma-glutamyl transferase, Pars test kit manufactured by Iran and BT3000 device were used. There has not been much research on kidney disorders in exercise so far. Much research has not been conducted on the kidney disorders in exercise so far. Obviously, the results of such studies can provide useful guidelines for trainers and coaches to plan the type of physical activity, intensity and duration of activity, nutrition and dietary supplements, antioxidants, fluid and electrolyte intake during exercise and before and after the match. Thus, given the importance of kidney tissue, it seems to be necessary to conduct research in order to identify the factors affecting the increase of the mentioned variables, each of which somehow indicates some injuries to the kidney tissue. In the present article, it was aimed to compare urinary protein, creatinine and gamma-glutamyl transferase concentrations as indicators for prognosis of some of the kidney injuries in Iranian football Premier League players who were under pressure of exercises and matches and performed two high-intensity exercises during the week.

2. Methodology

The present study aimed to compare the level of changes in urinary excretion of protein, creatinine, and gamma-glutamyl transferase 24 hours before and immediately after one session of acute exercise. Therefore, the research method in this quasi-experimental study was an applied method. The research population included all players in the Iranian Premier League. In the present study, due to the difficulties in coordinating with the football teams competing in the Premier League and considering the goals of the researcher, a total of 30 players in the Iranian Football League in different positions (goalkeeper, defender, midfielder, and forward) were selected as samples of the study using convenient sampling method. The independent variable included high-intensity exercises of teams (aerobic exercise with 85-75% of maximal oxygen consumption for 30 minutes and anaerobic exercises including lactic exercises such as 30-40m, 200m, 300m sprints, and explosive exercises, in which the heart rate is above 170 beats per minute and the exercises lasts for 30 minutes). The characteristics of the exercises have been presented in the protocol section of each exercise. The dependent variables in this study included changes in urine protein, creatinine, and gamma-glutamyl transferase levels. First, players' height and weight were measured. Then, their body mass index (BMI) (squared height (m)/weight (kg) = BMI) was calculated to ensure that players' height and weight were measured. The weight of players was measured three times during the day and the average of them was recorded. To measure the height of the subjects, a height meter with a precision of 0.5cm was used. To measure the weight of subjects, the SECA220 balance scale with a precision of 0.5kg was used. After recording the height and weight of the subjects, their body mass index was also calculated and recorded. The maximal aerobic power test was performed using the Bruce protocol on the treadmill. The Bruce Test or the Bruce Protocol is one of the most commonly used tests for assessing the ability of the cardiovascular system. It was developed by Bruce in 1973 and used widely in medicine under the title of "exercise testing for assessing the heart health." The tool was a treadmill with adjustable slope and speed and timer. The Bruce test is performed on a treadmill and has seven stages. At the beginning of the test, the person usually walks on the treadmill and he or she starts walking fast by increasing the slope and speed from third to fourth stages. The, he or she starts running if be able to continue. Each stage of the Bruce test takes 3 minutes. Table 1 shows the slope and speed of the treadmill. In the Bruce Maximal test, it is rare for a person to continue to work until the sixth and seventh stages, and only endurance runners show such an ability. The activity stops when the person becomes very tired and unable to continue it. Finally, activity time and heart rates are recorded. The maximal oxygen uptake rate is ml/kg of body weight per minute.

Table 1. Slope and speed in Bruce Maximal Test

stage*	Slope (%)	Speed		
		Miles/h	Km/h	m/min
1	10	7.1	7.2	45
2	12	5.2	4	67
3	14	4.3	5.5	92
4	16	2.4	8.6	113
5	18	5	8	133
6	20	5.5	8.8	147

7	22	6	6.9	160

According to an amendment made by Shepherd in 1985, the Bruce sub-maximal test was designed to stop activity when the subject's heart rate reached 150 beats per minute. Usually, an athlete stops in the third or fourth stage and a non-athlete stops at the end of the second or third stage. The exercise protocol consists of 7 to 10 minutes of general exercise, warm-up and stretching exercises, and 30 minutes of aerobic running (on track) with 75 to 85% of maximal oxygen consumption followed by 40 minutes of high-intensity anaerobic exercises, including lactic exercises such as 30 to 40 m, 200 m, 300 m sprints and explosive exercises at a rate above 170 beats per minute. It should be noted that 30 players were measured by pulse counter to determine the intensity of the heart rate exercise. Finally, the players will play 40 minutes of high-pressure football on standard football field.

Table 2. Football players' exercise program

Type of exercise	Duration of exercise
Warm-up (general exercises and stretching exercises)	7-10 min
Aerobic exercises (aerobic Running with 85-75% maximal oxygen consumption)	30 min
anaerobic exercises, including lactic exercises such as 30 to 40 m, 200 m, 300-m sprints and explosive exercises	40 min
Play football	35 min

The urine sample of players have been collected in two stages. In the first stage, players' urine sample was taken after 24 hours without doing exercise. In the second stage, it was taken immediately after exercise. High-intensive exercise causes hemodynamic changes in the kidney and increases protein excretion (Poortmans, 1994). This increase in protein excretion returns to normal level 24 to 48 hours after exercising. At each stage, the players were asked to excrete some of their urine and then their urine was collected in a tube used specifically for sampling. To measure the creatinine gamma-glutamyl transferase and urinary protein of athletes before and after the exercise session, the Pars Azmoon Kit (manufactured by Iran) and 3000 BT device were used. The laboratory methods used to measure creatinine, protein, and GGT were Jaffe, sedimentary and enzymatic, respectively. After completing the sampling and presenting the results of the urine test by the laboratory, the information obtained for statistical analysis were recorded in SPSS software. After collection of data, they were entered into SPSS, version 15, software. They were analyzed at descriptive and inferential levels. At descriptive levels, indicators such as mean deviation was used. In the inferential statistics section, to test hypotheses and especially to compare changes in urinary gamma-glutamyl transferase before and after exercise and to compare protein, creatinine, and gamma-glutamyl transferase levels between different positions, ANOVA test was used. To use these statistical methods, the data heuristic analysis was first performed to determine outliers and finite values. Then, Kolmogorov-Smirnov test was utilized to examine the normality of the data distribution. Moreover, for pairwise comparison of scores of variables in different positions, LSD post hoc test was used. Minimum level of significance was considered 0.05 in testing the relevant hypotheses and the statistical power of acceptance was considered 80%.

3. Results

Mean concentration of proteinuria, creatinine and gamma-glutamyl transferase enzyme excretion are assessed before and after exercise in general. Then, based on different playing positions ANOVA test and post-test LSD test results were used.

Table 3. Physical and physiological characteristics of the subjects

n	Age (year)	Height (cm)	Weight (kg)	BMI (kg / m 2)	Aerobic power (mm / kg/ min)
30	80.3 ± 1.26	39.01 ± 7.180	26.6 ± 9.78	13.87 ± 1.24	77.08 ± 2.49

Table 3 presents the mean and standard deviation of age, height, weight, body mass index and maximal aerobic power of the players. The subjects of the study were 22 football players who voluntarily participated in the study. The members of the sample group in this study had a mean age (26.1 ± 3.80), a mean height (180.01 ± 7.39), a mean weight (78.6 ± 9.26) and a mean body mass index (24.87 ± 1.13) and a maximal aerobic power 49.08

± 2.80. All subjects had good health and did not report any disease. Based on the significance level of Kolmogorov-Smirnov test, data of all the variables of physiological and biochemical characteristics had a normal distribution. Therefore, parametric tests can be used for analyzing the data.

Table 4. Kolmogorov-Smirnov test results in the studied variables

variable	Kolmogorov-Smirnov test	significance
Proteinuria	777.0	58.0
Creatinine	743.0	64.0
gamma-glutamyl transferase	509.0	96.0

Table 4 compares the level of urinary protein concentration before and after acute exercise using ANOVA test.

Table 5. Intra-group effects` comparison test of urinary protein variable before and after acute exercise

Statistical test	Sum of squares the third type	df	Mean squares	Coefficient f	significance	squares	Statistical power
Urinary protein	669.5203	1	669.5203	909.143	001.0	770.0	00.1
error	856.1554	43	159.361				

Table 5 shows the intra-group effects in the variable of urinary protein before and after acute exercise using a standard univariate test (p>0.05). The level of effect of acute exercise on urinary protein has been shown in first row of Table 4-3. 0.770 of total variance of urine protein in subjects was due to the effect of acute exercise. The statistical power in testing this hypothesis was 0.001 and the discriminative power of significant differences in this analysis was 100%.

Table 6. Intragroup effects` comparison test of urinary creatinine variable before and after acute exercise

Statistical test	Sum of squares the third type	df	Mean squares	Coefficient f	significance	squares	Statistical power
urinary creatinine	636.2795	1	191.5958	064.161	001.0	789.0	00.1
error	364.746	43	357.17				

Table 7. Intragroup effects` comparison test of gamma-glutamyl transferase variable before and after acute exercise

Statistical test	Sum of squares the third type	df	Mean squares	Coefficient f	significance	squares	Statistical power
gamma-glutamyl transferase	416.90	1	416.90	002.78	001.0	645.0	00.1
error	841.49	43	59.1				

Table 8. Mean and standard deviation of urinary protein before and after exercise separately for different positions

Variable	position	n	mean	SD
Protein before exercise	Defender	7	27.59	28.17
	Midfielder	8	17.70	63.14
	forward	9	13.56	39.10
	Goalkeeper	6	46.53	25.8
	total	30	71.60	67.14
Protein after exercise	Defender	7	18.78	91.16
	Midfielder	8	10.91	93.13
	forward	9	40.64	02.7
	Goalkeeper	6	67.64	50.6
	total	30	09.76	57.16

The real effect of acute exercise on urinary gamma-glutamyl transferase has been presented in the first row of the table above, which was equal to 0.645 in this variable. It means that 0.64% of the variance of urinary gamma-glutamyl transferase in subjects was due to the effect of acute exercise. The statistical power in testing this hypothesis was 0.001 and the discriminative power of significant differences in this analysis was 100%.

Based on the findings, the protein excretion in midfielders seemed to be higher than that in other positions at both times. The level of real effect before and after acute exercise on urinary protein was 0.344, meaning that 34% of the total variance of urinary protein levels among the subjects of different positions was due to the effect of acute exercise. The statistical power in testing this hypothesis was 0.968 and the significant discriminative power of this analysis was 0.97. In general, the results are as follows:

1. There was a significant difference between different positions in terms of urinary protein before and after exercise (p<0.001),

2. There was only a significant difference between midfield position and other positions in terms of urinary protein in post-test.

Table 9. LSD post-hoc test for the comparison of urinary protein in different positions

Compared positions			Mean significant difference
Midfielder	Defender	0.02	-11.91
Defender	Midfielder	0.02	11.91
Forward	Midfielder	0.001	20.37
	Goalkeeper	0.001	21.57
Midfielder	Forward	0.001	-20.37
Midfielder	goalkeeper	0.001	-21.57

There was only a significant difference between the midfielder and other positions in terms of urine protein (p<0.05). Results showed that urinary creatinine was significantly different in different positions. The real effect of pre- and post-acute exercise on urinary creatinine in the chi-square has been shown in first row of table, which was equal to 0.355 in this variable. It means that 36% of the total variance in urinary creatinine levels among the subjects of different positions was due to the effect of acute exercises. The statistical power of testing this hypothesis was 0.975 and the discriminative power of significant differences in this analysis was 0.98. In general, the results showed that there was a significant difference between urinary creatinine before and after exercises (p<0.01). A significant difference was seen only between goalkeeper and other positions and between midfielder and forward in post-test in terms of urine creatinine (p<0.05). The results showed that only the goalkeeper showed significant difference with other positions and midfielder and forward showed significant difference with other positions in post-test in terms of creatinine level (p<0.05).

Table 10. Mean and standard deviation of urinary gamma-glutamyl transferase before and after exercise separately for different positions

variable	position	n	mean	SD
	defender	7	4.72	1.43
	midfielder	8	4.31	10.02
Creatinine before exercise	forward	9	4.23	0.91
	goalkeeper	6	4.66	1.00
	total	30	4.46	1.06
	defender	7	6.27	2.24
	midfielder	8	5.98	1.50
Creatinine after exercise	forward	9	5.95	1.11
	goalkeeper	6	8.71	1.24
	total	30	6.48	1.85

The real effect of pre and post-acute exercise on urinary gamma-glutamyl transferase was 0.185. It means that 18% of the total variance in the urine creatinine level of the subjects in different positions was due to the effect of acute exercises. The statistical power of testing this hypothesis was 0.669 and the discriminative power of

significant differences in this analysis was 0.67. In general, there was a significant difference between urinary gamma-glutamyl transferase before and after in different positions. Only urinary gamma-glutamyl transferase of the goalkeeper position showed a significant difference with that of other position in the post-test (p<0.05).

Table 11. LSD post-hoc test for comparison of urinary gamma-glutamyl transferase at different positions

Compared positions			Mean Significant Difference
Goalkeeper	Defender	0.046	-1.91
Goalkeeper	Midfielder	0.01	-1.54
Goalkeeper	Midfielder	0.009	-1.59
Defender		0.046	1.19
Midfielder	Goalkeeper	0.01	1.54
Forward		0.009	1.59

Only a significant difference was observed between the urinary gamma -glutamyl transferase in goalkeeper position and that of other positions in the post-test (p<0.05).

Table 12. The significant results of the present study

	Significant level	Result (x=significance)
Proteinuria	$p \leq 0/001$	x
Urinary creatinine	$p \leq 0/001$	x
Gamma-glutamyl transferase	$p \leq 0/001$	x
Proteinuria in positions	$p \leq 0/001$	x
Creatinine in different positions	$p \leq 0/001$	x
Gamma-glutamyl transferase	$p \leq 0/04$	x

4. Discussion and Conclusion

Although it has been stated that exercise has good effects on people's health, research has shown in recent years that professional exercise and consequently high-intensive and continues exercise can cause problems for professional athletes. Repeated high-intensity exercise sessions, decreased kidney blood flow, inadequate recovery, dehydration, and blows imposed to athletes are some of the factors that can cause kidney tissue injury in professional athletes. Kidney tissue injury is associated with physiological symptoms.

These physiological indicators change based on the exercise pressures. The kidneys are responsible for the cleansing of some of the waste substances and toxins resulting from metabolism. Kidney function tests evaluate the ability of the kidneys to excrete these substances. The concentration of certain substances in the blood and urine is often measured to assess the kidney excretion power. Changes in urinary excretion of protein, creatinine and gamma-glutamyl transferase enzymes play a major role in the prognosis of kidney tissue injury.

The hypotheses of the present study included:

1-There is a significant difference between proteinuria levels before and after acute exercise.

2-There is a significant difference between creatinine levels before and after acute exercise.

3-There is a significant difference between the levels of gamma-glutamyl transferase before and after acute exercise.

4-There is a significant difference between the levels of gamma glutamyl transferase, creatinine and urinary protein in different positions. To test the hypotheses, 30 Iranian Premier League football players were selected purposefully using convenient sampling method. Variables of body height, weight, body mass index, maximal aerobic power and urinary protein concentration, urinary creatinine and gamma-glutamyl transferase enzyme were measured on the rest day after exercising (24 hours without exercise) and immediately after exercise, the urinary sample of the players was collected. To test the hypotheses and discover significant relationships, especially to compare changes in the levels of protein, creatinine, and gamma-glutamyl transferase before and after exercise and compare the level of excretion of protein, creatinine, gamma- glutamyl transferase between the different positions, ANOVA test was used. Moreover, LSD post hoc test was used for pairwise comparison of the variables.

The results in general and separately for research objectives showed that:

1- A significant difference was seen between the results of proteinuria excretion before and after the exercise $P \leq 0/001$

2- A significant difference was seen between the results of creatinine excretion before and after exercise $P \leq 0/001$

3- A significant difference was seen between the results of gamma-glutamyl transferase excretion before and after exercise $P \leq 0/001$

4- A significant difference was seen between the results of level of protein excretion in different positions $P \leq 0/001$

5-There was a significant difference between the results of creatinine excretion in different positions $P \leq 0/001$

6- There was a significant difference between the results of gamma-glutamyl transferase excretion in different positions $P \leq 0/04$

By comparing the consistent and inconsistent results in the present study, it was aimed to investigate the effect of one session of acute exercise on the level of changes in concentration of urinary gamma-glutamyl transferase, creatinine, and protein as indicators for the prognosis of some kidney injuries in Iranian Primer Football League players who were under pressure of exercises and competitions and played two heavy games during the week. In order to achieve this goal, specific factors were used as indicators of kidney function during physical activity. These indicators include proteinuria, urinary creatinine, and urinary gamma-glutamyl transferase. Results of the studies conducted in recent years have shown that professional exercise and consequently repetition of high-intensity sessions may lead into kidney tissue dysfunction in professional athletes. In such conditions, urinary proteinuria, creatinine, and gamma-glutamyl transferase (GGT) enzymes are non-invasive and are available tests for assessing kidney injury (Poortmans, 1985; Ayka, 2006). With regard to research hypotheses, the results of this study showed that there was a significant difference between the levels of protein excretion after exercise compared to before exercise. Results showed that 70-80% of athletes were affected by proteinuria after exercise. Proteinuria was observed not only in collision sports such as football, boxing, but also in non-collision sports such as running, swimming and sailing.

Ayka et al. (2006) investigated the effects of exercise on the level of proteinuria excretion and evaluated the effects of exercise on kidney function in male and female volleyball players. Their results showed that there was a significant difference between the proteinuria levels in males before and after exercising. In their study, Poortmans and Labiloy (1988) investigated the urinary excretion of total protein and albumin and its relationship with running distances. The study was conducted on 15 male runners at 100m, 400m and 3000m sprints. The results showed that total proteinuria excretion of 330, 1640, 565μg/min after 100m, 400m, 300m sprints, respectively, was significantly higher than its normal value (70μg/min). This study also showed a significant correlation (0.85) between blood lactic acid and albumin and total protein excretion after exercise.

It indicated that the intensity of activity had a greater effect on urinary protein excretion compared to the duration of exercise and there was a linear relationship between the intensity of activity and urinary protein excretion. This mechanism has not yet been clarified. Results of the present study with regard to proteinuria were in line with those of previous studies conducted by Ayka (2006), Ayka (2008), Turgut (2003), Grath (2000), Yaguchi (1998), Robert Shaw (1993), Poortmans (1991), Hiai (1990), Poortmans (1989), Ironing (1986), Poortmans (1978). In their study, they found that proteinuria increased as a result of physical activity. In their research, Poprtmans and Van Clock (1978) measured the levels of total protein, micro-albumin B2 in the urine of 15 healthy women before and after high-intensity and short-term exercise. A significant increase was observed in the level of urinary excretion of total albumin, albumin B2, and micro-albumin. This research revealed that the level of variety of proteins returned to their initial levels 45 minutes after the end of exercise.

However, albumin, albumin B2, and micro-albumin levels were still high. Turgut et al. (2003) in their study investigated the acute effect of exercise on the level of protein excretion in children. For this purpose, they examined 13 girls and 14 boys with an age range of 10 to 13 years who were members of the volleyball team in their school. Urinary samples were collected before and 2 hours after doing exercise. The results showed that there was a significant increase in urinary protein concentration in boys and girls after exercise. Based on the conducted studies, glomerular - tubular type of proteinuria excretion increases during short - term and high-intensity exercises and this increase in protein excretion results in an increase in glomerular permeability and a violation in single tubular reabsorption of plasma proteins. This phenomenon can occur immediately and

transiently in functional disorders of glomerular and tubular nephrons in children after doing exercise.

Poortmans et al. (1989) investigated 13 men under the high-intensity cycling exercise to determine the level of proteinuria during recovery. Total protein and albumin excretion levels were 581 and 315μg/min, respectively, which increased to 42 and 15g/min, respectively after doing exercise. However, urinary albumin and protein were elevated 2 hours after exercise. Urinary protein and albumin were still high 2 hours after doing exercise. Total protein and urinary albumin after exercise showed a logarithmic reduction of 54 minutes with the same life and it needed 4 hours to return to rest state level. This study showed a longer period to return initial state in protein control by the kidney compared to other biochemical parameters and provided accurate information on proteinuria kinetics after exercise. Senturk et al. (2007) showed that total protein levels before antioxidant administration in the athlete and non-athlete group 30 min and 24 h after exercise showed significant increase compared to steady state. Even with the consumption of antioxidants, total protein increased significantly 30 minutes after doing exercise. As the result of the study showed, it seems that exercise at the championship level and performing continuous activities to obtain fitness during a season may cause disabilities and affect the performance of athletes due to physiological and biochemical changes. In such conditions, evaluation of the changes in proteinuria, urinary creatinine, and urinary gamma-glutamyl transferase as the indicators of kidney function during physical activity can be useful. The results of such studies can provide useful guidance for coaches and trainers in planning the type, intensity and duration of exercises, type of nutrition and dietary supplements, use of antioxidants, fluids and electrolytes during and before and after the matches. It seems that the main reason for the increase in creatinine excretion following high-intensity exercise cen be a reduction in phosphocreatine and increased Pi and creatinine production. In addition, exercise increases the glomerular filtration ratio, as a result, it increases more creatinine passes through the glomerular network, leading to increased creatinine excretion. Results of the present study with regard to urinary creatinine were in line with those of the studies conducted by Ayka (2006), Turgut (2003), Poortmans (1996), Poortmans (1991), Ironing (1986) and Edes (1990), respectively. In line with the present study, they observed that creatinine excretion increased after physical activity. These results were inconsistent with those of the research conducted by Rasm et al. (1974), William (1970), Clerico (1990), and Poortmans (1978). It seems that difference can be due to differences between football field and other sports and differences between Football players and other sports players in terms of physical pressure imposed on athletes and exercise pressures.

Based on the results of the conducted studies, it seems that urinary creatinine excretion changes after exercise and these changes may eventually increase the glomerular filtration ratio and creatinine excretion rate. This increase in the level of creatinine excretion imposes pressure on kidney, and if this condition continues for a long term, it may cause irreparable injury to kidney tissue, leading to reduced filtration ratio. Exercise pressure and 35 minutes of playing high-pressure football in the present study stimulated creatinine. After collecting urinary samples after doing exercise, creatinine was above the normal levels. Therefore, the athletes in this study might be affected by creatinine complications and might experience kidney tissue injury and glomerular network damage. With regard to the third hypothesis of the study, there were few and different studies on the effect of exercise on the level of urinary excretion of gamma-glutamyl transferase before and after doing exercise. Miay and Ogata (1990) conducted a study on 17 high school baseball players. Results showed that physical activity did not increase significantly the ANG excretion level (2006). In a study on 12 male and 12 female volleyball players, Ayka (2006) found that urinary GGT excretion was significantly different only in male spiker players after doing exercise, whereas no significant difference was found in other positions. However, studies showed that exercise pressure increases gamma-glutamyl transferase after doing exercise. Baguchi (1998) examined and compared the rate of excretion of urinary enzymes before and after triple exercise. In a study on 12 male and 12 female volleyball players, Ayka (2006) found that urinary GGT excretion was significantly different only in male spiker players after exercise, whereas no significant difference was found in other positions. Ayka attributed this increase to high-intensity exercise pressure and jumps in spikes. Ayka (2000) also investigated the effects of high collision exercises such as taekwondo on kidney function. The results showed that gamma-glutamyl transferase increased significantly after the collision exercise.

It seems that these differences in the results on the level of excretion of the gamma-glutamyl transferase after exercise can be related to the type of exercise, the intensity and duration of the exercise. Based on the results of previous studies and the results of this study, it seems that gamma-glutamyl transferase can increase significantly in high collision sports such as taekwondo whose time is short as well as heavy exercises that require repetition and high pressure of exercises such as gymnastics. Therefore, exercise pressure and 25 minutes of high-intensity exercise in the present study stimulated gamma-glutamyl transferase. With regard to the fourth hypothesis of the research, the results of the present study showed that there was a significant difference in the level of urinary

protein excretion in different positions. Ayka et al. (2006) in their research examined the effects of exercise and level of urinary excretion of gamma-glutamyl transferase GGT, protein, and creatinine one hour after exercise. The results showed no significant difference between male and female players in terms of GGT level before and after doing exercise. Significant increase was seen in proteinuria excretion level for males. With regard to significant increase in urine creatinine levels in females as a result of exercise, it was observed that when urinary GGT levels were compared in setters and spikers, a statistical significant difference was found among them. Exercise increased the level of urinary GGT excretion. In this study, GGT showed significant difference only in make spikers. Ayka attributed this increase to the high-intensity exercise pressure and jumps by spikers. There was a significant difference in the level of creatinine excretion between different positions in this study. According to the results of the present study, the highest mean urinary protein excretion was observed in midfield and the highest mean urinary creatinine excretion was seen in midfield among different positions. The highest mean excretion of gamma-glutamyl transferase was observed in the goalkeeper.

Based on the findings of the studies conducted on football and the role of the players in the team and their activities, this result can be predicted. The midfield players are the most active among the players of four positions and they are required to assist the forward players when team attacks and moves forward and go back and assist the defenders when team defends. Thus, they have to travel longer distance during the match. Based on the studies, they travel almost 10-11 km in an official match, leading to an increase in the level of excretion of protein and creatinine. In addition, goalkeepers have long and frequent jumps to the side of the gate and explosive movements, leading to an increase in the levels of gamma-glutamyl transferase (Douglas, 1993).

In addition, as the present study has been designed for all players in general, the difference in creatinine and protein excretion levels in different positions can be attributed to 35 minutes of playing football at the end of exercise. Finally, it can be stated that due to the novelty of the research, completely convincing reason cannot be provided for differences in mean excretion of protein, creatinine, and gamma-glutamyl transferase enzymes among different positions. Finally, this study revealed that the kidneys, as other body organs, are affected by performing exercises at the championship level and performing heavy and continuous activities to gain physical fitness. Moreover, it seems that high-intensity and long-term exercise can increase the urinary creatinine, protein and gamma-glutamyl transferase levels, which can cause irreparable injury to the kidney if this condition continues over the long term. The results of the study also showed that the gamma-glutamyl transferase is more affected by the intensity of exercises and the collision of exercises and competition. Therefore, the use of certain factors such as proteinuria, urinary creatinine, and urinary gamma glutamyl-transferase enzyme can be useful as the indicators of kidney function during physical activity. According to the results of the present study, it is recommended that the present study can be conducted on athletes of other sports and their results can be compared with each other. It is also recommended that the similar studies to be conducted on different age groups in both genders. It is recommended that the similar study to be conducted on female athletes and its results to be compared with those of the present study in order to reveal gender differences. It is also recommended that the similar study to be conducted on athletes in exercise conditions, as this study was conducted in competition conditions. It is recommended that athletes consume electrolyte-containing fluids before, during, and after high-intensity and prolonged activity. It is also recommended for athletes to use antioxidants that contain vitamin E, C, and A. It is recommended that medical tests can be performed every few months to check the kidney function.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Investigation of the Relationship between Parental Mental Disorders and Autism among the Children of West Azerbaijan -Iran

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Abstract

Background: Autism disorders have increased over the last years. Autism is a neurological growth disorder associated with social communication disorders, growth retardation, and repetitive behaviors, along with serious consequences for children and families. The purpose of this research was to evaluate the relationship between parental mental disorders and autism among the children of West Azerbaijan Province.

Methods: This research was a case-control study in which the case group subjects were selected among the parents with autistic children and control group subjects were selected among the relative parents with healthy children and non-relative parents with healthy children. Both case and control groups were matched in terms of gender, living place, and age of children. Finally, the data were analyzed using SPSS-16 (Chicago, IL, USA) software.

Results: The current research results revealed that the frequency of mental diseases, including obsessive-compulsive, inter personality sensitivity, depression, anxiety, hostility, phobia, paranoid ideation, and psychotic disorder are different in fathers and mothers of the case and control groups. As Pvalue was lower than 0.05 in all scales, there was a significant relationship between the mental diseases of parents and the history of mental disorders in relatives and autism.

Conclusion: The prevalence of mental disorders in relatives and having a medical history can be a warning sign of autism in children.

Keywords: Autism, Parental Psychiatric Disorders, Case-Control, Scl-90-R, Mental Disorder

1. Introduction

Autism disorders have increased over the last years. Autism spectrum disorders with delayed or abnormal functioning occur at least in one of the areas of social interaction, the language used in social imaginative or symbolic communication (Yazdani et al., 2017). Autism is a neurological growth disorder associated with social communication disorders, growth retardation, and repetitive behaviors, along with serious consequences for children and the families (Ha et al., 2015; Lei et al., 2018). It has been investigated in various studies (MAKHADIYEVA, 2018). Based on the findings of the studies conducted over the last years, the root of more than 90% of brain function abnormalities and autistic behaviors is genetic. However, genetic is not the only factor involved in the development of all cases of autism, environmental factors are also involved in the development of autistic behaviors (Karimi et al., 2017; Yuen et al., 2019). Various studies have indicated that genetic factors play a major role in autism disorders, but the impact of these symptoms has not been well understood concerning environmental risk factors. Recent research suggests that environmental factors have had an effective role in about 40 to 50% of patients with autism (Deng et al., 2015; Gaugler et al., 2014; Kim & Leventhal, 2015; Yuen et al., 2019). Some experts reported that a wide range of parental mental disorders is associated with an autism spectrum disorder (ASD) (Bölte et al., 2007; Daniels et al., 2008; Jokiranta et al., 2013; Larsson et al., 2005). The results of the study conducted by Jokiranta et al. showed a significant relationship between parental emotional disorders and the incidence of autism in their children so that the possibility of having ASD children in the fathers with the emotional disorder is two times more than that of other fathers (Jokiranta et al., 2013). Based on the results of the study conducted by Sullivan et al., families whose first-degree relatives suffer from schizophrenia and bipolar

disorder are at a greater risk of autism for their children (Sullivan et al., 2012). The brain growing and developing in the uterus is sensitive to environmental factors from the beginning and evaluating the non-genetic factors affecting autism is critical for identifying potential risk factors and can help to reduce the risk of this disease (Modabbernia et al., 2017). It is estimated that approximately 1.7% of the world's population has autism, which almost 25% to 30% of children with this disease suffer from verbal problems and cannot speak. As the ability to communicate effectively is a vital skill, the inability to communicate effectively will cause many problems such as poor academic achievement, behavioral problems, and poor quality of life (Brignell et al., 2018). Due to the lack of an analytical study to evaluate the mental disorders of parents and relatives in the incidence of autism in children and as the studies conducted in this regard have been often cross-sectional and descriptive, this case-control research was carried out to evaluate the effects of parental mental disorders on autism, and accordingly, a major step was taken towards controlling and reducing the rate of these diseases, imposing high costs to the health system of the country.

2. Materials & Methods

This case-control study received its permission (No. IR.umsu.rec.1395.333-95/8/5) from the Ethics Committee of Urmia Medical Sciences University. It also secured letters of consent from the study parents. This research is a case-control study in which the case group subjects were selected among the parents with autism children (76 mothers and 76 fathers) and control group subjects were selected among the relative parents with healthy children (149 fathers and 149 mothers) and non-relative parents with healthy children (77 fathers and 77 mothers) and the groups were compared in terms of the study variables. The research population used in this research included all children who were diagnostic to be autistic in health care centers of West Azerbaijan province. In this research, 76 autistic children (with a 1% prevalence in the community) admitted to the centers providing pediatric services for autism children in Urmia city were selected and reevaluated by the psychiatrist. After meeting the research inclusion criteria, they were included in the research. Three children were considered in the control group per one child in the case group and for each child in the case group, two healthy children of first-degree relatives and one healthy child of non relatives were considered. Subjects were matched to control the potential confounding factors. Accordingly, both groups were matched in terms of gender, living place and age, and a total of 226 participants (149 relatives and 77 non-relatives) were selected for the control group. The method of selecting the case group was as follows: by referring to the centers providing service for autistic children in Urmia, those who were diagnosed with autism were included in the research. Selection of the samples was in this way: a list of all cases was obtained in each center. Then, they were selected based on weight ratio from each center using convenient sampling method. All of the selected children whose parents were willing to participate in the research were selected as the case group. The control group for this study included 3 children versus per child in the case group. The control group also included 2 children of the relatives` selected groups due to neutralizing the confounding factors which might be created due to different ethnicities and lifestyle and cultural factors, and so on and one child was selected from non-relatives to examine the possible family and genetic factors. The relative control group included first-degree families, including uncle and aunt and non-relative control group subjects were selected from the same living place. The samples were examined by the pediatric psychiatric specialist to approve the autism of children. The SCI-90_R questionnaire was used for a parental psychiatric disorder. The initial form of this questionnaire was designed by Liminen and Curie in 1973 to show the psychological aspects of patients with somatic and mental diseases (Leathem & Babbage 2000).The Scl-90-R checklist is the symptoms of mental disorders in the self-reporting questionnaire for screening and measuring the symptoms of mental disorders. This test was revised by Derogatis et al. based on clinical experiences and psychometric analyses. Its final form was prepared in 1976 and the internal validity of this test was reported by using alpha coefficient at the acceptable level (Derogatis et al., 1976). The reliability of this test was calculated by Cronbach's alpha method (94%). The correlation coefficient was estimated at 88% using the test-retest method with the time interval of one year, so its validity is at a high level (Simonds et al., 2008). The criterion validity coefficients of the nine dimensions of this test with the Minnesota multidimensional questionnaire, except for obsessive-compulsivescales, was reported between 36% and 73%, all of which were at the significant ($p < 0.05$) (Christensen et al., 2018). The data were analyzed based on descriptive methods such as statistical tables, calculation of central indices and distribution and percentage for qualitative variables. Analytical statistical methods were also used. All analyses were performed using SPSS-16 (Chicago, IL, USA) and the significance level was considered ($p < 0.05$).

3. Results

Table 1. Comparing the mean psychopathologic dimensions among the mothers of three studied groups a: Significant different with control1 (relative mothers with healthy children) group, b: Significant different with control2 (non-relative mothers with healthy children) group

		N	Mean±SD	95% Confidence Interval for Mean		P-Value[c]
				Lower Bound	Upper Bound	
Somatization	case	76	1.11±.60[b]	.97	1.25	F(2,299)=15.78 p<0.001
	control1	149	1.03±.45[b]	.96	1.11	
	control2	77	.71±.35	.63	.79	
Obsessive-Compulsive	case	76	1.30±.65[ab]	1.15	1.45	F(2,299)=33.63 p<0.001
	control1	149	1.22±.53[b]	1.13	1.30	
	control2	77	.68±.34	.60	.76	
Inter Personality Sensitivity	case	76	1.02±.57[ab]	.89	1.15	F(2,299)=38.14 p<0.001
	control1	149	1.06±.47[b]	.98	1.13	
	control2	77	.51±.30	.44	.58	
Depression	case	76	1.19±.66[ab]	1.04	1.34	F(2,299)=31.43 p<0.001
	control1	149	1.18±.53	1.09	1.26	
	control2	77	.64±.30	.57	.71	
Anxiety	case	76	1.10±.64[ab]	.95	1.24	F(2,299)=40.33 p<0.001
	control1	149	1.19±.59[b]	1.09	1.28	
	control2	77	.52±.26	.47	.58	
Hostility	case	76	1.14±.59[ab]	1.01	1.28	F(2,299)=51.86 p<0.001
	control1	149	1.09±.56[b]	1.00	1.18	
	control2	77	.42±.28	.35	.48	
Paranoid Ideation	case	76	1.53±.56[ab]	1.33	1.73	F(2,299)=39.70 p<0.001
	control1	149	1.28±.54[b]	1.19	1.36	
	control2	77	.69±.39	.60	.78	
Psychotic	case	76	.81±.54[ab]	.69	.93	F(2,299)=43.94 p<0.001
	control1	149	.87±.49[b]	.79	.95	
	control2	77	.29±.26	.23	.34	
Phobic	case	76	.90±.61[ab]	.76	1.04	F(2,299)=34.22 p<0.001
	control1	149	.99±.58[b]	.89	1.08	
	control2	77	.39±.27	.33	.45	
Global Severity Index	case	76	1.14±.50[ab]	1.02	1.25	F(2,299)=59.73 p<0.001
	control1	149	1.12±.40[b]	1.06	1.19	
	control2	77	.56±.21	.52	.61	

c: oneway ANOVA test

Table 2. Comparing the frequency of psychopathologic problems in the mothers of three studied groups

		Autism group		Control1[a]		Control2[b]		Odds ratio2[c] (CI 95%)	Odds ratio1[d] (CI 95%)	P-Value[e]
		n	%	n	%	n	%			
Global Severity Index	Normal	31	40.8	46	30.9	76	98.7	110.32 (14.56-	0.65 (0.37-	χ²(2,97.40)
	Psychopathologic	45	59.2	103	69.1	1	1.3	835.95)	1.15)	<0.001
Somatization	Normal	36	47.4	68	45.6	62	80.5	4.59	0.93 (0.54-	χ²(2,27.32)
	Psychopathologic	40	52.6	81	54.4	15	19.5	(2.23-9.45)	1.62)	<0.001
Obsessive-Compulsive	Normal	28	36.8	48	32.2	64	83.1	8.44 (3.96-	0.82 (0.46-	χ²(2,56.59)
	Psychopathologic	48	63.2	101	67.8	13	16.9	17.99)	1.45)	<0.001
Inter Personality Sensitivity	Normal	35	46.1	63	42.3	68	88.3	8.85 (3.86-	0.86 (0.49-	χ²(2,46.71)
	Psychopathologic	41	53.9	86	57.7	9	11.7	20.27)	1.50)	<0.001
Depression	Normal	28	36.8	47	31.5	65	84.4	9.29 (4.29-	0.79 (0.44-	χ²(2,60.77)
	Psychopathologic	48	63.2	102	68.5	12	15.6	20.10)	1.41)	<0.001
Anxiety	Normal	34	44.7	47	31.5	73	94.8	22.54 (7.48-	0.57 (0.32-	χ²(2,82.89)
	Psychopathologic	42	55.3	102	68.5	4	5.2	67.96)	1.01)	<0.001
Hostility	Normal	28	36.8	57	38.3	74	96.1	42.29 (12.18-	1.06 (0.60-	χ²(2,78.32)
	Psychopathologic	48	63.2	92	61.7	3	3.9	146.83)	1.88)	<0.001
Paranoid Ideation	Normal	11	14.5	37	24.8	59	76.6	19.37 (8.46-	1.95 (0.93-	χ²(2,79.02)
	Psychopathologic	65	85.5	112	75.2	18	23.4	44.36)	4.09)	<0.001
Psychotic	Normal	50	65.8	80	53.7	75	97.4	19.5 (4.43-	0.6 (0.34-	χ²(2,44.69)
	Psychopathologic	26	34.2	69	46.3	2	2.6	85.84)	1.07)	<0.001
Phobic	Normal	42	55.3	64	43	74	96.1	19.97 (5.78-	0.61 (0.35-	χ²(2,60.36)
	Psychopathologic	34	44.7	85	57	3	3.9	68.98)	1.06)	<0.001

In the present study, the mental disorders of the parents of autistic children were investigated as the case group (76 mothers and 76 fathers) and relative parents with healthy children (149 fathers and 149 mothers) and non-relative parents with healthy children (77 fathers and 77 mothers) were regarded as the control groups. Comparing the means of psychopathological dimensions showed significant difference among mothers of case and control groups in all mental dimensions (somatization, obsessive-compulsive, inter personality sensitivity, depression, anxiety, hostility, paranoid ideation, psychotic disorder, phobia) (Tables 1&2). Comparing the mean psychopathologic dimensions of the fathers studied in three groups showed a significant difference between the fathers of the case and control groups in all psychological dimensions (somatization, obsessive-compulsive, inter personality sensitivity, depression, anxiety, hostility, paranoid ideation, psychotic disorder, and phobia) (Tables 3&4). As shown in Table 4, comparing the frequency of psychopathologic problems among the fathers studied in the three groups showed a significant difference between the fathers of case and control groups in all mental dimensions, so that the odds of autism in the case group was more than that in the relative control group. However, in the non-relative control group, except for somatization, obsessive-compulsive, inter personality sensitivity and rest of the dimensions showed higher odds of autism. 180.

4. Discussion

The prevalence of ASD has increased remarkably over the last two decades. The Autism Diseases Control Center announced that the incidence of ASD in 2000 was 1 per 150 children (Christensen et al., 2019), while National Health Center reported it I child per 36 children in 2006, which is considered high rate (Zablotsky et al., 2017). In this research, the mental disorders were examined in parents of children with autism (case group), relative parents with healthy children, and non relative parents with healthy children (control groups). The results of this research showed that there was a significant relationship between mental disorders in the parents of children with autism (case group) and those of control group so that the odds of autism in the case group is higher than that in the relative control group. In a research conducted by Larson et al. (2005) and Jokiranta et al. (2013), in line with this study, parents' mental problems were associated with the incidence of autism in children. In a research carried out by Boukhris et al, it was reported that depression during pregnancy and the use of antidepressants during the second and third trimester of pregnancy increased the risk of ASD in children (Boukhris et al., 2016). In another study conducted by Khaiman et al, it was reported that mental disorders of parents, family history of psychiatric disorders, and high age of the father were among the most important environmental risk factors for ASD (Khaiman et al., 2015). Based on the results of this research, the odds of autism in children of fathers of case group was higher than that of non-relative and relative control groups with the dimension of obsessive compulsive disorder. Consistent with this research, Bolt et al. (2007) showed that obsessive compulsive disorder and schizophrenia are associated with autism (Bölte et al., 2007). Also, there PeerJ reviewing PDF | (2019:07:39830:0:1:NEW 10 Aug 2019) Manuscript to be reviewed is much other evidence suggesting that autism spectrum disorders, schizophrenia, bipolar disorder, and obsessive-compulsive disorder have a common molecular cause (O'Connell et al., 2018). The results of this research at the dimension of depression disorder revealed high odds of autism in relative mothers and relative and non-relative fathers in case group compared to that of the control group, but the odds of autism was low in non-relative mothers. In a study conducted by Hu et al, it was reported that the parents of children with ASD, experience more depressive disorder (Hu et al., 2018), compared to the parents of normal children. The results of this research also showed that the parents of children with autism will more likely have anxiety disorders. In another research conducted by Daniels et al, the findings revealed that depressive disorders are more common in mothers, but not common in fathers (Daniels et al., 2008). The results of this research revealed that mental disorder, hostility, phobia, paranoid ideation, and psychotic disorder have a significant difference between the groups of autism and their relatives and the control group. Thus, the frequency of mental disorders in relatives and having a medical history can be the risk factors of autism in children. The highest correlation was found between schizophrenic spectrum disorders in parents and non-classified pervasive developmental disorder. The results of several studies showed that there is a significant relationship between the emotional disorders of parents and the incidence of autism in their children. In the studies conducted in Sweden and Denmark, the results showed that there is a high correlation between emotional disorders and schizophrenia and ASD, and genetic studies have also proved that there is a high genetic association between these disorders (Daniels et al., 2008; Larsson et al., 2005). Families whose first-degree relatives have schizophrenia and bipolar disorder are at greater risk of autism in their children. Based on the results, these disorders have a high genetic association (Ghaziuddin, 2005).

Table 3. Comparing the mean psychopathologic dimensions of the fathers of three studied groups

		N	Mean±SD	95% Confidence Interval for Mean		P-Value[c]
				Lower Bound	Upper Bound	
Somatization	case	76	1.56±.88[ab]	1.36	1.76	F(2,299)=37.99 p<0.001
	control1	149	1.29±.52[b]	1.21	1.38	
	control2	77	.75±.31	.68	.82	
Obsessive-Compulsive	case	76	1.53±.71[ab]	1.36	1.69	F(2,299)=39.93 p<0.001
	control1	149	1.31±.57[b]	1.22	1.41	
	control2	77	.76±.32	.68	.83	
Inter Personality Sensitivity	case	76	1.36±.68[b]	1.21	1.52	F(2,299)=38.87 p<0.001
	control1	149	1.26±.53[b]	1.17	1.34	
	control2	77	.68±.32	.61	.76	
Depression	case	76	1.55±.72[ab]	1.38	1.71	F(2,299)=41.22 p<0.001
	control1	149	1.24±.56[b]	1.15	1.33	
	control2	77	.74±.30	.67	.81	
Anxiety	case	76	1.48±.87[ab]	1.28	1.68	F(2,299)=43.52 p<0.001
	control1	149	1.19±.53[b]	1.11	1.28	
	control2	77	.61±.30	.54	.68	
Hostility	case	76	1.14±.59[b]	1.01	1.28	F(2,299)=51.86 p<0.001
	control1	149	1.09±.56[b]	1.00	1.18	
	control2	77	.42±.28	.35	.48	
Paranoid Ideation	case	76	1.55±.68[ab]	1.39	1.70	F(2,299)=28.85 p<0.001
	control1	149	1.29±.74[b]	1.17	1.40	
	control2	77	.78±.33	.70	.85	
Psychotic	case	76	.81±.54[b]	.69	.93	F(2,299)=56.29 p<0.001
	control1	149	.87±.49[b]	.79	.95	
	control2	77	.29±.26	.23	.34	
Phobic	case	76	.98±.61[b]	.84	1.12	F(2,299)=20.26 p<0.001
	control1	149	.90±.45[b]	.83	.97	
	control2	77	.30±.18	.26	.34	
Global Severity Index	case	76	1.39±.56[ab]	1.27	1.52	F(2,299)=47.81 p<0.001
	control1	149	1.18±.39[b]	1.12	1.24	
	control2	77	.64±.18	.60	.68	

Table 4. Comparing the frequency of psychopathologic problems of fathers in three studied groups

		Autism group		Control1[a]		Control2[b]		Odds ratio2[c] (CI 95%)		Odds ratio1[d] (CI 95%)		P-Value[e]
		n	%	n	%	n	%					
Global Severity Index	Normal	21	27.6	37	24.8	76	98.7	199.05	(25.99-	0.87	(0.46-	χ^2(2,123.76)
	Psychopathologic	55	72.4	112	75.2	1	1.3	1524.49)		1.62)		<0.001
Somatization	Normal	24	31.6	37	24.8	57	74	6.18	(3.06-	0.72	(0.39-	χ^2(2,54)
	Psychopathologic	52	68.4	112	75.2	20	26	12.47)		1.32)		<0.001
Obsessive-Compulsive	Normal	18	23.7	36	24.2	57	74	9.18	(4.41-	1.03	(0.54-	χ^2(2,61.77)
	Psychopathologic	58	76.3	113	75.8	20	26	19.14)		1.96)		<0.001
Inter Personality Sensitivity	Normal	22	28.9	40	26.8	62	80.5	10.45	(4.79-	0.90	(0.49-	χ^2(2,66.59)
	Psychopathologic	54	71.1	109	73.2	15	19.5	21.50)		1.66)		<0.001
Depression	Normal	19	25	40	26.8	63	81.8	13.5	(6.20-	1.10	(0.59-	χ^2(2,73.72)
	Psychopathologic	57	75	109	73.2	14	18.2	29.39)		2.07)		<0.001
Anxiety	Normal	23	30.3	47	31.5	70	90.9	23.04	(9.20-	1.06	(0.58-	χ^2(2,82.53)
	Psychopathologic	53	69.7	102	68.5	7	9.1	57.72)		1.93)		<0.001
Hostility	Normal	28	36.8	57	38.3	74	96.1	42.29	(12.18-	1.06	(0.60-	χ^2(2,78.32)
	Psychopathologic	48	63.2	92	61.7	3	3.9	146.83)		1.88)		<0.001
Paranoid Ideation	Normal	15	19.7	41	27.5	58	75.3	12.41	(5.77-	1.54	(0.79-	χ^2(2,63.4)
	Psychopathologic	61	80.3	108	72.5	19	24.7	26.72)		3.02)		<0.001
Psychotic	Normal	39	51.3	77	51.7	77	100	-		1.02	(0.58-	χ^2(2,58.37)
	Psychopathologic	37	48.7	72	48.3	0	0			1.76)		<0.001
Phobic	Normal	34	44.7	74	49.7	71	92.2	14.62	(5.66-	1.22	(0.70-	χ^2(2,46.95)
	Psychopathologic	42	55.3	75	50.3	6	7.8	37.72)		2.12)		<0.001

a: control1: relative fathers with healthy children group, b: control2:non-relative fathers with healthy children group, c: odds ratio (abnormal/normal) ASD children fathers with relative fathers with healthy children group, d: odds ratio (abnormal/normal) ASD children fathers with non-relative fathers with healthy children group, e: chi-square test between three groups

5. Conclusions

As all mental disorders have a genetic history, and autism also has a high genetic history, exposure to mental risk factors can cause mutations in the genes and cause different genetic-related problems. This research suggests that autism can be a strong genetic potential, that mental factors can activate it.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Mid and Long Term Echocardiographic and Clinical Follow up of Treated Rheumatic Mitral Stenosis Patients

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Abstract

Introduction: Rheumatic mitral stenosis (MS) is a common valvopathy in developing countries that requires invasive or minimally invasive treatment. There is scarcity of data regarding the long term follow up of treated MS patients.

Aim: The aim of this study was to compare the long-term outcomes of biologic and mechanical prosthesis and percutaneous transvenous mitral commissurotomy (PTMC) in treated MS patients.

Materials and Methods: This historical cohort study was conducted on treated MS patients who underwent treatment in a tertiary hospital in Mashhad, Iran. Treated MS patients were identified and asked to refer to the center for follow up. Demographic characteristics and type of treatment intervention were obtained from patient records. The follow up assessment included history of PTMC, readmission and echocardiography to assess trans mitral gradient (MG), size and function of right ventricle (RV) and the presence and severity of other valvopathies.

Results: A total of 135 patients (21.5% males and 78.5% females) with the mean age of 43.68 ± 11.17 years old participated in the study. The most common intervention method was mechanical valve (61.5%) followed by PTMC (24.4%) and biologic valve (14.1%). Median follow up duration was 4 years. Majority of subjects (52.6%) remained asymptomatic and the functional class was reduced significantly compared to baseline. MG was significantly higher in PTMC and biological valve group compared to mechanical valve p<0.001). Mitral regurgitation (MR) was more prevalent in PTMC group (p<0.001) while abnormal RV size and function the least common in PTMC group (p=0.014 and p=0.002 respectively).

Conclusions: All intervention groups resulted in improved functional class of MS patients but high prevalence of MR and lower prevalence of abnormal RV size and function were observed in PTMS group compared to surgical groups.

Keywords: Percutaneous Transvenous Mitral Commissurotomy, Biologic Mitral Valve Prosthesis, Mechanical Mitral Valve, Follow up, Mitral Stenosis, Mitral Valvuloplasty

1. Introduction

Mitral stenosis (MS) is among the most common valvular heart diseases in developing countries including Iran (Saxena, 2018; Wunderlich, Beigel, & Siegel, 2013) affecting life expectancy and damaging the quality of life (Marzangi et al., 2018). The primary treatment for MS was partial commissurotomy (Selzer & Cohn, 1972). Balloon valvuloplasty was introduced by Inoue in 1982 and is being used ever since (Watanabe, 2016). There is scarcity of literature regarding the long term follow up of MS balloon valvuloplasty and its comparison with mitral valve replacement. In a study, balloon valvuloplasty patients were surveyed for 13 years and the preintervention mitral valve area and echocardiographic score were reported to be the predictors of mitral valve restenosis (Fawzy, 2009). In another study 15 year follow up of MS patients undergoing surgical valve replacement revealed that the need for valve replacement was significantly lower in mechanical valves compared to biologic prostheses (Al Mosa, Omair, Arifi, & Najm, 2016). In another study, mortality and morbidity due to repeated valvuloplasty and prosthesis replacement were significantly related to preintervention echocardiographic score (Fawzy et al., 2007). In another study echocardiography score above 8 in balloon valvuloplasty patients was associated with mitral insufficiency in follow up (Palacios, Sanchez, Harrell, Weyman, & Block, 2002). The aim of this study was to assess the mid term and long term follow up of patients with mitral stenosis who underwent valvuloplasty.

2. Materials and Methods

This historical cohort was performed on patients with the history of rheumatic MS who underwent mitral valvuloplasty in the Cardiology Department of the Imam Reza Hospital, Mashhad, Iran from March 2008 to March 2018. The demographic and baseline data prior to valvuloplasty and the type of valvuloplasty were extracted form patient records. Patients were then contacted by the researchers and were invited to the hospital to perform follow up assessment including physical examination and echocardiography. Patients with the history of endocarditis were excluded for the study. In the follow up assessment history of further hospitalization and the reason for hospitalization were also recorded. Other information included type and size of the prosthetic valve in cases of surgical valve replacement. A written informed consent was obtained from all patients who were willing to participate in the study.

The primary outcome measures included need for repeated valvuloplasty in each treatment group, while secondary outcome measures included number of further hospital admissions related to MS treatment, recurrence of mitral stenosis, status of other heart valves, left ventricular function and pulmonary artery pressure as well as the size and function of right ventricle. The results were then compared between treatment groups.

The study protocol was approved by the Ethical Committee of the Mashhad University of Medical Sciences (Reg. No. IR.MUMS.MEDICAL.REC.1397.270).

2.1 Statistical Analysis

Data was analyzed using the statistical package for social sciences (SPSS) software version 16 (IBM Inc., Chicago, Il, USA). The Kolmogorov-Smirnov test was used to assess the normality of continuous data. Normally distributed variables were presented using mean and standard deviation (SD) while non-normally distributed variables were presented using median and interquartile range (IQR). Categorical variables were presented using frequency and percentage. In order to compare normally distributed continuous variables between intervention groups, the one-way analysis of variance (ANOVA) test was used while the Kruskal-Wallis test was used to compare non-normally distributed variables between intervention groups. The chi square or Fisher exact test was used to compare the distribution pattern of the categorical variables between groups.

3. Results

A total of 135 patients (29, 21.5% males and 106, 78.5% females) agreed to participate in the study. The baseline characteristics of the study subjects are presented in Table 1. The mean age of the subjects at time of treatment intervention was 43.68 ± 11.17 years old. Mitral valve replacement using biological and mechanical valves were identified in 19 (14.1%) and 83 (61.5%) of the subjects respectively, while percutaneous transvenous mitral commissurotomy (PTMC) was performed in 33 (24.4%) of the subjects. The mean body mass index of the subjects was 27.82 ± 5.53 kg/m^2. Comparison of baseline characteristics of the subjects between intervention groups is presented in Table 1. There was a significant difference in the frequency of smoking ($p<0.001$), cardiac rhythm ($p=0.027$) and the extent of mitral regurgitation ($p<0.001$) between intervention groups at baseline (Table 1). The mean age of subjects in biological and mechanical valve groups were 41.47 ± 3.02 and 51.42 ± 1.14 years old respectively, while the mean age in the PTMC group was 45.12 ± 11.91 years old. There was a significant age difference between groups ($p<0.001$) indicating that the age in the mechanical valve group was significantly higher than biological ($p=0.002$) and PTMC ($p=0.021$) groups.

The median and IQR for follow up duration were 4.00 and 3.00 years respectively (ranging from 0 to 17 years). There was no significant difference in terms of follow up duration between intervention groups ($p=0.229$). The median and IQR for follow up intervals were 4.00 and 3.00 times respectively (ranging from 0.0 to 17.0 times). The characteristics of study subjects at follow up is presented in Table 2 and 3. The history of PTMC was present in 18 (13.3%) subjects among, whom 3 (16.7%) were in biologic valve group, 13 (72.2%) in mechanical valve group and 2 (11.1%) in PTMC group. There was no significant difference between groups in terms of the frequency of secondary PTMC ($p=0.368$). Mean trans mitral gradient was significantly different between intervention groups ($p<0.001$) indicating a significant difference in MG between biological and mechanical group ($p<0.001$) and mechanical and PTMC groups ($p<0.001$) (Table 2). Readmission was recorded in 36 (26.7%) of the subjects (Table 3). There was a significant difference in terms of mitral regurgitation severity ($p<0.001$), right ventricular size ($p=0.014$) and right ventricular function ($p=0.02$) between intervention groups (Table 3). The NYHA class was significantly improved at follow up compared to baseline ($p=0.039$) (Figure 1) but there was no significant difference between intervention groups ($p=0.505$).

Table 1. Baseline characteristics of study subjects

Variable		Total Frequency (%)	Biologic valve Frequency (%)	Mechanical valve Frequency (%)	PTMC Frequency (%)	p
Gender	Male	29 (21.5%)	3 (15.8%)	20 (24.1%)	6 (18.2%)	0.633
	Female	106 (78.5%)	16 (84.2%)	63 (75.9%)	27 (81.8%)	
Diabetes		17 (12.6%)	3 (15.8%)	11 (13.3%)	3 (9.1%)	0.749
Hypertension		21 (15.6%)	2 (10.5%)	14 (16.9%)	5 (15.2%)	0.787
Hyperlipidemia		20 (14.8%)	2 (10.5%)	15 (18.1%)	3 (9.1%)	0.400
Smoking		15 (11.1%)	0 (0.0%)	5 (6.0%)	10 (30.0%)	<0.001**
Cerebrovascular accident		13 (9.6%)	4 (21.1%)	9 (10.8%)	0 (0.0%)	0.039*
Chronic kidney disease		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Thyroid abnormality		6 (4.4%)	1 (5.3%)	5 (6.0%)	0 (0.0%)	0.358
Ischemic heart disease		8 (5.9%)	2 (10.5%)	6 (20.7%)	0 (0.0%)	0.074
Rhythm	NSR	81 (60.0%)	12 (63.2%)	43 (51.8%)	26 (78.8%)	0.027*
	AF	54 (40.0%)	7 (36.8%)	40 (48.2%)	7 (21.2%)	
MS severity	Paravalvular	6 (4.4%)	1 (5.3%)	5 (6.0%)	0 (0.0%)	-
	Progressive	10 (7.4%)	0 (0.0%)	0 (0.0%)	10 (30.3%)	
	Severe	18 913.3%)	0 (0.0%)	0 (0.0%)	18 (54.5%)	
	Very severe	5 (3.7%)	0 (0.0%)	0 (0.0%)	5 (15.2%)	
Type of intervention	MVR	51 (37.8%)	-	-	-	-
	PTMC	33 (24.4%)	-	-	-	
	MVR TV repair	17 (12.6%)	-	-	-	
	MVR AVR	15 (11.1%)	-	-	-	
	MVR CABG	6 (4.4%)	-	-	-	
	MVR TVR	5 (3.7%)	-	-	-	
	MVR AVR TV repair	5 (3.7%)	-	-	-	
	MVR AVR TVR	3 (2.2%)	-	-	-	
NYHA	Class I	7 (5.2%)	3 (15.8%)	3 (3.6%)	1 (3.0%)	0.080
	Class II	46 (34.1%)	6 (31.6%)	24 (28.9%)	16 (48.5%)	
	Class III	42 (31.1%)	5 (26.3%)	26 931.3%)	11 (33.3%)	
	Class IV	40 (29.6%)	5 (26.3%)	30 (36.1%)	5 (15.2%)	

NSR= normal sinus rhythm, AF= atrial fibrillation, MVR= mitral valve replacement, PTMC= percutaneous transvenous mitral commissurotomy, TV= tricuspid valve, AVR= aortic valve replacement, CABG= coronary artery bypass graft, TVR= tricuspid valve replacement, NYHA= New York Heart Association classification

* Significant at α=0.05

** Significant at α=0.01

Table 2. Characteristics of study subjects at follow up

Variable	Total Mean ± SD	Biological valve Mean ± SD	Mechanical valve Mean ± SD	PTMC Mean ± SD	p
BMI (kg/m^2)	27.82 ± 5.53	26.16 ± 3.14	27.60 ± 6.00	29.35 ± 5.09	0.111
Visit intervals	4.00 (3.00)	5.00 (3.00)	4.00 (4.00)	4.00 (3.00)	0.229
PAP (mmHg)	34.21 ± 8.60	32.63 ± 9.06	35.49 ± 9.11	31.88 ± 6.29	0.085
EF (%)	54.51 ± 7.36	55.62 ± 4.49	54.29 ± 7.91	54.33 ± 7.35	0.719
MG	4.81 ± 1.65	5.76 ± 2.07[a]	4.22 ± 1.29[ab]	5.73 ± 1.55[b]	<0.001**
TV annulus (??)	3.19 ±0.45	3.05 ± 0.36	3.21 ± 0.50	3.21 ± 0.34	0.355

BMI= body mass index, kg= kilogram, m=meter, PAP= pulmonary artery pressure, mm= millimeter, EF= ejection fraction, MG= trans mitral gradient, TV= tricuspid valve, PTMC= percutaneous transvenous mitral commissurotomy

[a] p<0.001, [b] p<0.001

* Significant at α=0.05

** Significant at α=0.01

Table 3. Characteristics of study subjects at follow up

Variable		Total Frequency (%)	Biological valve Frequency (%)	Mechanical valve Frequency (%)	PTMC Frequency (%)	p
Readmission		36 (26.7%)	5 (26.3%)	24 (28.9%)	7 (21.2%)	0.698
Hemodynamic motion	Normal	96 (07.0%)	17 (89.5%)	79 (98.8%)	0 (0.0%)	0.093
	Abnormal	3 (3.0%)	2 (10.5%)	1 (1.3%)	0 (0.0%)	
	Normal	117 (86.7%)	17 (89.5%)	70 (84.3%)	30 (90.9%)	
AS	Mild	1 (0.7%)	0 (0.0%)	1 (1.2%)	0 (0.0%)	0.844
	Progressive	13 (9.6%)	1 (5.3%)	9 (10.8%)	3 (9.1%)	
	Abnormal	4 (3.0%)	1 (5.3%)	3 (3.6%)	0 (0.0%)	
	Normal	73 (54.1%)	8 (42.1%)	48 (57.8%)	17 (51.5%)	
AI	Mild	41 (30.4%)	8 (42.1%)	23 (27.7%)	10 (30.3%)	0.209
	Moderate	20 (14.8%)	2 (10.5%)	12 (14.5%)	6 (18.2%)	
	Transvalvular	1 (0.7%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	
	No	101 (74.8%)	18 (94.7%)	78 (94.0%)	5 (15.2%)	
	Mild	15 (11.1%)	0 (0.0%)	0 (0.0%)	15 (45.5%)	
Mitral regurgitation	Moderate	12 (8.9%)	0 (0.0%)	0 (0.0%)	12 (36.4%)	<0.001**
	severe	1 (7.0%)	0 (0.0%)	0 (0.0%)	1 (3.0%)	
	Paravalvular	6 (4.4%)	1 (5.3%)	5 (6.0%)	0 (0.0%)	
	Progressive	10 (7.4%)	0 (0.0%)	0 (0.0%)	10 (30.3%)	
MS severity	Severe	18 913.3%)	0 (0.0%)	0 (0.0%)	18 (54.5%)	-
	Very severe	5 (3.7%)	0 (0.0%)	0 (0.0%)	5 (15.2%)	
Rheumatic TV involvement		38 (28.1%)	6 (31.6%)	28 (33.7%)	4 (12.1%)	0.061
	No	11 (8.1%)	1 (5.3%)	7 (8.4%)	3 (9.1%)	
TR	Mild	72 (53.3%)	13 (68.4%)	36 (43.4%)	23 (69.7%)	0.151
	Moderate	32 (23.7%)	4 (21.1%)	22 (26.5%)	6 (18.2%)	
	Severe	18 (13.3%)	1 (5.3%)	16 (19.3%)	1 (3.0%)	
Right ventricle size	Normal	104 (77.0%)	17 (89.5%)	57 (68.7%)	30 (90.9%)	0.014*
	Abnormal	31 (23.0%)	2 (10.5%)	26 (31.3%)	3 (9.1%)	
Right ventricular function	Normal	93 (68.9%)	17 (89.5%)	48 (57.8%)	28 (84.8%)	0.002**
	Reduced	42 (31.1%)	2 (10.5%)	35 (42.2%)	5 (15.2%)	
	None	71 (52.6%)	10 (52.6%)	46 (55.4%)	15 (45.5%)	
	Dyspnea	59 (43.7%)	7 (36.8%)	34 (41.0%)	18 (54.5%)	
Symptoms	Edema	2 (1.5%)	0 (0.0%)	2 (2.4%)	0 (0.0%)	0.106
	Chest pain	1 (0.7%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	
	Fatigue	1 (0.7%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	
	Palpitation	1 (0.7%)	0 (0.0%)	1 (1.2%)	0 (0.0%)	

AS= aortic stenosis, AI= aortic insufficiency, MS= mitral stenosis, TR= tricuspid regurgitation, PTMC= percutaneous transvenous mitral commissurotomy
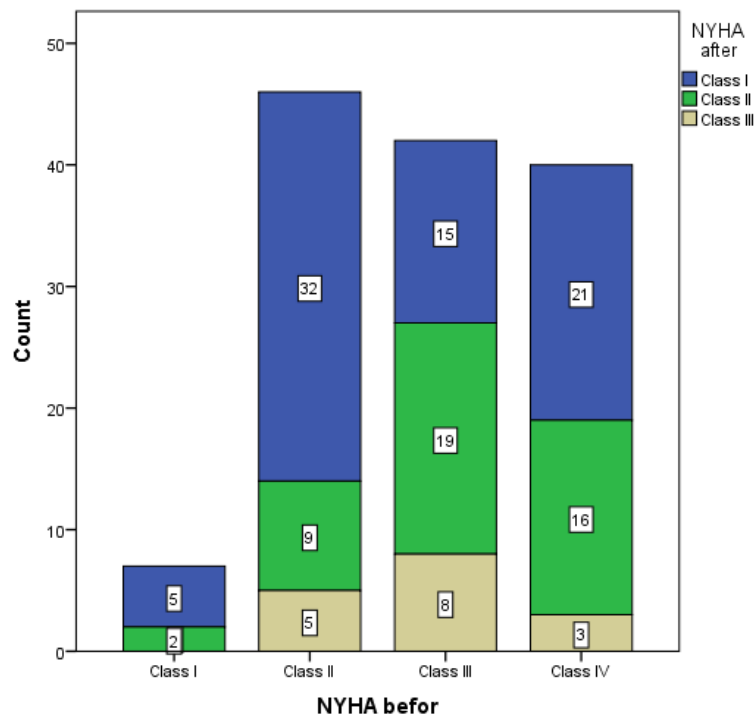
* Significant at α=0.05

** Significant at α =0.01

Figure 1. NYHA class distribution of subjects from baseline to the follow up

4. Discussion

The findings of this study revealed that majority of subjects underwent surgical valve replacement using mechanical valve (61.5%) followed by PTMC (24.4%). This finding was in contrast with the current trend in valvuloplasty, which indicates that PTMC is the first choice in developing countries (Kobayashi, 2019). As there was no significant difference in follow up duration between treatment interventions, the reason for the higher frequency of mechanical valve replacement might be due to the severity of MS and the existence of rheumatic involvement in other valves.

The findings of this study revealed that after a median of 4 years follow up 52.6% of the subjects were symptom free and the NYHA class was significantly improved in all treatment groups. This finding was in line with the findings of previous studies (Feldman et al., 2015; Bakir, Onan, Onan, Gul, & Uslu, 2013). Readmission occurred in 26.7% of the subjects in this study. As this study included three types of treatment interventions, the findings of this study were not comparable with the findings of the previous studies that assessed only one treatment method. The rate of readmission has been previously reported to range between 25% to 78% (Franzone et al., 2017; Vassileva, Ghazanfari, Spertus, McNeely, Markwell, & Hazelrigg, 2014).

This study also revealed that the prevalence of severe (7.0%) and progressive (4.4%) mitral regurgitation and severe tricuspid regurgitation (13.3%) were low. This finding was also in line with the findings of previous studies (Feldman et al., 2015; Bakir, Onan, Onan, Gul, & Uslu, 2013; Jackson et al., 2018).

Comparison of the outcomes between groups Echocardiographic findings of this study revealed that the size and function of the right ventricle were significantly higher in mechanical valve group at follow up compared to biologic valve and PTMC groups. Similar finding was observed in MS patients with comorbid and severe TR (Pubmeddev et al., 2019). Similarly, trans mitral gradient was significantly higher in biological valve and PTMC groups compared to mechanical valve group. This finding was in contrast with the finding of the study by Sharma et al. (2013), that reported no significant difference in trans mitral gradient between surgical valve replacement and PTMC in patients undergoing second time PTMC after prior valvuloplasty (Sharma et al., 2014). The reason for the difference in the findings of the studies might be due to the smaller sample size in the study by Sharma et al. (2013) and the inclusion of failed valvuloplasty cases. Furthermore, mitral regurgitation was significantly more prevalent in the PTMC group. This finding was in contrast with the findings of the study by Jackson et al. (2018) on 223 patients undergoing either PTMC or surgical repair and reported higher incidence of MR in the surgery

group (Jackson et al., 2018). The reason for this controversy might be due to the larger sample size in the study by Jackson et al. (2018).

One of the limitations of this study was lack of information regarding the pre-existing valvular abnormalities and baseline echocardiographic assessment records. It is recommended for further researches to include patients with available echocardiographic assessments in order to be able to identify changes in these parameters. The strength of this study was the duration of follow up and the inclusion of the three main methods of MS treatment in the study. but there is a need for larger and multicenter studies to identify the general pattern of long-term outcomes among these three intervention types.

5. Conclusion

The findings of this study revealed that after the median of 4 years follow up, the functional class of MS patients decreased significantly in all groups but PTMC and biologic valves were prone to increased trans mitral gradient, while surgical valve replacement methods were prone to abnormal right ventricle size and decreased right ventricular function.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Study of Anticancer Activity of Pratensein and Pratensein Glycoside Isolated from *Cuscuta kotchiana*

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Abstract

In the present paper, we demonstrate that extract of *Cuscuta kotchiana* is able to inhibit *in vitro* proliferation of two human breast cancer cell lines, MCF-7 and MDA-MB-231. The expression levels of p53, bcl-2, caspase-3 and bax genes at the mRNA and protein levels were evaluated using quantitative Real Time PCR and western blot analysis. The most active fractions of *C. kotchiana* were detected by NMR as pratensein and pratensein glycoside. The cytotoxic activity of pratensein glycoside was significantly more than pratensein. The expression level of bcl2 gene was decreased in cancer cells treated by both compounds at CC50 concentrations. But the expression levels of caspase-3, p53 and bax genes were increased in treated cancer cells. In conclusion, all the data demonstrated that the glycoside form of pratensein is important agent in inducing apoptosis in human breast cancer cells.

Keywords: Anticancer activity, pratensein, pratensein glycoside, apoptosis, bcl2, p53

1. Introduction

An increasing number of research papers appoint that medicinal plants exhibit a variety of therapeutic properties and could provide health security to rural people in primary health care (Buonaguro et al., 2007). Among medicinal plants, Cuscuta species appear to be relevant. The genus Cuscuta (Convolvulaceae family) also known as dodder is an obligate stem parasite. Cuscuta species cannot complete their life cycle without attachment to host plants and they are totally dependent on its host plant for assimilates, nutrients and water supply. The parasitic relationship of Cuscuta specious with its hosts has been subjected by numerous investigations (Cos et al., 2003 and De Clercq et al., 2009). About 270 species of Cuscuta have been reported throughout the world (Vermani et al., 2002 and Rates et al., (2001). Some specious of Cuscuta have been reported to have potent anti-cancer and anti-viral activities (De Clercq et al., 2009 and Alqasoumi et al., 2008). *Cuscuta kotchiana* is one of the most common species of Cuscuta (Vermani et al., 2002). It has broad geographical distribution and is one of the most damaging parasite worldwide (Singh et al., 2005; Wang et al., 2006). *Cuscuta kotchiana* is the most prevalent species in Iran. So in the present study, anti-cancer activity of *C. kotchiana* has been studied.

2. Methodology

2.1 Plant Material

The aerial parts of *C. kotchiana* were collected from University of Isfahan herbarium, Iran in Oct 2011. The plant material was carefully dried and powdered.

2.2 Extraction and Isolation of Compounds

Methanol extract (98%) of dried parts of *C. kotchiana* were prepared. The extraction was done thrice at 40°C. Then, the resulting liquid was collected, filtered and reduced through evaporation by a rotary evaporator (Stroglass, Italy) at 45°C and dried using a freeze dryer (Zirbus, Germany). Silica-gel column fractionation chromatography was carried out separately with the dried methanol extract of *C. kotchiana*. Dried methanol extract of *C.kotchiana* (6 g) was eluted with Chloroform: Aceton: Methanol: (10:0:0 – 0:0:10, v/v/v) and 100% methanol. Fractions 1–23 (0.20, 0.24, 0.2, 0.25, 0.23, 0.25, 0.21, 0.28, 0.22, 0.25, 0.20, 0.23, 0.24, 0.21, 0.23,

0.24, 0.23, 0.22, 0.25, 0.2, 0.25, 0.21, 0.20g) were obtained. Fraction 8 and 13 were found to have anti-cancer activity and were analyzed by NMR analysis.

2.3 NMR Analysis

NMR screening was used to approve structure of active compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 500 MHz spectrometer by use of CDCl$_3$ as residual solvent with chemical shifts expressed in parts per million (ppm)**.**

2.4 Culture Medium and Cell Lines

MCF-7 and MDA-MB-231 breast cell lines and Human embryonic kidney normal cell line (HEK-293T) were acquired from National Cell Bank of Pasture Institute, Tehran, Iran. Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 100U/ml penicillin and 100μg ml$^-$ streptomycin and 5mM L$^-$ glutamine. The cell lines were cultured at 37 ºC under 5% CO2 condition. All reagents and cell culture media were purchased from Gibco Company (Germany).

2.5 Cytotoxicity Assay

Cellular toxicity of pratensein and pratensein glycoside on cultured cells was determined using MTT assay (Twenty man et al., 1987). 5×10^4 cells per well were cultured in 96-well plates and incubated for 2 h. Then, cells were treated with different concentrations of extracts and incubated for 48 h. Later, MTT solution (25 μl of 5 mg/ml, Roche) was added to each well, and the plate was incubated for an additional 4 h. Finally, the medium was removed and 150 μl of DMSO was added to solubilize the formed formazan crystals. The amount of formazan crystal was determined by measuring the absorbance at 492 nm using a microplate spectrophotometer (Awareness Technology Inc., stat fax 2100). Then 50% cell cytotoxic concentration (CC50) values for both extracts isolated from *C. kotchiana* was calculated. All assays were carried out in triplicate.

2.6 Quantitative Real-Time Polymerase Chain Reaction Assay for p53, bcl-2, Caspase-3 and Bax

Expression levels of four widely established apoptotic-related mRNAs, p53, bcl-2, caspase-3 and bax were analyzed using Real Time PCR assay as described (Suzuki et al., 1999 and Ni et al., 2006). Real Time PCR was performed to quantify the amount of mRNA in untreated and treated cells. MCF-7 and MDA-MB-231 cells were treated with pratensein and pratensein glycoside at CC50 concentration for 6 and 12-h periods. Total cellular RNA was isolated from the untreated and treated cells using the Tri-Pure Isolation Reagent (Roche, USA), according to the manufacturer's instructions. A PCR reaction mixture of 50 μl containing 5 μl of dH$_2$O, 25 μl of Taq Man Universal PCR Master Mix, 5 μl of forward primer, 5 μl reverse primer, 5 μl FAM- TAMRA probes, 0.5 μl of reverse transcriptase, 2 μl random hexmer and 2 μl of purified RNA were used. Four pairs of primers were separately used to amplify the p53, bcl2 and caspase-3 and bax genes, the other pair for endogenous control gene, gapdh. The primers and probes have been shown in Table 1. Real-time PCR was carried out on Corbett Cycler. Cycling conditions were as follows: initial reverse transcription at 55°C for 45 min, 1 cycle denaturation at 95ºC with 10 min hold, followed by 40 cycles of 95ºC with 15 s hold, annealing temperature at 60ºC (p53, bcl2, caspase-3 and gapdh) with a 60 s hold. A negative control was included in each run to access specificity of primers and possible contamination. Primers and probes were synthesized by Metabion Company (Germany). Gene expression was normalized to gapdh using the comparative $2^{-\Delta\Delta CT}$ method, with expression levels in the untreated control.

Table1. The primer and probe sequences used in real-time PCR assay

Gene	Sequence
p53	Forward:5'-AGAGTCTATAGGCCCACCCC-3'
	Reverse: 5'-GCTCGACGCTAGGATCTGAC-3'
	Probe:5-FAM-TTGGGCAGTGCTCGCT-MGB-3
bcl-2	Forward:5'-TTCGATCAGGAAGGCTAGAGTT-3'
	Reverse:5'-TCGGTCTCCTAAAAGCAG GC-3'
	Probe:5′-(FAM)CCCAGAGCATCAGGCCGCCAC(TAMRA)-3′
gapdh	Forward:5'-CATGGGGAAGGTGAAGGTCGA-3'
	Reverse: 5'-TTGGCTCCCCCCTGCAAATGAG-3'
	Probe:5′-(JOE)CCGACTCTTGCCCTTCGAC(TAMRA)-3′
caspase-3	Forward:5'-TGCGCTGCTCTGCCTTCT-3'
	Reverse:5'-CCATGGGTAGCAGCTCCTTC-3'
	Probe:5-FAM-AGCTTCTTCATTTGTGTGCTCCGCTTTCA(TAMRA)-3′
bax	5′- CATGTTTTCTGACGGCAACTTC -3′
	5′- AGGGCCTTGAGCACCAGTTT-3'
	Probe:5′-(FAM) CCGGGTTGTCGCCCTTTTCTACTTTG(TAMRA)-3′

2.7 Western Blot Analysis

The expression levels of P53 and Caspase-3, Bcl-2 and Bax proteins in MCF-7 and MDA-MB-231 cells were assessed by western blot method as described by Fido et al. (1995). Both cells (5×10^6 cells/ml) were treated with pratensein and pratensein glycoside at CC50 concentrations for 48 h at 37 °C. Cells were lysed with 10 μl of lyses buffer (120 mmol/L Tris-HCl, 2 mmol/L N-ethylmaleimide, 2 mmol/L phenylmethyl sulfonylfluoride, 4% sodium dodecylsulfate, 4% dithiothreitol, 20% glycerol, 0.01% bromophenol blue, 2 mol/L urea and 10 mmol/L Na-EDTA at pH=6.8. Cell lysates were centrifuged at 16000 rpm/min for 20 min at 4 °C. 50 μg of each sample was separately resolved by SDS-PAGE and move onto a nitrocellulose membrane overnight at 30 mA. Membranes were blocked with 2% BSA diluted in PBS for 1 h at 37 °C. Membranes were incubated with saturating concentration of primary antibody (anti-P53; anti-Caspase-3, anti-Bcl2, anti-Bax) for 1 h under gentle agitation. The blots were washed three times and incubated with horse reddish peroxdase-conjugated anti-mouse IgG antibody for 1 h at 37 °C. Diaminobenzidine reagent was used to develop the immunoblots.

2.8 Statistical Analysis

Data from five independent experiments are presented as mean±SD. The CC50 values were calculated by Microsoft Excel 2003. One-way analysis of variance (ANOVA) test was used to assess significance between the test sample and solvent control. P value< 0.05 was considered to be statistically significant.

3. Results

3.1 NMR Analysis

The two active fractions obtained from *C. kotchiana* were fractions 8 and 13 which determined by NMR as pratensein and pratensein glycoside.

Pratensein:

^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.28 (s, 1H), 7.34 (d, 1H, J = 1.8 Hz), 7.02 (dd, 1H, J_1 = 7.8, J_2 =1.8 Hz), 6.88 (d, 1H, J = 8.4 Hz), 6.28 (d, 1H, J = 1.8 Hz), 6.16 (d, 1H, J = 1.8 Hz), 5.42 (s, OH), 3.81 (s, 3H). **^{13}CNMR (CDCl$_3$, 100 MHz):** δ = 181.32, 166.43, 162.80, 160.02, 154.58, 149.46, 148.01, 124.86, 123.75, 122.79, 116.81, 112.94, 105.85, 98.82, 94.02, 56.19.

Pratensein 7-O-glycoside:

^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.27 (s, 1H), 7.32 (d, 1H, J = 1.8 Hz), 7.02 (dd, 1H, J_1 = 7.8, J_2 =1.8 Hz), 6.88 (d, 1H, J = 8.4 Hz), 6.26 (d, 1H, J = 1.8 Hz), 6.16 (d, 1H, J = 1.8 Hz), 5.36 (s, phenolic OH), 4.87 (d, 1H, J = 7.3 Hz), 4.79 (dd, 1H, J_1 = 9.2, J_2 = 8.3 Hz), 4.38 (t, 1H, J = 7.5 Hz), 3.90 (m, 1H), 3.86 (s, 3H), 3.76 (dd, 1H, J_1 = 9.7 Hz, J_2 = 2.1Hz), 3.72 (d, 2H, J = 11.8 Hz), 3.60 (s, glycoside OH).

^{13}CNMR (CDCl$_3$, 100 MHz): δ = 181.30, 166.31, 161.00, 159.21, 154.50, 149.45, 148.00, 124.69, 123.75, 122.79, 116.81, 112.94, 105.85, 98.58, 98.13, 92.62, 78.45, 76.11, 72.32, 69.81, 61.51, 56.17.

3.2 Cytotoxicy Assay

Different fractions of *C. kotchiana* at 100 μg/ml were tested for cytoxicity against MCF-7 and MDA-MB-231 cell lines. Results showed that pratensein and pratensein 7-O-glycoside potentially inhibited viability of MCF-7 and MDA-MB-231 cell lines. Cytotoxic activity of these two extracts were further tested at different concentrations (2.5, 5, 10, 25, 50, 100, 150, 200 μg/ml). As shown in Fig. 1, cytotoxic activities of both extracts are dose-dependent. The CC50 values of pratensein and pratensein 7-O-glycoside were determined around 100 and 8.5μg/ml for MCF-7 cells, 125 and 23μg/ml for MDA-MB 231 cells respectively. The results showed that cytotoxic activity of pratensein 7-O-glycoside on MCF7 and MDA-MB 231 cells was more than pratensein. The results also demonstrated that cytotoxic activity of these extracts on both cancer cells were significantly more than HEK-293 cells.
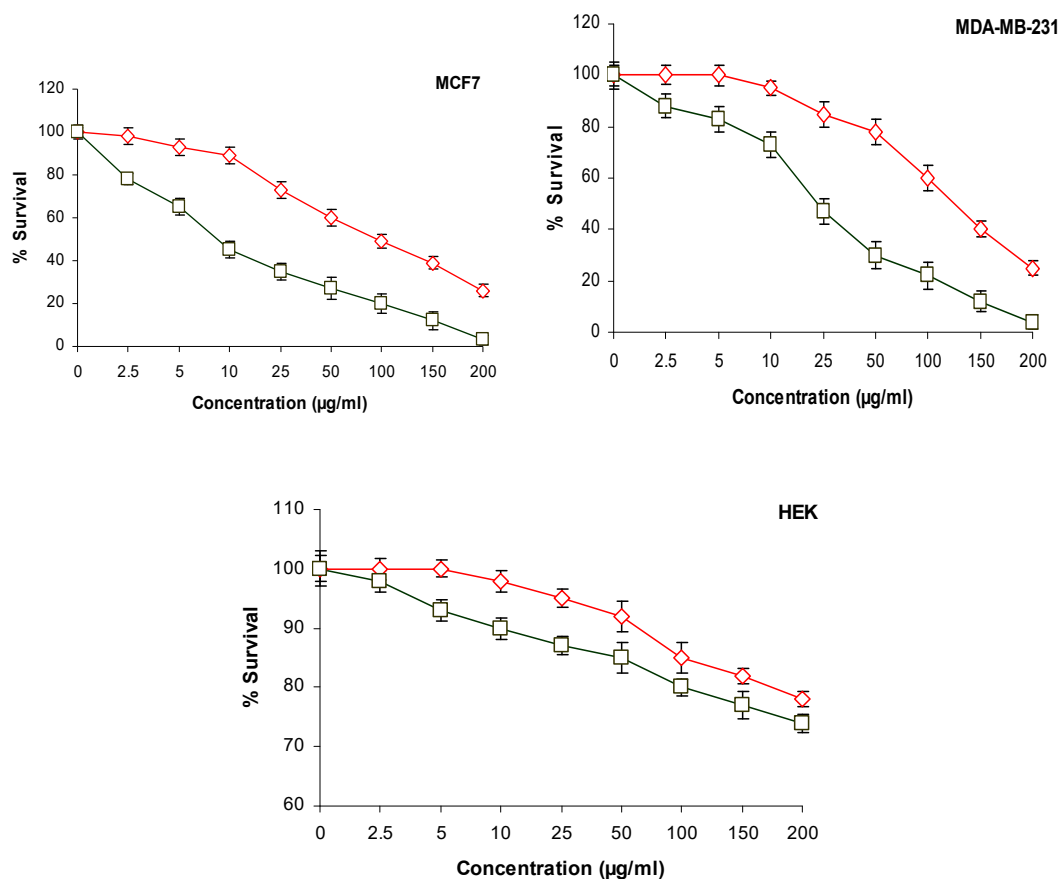
Figure 1. Cytotoxic activity of pratensein (◇) and pratensein glycoside □ against MCF7, MDA-MB-231 and HEK cell lines. CC50 values of pratensein and pratensein 7-O-glycoside were calculated around 100 and 8.5 µg/ml for MCF-7 cells, 125 and 23µg/ml for MDA-MB 231 cells respectively. Each value is the result of mean ± SD of three independent experiments. P value <0.05 was considered to be statistically significant.

3.3 Expression Level of Apoptosis-Related Genes

The relative quantification values of p53 and bcl-2, bax and caspase-3 genes in MCF7 and MDA-MB 231 cells which was induced by pratensein and pratensein 7-O-glycoside calculated based on the $2^{-\Delta\Delta Ct}$. Figures 2 and 3 showed that the expression levels of p53 and bcl2 respectively increased and decreased in cells treated with both extracts compared to untreated cells. The expression level of p53 and bcl2 in both breast cancer cells treated with extracts was time dependent. The strongest relative expression level of p53 after 12 h incubation, normalized to gapdh, was increased in MDA-MB 231 and MCF-7 cells treated with pratensein 7-O-glycoside up to 7 and 9 folds respectively (Fig. 2). Figure 3 indicated that the lowest relative expression level of bcl-2 was obtained in MCF-7 and MDA-MB 231 cells treated with pratensein and pratensein 7-O-glycoside up to 1 and 0.9 folds compared to control (1.8 fold). The relative expression of caspase-3 in MDA-MB-231 cancer cells treated with these two extracts was also increased as time-dependent to reach the maximum level at 12 h after stimulation. The maximum relative expression of caspase-3 was determined up to 5-7 folds in MDA-MB-231 cell line (Fig. 4). The absence of caspase-3 in MCF-7 cell leads to lack of any gene expression in treated and untreated cells (data not shown). The relative expression of bax in MDA-MB-231 and MCF7 cancer cells treated with both extracts was increased to reach the maximum of 7 to 8 folds at 12 h after stimulation.
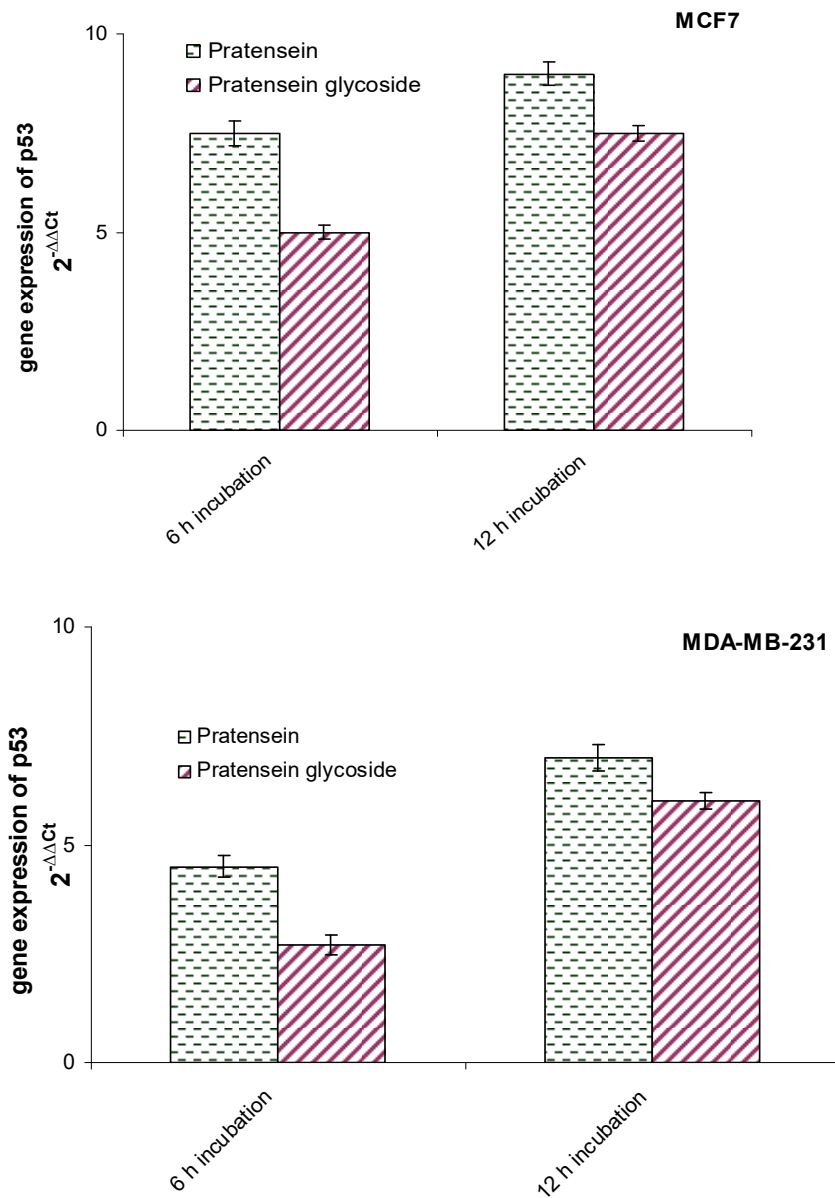
Figure 2. Changes in p53 mRNA expression in MCF7 and MDA-MB231 cells after treatment with pratensein (◇) and pratensein glycoside (□) at CC50 concentration for 6 and 12 h, in comparison with control cells. Detection and quantification of p53 mRNA in total cellular RNA was performed by RT-PCR. The value of $2^{-\Delta\Delta Ct}$ represents the expression of the p53 gene in treated cells normalized to gapdh relative to the normalized expression of p53 gene in control cells. P value <0.05 was considered significant.

Figure 3. Changes in bcl2 mRNA expression in MCF7 and MDA-MB231 cells after treatment with pratensein (◇) and pratensein glycoside (☐) for 6 and 12 h, in comparison with control cells. Detection and quantification of caspase-3 mRNA in total cellular RNA was performed by RT-PCR. The value of 2–ΔΔCt represents the expression of the caspase-3 gene in treated cells normalized to gapdh relative to the normalized expression of caspase-3 gene in control cells.
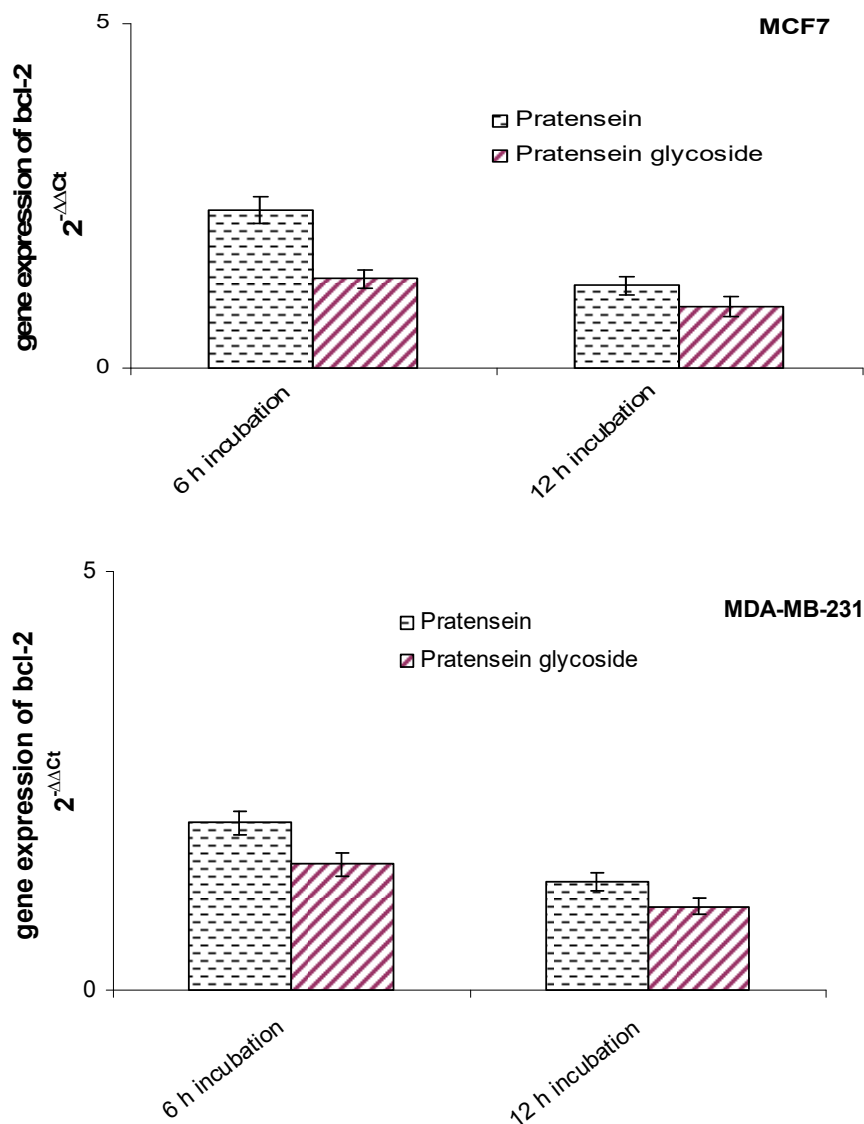
Figure 4. Changes in caspase-3 mRNA expression in MDA-MB231 cells after treatment with pratensein (◇) and pratensein glycoside (□) for 6 and 12 h, in comparison with control cells. Detection and quantification of caspase-3 mRNA in total cellular RNA was performed by RT-PCR. The value of 2–ΔΔCt represents the expression of the caspase-3 gene in treated cells normalized to gapdh relative to the normalized expression of caspase-3 gene in control cells.
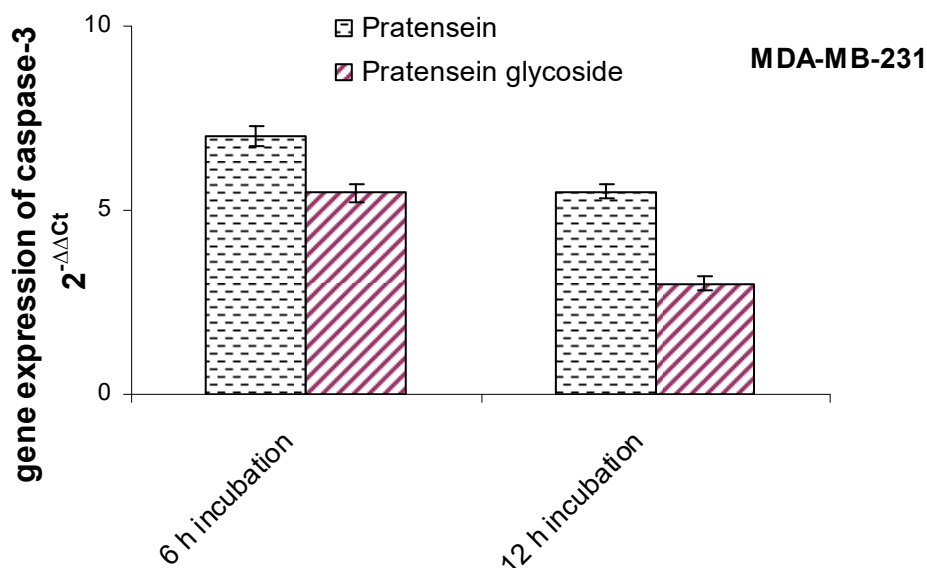
3.5 Western Blot Analysis

The expression level of Bcl2, Bax and P53 proteins in MCF7 and MDA-MB23 cells treated with pratensein and pratensein glucoside along with β-actin as an internal control are shown by western blotting analysis. p53 and bax genes expressed respectively 53-kda and 21-kda proteins on western blotting. MDA and MCF7 cells also encode a 32-kDa and 24-kda proteins whose associate with Caspase-3 and Bcl2 proteins. As shown in Fig. 6, Western blot analysis showed the increase in band intensity of P53 and Bax proteins in MCF7 and MDA cells when compared to the internal control β-actin. Caspase-3 was also increased in MDA cells compared to β-actin. The absence of caspase-3 gene in MCF-7 cell leads to lack of any Caspase-3 protein in treated and untreated cells. Bcl2 protein was decreased in cancer cells treated by both compounds at CC50 concentrations.

4. Discussion

In this study, pratensein and pratensein glucoside isolated from *C. kotchiana* were considered as anticancer compounds. Pratensein is a member of the flavonoid family which found in various plants such as *Trifolium pratense* (Wong et al., 1963). There are reports on biological activities of pratensein including antioxidant and anti-inflammatory effects (Chen et al., 2008). The cytotoxic activity of pratensein and its glycoside derivate has been report here for the first time. Our findings are in agreement with some previous studies showing that some flavones isolated from medicinal plants are able to diminish the growth of breast cancer cells (Rodgers et al., 1998 and Dolečková et al., 2012). In the present study, we have presented the antitumor effects of pratensein and pratensein glucoside in breast cancer cells through induction of Caspase-3, Bax and P53 expression levels and inhibition of Bcl2 activation in a time dependent manner. Most of the drugs currently used to treat cancer patients exert their anti-tumor activity through Caspase-3 and Bcl-2 mediated pathways. Therefore, the development of effective drugs which can reactivate wild-type P53, Bax and Caspase-3 is an attractive therapeutic strategy (Sano et al., 1997 and Issaeva et al., 2004). The previous results demonstrated that some isoflavone could induce apoptosis in cancer cells through the alteration of Bax/Bcl2 ratio, which associated with the release of cytochrome C and induction of apoptotic protease activating factor-1 (Apaf-1) (Chew et al., 2003 and kim et al., 1998). The present results also showed that the cytotoxic activity of pratensein glycoside was significantly more than pratensein. These observations indicated pratensein glycoside have a good potential to be used as anticancer agent

in patient. Nevertheless, further studies are needed to verify the molecular mechanism of pratensein glycoside on cancer cells.

5. Conclusion

Based on the findings of this study, it can be concluded that pratensein glycoside isolated from *C. kotchiana* is a new potential drug candidate for *in vivo* testing of patients with cancer.

Acknowledgments

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A Study on Testicular Development in *Apteronotus albifrons*

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Abstract

Apteronotus albifrons has its cloacal aperture located in the front of the body and on the back of the lower jaw. *A. albifrons* possess a pair of testes attached on the mesentery below its liver on the back of the abdominal cavity. In the early days of development, the testes are in translucent elongated thin strips in nude colour. As they develop, the testes will grow thicker and become white or creamy white in color. The rear end of the testes will split whereas the front end will merge to form the vas deferens which is connected to the external environment through the cloacal aperture. Testes of *A. albifrons* are lobular. There are six stages in its development. At 6 months of age, the testis structure shows clear medium with primary spermatocytes and abundant spermatogonia, suggesting that development is into the stageII. At 8 months of age, testes in male *A. albifrons* are in the shape of a thin rod in white with blood vessels in presence. Germ cells consist of spermatogonia, primary spermatocytes and spermatocytes, indicating stageIII development. At 11 months of age, testes in male *A. albifrons* grow fuller and are in creamy white with apparent blood vessels present. Primary spermatocytes, secondary spermatocytes, spermatids and a small amount of sperms are president, indicating stageIV of development. At 15 months of age, testes in male *A. albifrons* swells and consist of a large amount of sperms, reaching full maturity. This is the stageVIof development, after which is the stage featured by testes after spermiation.

Keywords: *Apteronotus albifrons*, Testis, Development

Gonadal development is vital to the reproduction and the existence of fish population. It is also one of the most crucial factors in fish reproduction. Research on testis development in fish species have been the interest of scholars for years. By far, histological and molecular biological methods have been used to look into things such as gonadal differentiation in fish, morphological features of each testes development stage and its time phasing as well as the molecular regulation in testicular development (Kaneko et al., 2015; Fan et al., 2017; Hsu et al., 2018).

Apteronotus albifrons is of *Gymnotiformes* order and *Apteronotidae* family (Wan et al., 2002). *A. albifrons* has a mild temperament and they are amazingly beautiful swimmers which gained popularity among hobbyists. *A. albifrons* has no dorsal fin and is weakly electric. They are very sensitive to water quality and are extremely difficult to breed in captivity. Research on *A. albifrons* reproduction is very few. Morphological and histological observations in terms of *A. albifrons* testicular development in captive breeding are aimed at understanding the patterns in its testicular development to enrich studies on its biological productivity. It also helps to get a solid grasp on *A. albifrons* reproduction patterns, thus providing theoretical foundation for artificial breeding of the species, which will boast significant relevance to the protection and development of this species resource.

1. Material and Methodology

1.1 Experimental Fish

Take 200 *A. albifrons* and keep in the following condition: water temperature 28±0.5℃, pH 7.5±0.5, lighting 16hr: 8hr (lighting: dark). Feed with nematode and bloodworm once daily respectively. Sex will be determined after anatomy. Take 10 fish each at 4 months of age, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 months of age respectively.

1.2 Sample Measurement and Morphological Observation of the Gonad

Measure individual body length, total length, body height, width and weight. Determine the sex via anatomy and observe gonad morphology. Take the gonad and keep it fixed in Bouin solution for 24 hours, then move to be preserved in 70% ethanol solution.

1.3 Tissue Section and Observation

Use paraffin wax and follow the steps: ① Dehydration. 70% ethanol (45 min), 80% ethanol (45 min), 90% ethanol (45 min), 95% ethanol (45 min), 100% ethanol 1 (30 min), 100% ethanol 2 (30 min). ② Clearing. 1:1 benzene-alcohol (40min), xylol 1 (40min), xylol 2 (40min). ③ Wax infiltration. 3:1 wax (30min), 1:1 wax (45min), purified wax 1 (30min), purified wax 2 (30min). ④ Embedding and blocking out. ⑤ Sectioning. Use KD-3358 microtome to section the tissues. Thickness is 4μm. ⑥ Flattening. Flatten and dry the slice with a temperature of 70℃. ⑦ HE staining. Conduct HE stain 12 hrs after the section is flattened. ⑧ Mounting with gum. Check the slice under microscope and preserve accordingly.

1.4 Data Analysis and Processing

Measure the biological parameters of the experimental fish, including body weight (BW), total length (TL), body length (BL) and gonadal weight (GW) etc. Calculate relevant gonadal development indices: Gonadosomatic index (GSI)= GW/BW x 100. Analyze the data with Microsoft Excel 2010. Mean ± SD is used for both measuring the data and calculating GSI. Conduct ANOVA analysis (SPSS software, V19.0) with the data above and check significance of difference. Note significance of difference (*P*) as 0.05. If $P \leq 0.05$ then there is statistical difference, if not, then no statistical difference.

2. Results and Analysis

2.1 Morphology and Structure of the Testes

A. albifrons has its cloacal aperture located in the front of the body and on the back of the lower jaw. *A. albifrons* possesses a pair of testes attached on the mesentery below its liver on the back of the abdominal cavity. In the early days of development, the testes are in translucent elongated strips in nude colour. As they develop, the testes will grow thicker and become white or creamy white in color. The rear end of the testes will split whereas the front end will merge to form the vas deferens which is connected to the external environment through the cloacal aperture.

2.2 Testicular Development Stages and Histological Observation

Referring to the testicular development staging methods by Yun Liu (Liu, 1933), Yaoguang Zhang (Zhang, Luo, & Zhong, 1992) and Yundong Lou (Lou, 1980) et al. and combined with the morphological features of *A. albifrons* testes and its germ cell distribution, six stages are identified during the development course of the testes:

At stageI, testes are in pairs and closely attached to the two sides of the abdominal cavity. They are in transparent and elongated thin strips which are hard to distinguish. Through histological studies, spermatogonia are randomly present and scattered among the connective tissues.

At stageII, testes are in translucent and elongated thin strips with nude color. Blood vessels are inapparent. However, testes and ovaries can be distinguished at this stage with naked eyes. Early seminiferous lobules can be visible. Primary spermatocytes are present with the number of spermatogonia increasing and apparent medium among tissues.

At stageIII, testes are in the shape of a thin rod in white with blood vessels present. Seminiferous lobule becomes larger. At this stage, germ cells in the testes generally consist of spermatogonia, primary spermatocytes and secondary spermatocytes. Primary spermatocytes are round or oval shaped. They have a smaller diameter than the spermatogonia with rich chromosomes in the nuclei and a violet colour pigmentation. The nucleus is going through the first meiosis. Secondary spermatocytes are smaller than primary spermatocytes with its nucleus in violet colour and basophilia continuing to enhance. Compared to the second stage, primary spermatocytes count is significantly increased.

At stageIV sees further development of the testes. They become larger, fuller and creamy white with clear distribution of the blood vessels. Seminiferous lobules continue to grow and so is the seminiferous lobule cavity. Primary spermatocytes, secondary spermatocytes, spermatids and a small amount of sperms along with others of the same cell types are piled up. Secondary spermatocytes, after the second mitosis, develop into spermatids that are irregular in shape with no apparent cytoplasm. Only the round nucleus is visible which has high basophilia and a violet colour. Sperm counts in mid and later stages gradually go up.

At stageV, testes are in creamy white blocks and reach maximum sizes. Blood vessels further develop, and the distribution is more distinctive. Testes are fully grown and reach full maturity. In this stage, the majority of germ cells in the testes are comprised of androcytes and sperms in metamorphosis. Sperms in great density swirled in and fulfilled the seminiferous lobule cavity, showing a deep blue colour. The sperm is the smallest cell in the

testes. It is a metamorphotic structure after the spermatid matures. The nuclei are clustered at the head of the sperm. Cells are in dark colours, in deep violet. A small number of spermatids and primary spermatocytes are visible on the edge of the seminiferous lobules. Male *A. albifrons* can discharge seminal fluid at this stage for multiple times. Emptied seminiferous lobule cavity can be visible.

At stageVI, testes become degenerated or have finished spermiation. Its size will decrease and the colour becomes light pink. Sperms in most seminiferous lobule cavities are discharged with a small amount of "old" sperms or undischarged sperms remaining to be absorbed. The rest are a very rare amount of spermatogonia and spermatocytes.

2.3 Male Gonadal Development Process
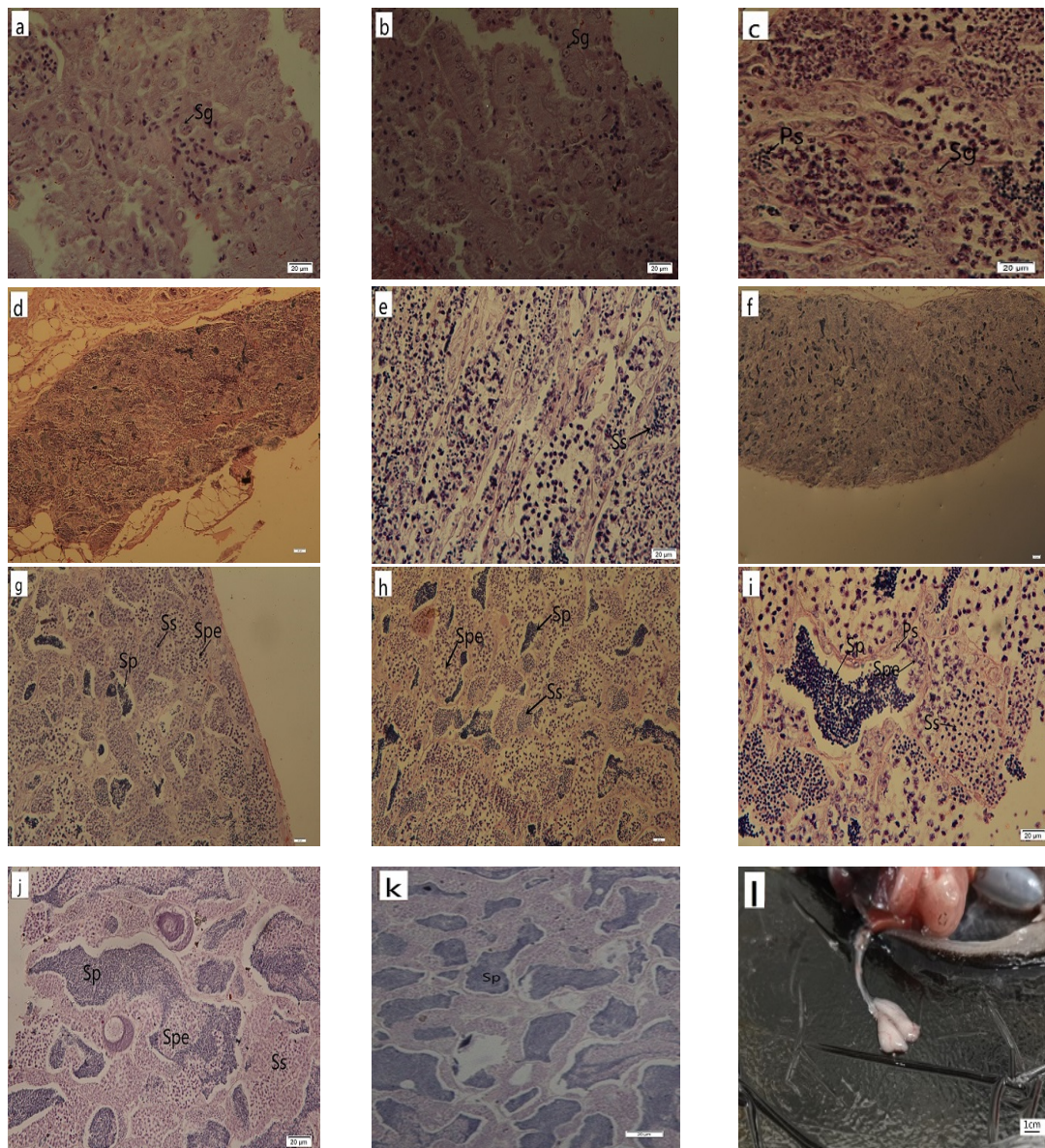
Figure 1. Testicular development of *Apteronotus albifrons*

a. stageI, showing testis of 4-month-old male fish; b. stageI, showing testis of 5-month-old male fish; c: stageII, showing testis of 6-month-old male fish; d: stageII,showing testis of 8-month-old male fish; e: stageIII, showing testis of 9-month-old male fish; f: stageIV, showing testis of 11-month-old male fish; g: stageIV, showing testis of 11-month-old male fish; h: g: stageIV, showing testis of 12-month-old male fish; i: stageIV, showing testis of 12-month-old male fish; j: stageIV,showing testis of 14-month-old male fish; k: stageV, showing testis of 15-month-old male fish; l: mature testis of 15-month-old male fish; Sg: spermatogonia; Ps: Primary spermatocyte; Ss: Secondary spermatocyte; Sp: spermatids; Sp: Sperms.

The correlation among male *A. albifrons* development parameters, its gonadal development and month of age can be seen from Table 1. At 6,7months of age, male *A. albifrons* is in testes development StageII; at 8,9,10months of age in StageIII at 11 to 14 months of age in Stage IV, and at 15th months of age in StageV. After spermiation, testes development then moves to StageVI.

Changes in male *A. albifrons* GSIs can be seen from Figure 2. As the gonad starts to develop, testes increase in weight and size and parent GSI starts to climb. Development accelerates in the StageIII and peaks during the StageV at 0.612±0.06 which is significantly higher than the 0.16±0.03 identified during the StageIII in ovaries.

Table 1. Correlation between Testicular Development and Months of age in *Apteronotus Albifrons*

| Months of age	Sample number	Overall length/mm	Body length/mm	Body height/mm	Body weight/g	Stage
4	10	66.3±1.8	52.6±1.8	10.4±0.5	0.80±0.08	I
5	10	74.6±3.8	64.3±2.1	14.7±0.5	1.48±0.06	I
6	10	82.9±2.4	72.5±0.2	17.9±0.8	2.19±0.15	II
7	10	89.9±2.0	79.6±2.8	19.6±1.4	3.69±0.05	II
8	10	103.6±2.5	86.6±1.8	20.6±1.3	5.39±0.57	III
9	10	126.4±5.3	102.2±4.7	25.4±1.3	7.98±0.53	III
10	10	156.3±6.2	139.9±6.4	34.6±1.3	15.49±0.76	III
11	10	198.3±18.3	175.7±16.2	42.7±6.9	20.99±3.24	IV
12	10	268.3±15.7	240.0±12.1	53.6±7.3	54.28±3.18	IV
15	10	323.7±19.5	282.0±18.1	66.6±8.9	69.26±5.27	V
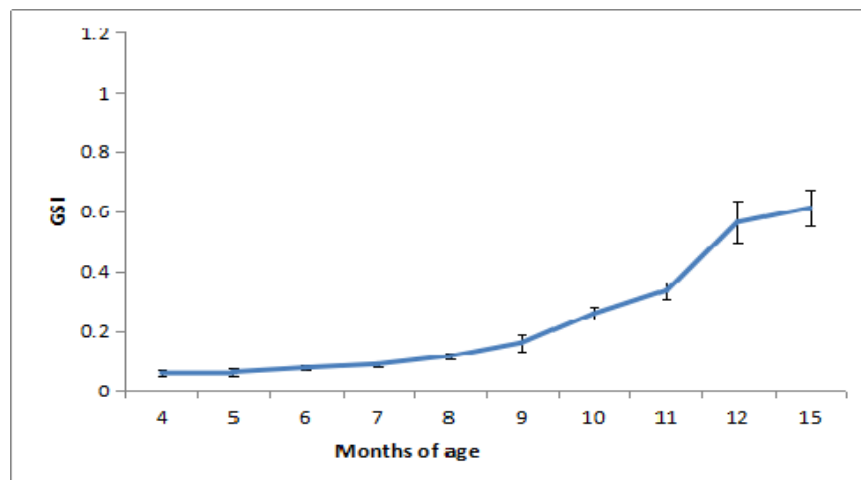
Figure 2. Monthly variations in GSI of male

3. Discussion

3.1 Male A. albifrons Gonadal Development Features

Studies on fish testicular development are far less detailed than those on ovarian development. There is yet to be a standard set of demarcations on the staging of testicular development. Based on testicular anatomy and histological features, some researchers have divided testicular development into the following five stages, i.e. immature, developing, maturing, spawning and spent (Akayli et al., 2013). Chinese researchers normally divide the process into 6 stages. As spermatogonia gradually mature, they go through five major stages, i.e. spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and sperm. *A. albifrons* testicular development demonstrates a "catch-up growth" phenomenon that goes from synchronized development to non-sync development and then to synchronized development again. This phenomenon is also present in the testicular development in teleost such as *Eleutheronema tetradactylum* (Lan et al., n. d.) and *Anguilla japonica* (D. Lin & H. R. Lin, 1984). The key features are that during the At stageIand stageII, spermatogonia and spermatocytes proliferate, and development is basically in sync. By the stageIII, the seminal vesicle near the seminiferous lobule cavity will first mature whereas the seminal vesicle near the intralobular interstitium

develops slowly which demonstrates the non-sync phenomenon. By the stageIV, seminiferous lobule cavity starts to see mature sperms and all sperms will gradually mature and continue to be released into the seminiferous lobule cavity. When developed to the stageV, the seminiferous lobule cavity, efferent tube, and vas deferens are filled with sperms. Development is then again in sync (Lan et al., n. d.).

Based on the histological structure and germ cell arrangement as well as development features, two main types of testicular structure are distinguished: a tubular and a lobular (Nagaham, 1983; Billard, 1986). There is no seminiferous lobule in tubular types which are not commonly seen in teleost fish species. Based on the arrangement of the seminiferous lobules, there are two subtypes of lobular testicular type, namely, radial type and ampulla type (Lan et al., n. d.). With radial type, the seminiferous lobules are specifically arranged in a radial layout in the testes, examples of which could be *Leiocassis crassilabris* (Chen et al., 2008) and *Eleutheronema tetradactylum* (Lan et al., n. d.)*,* whereas with ampulla type, the arrangement is usually irregular as seen in *spinibarbus caldwelli* (Lin, You, & Su, 2003). Research shows that *A. albifrons* testes are made up of many seminiferous lobules in which there are seminal vesicles where spermatids develop to maturity and then are released to seminiferous lobule cavity. Eventually, matured sperms are stored in the seminiferous lobule cavity and vas deferens. Seminiferous lobules are randomly arranged in *A. albifrons* testes. Its testicular structure falls under the ampulla type.

3.2 A. albifrons GSI and Reproduction

Compared to most teleost, male *A. albifrons* has relatively low GSI levels. Sexually matured male *Xenocypris davidi* (Bian & Yang, 1992) has a GSI as high as 7.8 whereas male *Schizopygopsismalacanthus baoxingensis* GSI can reach 5.41 during the peak of the breeding season. GSI of male *Oreochromis niloticus* ranges between 1.28 to 1.49. In contrast, male *A. albifrons* GSI is only 0.5 at the maximum. This has made it more difficult for the fish to be artificially bred. As the gonad is too small compared to the size and weight of the body, it is extremely difficult to obtain matured sperms externally (Xu et al., 2015). Gonadal development is regulated by individual hormone levels. Environmental factors also play a role in the reproduction of fish (Xu et al., 2017). Many environmental elements, such as meteorological and hydrological factors (water temperature, lighting), water physical and chemical factors (salinity, pH), fish feed and spawning substrates etc. can all impact fish reproduction (Wen & Lin, 2001; Song & Wen, 2005). With artificial breeding, feed and water quality are usually good. Gonads develop very fast in *apteronotus albifrons*, however, non-sync development between male and female *A. albifrons* were identified during the research which increased the difficulty for artificial breeding.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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The Curative Effect of a Second Curettage in Low-Risk Gestational Trophoblastic Neoplasia

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Abstract

Background: Gestational trophoblastic neoplasia (GTN), despite its widespread metastases, is a very common cancer in women that is curable. Although the GTN cases show a good response to chemotherapy, in an effort to reduce toxic drug exposure, the second curettage has been suggested for some patients. In the current study, we have aimed to compare the benefits of the second curettage in comparison with single-agent chemotherapy for low-risk GTN patients.

Methods: This retrospective observational study was carried out on GTN patients admitted to the gynecology department of Imam Khomeini Hospital in Ahvaz. The demographic profile of all participants was extracted. Patients' hospitalization records were also extracted from the files. Patients with an endometrial thickness above 10 mm were treated with re-curettage. The β hCG clearance time was estimated by the Kaplan Meier plot.

Results: In the present study, 148 patients with low-risk GTN stage 1 were studied. The time required for β-hCG clearance in patients undergoing re-curettage was significantly lower than the chemotherapy receiving group (7 months vs. 10 months, $p < 0.0001$). More than 50% of patients treated by re- curettage without needing chemotherapy. Moreover, the other 50% cases needed chemotherapy the number of courses was significantly lower than those received single-agent chemotherapy alone ($p < 0.0001$). The baseline β-hCG levels were significantly lower in those who did not need chemotherapy ($p = 0.012$). β-hCG resolution occurred more rapidly in patients undergoing re-curettage alone, while, those who received only chemotherapy had a longer duration for β-hCG clearance.

Conclusion: In general, the findings of this study showed that re-curettage could be used effectively in the treatment of GTN following molar pregnancy. This treatment reduces or eliminates the need for chemotherapy. Our findings also showed that the initial level of β-hCG could be considered as a predictive factor in response to curettage.

Keywords: GTN, Molar pregnancy, β-hCG; Second curettage

1. Introduction

Gestational trophoblastic diseases (GTD) include a variety of diseases during pregnancy that are commonly associated with abnormal trophoblastic proliferation and ranged from benign hydatidiform mole to malignant choriocarcinoma. Gestational trophoblastic neoplasia (GTN), despite its widespread metastases, is a very common cancers in women that is curable. GTN may be developed after molar, term, miscarriage, and even ectopic pregnancy. It is noteworthy that after the evacuation of molar pregnancy, persistent moles may remain in the uterus in more than in 15% of cases. Moreover, choriocarcinoma and also metastasis may develop in about 4% of cases (Khanlian, Smith, & Cole, 2003; Lurain, 2010; Seckl, Sebire, & Berkowitz, 2010).

Several staging and scoring systems have been developed for GTN. According to the Federation of Gynecology and Obstetrics (FIGO) system, GTNs are divided into low and high-risk groups. In the cases of low risk GTN the total scores obtained from the FIGO scoring system is less than 7. It is calculated based on the age of diagnosis-based, previous pregnancy outcome, interpregnancy interval, β-hCG serum level, the largest tumor size (uterus or metastasis, metastasis site, number of the identified metastases and number of drugs in previously failed chemotherapy) (Committee, 2002; van Trommel, Massuger, Verheijen, Sweep, & Thomas, 2005). The low-risk GTN patients treated with single-drug chemotherapy (Actinomycin - D or Methotrexate), while

high-risk GTNs are treated with multi-drug chemotherapy using Etoposide, Actinomycin-D, Cyclophosphamide, Vincristine, and Methotrexate) (Ng & Wong, 2003; R. Osborne & Gerulath, 2004).

When a molar pregnancy is diagnosed through routine pregnancy tests, the safest and standard way to empty the molar tissue is suction of curettage. After emptying the mole, some patients progress to a malignancy and needing chemotherapy (Kerkmeijer, Wielsma, Massuger, Sweep, & Thomas, 2007; Mangili et al., 2008). Although the GTN cases show a good response to chemotherapy, in an effort to reduce toxic drug exposure, the second curettage has been suggested for some patients (Pezeshki et al., 2004). Today, the gynecologists routinely use re-curettage for patients with persistent low-risk GTN in some medical centers. While, some other specialized centers believe that the risk of a second curettage is greater than its advantage, and therefore limit this procedure to patients with heavy bleeding (R. J. Osborne et al., 2016; van Trommel et al., 2005). In the current study, we aimed to compare the benefits of the second curettage in comparison with single-agent chemotherapy for low-risk GTN patients.

2. Material and methods

2.1 Study Design

This retrospective observational study was carried out on GTN patients admitted to the gynecology department of Imam Khomeini Hospital in Ahvaz. Women with low-risk GTN managed by second curettage or single-agent chemotherapy were included. Patients with incomplete hospital records were excluded from the study. The study has confirmed by the Ethical Committee of the Ahvaz Jundishapur University of Medical Sciences.

2.2 Measurements

The demographic profile of all participants was extracted. Patients' hospitalization records were also extracted from the files. GTN was defined as having one of the following criteria according to Table of Criteria for Diagnosis of Gestational Trophoblastic Neoplasia.

Table 1. Criteria for diagnosis of gestational trophoblastic neoplasia (Cunningham, Leveno, Bloom, Spong, & Dashe, 2014)

Criterion 1	Plateau of serum β-hCG level (± 10%) for four measurements during a period of three weeks or longer— days 1, 7, 14, 21
Criterion 2	Rise of serum β-hCG level > 10% during three weekly consecutive measurements or longer, during a period of two weeks or more—days 1, 7, 14
Criterion 3	Serum β-hCG level remains detectable for six months or more
Criterion 4	Histological criteria for choriocarcinoma

Patients with an endometrial thickness above 10 mm were treated with re-curettage, and there was no evidence in favor of uterine arteriovenous malformation (AVM) and the myometrial invasion to. Metastatic GTN patients were worked up by abdominal and pelvic sonography and Chest X-Ray.

2.3 Statistical Analysis

The data were described by descriptive statistics, including mean, median, standard deviation, frequency, and percentage. The means were compared by the independent t-test. The proportions were compared by the chi-square. The Kaplan Meyer analysis was used for determining the time for B-HCG resolution. All statistical analyses were performed using SPSS version 20. The *P-value* of less than 0,05 was considered significant.

3. Results

In the present study, 148 patients with low-risk GTN stage 1 were studied. Patients were divided into two groups as those receiving chemotherapy (group A) and re-curettage (group B). The mean age of the patients in groups A and B was 28.5 and 30 years, respectively (p = 0.13). Although most patients in group A were in the under-25 age group (69.1%), patients underwent second curettage were in the 25- to 35-year age group (64.8%). Endometrial thickness in all patients were above 10 mm. The initial β-hCG level did not show a significant difference in each group (p = 0.53). However, the time required for β-hCG clearance in patients undergoing re-curettage was significantly lower than the chemotherapy receiving group (7 months vs. 10 months, p <0.0001). More than 50% of patients in group B also needed chemotherapy. However, the number of chemotherapy courses was significantly lower than group A (p <0.0001) (Table 2).

Table 2. A comparison between patients receiving chemotherapy and patients undergoing recurettage

Characteristics	Second curettage N=74	Chemotherapy N=74	P-value
Age	30.01±5.4	28.5±6.26	0.13
<25	17 (30.9%)	38 (69.1%)	
25-35	46 (64.8%)	25 (35.2%)	
>35	11 (50%)	11 (50%)	
β-hCG Initial			
<500	1(100%)	0	P=0.53
500-5000	33(55%)	27(45%)	
5000-100000	39(45.9%)	46(54.1%)	
>100000	1(50%)	1(50%)	
B-hCG clearance	7(2-9)	10(6-12)	P<0.0001
Mol type			P=0.25
Complete Mole	59(53.2%)	52(46.8%)	
Partial Mole	15(40.5%)	22(59.5%)	
Chemo courses	4(1-7)	6(2-9)	P<0.0001

Furthermore, the patients, treated with re-curettage, were divided into two groups based on receiving or not receiving chemotherapy. The age distribution in both groups did not show any statistically significant difference (p = 0.37). However, baseline β-hCG levels were significantly lower in those which did not need chemotherapy (p = 0.012) (Table 3).

Table 3. A comparison between patients treated with second curettage and patients treated with second curettage plus chemotherapy

Characteristics	Second curettage	Second curettage+ chemotherapy	P-value
Age			P=0.37
<25	9(52.9%)	8(47.1%)	
25-35	22(47.8%)	24(52.2%)	
>35	3(27.3%)	8(72.7%)	
β-hCG Initial			
β-hCG Clearance	5(2-8)	8(4-10)	
<500	0(0%)	1(2.5%)	0.012
500-5000	22(64.7%)	11(27.5%)	
5000-100000	12(35.3%)	27(67.5%)	
>100000	0(2.5%)	1(2.5%)	
Mol type			P=0.38
Complete Mole	29(85.3%)	30(49.2%)	
Partial Mole	5(33.3%)	10(66.7%)	

β-hCG resolution occurred more rapidly in patients undergoing re-curettage alone, while, those who received only chemotherapy had a longer duration for β-hCG clearance (Figure 1).
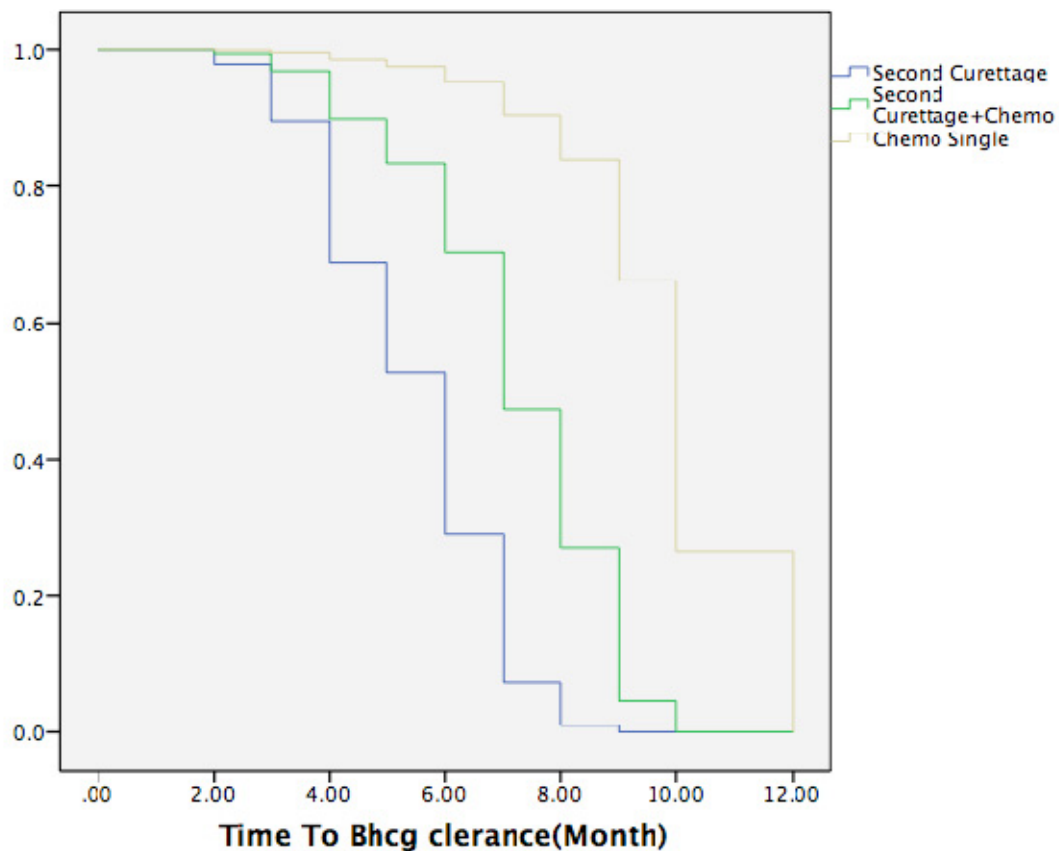
Figure 1. Comparison of β-hCG clearance duration in the studied patients.

In our study, there was not re-curettage complications such as perforation.

4. Discussion

In GTN following molar pregnancy, despite the removal of the hydatidiform mole, tumor activity persists and presented with no decrease or surging plasma B-hCG levels (van Trommel et al., 2005). Although chemotherapy is the first line treatment in these patients, re-curettage has been proposed as an alternative surgical treatment for them.

In the present study, it was shown that re-curettage caused non-chemotherapy treatment in about half of the patients and also a significant reduction with chemotherapy courses in the others. Previous findings have provided both positive and negative results. In a retrospective study, 37 GTN patients were evaluated and it was shown that only six patients recovered after curettage without the need for chemotherapy. The study also found that 8% of patients with curettage had uterine perforation. Therefore, the usefulness of curettage in this study was completely ruled out, the small sample size of this study has reduced the validity of its results (Schlaerth, Morrow, & Rodriguez, 1990). Moreover, in the study conducted by Van Trommel et al., the clinical efficacy of the second curettage was reported as 9.4%. Unlike the present study, they have examined patients with persistent trophoblastic disease (PTD) (van Trommel et al., 2005). However, in another study, Pezeshki et al. found that 60% of GTN patients recovered after re-curettage without the need for chemotherapy (Pezeshki et al., 2004). Also, in a phase II clinical trial, it was shown that re-curettage treated 40% of GTN patients without any side effects (R. J. Osborne et al., 2016). In addition, in another study, Ayatollahi et al. showed that 50% of patients were treated with re-curettage without the need for chemotherapy (Ayatollahi, Yekta, & Afsari, 2017).

Clinical centers for managing GTN patients have different approaches to the effectiveness of re-curettage. This treatment is mostly used to drain the remaining tumor tissue in the uterus or to control bleeding in newly

diagnosed patients. The risk of uterine bleeding, infection of the upper genital tract, or uterine perforation has always been cited as the main reason for discontinuation of curettage. However, the most complications have been managed by non-surgical treatments, and severe complication, including hysterectomy, had not been reported yet (Lorigan, Coleman, Ng, Coleman, & Hancock, 1996; Tidy et al., 2000). Of course, it should also be noted that delays in chemotherapy can lead to disease progression; as a result, some cases will need multi-drug chemotherapy. Hence, comprehensive multicenteric studies may help us to determine predictive factors for effectiveness of re-curettage therapy. In the present study, serum β-hCG levels were a factor in predicting the need for chemotherapy. Its level was significantly higher in patients requiring chemotherapy. These findings were consistent with the results of study conducted by Ayatollahi et al. (Ayatollahi et al., 2017), while, the study of Osbrone et al. did not show the predictive value of β-hCG in response to curettage therapy (R. J. Osborne et al., 2016). The studies are very limited to provide conclusive evidences; hence, more studies are needed to confirm the results.

5. Conclusion

In general, the findings of this study showed that re-curettage could be used effectively in the treatment of GTN follows molar pregnancy. This treatment reduces or eliminates the need for chemotherapy. Our findings also showed that the initial level of B-hCG could be considered as a predictive factor in response to curettage.

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Prevalence of Malnutrition (Overweight and Underweight) in Children Aged 10-13 Years in Schools in Manipal and Utopia, Karnataka

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Abstract

Background. Malnutrition is a major health problem in India. It mostly affects children of school going age with serious health problems putting them at an increased risk for developing chronic diseases later in life. This study aims to study the prevalence of malnutrition (overweight and underweight) in children aged 10-13 years in schools in Manipal and Utopia, Karnataka.

Materials and Methods. In this study, a total 400 students were studied. Their anthropometric measurements like height, weight, BMI and MUAC, dietary pattern, physical activity level, and child eating behavior. Questionnaire was used to note the student's information. The data collected was subjected to statistical analysis using SPSS version 21, Diet Cal, and Anthroplus1.0.4.

Results. It was observed that in the childhood category 10-13 years there were 222 (55.5%) male and 178(44.5%) female. According to the BMI for age 53.4% of them falls under the normal category; 22.3% falls under the mild wasting category; About 18.3% falls under the obese category and 8% falls under the moderate wasting category. There is no students falling under the severe wasting and overweight category according to the BAZ classification. When the height for age was done for all the children, about 36.3% falls under normal height; About 35.5% falls under mild stunting; 19.3% falls under the moderate stunting; 8.3% falls under the severe stunting category; and 1% falls under tall category according to the HAZ classification.

Conclusions. In conclusion, mild stunting and wasting was observed among children. There are no students falling under the severe wasting and overweight category according to the BAZ classification.

Keywords: Malnutrition, Children, Overweight, Underweight, Karnataka, India

1. Introduction

Malnutrition is a major health problem in India. It mostly affects children of school going age with serious health problems putting them at an increased risk for developing chronic diseases later in life. As per WHO as today's children are the citizens of tomorrow's world their existence security and development is the essential for the coming growth of humanity (WHO, 1996). The calculated global frequency of overweight and obesity in children 5-19 years is 10%. The occurrence arrays from 5.7%in Pakistan to over 40% in Mexico (Gupta, Goel, Shah, & Misra, 2012).

The average value for the mutual prevalence of juvenile and adolescent overweight indicates that it was greater in north, compared to south India. The combined data after 2010 estimated a combined occurrence of 19.3 per cent of childhood overweight and obesity which was an important increase from the earlier occurrence of 16.3 per cent stated in 2001-2005 (Ranjani et al., 2016). The study included of 2938 school children in the age group of 10 - 16 years from eleven randomly selected English schools of Udupi District, Karnataka State. Out of 2938 children, 1666 (57%) were males, 1216(42%) were living in a family with four family members, 1773 (60%) children were residing in the rural area, 1934(65.8%) were in the age group of 13 - 16 years, 2193(74.6%) were belonging to nuclear family, 1838 (63%) were Hindus. Occurrence of childhood overweight/obesity the statistics show that 1904 (65%) children were in the regular category of BMI, whereas 214 (7%) were overweight and 141 (5%) were obese (Nayak & VinodBhat, 2011).

According to WHO (2014) overweight and obese children are likely to stay obese in adulthood and more likely

to develop non communicable disease(Organization, 2014). Obesity and Underweight is becoming a major concern of children. Physical activity and proper diet intake is the only way to prevent overweight and obesity in childhood. Bowman et al. (2004) in his study indicates that Children with Undesirable nutrition also had higher BMI(Bowman & Vinyard, 2004).

Overweight, Obesity and chronic diseases affect all society and culture; still no group has been affected however, more so than lower socioeconomic status individuals are more affected by this problem(Berge, Arikian, Doherty, & Neumark-Sztainer, 2012). Chronic diseases are particularly preventable and can usually be categorized as lifestyle or behavioral diseases. Because chronic diseases are arguably lifestyle and behavioral disease.

There is a rise in the intake of bad quality foodstuffs together with the increase in sedentary activities and the decrease in physical activity (Ackard, Neumark-Sztainer, Story, & Perry, 2003; Baranowski & Domel, 1994; Cullen, Bartholomew, Parcel, & Koehly, 1998; Fulkerson et al., 2006). Bad quality food mainly includes fast foods, sugar-sweetened beverages (soda or soft drinks), and high fat and high sugar snacks. Soft drink consumption has raised 100% between 1981 to 1995 therefore the prevalence of BMI has increased in turn increases the overweight, obesity and malnourishment among children (Ludwig, Peterson, & Gortmaker, 2001).

Given the importance of the issue Malnutrition and the lack of a comprehensive study in recent years, the present study aimed to study the prevalence of malnutrition (overweight and overweight) in children aged 10-13 years in schools in Manipal and Utopia, Karnataka.

2. Materials and Methods

This is a descriptive cross - sectional study and carried out for seven months May 2017 to November 2017. Cross sectional studies are carried out at one time point or over a short period of time. This cross sectional study is conducted to identify the prevalence of malnutrition in children aged 10-13 Years in Schools in Manipal and Utopia, Karnataka. This study was conducted in schools located in Manipal and Udupi, Karnataka. The sample size was obtained using the result from previous study. Sample size was estimated to be 400. Purposive sampling was used as sampling technique. Inclusion Criteria in this study Including all School going children of age 10-13 years both boys and girls and exclusion criteria including children with known case of thyroid hormone abnormalities and children with co-morbid condition which can lead to overweight or underweight.

The following tools and materials were used to carry out the study:

Questionnaire: Questionnaire was used to note the student's information. The component of the questionnaire was the general information about the students like age, gender, religion, family income and academic performance etc. and also the anthropometric measurement and some question related to child eating behaviour. A pre-validated semi structured questionnaire will be used for the data collection related to personal information, Physical activity, Dietary behaviour, and 24-hour diet recall for students and socio -Demographic information Child eating Behaviour.

Anthropometric measurement: Height was measured using measuring, weight is measured using weighing scale, BMI is expressed by formula, MUAC by MUAC tape, waist circumference is measured with help of measuring tape and waist to hip ratio is found. The body fat percentage was calculated using the formula The BMI classification are as follows (Table 1).

Table 1. Classification of BAZ and HAZ

BMI for age	Z Score
Normal	+1 to -1
Mild Wasting	-1 to -2
Moderate Wasting	-2 to -3
Obese	>+2
Height for age	
Normal	+1 to 2
Mild Stunting	-1 to -2
Moderate Stunting	>3
Severe Stunting	<-3
Tall	2-3

Child eating behavior pattern questionnaire: The eating behavior questionnaire is a multidimensional questionnaire measuring child eating behaviors i.e., Enjoyment of food, Desire to drink, Satiety Responsiveness, Slowness in eating, Emotional under eating and food fussiness. The total sore of each category was added and computed with different variable(Carnell & Wardle, 2007).

Dietary assessment: Was done using 24-hour dietary recall and food frequency questionnaire.

The physical activity questionnaire: Was used to determine the physical activity of the subjects. Standard measuring spoons and cups: Measuring cups and spoons were used to ask the quantity of food the subjects were consuming.

School record: Students general information and academic performance were taken from school record.

The data collected was subjected to statistical analysis using SPSS version 21, Diet Cal, and Anthroplus1.0.4.

3. Results

It was observed that in the childhood category 10-13 years there were 222 (55.5%) male and 178(44.5%) female. In the case of religion, it was observed that 360(90%) were Hindu, 33(8.3%) were Muslims, 6(1.5%) of students were Christian and 1(.3%) were found to be Buddhist. Also in this study observed that physical activity ranges from sedentary to very active in the age group of 10-13 years. 159(39.8%) was observed to be sedentary. About 168(42%) falls under low active group, 68(17%) is active whereas 5(1.3%) of them are very active.

Anthropometric measurement mainly Height, Weight, Waist Circumference, Hip Circumference, Waist Hip Ratio, BMI, MUAC, skinfold thickness of three sites Biceps, Abdomen and Subscapular of all students were taken part in the study. The mean values of all anthropometric measurement has been tabulated in Table 2. The mean height and weight of the age group 10-13 years is 141.77 and 35.54 respectively. The mean value of waist circumference is 66.238 and hip circumference is 76.111. So proportionately the mean of waist hip ratio is 1.262. 17.4304 is the mean value for BMI. The mean MUAC is 21.70. The mean value for Skinfold Biceps, Skinfold Abdomen and Skinfold subscapular is 15.623, 16.175 and 13.553 respectively.

Table 2. Mean Anthropometric indices of children (10-13 years)

Anthropometric Parameters	(Mean)
Height	141±9.07
Weight	35.54±11.9
Waist Circumference	66.23±9.1
Hip Circumference	76.11±9.1
Waist Hip Ratio	1.26±5.53
Body Mass Index(BMI)	17.43±4.04
Mid Upper Arm Circumference(MUAC)	21.70±8.28
Skinfold Biceps	15.62±7.1
Skinfold Abdomen	16.17±11.21
Skinfold Subscapular	13.55±7.8
Body Fat Percentage	17.3±6.1

Table 3 shows prevalence of stunting in study children based on HAZ classification. When the height for age was done for all the children, about 145(36.3%) falls under normal height (+1 to-1 score) 141(35.5%) falls under mild stunting (-1 to 2 Z score), 77(19.3%) falls under the moderate stunting (-2 to -3 Z score). 33(8.3%) falls under the severe stunting category (<-3 Z score). 4(1%) falls under tall category (+1 Z score). Also according the results, the prevalence of wasting in study of children based on BAZ on age class According to the BMI for age classification 214(53.5%) of students falls under the normal category (+1 to -1Z score) of weight. 89(22.3%) falls under the mild wasting category (-1 to 2 Z score). 73(18.3%) falls under the obese category (-2 to -3 Z score). 24(6%) falls under the moderate wasting (-2 to -3Z score).

Table 3. Prevalence of stunting in study children based on HAZ classification

Height for age classification	Late Childhood(10-13years)	
	Frequency (n=400)	Percentage
Normal(+1 to 2 Z score)	145	36.3
Mild Stunting(-1 to-2 Z score)	141	35.3
Moderate Stunting(> 3Z score)	77	19.3
Severe Stunting(<-3 Z core)	33	8.3
Tall(2-3 score)	4	1

Dietary assessment of the students done by taking there dietary pattern and collecting 24 hours dietary recall from them and food frequency questionnaire by sending it to the parents. Dietary habits of the subject of the

study show that out of 400 samples 350(80.1%) of students eats breakfast regularly and 1(0.2%) sleeps immediately after breakfast. 153(37.5%) of students have the mid-morning snacks while 29(0.5%) of students sleeps immediately after mid-morning snacks. The frequency of students having lunch is 398(97.5%) whereas 1(.2%) sleeps immediately after lunch.372(91.2%) have evening snacks while 6(1.5%) sleeps immediately after evening snack. 391(95.8%) used to have the dinner and 22(5.4%) sleeps immediately after the dinner.82(20.1%) have bed time snack and 33(8.1%) sleeps immediately after the bed time snack.

Table 4. Food group adequacy classification of the subject of the study (10-13 yearrs)

Food group adequacy		N=400	
		Frequency	Percent
cereals adequacy class	Marginally inadequate	387	96.8
	Marginally adequate	11	2.8
	Adequate	2	0.5
Pulses adequacy class	Substantially inadequate	34	8.5
	Marginally inadequate	90	22.5
	Marginally adequate	97	24.3
	Adequate	81	20.3
	Excess	98	24.5
GLV adequacy Class	Substantially inadequate	371	92.8
	Marginally inadequate	28	7
	Marginally adequate	1	3
Roots and tubers adequacy class	Substantially inadequate	371	92.8
	Marginally inadequate	28	7
	Marginally adequate	1	0.3
Other Vegetable adequacy class	Substantially inadequate	400	100
	Marginally inadequate	0	0
	Marginally adequate	0	0
	Adequate	0	0
Meat and poultry adequacy class	Substantially inadequate	240	60
	Marginally inadequate	31	7.8
	Marginally adequate	16	4
	Adequate	28	7
	Excess	85	21.3
	Substantially inadequate	240	60
	Marginally inadequate	31	7.8
	Marginally adequate	16	4
	Adequate	28	7
	Excess	85	21.3
	Substantially inadequate	83	20.8
	Marginally inadequate	11	2.8
	Marginally adequate	21	5.3
	Adequate	12	3
	Excess	273	68.3
	Substantially inadequate	320	80
	Marginally inadequate	69	17.3
	Marginally adequate	11	2.8
	Substantially inadequate	217	54.3
	Marginally inadequate	37	19.3
	Marginally adequate	37	9.3
	Adequate	37	9.3
	Excess	105	26.3
	Substantially inadequate	88	22
	Marginally inadequate	42	10.5
	Marginally adequate	81	20.3
	Adequate	85	21.3
	Excess	104	26

From the Table 4 it observed that energy intake is marginally inadequate for most of the subjects that is 108(25.4%), the intake is excess for 86(20.2%) of the students. 73(17.1%) intake is adequate, 86(20.2%) is marginally adequate while 47(11.5%) is substantially inadequate. Protein intake is marginally inadequate for the subjects that is 36(9%), the intake is excess for most of the 63% of the students. 37(9.3%) intake is adequate, 30(7.5%) is marginally adequate while 45(11.3%) is substantially inadequate. Carbohydrate intake is marginally inadequate for most of the subjects that is 129(30.3%), the intake is excess for 49(11.5%) of the students.

53(12.4%) intake is adequate, 129(30.3%) is marginally adequate while 101(23.7%) is substantially inadequate. PUFA intake is marginally inadequate for the subjects that is 57(13.4%), the intake is excess for most 212(49.8%) of the students. 34(8%) intake is adequate, 35(8.2%) is marginally adequate while 62(14.6%) is substantially inadequate. Monounsaturated fatty acid is marginally inadequate for the subjects that is 106(24.9%), the intake is excess for 97(22.8%) of the students. 59(13.8%) intake is adequate, 59(13.8%) is marginally adequate while most of the subject 106(24.9%) is substantially inadequate. Thiamine intake is marginally inadequate for most of the subjects that is 138(32.4%), 24(5.6%) intake is adequate, 52(12.2%) is marginally adequate while most of the subject 101(23.7%) is substantially inadequate. Riboflavin intake is marginally inadequate for 35(8.2%) of the subject, the intake is excess for 5(1.2%) of the students. 3(0.7%) intake is adequate, 35(8.2%) is marginally adequate while most of the subject 346(81.2%) is substantially inadequate. B3 intake is marginally inadequate for the subjects that is 105(24.6%), the intake is excess for 27(6.3%) of the students. 24(5.6%) intake is adequate, 23(5.4%) is marginally adequate while most of the subject that is 221(51.9%) is substantially inadequate. B9 intake is marginally inadequate for most of the subjects that is 173(40.6%), 16(3.8%) intake is adequate, 97(22.8%) is marginally adequate while 104(24.4%) is substantially inadequate. Vitamin C intake is marginally inadequate for the subjects that is 59(13.8%), the intake is excess for 265(62.2%) of the students. 59(13.8%) intake is adequate, 40(9.4%) is marginally adequate while 9(2.1%) is substantially inadequate. Retinol intake is marginally inadequate for the subjects that is 27(6.3%), the intake is excess for 3(0.7%) of the students. 2(0.5%) intake is adequate, 3(0.7%) is marginally adequate while most of the subject that is 365(85.7%) is substantially inadequate. Beta carotene intake is marginally inadequate for the subjects that is 78(18.5%), the intake is excess for 23(5.4%) of the students. 78(18.3%) intake is adequate, 79(18.5%) is marginally adequate while most of the subject 181(42.5%) is substantially inadequate. Calcium intake is marginally inadequate for the subjects that is 121(28.4%), the intake is excess for 11(2.6%) of the students. 12(2.8%) intake is adequate, 95(22.3%) is marginally adequate while most of the subject 161(37.8%) is substantially inadequate. Carbohydrate intake is marginally inadequate for the subjects that is 5(1.2%), 2(0.5%) is marginally adequate while most of the subject 393(92.3%) is substantially inadequate. Total dietary fibre intake is marginally inadequate for most of the subjects that is 100(23.5%), the intake is excess for 87(20.4%) of the students. 69(16.2%) intake is adequate, 72(16.9%) is marginally adequate while 72(16.9%) is substantially inadequate. B9 intake is marginally inadequate for the subjects that is 36(9%), the intake is excess for most of the subject163 (40.8%). 127(31.8%) intake is adequate, 70(17.5%) is marginally adequate while 4(1%) is substantially inadequate.

From the Table 4 it is observed that cereal intake is marginally inadequate for 387(96.8%) of the subject.11 (2.8%) of the subject intake is marginally adequate. 2(0.5%) have adequate intake of cereals. Food group adequacy classification of the subject of the study (10-13 yearrs) presented in Table 4.

According to the Table 5, the mean intake of cereal is very less compared to RDA. The pulses and green leafy vegetable intake is almost meeting the RDA. Roots and tuber intake is inadequate compared to RDA. Other vegetables, Fruits and Milk intake is very high compared to the RDA. Nuts, Sugar, MFP intake is inadequate compared to RDA. Since most of the subject falls under the upper middle class the fruit and milk intake is seen to be very high (Table 5).

Table 5. The relation between mean food group intake and food group RDA

Food groups	Mean food group intake	Mean RDA intake	95% confidence interval		t-value	p-value
Cereals	178.58 ±31.74	349.95 ±44.78	-176.75	-165.97	62.471	P<0.001**
Pulses	62.56±68.32	68.32 ±7.46	-8.02	-3.48	-4.990	P<0.001**
Green Leafy Vegetables	27.52±14.53	27.52 ±14.53	.007	.0007	-	P<0.001**
Roots and Tubers	34.34±14.1	174.87 ± 25.03	-143.35	-137.69	97.714	P<0.001**
Other Vegetables	41.34±16.8	20.04±8.91	19.75	22.84	27.128	P<0.001**
Fruits	168.63±114.12	41.34±16.87	115.54	139.03	21.305	P<0.001**
Milk	338.91±98.00	200 ±0.00	129.28	148.55	28.350	P<0.001**
Nuts and Oil	9.18±14.91	20.67± 8.43	-13.05	-9.92	14.414	P<0.001**
Fats	19.32±4.28	168.63±114.12	-160.484	-138.14	26.279	P<0.001**
Sugar	20.41±4.82	100 ±0.00	-80.061	-79.11	329.91	P<0.001**
MFP	108.42±116.86	168.63± 114.12	-73.17	-47.253	-9.134	P<0.001**

Table 6 shows the comparison between the mean nutrient intakes with RDA. The macro nutrient considered are energy, protein, carbohydrate and total fat also micro nutrients like thiamine, riboflavin, B3, B6, B9, vitamin c, Retinol, Beta Carotene, Calcium and iron are added. From the paired t- test it is observed that all the micro and macro nutrients except poly saturated fatty acid have a significant difference compared to the RDA with the

mean intake p value for all the micro nutrient is 0.00 which is <0.001 but the p value for PUFA is 0.002 which is greater than 0.001 which states that there is no significant difference in the mean intake of mean intake compared to the RDA.

Table 6. Comparison of mean nutrient intake with RDA

Food groups	Mean Intake	Mean RDA intake	95% confidence interval		t-value	p-value
			lower	Upper		
Energy	1762.39±48.98	2109.26±718.18	-424.54	-269.18920	-8.779	p<0.001
Protein	53.49±20.82	40.928±13.59	10.03292	15.09587	9.757	p<0.001
Carbohydrate	249.38±127.54	350.73±116.70	-118.695	-84.00506	-11.48	p<0.001**
MUFA	10725.35±6526.57	13149.44±4397.	-3234.21	-1613.9707	-5.883	p<0.001
PUFA	9416.52±6204.33	6574.72±2198.7	2174.625	3508.98077	8.374	p<0.001
Total fat	52.622±22.06	65.75±21.03	-16.26	9.99	7.322	p<0.001
TSFA	16688±2.56	4602±15391.288	-10.02	7.373	-3.763	p<0.001
Thiamine	0.99±2.533	1.4±0.00	-0.650	-0.15267	-3.171	p<0.001
Riboflavin	0.80 ±2.741	1.6±0.00	-1.06452	-0.52555	-5.800	p<0.001
VitaminB3	9.13± 5.17	15±0.00	-6.37448	-5.35767	-22.68	p<0.001
VitaminB6	1.11±.425	1±0.00	0.07163	0.15520	5.336	p<0.001
VitaminB9	198.3717 ±55.27	300±0.00	-107.062	-96.19449	-36.76	p<0.001
Vitamin C	59.2343 ±24.18	45±0.00	11.85704	16.61164	11.771	p<0.001
Vitamin A	200.3654±171.26	600	235.35	212.88	12.11	p<0.001
Calcium	500.9399± 342.73	800±0.00	-332.750	-265.37011	-17.45	**p<0.001
Iron	8.5535± 3.20	32±0.00	-23.7617	-23.1314	-146.2	p<0.001
TDF	24.2454 ±8.62	30.2157±10.05	-7.30157	-4.63891	-8.816	**p<0.001

** Significant at 1% level.

Table 7 indicates the child eating behaviours. From the table we can see that the mean of food responsiveness score is 10.63 and the mean of emotional overeating is 10.09 with total score 25. The mean of the enjoyment of food is 10.4 that depicts that most of the student's falls under the high enjoyment of food. The mean of the desire to drink is 6.59 that means most of the students have a low desire to drink. Mean of satiety responsiveness is 11.45 that depicts most of the students have high satiety response. The mean of slowness in eating is 9.2 that depicts that most of the students are slow eaters. The mean of emotional under eating is 15.59 that depicts that most of the students fall under the high emotional under eating and the mean of food fussiness among students is 14.56 that means most of the students have food fussiness. Most of the subject have food avoidance more than food approach as the mean value of food avoidance is more than food approach.

Table 7. Eating Behaviour followed by child of age 10-13 years

Eating Behaviour	Mean±SD
Food Approach	9.03±1.48
Food Responsiveness	10.63±3.04
Emotional Overeating	10.09±0.94
Enjoyment of food	10.4±2.38
Desire to drink	6.59±2.11
Food Avoidance	11.63±1.63
Satiety Responsiveness	11.45±2.70
Slowness in eating	10.2±2.27
Emotional Under eating	8.59±3.64
Food Fussiness	14.56±3.06

4. Discussion

Given the importance of the issue Malnutrition and the lack of a comprehensive study in recent years, the present study aimed to study the prevalence of malnutrition (overweight and overweight) in children aged 10-13 years in schools in Manipal and Utopia, Karnataka. It was observed that in the childhood category 10-13 years there were 222 (55.5%) male and 178(44.5%) female. According to the BMI for age 53.4% of them falls under the normal category. 22.3% falls under the mild wasting category.18.3% falls under the obese category and 8% falls under the moderate wasting category. There are no students falling under the severe wasting and overweight category according to the BAZ classification. When the height for age was done for all the children, about 36.3% falls under normal height. 35.5% falls under mild stunting, 19.3% falls under the moderate stunting.8.3% falls under

the severe stunting category.1% falls under tall category according to the HAZ classification.

A cross sectional study carried out in the tea garden of Dibrugarh district of Assam assessed the dietary status of 605 adolescents based on their anthropometric measurements. Occurrence of stunting was 47.4% and 51.9% among boys and girls respectively in relation to NCHS reference, which reduced to almost 30% while Indian reference data was used. Incidence of thinness was higher among boys (59.5%) than girls (41.3%) counterparts(Medhi, Hazarika, & Mahanta, 2007). A research done to examine the commonness of malnutrition using anthropometric methods in tribal students appearing a school in rural south India proved that 59.4% students were stunted(Thomas, Srinivasan, & Sudarshan, 2013). A study found out that in rural Goa among adolescents (10-19 years) found out that 37.8% boys and 27.5% girls who joined the health camps were malnourished. According to the recommended daily allowance the energy intake of malnourished students was significantly lower (Banerjee, Dias, Shinkre, & Patel, 2011). Data from two NHANES (National Health and Nutrition Examination Survey) surveys (1976-1980 and 2003-2004) show that the occurrence of individuals who are overweight and obese is growing: for the age group 10-19 years, the incidence improved from 6% to 16% around 10%. There are several health problems at stake other than mere overweight/obesity. According to Swallen, Reither, Haas, and Meyer (2005), adolescents who have a higher than average BMI are more likely to have a poor physical quality of life(Swallen, Reither, Haas, & Meier, 2005). The rise in Body Mass Index can in turn lead to more complicated health issues, mainly for young people. Thus, the phase at which one becomes overweight/obese can be serious. Being overweight as a child is considered a danger for being overweight as an adult which means that overweight children are more likely to face physical and emotional health issues as adults(Dietz, 1998; Reilly et al., 2005; Whitaker, Wright, Pepe, Seidel, & Dietz, 1997). For children who are overweight, the somatic complications that can affect them has become a big issue. These adolescent are facing important mental complications. Obese adolescents are more likely to be harassed, oppressed, or discriminated against(Harper, 2006; McCrindle et al., 2007; Neumark-Sztainer et al., 2002). This can in turn lead to emotional distress, especially about looks(Viner et al., 2006). Li and colleagues (2007) found that girls who are obese, show a considerably higher depression rate than their ordinary weight counterparts, which can be explained by their disappointment(Li, Goran, Kaur, Nollen, & Ahluwalia, 2007). The raised threat of disappointment is noteworthy with obese adolescents(Daniels et al., 2005). A raised risk of depression will generally result in a decrease in both confidence and efficacy(Berry, Naylor, & Wharf-Higgins, 2005; Pinhas-Hamiel et al., 2006).

5. Conclusions

In conclusion, mild stunting and wasting was observed among children. There are no students falling under the severe wasting and overweight category according to the BAZ classification. According to the results of this study, about 36.3% falls under normal height; 35.5% falls under mild stunting; 19.3% falls under the moderate stunting; 8.3% falls under the severe stunting category; and 1% falls under tall category according to the HAZ classification.

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Study of the Relation between Parenting Style and Attitude with Physical Activity, Diet Behavior and Health of Children (10-13 Years): A Descriptive Cross - Sectional Study

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Abstract

Background. Due to the significant and Irrefutable effects of parental style on children and the lack of a comprehensive studies on this topic, the present study aimed to study the relation between parenting style and attitude with physical activity, diet behavior and health of children (10-13 years).

Materials and Methods. This study is a descriptive cross - sectional study and conducted in schools located in Manipal and Udupi (Karnataka, India) during May 2017 to November 2017. A well designed questionnaire was administered to children which included general information, along with physical activity questionnaire. The questionnaire also comprised of various other anthropometric measurements like BMI, MUAC, and Waist hip Circumferences etc. The data collected was subjected to statistical analysis using SPSS version 21. BMI has been calculated using anthroplus 1.0.4.

Results. The results of this study showed that there is a significant relationship between parenting style and diet behavior of the child (P< 0.001). Parents with stylistic dimension in both authoritative and authoritarian have more influence on child for food avoidance and food approach. Studies shows an association between authoritarian and permissive parenting style with food approach behaviors and child weight status of children. parenting style is not affected on Childs physical activity. 74.8% of variation in weight is explained by the independent factors like parenting style, Nutrient intake, Food group intake, Physical activity level, Anthropometric measurement, and child eating behaviour questionnaire. There was a significant relationship between weight and these factors at 1 % level (F=32.889, P< 0.001).

Conclusions. In conclusion, parents with stylistic dimension in both authoritarian and permissive style of parenting and parents with stylistic dimensions in all type of parenting style influences child physical activity. Parents with stylistic dimension in both authoritative and authoritarian have more influence on child for food avoidance and food approach behavior.

Keywords: Parenting style, Children, Physical activity, Diet behavior, Health

1. Introduction

Parents may unintentionally promote child weight gain by using wrong feeding methods such as stress, constraint, and control (Nobre, do Carmo Lessa, Lamounier, & Franceschini, 2017). Closely one-third of all mothers classified their overweight child has a normal weight status than the measured weight status. Mothers were also more possible to categorize their daughters who were really at a danger of overweight as being overweight than their sons (Maynard, Galuska, Blanck, & Serdula, 2003). Parents' observations of their own children's weight status are influenced by their children's features and do not seem greatly interrelated with their weight views of another child (Huang, Norman, Zabinski, Calfas, & Patrick, 2007). Many of the study show that parents are unable to classify their child as overweight or obese. Parents felt that it was the doctor's duty as a health qualified to educate the matter with the child if he/she was worried about the child's weight as long as the talk involved guidance about the problem. While, mothers also stated that they would get disappointed if the doctor states that their child is overweight. If the child is overweight as same as their parents or any of their family members who were also overweight, mothers find it extra difficult to categorize their child as being overweight (Pagnini, Wilkenfeld, King, Booth, & Booth, 2007).

Parenting style is an emotional construct in which parent's behavior are expressed in child rearing. Mostly a child's food intake and physical activity is controlled by parents, parents can act as good models in selecting healthy food providing children with care also they can check the obtainability and approachability to different kind of foods available and the activity chances. Four types of parenting styles are Authoritative, Authoritarian, Permissive and Neglectful. Parents who encourage, support and consider the wishes of the child with appropriate boundaries are more likely to have children with healthy eating pattern and less overweight. Studies show that children of more authoritarian parents were more likely to become overweight(Russell, Taki, Laws, et al., 2016). Pathways showing influence of Parents on childs overweight and obesity provided in Fig. 1(Russell, Taki, Azadi, et al., 2016).
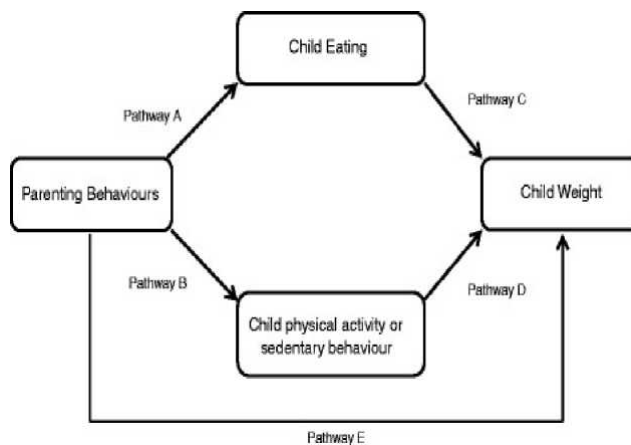

Figure 1. Pathways showing influence of Parents on Childs overweight and Obesity (Russell, Taki, Azadi, et al., 2016)

Findings on associations between parenting and child or infant weight and dietary intakes were generally suggestive of maternal feeding behaviors being in reaction to children's weight status. Parents with heavier children used more restriction and less pressure to eat. Parental feeding pressure and restriction were also associated with children's greater intakes of unhealthy foods and beverages in some, but not all(Russell, Taki, Laws, et al., 2016). Parental self-efficacy and parenting style are related with children's dietary comportment in early years of life. Parenting features matter for children's development of dietary behaviors. Parental self-efficacy and parenting style aimed interventions may be a promising approach to improve children's dietary behaviors. Interventions that address parental self-efficacy and parenting style have the potential to contribute to tackling childhood overweight and obesity(Xu, Wen, Hardy, & Rissel, 2016).

Due to the significant and unintended effects of parental style on children and the lack of a comprehensive studies on this topic, the present study aimed to study the relation between parenting style and attitude with physical activity, diet behavior and health of children (10-13 years).

2. Materials and Methods

This study is a descriptive cross - sectional study and conducted in schools located in Manipal and Udupi (Karnataka, India) during May 2017 to November 2017. This cross sectional study is conducted to study the relation between parenting style and attitude with physical activity, diet behavior and health of children. age group 10-13 years. According to the result from previous study, sample size of this study was estimated to be 400. The sampling technique used in this study was purposive sampling. Inclusion criteria in this study including all School going children of age 10-13 years both boys and girls and their parents and exclusion criteria including children with known case of thyroid hormone abnormalities and Children with co - morbid condition which can lead to overweight or underweight.

All parents are given subject information sheet to inform them about the study, subject of the study, study duration, location, benefits etc. A well designed questionnaire was administrated to children which included general information, along with physical activity questionnaire, 24 hour recall and food frequency of miscellaneous. The questionnaire also comprised of various other anthropometric measurements like BMI, MUAC, and Waist hip Circumferences etc.

Anthropometric measurement: Height was measured using measuring, weight is measured using weighing scale, BMI is expressed by formula, MUAC by MUAC tape, waist circumference is measured with help of measuring tape and waist to hip ratio is found. The body fat percentage was calculated using the formula The

BMI classification.

Child eating behavior pattern questionnaire: The eating behavior questionnaire is a multidimensional questionnaire measuring child eating behaviors i.e., Enjoyment of food, Desire to drink, Satiety Responsiveness, Slowness in eating, Emotional under eating and food fussiness. The total sore of each category was added and computed with different variable (Carnell & Wardle, 2007).

Parenting style questionnaire: was given to the parents to the parents to determine the parenting style adopted by each parents. Main categories of parenting styles are authoritarian, authoritative and permissive each category of parenting style was computed using total scores in each category. Parenting style is divided into 8 sub classes from the total scores obtained in each category like parents with low response to all parenting style, parents who are overall more permissive, parents who are overall more authoritarian, parents with stylistic dimension in both authoritarian and permissive, parents who are overall authoritative, parents with stylistic dimension in both authoritative and permissive, parents with stylistic dimension in both authoritative and authoritarian, and parents with stylistic dimension in all parenting style(Johnson, 2010).

The data collected was subjected to statistical analysis using SPSS version 21. BMI has been calculated using anthroplus 1.0.4.

3. Results

In this studySocio demographic classification is done considering gender, religion, education, occupation and income of the family. This has been tabulated in Table 1.

Table 1. Socio Demographic profile of the Subjects of the study

Socio Demographic Characteristics	n=400 Frequency	Percent
Gender		
Male	222	55.5
Female	178	44.5
Religion		
Hindu	360	90
Muslim	33	8.3
Christian	6	1.5
Buddhist	1	0.3
Fathers Education		
Middle School Certificate	104	26
High School Certificate	89	22.3
Intermediate Or Post High School Diploma	34	8.5
Graduate Or Postgraduate	160	40
Profession Or Honours	104	26
Occupation		
Unskilled Worker	21	5.3
Semi-Skilled Worker	117	29.3
Skilled Worker	50	12.5
Clerical, Shop Owner, Farmer	118	29.5
Semi Profession	39	9.8
Profession	46	11.5
Family Income		
13874-1879	57	14.2
1898-36996	91	22.8
5547-9248	25	6.3
9249-13893	51	12.8
18498-36996	38	9.5
<=1865	6	1.5
>=36997	132	33
Socio Economic Status		
Lower Middle	133	33.3
Upper Middle	257	64.3
Lower	10	2.5
Type of family		
Nuclear	290	72.5
Joint	94	23.5
Extended	16	4

Table 2 indicates the different type of parenting style adopted by parents. From the table it is observed that 31.64 is the mean value of warmth and involvement which is higher than the other parenting categories. The mean value of democratic participation is 4.7 whereas good natured is 2.8. For punitive strategy the mean value is found to be 8.6. Corporal punishment mean value is 4.47. 4.66 is the mean value for defectiveness. The mean value for verbal hostility is 4.59 while the mean value for follow through is 4.52. 5.97 is the mean value for self-confidence. Most of the parents adopt authoritarian type of parenting style as the mean value is 39.15. The mean value of authoritative type of parenting style is 22.33, and the mean score of permissive type of parenting style is 10.49.

Table 2. Mean scores of each categories of parenting styles

Parenting Style	Mean±SD
Authoritative Parenting Style	39.15±6.5
Warmth and involvement	31.64±6.27
Democratic Participation	4.7±1.30
Good Natured	2.8±1.09
Authoritarian Parenting Style	22.33±7.74
Non Reasoning, Punitive strategy	8.6±3.47
Corporal Punishment	4.47±2.28
Directiveness	4.66±2.34
Permissive Parenting Style	10.49 4.4
Verbal Hostility	4.59±2.20
Follow Through	4.52±2.18
Self Confidence	5.97±3.34

Table 3 indicates the different categories of parenting style group adopted by the parents. From the table it is observed that 60(15%) of the parents adopt stylistic dimension in all parenting style.61 (15.25%) of the parents choose stylistic dimension in both authoritarian and permissive style of parenting. 45(11.25%) of parents have low response to all parenting style. Parents who are overall more permissive is 42(10.5%). 51(12.75%) of parents have authoritarian type of parenting style. Parents who are overall authoritative are 59(14.75%). Parents with stylistic dimension in both authoritative and permissive style of parenting is 43(16.75%). 39(9.75%) have stylistic dimension in both authoritative and authoritarian type of parenting style.

Table 3. Parenting style groups adopted by the parents

Parenting style group	Frequency	Percentage
Parents with low response to all parenting style	45	11.25
Parents who are overall more permissive	42	10.5
Parents who are overall more authoritarian	51	12.75
Parents with stylistic dimension in both authoritarian and permissive	61	15.25
Parents who are overall authoritative	59	14.75
Parents with stylistic dimension in both authoritative and permissive	43	16.75
Parents with stylistic dimension in both authoritative and authoritarian	39	9.75
Parents with stylistic dimension in all parenting style	60	15

The results of this stud show that 74.8% of variation in weight is explained by the independent factors like parenting style, Nutrient intake, Food group intake, Physical activity level, Anthropometric measurement, and child eating behaviour questionnaire. There was a significant relationship between weight and these factors at 1 % level (F=32.889, P< 0.001). Multiple linear equation was performed to determine the anthropometric measurement on Childs weight status. The results show that as the waist circumference increase by one centimetre the weight is increased by 0.053 centimetre. Similarly, if the hip circumference increase by one centimetre the weight increases by .519 centimetre. The regression coefficient for body mass index is 1.661 which indicates that as the body mass index increases by one kg/m the weight is increased by 1.661. As the waist hip ratio increases by one centimetre the weight increases by 0.032. When the body fat percentage increase by one unit the weight increases by 0.034 kilograms. As the physical activity level increases by one unit the weight

increases by 2.456 units. It is observed that as the cereal intake increases by one gram the weight increases by .015 kilograms. Similarly, as the pulses intake increase by one gram the weight increases by 0.005 kilograms. The coefficient of green leafy vegetable is .008 as the green leafy vegetable increases by one gram the weight decreases by .008 kilograms. If the intake of roots and tubers increases by one gram the weight is decreased by .013 kilograms. As the intake of other vegetable increases by one gram the weight decreased by .014 kilograms. The regression coefficient of fruits is .002 which means as the fruit intake increases by one unit the weight is decreased by 0.002 kilograms. As the milk and milk product intake increases by one gram the weight decreased by .003 kilograms. Similarly, as the nuts and seeds intake increase by one gram the weight increases by .014 kilograms. The coefficient of fats is 0.117 as the intake of fats increases by one gram the weight increases by 0.117 kilograms. As the sugar intake increases by one gram the weight increases by .108 kilograms. As the meat fish poultry increases by one gram the weight decreases by .003 kilograms. As the protein intake increases by one gram the weight is decreased by 0.003 kilograms. As the intake of total dietary fibre increases by one gram the weight decreases by 0.021 kilogram. Similarly, as the carbohydrate intake increase by one gram the weight increases by .002 kilograms. As the total saturated fatty acid intake increases by one microgram the weight increases by 2.55 kilograms. If the calcium increase by one microgram the weight increases by .002. There is no relation between MUFA, and PUFA with the weight status of the children. From the results of this study it is observed that as the food Responsiveness increases the weight increases by .117 kilograms. Similarly, if the emotional overeating increases the weight decreases by -0.072 kilograms. If the enjoyment of food increases the weight increases by .323 kilograms. The coefficient of desire to drink is .206 which means as the desire to drink increases the weight increases by .206 kilograms. As the slowness in eating increases the weight increases by .004 kilograms if the satiety response increases the weight decreases by 0.014. As the food slowness in eating increases the weight increases by .004 kilograms. Similarly, if the emotional under eating increases the weight decreases by .044 kilograms. If the food fussiness increases the weight increases by 0.061 kilograms.

The results of this study show a significant relation between authoritarian parenting style and educational success. Chi Square is used to find the relation between parenting style and academic performance of the child from the table it is observed that parents with who are overall authoritative and parents with stylistic dimension in all type of parenting style got the maximum score. Parents with stylistic dimension in both authoritative and authoritarian type of parenting style got the least score. Since the p value is 0.49 and chi-square value is 34.48 there is no significance between parenting style in child's academic performance.

Studies show that parents with more permissive style of parenting have child with more physical activity. Chi square is used to find the relation between parenting style and Childs physical activity of the child from the table 4.7.2 it is observed that parents with stylistic dimension in both authoritarian and permissive style of parenting and parents with stylistic dimensions in all type of parenting style have more percentage of score that is 15%. Since the p value is 0.68and chi-square Value is 17.7 there is no significance between parenting style in child's physical activity (Table 4).

Table 4. Association of parenting style and child physical activity level

Parenting style group	Sedentary	Low Active	Active	Very Active	Total	Chi.Sq	Sig
Parents with low response to all parenting style	0%	9.7%	7.5%	15%	45%		
Parents who are overall more permissive	0%	11.6%	7.5%	8%	43%		
Parents who are overall more authoritarian	0%	13.4%	12.5%	9%	51%		
Parents with stylistic dimension in both authoritarian and permissive	40%	14.1%	15%	13%	60%		
Parents who are overall authoritative	0%	15.5%	20%	8%	59%	17.7	0.68[ns]
Parents with stylistic dimension in both authoritative and permissive	20%	10.1%	10%	10%	43%		
Parents with stylistic dimension in both authoritative and authoritarian	0%	10.5%	10%	6%	39%		
Parents with stylistic dimension in all parenting style	0%	15.2%	17.5%	9%	60%		

The results of this study show that the different group of parenting style is statistically significant with the food responsiveness as the p value is less than 0.001 (p<0.001). The different alphabets indicate significant difference

in between the parenting style groups. In this study observed that parents with stylistic dimension in both authoritarian and permissive style of parenting have more mean score. There is a significant association between authoritarian and permissive style of parenting with food responsiveness (p<0.001).

The mean score of authoritarian and permissive style of parenting is 2.45. From the table it is observed that the different group of parenting style group is statistically significant with the emotional overeating as the p value is less than 0.001(p<0.001). The different alphabets indicate significant difference in between the parenting style groups. Parents with stylistic dimension in authoritarian and permissive type of parenting style have more influence on emotional over eating (Table 5).

Table 5. Comparison of parenting style on emotional overeating

Parenting style group	Mean±SD	95% CI		f value	p value
Parents with low response to all parenting style	2.0444[a]	0.9524	1.7583	2.330	
Parents who are overall more permissive	2.1628[a]	0.8709	1.8947	2.430	
Parents who are overall more authoritarian	2.3333[a]	0.9092	2.0776	2.589	
Parents with stylistic dimension in both authoritarian and permissive	2.45[a]	0.9987	2.192	2.708	
Parents who are overall authoritative	1.7288[ab]	0.8676	1.5027	1.954	3.44 p<0.001**
Parents with stylistic dimension in both authoritative and permissive	2.1163[a]	0.956	1.8219	2.410	
Parents with stylistic dimension in both authoritative and authoritarian	1.9744[ab]	0.9593	1.6634	2.285	
Parents with stylistic dimension in all parenting style	1.9333[ab]	0.9181	1.6962	2.170	

According to the results of this study the different parenting style group is statistically significant with the enjoyment of the food as the p value is less than 0.001 (p<0.001). The different alphabets indicate significant difference in between the parenting style group. Parents with stylistic dimension in both authoritative and authoritarian have the highest mean score. Which means there is an association between authoritative and authoritarian type of parenting style with food enjoyment.

From the Table 6 it is observed that the different group of parenting style group is statistically not significant with desire to drink (p<0.001). The mean score or parents who are permissive is 7.23 which is the highest score which indicates a relation between permissive type of parenting and desire to drink. The different alphabets indicate significant difference in between the parenting style group (Table 6).

Table 6. Comparison of mean parenting style on desire to drink

Parenting style group	Mean±SD	95% CI		f value	p value
Parents with low response to all parenting style	6.288[a]	2.1492	5.6432	6.9346	
Parents who are overall more permissive	7.232[b]	2.2129	6.5515	7.9136	
Parents who are overall more authoritarian	6.745[a]	1.9271	6.2031	7.2871	
Parents with stylistic dimension in both authoritarian and permissive	6.65[a]	1.8578	6.1701	7.1299	1.335 0.232
Parents who are overall authoritative	6.152[a]	2.0495	5.6184	6.6866	
Parents with stylistic dimension in both authoritative and permissive	6.651[a]	2.2348	5.9634	7.3389	
Parents with stylistic dimension in both authoritative and authoritarian	6.897[a]	2.3596	6.1325	7.6624	
Parents with stylistic dimension in all parenting style	6.366[a]	2.1547	5.81	6.9233	

** indicates significance at 1% level Different alphabet indicates significance between different groups

According to the results, the different group of parenting style group is statistically not significant with the satiety responsiveness (p value= 0.003). The different alphabets indicate significant difference in between the

parenting style groups. Parents with stylistic dimension in both authoritarian and authoritative parenting style have more influence in satiety responsiveness. The results of this study show that the different group of parenting style group is statistically significant with the slowness in eating (p<0.001). The different alphabets indicate significant difference in between the parenting style groups. Parents with stylistic dimension in both authoritative and authoritarian have more significance with slowness in eating. In this study it is observed that the different group of parenting style group is statistically not significant with emotional under eating (p= 0.003). The different alphabets indicate significant difference in between the parenting style groups. Parents who are overall authoritarian and parents with more stylistic dimension in both authoritarian and permissive have more relation emotional under eating. About the comparison of mean parenting style on food fussiness, the results showed that the different group of parenting style group is statistically not significant with emotional under eating (p value= 2.52). The different alphabets indicate significant difference in between the parenting style groups. Parents who are more authoritarian have influence on food fussiness.

Table 7 indicates parenting style with food approach. From the table it is observed that the different group of parenting style group is statistically significant with food approach (p<0.001). Parents with stylistic dimension in both authoritative and authoritarian parenting style have more influence on food approach since their mean value is more compared to the other parenting style group. The different alphabets indicate significant difference in between the parenting style groups.

Table 7. Comparison of mean parenting style on food Avoidance

Food Avoidance	Mean±SD	95% CI	f value p value
Parents with low response to all parenting style	10.8667[a] 1.82906	10.3172 11.4162	
Parents who are overall more permissive	11.6512[b] 1.75775	11.1102 12.1921	
Parents who are overall more authoritarian	12.2157[ab] 1.28552	11.8541 12.5772	
Parents with stylistic dimension in both authoritarian and permissive	12.1[ab] 1.18893	11.7929 12.4071	
Parents who are overall authoritative	11.2373[b] 1.60107	10.82 11.6545	4.721 p<0.001**
Parents with stylistic dimension in both authoritative and permissive	11.4419[b] 1.62279	10.9424 11.9413	
Parents with stylistic dimension in both authoritative and authoritarian	12.2051[ab] 1.64124	11.6731 12.7372	
Parents with stylistic dimension in all parenting style	11.4167[b] 1.75916	10.9622 11.8711	

The results of hypothesis testing presented in Table 8.

Table 8. Hypothesis testing

HYPOTHESIS (Ho)	TEST	P-VALUE	INFERENE
There is no significant relationship between parenting style, Diet Behaviour, food group, Physical Activity Level, Anthropometric Measurement, Income of family and child eating behaviour	Multiple Linear Regression	P<0.001	H0 not accepted. Alternate hypothesis is accepted

Discussion

The present study aimed to study the relation between parenting style and attitude on physical activity, diet behavior and health of children. The results of this study showed that there is a significant relationship between parenting style and diet behavior of the child. Parents with stylistic dimension in both authoritative and authoritarian have more influence on child for food avoidance and food approach. Studies shows an association between authoritarian and permissive parenting style with food approach behaviors and child weight status of children. parenting style is not affected on Childs physical activity. 74.8% of variation in weight is explained by the independent factors like parenting style, Nutrient intake, Food group intake, Physical activity level, Anthropometric measurement, and child eating behaviour questionnaire. There was a significant relationship

between weight and these factors at 1 % level.

Parenting consist of a countless responsibility in shaping the behaviors' in children. Behavior's comprises of activity habits, diet, sleep and television viewing time. Parents can be a good role model to their child. Most children are likely to mimic or repeat the activities of their parents(Francis, Lee, & Birch, 2003), so the parents have a direct impact on child's diet and physical activity. Parental influence may have an effect on over eating in children. While parents are the caretakers to the meals provided to the child, there can be variations in the child's fondness for meals(Costanzo & Woody, 1985). On the other hand, parents who have more control on the diet behavior of their girl child had lower weight and BMI. Parenting style and restriction has been related with child's BMI(Joyce & Zimmer-Gembeck, 2009). This topic need to be studied more to attain more specific result about relation between parenting style on physical activity and diet behaviour of the child. A longitudinal research linked parent's Body Mass index with their child's Body Mass Index, snacking habits, and television viewing both parent and child who are overweight had a raised BMI, along with rise in snacking and TV viewing time (Francis et al., 2003; Whitaker, Pepe, Wright, Seidel, & Dietz, 1998).

Further features such as parent's literacy level, Salary, and the neighbourhood safety are in reversely linked to child Body Mass Index, with watching television has been known as the primary mediator(Cecil-Karb & Grogan-Kaylor, 2009; Morgenstern, Sargent, & Hanewinkel, 2009). Parenting styles has a significant part in the lifestyle factors and Body Mass Index of children. Four original parenting styles including: Authoritative (Provide clear approach and warm discipline), Authoritarian (Have clear power over the child and expects unquestioning obedience), Permissive (Allow child to make their own decisions and provide minimal punishment), and Permissive (Allow child to make their own decisions and provide minimal punishment)(Baumrind, 1966). The fourth typology, straightforward, characterized parents who had qualities of tolerance and provided the child slight warmth. These types of parents did not expect much from the child and had slight control over the child's behaviour (Maccoby, 1983). Parents who showed authoritative parenting style lead to better food choices and more activity levels in children(Golan & Crow, 2004), while children of permissive parents had poorer intake of food and less control to unhealthy diet behavior. Research done to study parenting styles of low-income group sections have found that these parents have more authoritarian type of parenting style, irrespective of their culture(Pinderhughes, Dodge, Bates, Pettit, & Zelli, 2000). This supports the power of effect of income that has on parent pressure, control, and feelings.

Conclusions

From the study it is observed that the parenting style is not affected on Childs physical activity but parents with stylistic dimension in both authoritarian and permissive style of parenting, parents with stylistic dimensions in all type of parenting style influences, parent who are overall more permissive, parents who are overall authoritarian and parents with stylistic dimension in both authoritative and permissive shows more relation with Childs physical activity. There is an influence of parenting style on child diet behaviour but there is a significance association between all the parenting style on food approach and food avoidance. Influence is seen in parents who adopt stylistic approach in both authoritative and authoritarian type of parenting with food approach and food avoidance.

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The Relationship between Abnormal Echocardiography Findings in Fetus with Small Gestational Age

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Abstract

Background: Intrauterine growth restriction (IUGR), refers to a condition in which the fetus does not grow well according to its genetic potential. Development of the cardiovascular system and impairing fetal developmental planning ultimately caused long-term cardiovascular morbidity. In the present study, the incidence of cardiac abnormalities in fetuses affected by IUGR as well as factors related to the occurrence of these abnormalities were evaluated.

Material and Methods: In this descriptive-analytical study, pregnant women referred to the Imam Khomeini Hospital in Ahvaz during February 2018 to January 2020 were recruited. Complete clinical information was recorded using a questionnaire. The recorded information included age, birth weight, gestational age, weight and BMI and comorbidities. Then, echocardiographic data; structural abnormalities including septal condition, valves and outflow of large arteries, and the presence of mass or oxygen in the heart and pulmonary arteries, EF and HR, were extracted from the medical records.

Results: In the present study, 152 patients were studied. The mean age of patients was 28.2 ranged from 17- to 40year. Fetal echocardiography showed that nearly 60% of fetuses had heart abnormalities. The most common disease was **ventricular septal defect (**VSD) (n = 21,13.8%), followed by **atrial septal defec**(ASD) (n = 15,9.9%) and concurrent ASD and VSD was observed in of 11.2% cases (n = 17). The incidence of echocardiographic abnormalities in IUGR fetuses was significantly higher than in SGA (p = 0.0001). However, the type of IUGR did not have a significant relationship with the incidence of echocardiographic findings in terms of time of performance (Early or late). Among the Doppler ultrasound factors, uterine artery PI (p = 0.001) and umbilical artery PI (p = 0.008) were associated with fetal heart abnormalities.

Conclusion: In general, the findings of this study showed that the incidence of heart disorders in IUGR fetuses is high and is related to the severity of fetal growth restriction. Cardiac abnormalities are more common in IUGR fetuses than in SGA. Our results have also shown that the umbilical artery PI and uterine artery PI can be used as predictive factors in diagnosing fetal heart abnormalities. Our findings suggest an evaluation of echocardiography in IUGR fetuses.

1. Introduction

Intrauterine growth restriction (IUGR), refers to a condition in which the fetus does not grow well according to its genetic potential (Sharma, Shastri, & Sharma, 2016). One of the main causes of IUGR is vascular insufficiency of the placenta (Krishna & Bhalerao, 2011). Under these conditions, the deprivation of oxygen and nutrients causes the IUGR fetus to make certain changes in its blood circulation. These changes are due to adaptability to the intrauterine suboptimal environment. These adaptations are aimed at providing oxygen and nutrients to vital organs such as heart, brain, and adrenal glands. These adaptations in blood circulation may alter the development of the cardiovascular system and impair fetal developmental planning and ultimately caused

long-term cardiovascular morbidity (Cohen, Wong, Horne, & Yiallourou, 2016).

Congenital heart defects are currently the most common congenital anomalies at birth, with an incidence of 8 to 10 per 1,000 live births (Drose, 2013). According to the World Health Organization, between 1950 and 1996, 72% of infant deaths were due to heart problems (Carvalho, Allan, Chaoui, Copel, …, & Yagel, 2013). One of the most difficult prenatal diagnoses is screening for fetal heart defects (Satomi, 2015). Using screening tests and with prescribing prophylactic folic acid have gradually eliminated major neural tube defects and common trisomy, and lead to rise the cardiac anomalies from third to first congenital anomalies. Of course, the presence of known and unknown teratogens and the increase in age of first pregnancy should also be considered. On the other hand, despite the invention of new diagnostic methods during pregnancy, diagnosis, identification, and treatment of patients with these disorders remain a serious problem in fetal and maternal medicine (Resnik, Lockwood, Moore, Greene, Copel, & Silver, 2018). Prenatal diagnosis of heart abnormalities is vital step in managing this problem. In this regard, fetal echocardiography is suggested as a standard part of screening for the following reasons (Rychik, Ayres, Cuneo, Gotteiner, Hornberger, Spevak, & Van Der Veld, 2004):

1- Decide to terminate or continue the pregnancy (termination of pregnancy in incurable and debilitating anomalies).

2- Identify and diagnose associated syndromes such as DJ, Down syndrome, sclerosis, tuberculosis, etc.

3- Starting intrauterine therapies such as treatment of fetal arrhythmias, pericardial fluid drainage, etc.

4- Early treatment of the disease in the first days of life (before causing complications) such as arterial switching in the movement of large arteries.

5- Diagnosis and management of maternal diseases such as diabetes and lupus.

6- Psychological support of the parents.

Today, one of the most important parts of prenatal screening is fetal echocardiography, which is used to diagnose fetal heart disorders. Assessing the incidence of cardiac abnormalities in IUGR fetuses can well illustrate the relationship between these two complications. However, to the best of our knowledge, no studies have evaluated echocardiographic findings in IUGR fetuses. In the present study, the incidence of cardiac abnormalities in fetuses affected by IUGR as well as factors related to the occurrence of these abnormalities were evaluated.

2. Material and Methods

2.1 Study Design

In this descriptive-analytical study, pregnant women referred to the Imam Khomeini Hospital in Ahvaz during February 2018 to January 2020 were recruited. Confirmed cases with diagnosis of IUGR or SGA were included. While those with chromosomal or structural disorders, premature rupture of membrane and chorioamnionitis were excluded. The study has reviewed and confirmed by Ahvaz Jundishapour University of Medical Sciences Ethics Committee. Signed inform consent were obtained from all participants.

2.2 Measurements

Complete clinical information was recorded using a questionnaire. The recorded information included age, birth weight, gestational age, weight and BMI and comorbidities. Then, echocardiographic data; structural abnormalities including septal condition, valves and outflow of large arteries, and the presence of mass or oxygen in the heart and pulmonary arteries, EF and HR, were extracted from the medical records.

2.3 Statistical Analysis

The data were presented by descriptive statistics including mean, median, standard deviation and frequency. The means were compared by independent t-test. The proportions were compared using Chi-Square tests. In order to determine the predictive factors of fetal heart failure, univariate and multivariate regression analyses were performed. All statistical analyses were carried out using SPSS version 20. The p value less than 0.05 considered significant.

3. Results

In the present study, 152 patients were studied. The mean age of patients was 28.2 ranged from 17- to 40year. The mean body mass index (BMI) was 28.4 with the range of 18 to 39.5. The mean gestational age at the time of admission was 32.7 weeks (22.2 to 36.5 weeks). Pregnancy hypertension (GTHN) with a frequency of 17.1% and gestational diabetes with a frequency of 16.4% were the most common underlying diseases. Seventeen cases (11.2%) were pregnant using assisted reproductive techniques.

Table 1. Characteristics of the studied mothers

Characteristics	Mean(range) ± SD or Frequency(%)
Age (y)	28.2(17-40)±6.7
BMI (kg/m²)	28.4(18-39.5)±4.8
Gestational Age(week)	32.7(22.29-36.57)±2.57
Comorbidities	
GTHN	26(17.1%)
GDM	25(16.4%)
Hypothyroidism	8(5.3%)
Hyperthyroidism	9(5.9%)
Normal	84(55.3%)
Pregnancy	
Spontaneous	135(88.8%)
ART cycles	17(11.2%)

According to fetal ultrasound, it was found that **Abdominal circumference** (AC) levels were abnormal in 65.1% of cases. Abnormal Estimation of Fetal Weight (EFW) was also observed in 38.1% of cases. On the other hand, during doppler ultrasound it was found that 55.9% had an abnormal umbilical artery Perfusion Index (PI), 11.8% had an abnormal central PI artery, and 34.2% had an abnormal uterine artery PI. Finally, 50 fetuses (32.9%) had SGA and another 102 were identified as IUGR.

Table 2. Ultrasound characteristics of the studied participants

Characteristics	Median(range) - Frequency(%)
Abdominal Circumference	
Normal	53(34.8%)
Abnormal	99(65.1%)
Estimated Fetal Weight	
Normal	93(61.1%)
Abnormal	59(38.81%)
Umbilical artery PI	1.17(0.65-2.1)
Normal	67(44.1%)
Abnormal	85(55.9%)
Middle cerebral artery	1.99(0.55-3.37)
Normal	134(88.2%)
Abnormal	18(11.8%)
Mean Uterine artery PI	0.67(0.44-1.67)
Normal	100(65.78%)
Abnormal	52(34.2%)
IUGR/SGA	
IUGR Stage I	79(52%)
IUGR Stage II	14(9.2%)
IUGR Stage III	8(5.3%
IUGR Stage IV	1(0.7%)
SGA	50(32.9%)

Table 3. Spectrum of fetal heart abnormalities

Characteristics	Frequency	Percent
Normal	63	41.4
VSD	21	13.8
ASD	15	9.9
ASD & VSD	17	11.2
Mild RVE	4	2.6
AS & MS	5	3.3
TGA&MR&MS	4	2.6
Mild PS	8	5.3
Hydrops fetalis	4	2.6
Large PFO	3	2.0
Arrhythmia	3	2.0
LVE and LVH	3	2.0
TR and MR	2	1.3

Table 4. Comparison of fetus with and without abnormal echocardiographic feature

Characteristics	Echo Cardiography findings		P value
	Normal	Abnormal	
SGA/IUGR			0.0001
SGA	28(56%)	22(44%)	
IUGR Stage I	34(43%)	45(57%)	
IUGR Stage II	0(0%)	14(100%)	
IUGR Stage III	1(12.5%)	7(87.5%)	
IUGR Stage IV	0(0%)	1(100%)	
IUGR type			0.164
Early	8(22.2%)	28(77.8%)	
Late	20(37.7%)	33(62.3%)	
Mean PI Uterine			0.001
Normal	51(51%)	49(49%)	
Abnormal	12(23.1%)	40(76.9%)	
PI umbilical			0.008
Normal	36(53.7%)	31(46.3%)	
Abnormal	27(31.8%)	58(68.2%)	
PI MCA			0.12
Normal	59(44%)	75(56%)	
Abnormal	4(22.2%)	14(77.8%)	

Table 5. Univariate and multivariate regression model

Variables	Univariate		Multivariate	
	OR(95% CI)	P-Value	OR(95% CI)	P-Value
Age	1.0(0.96-1.05)	0.74		
BMI	0.94(0.87-1)	0.079	1.1(01.001-1.3)	0.052
ART cycle	1.8(0.6-5.4)	0.29		
AC	0.74(0.38-1.4)	0.36		
EFW	0.9(0.63-4.4)	0.819		
PI UMB	2.49(1.28-4.8)	0.007	3.39(1.4-8.2)	0.007
Mean PI uterine	3.46(1.6-7.3)	0.001	6.04(2.07-17.5)	0.001
Early IUGR	2.17(1.02-4.6)	0.042	2.17(0.94-5.04)	0.069

Fetal echocardiography showed that nearly 60% of fetuses had heart abnormalities. The most common disease was **ventricular septal defect (**VSD) (n = 21,13.8%), followed by **atrial septal defec** (ASD) (n = 15,9.9%) and concurrent ASD and VSD was observed in of 11.2% cases (n = 17) (Table 3). Based on that, the patients were divided into two major groups; normal and abnormal fetal heart. It was found that the incidence of

echocardiographic abnormalities in IUGR fetuses was significantly higher than in SGA (p = 0.0001). However, the type of IUGR did not have a significant relationship with the incidence of echocardiographic findings in terms of time of performance (Early or late). Among the Doppler ultrasound factors, uterine artery PI (p = 0.001) and umbilical artery PI (p = 0.008) were associated with fetal heart abnormalities (Table 4).

In order to determine the predictive factors of fetal heart failure, univariate and multivariate regression analyses were performed. Based on this, it was found that impaired uterine artery PI and umbilical artery PI independently predict fetal echocardiographic abnormalities (Table 5).

4. Discussion

The findings of the present study showed that the occurrence of cardiovascular disorders is associated with IUGR. Thus, its incidence was significantly higher in IUGR than in normal fetuses. In line with our results, previous studies have also shown association of IUGR with many cardiovascular disorders. Niewiadomska et al. showed that children with IUGR had subclinical myocardial disorders (Niewiadomska-Jarosik, Zamojska, Zamecznik, Stańczyk, Wosiak, & Jarosik, 2017). Fouzas et al. Reported that IUGR infants showed changes in subclinical changes in cardiac morphological that may lead to altered adaptation pattern of cardiovascular system with extra uterine life (Fouzas, Karatza, Davlouros, Chrysis, Alexopoulos, Mantagos, & Dimitriou, 2014). Another study of 209 IUGR or SGA, and 150 healthy fetuses as a control group showed that IUGR and SGA fetuses had larger and more spherical hearts compared to the control group. Impaired systolic and diastolic performance including decreased Tricuspid annular plane systolic excursion (TAPSE) and increased left ventricular performance index (LVGFI) was reported (Pérez‑Cruz, Cruz‑Lemini, Fernández, Parra, Bartrons, Gómez-Roig, Crispi, & Gratacós, 2015). Sehgal et al. In their study of heart function and biochemical characteristics of arteries in SGA infants showed that the Ponderal index was significantly lower and the blood pressure was significantly higher in SGA infants than AGA. Ventricular output is lower in SGA infants and the diastolic function impairment is higher. Moreover, the isovolumic relaxation time is longer in the SGA infants (Sehgal, Doctor, & Menahem, 2013). In the present study, unlike to the Segah et al's study, different types of IUGR and SGA were studied and compared separately.

Our findings also showed that uterine artery PI and umbilical artery PI could independently predict the incidence of heart disorders in IUGR/SGA fetuses. Graupner et al. Showed that congenital heart disease is associated with changes in Doppler patterns, especially with increased uterine artery PI (Graupner, Koch, Enzensberger, Götte, Wolter, Müller, Kawecki, Herrmann, & Axt-Fliedner, 2019). Abnormal uterine artery Doppler suggests impaired placentation and poor placental function. In the present of uteroplacental insufficiency, increased placental resistance reduces end-diastolic flow in the umbilical artery. Then during pregnancy, the subsequent further increase in placental resistance, end-diastolic flow may be absent or even reverse. As a result of these changes, cardiac damage may occur, leading to an increase in central venous pressure, which manifests itself with an increase in PI or even reversibility of the flow of ductus or umbilical veins (Baschat & Harman, 2001; Turan et al., 2008; Spinillo, Gardella, Bariselli, Alfei, Silini, & Bello, 2014; Mari, Hanif, Kruger, Cosmi, Santolaya-Forgas, & Treadwell, 2007; Brantberg & Sonesson, 1999).

5. Limitations of the Study

Given the very few studies that have been done in this area, justifying the findings of this study requires further investigation.

6. Conclusion

In general, the findings of this study showed that the incidence of heart disorders in IUGR fetuses is high and is related to the severity of fetal growth restriction. Cardiac abnormalities are more common in IUGR fetuses than in SGA. Our results have also shown that the umbilical artery PI and uterine artery PI can be used as predictive factors in diagnosing fetal heart abnormalities. Our findings suggest an evaluation of echocardiography in IUGR fetuses.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Risk of Fetal Loss after Amniocentesis in Twins Comparing with Singleton Pregnancy

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Abstract

Introduction: Amniocentesis is one of the most widely used diagnostic interventions but despite its many advantages, it has side effects such as fetal loss. The importance of fetal loss after amniocentesis in twin pregnancies is very important and some researchers believe that in twin pregnancies, the risk of fetal loss after amniocentesis is higher than singleton. Therefore, the present study examined the rate of fetal loss after amniocentesis in twin pregnancies compared to singleton pregnancies.

Methods: This prospective descriptive-analytical study was performed on 712 mothers with singleton (628 cases) and twin (84 cases) pregnancies who referred to the obstetrics and gynecology clinic of Imam Khomeini Hospital in Ahvaz with Amniocentesis due to several reason during 2016-2020. A questionnaire containing information such as age, gestational age, number of pregnancies, childbirth and abortion, the cause of the diagnostic test, quarterly screening and NT ultrasound, and the result of amniocentesis were completed for patients. Patients were followed up during the first week, two weeks, and 60 days after amniocentesis, and finally data were analyzed by SPSS version24.

Results: The mean age of the patients was 31.96 ± 6.33 years and the mean gestational age at amniocentesis for all patients was 15.37 ± 0.7. The mean NT in patients in the two groups did not differ significantly (1.60 ± 0.44 vs 1.54 ± 4.64, p=0.56). the prevalence of preterm in twin mothers was 13.1% and, in the singleton was 1.75% (p = 0.01). The occurrence of fetal loss was 2.4% in the twin group and 2.07% in the singleton group which was not statistically significant (p = 0.56). in the twin group, one (50%) fetal loss occurred in the first week and another one (50%) occurred between 15 and 60 days. But in the singleton group, 7 cases (63.6%) in the first week, 1 case (9%) in 15 to 60 days, and 3 cases (27.3) after more than 60 days lost the fetus. Previous pregnancy records in twins showed that the mean Gravid (p = 0.01), Parity (p = 0.01) and Living child (p = 0.02) in preterm twins' patients were statistically significantly lower than in the term patients.

Conclusion: Our findings show that fetal loss following amniocentesis in twin pregnancies does not increase significantly compared to singleton pregnancies. However, our findings require repeated study in bigger sample size and as multi-centered as possible then It can be generalized to wider communities.

Keywords: Amniocentesis, Fetal Loss, Twins, Pregnancy

1. Introduction

Amniocentesis is the first invasive procedure used to diagnose and treat fetal diseases during pregnancy. (Connolly & Eddleman, 2016) It is currently the most common invasive genetic test in pregnant women. Amniocentesis is used as a basic invasive method for early detection of various pregnancy-related conditions, such as fetal karyotype, diagnosis of metabolic or enzymatic diseases, evaluation of severe hemolytic diseases, development of pulmonary maturation and diagnosis of fetal infections (Jummaat, Ahmad, & Ismail, 2019; Drukker, Sela, Ioscovich, Samueloff, & Grisaru-Granovsky, 2017). In addition, amniocentesis is used to inject various drugs into the amniotic cavity, to determine the composition of the amniotic fluid, and finally to drain Amniotic fluid (Theodora, Antsaklis, & Antsaklis, 2015). Despite the many benefits of amniocentesis, this procedure has side effects, including fetal loss. However, the actual risk of fetal loss with this method is not yet clear (Theodora, Antsaklis, Antsaklis, Blanas, Daskalakis, Sindos, Mesogitis, & Papantoniou, 2016). Therefore,

more research is needed to obtain clearer information. Amniocentesis is performed in pregnant women aged 35 years and older because the mother's chances of having a child with chromosomal abnormalities increase (Xiao, Yang, Zhang, Liao, Zhao, & Liao, 2016), as well as in mothers who have previously had a child with problems such as Down syndrome (Mukherjee & Chaudhury, 2015). Another important application of amniocentesis is in twin pregnancies. It has previously been shown that twin pregnancies have a higher risk of developing chromosomal abnormalities. In addition to genetic testing, amniocentesis is used in third-trimester screening tests to check for markers of fetal lung maturation in amniotic fluid (Boyer, Cameron, Munoz-Maldonado, Bronsteen, Comstock, Lee, & Goncalves, 2014; Ramirez-Montiel, Casillas-Barrera, Morales-Morales, Ortiz, Carrasco-Blancas, & Morales, 2017). The main concern is fetal complications. These complications include fetal loss, placental abruption, preterm delivery, and premature rupture of fetal membranes. Amniocentesis may also cause infection within the amniotic fluid, meaning that microorganisms can enter the amniotic cavity through the needle. The risk of fetal loss after amniocentesis has been assessed by several centers. The different results of these studies indicate the difference between the assessment methods and the studied populations (Theodora, Antsaklis, Antsaklis, Blanas, Daskalakis, Sindos, Mesogitis, & Papantoniou, 2016; Shirazi, Mohseni, & Ghajarzadeh, 2015). In general, the rate of fetal loss loss is based on three main studies in the 1970s that were not randomized. The Centers for Disease Control and Prevention (CDC) estimates that the rate of fetal loss after amniocentesis is 0.5%. Despite the fact that these studies were not random and that amniocentesis was not performed using ultrasound at the same time, this CDC estimate of post-amniocentesis abortion is still being used (Connolly & Eddleman, 2016). The importance of fetal loss after amniocentesis in twin pregnancies is very important to consultants, but few studies have examined the risk of fetal loss in amniocentesis in twin pregnancies, and some researchers believe that in twin pregnancies, the risk of fetal loss after amniocentesis is higher than singleton (Enzensberger, Pulvermacher, Degenhardt, Kawecki, Germer, Weichert, Krapp, Gembruch, & Axt-Fliedner, 2014; Wilson, Gagnon, Audibert, Campagnolo, Carroll, Brock, Chong, Johnson, MacDonald, Okun, & Pastuck, 2015). Due to the significant effect of amniocentesis on maternal and fetal health during pregnancy and the importance of public health in this regard, preventive strategies are recommended. Therefore, early detection of high-risk mothers for amniocentesis is critical from the perspective of therapeutic interventions (Lenis-Cordoba, Sánchez, Bello-Muñoz, Sagalá-Martinez, Campos, CarrerasMoratonas, & Cabero-Roura, 2013). Recent advances in amniocentesis have facilitated further analysis of singleton and twin pregnancies Therefore, the present study examined the rate of fetal loss after amniocentesis in twin pregnancies compared to singleton pregnancies.

2. Material and Methods

After obtaining permission from the ethics committee of Ahvaz Jundishapur university of medical sciences , in a prospective descriptive-analytical study, This research was performed on 712 mothers with singleton (628 cases) and twins (84 cases) pregnancies who referred to the obstetrics and gynecology clinic of Imam Khomeini Hospital in Ahvaz with Amniocentesis due to several reason during 2016-2020. The information was based on the questionnaire, the results of amniocentesis and follow-up of patients on 7, 14 and 60 days after amniocentesis until term and their comparison. The sampling method in this study was a simple census of pregnant women who referred to the obstetrics and gynecology clinic of Imam Khomeini Hospital in Ahvaz during 1997-98. The study was performed on pregnant women who underwent amniocentesis at different times during preterm labor. Pregnant women were divided into singleton and twin groups. After receiving written consent to enter the study, the patients were given a questionnaire containing information such as: age, gestational age, number of pregnancies, childbirth and abortion, the reason for the diagnostic test, first trimester screening, NT ultrasound and amniocentesis results. Patients were monitored through pregnancy during the first week, two weeks, and 60 days after amniocentesis, and in case of abortion or preterm delivery, the age of fetal loss was compared with the information in the questionnaire and compared to the standard values of these parameters. Methods of performing amniocentesis: The surface of the mother's abdomen in the area of the uterus was disinfected and anesthetized. The specialist inserted a needle from the abdomen into the uterus and removed some amniotic fluid from the uterus with a syringe (1 cc per week of pregnancy). Then he gently removed the needle from his abdomen. All the time with the insertion of the needle, the needle path was controlled by ultrasound so as not to harm the fetus. After collecting the required information, data analysis was performed using SPSS statistical software (version 24). For quantitative variables, the mean and standard deviation were determined and for qualitative variables, absolute and relative abundance were determined. The chi-square, Fisher, and T-independent tests were used to compare different variables between the two groups, with a significance level of 0.05.

3. Results

Patients in the present study included 776 pregnant mothers with singleton (686) and twin (90) pregnancies who underwent amniocentesis. However, during the study, 6 twin pregnancy were excluded from the study due to medical abortion as well as 58 singleton pregnancy, and finally 712 pregnant patients with singleton pregnancies (628) and twins (84) entered the final analysis.

The mean age of the patients was 31.96 ± 6.33 years and no statistically significant difference was found between the ages of the two groups (p = 0.79). Also, the mean gestational age of patients during amniocentesis was 15.37 ± 0.7 weeks and no significant statistical difference was found between the two groups (p = 0.69). Previous pregnancy records in the studied patients showed that the mean of twin Gravid was statistically significantly higher than the singleton group (p = 0.01). But the mean of Parity (p = 0.001), Living child (p = 0.03), and Abortion (p = 0.01) were statistically significantly lower in twin patients than in singleton group. Also, the mean NT for all patients was 1.55 ± 0.15 and no statistically significant difference was found between the two groups (p = 0.56).

3.1 Results of Fetal Loss and Preterm Delivery in Both Groups

In the twin group, the incidence of preterm was 13.1% and in the singleton group it was 1.75%, which is statistically significant in the group of twins than singleton group (p = 0.01). The occurrence of fetal loss was 2.4% in the twin group and 2.07% in the singleton group, which did not have a statistically significant difference between the two groups (p = 0.56). Evaluation of fetal loss after amniocentesis also showed that of the two fetal loss cases in the twin group, one (50%) occurred in the first week and another one (50%) occurred between 15 and 60 days. But in the singleton group, 7 cases (63.6%) in the first week, 1 case (9%) in 15 to 60 days after the test, and 3 cases (27.3) after more than 60 days lost the fetus.
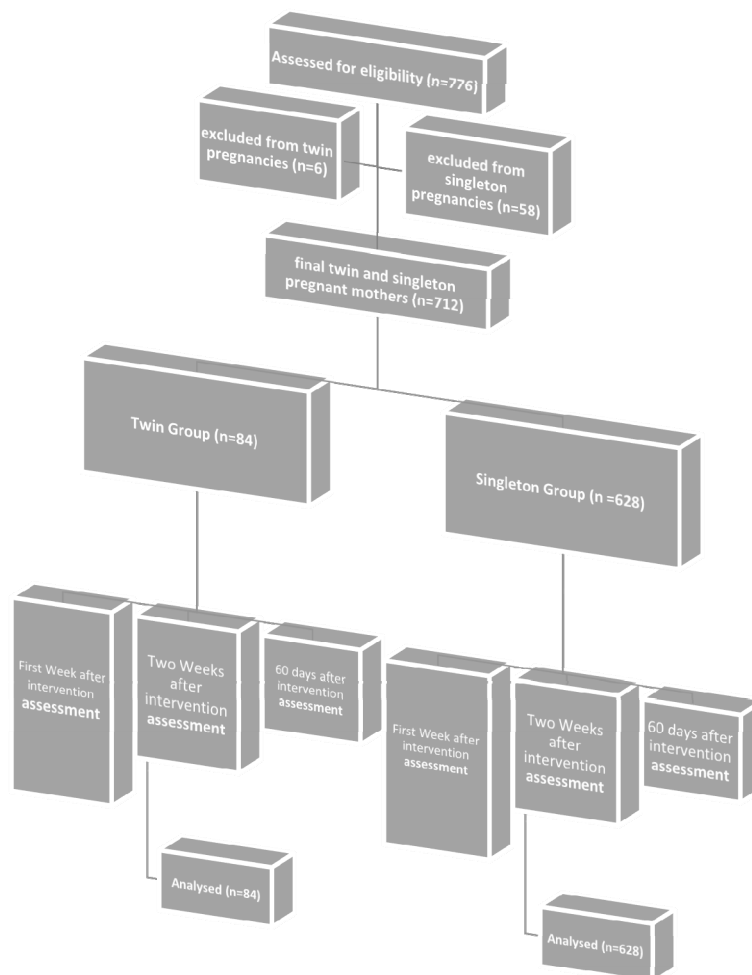

Figure 1. Schematic Material and Methods

Table 1. Patient's characterization in two groups of twin and singleton

Variables		Group		P value
		Twin (n=84)	Singleton (n=628)	
Age (M±SD)		32.08±4.68	31.95±6.89	0.79
Gestational age (M±SD)		15.4±0.77	15.37±0.69	0.69
History of pregnancy	Gravid	1.68±1.30	1.23±0.70	0.01
	Parity	0.96±0.97	1.32±0.47	0.001
	Living child	0.94±0.90	1.05±0.22	0.03
	Abortion	0.85±0.37	1.38±0.97	0.01
NT		1.60±0.44	1.54±4.64	0.56
Preterm N (%)		11 (13.1%)	11 (1.75%)	0.01
Fetal Loss N (%)		2 (2.4%)	13 (2.07%)	0.65
	Week 1	1 (50%)	7 (63.6%)	
	15 to 60 days	1 (50%)	1 (9%)	
	After 60 days	0	3 (27.3%)	

Time of fetal loss

3.2 Comparison of Twin Patients Based on Term and Non-Term

Of the 84 patients, 71 (84.5%) had term births, 11 (13.1%) had preterm births, one (1.2%) had fetal loss and 1 case was preterm, who also lost the fetus (1.2%). Also, one of the cases was a 16-week abortion due to the rupture of the membrane, and the other was a 24-week preterm. Also, a study of patients showed that most mothers (73.8%) underwent amniocentesis due to high-risk screening. Also, 2 cases (2.4%) underwent amniocentesis due to the result of abnormal ultrasound in one of the fetuses, which was reducted due to anomaly, and the fetus remained healthy and term. The mean age of pre-term patients was less than the term patients (p = 0.04) and the mean gestational age at amniocentesis did not show a statistically significant difference between the two groups (p = 0.77). Previous pregnancy records showed that the mean Gravid (p = 0.01), Parity (p = 0.01) and Living child (p = 0.02) in preterm patients were statistically significantly lower than in the term patients. but no significant statistical difference was observed for the Abortion (p = 0.09).

Table 2. Comparison of twin patients based on Term and non-Term

Variables		Group		P value
		Term (n=84)	Pre-Trem (n=628)	
Age (M±SD)		32.52±4.53	29.69±4.92	0.04
Gestational age (M±SD)		15.39±0.7	15.46±1.12	0.77
History of pregnancy	Gravid	1.83±1.27	0.84±1.21	0.01
	Parity	1.07±0.98	0.38±0.76	0.01
	Living child	1.00 ± 0.89	0.38 ± 0.76	0.02
	Abortion	0.80 ± 0.44	1.00 ± 0.00	0.09
NT		1.62±0.45	1.50±0.42	0.37

4. Discussion

The present study was conducted to compare the rate of amniocentesis fetal loss in twin and singleton pregnancies and, as previously presented, 2.4% in the twin group and 2.07% in the singleton group had fetal loss. There is no statistically significant difference between the two groups (p = 065). However, in the twin group, the prevalence of preterm was 13.1% and in the singleton group it was 1.75% and in the twin group, it was statistically significantly higher than in singleton group (p = 0.01). In general, the chances of preterm pregnancies in twins are much higher than in single twins, and the side effects are greater in twin pregnancies. In the following, due to the small sample size and single centrality of the present study, we will examine other studies conducted by other researchers to review their achievements, based on the reported results, to have a better understanding of the implications of the study hypothesis. In one study, Kalogiannidis et al., 2011 in Greece, compared the effects of amniocentesis on 6270 twin and singleton pregnancies. Maternal age was the main indicator of amniocentesis in both twins and singleton. The results showed that there was no difference in the rate of abortion (0.24% in singleton and 0% in twins). According to these results, the incidence of

amniocentesis side effects in twin pregnancies did not increase compared to singleton pregnancies (Kalogiannidis, Petousis, Prapa, Dagklis, Karkanaki, Prapas, & Prapas, 2011). In the present study, no statistically significant difference was observed about fetal loss between the two groups. Also, the sample size of the two studies was almost the same and indicates the similarity of the consequences of the intervention in both Asian and European societies. In another study, Lenis-Cordoba et al., 2013 in Spain, assessed the risk of fetal loss after second trimester amniocentesis in twin pregnancies. The findings showed that the rate of fetal loss 4 weeks after amniocentesis (2.7 vs. 2.6%) and the rate of fetal loss before 24 weeks of gestation (1.2 versus 1.1%) between the two groups is close to each other without statistically significant difference (Lenis-Cordoba, Sánchez, Bello-Muñoz, Sagalá-Martinez, Campos, CarrerasMoratonas, & Cabero-Roura, 2013). Although in their study the control group was designed and in the present study the singleton group was compared, the final result of both studies shows that amniocentesis will not increase the risk of fetal loss in patients. Other studies have reported a very low rate of fetal loss following amniocentesis in twin pregnancies. For example, Kan and colleagues in Hong Kong reported in 2012 that out of 105 twin pregnancies, 102 gave birth to live babies (Kan, Lee, Leung, Chan, Tang, & Chan, 2012). However, in some other studies, the results have been reported to be inconsistent with the present study, and contrary to our findings, amniocentesis has increased the rate of fetal loss in patients. In one study, Hanprasertpong et al., 2008 in Thailand, found that the rate of fetal loss 14 days after amniocentesis in pregnant women with twin pregnancies was 1.4% which showed Fetal loss in twin pregnancies after amniocentesis was higher than in single pregnancies (Hanprasertpong, Kor-anantakul, Prasartwanakit, Leetanaporn, Suntharasaj, & Suwanrath, 2008). In fact, contrary to the present study, fetal loss occurred in twin patients more than in single patients, but one of the reasons for this difference between the present study and their study could be the difference in the history of patients and the reasons for amniocentesis. Because in their study, all patients were intervened due to old age, while in the present study, patients for reasons including 62 people (73.8%) high-risk amniocentesis, 13 people (15.5%) due to parents with blood diseases, 7 people (8.3%) due to The history of children with genetic defects and 2 people (2.4%) also underwent amniocentesis due to abnormal ultrasound of one of the fetus. Inconsistent results are also seen in other studies. For example, Cahill et al. (2009) reported that people with twin pregnancies who underwent amniocentesis were more likely to lose their pregnancies than those who did not have amniocentesis (3.2% Compared to 1.4%) (Cahill, Macones, Stamilio, Dicke, Crane, & Odibo, 2009) and their findings on creativity are the results of our study which has been inconsistent with our results.

5. Conclusion

Our findings show that fetal loss following amniocentesis in twin pregnancies does not increase significantly compared to singleton pregnancies. However, our finding requires repeated study in bigger sample size and as multi-centered as possible then It can be generalized to wider communities.

Acknowledgements

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Relationship between Pre-Cystoscopy Anxiety and Post-Cystoscopy Pain: A Cross Sectional Study

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Abstract

Introduction: Cystoscopy is one of the common procedure in diagnosis and treatment of problem related to urinary tract, pre-cystoscopy anxiety is a very common problem, despite evidences shows suitable intervention for post-cystoscopy pain but enough pain relief still remains as a problem, the aim of this study was to investigate the relationship between pre-cystoscopy anxiety and post-cystoscopy pain among patients who was hospitalized for cystoscopy.

Methods: This was a cross sectional study and sampling of this study was done in April 2017 to April 2018 in surgical unit of amiralmoemenin hospital in zanjan, Iran, using non-probability convenience Sampling method, 61 patients aged 16-45 years old who was hospitalized for cystoscopy was recruited in this study. Pre-cystoscopy anxiety was assessed by spilberger (STAI) questionnaire and post-cystoscopy pain measured by pain visual analogs scale (VAS). **Results:** The mean (SD) age of men and females were 43.81 (14.68), 44.88 (13.61) years old, respectively The relationship between state anxiety and post cystoscopy pain was direct and significant ($p<0.05$). The relationship between trait anxiety and post cystoscopy pain was direct and significant too ($p<0.05$).

Conclusion: the state and trait Anxiety of patients who were hospitalized for cystoscopy could increases the intensity of patients' pain in post-cystoscopy.

Keywords: Anxiety, Pain, Cystoscopy, Cross-Sectional Studies

1. Introduction

Cystoscopy is an important diagnostic method over the past 150 years. Each person typically experiences cystoscopy throughout his life, but since it is main method for assessment and diagnosis bladder tumor, cystoscopy is more common in individuals with bladder tumors (Jellinghaus, 1979).

The main goal of cystoscopy is to evaluate the patients thoroughly and accurately, without discomfort and without using sedative medications. The success of cystoscopy depends on insertion of the instrument into the bladder, precise observation and minimize patient discomfort during the procedure, although Technical advancements have reduced pain and discomfort in cystoscopy, but several studies have demonstrated significant pain and discomfort in cystoscopy due to the procedure and environmental status (Burke, Shackley, & O'reilly, 2002; Grossfeld et al., 2001). Recognition and treatment of pain is one of the oldest sciences which humans to find out more about it. From the beginning of creation, humans have suffered from pain and have achieved tremendous achievements in dealing with it. But pain is still one of the major problems in global health that people have been terrified throughout life, which may also be eradicated by pain (Nikbakht Nasrabadi, 1994). various ways have been used to pain relief (Bonica, 1990), Because effective pain treatment prevents the physiological and psychological harmful results of this phenomenon, the effective pain treatment is very considerable both in patient's recovery and in patient's economic condition (Chung, Hou, & Pan, 2004).

Despite the evidences shows suitable intervention for post-operative pain, but the enough pain relief treatments

still remains as a problem (Watt-Watson, Stevens, Garfinkel, Streiner, & Gallop, 2001). Acute post-operative pain is one of the worse pain that human tolerates and this pain be more intense, it creates more unfavorable hemodynamic and metabolic responses (Dabbagh et al., 2010). Post-operative pain can cause to harmful results, for example: thromboembolism, reduce of myocardial perfusion, increase of surgical site infection, irregular heartbeat, Urinary retention, bowel obstruction, delay in wound healing, delay in return stomach movements, increase of nausea, severe inability, and delay in starting oral nutrition (Andaroodi, Mahoori, Abbasivash, Noroozinia, & Heshmati, 2006; Mokhtari, Sirati, Sadeghi, Ghanbari, Babatabar, & Mahmoudi, 2010).

One of the factors that may effect on patients' post-operative pain is rate of pre-operative anxiety (Kim, Byeon, Song, & Lee, 2010). Studies showed that the patients are very agitated before surgery (Gunnarsdottir & Jonsdottir, 2007). Cystoscopy can cause to anxiety in patients too (Morgan, Roufeil, Kaushik, & Bassett, 1998).

Anxiety is a vague feeling, worry that often creates due to exposure to unfamiliar situations, the risk of death, fear, changes of body shape or function, increase of dependency, familial concerns and lifestyle acute changes (Zakerimoghadam, Aliasgharpoor, Mehran, & Mohammadi, 2010). Pre-operative anxiety is a very common problem among hospitalized patients (Bassampour, Nikbakht Nasrabadi, Mehran, Poresmaeil, & Valiee, 2008).

Boker et al. (2002) stated that the rate of pre-operative anxiety is 60 percent (Boker, Brownell, & Donen, 2002) Pre-operative anxiety is a challenging aspect in pre-operative care, most patients who waiting for elective surgery are anxious and it widely accepted as an expected response (Badner, Nielson, Munk, Kwiatkowska, & Gelb, 1990).

Studies showed that high level of pre-operative anxiety can lead to increase of post-operative analgesic demand, increase of hospitalization time, and reduce the patients' satisfactions (Hobson, Slade, Wrench, & Power, 2006). Because anxiety effects on the patient's mental state and it may have an adverse effect on treatment outcomes, it is very important that nurses consider the strategies to increase comfort and reduce the stress and anxiety in patients (Gagner-Tjellesen, Yurkovich, & Gragert, 2001).

In a study conducted by Stein et al. (1994) the anesthesia effect of intraurethral lidocaine gel and anxiety of patient on pain during cystoscopy was measured. In this study they found no decrease in pain perception in men or women following lidocaine gel instillation with a 5 or 10-minute dwell time compared to instillation of the plain lubricant. Increased pre procedure anxiety was correlated with increased pain perception in women. Personal experience with prior cystoscopy procedures significantly decreased the current pain perception in men (Stein, Lubetkin, Taub, Skinner, Haberman, & Kreutzer, 1994).

Soomro et al. (2011) evaluated the effect of patient's self-viewing on cystoscopy's pain, so the samples viewed the cystoscopy's procedure on a video then the pain of patients was evaluated. The results showed that the patients who viewed the cystoscopy procedure on a video monitor, reported less pain on a visual analog scale than the control group (Soomro, Nasir, & Ather, 2011).

Ganei et al. (2013) evaluated the relationship between pre-Caesarean anxieties with post-caesarean pain. The results showed pre-operative anxiety significantly was related to post-operative pain (Ganei, Rezai, & Mohamady, 2013). According to this study, it can be concluded that there is likely a relationship between the pre-cystoscopy's anxieties and the post-cystoscopy's pain.

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Table 1. Inclusion and exclusion criteria

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	Occurrence of post-surgical Complications such as bleeding
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The project proposal legislated with the ethics code (ZUMS.REC.1396.09) in Zanjan University of Medical Sciences. Then an introduction letter from Zanjan University of Medical Sciences was taken and referred to surgical unit of amiralmomenin hospital in Zanjan, Iran, for sampling and getting information of them This study was done in tree steps. First step; getting information about the goal of study and confidentiality of information was given to the samples, then the consent forms was taken from the patients and demographic characterizes of samples was recorded, second step; spillberger questionnaire (questionnaire for pre-cystoscopy's anxiety of patients) were completed by cystoscopic candidates before cystoscopy. third steps; secondary questionnaire (VAS; Visual Analogs Scale) for post-cystoscopy's pain were completed by them two hours after cystoscopy (Ganei, Rezai, & Mohamady, 2013). The two questionnaires were given to 61 patients that had inclusions criteria, all of the patients completed questionnaires correctly and finally, 61 samples participate for final analysis.

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For data analysis, the $SPSS_{21}$ (Chicago, IL, USA) was used .To determine the normal distribution of data, the Kolmogorov-Smirnov test (K-S) was used. Spearman correlation test was used to measure the relationship between pre-cystoscopy's anxiety and post- cystoscopy's pain.

3. Results

The mean (SD) age of men and females were 43.81(14.68), 44.88(13.61) years old, respectively. other demographic characters has been given in Table 2.

Table 2. Demographic Characteristic of qualitative variables

		Frequency	Percent (100%)
Gender	male	44	72.1
	female	17	27.9
Marital status	Married	48	78.7
	single	13	21.3
Living area	urban	21	34.4
	village	40	65.6
Surgical history	Yes	38	62.3
	No	23	37.7
Educational level	illiterate	32	52.5
	middle school degree	10	16.1
	High school diploma	9	14.8
	Upper High school diploma	10	16.4
Job	Unemployed	19	31.1
	Employee	8	13.1
	Self-employed	34	55.7

The distribution of data was not normal ($P<0.05$) .therefore, the spearman test was used for analyzing the relationship between variables. The results of analysis of the relationship between patients' state and trait anxiety with post-cystoscopy's pain are given in Table 3. There was direct and significant relationship between patients' state anxiety with post-cystoscopy's pain (p=0.005). There was direct and significant relationship between patients' trait anxiety with post-cystoscopy's pain (p=0.025) and there was direct and significant relationship between patients' state and trait anxiety too (P=0.000).

Table 3. Relationship between patients' state and trait anxiety with post-cystoscopy pain

Patients' pain and Anxiety		SD	Mean	N	P (Spearman)		R (spearman)	
Patient's pain		2.49	2.14	61				
Patient's anxiety	0.355[a]	10.32	41.81	61	State anxiety	0.628[b]	0.005[c]	0.000[d]
	0.286[a]	7.70	41.42	61	Trait anxiety		0.025[c]	

4. Discussion

The aim of this study was to determine the correlation between pre-cystoscopy's anxieties with post-cystoscopy's pain. results of this study showed that the correlation between pre- cystoscopy's state anxiety and post-cystoscopy's pain is significant and direct.

Cystoscopy is one of common process that urologists use it (Yoshimura, Wada, & Kishimoto, 1999). One of the challenging concept in pre-operative caring is anxiety (Jawaid, Mushtaq, Mukhtar, & Khan, 2007) Most of patients awaiting for elective surgery are anxious (Badner, Nielson, Munk, Kwiatkowska, & Gelb, 1990). In This study, fifty percent of patients have moderate score of trait and state of anxiety that is similar to study of Jawaid et al. (Jawaid, Mushtaq, Mukhtar, & Khan, 2007), that showed there was significant anxiety in patients before surgery.

Studies have shown significant pain and discomfort in patients because of cystoscopy (Burke, Shackley, & O'reilly, 2002; Grossfeld et al., 2001), in several studies, relationship between pre-cystoscopy factors with cystoscopy pain have been assessed (Ozdemir, Altinova, Koyuncu, Serefoglu, & Balbay, 2014).

In this study, the pre-cystoscopy's anxiety (state and trait anxiety) and post-cystoscopy's pain of patients were assessed. The results showed that there was significant correlation between pre-cystoscopy's anxiety and post-cystoscopy's pain. in the study of stein et al., there was significant correlation between women's pre-cystoscopy's anxiety and their pain that is similar to the results of present study but results of their study showed that men's pre-cystoscopy's anxiety had no correlation with cystoscopy's pain(20). The reason of their findings maybe was related to the race of the participants, because their participants were Blacks and other race, difference of races may lead to difference in results.

In current study the correlation between state anxiety and cystoscopy's pain was stronger than the correlation between trait anxiety and cystoscopy's pain. The reason for this findings maybe is because that state anxiety creates with some factors like Imagine about procedure, lack of information about it and factors related to hospital conditions and nursing condition (Salehi, Amini, Frizzy-Amiry, & Pakpour, 2017) and it creates in the same moment. Those factors may lead to sense pain after cystoscopy. The results of this study is similar to study of Soomro et al., jowang king yu et al., and kasra et al., (Soomro, Nasir, & Ather, 2011; Yeo, Cho, Oh, Park, & Park, 2013; Kesari, Kovisman, Cytron, & Benjamin, 2003). In these studies cystoscopy's pain has reduced by using several anxiety reducing methods like showing cystoscopy process with video projector to patients and listening to music during cystoscopy (Soomro, Nasir, & Ather, 2011; Yeo, Cho, Oh, Park, & Park, 2013; Kesari, Kovisman, Cytron, & Benjamin, 2003)). These studies confirmed that by reducing the cystoscopy's state anxiety, the cystoscopy's pain can be reduce significantly.

Chiu et al., has studied about the stress management in patients who had prostate biopsy, in this study patient divided into two groups (case and control). Case group received intervention for reduce of stress at pre procedure, results of this study show reducing of patients anxiety and pain in case group after procedure (Chiu et al., 2016). Result of this study have confirmed this issue that by removing stress which is one of the factors contributing to pain ,we can prevent of post - operative pain.

According to the mentioned studies and results of present study, state anxiety is major factor that increases patient's pain, so it is recommended that an anxiety reduction programs for patient undergoing cystoscopy should be set up. The future studies can include study that compares different types of interventions to reduce

patient's anxiety. The Limitation of this study is related to selfn - reporting of anxiety and pain by means of patients because the VAS scale and spilberger questionnaire are self - reporting instruments. Low sample size was other limitation of this study, because the researchers' time had one year's permission for collecting data, they had to end the project just with 61 participants.

5. Conclusion

Results of this study showed that the correlation between pre-cystoscopy's anxiety and post-cystoscopy's pain was significant, and the state anxiety which occurs due to the process itself, had stronger correlation with cystoscopy's pain. Therefore by using anxiety reducing methods before cystoscopy the patient's pain can be prevent and reduce significantly. The medications that have harmful results for patients can be reduce to use too which can help patients and governments economy.

Acknowledgement

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Relationship between Pre-Cystoscopy Anxiety and Post-Cystoscopy Pain: A Cross Sectional Study

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Abstract

Introduction: Cystoscopy is one of the common procedure in diagnosis and treatment of problem related to urinary tract, pre-cystoscopy anxiety is a very common problem, despite evidences shows suitable intervention for post-cystoscopy pain but enough pain relief still remains as a problem, the aim of this study was to investigate the relationship between pre-cystoscopy anxiety and post-cystoscopy pain among patients who was hospitalized for cystoscopy.

Methods: This was a cross sectional study and sampling of this study was done in April 2017 to April 2018 in surgical unit of amiralmoemenin hospital in zanjan, Iran, using non-probability convenience Sampling method, 61 patients aged 16-45 years old who was hospitalized for cystoscopy was recruited in this study. Pre-cystoscopy anxiety was assessed by spilberger (STAI) questionnaire and post-cystoscopy pain measured by pain visual analogs scale (VAS).

Results: The mean (SD) age of men and females were 43.81 (14.68), 44.88 (13.61) years old, respectively The relationship between state anxiety and post cystoscopy pain was direct and significant ($p<0.05$). The relationship between trait anxiety and post cystoscopy pain was direct and significant too ($p<0.05$).

Conclusion: the state and trait Anxiety of patients who were hospitalized for cystoscopy could increases the intensity of patients' pain in post-cystoscopy.

Keywords: Anxiety, Pain, Cystoscopy, Cross-Sectional Studies

1. Introduction

Cystoscopy is an important diagnostic method over the past 150 years. Each person typically experiences cystoscopy throughout his life, but since it is main method for assessment and diagnosis bladder tumor, cystoscopy is more common in individuals with bladder tumors (Jellinghaus, 1979).

The main goal of cystoscopy is to evaluate the patients thoroughly and accurately, without discomfort and without using sedative medications. The success of cystoscopy depends on insertion of the instrument into the bladder, precise observation and minimize patient discomfort during the procedure, although Technical advancements have reduced pain and discomfort in cystoscopy, but several studies have demonstrated significant pain and discomfort in cystoscopy due to the procedure and environmental status (Burke, Shackley, & O'reilly, 2002; Grossfeld et al., 2001). Recognition and treatment of pain is one of the oldest sciences which humans to find out more about it. From the beginning of creation, humans have suffered from pain and have achieved tremendous achievements in dealing with it. But pain is still one of the major problems in global health that people have been terrified throughout life, which may also be eradicated by pain (Nikbakht Nasrabadi, 1994). various ways have been used to pain relief (Bonica, 1990), Because effective pain treatment prevents the physiological and psychological harmful results of this phenomenon, the effective pain treatment is very considerable both in patient's recovery and in patient's economic condition (Chung, Hou, & Pan, 2004).

Despite the evidences shows suitable intervention for post-operative pain, but the enough pain relief treatments

still remains as a problem (Watt-Watson, Stevens, Garfinkel, Streiner, & Gallop, 2001). Acute post-operative pain is one of the worse pain that human tolerates and this pain be more intense, it creates more unfavorable hemodynamic and metabolic responses (Dabbagh et al., 2010). Post-operative pain can cause to harmful results, for example: thromboembolism, reduce of myocardial perfusion, increase of surgical site infection, irregular heartbeat, Urinary retention, bowel obstruction, delay in wound healing, delay in return stomach movements, increase of nausea, severe inability, and delay in starting oral nutrition (Andaroodi, Mahoori, Abbasivash, Noroozinia, & Heshmati, 2006; Mokhtari, Sirati, Sadeghi, Ghanbari, Babatabar, & Mahmoudi, 2010).

One of the factors that may effect on patients' post-operative pain is rate of pre-operative anxiety (Kim, Byeon, Song, & Lee, 2010). Studies showed that the patients are very agitated before surgery (Gunnarsdottir & Jonsdottir, 2007). Cystoscopy can cause to anxiety in patients too (Morgan, Roufeil, Kaushik, & Bassett, 1998).

Anxiety is a vague feeling, worry that often creates due to exposure to unfamiliar situations, the risk of death, fear, changes of body shape or function, increase of dependency, familial concerns and lifestyle acute changes (Zakerimoghadam, Aliasgharpoor, Mehran, & Mohammadi, 2010). Pre-operative anxiety is a very common problem among hospitalized patients (Bassampour, Nikbakht Nasrabadi, Mehran, Poresmaeil, & Valiee, 2008).

Boker et al. (2002) stated that the rate of pre-operative anxiety is 60 percent (Boker, Brownell, & Donen, 2002) Pre-operative anxiety is a challenging aspect in pre-operative care, most patients who waiting for elective surgery are anxious and it widely accepted as an expected response (Badner, Nielson, Munk, Kwiatkowska, & Gelb, 1990).

Studies showed that high level of pre-operative anxiety can lead to increase of post-operative analgesic demand, increase of hospitalization time, and reduce the patients' satisfactions (Hobson, Slade, Wrench, & Power, 2006). Because anxiety effects on the patient's mental state and it may have an adverse effect on treatment outcomes, it is very important that nurses consider the strategies to increase comfort and reduce the stress and anxiety in patients (Gagner-Tjellesen, Yurkovich, & Gragert, 2001).

In a study conducted by Stein et al. (1994) the anesthesia effect of intraurethral lidocaine gel and anxiety of patient on pain during cystoscopy was measured. In this study they found no decrease in pain perception in men or women following lidocaine gel instillation with a 5 or 10-minute dwell time compared to instillation of the plain lubricant. Increased pre procedure anxiety was correlated with increased pain perception in women. Personal experience with prior cystoscopy procedures significantly decreased the current pain perception in men (Stein, Lubetkin, Taub, Skinner, Haberman, & Kreutzer, 1994).

Soomro et al. (2011) evaluated the effect of patient's self-viewing on cystoscopy's pain, so the samples viewed the cystoscopy's procedure on a video then the pain of patients was evaluated. The results showed that the patients who viewed the cystoscopy procedure on a video monitor, reported less pain on a visual analog scale than the control group (Soomro, Nasir, & Ather, 2011).

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	village	40	65.6
Surgical history	Yes	38	62.3
	No	23	37.7
Educational level	illiterate	32	52.5
	middle school degree	10	16.1
	High school diploma	9	14.8
	Upper High school diploma	10	16.4
Job	Unemployed	19	31.1
	Employee	8	13.1
	Self-employed	34	55.7

The distribution of data was not normal ($P<0.05$). Therefore, the spearman test was used for analyzing the relationship between variables. The results of analysis of the relationship between patients' state and trait anxiety with post-cystoscopy's pain are given in Table 3. There was direct and significant relationship between patients' state anxiety with post-cystoscopy's pain (p=0.005). There was direct and significant relationship between patients' trait anxiety with post-cystoscopy's pain (p=0.025) and there was direct and significant relationship between patients' state and trait anxiety too (P=0.000).

Table 3. Relationship between patients' state and trait anxiety with post-cystoscopy pain

Patients' pain and Anxiety		SD	Mean	N	P (Spearman)		R (spearman)	
Patient's pain		2.49	2.14	61				
Patient's anxiety	0.355[a]	10.32	41.81	61	State anxiety	0.628[b]	0.005[c]	0.000[d]
	0.286[a]	7.70	41.42	61	Trait anxiety		0.025[c]	

4. Discussion

The aim of this study was to determine the correlation between pre-cystoscopy's anxieties with post-cystoscopy's pain. results of this study showed that the correlation between pre- cystoscopy's state anxiety and post-cystoscopy's pain is significant and direct.

Cystoscopy is one of common process that urologists use it (Yoshimura, Wada, & Kishimoto, 1999). One of the challenging concept in pre-operative caring is anxiety (Jawaid, Mushtaq, Mukhtar, & Khan, 2007) Most of patients awaiting for elective surgery are anxious (Badner, Nielson, Munk, Kwiatkowska, & Gelb, 1990). In This study, fifty percent of patients have moderate score of trait and state of anxiety that is similar to study of Jawaid et al. (Jawaid, Mushtaq, Mukhtar, & Khan, 2007), that showed there was significant anxiety in patients before surgery.

Studies have shown significant pain and discomfort in patients because of cystoscopy (Burke, Shackley, & O'reilly, 2002; Grossfeld et al., 2001), in several studies, relationship between pre-cystoscopy factors with cystoscopy pain have been assessed (Ozdemir, Altinova, Koyuncu, Serefoglu, & Balbay, 2014).

In this study, the pre-cystoscopy's anxiety (state and trait anxiety) and post-cystoscopy's pain of patients were assessed. The results showed that there was significant correlation between pre-cystoscopy's anxiety and post-cystoscopy's pain. in the study of stein et al., there was significant correlation between women's pre-cystoscopy's anxiety and their pain that is similar to the results of present study but results of their study showed that men's pre-cystoscopy's anxiety had no correlation with cystoscopy's pain(20). The reason of their findings maybe was related to the race of the participants, because their participants were Blacks and other race, difference of races may lead to difference in results.

In current study the correlation between state anxiety and cystoscopy's pain was stronger than the correlation between trait anxiety and cystoscopy's pain. The reason for this findings maybe is because that state anxiety creates with some factors like Imagine about procedure, lack of information about it and factors related to hospital conditions and nursing condition (Salehi, Amini, Frizzy-Amiry, & Pakpour, 2017) and it creates in the same moment. Those factors may lead to sense pain after cystoscopy. The results of this study is similar to study of Soomro et al., jowang king yu et al., and kasra et al., (Soomro, Nasir, & Ather, 2011; Yeo, Cho, Oh, Park, & Park, 2013; Kesari, Kovisman, Cytron, & Benjamin, 2003). In these studies cystoscopy's pain has reduced by using several anxiety reducing methods like showing cystoscopy process with video projector to patients and listening to music during cystoscopy (Soomro, Nasir, & Ather, 2011; Yeo, Cho, Oh, Park, & Park, 2013; Kesari, Kovisman, Cytron, & Benjamin, 2003)). These studies confirmed that by reducing the cystoscopy's state anxiety, the cystoscopy's pain can be reduce significantly.

Chiu et al., has studied about the stress management in patients who had prostate biopsy, in this study patient divided into two groups (case and control). Case group received intervention for reduce of stress at pre procedure, results of this study show reducing of patients anxiety and pain in case group after procedure (Chiu et al., 2016). Result of this study have confirmed this issue that by removing stress which is one of the factors contributing to pain, we can prevent of post - operative pain.

According to the mentioned studies and results of present study, state anxiety is major factor that increases patient's pain, so it is recommended that an anxiety reduction programs for patient undergoing cystoscopy should be set up. The future studies can include study that compares different types of interventions to reduce

patient's anxiety. The Limitation of this study is related to selfn - reporting of anxiety and pain by means of patients because the VAS scale and spilberger questionnaire are self - reporting instruments. Low sample size was other limitation of this study, because the researchers' time had one year's permission for collecting data, they had to end the project just with 61 participants.

5. Conclusion

Results of this study showed that the correlation between pre-cystoscopy's anxiety and post-cystoscopy's pain was significant, and the state anxiety which occurs due to the process itself, had stronger correlation with cystoscopy's pain. Therefore by using anxiety reducing methods before cystoscopy the patient's pain can be prevent and reduce significantly. The medications that have harmful results for patients can be reduce to use too which can help patients and governments economy.

Acknowledgement

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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The Effect of TNF-α on the Expression of MMP9 in Human Mesenchymal Bone Marrow-Derived Stem Cells

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Abstract

Matrix metalloproteinase 9 (MMP9) as the enzyme of adult stem cells secreted from damage cells. In spite of low level of MMP9 enzyme in the mesenchymal stem cells, many inflammatory cytokines stimulation such as TNF-α could increase MMP9 level in cells.

Current study evaluated the expression of the MMP9 enzyme under the influence of TNF-α in human bone marrow mesenchymal stem cells.

The human bone marrow mesenchymal stem cells were classified into control and experimental groups. In the experimental groups, various concentrations of the TNF-α (1ng/ml and 10ng/ml) were administrated in different times (10 and 24 hours), whereas the control group was not treated with TNF-α. MMP9 gene expression was evaluated by Real-Time PCR. TNF-α administration in 1ng/ml and 10ng/ml dosage for 10 hours, induced the expression of MMP9 1468.3 and 1782.8 times more than the control group, respectively.

After 24h, in comparison between 1ng/ml and 10ng/ml with control groups, MMP9 expression were 442.64 and 1184.4 times more than control group, respectively.

In conclusion, the expression rate of the MMP9 gene in bone marrow mesenchymal stem cells might be effected by dosage and time of exposure to TNF-α. Furthermore, the time of exposure might have the prominent role in alteration of MMP9 gene expression induction in the mesenchymal stem cells.

Keywords: Bone Marrow, Mesenchymal Stem Cell, MMP9 Enzyme, TNF-α, Cell Migration

1. Introduction

Mesenchymal stem cells (MSCs) participate to repair of their originated tissues such as bone, cartilage, muscle, tendon, and fat. Moreover, these are supportive cells for the hematopoietic cells production (Friedenstein et al., 1966). Typical mesenchymal stem cells were specialized to differentiated cells in the same texture of originated tissues when abnormal tissue proliferation occurs, as a result, they called them adult stem cells (Ullah et al., 2015).

According to International Society of Cell Therapy (ISCT) definition Human MSCs attache to the bottom of plastic culture dishes in the normal culture conditions. They are negative for the expression of the surface markers CD105, CD73 and CD90, hematopoietic markers CD45, CD34 and other markers such as CD19, CD79, CD11b, CD14 as well as HLA-DR; and should have been able to differentiate into adipocytes, cartilage and bone in vitro (Keating, 2006).

Many studies have shown that MSCs are able to escape from immune system and inhibit the immune response which have key role in transplantation and cell therapy (Zhao et al., 2011). MSCs have been used for tissue regeneration in several clinical studies especially in wound healing (Perry et al., 2008). Also, Human and mouse MSCs have been used in the mouse models of spinal cord injury healing as well as damaged heart tissue recovery (Rojas et al., 2005).

Matrix metalloproteinases (MMPs), as a calcium-dependent zinc-containing endopeptidases, express in the most damaged tissues such as cardiovascular and liver injury. The activity of these enzymes is regulated by tissue inhibitors. Changes in the expression levels of these enzymes have been reported in liver disease and

development as well as damaged body tissue treatment such as liver transplantation or stem cell transplantation. These enzymes digest many components of the extracellular matrix (ECM) and basement membrane structure, and their role is significant in physiological and pathological processes (Duarte et al., 2015).

MMPs have been reported to be either proenzyme or enzymes in biological samples. The activity of this protein is required for the precise regulation and control of cells including keratinocytes, fibroblasts, endothelial cells, macrophages, neutrophils, mast cells, acidophilic and CD34^{+} bone marrow, and umbilical cord. MMPs protein activity and degeneration must be controlled by the specific inhibitors. Inhibitors of this enzyme family (TIMPs) have a high concentration in the serum. (Behrendtsen & Werb, 1997).

Matrix metalloproteinase 9 (MMP9) gene family, the enzyme of adult stem cells in all tissues (especially in bone marrow, umbilical cord, and liver), is secreted from damaged cells. Kuppfer cells are important group of cells in the liver with macrophage-like behavior. Expression of MMP9 enzyme is increased with enhanced activity of Kuppfer cells. Progelatinaze B is activated by plasmin and estomelesine through protease activity, leading to a 92 kDa protein production. One of the most important members of Progelatinaze B gene family, gelatinase, is the major basement membrane composition in human cells. Gelatinase is the only member of this family with capability of binding to fibronectin and digesting collagen. Human MMP-9 gene is located on chromosome 20q and contains 13 exons which is a polymorphism C/T in the promoter region and leading to changes in the expression of this gene (Luo et al., 2004).

TNF-α belongs to the superfamily of TNF which comprises at least 19 members. TNF-α is produced by activated mononuclear phagocytic cells, antigen-stimulated T cells, NK, mast cells, and fibroblasts. TNF receptors are TNF-RI and TNF-RII that are found in most cells. TNF receptor binding strength is weaker than the other cytokines. TNF is a member of large proteins family involved in immune response and inflammation. Cytokines bind to the cytoplasmic domain of the TNF receptors induced activation of the transcription factors particularly nuclear factor κB (NF-κB) and activation protein (AP-1) which leading to caspase activation, apoptosis, and cell death (Hong et al., 2013).

The main physiological action of TNF is recruiting the monocytes and neutrophils to the site of infection. On the other hand, TNF affects the vascular endothelial cell adhesion molecules. TNF also stimulates the endothelial cells and macrophages to secrete the enzymes and cause leukocyte chemotaxis (Hong et al., 2013).

In the current study, we identified human umbilical cord mesenchymal stem cells (hUC-MSCs) by flow cytometry assay and induced differentiation assay to investigate whether the inflammatory environment could affect MSCs proliferation, migration and cytokines secretion. Also MSCs stimulation were examined by both TNF-α (20 ng/ml) and IFN-γ (50 ng/ml) as well as cell viability by MTT assay, migration by trans well assay, and cytokines expression level by real-time PCR analysis.

2. Material and Methods

### 2.1 Isolation and Culture of Human MSCs

Human MSCs were obtained from 5-ml iliac crest aspirates of about 15normal donors within the age range of 20-50 years who underwent bone marrow collection for a related patient after obtaining approval of the ethics committee. The code of ethics was taken from Shiraz University of Medical Sciences. Written informed consent was collected for clinical data collection and paraclinical examination. Each sample of aspirate was diluted 1:1 with Dulbecco's modified Eagle's medium (DMEM)-low glucose (1,000 mg/l glucose) (Invitrogen, Merelbeke, Belgium) and layered over about 5 ml of ficoll (Lymphoprep; Oslo, Norway). The isolation method was based on a previously reported method with slight modifications (Ayatollahi et al., 2011).briefly. after centrifugation harvested sample at 939g for 20min, the mononuclear cell layer was removed from the interface. The cells were suspended in DMEM and centrifuged again at 338g for 15 min. Then, centrifuged cells were suspended in basal DMEM medium containing 10% fetal calf serum (Invitrogen, Merelbeke, Belgium), 1% penicillin (Invitrogen, Merelbeke, Belgium), 1% streptomycin (Invitrogen, Merelbeke, Belgium), and 2 mM glutamine (Invitrogen, Merelbeke, Belgium). The cells were seeded at a density of 80.000/cm^2 in 25 cm^2 T-flasks and maintained at 37°C in an atmosphere of 5% CO2. After 4 days, the non-adherent cells were removed and the media changed every 3 days. In order to expand the MSCs cells, the adhered monolayer was detached with trypsin-EDTA (Invitrogen, Merelbeke, Belgium) for 5 min at 37°C, after 14 days for the first passage as well as every 4-5 days for successive passages sample. During *in vitro* passaging, the cells were seeded at a density of 5-10×10^3 cells/cm^2 and expanded for several passages until they no longer reached confluence.

2.2 Characterization of MSCs

At each stage of passage, the cells were counted and analyzed for viability by trypan blue staining analysis.

Cultured MSCs have been analyzed both morphologically and surface markers examination. Functional ability of differentiation into osteocyte and adipocyte were achieved in response to specific culture conditions. Each experiment described here was replicated three times.
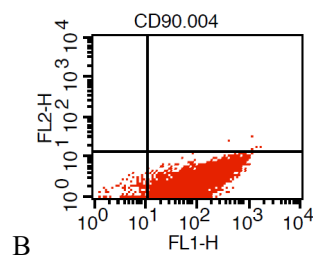
2.3 Flow Cytometric Analysis

A
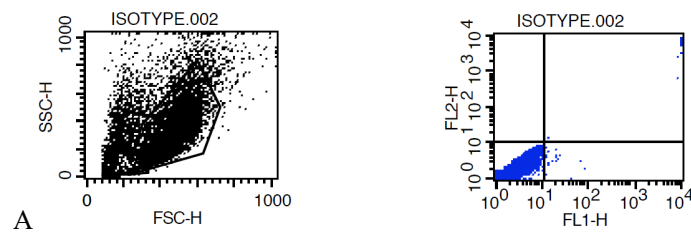
Quadrant Statistics

File: ISOTYPE.002   Log Data Units: Linear Values
Sample ID: ISOTYPE   Patient ID:
Gate: G6   Gated Events: 7147
Total Events: 10000   X Parameter: FL1-H (Log)
Y Parameter: FL2-H (Log)   Quad Location: 11, 11

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	14	0.20	0.14
LL	7110	99.48	71.10
LR	23	0.32	0.23

B

Quadrant Statistics

File: CD90.004   Log Data Units: Linear Values
Sample ID: CD90   Patient ID:
Gate: G1   Gated Events: 7073
Total Events: 10000   X Parameter: FL1-H (Log)
Y Parameter: FL2-H (Log)   Quad Location: 11, 14

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	7	0.10	0.07
LL	158	2.23	1.58
LR	6908	97.67	69.08

C
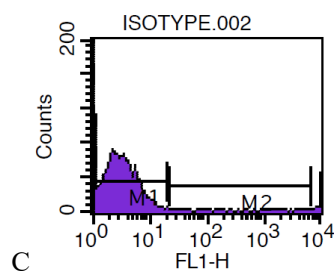
Histogram Statistics

File: ISOTYPE.002   Log Data Units: Linear Values
Sample ID: ISOTYPE   Patient ID:
Gate: No Gate   Gated Events: 10000
Total Events: 10000   X Parameter: FL1-H (Log)

Marker	Events	% Gated	% Total	Mean	Peak Ch
All	10000	100.00	100.00	163.74	1
M1	9740	97.40	97.40	3.30	1
M2	100	1.00	1.00	1919.57	21

D
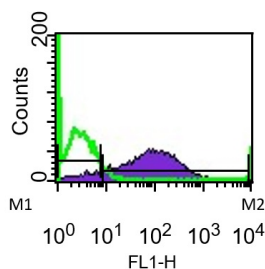
Histogram Statistics

File: CD90.004 Log Data Units: Linear Values Sample
ID: CD90   Patient ID:

Gate: No Gate   Gated Events: 10000

Total Events: 10000   X Parameter: FL1-H (Log)

Marker	Events	% Gated	% Total	Mean	Peak Ch
All	10000	100.00	100.00	133.58	73
M1	670	6.70	6.70	4.39	1
M2	9255	92.55	92.55	142.88	73

Figures 1. Use Two types antibody on hMSC, graph A and histogram C ellustrate no antigen receptores for isotype 002 on hMSC but graph B and histogram D ellustrate exist antigen receptores for CD90 on hMSC

The identification of adherent cells was performed by flow cytometric analysis. At the third passage, the cells were detached from the culture flasks with trypsin-EDTA and counted. About 1×10^6 of cells were incubated on ice for 30 min with goat serum, re-suspended in phosphate-buffered saline (PBS), and pelleted by centrifugation for 4 min at 2100 rpm. Subsequently, the cells were stained for 30 min at 4°C with a fluorescent isothiocyanate (FITC)-coupled or phycoerythrin (PE)-conjugated isotype 002 (negative control), HLADR, CD45, CD90, CD80, and CD40. The labeled cells were thoroughly washed with PBS and analyzed on a flow cytometer system (FACS Calibur Becton, Dickinson, USA), by WinMidi software (Scripps Research Institute; San Diego, USA). The percentage of positive cells was calculated by the cells stained with Ig FITC/PE as a negative control.

2.4 Experimental Groups

The MSCs of the six passages were trypsinized with 0.25% trypsin-EDTA and the cell suspension was centrifuged at 1200×g for 5 min. The cells were then treated with TNF-α at different concentrations and incubated at different times. Differences in the expression levels of MMP9 were compared between TNF-α-treated and untreated MSCs. Also, differences in the expression levels of MMP9 were compared in different concentrations and times of the treatment by TNF-α. The cells were divided into the following groups: 1) without TNF- α treatment; 2) stimulated for 10hr with 1ng/ml TNF-α; 3) stimulated for 24hr with 1 ng/ml TNF-α; 4) stimulated for 10hr with 10 ng/ml TNF-α; and 5) stimulated for 24hr with 10 ng/ml TNF-α.

2.5 RNA Extraction

Total RNA was extracted from TNF-α treated and untreated MSCs groups by RNX Plus solution kit (Cinna Gene- Iran) with following protocol: 1000 µl cultured MSCs were centrifuged at 12000 rpm for 5 min. The liquid phase was removed and mixed with 100 µl phosphate buffered saline (PBS). Then, 400µl RNX Plus solution and 200µl cold chloroform were added and centrifuged at 13500 rpm for 20 min in 4ºC condition which kept overnight at -20ºC. Finally, the pellet was washed twice with ethanol 75% and dissolved in the 25µl DEPC water.

2.6 cDNA Synthesis

cDNA was synthesized by in-house optimized protocol in total volume of 23µl. first of all, 1µl (0.2 µg/µl) of Random hexamer (Cinna Gene-Iran), 2µl (200 U/µl) of M-MuLv reverse transcriptase (ViVantis-Indonesia), 2µl (10x) of reverse transcriptase buffer, 2µl (10 mMol) of dNTP (Cinna Gene-Iran), 1.3µl (40U/µl) RNase inhibitor (ViVantis-Indonesia), and 14.7µl DEPC water. Finally, cDNA was synthesized in 42ºC for 90 minutes followed by 85ºC for 5 minutes.

2.7 Real-Time Polymerase Chain Reaction

The relative quantification of MMP9 in comparison with β-actin mRNA expression was performed after the designation of the specific primers for both MMP9 and β-actin gene transcripts. The MMP9 and β-actin primer sequences were as follows respectively:

Forward primer: 5'-GGACAAGCTCTTCGGCTTCT-3`; Reverse primer: 5`-

TCGCTGGTACAGGTCGAGTA-3`and Forward primer: 5`-

GGGCGGCACCACCATGATCC-3`; Reverse primer: 5`-

GACGATGGAGGGGCCCGACT-3`.

اشاره نشده است؟ housekeeping gene چرا در اینجا به

Table 1. Real-time PCR reactions for MMP9 and β-actin genes

Component	Amount (µl)	Concentration
SYBR premix Ex Taq II	10	1x
Dye	0.4	1x
Forward primer	0.4	5 pM
Reverse primer	0.4	5 pM
DEPC water	6.8	-
cDNA	2	100 ng
Total	20	-

Table 2. Real-time PCR programs for MMP9 and β-actin genes

Gene	Step	Temperature (ºC)	Time	Cycle
	Denaturation	95	2 min	1
	Denaturation	95	30 s	
MMP9	Annealing	57.5	20 s	40
	Extension	72	30 s	
	Final extension	72	5 min	1
	Denaturation	95	2 min	1
	Denaturation	95	30 s	
β-actin	Annealing	64	20 s	40
	Extension	72	30 s	
	Final extension	72	5 min	1

Upon making the PCR reaction mixes, the expression analysis was performed by the real-time PCR thermocycler (Step one plus Applied Biosystems-U.S.A) (Table 1 and 2). The fold change of relative mRNA expression was determined by using Livak method ($2^{-\Delta\Delta Ct}$).

3. Results

3.1 Isolation and Expansion of Human MSCs

Adherent cells were observed in all samples after 3 days of culture and 15 days later, adherent monolayer was achieved (Figure 2A). The rapid expansion of the MSCs in the culture might be related to the presence of single-cell-derived colonies composed of a few fibroblast-like cells (Figure 2B). Bone marrow cells rapidly generated a confluent layer of cells with an elongated, fibroblastic shape. These cells contained two types: a type of cells with large and flat morphology, and a type of smaller spindle-shaped cells (Figure 2C). The cells were increased in size and showed a polygonal morphology with evident filaments in the cytoplasm, especially when early passage cells were compared with late passage cells. MSCs isolated from healthy donors were expanded for up to 10 passages.

3.2 Viability Evaluation

At each passage, the cells were counted and analyzed for viability by trypan blue staining analysis, showing a viability of 98- 100% in the samples.
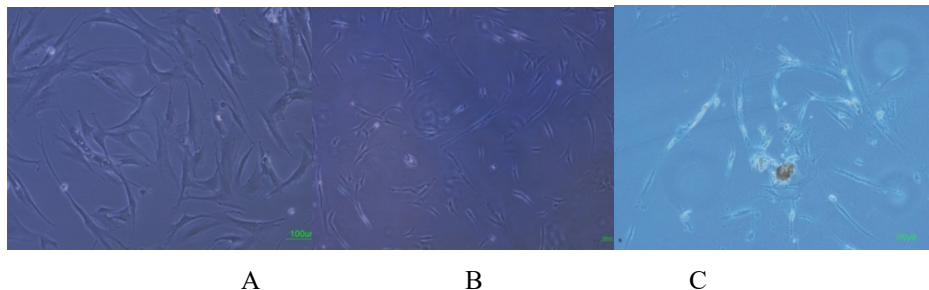

A   B   C

Figure 2. Isolation and culture of human bone marrow derived MSCs

A) Adherent monolayer was achieved in the following 6-7 days;

B) The presence of single cell-derived colonies composed of a few fibroblast-like cells;

C) As the culture proceeded, the cells were both of small spindle, and wide-shaped morphology. Scale bar for the Figures A-C is 100 um.
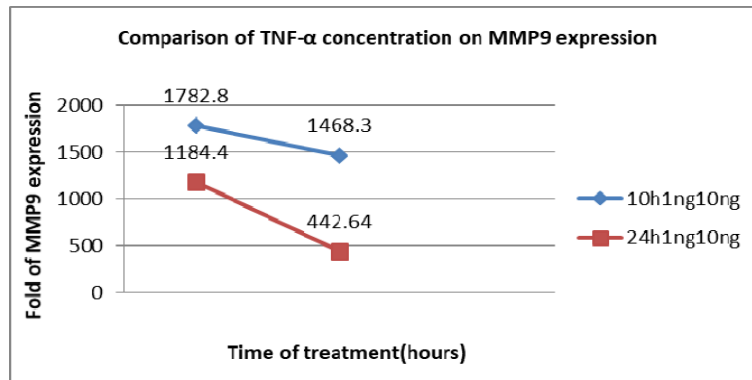
3.3 MMP9 Expression

The expression level of MMP9 gene in the untreated MSCs was lower than treatment groups (Table 3). The expression level of MMP9 gene was compared for various treatment conditions with different times and concentrations that was highly increased in the two treatments including 1ng/ml ($2^{-\Delta\Delta Ct}$=1468.3) and 10 ng/ml ($2^{-\Delta\Delta Ct}$=1782.8) TNF-α for 10 h (Table 3 and Figure 2). However, the highest level of MMP9 gene expression was found upon treatment with 10 ng/ml TNF-α for 10 h ($2^{-\Delta\Delta Ct}$=1782.8) (Table 3 and Figure 3).

Table 3. Gene expression level of MMP9 and β-actin in MSCs after treatment with TNF-α

Conditions of treatment with TNF-α	Ct MMP9	Ct β-actin	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$
1 ng/ml–10 hours	17.28	17.69	- 0.41	-52.10	1468.3
1 ng/ml–24 hours	14.89	13.57	1.32	-8.79	442.64
10 ng/ml–10 hours	19.78	20.47	- 0.69	- 10.8	1782.8
10 ng/ml–24 hours	18.14	18.97	- 0.83	- 10.21	1184.4
Negative Control (Untreated)	32.46	22.35	10.11	-	-

A) Comparison of TNF-α concentration on MMP9 expression

B) MMP9 expression in treated and untreated hBMSCs
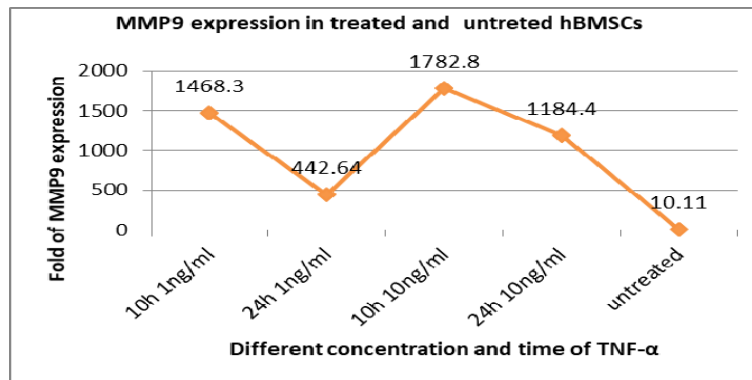
C) Histogram chart effect of concentration TNF-α on expression mmp9 in hBMSCs
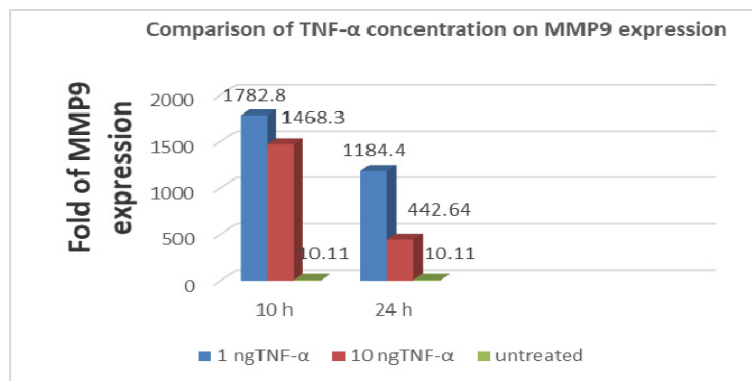
Figure 3. Comparison of TNF-α concentration on MMP9 gene expression in the treated and untreated MSCs

4. Discussion and Conclusion

Mesenchymal stem cells are nonhematopoietic stem cells with multi-proliferative and multi-differentiative potentials. The ability of MSCs to modulate the immune responses and migration to the site of inflammation makes these cells a promising source of cells for cell-based therapeutic strategies (Tsai et al., 2011). Chemokine receptors, ligands, and adhesion molecules play key roles in tissue-specific homing of the leukocytes and have also been implicated in trafficking of hematopoietic precursors into the tissues. The migration of MSCs to the sites of injury or inflammation is also mediated by chemotactic factors produced by the immune cells. It has been demonstrated that human MSCs show significant chemotaxis responses to several factors, including PDGF, VEGF, IGF-1, IL-8, bone morphogenetic protein (BMP)-4 and BMP-7 (Yorsangsukkamol et al., 2011; Kollet et al., 2003). The mechanism of MSCs migration and homing has not been fully understood. Although it seems that homing in MSCs occurs by tethering, rolling and endothelial transmigration similar to the leukocytes; however, they differ in the types of molecules involved in the migration process. For example, MSCs do not utilize E or P-selectin as a tethering mechanism since they do not express the fucosyltransferase IV or VII, and use other adhesion molecules such as CD44, VLA-4 and VCAM (Yorsangsukkamol et al., 2011). MSCs are also large cells, so they can hold up on capillary beds which allow them to transmigrate across the endothelium in response to chemokine gradient (Ullah et al., 2015).

In this study, we analyzed different gene expression patterns of chemokine receptor MMP9 on MSCs which have a critical role in migration and engraftment. It was shown that human bone marrow MSCs expressed low level of MMP9. Our observation was confirmed by other studies such as that of Wynn *et al.*; they reported that a small portion of MSCs expressed MMP9 which contributed to their migration *in vitro*. They showed that less than 1% of human MSCs express CXCR4 (Wynn et al., 2004). Ahmadian Kia *et al.* observed low or no detectable mRNA expression of MMP9 in the bone marrow MSCs, whereas Sordi *et al.* demonstrated that 26% of human bone marrow MSCs express CXCR4 (Ahmadian Kia et al., 2011; Sordi et al., 2005). There are controversial reports about expressing chemokine receptors on MSCs (Macfarlan et al., 2012; Nakagawa & Yamanaka, 2010; Meriane et al., 2006).

Considering the important role of the couple of ligand-receptor SDF-1/ MMP9 in cell migration and cell engraftment, overexpression of chemokine receptor MMP9 on MSCs increases the ability of migration and engraftment for clinical application of these cells and this can be one of the strategies for enhancing the potential of MSCs in cell-based therapies. On the other hand, there are various inflammatory cytokines and chemokines at the injured sites that can lead to movement of MSCs to the injured and inflamed tissues (Meriane et al., 2006). These cytokines can regulate the expression of genes in various cells depending on the cell type and differentiation stage (Nombela-Arrieta et al., 2011).

TNF-α is a pro-inflammatory cytokine that has been shown to affect the gene expression in cells and can influence the expression of molecules involved in the migration process (Meriane et al., 2006; Tsai et al., 2011). Recently, it has been demonstrated that MMP9 expression increase in gastric cancer cells is induced by TNF-α (Zhao et al., 2010). Croitoru-Lamoury *et al.* demonstrated that IFN-γ and IFN-1β up-regulated the chemokines and chemokine receptors in human MSCs (Croitoru-Lamoury et al., 2007). Esteve *et al.* observed that MMP9 expression in treated human astroglioma cells was enhanced by TNF-α and IL-1β (Esteve et al., 2002). However, it has also been reported that TNF-α reduces chemokine receptor expression in some types of cells. Tikhonov *et al.* have found downregulation of MMP2 expression by TNF-α in polymorphonuclear leukocytes (Tikhonov et al., 2001).

Our data suggest that TNF-α can up-regulate the MMP9 expression in human bone marrow MSCs in a time and concentration-dependent manner. We observed that the highest level of MMP9 gene expression was treated with 10 ng/ml TNF-α for 10 hours. We revealed that the optimum time of TNF-α- treatment for enhancing MMP9 expression was 10 hours.

Previously, Kulbe *et al.* treated the ovarian cancer cells with 1, 10 and 100 ng/ml TNF-α in different incubation times (Kulbe et al., 2007). They have indicated that the most rate of MMP9 expression level occurred in 10 ng/ml TNF-α between 6 to 24 hours. They also have demonstrated that there is a relationship between TNF-α/NF-κB and MMP9 expression (Cheng et al., 2008). Effect of TNF-α on MMP-9 expression in HT1376 cells, the experiment showed the expression of mmp9 in 100 ng/ml and time 24h of TNF-α (Se-Jung Lee et al., 2007).

TNF-α- induced production of matrix metalloproteinase-9 by human bronchial epithelial cells. After stimulation with TNF-a (10 ng/ml), the level of matrix metalloproteinase-9 mRNA was increased in a time-dependent manner and the expression peaked at 24h (Hozumi et al., 2001). To determine the effect of TNF-α on MMP-9

expression, MC3T3-E1 cells were incubated with various concentrations of TNF-α for the indicated time intervals. The experiment also showed expression of mmp9 in 30 ng/ml and time 48h of TNF-α(Chia-Lan Tsai et al., 2014).

The results of current study suggested that *in vitro* control of environmental factors both in the concentration and time level may be important in the stem cell migration capacity and perhaps it is crucial in stem cell transplantation therapies.

Acknowledgements

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Assessment of the Consistency of Tetabulin Injection to the Patients with an Open Fracture Referred to the Khatamolanbia Hospital, Zahedan in 2017 with the National Guidelines

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Abstract

Background and Goal: Open fractures are at risk of infection with Clostridium tetani and severe traumatic infections. Tetabulin injection is strongly recommended for the patients with an open fracture and severe wounds. The goal of this study is to assess the consistency of tetabulin injection to the patients with an open fracture referred to the Khatamolanbia hospital in Zahedan in 2017 with the national guidelines.

Materials and Methods: This study is a cross-sectional descriptive study. 300 patients with an open fracture referred to the ER of the Khatamolanbia Hospital in Zahedan in 2017 were selected as the sample. Their fracture type and severity were assessed. The data were classified in the tables and statistically analyzed using Chi-square, pared t-test, Pearson correlation, and regression in SPSS 26.

Findings: Among 300 patients, 275 patients (91.7%) were male and 25 patients (8.3%) were female. The most frequent age range was 20 to 30 years old (31.7%), and the least frequent ones were 5 to 10 years old (10%) and more than 50 years old (11.6%). The results showed that gender has no significant effect on the predictability of the need of tetabulin injection for the patients with open fractures (P=0.780). However, age has a significant positive effect on the predictability of the need of tetabulin injection for the patients with open fractures; as the age increases, the need for tetabulin injection also increases, and it must be injected in the 50 years and older patients (P=0.05).

Conclusion: The results showed that age was effective on the decrease of the serum level of anti-tetanus antibody, however, gender had no significant effect on it. Therefore, it is concluded that tetabulin injection for open fractures is consistent with the national guideline.

Keywords: Tetabulin, Open Fracture, Tetanus Toxin, Trauma

1. Introduction

Accident is the most frequent cause of an open fracture and severe wound in Iran (Jafari, Abolhasani, Naghavi, Pourmalek, & Moradi, 2009). The wounds due to accident are one of the most common complication in the ERs. Open fractures and wounds are at risk of infection with bacteria such as Clostridium tetani. Tetanus is a dangerous disease which is caused by the entrance of Clostridium tetani (tetanus) into the wound (Mokhayeri et al., 2016). This bacterium produces a dangerous toxin in the wound which harms the nervous system and leads into loose paralysis or spasm of the muscles (especially the paralysis of the respiratory muscles). Severe muscular spasm due to tetanus leads to the serious health complications such as respiratory problems due to the spasm of the vocal cords (laryngospasm) and muscles that control respiration, pneumonia (lung infection), brain injuries due to the lack of oxygen, cardiac arrhythmia, bone and spinal fracture due to muscular spasm, and seizure (Qadir & Komal, 2019). Due to the high rate of mortality of tetanus, the assessment of the immune condition of the patients and making decision about the need for the injection of tetabulin necessary. This

assessment is performed in different ways, such as doctor's judgment about the condition of the infected wound, the time of the previous vaccination based on the recommended protocols in the reference books and world health organization (WHO), rapid diagnostic methods, and ELISA test (Dong, Masuyer, & Stenmark, 2019). Therefore, since the treatment of tetanus is very difficult, prevention by vaccination is the best thing to do. The most important method to prevent tetanus infection in the open fractures and wounds, especially in those who have unknown or incomplete (less than 3 times) tetanus vaccination history, is Tetanus Immune Globulin (TIG) injection under the brand name tetabulin (Finkelstein, Teisch, Allen, & Ruiz, 2017). Tetabulin is a hyper immune sterile solution that creates passive immunity against tetanus. In fact, this treatment is a type of immune transmission against the tetanus toxin in a passive manner (O'Dwyer, 2017). Therefore, in patients with an open fracture and severe wounds, tetabulin injection is strongly recommended to prevent tetanus infection. The mechanism of tetanus immunoglobulin is that the immunoglobulins attach to the tetanus toxoids and prevent the normal attachment of them to the body tissues. Therefore, the immunoglobulins inhibit the entrance of the toxoids into the nervous system and prevent the painful muscular spasms and brain function disorders. Clostridium tetani is destroyed by the antibiotic produced by the host body. Furthermore, the immunoglobulin attached to the bacterial toxoid destroys the bacteria by inducing phagocytosis in the immune system of the host (Graham & Ambrosino, 2015).

Tetanus immunoglobulin is a product produced by the human plasma, and it consists of the antibodies against the tetanus toxoids. Tetabulin is generally used to prevent tetanus in the patients with severe wounds. Tetanus toxin produced by Clostridium tetani leads to severe spasm, and in 30-40% of the cases, it leads to spinal fracture and death. Tetabulin injection is used for protection and prevention against the tetanus toxin in the unimmunized individuals. Also, tetabulin injection is necessary while there is an open fracture in the patients with immunodeficiency and the individuals who hasn't been vaccinated with the anti-tetanus vaccine during the last 5 years. The recommended dose for these individuals is 250 I.U, and it is doubled (500 I.U.) for those whose weight is more than 90 Kgs and the individuals who have an open fracture for more than 24 hours (Yen, Murray, Zipprich, Winter, & Harriman, 2015). Tetanus immunoglobulin is contraindicated for those with thrombocytopenia, coagulation disorders, and hypersensitivity to Gamma globulin and immunoglobulin A. The side effects of tetanus immunoglobulin include nephrotic syndrome, anaphylaxis, nausea, vomiting, hives, fever, chest pain, lethargy, pain in the injection site, and angioneurotic edema. Therefore, the injection of live viral vaccines is contraindicated up to 3 months after tetanus immunoglobulin injection. Since tetanus immunoglobulin is made of human plasma, there is a possibility of the transmission of viral infection with the reactions such as wheezing, heavy feeling in the chest, fever, itching, severe cough, cyanosis, seizure, and edema in the face, lips, tongue, and throat. The side effects of this medicine have not yet been rejected, and there are no thorough human researches in this regard. Therefore, it is only prescribed when the doctor believes that its advantages surpass the possible disadvantages, and its use is necessary for the patient (Erdem & Blankson, 2013).

In a study performed by Venturini et al., the immunity against tetanus toxin and the need for booster injection was assessed in the students and workers at risk of tetanus in a university in Italy. 95% of 1433 individuals who were vaccinated during the past 10 years had high levels of blood antibody titer (more than 1.0). Also, those who got 5 doses of booster had very high and long-term immunity (more than 1.97 IU/ml). This study suggested that if the individuals complete the vaccination period and then get 5 doses of boosters, they can be immune to tetanus for up to 20 years (Borella-Venturini et al., 2017). In study conducted by Nemati et al. (2015), the anti-tetanus antibody level in the patients with type II diabetes was assessed. The average antibody titer in these patients was lower than the normal population, and it was even lower in the female diabetic individuals in comparison to the male diabetic patients. They also found that there is a direct relationship between the decrease of the anti-tetanus antibody titer and the duration of diabetes, and it was lower in the patients with more than 5 years of diabetes. Therefore, they concluded that tetabulin injection in the diabetic patients with diabetic ulcers referred in the ERs is essential (Nemati et al., 2014). In a study administered by Khurana et al. (2014), they assessed the demographic data (gender and age) of the patients with tetanus and compared the treatment outcomes of intramuscular and spinal injection of tetabulin. They found that simultaneous injection of tetabulin (intramuscular and spinal) significantly increases the survival of the patients with tetanus (Narang, Khurana, Gomber, & Choudhary, 2014).

Since there were not many related studies in this regard in Iran, the goal of this study was to assess the consistency of tetabulin injection to the patients with an open fracture referred to the Khatamolanbia hospital in Zahedan with the national guidelines.

2. Materials and Method

The present study is a cross-sectional descriptive study. Its population is all the individuals with an open fracture. The inclusion criteria are having an open fracture and wound and no hypersensitivity to tetabulin, and the exclusion criterion is the lack of physical health of the patient that disables them from answering the questions properly. 300 individuals who had the inclusion criteria were selected using simple and available sampling from the patient cases in the hospital archive, and they were recorded in the researcher made checklists. This study is a prospective cross-sectional (descriptive-analytic) study to assess the consistency of tetabulin injection to the patients with an open fracture referred to the ER of the Khatamolanbia hospital in Zahedan in 2017 with the national guidelines. All the Helsinki principles are observed through this study, and it was approved by the ethics committee of the Zahedan University of Medical Sciences. First, the individuals with the inclusion/exclusion criteria were selected, then the tools used in this study were completely explained to them and their consent was taken.

Each patient was assessed according to the open fracture severity and tetabulin injection, and their data were recorded in the checklists and entered in the SPSS 26. Then, the results were statistically analyzed. The qualitative and quantitative variables were described by number (percent) and mean (standard deviation) respectively. To compare the mean score of the analytical experiences and psychological traumas, independent t-test and ANOVA were used. Finally, Pearson correlation and linear regression were used to assess the relationship between the analytical experiences and psychological traumas. The significance level of the tests was considered to be 0.05 (P=0.05).

3. Findings

Table 1. Patents' characteristics and the assessed variables (The frequency of the patients based on their gender, age, cause of fracture, the need for tetabulin injection, and the history of tetabulin injection)

Frequency (percent)	Group	Variable
33 (10.06%)	5-10	
77 (24.7%)	10-20	
99 (31.7%)	20-30	Age
58 (18.03%)	30-40	
36 (11.60%)	≤40	
25 (8.06%)	Female	Gender
275 (91.70%)	Male	
58 (19.1%)	Finger imputation	
154 (50.8%)	Accident	
70 (23.1%)	Being shot	
11 (3.6%)	Falling	Cause of fracture
3 (0.7%)	Smashing	
7 (2.3%)	Caught in door	
58 (19.1%)	Finger imputation	
154 (50.8%)	Accident	
297 (97.04%)	No (immunized)	Needing tetabulin injection
6 (2.00%)	Yes (unimmunized)	
81 (29.04%)	>5	
36 (11.88%)	5-10	
61 (20.13%)	10>	History of tetabulin injection
92 (30.36%)	Not remembered	
33 (10.89%)	Not vaccinated	
110 (36.60%)	10-15	
48 (15.08%)	20	
85 (28.01%)	30	
9 (3.09%)	45	Tetabulin injection duration
40 (13.02%)	60	
5 (1.70%)	90	
5 (1.70%)	120	
110 (36.60%)	10-15	

✓ The frequency is shown as number (percent)

In this study, 300 patient cases with an open fracture referred to the ER of the Khatamolanbia Hospital in Zahedan in 2017 were assessed. Among 300 patients, 275 patients (91.7%) were male and 25 patients (8.3%) were female. The most frequent age range was 20 to 30 years old (31.7%), and the least frequent ones were 5 to 10 years old (10%) and more than 50 years old (11.6%). Regarding tetabulin injection history: 29.04% of the patients injected tetabulin in less than 5 years, 11.88% injected it in 5 to 10 years, 20.13% injected it in more than 10 years, 30.36% didn't remember whether they had received it or not, and 10.89% had no history of tetabulin injection.

The most frequent cause of an open fracture among the patients in the ER of the Khatamolanbia hospital in Zahedan during 2017 was accident (50.8%), and its lowest frequency was smashing (less than 1%). The frequency of the unimmunized and immunized patients was 2% and 97.04% respectively. It means that according to the injection history and severity of the injury, 97% of the patients didn't need tetabulin injection and only 2% of them strongly needed tetabulin injection. The frequency of the patients based on their gender, age, cause of fracture, the need for tetabulin injection, and the history of tetabulin injection are listed in Table 1.

In order to determine the frequency of the patients with an open fracture in the Khatamolanbia hospital in 2017 who needed tetabulin injection based on their gender, Kappa Cohen's correlation coefficient (also known as Kappa coefficient correlation) was used. The results showed that the Kappa coefficient is 0.004 (p=0.339). Kappa coefficient ranges from 0 to 1, and when it is closer to 1, it implies more agreement. Kappa coefficient less than 0.4, 0.4 to 0.75, and more than 0.75 imply the weak, good, and excellent agreement respectively. Therefore, the agreement between the frequency of the patients with an open fracture who needed tetabulin injection and their gender is 0.4, and it is weak (Table 2).

Table 2. Determination of the frequency of the patients with an open fracture who needed tetabulin injection based on their gender using Kappa correlation coefficient

Variable		The need for tetabulin injection		Kappa test	p
		Yes Frequency (percent)	No Frequency (percent)		
Gender	Male	5 (83.33%)	274 (92.25%)	0.004	0.399
	Female	1 (16.66%)	23 (7.74%)		
	Total	6	297		

To determine the frequency of the patients with an open fracture in the Khatamolanbia hospital in 2017 who needed tetabulin injection based on their age, Kappa Cohen's correlation coefficient (also known as Kappa coefficient correlation) was used. The results showed that the Kappa coefficient is 0.043 (p=0.04). Kappa coefficient ranges from 0 to 1, and when it is closer to 1, it implies more agreement. Kappa coefficient less than 0.4, 0.4 to 0.75, and more than 0.75 imply the weak, good, and excellent agreement respectively. Therefore, the agreement between the frequency of the patients with an open fracture who needed tetabulin injection and their age is 0.4 to 0.75, and it is good (Table 3). In the other words, as the age increases, the need for tetabulin injection for the patients with an open fracture also increases. This correlation is statistically significant (p=0.04).

Table 3. Determination of the frequency of the patients with an open fracture who needed tetabulin injection based on their age using Kappa correlation coefficient

Variable		Need for tetabulin injection		Kappa test	p
		Yes Frequency (percent)	No Frequency (percent)		
Age	0-30	4 (66.66%)	211 (71.04%)	0.043	0.04
	>30	2 (33.33%)	86 (28.95%)		
	Total	6	297		

To determine the frequency of the patients with an open fracture in the Khatamolanbia hospital in 2017 who received tetabulin based on their gender, Kappa Cohen's correlation coefficient (also known as Kappa coefficient correlation) was used. The results showed that the Kappa coefficient is 0.014 (p=0.780). Kappa coefficient less than 0.4 implies the weak agreement. Therefore, the agreement between the frequency of the patients with open fracture who received tetabulin and their gender is less than 0.4, and it is weak (Table 4).

Table 4. Determination of the frequency of the patients with an open fracture who received tetabulin based on their gender using Kappa correlation coefficient

Variable		Need for tetabulin injection		Kappa test	p
		Yes Frequency (percent)	No Frequency (percent)		
Gender	Male	224 (91.80%)	55 (93.22%)	0.014	0.780
	Female	20 (8.19%)	4 (6.77%)		
	Total	244	59		

Finally, in order to determine the frequency of the patients with an open fracture in the Khatamolanbia hospital in 2017 who received tetabulin based on their age, Kappa Cohen's correlation coefficient (also known as Kappa coefficient correlation) was used. The results showed that the Kappa coefficient is 0.046 (p=0.05). Kappa coefficient 0.4 to 0.75 implies the good agreement. Therefore, the agreement between the frequency of the patients with an open fracture who received tetabulin and their age is good (Table 5). In the other words, as the age increases, the frequency of tetabulin injection for the patients with an open fracture also increases. This correlation is statistically significant (p=0.05).

Table 5. Determination of the frequency of the patients with an open fracture who received tetabulin based on their age using Kappa correlation coefficient

Variable		Need for tetabulin injection		Kappa test	p
		Yes Frequency (percent)	No Frequency (percent)		
Age	0-30	171 (70.08%)	44 (74.57%)	0.046	0.05
	>30	73 (29.91%)	15 (25.42%)		
	Total	244	59		

4. Discussion and Conclusion

The goal of this study was to assess the consistency of tetabulin injection to the patients with an open fracture. This was a cross-sectional descriptive study. The population of the study was all the patients with an open fracture referred to the Khatamolanbia hospital in Zahedan in 2017. The statistical sample included 300 patients with an open fracture who were selected by random and available sampling. In this section of the study, the goal, research problem, and method of the study will be briefly discussed and conclusion and comparison will be made.

As mentioned in the previous section, the results showed that the Kappa coefficient for the frequency of the patients with an open fracture who needed tetabulin injection based on their gender is 0.004. Kappa coefficient ranges from 0 to 1, and when it is closer to 1, it implies more agreement. Kappa coefficient less than 0.4, 0.4 to 0.75, and more than 0.75 imply the weak, good, and excellent agreement respectively. Therefore, the agreement between the frequency of the patients with an open fracture who needed tetabulin injection and their gender is 0.4, and it is weak. In a study performed by Afzali et al. (2015) with the goal to assess the level of anti-tetanus antibody in the trauma patients referred to Shahid Beheshti hospital in Kashan, they realized that gender is not an effective factor on the level of anti-tetanus antibody in these patients (Afzali, Sharif, & Mousavi, 2015). In their study, Nemati et al. (2014) also concluded that there was no significant relationship between the gender and anti-tetanus antibody level in the patients with type II diabetes (Nemati et al., 2014). In contrast, in a study performed by Wu et al. (2009) to assess the relationship between the decrease of tetanus antitoxin and age in Taiwan, they realized that the need for tetabulin injection was higher in men in comparison to the women. Therefore, they concluded that gender has a significant positive effect on the decrease of tetanus antitoxin in the serum (Wu, Ko, Lee, Tsai, Li, Pao, Lee, Chang, Shih, & Ko, 2009). Also, in a study conducted by Kader et al. (2016) in Yazagat, Turkey, they concluded that gender has a significant positive effect on the decrease of tetanus antitoxin in the serum, and it is lower in men (Kader, Balci, & Erbay, 2016).

As mentioned in the previous section, the results showed that the Kappa coefficient for the frequency of the patients with an open fracture who needed tetabulin injection based on their age is 0.043. Kappa coefficient ranges from 0 to 1, and when it is closer to 1, it implies more agreement. Kappa coefficient less than 0.4, 0.4 to 0.75, and more than 0.75 imply the weak, good, and excellent agreement respectively. Therefore, the agreement between the frequency of the patients with an open fracture who needed tetabulin injection and their age is 0.4 to

0.75, and it is good (table 3). In the other words, as the age increases, the need for tetabulin injection for the patients with an open fracture also increases. This correlation is statistically significant. Toker et al. (2017) assessed the immunity of the adult trauma patients against tetanus and concluded that as the age of the patients increases, their immunity against tetanus decreases. Therefore, the older trauma patients need tetabulin injection more than the younger ones (Toker et al., 2017). In their study, Mizuno et al. (2014) also concluded that there is a significant positive relationship between the age and decrease of anti-tetanus antibody level, which means as the age increases, the anti-tetanus antibody level in the serum decreases. This is more significant in the 50 years and older patients (Mizuno, Yamamoto, Takeshita, & Takahashi, 2014).

According to the results obtained in this study, age is effective on the decrease of the serum level of anti-tetanus antibody. However, gender has no significant effect on the level of anti-tetanus antibody level. Thus, it can be concluded that the amount of tetabulin injection for open fractures is consistent with the national guideline.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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