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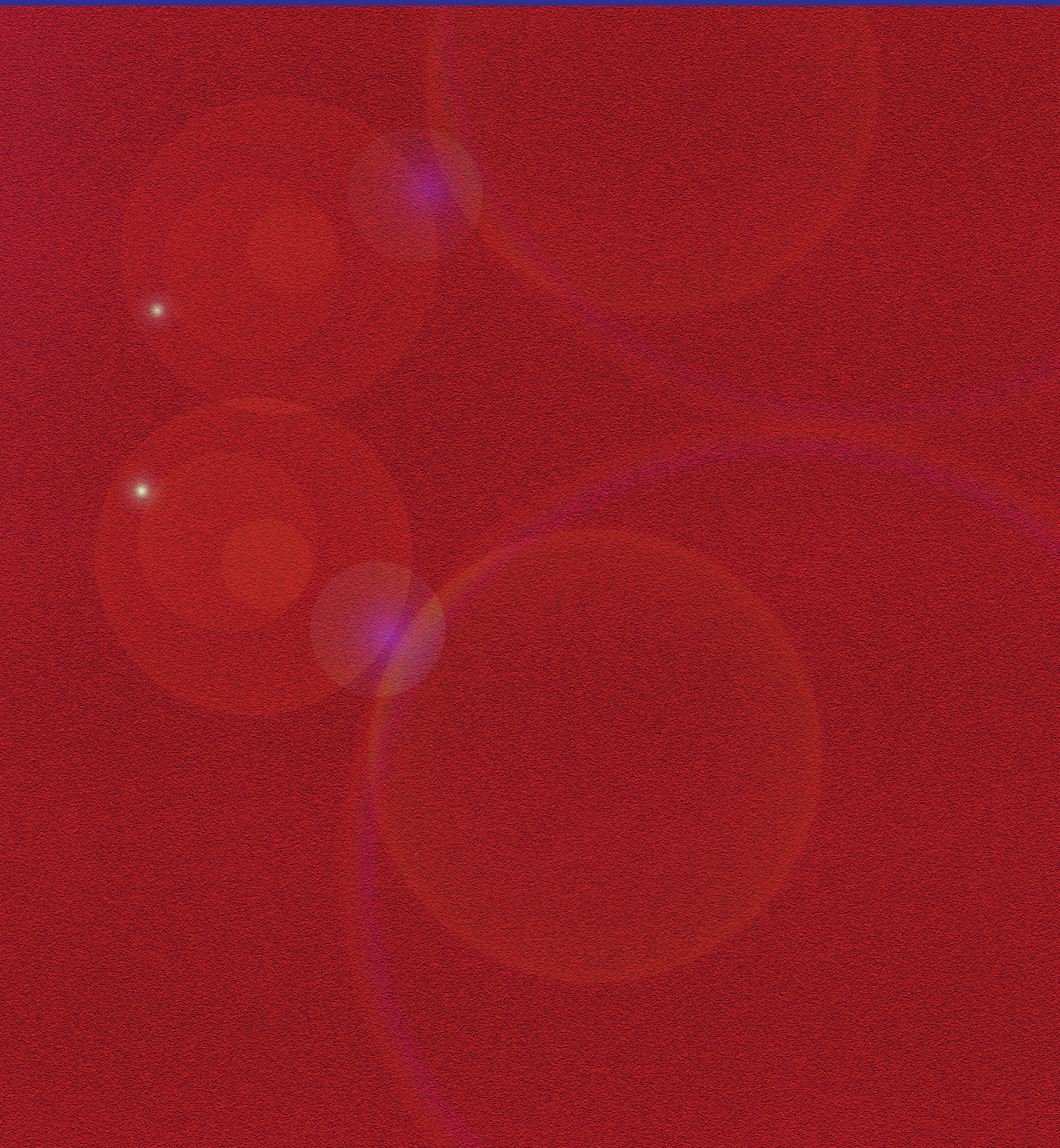

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Anti-retroviral therapy and incidence of adverse drug reactions among cohort of Malaysian HIV/AIDS patients

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Abstract

Background: Adverse drug reactions (ADRs) affect patients' compliance and health related quality of life. HIV patients face challenges in adherence to their medications and experience side effects affecting their overall well-being, a primary outcome of HIV/AIDS treatment.

Methods: Four hundred and forty three Malaysians with a known diagnosis of HIV/AIDS, using Antiretroviral Therapy (ART) for at least past three months, were invited to participate in this study. The data were analyzed using the Statistical Package for Social Sciences (SPSS®) version 18 and STATA IC® version 12.

Results: About 44% (n=194) of total 443 HIV patients reported ADR, among them 131 (67.5%) were male while 63 (32.4 %) were females. Weight loss (12.6%), lipodystrophy (12.4%), peripheral neuropathy (12%), itchiness (11.7%) and skin problems (8.6%) were frequently found ADRs. Almost similar numbers of patients experienced ADRs who were combivir based (21.7%) and other drug therapy (22.1%) and this translated insignificant association between type of therapy and ADRs. However the poor CD4 counts (OR 1.72, 95% CI: 1.04 — 2.86), and poor viral suppression (OR 1.87, 95% CI: 1.04 — 3.36) did increase the odds of experiencing adverse drug reactions.

Conclusion: Uninformed and unpredictable ADRs may lead to non-adherence, poor health related quality of life and refusal to take medications which can ultimately affect the outcomes of HIV program. This poses a great necessity for optimizing pharmacovigilance services by addressing areas that require more attention.

Key Words: HIV & AIDS, Adverse Drug Reactions, anti-retroviral therapy

Introduction

According to Joint United Nations Programme on HIV/AIDS (UNAIDS), globally; 34.0 million [31.4 million–35.9 million] people were living with HIV at the end of 2011. The number of people newly infected globally is continuing to decline, although the burden of the epidemic continues to vary considerably between countries and regions [1].

During the recent years there has been an unprecedented increase in access to HIV treatment even in resource-limited settings where antiretroviral medications were previously unavailable. This has definitely contributed towards a global improvement in epidemics and HIV/AIDS related morbidity and mortality. There is no doubt about the overall positive impact of HAART in managing HIV & AIDS, however this evolution of ART treatment of HIV/AIDS as a chronic infection/disease presents challenges for patients and health-care professionals, and measures of QOL can provide important information in behavioral and clinical studies of ART [2].

Similar to other chronic illnesses, HIV/AIDS patients experiences many adverse drug reactions which definitely affect their adherence and health related quality of life, a prime therapeutic objective of HIV treatment. These medicines are associated with significant safety concerns including serious ADRs, with both short- and long-term effects, affecting many organ systems. These may damage confidence in any national ARV program and affect patient adherence [3,4]. It is logical and expected that most of the time these patients are provided

with expert care on the co-morbidities in addition to disease itself, however a routine assessment and evaluation of adverse drug reactions will help in devising more effective treatment programs which in turn can help in addressing issues like adherence and poor quality of life among HIV patients, since Long-term complications of this disease are multifactorial and can be related to the virus itself or to adverse effects of antiretroviral therapy [5]. Adherence to treatment is closely linked to adverse drug reactions and it would be futile simply changing the medications without addressing the adherence barriers. It is thus imperative that clinicians clearly understand ADRs, readily recognize them in patients and manage them effectively [6, 7].

By the end of 2011, about 81000 patients were living with status of HIV and AIDS in Malaysia with an estimation of 37,306 eligible for HIV treatment [8]. With best of our knowledge and search not many studies have done so far to evaluate ADRs among patients on HIV treatment in Malaysia, except by Hasan et *al* [9] which has provided some basic information by utilizing a cross sectional approach. However in present study we have utilized a case-control approach towards ADR evaluation in which we recruited patients who were on antiretroviral treatment for at least past three months as it has been reported that ADRs are more likely to occur within the first six months on treatment [6], unlike to earlier study which recruited patients with at least one month treatment [9]. This provides us confidence that our findings has shown detailed picture of ADR experienced by Malaysian HIV/AIDS patient.

The Malaysian AdverseDrug Reaction Advisory Committee (MADRAC) is responsible for collecting ADRs reports in Malaysia which was formed in 1987 however the number of reports received by MADRAC remains low compared to other countries [10]. In addition this has another important implication as according to a study important predictor variables, which limit physicians from reporting ADRs, were related to uncertainty of types of reaction to report, lack of awareness about the existence, function and purpose of national ADR reporting [11].

Materials and methods

Four hundred and forty three Malaysians with known diagnosis of HIV/AIDS,who were using Antiretroviral Therapy (ART) for at least past three months, and treated as outpatients at medication therapy adherence clinics (MTAC) at Hospital Sungai Buloh, were invited to participate in this study. Hospital Sungai Buloh, is the largest infectious disease referral center in the country. The study was conducted according to the principles expressed in the Declaration of Helsinki, and was approved by the Medical Research & Ethics Committee (MREC) and Clinical Research center (CRC),Ministry of Health Malaysia. The study was also registered with National Medical Research Registry (NMRR).

Patients were categorized as having HIV/AIDS if they were attending MTAC for the management of HIV/AIDS, and every second patient with HIV/AIDS on the respective patients' list at the clinic sites was recruited. Prior to information gathering a study information sheet was provided and verbal or oral consents were taken. A data collection form was used to gather demographics and ADR descriptions including relevant clinical details from patients' medical records. A Naranjo scale and hospital medical records were used to establish the likelihood of adverse drug reactions. Based on responses patients were divided into two clusters' i.e with ADR and without ADR. Other relevant clinical information including details of medications, viral load and CD4 cell counts were also obtained from patients' medical records.

Face-to-face interviews were conducted at the outpatient clinics, using a designed questionnaire to seek relevant information including patient demographics, perceived ADRs and clinical parameters as described earlier. The questionnaire had been subjected to content and faces validation and reviewed by individuals who were not experts in the design and testing of the instrument. The validated English version of the questionnaire had been translated into the Malay language (Bahasa Malaysia) with forward/backward translation, and piloted on a sample of 30 participants.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS®) version 18 and STATA IC® version 12, with a significance level of ≤ 0.05. Descriptive statistics were used to calculate percentages frequencies, means and standard deviations. The relationship between variables for cate-

gorical data was performed using the X^2 (Chi-Sq). Fisher Exact test was applied in cases where sample size was small. Similarly, on occasions where we had less than 5 readings per cell for Chi-Sq, Likelihood Ratio test was applied. Comparisons between groups with normal distribution were performed using the Student's t-test. Pearson's correlation test was used to verify the existence of a correlation between instruments' mean scores or other values.

We presented our results in the form of crude odds ratios (ORs) to establish the association between adverse drug reactions and anti-retroviral therapy. The crude odds ratio is an unadjusted measure of the association in the aggregate form and it does not control for possible confounding. Therefore, a series of multiple logistic and multinomial regression models (see footnotes of Tables 4 and 5) were used to determine the association of adverse drug reactions with anti-retroviral therapy and socio-demographic factors assessed as categorical outcomes. Data on adverse drug reactions were collected through a self-administered questionnaire, where participants were asked "Have you EVER experienced adverse effects after taking your HIV medications (anti-retroviral therapy)?" with response options "yes" or "no." Subjects were categorized as having adverse drug reactions if they answered "yes" to this question. This information was further confirmed by patients' medication records. The information about the anti-retroviral therapy and individual drug was collected from patients' medical records.

For logistic regression, the main outcome variable, adverse drug reaction was used as binary outcome.The variables,CD4 counts and viral load were also used as binary variables. We used 500 cells/μLor less as the cut-off point to define poor optimal viral suppression. Similarly > 75 copies/ml was used as the cut-off to define poor viral load control. The effects were adjusted for age, gender, ethnicity, education, marital status, occupation, comorbidity, and type of HIV treatment. For potential confounding, the unadjusted associations of anti-retroviral therapy and socio-demographics with adverse drug reactions were compared with the adjusted associations, with confounding confirmed when the unadjusted effect size and adjusted effect size estimates differed.

Results

Socio-demographic characteristics

Out of 475 respondents that we approached, a total of 443 respondents agreed and participated in this study. Out of which 72% (n=319) were male with 59.4% (n=263) from age group between 31-40 years.Almost 50% of respondents were Chinese, while secondary school education levelwas found to be highest (50.6%) education level, with 63.0% working adults (Table 1).

Table 1. Socio-demographics of the study population (N = 443)

Variables	N	%
Age		
18 – 30	54	12.19
31 – 40	194	43.79
41 – 50	126	28.44
> 50	69	15.58
Gender		
Male	319	72.01
Female	124	27.99
Ethnicity		
Malay	134	30.25
Chinese	220	49.66
Indian	37	8.35
Others	52	11.74
Marital Status		
Married	216	48.76
Unmarried	185	41.76
Divorced	36	8.13
Widowed	6	1.35
Education level		
No formal education	56	12.64
Primary education	98	22.12
Secondary education	224	50.56
Graduate	54	12.19
Postgraduate	11	2.48
Occupation		
Unemployment	118	26.64
Labor	188	42.44
Professional	26	5.87
Housewife	34	7.67
Retired	14	3.16
Others	63	14.22
Co morbidities		
No	394	88.94
Yes	49	11.06
Adverse effects		
No	249	56.21
Yes	194	43.79
Type of HIV Treatment		
Combivir based therapy	227	51.24
Other therapy	216	48.76

Table 2. Socio-demographics of the study population, by adverse drug reactions (N = 443)

Variables	Did not experienced Adverse Effects		Experienced Adverse Effects		p-value
	N	%	N	%	
Age					
18 – 30	30	12.05	24	12.37	Pr = 0.599
31 – 40	114	45.78	80	41.24	
41 – 50	71	28.51	55	28.35	
> 50	34	13.65	35	18.04	
Gender					
Male	188	75.5	131	67.53	Pr = 0.064
Female	61	24.5	63	32.47	
Ethnicity					
Malay	79	31.73	55	28.35	Pr = 0.520
Chinese	126	50.60	94	48.45	
Indian	19	7.63	18	9.28	
Others	25	10.04	27	13.92	
Marital Status					
Married	113	45.38	103	53.10	Pr = 0.414
Unmarried	111	44.58	74	38.14	
Divorced	22	8.84	14	7.22	
Widowed	3	1.20	3	1.55	
Education level					
No formal education	23	9.24	33	17.01	Pr = 0.016
Primary education	55	22.10	43	22.16	
Secondary education	139	55.82	85	43.81	
Graduate	29	11.65	25	12.89	
Postgraduate	3	1.20	8	4.12	
Occupation					
Unemployment	59	23.70	59	30.41	Pr = 0.027
Labor	120	48.19	68	35.05	
Professional	13	5.22	13	6.70	
Housewife	13	5.22	21	10.82	
Retired	6	2.41	8	4.12	
Others	38	15.26	25	12.88	
Co morbidities					
No	224	89.96	170	87.63	Pr = 0.438
Yes	25	10.04	24	12.37	
Type of HIV Treatment					
Combivir based therapy	129	51.81	98	50.52	Pr = 0.787
Other therapy	120	48.19	96	49.48	
CD4 Counts					
Normal	61	24.50	33	17.01	Pr = 0.093
Poor CD4 counts	172	75.50	140	72.16	
Viral load counts					
Optimal viral suppression	182	73.10	116	59.79	Pr = 0.076
Poor viral suppression	33	13.25	34	17.52	

Note: p-value was obtained by Chi-Sq test

Table 3. Adverse drug reactions associated with anti-retroviral therapy (N=443)

Adverse effects	N	%
Weight loss	56	12.64
Lipodystrophy	55	12.41
Peripheral neuropathy	53	11.96
Itch	52	11.74
Skin problems	38	8.58
Lost appetite	34	7.67
GI discomfort	25	5.64
Dizziness	24	5.42
Anaemia	24	5.42
Fatigue	22	4.97
Rashes	18	4.10
Vomit	15	3.39
Mood swing	13	2.93
Headache	13	2.93
Diarrheoa	7	1.58
Gynaecomastia	3	0.68

Upon further evaluation 43.8%(n=194) respondents reported ADR, of which almost67.5% (n=131) were male while 32.5% (n=63) of them were females. Interestingly most (41.24%)of the respondents from ADR group were from age group of 31-40 years and were male (67.53%) with secondary school education as the highest level among majority (43.81%) of this group respondents (Table 2). The most commonly found adverse drug reactions associated with anti-retroviral therapy were weight loss (12.6%), lipodystrophy (12.4%), and peripheral neuropathy (11.9%) (Table 3).

Odds of adverse drug reactions

The associations between demographics and clinical parameters, and odds of adverse drug reactions are presented in (Table 4). Age groups, gender, ethnicity, and co morbidities were not significantly associated with the occurrence of ad-

Table 4. The association between demographics and clinical parameters, and odds of adverse drug reactions (N = 406)

Variables	Did not experienced Adverse Effects		Experienced Adverse Effects		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
	N	%	N	%		
Age						
18 – 30	27	11.59	20	11.56	1.0	1.0
31 – 40	105	45.06	69	39.88	0.88 (0.48-1.61)	0.71 (0.33-1.51)
41 – 50	70	30.04	52	30.06	0.97 (0.51-1.84)	1.02 (0.46-2.25)
> 50	31	13.30	32	18.50	1.29 (0.63-2.63)	1.14 (0.46-2.84)
Gender						
Male	174	74.68	118	68.21	1.0	1.0
Female	59	25.32	55	31.79	1.48 (0.98-2.25)	1.41 (0.77-2.56)
Ethnicity						
Malay	72	30.90	50	28.91	1.0	1.0
Chinese	121	51.93	84	48.55	1.10 (0.69-1.66)	1.20 (0.70-2.10)
Indian	17	7.30	16	9.25	1.36 (0.66-2.83)	1.46 (0.57-3.74)
Others	23	9.87	23	13.29	1.55 (0.82-2.95)	1.05 (0.44-2.53)
Co morbidities						
No	208	89.27	150	86.71	1.0	1.0
Yes	25	10.73	23	13.29	1.28 (0.70-2.33)	1.20 (0.62-2.33)
CD4 Counts						
Normal	61	26.18	33	19.08	1.0	1.0
Poor CD4 counts	172	73.82	140	80.92	1.50 (0.93-2.42)	1.72 (1.04-2.86)*
Viral load counts						
Optimal viral suppression	182	78.11	116	67.05	1.0	1.0
Poor viral suppression	33	14.16	34	19.65	1.62 (0.95-2.75)	1.87 (1.04-3.36)*

*ORs were adjusted for age, gender, ethnicity, education, marital status, occupation, adverse drug reaction, comorbidity, and type of HIV treatment. Logistic regression, * = statistically significant*

verse drug reactions and remained insignificant after adjustments for the effects of confounders. However the poor CD4 counts (OR 1.72, 95% CI: 1.04 —2.86), and poor viral suppression (OR 1.87, 95% CI: 1.04 — 3.36) did increase the odds of experiencing adverse drug reactions.

There were no significant difference between combivir based and other drug therapy (OR 1.10, 95% CI: 0.68—1.71) in increasing the odds of adverse drug reactions. Similarly none of the protease inhibitors, nucleoside reverse transcriptase inhibitors, and non nucleoside reverse transcriptase

Table 5. The association between anti-retroviral therapy, and odds of adverse drug reactions (N = 443)

Variables	Did not experienced Adverse Effects		Experienced Adverse Effects		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
	N	%	N	%		
Type of HIV Treatment						
Combivir based therapy	129	51.81	98	51.52	1.0	1.0
Other therapy	120	48.19	96	49.82	0.95 (0.65-1.38)	1.10 (0.68-1.71)
Protease Inhibitors (PI)						
Indinavir						
No	246	98.80	190	97.94	1.0	1.0
Yes	3	1.20	4	2.06	1.73 (0.38-7.80)	1.33 (0.27-6.57)
Ritonavir						
No	239	95.98	192	98.96	1.0	1.0
Yes	10	4.02	2	1.04	0.25 (0.10-1.15)	0.22 (0.10-1.10)
Kaletra (Lopinavir/Ritonavir)						
No	247	99.20	189	97.42	1.0	1.0
Yes	2	0.80	5	2.58	3.27 (0.63-17.02)	3.10 (0.55-17.48)
Nucleoside reverse transcriptase inhibitor (NRTI)						
Didanosine						
No	245	98.39	191	98.45	1.0	1.0
Yes	4	1.61	3	1.55	0.96 (0.21-4.35)	0.75 (0.14-4.04)
Zidovudine						
No	242	97.20	193	99.48	1.0	1.0
Yes	7	2.80	1	0.52	0.18 (0.02-1.47)	0.13 (0.01-1.29)
Stavudine						
No	226	90.76	165	85.05	1.0	1.0
Yes	23	9.24	29	14.95	1.72 (0.96-3.10)	1.79 (0.93-3.48)
Lamivudine						
No	221	88.76	162	83.51	1.0	1.0
Yes	28	11.24	32	16.49	1.56 (0.91-2.69)	1.53 (0.81-2.88)
Non nucleoside reverse transcriptase inhibitor (NNRTI)						
Nevirapine						
No	178	71.49	144	74.23	1.0	1.0
Yes	71	28.51	50	25.77	0.87 (0.57-1.33)	0.88 (0.55-1.41)
Efavirenz						
No	141	56.63	94	48.45	1.0	1.0
Yes	108	43.37	100	51.55	1.40 (0.96-2.04)	1.41 (0.95-2.10)
Bactrim						
No	223	89.56	178	91.75	1.0	1.0
Yes	26	10.44	16	8.25	0.77 (0.40-1.48)	0.73 (0.36-1.50)

*ORs were adjusted for age, gender, ethnicity, education, marital status, occupation, co-morbidity, and type of HIV treatment. Logistic regression, * = statistically significant*

inhibitors was found significantly associated with the occurrence of adverse drug reactions (Table 5).

Discussion

The study was carried out the largest infectious disease centers of Malaysia that caters huge percentage of HIV & AIDS patients in the country, with a primary aim to assess incidence of reported ADRs among patients on antiretroviral medications. In terms of socio-demographics, interestingly our findings confirmed that majority of the respondents under ADR group represented the current epidemics of HIV/AIDS in the country as it was reported in a report that there is an increase trend of HIV prevalence among adults of 30-39 years while male represented majority of HIV cases in the country [8]. Most of the experienced ADRs were not very different to what has been reported in a number of other studies across the different parts of the world [3, 5, 6, 9, 13-14, 16-18, 23]. Almost half of the studied patients reported ADRs which was lower compare to a previously reported study in Malaysia [9].

Adverse drug reactions have been regarded as single most common reason for poor patient adherence towards medication treatment, thus identifying risk factors for the occurrence of ADRs is of crucial importance to optimize the initial choice of ARVs regimen before initiating therapy and to adapt the pace of surveillance to each unique situation [12]. We found efavirenz(EFV) and nivirapine (NVP) as the two most common drugs associated with adverse drug reactions. Both of these non-nucleoside reverse transcriptase inhibitors (NNRTIs) are well known for causing side effects e.g. dizziness, insomnia, headaches, abnormal dreams and mood changes with efavirenz while skin problems and headaches are commonly associated with nivirapine use [3, 5-6, 9, 16-17, 22]. In addition to these incidence of hepatotoxicity (16% and 8%) for patients on NVP and EFV respectively was also reported [28].

It is also vital to understand that though we did not attempt to report metabolic, cardiovascular, liver and other organ and system specific toxicities established with the use of antiretrovirals [5, 18-20, 22, 23], many of reported ADRs were found affecting patient adherence resulting in poor health related quality of life and virological failure [2, 7, 22]. In addition ADR related issues such as poor adherence, treatment discontinuation etc. are affecting HIV programs in many countries thus devising methods for closer monitoring in community setting may help in preventing these toxicities and improved antiretroviral adherence since little is known about the adverse drug reactions (ADR) of ARVs in many HIV programs in the public health sector of developing countries [24, 25]

Our study found that poor viral load suppression and lower CD4 cell counts were associated with likelihood of experiencing adverse drug reactions. This is unlike to a recently published study in Nigeria where both above mentioned clinical parameters along with other patient demographics were found to have no association with the occurrence of ADRs [6]; however some studies have associated low CD4 count at treatment initiation as well as prolong antiretroviral treatment as a risk factor for ADRs [26]. Many confounding factors such as concurrent disease conditions, medication use, use of complementary and alternative medicines could also be an explanation of this [15]. Also with lower CD4 values AIDS defining symptoms become more evident which definitely affects patients understanding of adverse drug reactions, hence a lower CD4 value could be a potential bias where patient is unable to differentiate between these symptoms and adverse drug reactions. Another potential bias would be the fact that we did not attempt to categorize CD4 count as per guidelines of center of disease control (CDC) where each of three categories A, B and C are further divided into three sub categories i.e. 1, 2 and 3; to reflect disease staging. However we have used CD4 count <500 to denote poor control from ART usage perspective since treatment recommendations varies for asymptomatic and symptomatic patients with CD4 counts > 500 [29].

There is no doubt that advent of HAART has transformed HIV/AIDS to a chronic illness where one of the main outcomes of the treatment is to improve patient's quality of life. Antiretrovirals have definitely change the face of this illness in present era; however beside their effectiveness there are still issues like non-adherence, refusal to take ARVs etc. which often found to be associated with incidence as well as fear of adverse drug reactions [27]. We do understand that poor adherence to any medications has multi facet reasons, ranging from those derived

from patient to those resulted due to insufficient drug counseling or lack of support system. Realizing these disparities among health care systems in developed and developing world there is a need optimize pharmacovigilance services which warranted a multidisciplinary approach. Such vigilance has greater implications in developing countries for many reasons since important predictor variables, which limit physicians from reporting ADRs, were related to uncertainty of types of reaction to report, lack of awareness about the existence, function and purpose of national ADR reporting [11].

Study Limitations

This study has strength as the data generated represents the required sample size which the best of our knowledge is the largest cohort of HIV/AIDS patients who have been surveyed in Malaysia for ADR surveillance. At the same time it has some limitations as the generated data may not be able to provide information on long term adverse effects including early onset ADR from these patients which we may have missed. In addition our ADR reporting tool was not intended to find further details of ADRs, thus we believe that the actual picture of total ADR experienced by this cohort could be under reported.

Conclusion

In conclusion, the study has provided some basic data of ADRs experienced by Malaysian HIV/AIDS patients, though they were not found to be different and unique than what has been reported in other parts of the world. One of the important implications of these finding would be to optimize methods of ADR monitoring by addressing issues related to pharmacovigilance program, this in turn can help in optimizing over all HIV care.To optimize adherence, and hence efficacy, clinicians must focus on preventing adverse effects, when possible, and distinguishing those that are self-limited from those that are potentially serious [12].

Future Directions

Longitudinal studies are needed to help better understand ADR related issues, without this understanding, the success of our current therapies can, for a substantial number of individuals, be assumed to be short-lived [21]

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Restoration of shoulder abduction by spinal accessory nerve transfer to suprascapular nerve using posterior approach

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Abstract

Introduction: the aim of this study is to investigate the efficacy of using posterior approach for spinal accessory nerve transfer in patients with complete brachial plexus injuries.

Methods: it is a cohort study conducted between July 2012 to July 2013. All the patients with complete brachial plexus injuries, whose injuries were less than one year old and their age were not more than 60 years were enrolled in the study. The records (including age, sex, education clinical data about the range of motion and strength of involved joint and muscles) were collected before and after posterior approach operation.

Results: totally 14 patients with complete brachial plexus injury were enrolled. The mean of age of all patients studied was 26. 28 years (SD: 6. 7, range 10-38), 11 patients were male, the mean of shoulder abduction was 4. 28 degrees before operation. (SD: 10. 89, range 0-30). The mean of external rotation was 17. 69degrees before operation(SD: 20. 47, range 0-50). The mean of muscle strength for supraspinatus muscle was 0. 78 (SD: 0. 57, range 0-2) and it was the same for infraspinatus muscle. The strength of trapezius muscle for all patients was 5 before operation. The mean of shoulder abduction was increased to 117. 00degrees after operation (SD: 74. 08 range 0-180). The mean of external rotation was increased to 48. 21 degrees (SD: 43. 12, range 0-100) after operation(P value=0. 008). The strength of supraspinatus and infraspinatus muscles were increased to 1. 85 (SD: 1. 35, range 1-5) and 1. 42 (SD: 1. 55, range: 0-5) respectively. (p value=0. 007 and 0. 071 respectively).

Conclusion: it seems using posterior approach for transferring the spinal accessory nerve to suprascapular nervee in patients with brachial plexus injury can have a relatively satisfying result in reversing the abduc tion and external rotation of the shoulder and strength of supraspinatus and infraspinatus muscles of the patients.

Key word: spinal accessory nerve transfer, shoulder abduction, shoulder external rotation

Introduction

Brachial plexus injuries are among the destructive injuries which cause unilateral or bilateral loss or malfunction of upper extremities and consequently considerable disability in daily activities. It was between 1970 and 1980 when surgeons first began to use microsurgical techniques to reconstruct brachial plexus injuries. In the past decade extraplexal nerve transfers have been defined as one of the most efficient and valuable procedures of brachial plexus damages specially shoulder abduction(1, 2). Narkas investigated the historical and anatomical basis of nerve transfer in recent decades(3). After that, nerve transfers have been used increasingly in repairing the brachial plexus injuries. Nerve transfer or neurotization means transfer of a normal or almost normal nerve to a main nerve which is damaged.

The most frequent indications of neurotization are preganglionic injuries and reinnervation of FFMTs. It is important to do the repair outside the zone of trauma. It is better to select nerves closer to the damaged nerve, and also avoid nerve grafts if possible. BY using this method there has been a great advance in management of plexus trauma and there has been there has been a considerable improvement in results (4-8).

Spinal accessory, ulnar, median, triceps branch of radial, medial pectoral, thoracodorsal, inter-

costals, Phrenic and c7 are of the most used nerves in nerve transfer procedures (1, 2).

For shoulder abduction spinal accessory nerve (cranial nerve 11) is defined as an efficient modality. the external branch of this nerve supplies sternocleidomastoid and trapezius muscles. After giving branches to sternocleidomastoid muscle it descends to trapezius muscle in the posterior triangle. First it has 2 or 3 branches in the upper part of this muscle and then goes obliquely to the middle and lower parts and gives off several branches to them. Traditionally there are some reports of transferring spinal accessory nerve to supra scapular nerve using anterior approach for restoration of the shoulder abduction. In this method both spinal accessory and suprascapular nerves are approached anteriorly at posterior border of sternocleidomastoid with classic or transverse cervical incision which is usually anaesthetic and long. This procedure may have good results for shoulder abduction but can weaken the function of superior part of trapezius muscle, the other problem is that, sometimes when this nerve is sacrified more proximally, so it needs nerve grafts to reach suprascapular nerve. The other point is that sometimes there is a concomitant segmental injury in suprascapular nerve at the suprascapular notch and neurotization proximal to this injury would ruin the results. These problems can be solved by using posterior approach. In such approach the spinal accessory nerve is explored via a transverse posterior incision at superior border of scapula, so the proximal branches are better saved, the incision is aesthetically acceptable and second point damage is obviated and probable grafting is avoided.

There are a little reports about this approach, so the aim of this study is to investigate the results of spinal accessory nerve transfer to suprascapular using posterior approach.

Method and material

This cohort study was conducted from July 2012 to July 2013. Patients with total brachial plexus injury enrolled in the study. Inclusion criteria were: total brachial plexus injuries without limitation in passive motion of shoulder, age less than or equal to 60 years, passing less than one year from their injuries, filling informed consent. The study was approved by research ethical committee in our center. Demographic data including age, sex, and education, were collected. Patients were assessed for abduction and external rotation of shoulder and power of supraspinatus, infraspinatus and trapezius muscles two times before and after spinal accessory nerve transfer to suprascapular nerve using posterior approach. The strength of muscles measured based on The Medical Research Council System. Data were reported using mean, SD, min, max, frequency and relative frequency. For comparing the measurement of before and after operation Wilkason test was used. SPSS version 13 was used for statistical analysis.

Operation technique

The patients recline in prone position, and both the suprascapular and spinal accessory nerves are explored by a transverse incision at the superior border of scapula. The suprascapular nerve is located midway between the superior angle of the scapula medially and the acromion laterally (figure 1).

Figure 1. Surface anatomy of spinal accessory nerve(SA) and suprascapular nerve (SS)

It is identified at the superior notch, the superior transverse ligament is opened and the nerve is released as far proximally as possible (figure 2). The spinal accessory nerve is located at a point 40% of the distance between the dorsal midline (spinous process) and the acromion. (figure 1) It can be identified deep to the trapezius muscle, which is separated parallel to the course of its fibers (figure 3). The spinal accessory nerve is divided as far distally as possible to achieve a coaptation without tension (figure 4-5) the nerves are connected with 10-0 prolen (figure 6-7) (9).

The patient had to strap their arms to chest for 21 days and then were scheduled to attend physical practice and nerve stimulations continually. They had monthly visits to clinic to measure improvements.

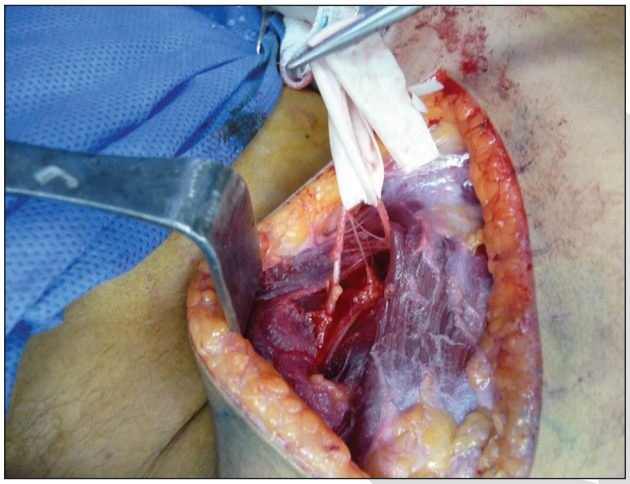

Figure 2. Dissection of suprascapular nerve

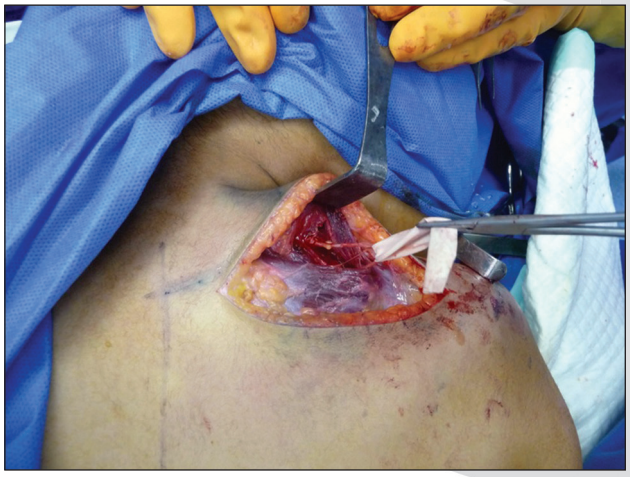

Figure 3. Dissection of spinal accessory nerve

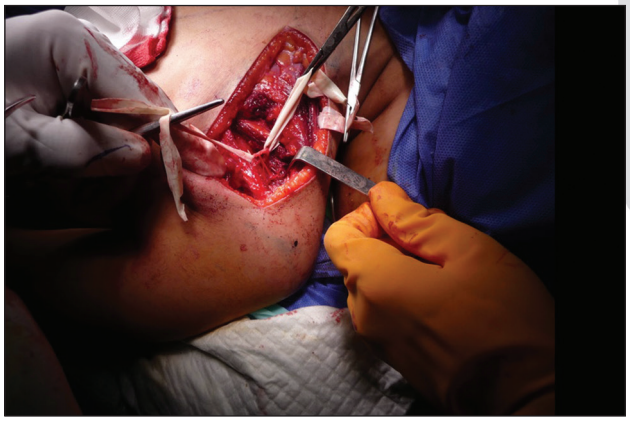

Figure 4. Dissection of suprascapular nerve as far distally as possible

Figure 5. Apposition of both nerves without tension

Figure 6. Coaptation of the nerves without tension

Figure 7. Nerve transfer is completed by end to end suturs

Results

14 patient with complete brachial plexus injury were enrolled in this study, Mean age of the patients was 26. 28 years (SD: 6. 7, range 10-38), 11 of them were male and 3 of them were female. The chief complain of all patients was lack of ability for shoulder abduction. The mean of follow up period was 9. 26 months. (SD: 2. 66, range 5. 20-13. 23, median 9. 55). The total characteristics of patients are shown in table 1. By comparing of the range of motion and strength of muscles before and after the procedures, only the strength of trapezius muscle was decreased which this difference was not statistically significant, whereas other measures including: supraspinatus and infraspinatus muscles strength, shoulder external rotation and abduction were increased, and these changes were statistically significant (table 2, figure 8, 9). We showed the results of operation in a patient figure (10, 11.)

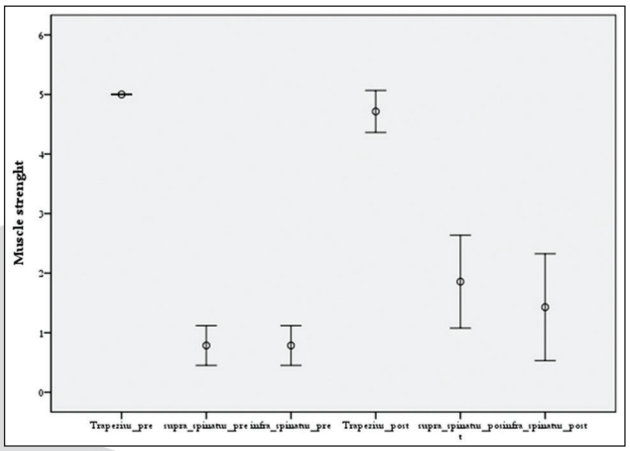

Figure 8. Changes of muscles strength before and after operation

Table 1. Characteristic of total patients

id	age	Sex	Trapezius_pre	supra_spinatus_pre	infra_spinatus_pre	ext_rot_pre	int_rot_pre	abduction_pro	Trapezius_post	supra_spinatus_post	infra_spinatus_post	ext_rot_post	int_rot_post	abduction_post	Follow up (months)
1	31	male	5	2	2	40	.	30	5	5	5	100	.	180	9. 10
2	26	male	5	1	1	.	0	0	3	1	0	0	0	0	7. 13
3	21	male	5	0	0	0	0	0	5	2	0	0	.	48	6. 10
4	31	male	5	0	0	0	.	0	4	2	1	0	.	30	8. 33
5	38	female	5	1	1	0	.	0	5	2	1	5	.	70	6. 77
6	10	female	5	1	1	50	.	30	5	4	4	80	.	180	13. 23
7	25	male	5	1	1	0	.	0	4	2	1	0	.	40	6. 10
8	28	female	5	1	1	30	.	0	5	2	3	90	.	180	12. 17
9	19	male	5	1	1	0	.	0	5	2	2	80	.	180	10. 57
10	31	male	5	1	1	0	.	0	5	2	1	0	.	30	5. 20
11	28	male	5	0	1	30	0	0	5	0	1	80	0	180	12
12	28	male	5	1	0	40	0	0	5	1	0	90	0	180	12
13	30	male	5	0	0	40	0	0	5	0	0	80	0	180	11
14	22	male	5	1	1	0	0	0	5	1	1	70	0	160	10

Table 2. The status of range of motion and strength of muscles before and after operation

Measurement	Before operation				After operation				P* values
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Trapezius strength	5. 00	. 00	5. 00	5. 00	4. 71	. 611	3. 00	5. 00	. 102
supraspinatus strength	. 78	. 57	. 00	2. 00	1. 85	1. 35	. 00	5. 00	. 007
infraspinatus strength	. 78	. 57	. 00	2. 00	1. 42	1. 55	. 00	5. 00	. 071
Shoulder external rotation (degree)	17. 69	20. 47	. 00	50. 00	48. 21	43. 12	. 00	100. 00	. 008
Shoulder abduction (degree)	4. 28	10. 89	. 00	30. 00	117. 00	74. 08	. 00	180. 00	. 001

**Wilkason test was used*

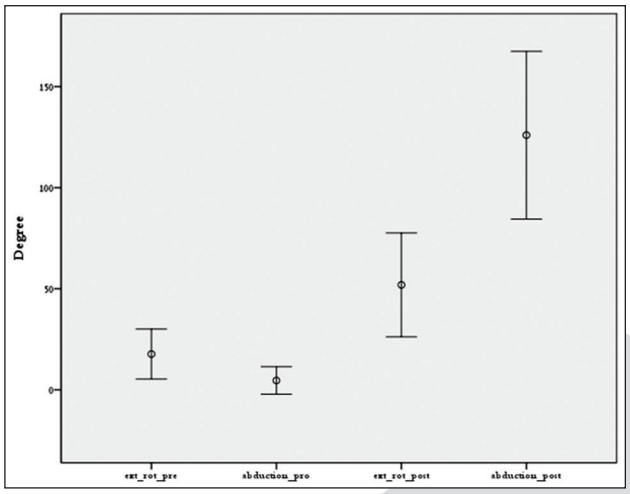

Figure 9. Changes of external rotation and abduction of shoulder before and after operation

Figure 10. Status of shoulder rotation a 10 years old girl (case number 6) before operation

Figure 11. Status of shoulder rotation a 10 years old girl (case number 6) after operation

Discussion

Restoration of shoulder abduction in an important goal of plexus surgery. For this purpose spinal accessory nerve and axillary nerves are the two main targets to achieve this goal. In a meta-analysis by Merrell et al spinal accessory nerve is known to be the best donor to be transferred to suprascapular nerve[12]. Nagano and in a separate study Mikami noted that double or triple level injury in SSN is frequent and explains the poor outcomes in the force of supra and infra spinatus muscles in the anterior approach. [12]. In a similar study In the present study we detected a significant improvement in our patients results, our finding is comparable with similar studies. In a study conducted by Shibing et al, they compared the results of spinal accessory nerve transfer to suprascapular between anterior and posterior approaches in 18 to 26 month follow ups. They reported the results of shoulder abduction 62. 8°± 12. 6° in posterior approach which was better than results of anterior approach 51. 6°±15. 7°(10). In our study 7 patients had up to 180 degree abduction and the mean abduction was 117. 00 which is considerably better than them, but our results in achieving external rotation was less than Shibing study in which the external rotation got to normal value. In our study only one patient got to 100 degree external rotation and its mean was 48. 21 degree. Bhandari et al in another study compared the results of shoulder abduction and external rotation in posterior approach with a control group of anterior approach. He reported the range of abduction and external rotation in posterior approach 70-170 and 30-80 degrees and in anterior approach 65-160 and 22-55 degrees respectively(11).

Ren et al in a study investigated the nerve transfer using posterior approach, and reported the mean of abduction 92. 5 degrees (rang 40-160) which is a better result in comparison with other studies but our results are still better(12). In another study by Bertelli with anterior approach 21 patients of 22 recovered some shoulder abduction with mean range of 57 deagrees and external rotation was restored in 2 of 22 to mean of 90 degrees. [14]which showed inferior results compare to our study which can be defined by some reasons like multi -level injuries or far more distance to target muscles.

Spinal accessory nerve transfer to supra scapular region can weaken the function of trapezius muscle. Using posterior approach can decrease this side effect and as we observed in our study this decrease was not significant, this issue can be the main advantage of this method. In a study by Bhandari et al compared the strength of trapezius muscle in posterior approach (14 patients) vs anterior approach (21 patients). In anterior approach group the strength of trapezius decreased from 5 to 3 whearas in posterior group in 13 patients it decreased from 5 to 4 and only in a patient decreased to 3 (11). In our study in 11 patients the strength remained 5, in 2 patients decreased to 4 and only in a patients decreased to 3 (table 1), our finding is in agree with Bhandari, s study(11).

Our study was conducted with limited numbers of patients with a different amount of brachial plexus injury. It is expectable that many factors like experience of the surgeon, mechanism of trauma and concomitant insults to brachial plexus, rehabilitation program, general comorbidities … can influence on final outcome, which considering their role is difficult with limited numbers of patients, so it seems that using a multicenteric studies is reasonable in this regard, furthermore conducting a systematic review for summarizing the present evidences will be useful for providing better guideline in this regard.

Conclusion

It seems that using posterior approach for transferring the spinal accessory nerve to suprascpular nerve in patients with complete brachial plexus injury can have a relatively more satisfying result in reversing the the amount of abduction and strength of muscles of the patients, and its weakening effect on trapezius muscle is not clinically important.

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The impact of the thyroidectomy on the severity of osteoporosis and bone minerals metabolism in postmenopausal women

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Abstract

Aim: The purpose of this study is examining the effects of total thyroidectomy (TT) on bone mineral density in postmenopausal women.

Methods: 50 women who had total thyroidectomy and 50 women who had unilateral or subtotal thyroidectomy in between 2002 and 2007 for benign reasons were included in these study. Patients all are in their postmenopausal period for at least 5 years. Both groups were euthyroid with hormon replacement therapy if they needed. Calcium (Ca), Phosphate (P), Magnesium (Mg), Parathyroid hormone (PTH), Alkaline phosphatase (ALP), 24-h urinary Ca excretion were evaluated between two groups. To evaluate the bone mineral density of the groups, L1, L2, L3, L4, femoral neck; trochanteric region, and Ward's triangle regions were chosen to be compared.

Results: Comparison of Ca, P, Mg, PTH, ALP, and 24-hour urinary Ca excretion did not show any statistical significance . In TT group, 4 patients (8%) had permanent hypocalcemia whereas there was no permenant hypocalcemia in control group. 25 patients of TT group (50%) had osteoporosis, whereas in control group only 8 patients had osteoporosis and this was statistically significant ($p = 0.001$).

Conclusion: Consequently, the frequency of osteoporosis in postmenopausal women after total thyroidectomy significantly higher than the control group.

Key Words: thyroidectomy, osteoporosis, bone minerals, metabolism, postmenopausal women.

Introduction

Benign enlargement of the thyroid gland (goiter) is a common endocrine disorder.Thyroid surgeries of benign reasons are frequent (1,2).Today, the boundaries of thyroidectomy are still being discussed. Unilateral, subtotal and total thyroidectomy are the surgical methods are used in thyroid surgery. These are still often used surgical procedures, and each one has its own advantages and disadvantages. TT for benign thyroid diseases has been popularized in recent years and in some centers (2). Thyroid hormone replacement in patients after total thyroidectomy is a lifetime need. Osteoporosis is the most common metabolic bone disease. Osteoporosis is seen in 70% of women over 80 years of age. Osteoporosis and fractures due to osteoporosis has become a major public health issue. Especially the hip and spine fractures resulted in increased mortality and diminished quality of life.

Ischemia in parathyroid glands after total thyroidectomy or iatrogenic parathyroidectomy may be incidental. A single parathyroid gland is known to be sufficient in a normal healthy adult. Since there is a tendency to osteoporosis in postmenopausal women, we try to understand whether damage to parathyroid glands or more severe hypothyroidism after total thyroidectomy may increase in the severity and frequency of osteoporosis.

The purpose of this study is assessing the impact of thyroidectomy on severity and frequency of osteoporosis in postmenopausal patients.

Materials and methods

This is a retrospective randomized study. A total of 100 postmenopausal women patients were included in this study in General Surgery Department of Haydarpasa Numune Training and Research Hospital. 50 women who had total thyroidectomy and 50 women who had unilateral or subtotal thyroidectomy in between 2002 and 2007 for benign reasons were included in these study.

Inclusion criteria for the patient group: The TT Group member

1 Should undergo total thyroidectomy due to a benign disease,
2 Post-thyroidectomy period should be more than 5 years,
3 Should be in post-menopausal period during total thyroidectomy surgery,
4 Should not take any hormonal treatment or not use a drug that might affect bone metabolism other than thyroid replacement therapy,
5 Should have normal thyroid function tests (T3, T4, TSH) within the last 5 years.

Inclusion criteria for the control group: The Control Group member,

1 Should undergo thyroidectomy due to a benign disease but surgical interventions must be unilateral,subtotal or near total thyroidectomy,
2 Post-thyroidectomy period should be more than 5 years,
3 Should be in post-menopausal period during thyroidectomy surgery,
4 Should not take any hormonal treatment or not use a drug that might affect bone metabolism other than thyroid replacement therapy,
5 Should have normal thyroid function tests (T3, T4, TSH) within the last 5 years.

All pathology reports and operative reports the patient group members were examined.

Parameters evaluated:

1 Age,
2 Weight,
3 Height,
4 Menopause starting time,
5 Dose of thyroid replacement therapy in patient group,
6 Ca, P, Mg, ST3, ST4, TSH, PTH, ALP blood values and 24-hour urine excretion of Ca,
7 Bone densitometry of L1, L2, L3, L4, femoral neck, trochanteric region of femur and wards region.

Patients' blood and urine tests were studied in Haydarpasa Numune Training and Research Hospital, Biochemistry Laboratory. Bone density measurements of patients were conducted in our hospital Norland brand Dual Energy X-Ray Absorptiometry (DEXA) device. In the patient and control groups DEXA results that were taken in the last 6 months at other centers were also approved. While assessing the results of densitometry of patients World Health Organization's (WHO) T-score base were taken as a criterion for diagnosis of osteoporosis.

Statistical Investigations

The findings of this study were evaluated with SPSS for Windows 10.0 statistical package for statistical analysis. Student's test was used to compare average ages. Mann-Whitney U test was used to compare menopause times. Chi-square test was used for the frequency of osteoporosis comparison between total thyroidectomy patient group and control group. Student's t test was used to compare the average values of Ca, P, Mg, PTH, ALP, 24-hour urinary excretion between total thyroidectomy patient group and control group. Mann-Whitney U test was used for bone mineral density measurement of L1, L2, L3, L4, femoral neck, trochanteric region of femur and wards region to compare the averages between total thyroidectomy patient group and control group.

Results

Age, weight, height and menopause time values of total thyroidectomy patient group and control groups are shown in Table 1. In terms of age, weight, height and menopause times there was no statistically significant difference between the two groups. 25 patients (50%) in TT group, 8 patients (16%) in control group had osteoporosis (Table 2). In TT group osteoporosis was more common inL1, L2, and L4 vertebral bone but there were no significant differences in the frequency of osteoporosis. In L3 vertebrae, femoral neck, trochanteric regions and Wards triangle frequency of osteoporosis was significantly higher in patient group then control group (Table 3). For L1, L2, L3, L4, femoral neck, trochanteric region and Wards triangle bone mineral density comparison of the average values of both groups are shown in Table 4. There was no significant difference between the average bone mineral densities of L1, L2, L4 regions between two groups. Statistically, average bone mineral density of L3, femoral neck,

Table 1. Comparison of age, weight, height and menopause period values of both groups. Standard deviation (SD)

	TT Group		Control Group		P
	Mean	SD	Mean	SD	
Age	57,36	7,23	59,34	7,04	0,17
Weight (kg)	67,3	6,19	64,7	8,55	0,30
Height (cm)	157	18,4	159	21,2	0,24
Menopause Time	7,08	6,89	6,96	5,75	0,93
Avarage dose of levothyroxine replacement therapy (mcgr/day)	172,75	13,5	64,5	7,35	0,01

Table 2. Comparison of both groups for the frequency of osteoporosis

T score	TT Group	Control Group	P
>-2,5	25(50)	42(84)	
<-2,5	25(50)	8(16)	0,001

T score; Normal between +1 and -1; Osteopenia between -1 and -2,5; Osteoporosis <-2,5

Table 3. Comparison of both groups for the frequency of osteoporosis in bone mineral density distribution based on regions

	TT Group	Control Group	P
	n(%)	n(%)	
L1	10(20)	4(8)	0,244
L2	11(22)	3(6)	0,065
L3	10(20)	1(2)	0,003
L4	8(16)	3(6)	0,193
Femoral Neck	14(28)	2(4)	0,001
Trochanteric Region	11(22)	3(6)	0,006
Wards triangle	24(48)	5(10)	0,001

Table 4. Comparison of both groups for bone mineral density averages of L1, L2, L3, L4 and femoral neck and trochanteric regions and Wards triangle.

	TT		Control Group		P
	Mean	SD	Mean	SD	
L1	-0,50	1,61	0,09	1,43	0,053
L2	-0,69	1,50	-0,17	1,33	0,073
L3	-0,72	1,39	-0,14	1,24	0,029
L4	-0,57	1,21	-0,45	1,05	0,595
Femoral Neck	-1,16	1,66	-0,16	1,36	0,001
Trochanteric Region	-1,07	1,55	-0,28	1,20	0,005
Wards triangle	-1,91	1,43	-0,61	1,21	0,001

Table 5. Comparison of both groups for averages of Ca, P, Mg, PTH, ALP, and 24-hour urinary Ca excretion values

	TT Group		Control Group		P
	Mean	SD	Mean	SD	
Ca (mg/dl)	9,08	0,55	9,19	0,46	0,276
P (mg/dl)	3,76	0,74	3,43	0,35	0,007
Mg (mg/dl)	1,92	0,22	2,08	0,35	0,010
PTH (pg/ml)	62,9	42,69	64,83	18,15	0,769
ALP (U/L)	74,8	21,05	75,76	18,53	0,809
24-hour urinary Ca excretion (mg/day)	153,72	98,73	142,67	62,77	0,506

trochanteric regions and Wards triangle in patient group was significantly lower than the control group. The comparison of average values of Ca, P, Mg, PTH, ALP, 24-hours urinary Ca excretion of both groups are summarized in Table 5. Between the two groups considering the average values of Ca, P and Mg, ALP, PTH, 24-hour urine excretion of Ca there is no statistically significant difference between two groups.

Discussion

There is an increase in the number of centers and surgeons performing total thyroidectomy surgeries due to benign reasons. Aside from two major complications that are paralysis of recurrent laryngeal nerve (RLS) and hypocalcemia, total thyroidectomy has a permanent effect on patients as using high dose of thyroid hormone for lifetime. The most important reason for TT defending surgeons is 50% recurrence rate and the possibility of incidental thyroid cancer at an average rate ranging from 3% to 16% after subtotal thyroidectomy (STT) whereas the high complication rates are surgeons' nightmare in complementary thyroidectomy (5). Vaiman et al. evaluated 6223 TT and STT patients and the study has shown there is no significant difference between RLS paralysis and permanent hypocalcemia in TT, STT or lobectomy patients(6). In another study by Vaiman et al, it has been claimed that 5195 patients with benign reasons should undergo TT and stated that "TT is a reliable procedure with the appropriate surgical technique and in the hands of well-trained surgeons"(7). In the general surgical clinics of our hospital TT is recently populer. Between 2002 and 2007 out of 973 thyroidectomies, TT was preferred for 360 (36,9 %) patients with benign thyroid diseases. In our study between the patient and the control group, no significant differences were noted for PTH, ALP, Ca, P, Mg, and the 24-hour urine Ca level.

Sitges-Serra et al. reported that the hypocalcemia developed in post TT patients was 50% and permanent hypocalcemia was around 1% (8). Literature is showing that postoperative hypoparathyroidism is between 6% to 12% whereas permanent hypoparathyroidism rate after one year is 1% (8, 9). It is reported that after total thyroidectomies incidental parathyroid gland excision varies from 8 to 19% (10, 11). Sasson et al. reported that the incidental parathyroidectomy rate was 15% in their study on 141 cases (11).The same study shows 50% of the parathyroid glands were found in the intrathyroidal region, yet only 1 patient developed permanent hypocalcemia (11).

Sakorafas et al. evaluated incidental parathyroidectomies in a study of 158 patients who underwent total thyroidectomy. In this study incidental parathyroidectomy rate was found as 17.7%. Single-gland excision was determined as 86% whereas multiple-gland excision was 14% (12). It's obvious that during the TT, parathyroid glands are being damaged (9, 13). During TT as a result of inferior thyroid arter ligation parathyroid glands can remain ischemic and because of this reason, it's recommended that the intact glands are to be divided into small pieces for auto-transplantation. In our study, hypoparathyroidism after total thyroidectomy is observed in 4 patients (8%). In the literature, 1-29% of permanent hypoparathyroidism after thyroidectomy has been reported, this wide range is said to be related to the surgical technique, whether central resection is done or not and whether the patient underwent medical intervention or not (3). There is an interesting relationship between PTH and osteoporosis. The main effect of PTH is to increase bone resorption and the renal reabsorption of calcium. It is proved that these effects are shown when there is continuous PTH secretion and in case of intermittent secretion anabolic effects on bones are recorded. The recombinant PTH peptides are now started to be used in the treatment of osteoporosis after the effects of PTH are shown (14).

Robert M. Neer et al. found that parathyroid hormone treatment decreased vertebral and non vertebral fractures significantly and at the end of 2 years it increased total bone mineral density in his study of 1637 postmenopausal patients with osteoporosis in 12 different centers (15). In our study, after total thyroidectomy , osteoporosis is more frequently seen especially in femur. Some studies have shown that a single parathyroid gland is sufficient for calcium absorption in a healthy adult unless there is a distortion of absorption in gastrointestinal tract (16, 17). It is not entirely clear for how many parathyroid glands maintain

normal serum calcium level. In the literature, many researchers have an overall view that functional normal parathyroid gland activity alone is sufficient whereas some other researchers argue that there is a need for two or more parathyroid glands (18). The parathyroid glands consist of four each weighing 40-50 mg but function as one. Therefore, the loss of residual tissue causes either hyperplasia or parathyroid function insufficiency.

Being within the normal range of blood PTH level in postmenopausal period; increasing bone density at femoral neck, hip and spine and also the decrease in spinal fractures due to use of PTH can be associated with damaged parathyroid of the patients who had TT. The defective parathyroid hormone secretion from the damaged parathyroid glands after TT in postmenopausal period may be one of the possible hypotheses to explain the increasing frequency of osteoporosis in our series .

Hyperthyroidism is known to increase bone turnover and particularly osteoclastic activity. There is an increasing incidence of osteoporosis in patients with hyperthyroidism (4). In this study, both patients group and also the control group were euthyroid. Additionally, thyroid function tests remained normal in total thyroidectomy group during the last 5 years. However, different than the control group, the patient group was receiving thyroid hormone therapy a replacement level. There is a number of studies examining the effects of thyroid hormone replacement on bone density.

Schneider et al. examined 63 separate studies. In 31 studies amongst all it was concluded that levothyroxine replacement therapy on bone density were not effective. 23 studies concluded that it might be partially harmful. In 9 of these studies it was concluded that a significant reduction in bone density was noticed even at the dose of replacement (19). Schneider et al. concluded that there was a general tendency for peripheral cortical bone loss when analyzing these studies .

Paul T. L. et al's study concluded that even at the replacement dose of levothyroxine it might lead to osteoporosis (19). In our study, osteoporosis is more frequently seen especially in the femur within the patient group. Difference in bone density between patients and control groups was more significant than other studies examined the effect of levothyroxine on bone density. This made us consider that in our study higher frequency of osteoporosis in total thyroidectomy group was not only due to levothyroxine treatment.

As a result we found post-menopausal osteoporosis development in half of the patients who underwent TT and this was significantly different than the control group. It is possible to say that thyroid hormone replacement therapy after TT and PTH secretion disorders were among the possible causes. TT for benign thyroid diseases is an issue that is still being discussed.

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The BCG scar size in asthmatic children

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Abstract

Introduction: This cross sectional study evaluate relationship between size of BCG scar and occurrence of asthema in children

Methods: The diameter of BCG scar of two groups (children with asthma and healthy children) was measured in length and and width and then compared.

Results: Total 400 children were included for the study, 200 children in each group. The mean diameter of BCG scar was 4.68mm in the asthmatic group and 4.62mm in nonasthmatic group. There was no significant difference between two group.

Conclusion: Asthmatic children have BCG scar size similar to nonasthmatic children.

Key words: BCG, BCG scar, Asthma, Children.

Introduction

BCG vacccine was used for prevention of severe form of tubercolosis infections specialy in endemic regions. It is currently suggested that BCG vaccine can decreases the incidence of asthma and other allergic diseases. This effect of vaccine explained by immunological responses. Th-2 type lymphocytes enhanced production of class E immunoglobulin (IgE) and generation of eosinophils by secrete interleukins IL-4 and IL-5 resulting in allergic disorders disorders such as asthma. Immunization by BCG induce characteristic Th-1 responses which produce interferon gamma (IFN-Ɣ) as a suppresser of Th-2 activity. On the other hand some studies have shown that tissue reactions at the site of the BCG vaccination are dependent to level of INF-Ɣ in response to the vaccine. Therefore, the BCG scar size may be a useful prognostic factor for occurrence of asthma in children (1-7).

Methods

This cross-sectional case control study was carried out in Ghaem Hospital, Mashhad university of medical science. Case group consist of 200 patients with asthma diagnosed by a pediatric immunologist at least six months later. Control group consist of 200 healthy child without history of asthma and allergy. Inclusion criteria for both groups were age between 2 to 14 years, history of BCG vaccination in the first month of life, wegith and hegith between percentile of 10 to 90 .exclusion criteria for both groups were history of febrile illnesss or recieving viral live virus vaccine at last month, immuno-suppression by disease or drugs and history of admission during neonatal age in the hospital. Patient data including age, sex, weight, hegith,lodging, time of breast feeding were recorded. Two different observers recorded the diameter of the BCG scar, using a transparent millimeter ruler. Transverse and longitudinal diameters and their average was calculated. After entering data into SPSS version 16.0 software, quantitative data were analyzed by the Wilcoxon signed rank test and qualitative data by the McNemar test. P value < 0.05 was considered significant.

Result

Mean age of patients was 6/7 8/2 ± years in asthmatic group and 21/8 7/2 ± years in control group. 37.5% of case and 58.5% of control group were female. Data of case and control group was summarized in Table 1. There were no significant relations between age, sex and birth weight in two groups. Also there was no significant relation between BCG scar diameter in two groups. Whereas there was significant difference between BMI, lodging and time of breast feeding between case and control groups.

Table 1. General and clinical characteristics of patient and control groups

	Case group	Control group	P value
Age,mean (year)	7.6±2.8	8.12±2.7	0.053
Sex, F(%)	37.5	58.5	0.47
Bearth weight, no(%) <2500g ≥2500g	 (3.5)7 (96.5)193	 (1)2 (99)198	0.17
BMI	16.3±3.3	15.4±2.2	0.001
Lodging, no(%) Urban Rural	 (89)178 (11)13	 (96.5)193 (3.5)14	0.004
Time of breast feeding (month)	21.2±6.0	22.3±4.5	0.001
Size of BCG scar vaccine (mean,mm)	4.68	4.62	0.45

Disscusion

The prevalence of asthma has a dramatic rise in recent decades. It may be due to changes in lifestyle and exposure to certain infections (8, 9). Hygiene hypothesis has been suggested as a possible reasons for this increase. According to this hypothesis suggest the protective effect of infections in early childhood against allergic disease. Intracellular bacteria and viruses induce a TH1-mediated immune response, whereas allergens are associated with TH2 immune responses, and dominant pathway is established in early childhood. BCG vaccine is thought to be capable of manipulating the immune system toward TH1 and reduce the risk of atopic disease (10).

Previous studies have conflicting results about association of BCG vaccine and risk of asthma (10). In this study we didnot find significant association between BCG vaccination and reduce risk of asthma. It is similar to results of a study in adult patients (11). Vargas showed that BCG vaccination was unable to cause a long-term reinforcement of Th1 response in asthmatic children, although it could avoid the increased Th2 response in control group (6). However, some studies showed that children with asthma have significantly greater BCG scar (1, 8, 12).

Other studies demonstrated that BCG scar size depends on the strain and vaccination dose, gender, age, training of the health professional, response to the vaccine and environment (urban or rural) (12, 13). Some of the above confounding factors were similar in both case and control group but some were different in two group that may influence the results.

In the other hand it is suggested that BCG scar is more related to the mechanism of wound healing rather than potency of TH1-immunity (14).

Genetic suseptibility for atopy is important risk factor for asthma. Also environmental factors such as tobacco exposure, air pollution and viral respiratory infections can affect risk of asthma development. This multifactorial pathogenesis of asthma could explain the controversial results in studies that assessed the relationship between BCG scar size and asthma development (11).

The present study has limited informations on envirmental exposures. The confounding factors that may affect the results of our study include time of breast feeding, lodging and BMI of the individuals studied. We did not classify asthma severity. It is probably that BCG scar is associated with severity of asthma. It is appropriate to evaluate this factor in further studies.

Conclusion

Asthmatic children have BCG scar size similar to nonasthmatic children.

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Chlamydia trachomatis and genital mycoplasmatic infections in female students at the University of Sarajevo

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Abstract

Objectives: The aim of the study was to determine prevalence of chlamydial and mycoplasmatic urogenital infections and to study associations with sexual behavior risk factors, among female students at the University of Sarajevo.

Methods: This cross-sectional study was performed on endocervical swabs and questionnaires obtained from 100 randomly selected female students from University of Sarajevo. All participants completed a self-administered questionnaire on sexual knowledge, attitudes and behaviors. Detection of *Chlamydia trachomatis* (CT) in endocervical swabs was performed by direct immunofuorescence assay (DFA), Chlamydia Direct IF (ID), BioMérieux, France. Urogenital mycoplasmas (*Mycoplasma hominis, Ureaplasma urealyticum*) in endocervical swabs were detected by selective cultivation in broth Mycoplasma IST 2, BioMérieux, France.

Results: The mean age of students was 23 (22.96 ± 2.0) years, and 98% of them reported ever having had sexual intercourse. Prevalence of CT infections was 7.0%, and mycoplasmatic infections 8.0%. Proportion of asymptomatic chlamydial infections was high (71.4%) in participants tested positive. Risk factor significantly associated with chlamydial urogenital infections at $p<0.05$ level was inconsistent (rare) use of condoms in previous year. Average risk factors score values were significantly higher in CT positive participants compared to negative ones (3.0 ± 0.8 vs 2.0 ± 0.9; $p<0.01$).

Conclusion: The prevalence in our survey resembles those found in other countries in Europe. Screening for *Chlamydia trachomatis* and urogenital mycoplasmas infection in young sexually active women would be cost-effective in Bosnia and Herzegovina given the prevalence of both infections measured by this study. It is necessary to conduct larger, population-based studies in the future, which would obtain comprehensive epidemiological data in order to get real situation insight and estimate the true burden of STIs.

Key words: *Chlamydia trachomatis*; urogenital mycoplasmas; prevalence; sexual behaviour; female students.

Introduction

Chlamydia trachomatis (CT) infections are the most frequently reported urogenital, bacterial sexually transmitted infections (STIs) worldwide. Latest figures (2008) from the WHO show that there were an estimated 105.7 million new cases per year of CT infections among adults aged 15 to 49 years (1). CDC data shows chlamydial infection prevalence of 8.3% among sexually active women under 25 years of age (2). The National Chlamydia Screening Programme (NCSP) in England offers opportunistic screening to sexually active men and women under 25 years of age attending healthcare and non-healthcare settings; found prevalence is around 10% (3). Chlamydial infections are asymptomatic in up to 90% of women, and if left untreated may cause severe complications, permanent consequences and profound impact on sexual and reproductive health (2,4). WHO estimates that 10 – 15% of women with untreated infections develop symptomatic pelvic inflammatory disease (PID) or other severe sequelae such as infertility or extrauterine pregnancies (5). Because of the large burden of asymptomatic cases and risks associated with infection, CDC recommends screening for CT infection for all sexually active young women un-

der the age of 25 years, and older women who are at increased risk (2). Urogenital mycoplasmas are emerging STIs pathogens and have recently been proven to be similar to CT in several aspects, such as preference to the genital tract, mode of transmission, mostly asymptomatic infections and complications. Over 27,000 women from 48 published reports have been screened for *M. genitalium* urogenital infection in high- or low-risk populations worldwide with an overall prevalence of 7.3% and 2.0%, respectively (6).

Sexually active young women under the age of 25 are high risk population for acquiring STIs. Risk factors for genital CT infection, besides age under 25 years and female sex, are: lower socioeconomic status, inconsistent condom use, early age of sexual debut, multiple lifetime sexual partners, two or more partners in the past year, having new or casual sexual contacts, cervical ectopy and associated other STIs (7).

Several studies have reported the prevalence of genital CT and mycoplasmatic infections in young sexually active women. In a population-based survey in Croatia among young people aged 18-25 years, by *Bozicevic et al.* (2011), the prevalence of CT infections was 5.3% in women (8). *De Maria et al.* (2013) have observed high prevalence rates, 9.9% among women aged 14–24 years (9). *Kløvstad et al*. (2012) found that the prevalence of CT infection was 5.8% among women under the age of 25 years in Norway (10). CT prevalence of 4.8% among female students attending higher education in the Republic of Ireland was reported by *O'Connell at al.* (2009) (11). *M. genitalium* prevalence was 3.3% in sexually active female students, in the study by *Oakeshott et al.* (2010) (12). *Andersen at al. (*2007) found that in women aged 21–23 years, the prevalence of *M. genitalium* and CT infection was 2.3% and 8.4%, respectively (13).

In Bosnia and Herzegovina, as in the other countries of Eastern Europe, there is a lack of data on prevalence of CT in the general population, including young adults. Other authors published that there has been 286 cases of CT infections in 2006, 112 in 2007, 194 in 2008 and 84 in 2009 (14). However, those figures are based on case reporting, and are believed to substantially underestimate true incidence. Although compulsory, reporting of CT infections in Bosnia and Herzegovina is mostly done by practicing physicians in public institutions and tends to be incomplete. *Mahmutovic Vranic at al.* (2013) have recently published a study conducted among university students of both genders at the University of Sarajevo and found overall prevalence of 9.5% (15).

The aim of the study was to determine prevalence of chlamydial and mycoplasmatic urogenital infections and to study associations with sexual behavior risk factors, among sexually active young women, undergraduate students at the University of Sarajevo.

Methods

This study was performed on specimens and questionnaires obtained from one hundred (100) randomly selected female students from University of Sarajevo participating in a cross-sectional study carried out from March to September 2013. Students who attended the Insitute of Health Protection of Students at University of Sarajevo in this period of time were approached. If they indicated an interest in participating, the significance of a positive chlamydia and mycoplasmas test, as well as the subsequent management of same were explained to them by a gynecologist. After providing written informed consent, all participants received a physical examination, including a pelvic examination by a gynecologist. Endocervical swabs were obtained from every student, and all tested for CT infection; 25 swabs were tested for genital mycoplasma infections as well. All participants completed a self-administered questionnaire on sexual knowledge, attitudes and behaviors. The questionnaire consisted of 20 mostly dichotomous yes/no option and the forced choice questions. It included demographic questions, questions about sexual health, sexual lifestyle, suggestive symptoms at the time of testing and other STIs-associated risk factors.

Detection of CT in endocervical swabs was performed by direct immunofuorescence assay (DFA), Chlamydia Direct IF (ID), BioMérieux, France. Urogenital mycoplasmas (*Mycoplasma hominis, Ureaplasma urealyticum*) in endocervical swabs were detected by selective cultivation in broth Mycoplasma IST 2, BioMérieux, France. Both tests were performed according to the manufacturer's instructions.

Students who tested positive for chlamydial and mycoplasmatic infections were managed by the clinic where they were tested. The treating gynecologist were provided with up-to-date treatment guidelines (1 g of azithromycin for uncomplicated infections, if there were no symptoms to suggest PID). Patients were informed about importance of partner(s) notification and treatment.

All data analyses were performed using SPSS software, version 16. Statistical significance was determined by Fisher's exact test. Any P value < 0.05 was considered significant.

Data protection was assured. Unique identification number was given to speciments and questionnaire obtained from the same student, and it was used in all data analyses. The study protocol

Table 1. Socio-demographic and sexual behavioural characteristics of students included in the study

Variables	Details	Participants (%)
Age	< 25 years	85
	≥ 25 years	15
Year in higher education	1st Year	8
	2nd Year	22
	3rd Year	19
	4th Year	28
	5th Year	23
Socioeconomic status	Inferior to others	4
	Equal to others	90
	Better than others	6
Ever engaged in sexual intercourse	Yes	98
	No	2
Age at first intercourse	≤ 16	4
	≥ 17	94
	never had	2
The number of lifetime partners	0-2	71
	≥ 3	29
The number of partners in the past year	0 – 1	82
	≥ 2	18
In a steady relationship	Yes	85
	No	15
Condom use during intercourse in the past year	Always	17
	Often (more than 70%)	20
	Sometimes (30-70%)	24
	Rarely (less than 30%)	22
	Never	15
	Never had intercourse	2
Previously diagnosed with an STI	No	86
	Doesn't know	5
	Bacterial vaginosis	1
	HPV	6
	Trichomonas vaginalis	2
Previous STIs treatment	No	91
	Yes, with partner	6
	Yes, without partner	3
Previous STIs screening	Yes	39
	No	61
Have suggestive symptoms	No	35
	Yes	65

received ethical approval from the Ethical Committee of Faculty of Medicine, University of Sarajevo.

Results

The questionnaire was completed by 100 female students who had also been tested for CT infection; 25 of them were tested both for chlamydial and mycoplasmatic infections. The mean age of participants was 23 (22.96 ± 2.0) years. 98% of participants reported ever having had sexual intercourse. Only 4.0% of them reported their socio-economic status to be inferior, comparing to others in their surroundings, which is risk factor for acquiring STIs. Early sexual debut (age ≤ 16 years) had 4.0% of students, and the mean age for first sexual intercourse was 19.4 (SD = 1.8) years. The mean number of lifetime sexual partners was 2.4 (SD = 2.2, range 1-17, median 5). 18.0% of the participants had two or more new male sexual partners in the previous 12 months. The mean number of sexual partners in previous year was 1.2 ± 0.6. 85.9% of all students confirmed current steady relationship. Only 17.0% of students indicated that partner always used condom in past 12 months, the rest of them answered often (20.0%), sometimes (24.0%), rarely (22.0%) and never (15.0%). A previous STI was reported by 9.0% of whom 6.0% reported previous HPV infection; 3.0% of students were not advised about partner notification and treatment. Socio-demographic and sexual behavior variables among participants are showed in Table 1.

Prevalence of CT infections was 7.0%, and mycoplasmatic infections 8.0%. Proportion of asymptomatic chlamydial infections was high (71.4%) in participants tested positive. 28.6% of CT positive students reported previous HPV infection. CT and genital mycoplasmas test results in association with sexual bihevioural factors are presented in Table 2 and Table 3. We investigated CT and urogenital mycoplasmas positivity by different determinants, socio-economic and sexual behaviour characteristics, but only risk factor significantly associated with chlamydial urogenital infections at $p < 0.05$ level was inconsistent (rare) use of condoms in previous year. Risk factors score was 3 in 60.0% and 4 in 20.0% of CT positive students which was significantly higher compared to CT negative students. Average risk factors score values were also significantly higher in CT positive participants compared to negative ones (3.0 ± 0.8 vs 2.0 ± 0.9; $p < 0.01$).

Table 2. CT test results in association with sexual behavioural factors

Variables	CT test positive	CT test negative
Mean age (years)	23.3 ± 2.8	22.9 ± 2.45
Average age of sexual debut (years)	18.4 ± 1.1	19.5 ± 2.4
Average number of lifetime partners	3.0 ± 1.5	2.4 ± 2.2
Average number of partners in the last year	1.6 ± 0.9	1.2 ± 0.6
Condom use during intercourse in the past year (% of students)		
Always	0 %	18 %
Often (more than 70%)	20 %	20 %
Sometimes (30-70%)	20 %	26 %
Rarely (less than 30%) ‡	60 %	20 %
Never	20 %	16 %
Previously diagnosed with an STI (% of students)		
No	42.8 %	89.2 %
Doesn't know	28.6 %	3.2 %
Bacterial vaginosis	0.0 %	1.1 %
HPV	28.6 %	4.3 %
Trichomonas vaginalis	0.0 %	2.2 %
Previous STIs treatment (% of students)		
No	71.4 %	92.4 %
Yes, with partner	14.3 %	2.2 %
Yes, without partner	14.3 %	5.4 %
Previous STIs screening (% of students)		
Yes	71.4 %	36.6 %
No	28.6 %	63.4 %
Have suggestive symptoms (% of students)		
Yes	28.6 %	67.7 %
No	71.4 %	32.3 %
Average risk factors score value †	3.0 ± 0.8	2.0 ± 0.9
Risk factors score value (% of students)		
0	0.0 %	3.2 %
1	0.0 %	22.6 %
2	20.0 %	49.4 %
3	60.0 %	22.6 %
4	20.0 %	2.2 %

‡ $p < 0.05$ † $p < 0.01$
CT – Chlamydia trachomatis

Table 3. Genital mycoplasmas test results in association with sexual bihevioural factors

Variables	Genital mycoplasmas test positive	Genital mycoplasmas test negative
Mean age (years)	21.5 ± 2.4	23.5 ± 1.2
Average age of sexual debut (years)	17.5 ± 0.7	19.6 ± 2.4
Average number of partners in the last year	1.5 ± 0.8	1.2 ± 0.6
Condom use during intercourse in the past year (% of students)		
Always	0 %	5 %
Often (more than 70%)	0 %	19 %
Sometimes (30-70%)	50 %	24 %
Rarely (less than 30%)	50 %	33 %
Never	0 %	19 %
Previously diagnosed with an STI (% of students)		
No	100 %	56.6 %
Doesn't know	0 %	8.7 %
Bacterial vaginosis	0 %	4.3 %
HPV	0 %	26.1 %
Trichomonas vaginalis	0 %	4.3 %
Previous STIs treatment (% of students)		
No	100 %	65.3 %
Yes, with partner	0 %	21.7 %
Yes, without partner	0 %	13.0 %
Previous STIs screening (% of students)		
Yes	50 %	52.2 %
No	50 %	47.8 %
Have suggestive symptoms (% of students)		
Yes	100 %	56.5 %
No	0 %	43.5 %
Average risk factors score value	2.50 ± 0.68	2.43 ± 0.84

Discussion

The aim of this study was CT and urogenital mycoplasmas screening of the vulnerable population, young sexually active women. In Bosnia in Herzegovina there are no national guidelines regarding the screening programme or oportunistic testing of STIs. The mean age of participants was 23 (22.96 ± 2.0) years, and 98% of them reported ever having had sexual intercourse; these results classifed them in high risk population of acquiring STIs. CT was detected in 7.0% and urogenital mycoplasmas in 8.0% of female University students. None of them were positive both for CT and urogenital mycoplasmas. *Mahmutovic Vranic et al.* found similar prevalence (8.9%) among female students at University of Sarajevo (15). In Croatia *Bozicevic et al.* (2011) reported that prevalence of CT infections was 5.3% in women aged 18-25 years (8). In Slovenia, *Kobal et al.* (2006) found CT prevalence of 6.5% in young people under the age od 25 years (16). *Tomanovic et al.* (2013) reported chlamydia prevalence of 5.8% (DFA testing) and 8.7% (PCR testing) among female students at University of Belgrade (17). The similarity with our findings may be explained by the fact that Croatia, Slovenia and Serbia are neighboring countries and might have a similar socio-cultural profile. Compared to the similar European studies, the prevalence of both pathogens found in this study is considerable (3,6,9-13,18-20). Our results are, as well, comparable to actual CDC data (2). This prevalence may support a screening strategy as models have shown screening to be cost effective at prevalences of 3.1 - 10.0%, and cost saving at a prevalence as low as 1.1% (21).

At the time when the sample was taken, 28.6% of students positive for CT infection had suggestive symptoms, a finding that must be considered when documenting the presence of asymptomatic CT infections. This is relevant since this silent infections can lead to severe complications and also represents a potential source of infection for sexual partners. CDC estimates that 80 - 90% percent of women have asymptomatic CT infections (2). Our results confirmed high proportion (71.4%) of asymptomatic chlamydial infections. The particular challenge for chlamydia control is reducing the duration of infection through diagnosis and treatment. Since most cases are asymptomatic, infected individuals may have no reason to present to clinical services. Unless the person her/himself comes for medical check up, is notified by a partner who has developed symptoms, or is actively screened, the infected person will remain infectious for a long period. This emphasises need for CT screening, especially among population at risk, with the

aim of identifying asymptomatic cases and reducing chlamydia prevalence in the population.

We found that risk factor significantly associated with chlamydial urogenital infections at $p < 0.05$ level was inconsistent (rare) use of condoms in previous year. This finding is also comparable to international data. *Kobal et al.* found significant correlation between asymptomatic chlamydial infections and inconsistent use of condoms (16). *Bozicevic et al.* (2006) reported that not using condoms during the first sexual intercourse was correlate of genital discharge in women aged 18 – 24 years (22). In the study by *Fernández-Benítez et al.* (2013), there was an increase in the risk of CT infection when barrier contraceptives were not routinely used (23). Our results suggest that prevention of CT with routine condom use could lead to a reduction in the observed CT infection rates. According to WHO, when used correctly and consistently, condoms offer one of the most effective methods of protection against STIs (5). Condom use should be encouraged and promoted in educational programs that targets population at risk, as part of good primary preventive interventions.

When compared to students with CT and urogenital mycoplasmas negative tests, in those tested positive average age of sexual debut was lower, and average number of lifetime partners and partners in the past year was higher. Other authors found that two or more partners in the past 6 or 12 months correlated with CT infection (9,10,20,24,25). Although our findings were not statistically significant, it is suggested that positive students displayed generally more high-risk behaviour. Risk factors score was 3 in 60.0% and 4 in 20.0% of CT positive students which was significantly higher compared to CT negative students. Average risk factors score values were also significantly higher in CT positive participants compared to negative ones (3.0 ± 0.8 vs 2.0 ± 0.9; $p < 0.01$). Those results, along with finding that previous HPV infection reported 28.6% CT positive students, again suggest high-risk behavior of young sexually active women under the age of 25 years.

Steady relationship confirmed 71.0% of CT positive and 50.0% of mycoplasma positive students. The fact that 3 of 9 students (33.3%) with previous STI were not advised about partner notification is also of great concern. The notification and management of sexual partners is an essential component of chlamydia case management. Contacting sexual partners of people with STIs has been shown to reduce rates of reinfection and provides a mechanism for targeting those who are already infected or may be at high risk of becoming infected. In Bosnia and Herzegovina partner notification and tracking is not compusory for health providers. There is a number of approaches to partner management, and guidelines should be developed for local practice and the appropriate procedures put in place to ensure that it is carried out effectively.

There are some limitations in our survey. The main limitation is that the sample size was restricted to 100 female students. Higher education students are not representative of the general population. Students attending the Insitute of Health Protection of Students at University of Sarajevo may not be representative of all students as some students may not seek any medical care and others may seek care at their family doctor or in private clinics. Voluntary recruitment may also have favoured participation by students already concerned regarding STIs. The potential self-reporting bias should also be taken into consideration.

Conclusion

Our results suggest that chlamydial and mycoplasmatic infections exist as a current issue in Bosnia and Herzegovina, considering young sexually active women. It is necessary to conduct larger, population-based studies in the future, which would obtain comprehensive epidemiological data in order to get real situation insight and estimate the true burden of STIs. These findings would provide stronger evidence for the implementation of screening policies for CT and other STIs in Bosnia and Herzegovina, as important factor in secundary prevention considering large proportion of asymptomatic infections. The prevalence in our survey resembles those found in other countries in Europe. Screening for CT and urogenital mycoplasmas infection in young sexually active women would be cost-effective in Bosnia and Herzegovina given the prevalence of both infections measured by this study. Effective STI surveillance system depends on complete, reliable and routine reporting of STIs, including laboratory based surveillance and optimized management in

identification, notification and treatment of sexual partners, and should be evaluated in Bosnia and Herzegovina to guide future, hopefully improved policies in national STIs control strategy.

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Novel R42C mutation identified in Early Onset of Parkinson's disease in Pakistani Patients

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Abstract

Parkinson's disease is the most common neurodegenerative disease. In this disease dopaminergic neurons are lost in substantia nigra. Mutations in the *parkin* gene cause Early Onset of Parkinson's disease (EOPD) in 50-60% of patients. *Parkin* gene encodes for Parkin protein which consist of four domains (Ubl, RING1, IBR, RING2). 1-3 exons form Ubiquitine-like (Ubl) domain, which help in the attachment with substrates like Rpn10 subunit of 26S proteasome. To observe if there is any mutation involved in the exons of *Parkin gene* in EOPD in patients from oriental region. 30 patients below age 45 years were selected and DNA was isolated from their blood samples. Amplification of exons was done by sequencing PCR**:** In current study, one transition C→T mutation has been found in 2nd exon of Ub1 domain which resulted in the amino acid substitution from Arginine to Cysteine at position 42. Wild-type amino acid was involved in the attachment of Rpn10 subunit of 26S proteasome which selectively binds with the poly-ubiquitin chain of damaged proteins and degrades them. So, this amino acid change resulted in the decreased or no attachment of 26S proteasome via its Rpn10 subunit which results in no degradation of misfolded and defected protein in the cytosol. As a consequence, these defected proteins start forming aggregates within the cell which eventually decrease cell's function and cells start dying. The data suggests that there is a strong possibility that the above mentioned mutation is one of the primary causes of EOPD.

Key Words: Parkinson's disease, Novel mutation, Pakistani population

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease (Lesage and Brice 2009). PD has doricaly been pondered as a disease of motor dysfunction, but psychiatric symptoms in addition to other symptoms are also included (Vaughan 2000). Four motor symptoms contemplated pivotal in PD are tremor, rigidity, slowness of movement and postural instability. Numerously reported non-motor symptoms include autonomic dysfunction, neuropsychiatric problems, sleep and digestive problems (Park and Stacy 2009).

PD is caused by the loss of neurons in the substantia nigra and formation of a-synuclein aggregates known as Lewy bodies (Wauer and Komander 2013). This loss of dopaminergic neurons results in the degeneration of dopaminergic terminals with dopaminergic depletion in striatum. These dopaminergic terminals are required for normal motor function (Quesada, Lee et al. 2008). A fundamental biological function of the nigrostriatal unit is the control and fine tuning of motor performance at the extrapyramidal level (Karakaya, Kipp et al. 2007). To control and coordinate the muscle activity, dopaminergic neurons send signals in the form of neurotransmitter known as dopamine (Figure 1) (Fung, Scholz et al. 2006). The loss of dopaminergic neurons cause changes in the sensitivity and density of dopamine receptors specially D1 and D2 receptors. The sign and symptoms of PD start appearing when 70% of the nigrostriatal neurons are depleted (Galvan and Wichmann 2008).

Usually, motor symptoms begin from one side of the body, later affecting the other side as well. As the time rolls on, more degeneration of dopaminergic neurons takes place, worsening the PD symptoms. Severity of the disease is measured by motor symptoms and the assessment of ability to perform daily functions (Perlmutter 2009). Although most patients have sporadic or idiopathic Parkinson's disease but its etiology is still not clear. 5–10% of patients have a positive family hi-

story of this progressive neurological movement disorder (Funayama, Li et al. 2008). 1% individuals are affected with PD who have age ≤50 years while occurrence rate increases up to 3% in individuals of ≥ 75 years. Risk for the disease increases proportionally with the increase in age (De Rijk, Breteler et al. 1995) and gender also contributes to the disease. Many studies have reported that males are usually more affected than females. Symptoms are also severe in males as compared to the females. Combined incidence rate related to age and gender is approximately 1.34 per million of individuals. This incidence rate among men is 1.9 per million while 0.99 per million in women (Simunovic, Yi et al. 2010).

Molecular studies of PD have shown that this disease has two forms of inheritance. One is autosomal dominant (AD) which is very rare. Missense mutation (A53T, E46K and A30P), duplications and triplications of gene α-synuclein *SNCA* (present on chromosome 4q22.1) are cause of autosomal dominant familial PD but its prevalence is very low. More than 40 nonsense and missense mutations have been reported in leucine-rich repeat kinase 2 (*LRRK2*) which is present on chromosome 12p12. Mutations present in other genes like nuclear receptor subfamily 4, group A, member 2 (NR4A2), microtubule-associated protein tau (MAPT) and ubiquitin carboxyl-terminal esterase L1 (UCH-L1) are also responsible for PD (Le, Xu et al. 2002; Edwards, Scott et al. 2010; Kessler 2013). Genes associated with Autosomal recessive PD are *ATP13A2,* PTEN-induced putative kinase 1 (*PINK1*), *DJ-1, and parkin mapped on chromosome 1p36, 1p36.12, 1p36.23 and 6q26 respectively (A. Wood-Kaczmar 2006; Bekris, Mata et al. 2010)*. But mutations present in *SNCA*, *parkin*, *PINK*1, *DJ*-1 and *LRRK*2 are responsible for most of the cases of monogenic PD in different populations (Filatova, Shadrina et al. 2013). From these autosomal recessive genes, *parkin* gene with more than 100 mutations including missense, nonsense, deletions as well as exonic deletion, rearrangements and duplication is the most frequent cause of PD. HomoloGene of NCBI database showed that orthologs of this gene are also present in nematodes, arthropods and chordates and plays the same role as it plays in *Homo-sapiens*. *Parkin* is an ancient gene and EggNog (version 2.0) database shows (Figure 2) that this gene appeared at the level of bacteria and archea. It is present even in very simple and quite ancient organism. It is present in all the eukaryotes and prokaryotes. It also shows that PARK2 (*parkin*) gene is duplicable and has multiple copies or paralogs (Muller, Szklarczyk et al. 2010).

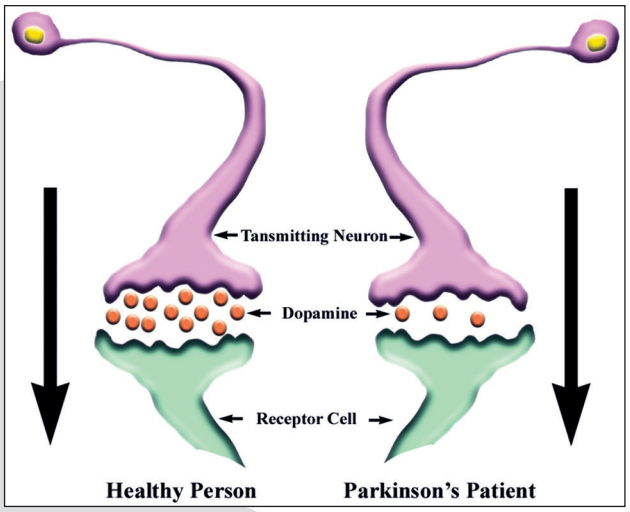

Figure 1. Dopamine level in normal and Parkinson's disease affected neuron

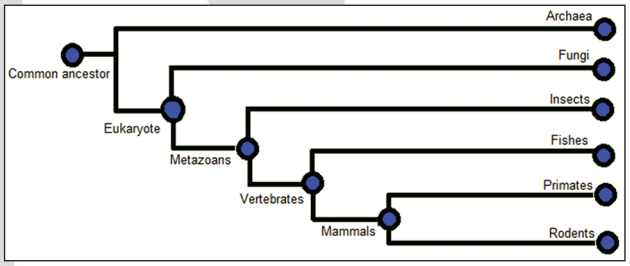

Figure 2. Evolutionary history and duplicability of PARK2 (parkin) gene (EggNog Version 2.0)

The mutations spread over the entire length of *parkin. Parkin* gene consists of 12 exons (Figure 3) from which exon 2 is considered as one of the hot spot for mutation with more than 28 point mutations whereas exonic rearrangements mostly occur in introns 2 through 4.

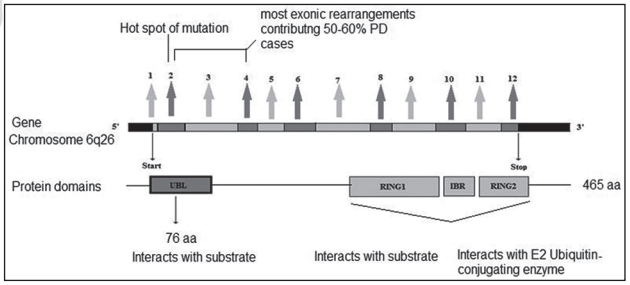

Figure 3. Structure of parkin gene and its protein domain

These mutations make it susceptible for 50-60% cases of PD usually occurs at the age between childhood to 45 years and its occurrence decreases to <1% as the age increases (Bekris, Mata et al. 2010).

The protein, which is encoded by the *parkin* gene, is a member of protein family which have conserved Ubiquitin-like (Ubl) domain, Really Interesting New Gene (RING) finger motifs and In-between-RING (IBR) motif (figure 3). 2 RING motifs linked by cysteine rich IBR motifs make RBR domain which interact with E2 enzymes to catalyze the tagging of proteins with ubiquitin for destruction by proteosome. Parkin protein monoubiquitinates and polyubiquitinates different proteins. The polyubiquitin chain, which becomes a degradation signal for proteasome synthesized by a cascade reaction involving the 3 enzymes ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, and ubiquitin-ligating enzyme, which act as substrate-recognition molecules (Shimura, Mizuno et al. 2012). Parkin is mainly present in cytosol, but it is also present in Golgi complex, synaptic vesicles and endoplasmic reticulum. In PD, very important pathogenic role is played by Parkin E3 ligase. Mutations found in *parkin* gene change the cellular localization, propensity to aggregate, or solubility of wild-type Parkin protein (Bekris, Mata et al. 2010; Dawson and Dawson 2010).

Materials and Methods

Patients

Thirty isolated patients were selected for this study according to the following criteria; 1) age at onset (≤ 45 years), 2) showing at least two cardinal signs like resting tremors, rigidity or bradykinesis and 3) responding to levodopa. Expert neuro physicians of Nishtar Hospital, Multan and Sheikh Zayed Hospital, Rahim Yar Khan, Pakistan helped us in selection of positive patients who have been fulfilling the above mentioned criteria. This study was in accordance with the Declaration of Helsinki (2008). More patients were males (25/30) and only 5 had family history to this disease and all of them were males (table 1). Mean age of the patients was 35.8 years. Most of the patients fall in the age range of 31-40 years (table 1).

Molecular analysis

After the approval from the Ethical Review Committee of University of Veterinary and Animal Sciences and informed consent from the selected patients, blood samples were collected from patients. DNA was isolated by following the organic procotol established by Methew (Mathew 1985). Amplification of exons 1, 2 and 3 which encodes the ubiquitin-like (Ubl) domain of Parkin protein was done by using the following primers (table 2)

PCR reactions were separately carried out for each primer in volume of 25µl consisting on 50ng of genomic DNA, 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 uM dNTPs, 10 pmoles of each primer and 5 U Taq Polymerase (Vivantis). Thermal program for PCR was settled as initial denaturation at 94°C for 4 min, for next 35 cycles following setting was used: denaturaion at 95°C for 30 sec, annealing at 58°C, 56°C, 57°C for exon 1,2 and 3 respectively for 45 sec, extension at 72°C for 45 sec and final extension was also carried out at 72°C for 10 min. Specific

Table 1. Demographics of Study population

Age Range (years)	Total patients		Patients with family history	
	Male	Female	Male	Female
21-30	7	2	2	0
31-40	12	3	1	0
41-50	6	0	2	0
Total	**30**		**5**	

Table 2. Primer sequences and product size

Gene/Exon	Forward primer	Reverse primer	Product Size
Parkin/1	AGGAGGCGTGAGGAGAAACT	GGTCTTCATGAGAACGCTCAG	460
Parkin/2	CTATCACCATTTAAGGGCTTCG	TTCTCACTCGCAGTTGTTCTTC	448
Parkin/3	AAGGAGCACCTAAGTTGGTCAG	CTCACCGGGTGCATATTTAGTT	471

PCR products of three exons were purified by using 80% ethanol at final concentration and were used in Chain termination sequencing reactions. Above mentioned primers were used in the sequencing PCR reactions. The amplified products of sequencing PCR were again purified by using 70% ethanol and then resolved on an ABI-3130 capillary DNA analyzer (Applied Biosystems, Inc.,Foster City,CA). The final sequences of three exons encoding Ubl domain were inspected on the Chromas version 2.40 then transported to BLAST for their alignment with reference sequence obtained from ENSEMBLE Genome Browser with Gene ID "ENSG00000185345".

Results

From thirty patients, 3 patients (10%) have been found to carry a novel mutation in exon 2 of *parkin* gene. This mutation was a substitution of C → T at position 85 of exon 2 and 284415 in parkin gene.

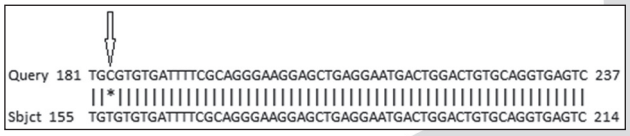

Figure 4. SNP found in Ubl domain
Query = Wild-type Sequence,
Subject = Mutated Sequence

As already mentioned that exon 2 encodes Ubl domain by combining with exon1 and 3. So, the mutation found was also involved in the amino acid substitution R→C which can cause structural change of Ubl domain.

Although there are no changes observed in the predicted Secondary and Tertiary Structure of Ubl domain it can be said that this mutation in the wild-type Ubl domain can cause conformational change in the structure which will result in the impaired proteasomal binding of Parkin protein. It will decrease the degradation of denatured proteins.

Discussion

Parkinson's disease (PD) is a frequent neurodegenerative disorder with prevalence near to 2% after age 65(Abbas, Lücking et al. 1999). Many studies have reported that males are normally 1.5 times more affected than females; the probable reason could be the neuroprotective effect of Estrogen hormone. But still researches are going on to confirm this hypothesis (Wooten, Currie et al. 2004; Simunovic, Yi et al. 2010; Al Sweidi, Sanchez et al. 2012). Numerous researches have been conducted to know mechanism that how point mutations associated with PD, affects its function. Many mutations have been discovered which causes PD. It is observed that point mutations are more common as compared to homozygous exon deletions. According to the reported literature, single amino acid substitutions rarely affect the function of Parkin protein; these substitutions can only decrease the stability of Parkin protein or may cause formation of aggregates (Abbas, Lücking et al. 1999).

In *parkin* gene, exon 2 is considered as one of the hotspot for point mutations resulting in PD. Mutations present in Ubl domain effect the stability of Parkin protein. Most frequent point deletion mutation 255A present in exon 2 affect the Asn52 amino acid and produce a truncated protein by inserting stop codon at position 81. Periquet *et al*. has also reported heterozygous duplication of exon 2 and 3. Heterozygous deletion of exon 2 is also reported in French patient. Duplication of exon 2 and exons 2–4 would predict a frameshift and a stop codon at position 98 and 226, respectively (Periquet, Latouche et al. 2003).

Table 3. Codon changed in Ubl domain

Sr.No.	Chromosomal Position	Wild-type Nucleotide	Mutated Nucleotide	Wild-type codon	Mutated codon	Remarks (Mutation type)
1	162864407	C	T	CGT	TGT	Transition

Table 4. Amino acid changed in Ubl domain

Sr. No.	Chromosomal Position	Position in Ubl domain	Reference Amino acid	Changed Amino acid	Remarks (Mutation)
1	162864407	42	Ariginine	Cysteine	Non-Synonymous

R42P substitution in Ubl domain leads to the conformational changes in the Rpn10 binding site, which results in impaired proteasomal binding of Parkin protein (Sakata, Yamaguchi et al. 2003). Some other missense mutations R33Q, K48A and V56E were found of decreasing stability of Parkin protein in cytosol when compared with wild-type Parkin protein (Hoenicka, Vidal et al. 2002; Dev, van der Putten et al. 2003; Oliveira, Scott et al. 2003). In exon 2 and 3, Abbas *et al*. (Abbas, Lücking et al. 1999) has identified 255-256delAG and 321-322insGT frameshift mutation, it also produces truncated protein by inserting stop codon at position 37 and 81, respectively

In present study, those patients were selected for mutational analysis of *parkin* that have Juvenile or Early-onset Parkinsonism. About 10% (3/30) have shown mutation in their exon 2, which results in the amino acid substitution at position 42 in Ubl domain. Previously, substitution of Arginine→ Proline has been reported, with results in the impaired proteasomal binding of Parkin protein (Sakata, Yamaguchi et al. 2003). But substitution of Arginine → Cysteine at the same position has not been reported. So, to get insight into its effect on the protein biochemical nature of both Arginine and Cysteine should be discussed.

Arginine is a polar amino acid with guanidino group which has positive charge on it. As a cation, it plays a role in maintaining the overall charge balance of a protein Cysteine which is a polar uncharged sulfur containing amino acid. Cysteine plays an important role in stabilizing extracellular proteins. Because of sulfur, Cysteine can form disulfide bond by oxidation which results in the formation of dimer. Disulfide bond stabilizes the folded form of protein. Internal environment of cell is strongly reducing. So, it inhibits the addition of oxygen to Cysteine and prevents formation of disulfide bond.

Conclusion

In conclusion, it can be said that this novel substitution can be one of the cause of EOPD in Pakistani patients. Because change of amino acid in Ubl domain at position 42, which is the critical site for the binding of Rpn10 subunit of 26S proteasome; will not result in degradation of misfolded and defected protein in the cytosol. As a consequence, these defected proteins will start forming aggregates within the cell which will eventually decrease cell's function and they will start dying.

Acknowledgments

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The effect of a 12-week physical exercise program in adults on satisfaction with life, self-esteem, healthy lifestyle behaviors and perceived social support

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Abstract

The aim of this study was to examine the effect of a 12-week physical exercise program in adults on satisfaction with life, healthy lifestyle behaviors, perceived social support and self-esteem. The sampling of the study was composed of a total of 84 volunteers, with 42 individuals who were members at different healthy life centers in Izmir Province. The "Satisfaction with Life Scale", "Multi-dimensional Perceived Social Support Scale", "Self-esteem Scale" and "Healthy Lifestyle Behaviors Scale" were used for obtaining the data. First of all, the descriptive statistics were applied in the statistical analysis and evaluation of the data and subsequently, the "Analysis of Variance (ANOVA)", "unpaired t test" and "paired t test" tests were implemented. The main findings of the present study showed that an interaction effect for group and time existed in self-esteem, physical activity, friend and special person ($p<.05$). When the analyses made for the concept of perceived social support were examined in the study, while a statistically significant difference was observed in a positive direction in the pre-test and final test point averages for the sub-dimensions of "friend" and "special person" of the experimental group, it was observed that there was no difference at all in the "family" sub-dimension. In conclusion, it is thought that participating in regular physical activity is effective in preserving the bodily functions of individuals and in being psychosocially healthy individuals.

Key words: Satisfaction with life, self-esteem, healthy life style behavior, perceived social support

Introduction

It is known that physical exercise programs made based on conscious, regular and scientific foundations plays a significant role in individuals being healthy throughout their lives as well as engaging in social support in societal life, because all kinds of physical exercise has the individual get together with other persons and groups. On this point, it is also known that physical exercise is a societal phenomenon, which is under the influence of the fact of the social surroundings of people. Individuals, along with participating in sports activities, are decreasing the intensive stress created by city life and the problems in daily life and are receiving social support in response to situations that could pave the way for spiritual isolation (1, 2, 3). With a general perspective, social support is known as sociopsychological assistance, which is provided to the individual by an environment in which there are communications with family, friends and neighbors and within work life. This concept, which explains the basic social requirements of individuals, such as love, attachment, self-esteem and belonging to a group, is examined in the three basic dimensions of receiving support from "family", "friends" and "special persons" (4). Individuals who are healthy both physiologically and psychologically are successful in interpersonal support and feel self-confidence, which also strengthens their egos (5). It is known that in parallel with the feeling of "me", self-respect develops and persons have a number of beliefs and images about themselves. That is, self-esteem is a situation of liking, which is created from the approval of the concept of ego reached at the conclusion of self-evaluation by the person (6). Individuals who have high self-esteem, who have more confidence in themselves are individuals who can be effective in solving problems encountered and who can be influenced in a positive direction from the psychological and social aspects of life (7). The relationship of physical exercise with self-esteem and level of social support has been included in many studies. It has been observed that these

phenomena can be decreased or increased connected to many different variables, such as physical exercise (8, 9). The compilation study made by Fox (10) from 79 studies, stated that physical exercise affected mental health and esteem in a positive direction as a contribution to field and experimental applications. When the entire life of the individual is taken into consideration, it is thought that the fact that self-esteem is high, social support is sufficient and physical exercise is regular, raises the psychosocial health of the individual (11). We are confronted with an important component along with the fact that mental, physical and social health is influenced by each other: the attitudes for a healthy lifestyle of individuals. Healthy lifestyle behaviors are defined as individuals controlling their health habits and organizing their daily activities by selecting the behaviors in accordance with their own health status (12). It is known that the concepts of self-actualization, health responsibility, physical activity, nutrition, interpersonal support and stress management, which determine the levels of behavior and preserve the health of the individual, are the indicators of a healthy lifestyle (13, 14, 15). Studies are conspicuous, which set forth that different variables, such as socioeconomic status, family and surroundings experienced, educational status and gender, affect healthy lifestyle behaviors (16, 17). In a study made by Ulla Diezi and Peres-Fortis (18) on university students in Mexico they stated that sociodemographic attributes, such as gender and educational level of mother, had different influences on healthy lifestyle behaviors. Recently, "satisfaction with life" is one of the subjects that have been examined together with the concepts of healthy and high quality of life, psychological well-being, positive social relations, etc. The positive and negative feelings obtained as a result of comparing happiness, the expectations of individuals (what they want) and what they have, is defined with the name known as satisfaction with life (19, 20). There are studies that explain the relationship between many concepts, such as satisfaction with life with depression, self-esteem, frequency of illness and physical health (21, 22). In this context, it is known that the lives of persons are negatively affected by a deficiency of attitudes and behaviors related to health and many different problems stemming from an inactive life. This study was made with the objective of examining the effect of a 12-week physical exercise program on satisfaction with life, healthy lifestyle behaviors, perceived social support and self-esteem for determining whether or not the quality of the life of the individual increased or decreased.

Methods

Participants

The experimental group of the study was composed of 42 individuals of whom 20 (47.6%) were females and of whom 22 (52.4%) were males above 18 years of age and who were members of Healthy Life Centers at Izmir Province. The control group of the study was composed of 42 individuals of whom 25 (59.5%) were females and of whom 17 (40.5%) were males above 18 years of age who led a sedentary life at Izmir Province. Selecting them with the random method provided the voluntary participation of the sampling group composed of a total of 84 participants. The average age of the experimental group was determined to be 27.50±11.31 and the average age of the control group was determined to be 21.00±8.20.

Measures

Satisfaction with Life Scale: This scale was developed by Diener and et.al. (23) and adapted into Turkish by Köker (24). The scale consists of 5 items. The scale is designed in seven evaluation steps (1 = strongly disagree ve 7 =strongly agree). Total score that can be obtained from the scales could be 5 at minimum and 35 at maximum. Results in the study of Köker (24) showed test-retest reliability coefficient of .85 (25).

Healthy Life Style Behavior Scale II: This questionnaire was developed by Walker et al. (26). Later, the scale was revised again by Walker et al. in 1996 (13, 15). This scale was adapted into Turkish by Bahar et al. (15). The scale consists of 52 items and six subscales. Health Responsibility (9 item), Physical Activity (9 item), Nutrition (9 item), Spiritual Growth (9 item), Interpersonal Relations (9 item) and Stress Management (8 item). The scale is designed in four evaluation steps (1= never, 4=regularly). General scores of the scale indicated the score of healthy life style behaviors. Total score that can be obtained from the scales could be 52 at minimum and 208 at maximum.

The Cronbach alfa internal consistencies of scale is .92. Alpha values of the scale's subscales vary between .64 and .80.

Multidimensional Scale of Perceived Social Support: This scale was developed by Zimet et al. (27) and adapted into Turkish by Eker et al. (4). The scale consists of 12 items and three subscales. Family (4 item), Friend (4 item) and Special Person (4 item). Personal responded to a seven-point Likert-type (1 = Very Strongly Disagree; 7 = Very Strongly Agree) scale. Total is sum of all 12 items, possible range for total is 7-84. High scores and low scores obtained from this subscale represent high and low level perceived social support, respectively. Total score that can be obtained from each subscale could be 4 at minimum and 28 at maximum. The Cronbach alfa internal consistencies among the subscales of the scale are in the range of [.80-.95] (4).

Self-Esteem Scale: This scale was developed by Morris Rosenberg and adapted into Turkish by Çuhadaroğlu (28). Self-esteem scale is designed for individuals to make a general evaluation on their self-esteem. The scale consists of 63 items and 12 subscales. However, only the 10-item self esteem part of the adapted scale was used in this study. The scale of each item has a four-step evaluation. High and low scores obtained from the scale represent high and low level of self-esteem, respectively. Individuals with low level of self-esteem have also low level of self-confidence (29). Results in the study of Çuhadaroğlu (28) showed an Internal consistency reliability coefficient of .71 and test-retest reliability coefficient of .75 (28).

Procedures

A total of 42 individuals above 18 years of age who participated in an exercise program for the first time formed the experimental group of this study. The persons in this random group implemented fitness programs for 12 weeks that were organized for them individually and that were continuously monitored. The other group, which did not participate in any exercise program, formed the control group. Both groups implemented pre-tests and final tests for the Healthy Lifestyle Behaviors, Multi-dimensional Perceived Social Support and Self-esteem scales. The scales were implemented face-to-face by researchers accompanied by the required explanations to individuals by going to the healthy life centers determined randomly at Izmir Province. The results were limited to the responses expressed by the participants. It was assumed that those who participated in the study responded with sincerity to the scales.

Statistical Analysis

The data of this study was realized by using the SPSS software (version 15). First of all, the descriptive statistics for the changes taken as the basis in the study were made during the analysis and evaluation of the statistical data. Subsequently, the main effects on the group and time data and whether or not there was an interaction between the group and time was evaluated with the 2 × 2 (group × time) Generalized Linear Model Two-Factor Mixed Model Analysis of Variance (GLM TFMM ANOVA) test. The differences in the variables of the inter-group performance were determined with the unpaired t test, whereas, the differences in the intra-group pre-test and final test were determined with the paired t test. The magnitudes of effect for the related tests were reported according to Cohen's classification (0.2=small, 0.5=medium and 0.8= large effect size). A level of significance of $p \leq 0.05$ was accepted in all of the statistical evaluations. These values were visualized with scatter plots for giving an idea about the pre-test and final test results obtained in different tests by the participants.

Results

The main findings of the present study showed that an interaction effect for group and time existed in Self-esteem, Physical activity, Friend and Special Friend ($p<.05$). In other words, exercises showed positive effects on individuals in terms of above mentioned characteristics. Results were demonstrated in figure 1-6.

No significant main effect for both group and time was detected in satisfaction with life ($p=0.56$, and $p= 0.15$) and self–esteem ($p=0.42$ and $p=0.40$) scales. A significant interaction effect was found between "group and time" for self-esteem scale ($p=0.03$) indicating that while self-esteem level increased by 4.4% in exercises group, a decrease was detected in control group by 1.5%.

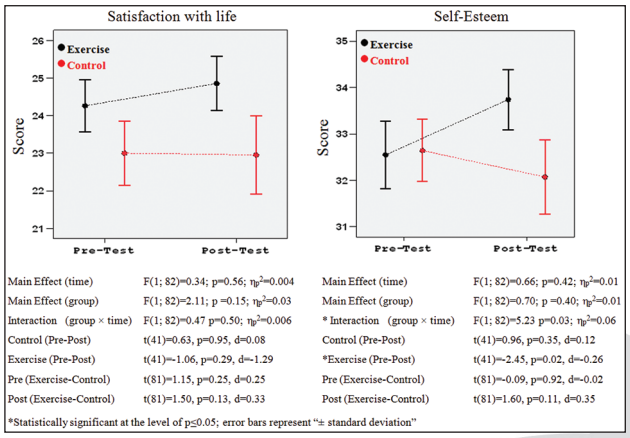

Figure 1. Effects of exercise on satisfaction with life and self-esteem scores

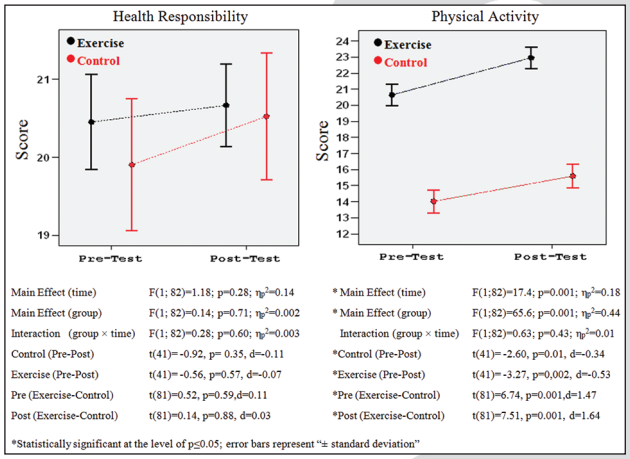

Figure 2. Effects of exercise on health responsibility and physical activity scores

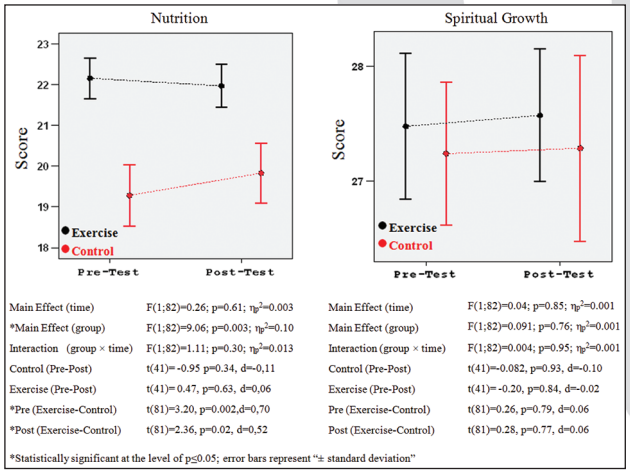

Figure 3. Effects of exercise on nutrition and spiritual growth scores

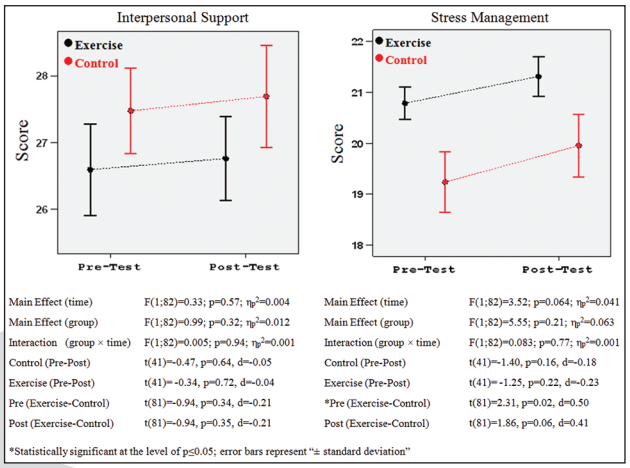

Figure 4. Effects of exercise on interpersonal support and stress management scores

No significant main effect for both group and time was detected in health responsibility (p= 0.71 and p= 0.28), nutrition (p= 0.003 and p= 0.61), spiritual growth (p=0.76 and p= 0.85), Interpersonal support(p= 0.32 and p=0.57) and stress management(p= 0.21 and p=0.064) . A significant interaction effect was found between "group and time" for physical activity subscale (p=0.001) indicating that while physical activity level increased by 15.2% in control group, a decrease was detected in exercises group by 14.3%.

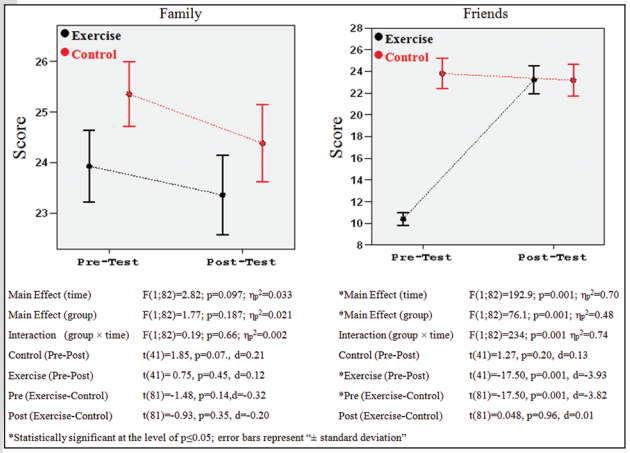

Figure 5. Effects of exercise on family and friends scores

No significant main effect for both group and time was detected in family (p= 0.18 and p= 0.09),. A significant interaction effect was found between "group and time" for friends and special person subscales (p=0.001) indicating that while friends and special person subscale level, respectively, increased by 132.6% and 108.2 % in exer-

cises group, control group showed a decrease of 14.3% and 27.5% respectively.

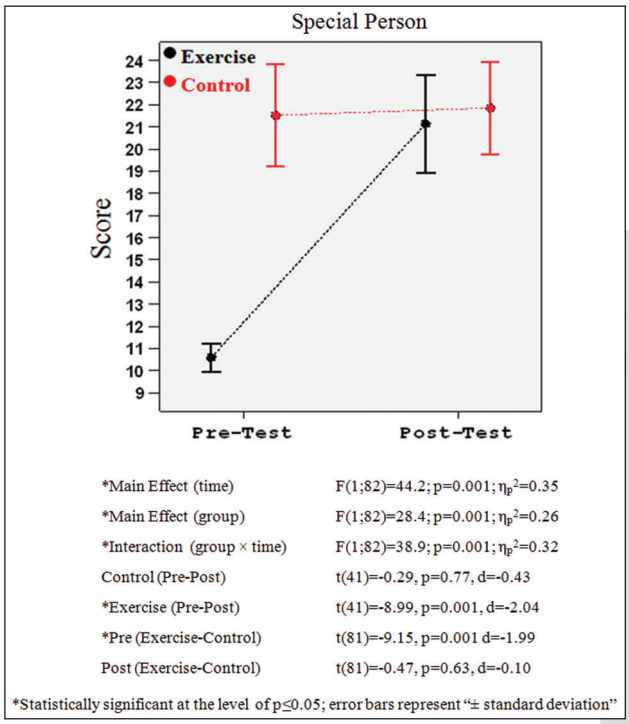

Figure 6. Effects of exercise on special friends scores

Discussions

Physical activity is generally thought to be a strategy realized on individuals for forming healthy lifestyle behaviors and for increasing the quality of life. Satisfaction with life, which is an important factor in raising the quality of life, is accepted in the present-day as an important indicator of physical and mental health (30). In this context, the effect of a 12-week physical exercise program implemented in adults on satisfaction with life, self-esteem, healthy lifestyle behaviors and perceived social support was examined in this study.

At the conclusion of the analyses made, it was observed that there was not a statistically significant change in satisfaction with life after the 12-week physical exercise program of the individuals in the control and experimental groups. In the study made by Pori et al. (31) on adult recreative runners and runners who participated in the Ljubljana marathon, they reached the conclusion that the recreative runners were more pleased with their lives. At the same time, Grant et al. (32) stated that individuals received greater pleasure from activities formed from simple basic components in receiving satisfaction with their lives. Thogersen et al. (33) stated that participating in physical activities a minimum of once/week was the cause of a high satisfaction with life. Whereas, Blace (34) stated that participating in more physical activities could play a role in individuals having better health conditions and in having higher satisfaction with live. In parallel with our study, in the study by Blacklock et al. (35) they stated that there was not a significant relationship between quality of life with regular walking (slow, medium, fast) and physical activity. In this context, the fact that there are many factors, which influence the satisfaction with life of the individual, such as gender, age, economic status, social relations, environmental factors, etc. it can be thought that the effect created by feeling oneself physically well could be made meaningless.

When the analyses made with the concept of self-esteem were examined in the study, while a statistically significant increase ($p<.05$) in the point averages of the experimental group was determined after the 12-week physical exercise program, it was observed that there was not a statistically significant change in the level of self-esteem of the control group. In parallel with this study, in the study made by McAuley et al. (36) on middle-aged individuals, they stated that there was an increase in a positive direction in the self-esteems of the individuals at the end of a 20-week exercise program. Also, in the study made by McAuley, Blismer, Katula, Duncan and Mihalko (37), they observed that there was a rise in the levels of self-esteem of the individuals with an average age of 65 at the end of an exercise program made in 2 periods for 12 months. In the study made by Spence et al. (38), they determined that exercise made a positive effect on self-esteem. There are many studies, which state that individuals who participate in daily physical activities and sports have higher self-esteem compared to individuals who do not engage in sports (39, 40). These studies made support our study. As it can be observed, it has been stated that physical exercise in individuals has effects in a positive direction on the concepts related to self-esteem, such as feeling self-confident and being able to establish more balanced communications and social relations, etc.

In the study, a statistically significant difference was not found between the pre-test and final test average points for the sub-dimensions of Health Responsibility, Nutrition, Self-actualization, Interpersonal Support and Stress Management for the group participating in physical activity and the group that led a sedentary life. In the study made by Song and Lee for examining the effect on motivation and healthy lifestyle behaviors of a 12-week exercise program for cardiac patients, positive changes were shown in the 12-week period within both groups who exercised and did not exercise for general lifestyles, but they stated that there were no significant differences (9). In contrast to this, in the study made by Türkmen et al. (41) on 2,218 university students, they observed that there was a positive relationship among all of the sub-dimensions for the healthy lifestyle behaviors with different physical activity levels. They also stated that regular physical activity could create many positive effects physically, mentally, socially and psychologically on the life of university students. In the study realized by Ay et al. (42) on 1,007 university students, they stated that the students attending the Department of Physical Education and Sports had higher averages for the sub-dimensions of healthy lifestyle behaviors, health responsibility, nutrition, interpersonal support and stress management and a higher average in total points compared to the students attending other departments. When the physical activity sub-dimension data in our study were examined, a significant difference in favor of the experimental group was found between the pre-test and final test average points. Çoban et al. (43) stated that students who participate in regular physical activities had internalized the healthy lifestyle behaviors more compared to students who led a sedentary life. It takes a definite period of time and efforts for individuals to acquire a behavior and for them to transform this behavior into a lifestyle. According to the results of our study, a change was not experienced in the concepts of "Health Responsibility", "Nutrition", "Self-actualization", "Interpersonal Support" and "Stress Management", which belong to the healthy lifestyle. It can be thought that this result stemmed from the habits previously acquired by individuals. It is thought that if suitable strategies and periods unique to the individual are provided, then positive changes could be experienced.

When the analyses related to the perceived social support concept were examined in the study, while a statistically significant difference in a positive direction was observed between the pre-test and final test point averages for the "friend" and "special person" sub-dimensions of the experimental group, no difference at all was observed in the "family" sub-dimension. Whereas, it was determined that no significant difference at all was formed for the results related to the control group in all of the sub-dimensions. In the study made by Darlow and Xu (44), it was determined that the exercise habits of individuals were related to the perceived exercise habits of the special persons and friends in their lives. On this point, it is thought that friends or special persons could be a model in acquiring an exercise behavior to the individual. In the study realized by Sasidharan et al. (45) in individuals past middle-age, they observed that the participation in physical activities during free time were composed of different effects of the perceived social support sub-dimensions upon the health perceptions and satisfaction with life levels. At the same time, especially the importance was stressed of the "friend" sub-dimension in perceived social support among individuals past middle age who participated in free time and recreational physical activities. In contrast to this, in the study made by Yılmazel (46), it was stated that there was no significant difference related to the sub-dimensions of perceived social support of the university students who participated or did not participate in physical activities. There are many factors, such as sociocultural area, environmental status, heredity, etc. included in the participation of individuals in physical activities. At the conclusion of our study, it can be stated that along with participants engaging in regular physical activities, it paves the way for them establishing special relations with different persons in the environment in which they are found and for them acquiring friends. It can be thought that individuals who participate in physical exercise programs evaluate this situation as an environment where friends and special relations are developed and that it is a cultural attribute of the Turkish society.

In conclusion, it can be stated that participation in regular physical activities preserves the bodily functions of individuals and makes them psycho-

socially healthy individuals, in other words, it could create significant differences for increase in the quality of life.

Proposals

It is proposed that the required environment and conditions are created for providing for participation in physical activity from an early age by taking into consideration the difficulties of changing a habit acquired by individuals or of acquiring new habits at advanced ages.

It is proposed that many attractive activities aimed at increasing self-esteem by providing for the participation in physical activities of individuals in our society should be organized in accordance with the objective together with the mass communications media.

It is proposed that it would be meaningful to provide education to individuals for healthy lifestyle behaviors along with physical activities and to make them aware of all its dimensions.

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Access to medicines within the state health insurance program for pension age population in Georgia (country)

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Abstract

Objectives. Pension age population belong to the largest risk group within insurance sphere. Healthcare for pensioners often represents catastrophic expenses and has become the major reason for their impoverishment. This paper examines financial access to medicines and the satisfaction with the insurance policy within the Georgia State Health Insurance Program for pension age population.

Methodology. Using a randomized selection method, 500 pension age persons were interviewed by means of direct questioning.

Results: Reduction of catastrophic healthcare expenses should become the chief aim of the government health policy. It applies to the high risk population, those under poverty line and pension age population, as well as those with chronic diseases. Accordingly, based on the findings, it is reasonable to expand the insurance program for the pension population and also provide more reasonable coverage of medication expenses.

Key words: Georgia; Health insurance; Catastrophic out-of-pocket payments; Financial access to healthcare; Pension age population.

Introduction

Georgia is a country in the Caucasus, located at the crossroads of Eastern Europe and Asia, with a population of almost five million. The capital is Tbilisi with a population of about 1.2 million. Georgia is a relatively new country, gaining independence after the collapse of the Soviet Union in 1991. The country went from almost complete public ownership of healthcare services to economic collapse due to civil wars, corruption, rapid marketization and hyperinflation (Chanturidze, Ugulava, Durán, Ensor, & Richardson, 2009). Georgia's population decreased by nearly a fifth, the healthcare system was severely damaged, and the standard of living fell sharply. According to Gamkrelidze et al., there was a drastic reduction in public funding to run a system almost totally dependent on public resources. Between 1990 and 1994, "real per capita public expenditures on health declined from about US $13.00 to less than a dollar in 1994" (Gamkrelidze, Atun, Gotsadze, & MacLehose, 2002. p. 3).

Since the early nineties, a variety of reforms have taken place with the emphasis on improving equity, accessibility and affordability of health services (Rukhadze, 2013). While there have been attempts to increase the portion of state funding, direct out-of-pocket payments comprise most health expenditure in Georgia. A major problem is that government sources cover only 18.4% of health expenditure while the out-of-pocket payment (70.9%) represents the main source of funding in Georgia (Chanturidze et al., 2009). This payment significantly reduces access to health services for the majority of people, especially the access to pharmaceuticals. The World Bank claims that the illness of a family member is a major source of poverty in Georgia (World Bank, 1999).

Research shows that families in Georgia spend about 60% of all their healthcare expenses on medicines, this being one of the highest rates across the world (Ministry of Labour Health and Social Affairs of Georgia, 2013). There is some evidence that the high costs of medicines are due to a combination of factors, including insufficient usage/administering of generic medicines, lack or inadequate utilization of prescription mechanisms, insufficient financial limit for medicines within the state healthcare programs, and aggressive marketing by the pharmaceutical industry (Ministry of Labour Health and Social Affairs of Georgia, 2013).

The government has been trying to decrease the out-of-pocket spending via different strategi-

es, including universal health coverage (UHC). Since February 2013, the Universal Health State Program came into force, targeting two million of uninsured population and providing a basic package for primary health care and emergency services (World Health Organization, May 2013).

Pension age population form the largest at risk group within the insurance sphere. Healthcare for pensioners often represents catastrophic expenses and has become the major reason for their impoverishment. The most rapidly increasing and unaffordable share of healthcare expenses falls upon medicines. This paper examines financial access to medicines and the satisfaction with the insurance policy within the Georgia State Health Insurance Program for pension age population.

Methodology

The research covered Tbilisi population involved in the State Health Insurance Program for the pension age population. Using a randomized selection method, 500 pension age persons were interviewed by means of direct questioning. The semi-structured interview was designed to examine the types of the medical services paid for out-of-pockets, access to prescribed medicines after consultation with family or policlinic doctor, and satisfaction with the provided medical services. The study was implemented in the May – April period of 2013.

Results

According to the Social Service Agency, there are 45,455 pension age recipients of medical insurance in Tbilisi with a total of 673,183 pension age population across the country (National Statistics Service of Georgia, 2013). Out of the 500 persons (62.4% women and 37.6% men) interviewed. The sample population covered all the districts of Tbilisi. The interviews took place in clinics. The largest segment of the interviewed population was between the ages of 71-75 (35.2%). The second largest group was aged from 66 to 70 (27.2%), followed by pensioners aged 75 and over (24%). The group between the ages of 60-65 was represented by only 68 persons (13.6%).

The family income of 38.2% of those interviewed exceeded 300 GEL; 27% people had income between 200-300 GEL, 32.6% between 100-200 GEL, and 2.2% below 100 GEL [100 GEL is about 58 USD]. The results indicate that only 2.4% pensioners lived alone; 47.6% respondents lived in households of four and more, 23.6% in households of three, and 26.4% in households consisting of two people. When asked about the major problems, the majority of the interviewed identified buying medicines (36.6%), followed by unemployment (31.6%), inaccessibility of relevant medical services (22.8%), and buying food (8.2%). Other problems mentioned included small dwelling space, heavy taxes and poor living conditions.

The results showed that the majority of respondents purchased medications once or twice a month (55.2%) and 34.4% bought medications every week. Only 2.8% buy medicines once in six months while 7.2% buy medications only rarely. In terms of expenses, 13.2% spent between 10 and 30 GEL on medicines out of their pockets (1 USD is about 1.73 GEL); 22.8% spent between 31 and 50 GEL; 43.6% between 51 and 80 GEL; and 20.4 % spent over 80 GEL. The findings also show that in addition to medicines, 58% of the respondents had to provide out-of-pocket payments for certain types of medical services covered by the state insurance program. A large number of the respondents (42%) did not pay for any additional medical service.

When investigating the awareness about the compensation of the cost of the medicines, the majority of those interviewed did not know that insurance companies compensate medicine expenses within the annual insurance policy limit in the amount of 100 GEL, with 50% co-payment from users. Only 32.8% of the respondents were aware of that information. The data also showed that the affordability of medicines was low. A large proportion of the respondents (38.4%) were not able to buy all medicines prescribed by family or policlinic doctors while 32.4% individuals could not purchase any medicine due to high price. Only 28% pensioners were able to buy all the prescribed medicines. Four respondents (0.8%) did not visit a doctor at all.

In terms of satisfaction with the services provided by the insurance policy, 2.8% pensioners expressed their total satisfaction and 18.4% general satisfaction. Partial satisfaction was experienced by 61.2% respondents, while 17.6% pensioners were not satisfied at all.

Discussion

While the Georgia State Health Insurance Program for pension age population includes some compensation for medicines, medical insurance does not provide guarantee for financial accessibility to medical service. The results show that out-of-pocket payment is the most common health financing mechanism in Georgia. The cost of healthcare for pensioners often represents catastrophic expenses and becomes the major reason of their impoverishment. Based on the results, medication expenses represent one of the most important components of the medical service for the insured pensioner population. Over 55% of the pensioners have to buy medications at least once a month and 34% purchase them at least once or twice a week. Most of the interviewed insured population believes that buying medicines, combined with low financial accessibility to relevant medical services, is the most significant problem for their families.

The awareness level of the insured persons about medication coverage and payments for medical services provided by the insurance package within certain limit is low (100 GEL - about 57 USD). Major proportion of those interviewed (64%) stated that their out-of–pocket expenses exceeded these limit; 20.4% exceeded that amount by 80 GEL or more. The fact that 32.8% of the insured population cannot afford medications prescribed by their doctors also points out at low financial accessibility for medicines. The majority of those insured did not know that the insurance company compensates medicine expenses within the policy annual insurance limit. Additionally, 58.8% of those who have to pay for certain types of medical services covered by the state insurance program out of their pockets were not aware about the limits.

Conclusion

Reduction of catastrophic healthcare expenses should become the chief aim of the government health policy. It applies to the high risk population, those under poverty line and pension age population, as well as those with chronic diseases. Accordingly, based on the findings, it is reasonable to expand the insurance program for the pension population and also provide more reasonable coverage of medication expenses.

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A study into the mineral water consumption of individuals

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Abstract

Purpose: The current study was carried out to investigate the effect of educational status and BMI of the young, adult, middle aged and elderly on the mineral water consumption of individuals.

Settings: It is collected data from individual, living in the district of Beypazarı in Ankara, in Turkey.

Design: This was a descriptive study.

Methods and Method: The data collection instrument prepared to determine "the consumption status of mineral water" by the individuals was developed by the researcher and made up of two sub-division. The first part was made up of the questions regarding the consumption status of mineral water by the individuals and second part was comprised of personal questions. In order to apply data collection tools, necessary consents were taken from the supermarket owners in the district. Making a pre-interview with the customers coming for shopping, the researchers collected the data on a voluntary basis.

Results: It was found for the mineral water consumption times for age groups that the mean scores of the young and adult age group was higher in the item of "I consume mineral water after sport", while it was found lower in the middle and over age group. The graduates of secondary education and high school had a higher score for the item "I think pills could be taken with mineral water", while university graduates had lower score. This difference was found statistically significant. For the item of "it would help to lose weight", the scores for underweight, slightly overweight and overweight people were higher compared to those are at their normal weight.

Conclusion: In order that necessary and correct information is told to individuals over mineral water, people should be trained over drinks through media, TVs and public service announcement.

Key words: Mineral water consumption, age, education, weight

1. Introduction

Mineral water is defined as natural water obtained by digging wells from the underground waters conserved geologically and physically or getting from the spring and having a pH value between 2.38 and 4.03[1,2]. Of the minerals necessary to take daily are calcium, magnesium, sodium and chlorine. Also, other minerals that are required for the body but in less amounts are potassium and fluoride and sometimes iodine. Lack of magnesium and the accumulation of fat and calcium in the vessels could lead to arteriosclerosis [3] and therefore to cardiovascular diseases and hypertension [4].

With the calcium mineral in mineral water, it helps to the structure of bones besides recovery at muscle spasms, transmitting the nerve stimulations, iodine change at the cell membrane and the secretion of hormones, digestive enzymes and neurotransmitters [5]. Fluoride in the mineral water will prevent tooth decays and it is necessary for bone and tooth health. Chloride serves for the balance of water and electrolytes and with sodium in digestion [6,7].

While mineral water is defined as natural water, soda is described as the drink obtained by pressing carbon dioxide gas into the water or the drink made of water during production process [1]. Fruit flavored drink is defined as the fizzy cold drink obtained with an addition of various fruit aromas and refined sugar into the mineral water [8].

It is recommended for the diabetics and the patients of type 2 mellitus disease to consume mineral water prepared with artificial sweeteners and fruit aromas; since preference for mineral waters with fruit aroma containing such artificial sweeteners as aspartame and acesulfame K will decrease refined sugar intake [9]. It is also recommended for the consumers to prefer those having lower sodium and higher magnesium and calcium content in their choices [10]. The current study that was

planned depending on the contributions made to the field by related studies with regard to mineral water was carried out to determine the effect of the views of the individuals residing in the district of Beypazarı, Ankara over the status of consuming mineral water, the relation with the diseases mentioned above on the age group of youth, adult, middle age and over and on their educational status and Body Mass Index (MBI).

2. Method

2.1. Participants

The current study was carried out on 598 individuals in total, 328 women and 270 men, living in the district of Beypazarı in Turkey, volunteering to participate in the research. The individuals included in the study were chosen through random sampling method.

Of the individuals, 54.8 % were women and 45.2 % were men and the mean age of those at the age of 19-51 was 34.03. The individuals were grouped as young 34.4 % (19-29), adult 54.2 % (30-40), middle age and over 10.9 % (41-51). As for the education, 30.9 % of the participants were a graduate of a secondary school, 25.4 % were graduated from a high school and 43.6 % were university graduates. It was determined that 3.3% were underweight, 43.5 % were at their normal weight, 42.6 % were slightly overweight and 10.5 % were overweight.

2.2. Materials

The data collection instrument prepared to determine "the consumption status of mineral water" by the individuals was developed by the researcher and made up of two sub-division. The first part was made up of the questions regarding the consumption status of mineral water by the individuals and second part was comprised of personal questions. Each question in the questionnaire is a five point evaluation scale; (1)=never, (2)=rarely, (3)= occasionally, (4)=often and (5)=very often. While scoring, all questions are recoded again code (1=5, 2=4, 3=3, 4=2, 5=1). Body Mass Index(BMI) is the value obtained by dividing the body weight (kg) by the square of height (m^2) (BMI kg/ m^2). It is classified as (BKI< 18.05) Underweight (1), (BKI 18.5–24.9) Normal range (2), (BKI 25.0- 29.9) Overweight (3), (BKI ≥ 30.00) Obesity (4) [11]. Cronbach Alfa reliability coefficient of the instrument is found r=.490. To test validity, factor analysis is done. Compenent matrix coefficients are found between r=.40 and r=.73. KMO measure of sampling adequacy was found .776, and Bartlett's test of sphericity is found significant ($p<0.001$).

2.3. Procedure

In order to apply data collection tools, necessary consents were taken from the supermarket owners in the district. Making a pre-interview with the customers coming for shopping, the researchers collected the data on a voluntary basis. The application of the data collection tools was made by the researcher. The individuals participating in the study were only explained the purpose of the study but no explanation was made concerning the question in the data collection tool.

2.4. Analysis

The demographic information of the individuals was given as frequencies in the analysis. The distribution of mineral water types preferred by the consumers was examined. The time of mineral water consumption was compared through one-way ANOVA in terms of ages. The views regarding the educational status of mineral water consumptions of the individuals were evaluated through one-way ANOVA. Between which groups there was a difference was determined through Post-hoc Tukey test. The views of the individuals regarding the situations they feel after the consumption of mineral water in their body were compared with one-way ANOVA depending on their BMIs. The difference between the groups was tested through Post-hoc Tukey test. The statistical significant level was taken as $p<0.05$.

3. Results

It was found that 46.8 % of the individuals preferred plain mineral water, 35.6 % preferred fruit flavored mineral water and 17.6 % of them preferred both plain and fruit flavored mineral water (Table 1).

Table 1. The distribution of the mineral water types preferred by individuals to consume

Preferred status	n	%
Spring with mineral water	280	46.8
Mineral water with fruit flovered	213	35.6
Mineral water without additional ingrediants	105	17.6
Total	598	100.0

Table 2. The distribution of the views of the individuals over the health problems they had after the consumption of mineral water and fruit flavored mineral water (n=183)

Living health problems	n	%
Over consumed mineral water		
Gastrit	63	34.4
Blood pressure	35	19.1
Kardio vascular	25	13.7
Consumed mineral water with fruit flovered		
Obesity	40	21.9
Tip 2 diyabetics mellitus	20	10.9
Total	183	100.0

Table 3. The results of One-Way ANOVA of the mineral water consumption time of the individuals in terms of age groups (n=598)

When consuming mineral water	Age groups	n	Mean	SD	F	p	Tukey
While an empty stomach in the morning	Young	206	3.71	1.84	.22	.797	-
	Adult	327	3.62	1.88			
	Midle age and over	65	3.73	1.80			
After meals	Young	206	4.50	1.08	.99	.372	-
	Adult	327	4.41	1.18			
	Midle age and over	65	4.61	1.11			
When thirsty	Young	206	4.03	1.41	3.73	.024	(1-2)*
	Adult	327	4.35	1.27			
	Midle age and over	65	4.27	1.26			
When it comes to in my mind	Young	206	4.11	1.44	12.13	.000	(1-2)* (1-3)*
	Adult	327	3.51	1.74			
	Midle age and over	65	3.13	1.80			
While dieting on	Young	206	4.14	1.48	69.16	.000	(1-2)*
	Adult	327	2.63	1.76			(1-3)*
	Midle age and over	65	2.00	1.40			(2-3)*
When under pressure	Young	206	3.26	1.82	12.35	.000	(1-2)*
	Adult	327	2.75	1.80			(1-3)*
	Midle age and over	65	2.04	1.69			(2-3)*
After doing exercise	Young	206	3.78	1.65	37.99	.000	(1-2)*
	Adult	327	2.55	1.81			(1-3)*
	Midle age and over	65	2.16	1.71			(2-1)*
Before going to bed	Young	206	1.96	1.56	2.85	.058	-
	Adult	327	2.16	1.49			
	Midle age and over	65	2.46	1.64			
After smoke	Young	206	1.73	1.41	.91	.401	-
	Adult	327	1.59	1.32			
	Midle age and over	65	1.53	1.29			

In particular, 34.4 % of the individuals expressing that consuming too much mineral water causes a problem pointed out that their gastritis problem increased, 19.1 % expressed

their blood pressure problem increased. Regarding the consumption of fruit flavored mineral water, 21.9 % of them pointed out that they had obesity problem and 10.9 % had type 2 diabetics mellitus (Table, 2).

Another important finding was that the difference between the mean score between the item of "I consume mineral water during my diet during the time of mineral water consumption" was statistically significant ($F_{(1.76, 1.40)}$=69.16, p<0.05). In the comparisons made through Tukey HSD Post-Hoc test applied to determine the source of the difference between groups, it was found that while the mean score of the young age group (M=4.14) was higher, the mean scores of middle age and over age group were (M=2.63) and (M=2.00) were lower, respectively. This difference was found statistically significant (p<0.05).

Another important finding is that the difference between the mean score of the item "I become stressful" while consuming mineral water was found statistically significant ($F_{(1.82, 1.69)}$=12.35, p<0.05). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the young and adult age group were higher (M=3.26), (M=2.75) respectively, while those of the middle and over age group (M=2.04) was found lower. This difference was found statistically significant (p<0.05).

Another significant finding is that the difference between the mean score of the item I consume mineral "after sport" during the consumption times was found statistically significant ($F_{(1.81, 1.65)}$=37.99, p<0.05). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the young and adult age group were higher (*M*=3.78), (*M*=2.55) respectively, while those of the middle and over age group (*M=2*.16) was found lower. This difference was found statistically significant (p<0.05, Table 3).

A significant statistical difference was found between the mean scores for the item of "I believe mineral water is beneficial for the body" in their views over mineral water consumption in terms of the educational status of the individuals. ($F_{(1.71, 1.28)}$=13.76, p<0.05). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the university and high school graduates were higher

Table 4. One-Way ANOVA results of the views of individuals over mineral water consumption with regard to the educational status (n:598)

Views over mineral water consumption	Educational Status	n	Mean	SD	F	p	Tukey
I believe mineral water is beneficial for the body	1	185	1.83	1.28	13.76	.000	(2-3)* (2-4)*
	2	152	2.38	1.49			
	3	261	2.60	1.71			
I believe there is a difference between mineral water and soda	1	185	2.05	.72	47.99	.000	(2-3)* (2-4)*
	2	152	2.75	1.27			
	3	261	3.09	1.22			
I think I have an idea about the components of mineral water	1 2 3	185 152 261	2.14 2.45 3.30	1.14 1.35 1.75	36.52	.000	(2-4)* (3-4) *
I believe mineral water is the alternative of still water	1	185	2.05	1.09	91.48	.000	(2-3)* (2-4)* (3-4)*
	2	152	2.75	1.61			
	3	261	3.76	1.32			
I believe that pills can be taken with mineral water	1	185	2.36	1.28	40.60	.000	(2-3)* (2-4)* (3-4)*
	2	152	1.96	1.34			
	3	261	1.38	.89			

p<0.05 1: Secondary school graduate 2: High school graduate, 3: University graduate

(M=2.60), (M=2.38) respectively, compared to those of secondary school graduates (M=1.83). This difference was found statistically significant ($p<0.05$). Another important finding of the study was that the mean scores for the item of "I believe there is a difference between mineral water and soda" were found significantly different ($F_{(1.27, .72)}=47.99$, $p<0.05$). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the university and high school graduates were higher (M=3.09), (M=2.75) respectively, while those of the secondary education graduates (M=2.05) were found lower. This difference was found statistically significant ($p<0.05$).

Another striking finding of the study was that a significant difference was found between the scores for the item of "I believe that pills can be taken with mineral water". ($F_{(1.34, .89)}=40.60$, $p<0.05$). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the secondary school and high school graduates were higher (M=2.36), (M=1.96) respectively, while those of the university graduates (M=1.38) were found lower. This difference was found statistically significant ($p<0.05$, Table 4).

A significant statistical difference was found between the mean scores for the item of "It meets the liquid need" in their views over the cases felt in the body after the consumption of mineral water. ($F_{(1.53,.29)}=4.51$, $p<0.05$). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the underweight and normal weight individuals were higher (M=4.30), (M=3.45) respectively, while those of the slightly overweight and overweight individuals were lower respectively (M=3.14),

Table 5. One-Way ANOVA results of the views of individuals over what they feel in their bodies after mineral water consumption

The cases felt in the body after the consumption of mineral water	BMI (kg/m^2)	n	Mean	SD	F	p	Tukey
Makes my stomach relaxed	1	20	4.80	.52			
	2	260	4.74	.82	1.27	.283	--
	3	255	4.68	.89			
	4	63	4.90	.34			
Meets my liquid need	1	20	4.30	.29			
	2	260	3.45	1.53	4.51	.004	(1-3)*
	3	255	3.14	1.52			(1-4)*
	4	63	3.26	1.53			
Makes my skin look bright	1	20	3.65	1.72			
	2	260	3.46	1.74	1.46	.223	--
	3	255	3.21	1.85			
	4	63	3.06	1.90			
Helps the digestion system work better	1	20	3.60	1.72			
	2	260	2.95	1.77	1.80	.146	--
	3	255	3.24	1.73			
	4	63	3.23	1.79			
Helps to keep the body weight balanced	1	20	4.85	.36			(1-2)*
	2	260	3.44	1.75	8.89	.000	(1-3)*
	3	255	2.97	1.80			(1-4)*
	4	63	3.43	1.73			(2-3)*
Helps to lose weight	1	20	3.83	1.81			
	2	260	2.60	1.37	5.80	.001	(1-2)*
	3	255	2.65	1.19			(1-3)*
	4	63	2.63	1.23			(1-4)*

(*M=3*.26). This difference was found statistically significant (p<0.05).

The difference between the mean scores was statistically significant different for the item of "It helps to lose weight" in their views over the cases felt in the body after the consumption of mineral water ($F_{(1.81,\ 1.19)}$ =5.80, p<0.05). In the comparisons made with Tukey HSD Post-Hoc test applied to determine the source of the difference between the groups, it was found that the mean scores of the underweight, slightly overweight and overweight individuals were higher (*M*=3.83), (*M*=2.65), (M=2.63) respectively, while those of the normal weight ones were lower (*M=2*.60). This difference was found statistically significant (p<0.05, Table 5).

4. Discusion

It was found in the research that individuals preferred to consume plain, fruit flavoured and both fruit flavoured and plain mineral water. Similar to these findings, it was found by [12] in a study that individuals preferred plain mineral water in the first place and that they did not make a difference for mineral water and fruit flavoured mineral water. The fact that there is a mineral water production in the district of Beypazarı where the research was carried out could lead to their having a habit of consuming mineral water and adopting fruit flavoured mineral water consumption in due course.

Another significant finding of the research was that individuals stated in their views over the chronic health problems they experienced after consuming mineral water that such diseases as gastritis, high blood pressure and coronary heart problems increased. The individuals expressed that they experienced obesity and type 2 diabetes mellitus problem with the consumption of fruit flavoured mineral water. According to [6], mineral waters with high sodium bicarbonate content are not good for people with hypertension risk and that mineral waters with calcium bicarbonate and magnesium bicarbonate content are not good for the individuals with hypertension risk and heart diseases. It was found by ([13,14]that individuals experienced hypertension, atherosclerosis and Type 2 diabetes mellitus had a higher prevalence with low level serum magnesium in diet. However, no cardiovascular and hypertension was found with the low level serum magnesium in blood in another study [4, 15]. In another study, it was stated that overconsumption of fruit flavoured mineral water increased obesity and type 2 diabetes mellitus problem [10]. It was found that the individuals consuming one or more than one bottles of sugar sweetened fruit flavoured a day have a risk of catching type 2 diabetes mellitus twice as high as the ones consuming one bottle or less in a month [16]. It is believed that the health problems individuals experienced after the consumption of more mineral water and fruit flavoured mineral water could trigger their inherent chronic health problems.

Another important finding was that the mean scores of the young age group in the item of consuming mineral water "on diet" were higher compared to those of the adult and middle and over age group. The difference between the groups was found statistically significant. This case could be resulting from the fact that young people pay more attention to their body images compared to adult and middle and over age group. [17] expressed that the idea of being slim is regarded as the indicator of beauty; the view of "the thinner, the more beautiful" could lead to adopting wrong eating attitudes and behaviours.

In the item of consuming more mineral water "in stressful cases", mean scores of the young and adult group were higher compared to those of the middle age and over group. Even though this difference was found statistically significant, it is believed that this could be resulting from the fact that young and adult age groups have more intensified life stresses compared to middle and over age groups. [18] stated that there became a 50.0 % increase in the consumption of individuals while they are on diet and stressful. [19] found that individuals consume more food and drink in such cases as trouble, hatred, joy and depression.

Another significant finding was that the mean scores of young and adult age groups were higher again compared to those of the middle and over age group in the item of consuming mineral water "after sport". This difference was found statistically significant. It is likely to say that individuals are aware of consuming mineral water after sport for young and adult age group compared to middle and over age group. [20] stated that 13.0 % of the individuals doing team sports and 5.0 % of tho-

se doing individual sports preferred mineral water to tolerate the liquid they lost with sweating. In another study, it was found that mineral water consumed after sport is of important in terms of compensating the water supplying water and mineral loss through sweating and making a balance of water electrolyte [21].

Another finding in the study with regard to the educational study was that the mean scores of the university graduates in the item of "I believe there is a difference between mineral water and soda" were higher compared to secondary school graduates. This different was found statistically significant. This case could be resulting from the fact that university graduates are both well-informed and aware of the fact.

Another striking finding in the study in the item of "I believe that pills can be taken with mineral water" were higher for the secondary and high school graduates compared to those of the university graduates. This different was found statistically significant. This case could be resulting from the fact that university graduates are both well-informed and more attentive about mineral water and taking pills. It was stated in some other studies that [22, 23, 24] there could have some negative interaction in the absorption of use of the pill or mineral or both when the sodium, potassium, calcium, magnesium and chlor mineral in the mineral water are taken with pill.

In the item of "It meets my needs of liquid" in the views what is felt in the body after the consumption of mineral water consumption in terms of the BMIs of the individuals, the mean scores of the underweight and normal weight individuals were found higher, while those of the slightly overweight and overweight ones were lower. This difference was found statistically significant.

5. Conclusion

In the current study, it was found that the preference of the individuals in the priority of consuming mineral water and fruit flavoured mineral water. However, it was also found that when mineral water is consumed more than necessary, there could appear some health problems such as gastritis, hypertension and cardiovascular diseases. Besides, it was determined that when fruit flavoured mineral water is consumed more than daily needs, some problems such as obesity and Type 2 diabetes mellitus risk could occur. It was found that young and middle and over age individuals were more informed over mineral water consumption after sport compared to adult group. As for the item of "I believe that pills can be taken with mineral water", the reason why the mean scores of the university graduates were lower could be resulting from the fact that they are more informed and educational compared to secondary and high school graduates.

6. Recommendation

Individuals should consume mineral water by checking the labels of the products depending on their age, educational status and BMIs. In particular, the individuals with weight problem and diabetes should avoid from consuming too much fruit flavoured mineral water. On the other hand, mineral water is not a drink that helps people lose weight, so drinking more than 2 bottles a day is not recommended. In order that necessary and correct information is told to individuals over mineral water, people should be trained over drinks through media, TVs and public service announcement. The training to be given to individuals over drinks is of great importance for the correct consumption behaviours and information change for individuals.

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Implementation of descriptive algorithm on infertility data (Data Mining case study)

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Abstract

Nowadays, leading-edge advanced medical tools and new ways of communication are two of the important topics being considered in any medical discussions. Many algorithms introduced in the field of data mining are some of these advanced solutions which makes us able to appropriately interpret and analyze a variety of problems. In the medical field, the data mining has been employed successfully in diagnosis and treatment of disease. Along with the advances in medical science in the world, researchers and medical society in Iran have been progressed notably in discovering new methods of dealing with infertility. This great leap is due to the fact that infertility is one of the issues at the concern of academia, industry, and the community who are suffering from this inability. This work of study uses data mining algorithms to extract a descriptive model from data collected in Sarem Hospital. This data has been collected from patients with infertility being treated with Intra cytoplasmic sperm injection (ICSI) method. Moreover, this work of study enhanced and employed K-means clustering algorithm to improve the result. Descriptive results on Sarem Hospital data are presented.

Key words: Infertility, Clustering, ICSI method, Data Mining

1. Background and Objectives

By considering the social and economical issues of developing communities, one of the critical programs would be control of population and fertility. On the other hand, there are over millions of infertile couples included as one of the crucial problems of the society. Besides the effects of infertility on the relations of couples with each other, it also endangers the health of their mind and body. The objective by infertility expertise centers and hospitals is to help infertile couples to have healthy children and finally solving a problem of the society by taking advantage of the new technology. [1]

During recent years, many data has been gathered on infertile couples from hospitals and different medical centers; that appropriate use of this information and their study and analysis can be applied along increasing treatment rate of referrers and also reduction in the required time for medical process by selecting the appropriate method for each of the referrers. In this project, some records of patients in hospitals have been clustered for introduction with infertility medical processes and facilitation and speedup of treatment procedures.

1.1. Knowledge discovery & Data Mining

Knowledge discovery from data bases is included of the process for distinguishing patterns and models which are valid, novel, and potentially efficient and completely comprehensible. Data mining is a step of the process of knowledge discovery that finds the patterns or models by help of specific data mining algorithms with acceptable computational efficiency. The objective by Knowledge Discovery and Data Mining (KDD[1]) is to find the patterns or models available in data bases which are buried in a large mass of data. KDD is an intermediary field which is in relation with the fields such as statistics, data base, algorithms, machine learning, etc. based on the overlook that finds data mining a part of knowledge discovery process, the knowledge discovery process is included of following steps:

- Detection and problem defining
- preparation and pre-process
- discovery of patterns (data mining)
- interpretation and evaluation
- using the knowledge discovered

1 Knowledge Discovery & Data Mining

The step of discovery of patterns is included of data mining phase in knowledge discovery process. Data mining process encompasses the algorithms specific for data mining in a way that it discovered patterns or models under efficient and acceptable computational limitations [2]. The main methods of data mining are categorized in two descriptive and predictive groups. Descriptive methods are applied in order to find interpretable patterns for human being and predictable algorithms are used for predicting future behaviors. Among the prevalent methods in descriptive algorithms, we can mention to cluster method. Cluster means dividing the data to similar groups. The data are clustered based on maximization of similarity inside groups and minimization of similarity between groups. Cluster is a prevalent descriptive method that is seeking to distinguish a limited number of clusters for describing the data [3].

In this project, the prevalent cluster algorithm is used for study and analysis of data. The data studied in this project are the information related to infertile couples from "Sarem" infertility specialized hospital. This data is included of individual description of couples and their medical process.

1.2. Infertility

Based on the definition of World Health Organization (WHO), infertility is the inability to conceive a child. A couple may be considered infertile if, after two years of regular sexual intercourse, without contraception, the woman has not become pregnant[4][5]. By taking to account the importance and specific consideration of families to the issue of infertility, different medical methods have been used all over the world in order to solve the problem of infertile ones and it has brought different results in different situations. The most prevalent reason for infertility reported in researches and different projects is included of male factor such as sperm disorder [6][7], female factor such as ovarian dysfunction [8][9] and tubal factor [8][10], both male and female factors [11][12] and infertility by unknown reason. Among the most important medical activities in the field of infertility, we can mention to Assisted Reproductive Technology (ART) which is applied in medical centers and specialized hospitals. The success rate of ART depends on investigating the reasons and treatment of infertility [13]. The specifications and reasons of infertility vary in developing countries [14]. Among the prevalent ART techniques in treatment of infertility, we can mention to Intra Uterine Insemination (IUI), In Vitro Fertilization (IVF), ICSI, Gamete Intra Fallopian Transfer (GIFT) and Zygote Intra Fallopian Transfer (ZIFT). The data related to ICSI medical process on patience is studied in this project.

One of the prevalent and valuable methods which has opened new doors in treatment of infertility is ICSI method.

1.3. Literature Review

Since the first gestation reported by Palermo and his Colleagues[15], ICSI has been the first choice as a standard treatment employed for patients with male factor and Patients with failed IVF technique.[16] The most common cause of infertility reported in various research projects are as follows: male factor such as Sperm Disorders, Female Factor such as Ovarian factor, Tubal Factor and Unexplained factor infertility.

Aspraf and Fritz argued that the success rate of assisted reproductive techniques depends on the causes and treatment of infertility. Also Iunefeild and B.Ishor showed that specifications and causes of infertility varies among developing countries. [17] in Bayesian classification paper – for embryo selection in IVF- DinoraAraceli and her colleagues using profile of couples, selected embryos with the highest quality and which enhanced the likelihood of success in IVF.[18] Another study uses the number of embryos transferred in ART cycles to find the relation between the number of transferred embryos in pregnancy using IVF.[19] In addition, Researchers reported the results of combining genetic algorithm and decision tree in order to improve the prediction of IVF outcome.[20] Also, Kaufmann and his colleagues worked on a method based on neural network to predict IVF outcome.[21]

The above mentioned method was only a brief list employed in this context.

2. Methods

By considering the phases of knowledge discovery process propounded, after the steps for co-

gnition of project environment, data selection and data preparation, there stands the step for data modeling which is the core process of data mining. In order to conduct the project process step by step and specifying required phases, Cross Industry Standard Process for Data Mining (CRISP-DM) was applied[2]. Different phases of this model are displayed in figure (1).

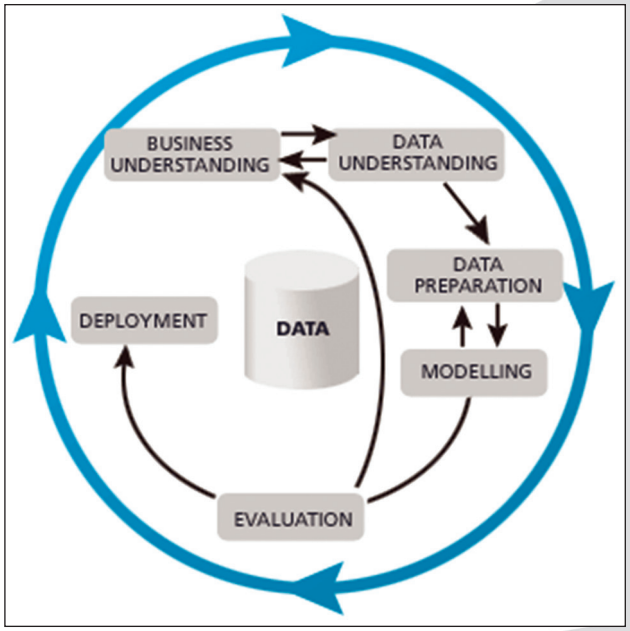

Figure 1. CRISP-DM Model
Reference: Shearer C., The CRISP-DM model: the new blueprint for data mining, J Data Warehousing (2000).

According to what is observed in figure (1), different phases of CRISP-DM model are specified which will be explained below.

The first step from this process is to understand the respective application area and the relation categorization of the issue. The objective is to get familiar with the project environment and the processes conducted in it. The data for this project are extracted from patients' records in Sarem hospital data base. The process for infertility treatment in this hospital is conducted by the couples referring to the hospital, counseling meetings with them for investigating the problem and selecting the appropriate treatment method. By couples referring to the hospital, a profile is registered for them that in all future referring there, all tests and their results are recorded in this profile. In order to diagnose the reason of infertility, the physician studies the medical background and the general situation of the body of couples and then among the ART treatments conducted in this hospital, the appropriate treatment would be selected. In selection of the type of treatment, even the previous treatments conducted in other centers will be considered and the method is selected having the maximum probability of success by the opinion of hospital physicians. In the first phase of project process, some processes and activities such as studying the profiles and negotiation with respective physicians was performed in order to get familiar with the hospital environment. Along this, some algorithms were drawn on the activities followed during the medical procedure of infertile couples and all medical procedures were explained.

The objective of second step of project process is to understand the hospital data base and study of all saved data and selection of required data among them. In order to facilitate the project and the results being objectively, the required data shall be selected and studied among the registered records at hospital from different treatment fields and in different time periods and then they have to be analyzed. In this phase, in order to confine the analysis area, one of the different types of infertility techniques was considered and the respective data was extracted from the hospital data base. Since ICSI treatment method has been most prevalent in Sarem hospital and the data regarding the patients taking this treatment method have had sufficient number for performing data mining analysis, the data respective this method were extracted from the data base and the project's executive phase started.

The third phase of the project process takes the most time of the whole data mining process and over 70% of the success of data mining projects depends on this phase [22]. Gathering and preprocess of data include selection of data resources, omission of outliers or noise data, way of treating missing data, transmission, discrimination and reduction of data. Data preprocess is necessary for improving quality of real data of data mining. Data with quality is used to be correct, complete, compatible, up-to-date, acceptable, valuable, interpretable and accessible. Some of important activities performed for having data with quality are as follow:

2 http://www.crisp-dm.org/Process

- Study and investigation on researches related to infertility in order to select specifications effective in selection of treatment method
- Study and investigation on specifications and understanding of the amounts recorded at data base for them
- Amendment or omission of data without value from the sum of data to make better interpretation of the results
- Determining the permitted range for each of the specification by consulting hospital physicians and amendment of amounts staying in unpermitted range (for example age, weight or height range)
- Matching the amounts recorded for each of the specification at data base in terms of criteria unit for that specification (for example, for infertility duration)
- Performing re-computation in order to create new fields (like age from the date of birth, referring date, Body Mass Index (BMI) from weight and height)

The items mentioned are a short number of actions taken along preprocess of data and explaining all processes are not considered as the main subject of this paper. By passing through preprocess, 702 qualified data were extracted from the records of patients experiencing ICSI treatment method. During the process of repeated referring to hospital physicians, the ten more efficient specifications were selected. These items are displayed in table (1).

Table 1. Attributes of selected infertility data

Data Range	Description	Attribute	Number
20-51 years old	This attribute are calculated using the birth date & ART date	Woman`s age	1
1-30 years old	According to World Health Organization (WHO) : higher than one year	Infertility duration	2
Less than 18.5: 6 records Between 18.5 & 24.9: 372 records Between 25 & 29.9: 242 record Above 30: 82 records	BMI, is a heuristic proxy for human body fat based on an individual's weight and height & is defined as the individual's body mass divided by the square of his or her height. The formulae universally used in medicine produce a unit of measure of kg/m^2. $BMI = \frac{mass(kg)}{(height(m))^2}$ • The WHO regards a BMI of less than 18.5 as underweight and may indicate malnutrition, an eating disorder, or other health problems. • Between 18.5 & 24.9 is normal. • a BMI greater than 25 is considered overweight. • above 30 is considered obese.	Body Mass Index (BMI)	3
Male Factor (MF): 655 records Ovary Factor (OF): 206 records Fallopian Tubes Factor (TF): 136 records	Infertility is a couple's problem, and is commonly due to some contribution from both the male and female partners. In this database, the causes of infertility involves male factor, ovary factor and fallopian factor.	Infertility Cause	4
Primary infertility: 558 couples Secondary infertility: 144 couples	There are two types of infertility: • Primary Infertility is defined as neither partner having achieved a pregnancy. • Secondary Infertility is defined as when a pregnancy has previously been achieved by the couple but regular, unprotected intercourse has not resulted in a second pregnancy.	Infertility type	5

0 – 7 embryos	In ICSI method, 0 to 7 embryos transferred. (0 embryo means that treatment protocol was canceled before transfer step.)	Number of transferred embryos	6
FSH: 0.1 - 76 LH: 0.1 - 101 Estradiol: 0.6 - 673	Effective hormonal test in ARTs are FSH, LH, Estradiol	Hormonal test	7
1 – 38 follicles	Ovarian follicles are the basic units of female reproductive biology, each of which is composed of roughly spherical aggregations of cells found in the ovary. They contain a single oocyte	Number of Ovarian Follicle	8
0 – 46 ovum	An ovum is a haploid female reproductive cell or gamete.	Number of ovum	9
4 – 17	Reproductive potential is increased with an increase in endometrial thickness.	Endometrial thickness	10

The most important phase is modeling phase. The objective of this phase of project process is to extract hidden patterns or models. Among the prevalent descriptive algorithms is the cluster algorithm that in this project k-means algorithm was applied.

This algorithm is from the types of partitioning descriptive algorithms. Partitioning algorithms organize the items in K partitions in a way that each partition displays one cluster. K-means algorithm assumes the parameter K as the input and partitions N items to K clusters, in a way that the interior similarity of clusters is high and the exterior similarity of them is low. Similarity of each cluster is measured proportioned to the average of items of that cluster that this average is called the center of cluster. In K-means algorithm, K desired spots are selected randomly as the centers of primary clusters and the rest items are dedicated based on the maximum similarity to the cluster centers. After clustering with the specified clusters, clustering evaluation indicators shall be applied for selecting the best number of clusters. In this project, Silhouette indicator was used in order to find the optimized number of clusters. Silhouette indicator is defined as follow: [23]

$$S = \frac{1}{k}\sum_{k=1}^{k}\left[\frac{1}{n_k}\sum_{x\in C_k} S(x)\right]$$

In above formula, the amount for S(x) is equal to the amount of Silhouette indicator in the spot x of a cluster. For all Xs, the higher amount of S(x) results in increase of Silhouette indicator in general which would bring desired results. From the defections of K-means algorithm is that due to random selection of centers of primary clusters, the gained clusters change in each time performing the algorithm. In order to improve the algorithm's situation and achieving better results, 100 times of K-means algorithm is performed for each clustering with specific number of clusters and after each time of performing, Silhouette evaluation indicator is calculated. In this procedure, for each of the 3 to 10 clustering, random spots are selected 100 times and the Silhouette indicator amount is calculated a 100-time. Among the amounts gained for indicator, the best state for Silhouette indicator which is the highest amount will be selected for each of the clustering. The optimized amounts of indicator in each clustering with 3 to 10 cluster is displayed in table (2) and chart (1).

Table 2. Optimal values of Silhouette indicator

Indicator value	Cluster count
0/207002961	3
0/209786511	4
0/230497583	5
0/202479161	6
0/198980788	7
0/221558173	8
0/218181227	9
0/217420429	10

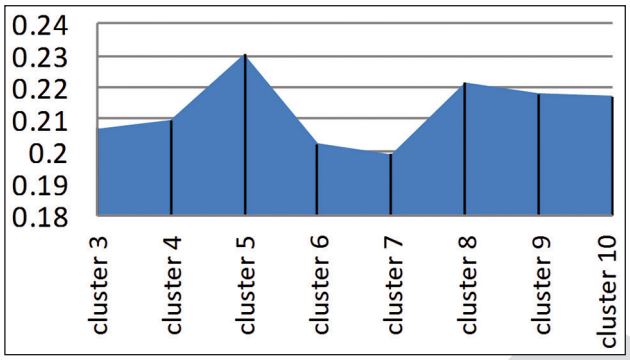

Figure 2. Silhouette indicator values comparison

According to the results achieved, the number of optimized cluster in this project is 5 clusters.

3. Results

Two last phases of CRISP-DM model focused on Interpretation and evaluation and use of earned knowledge.

The core and critical issue in infertility treatment process is the rate of success in this process and the gained results. After clustering and achieving the 5 respective clusters, we would require treatment results in each of these clusters in order to analyze the data. In Sarem hospital data base, the treatment results of patients was registered in two steps, each indicating a rate of success in treatment process which are explained below:

- Chemical pregnancy: in this step, the first pregnancy test is performed and the result is recorded.
- Clinical pregnancy: in this step, in case of observing uterine pregnancy, the treatment has been completely successful.

In order to analyze and study the gained clusters, the specification of clusters and the results of treatment processes about each cluster will be analyzed separately. This information is displayed in table (3).

4. Discussion

Each of the clusters 1 to 5 are in some cases different to other clusters and they have remarkably appeared in these specifications. The remarkable specifications of these clusters are explained bellow.

Cluster 1: this cluster with 100 records has the highest age mean among other clusters (about 35.7) and the least infertility duration (4.5 years). Types of infertility in all records of this cluster were from secondary type and the least rate for the number of follicle and ovule and endometrial thickness. The result of treatment in this cluster at the first step has been the least amount proportioned to other clusters. However, among 15 persons with success in chemical pregnancy step, 11 persons made it successfully to clinical pregnancy which is indicative of this fact that 73% of the ones successful in chemical pregnancy have also been successful in clinical pregnancy. At the end, the maximum rate of final success was observed in the members of this cluster.

Cluster 2: this cluster with 68 records has the longest infertility duration (about 10 years). The state of BMI in members of this cluster is over 30 which means overweight and 51% of the records of this cluster have the same situation. 77% of the members of this cluster have primary infertility and the number of transmitted embryo has been 3 which include 42% of the records. In this cluster, 12 persons which are 17.64% of the whole members of the cluster have been successful in the first step and among them, 5 persons (41%) had successful result in clinical step and finally the rate of total success in this cluster has been lower than other clusters.

Cluster 3: this cluster with 118 records had the minimum age mean (about 29.37 year) and 87% of the members of this cluster had primary infertility. The dominant reason for infertility in this cluster has been the ovary problem which included all records of this cluster. This cluster had the highest mean for the number of follicle and ovule among other clusters and the results of FSH and LH hormone tests has been the highest amounts with 6.06 and 11.99. In this cluster, the maximum success has been in the first step and 21 persons have been successful in the first treatment step which included 17.79 % of the data of this cluster and in the second step, 11 persons were successful which is indicative that 52% of the ones having successful chemical pregnancy have been also successful in clinical pregnancy. As it is observed from the data of this cluster, due to problem of ovary in fertilizing the ovule of the members of this cluster, by taking ovulation stimulating medications,

this problem has been removed and the number of follicles and ovules has increased and this has brought good results for the couples. Thus, about the members of this cluster, it can be said that the success in the first step is achieved by prescription of medications appropriate for ovulation.

Cluster 4: this cluster with 240 records has the maximum number of members compared to other four clusters. The dominant (91%) BMI state of the members of this cluster has been 18.5 to 24.9 which is indicative of normal body rate. All records of this cluster have the primary infertility type and none of the members of this cluster have ovary problem. The rate of success for the members of this cluster in the first step is 17.5% of the whole data and among them, 10% have been successful in clinical pregnancy step. The success rate in the second step is in this way that about 57% of the ones successful in the first step have been also successful in the second step. Among the specifications of this cluster, we can mention to the high number of records, BMI state and infertility duration and the members of this cluster have been the ordinary and normal referrers.

Cluster 5: this cluster with 176 records had the dominant BMI state of 25 to 29.9 which means overweight. 97% of the members of this cluster have primary infertility. The maximum mean of endometrial thickness belongs to the members of this cluster. The results of estradiol test in the members of this cluster had the highest mean. Treatment in the first step has been successful for 29 members of this cluster which include 16.47% of the whole records and among the ones being successful in the first step, 44% have been also successful in the clinical step too.

5. Suggestions

By considering the specifications of each cluster and the success rate in each cluster, by comparing the patients with the specifications of each cluster the situation of treatment result can be predicted in future studies.

Table 3. Clusters Specifications and results

Cluster 5	Cluster 4	Cluster 3	Cluster 2	Cluster 1	Clusters / Attributes	
176	240	118	68	100	Number of clusters record	
31/84	30/67	29/37	34/22	35/74	Mean of Woman`s age	
9/5	7	7/5	10	4/5	Mean of Infertility duration	
Between :25 & 29.9 %97	Between 18.5 & 24.9 %92	Between 18.5 & 24.9 %69	Above 30 %51	Between 18.5 & 24.9 %57	BMI status	
Primary: %92	Primary: 100%	Primary : %87	Primary : %77	Secondary : %100	Infertility type	
%97	%96	%88	%83	%92	MF	Infertility cause
%8	%0	%100	%80	%19	OF	
%25	%10	%11	%23	%37	TF	
8/8	9/1	12/6	8/5	7/8	Number of Ovarian Follicle	
10/4	10/4	14/8	8/5	8/1	Number of ovum	
9/77	9/34	9/08	9/19	8/58	Endometrial thickness	
4/73	5/26	6/06	4/64	5/19	FSH	Hormonal Test
7/24	8/95	11/99	7/84	6/80	LH	
94/53	81/82	80/48	72	92/24	Estradiol	
4 embryos : %44	4 embryos : %45	4 embryos : %63	3 embryos : % 42	4 embryos : %41	Number of transferred embryos	
%16/47	%17/5	%17/79	%17/64	%15	Chemical Pregnancy	Successful outcome
%7/38	%10	%9/32	%7/35	%11	Clinical Pregnancy	

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Effects of glucosamine and ginger on pain reduction and function improvement in patients with the knee osteoarthritis

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Abstract

Background: Knee osteoarthritis is the most common joint disease in humans that make impaired mobility in life. Various treatments are used to reduce the pain and stop the disease process. We aimed to compare the effect of ginger powder (zingiber officinale), glucosamine sulfate and placebo in patients with knee osteoarthritis referred to rheumatology clinic of Rasool Akram general hospital, Tehran, Iran.

Methods: This study was Double-Blind Randomized Clinical Trial on 225 patients with knee osteoarthritis diagnosed according to ACR criteria. Patients were divided into three groups of 75 cases that received glucosamine sulfate, ginger and placebo for 12 weeks. Pain was assessed by VAS-100mm criteria and functions by WOMAC questionnaire at time zero, 6 and 12 weeks.

Results: 190 of 225 patients completed the study. After 6 weeks treatment, all three groups improved about rest pain severity and the differences were statistically significant in all groups. Although, this pain reduction continued to 12 weeks in all groups, the difference in placebo group was not significant. At rest pain reduction in both glucosamine and ginger groups was significantly more than placebo patients. Functional improvement was 66% in glucosamine sulfate, 56% in ginger group and 32% in placebo group that this difference was statistically significant.

Conclusion: Glucosamine sulfate and ginger are effective in reducing at rest pain and improving activity in patients with the knee osteoarthritis.

Key words: Osteoarthritis of the knee, Ginger, Glucosamine

Background

Osteoarthritis (OA) is among the most disabling and costly chronic conditions (1-2). It is a degenerative and progressive joint disease which mainly affects people in the older age groups (3). Although OA can occur in any *joint*, because knees are primarily weight bearing*s*, they are very commonly involved. Education of patients, social support, changes in lifestyle such as weight reduction and exercise are effective and recommended treatments for knee OA (4). But definitive therapy for OA has not yet been identified.

Treatment of OA is aimed at relief of pain and stiffness, preserving and increasing joint mobility, decreasing physical disability, retarding the progression of joint damage and improving the quality of life (5). The mainstay of this management is non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics which are associated with serious side effects (6-7). Thus, many patients with osteoarthritis often look for complementary and alternative medicine (CAM) therapies.

The use of cartilage constituents such as chondroitin and glucosamine have been increased in the past 10 years. Glucosamine sulfate alters the metabolism of cells derived from synovium *in vitro*. Beneficial effect of glucosamine sulfate has also reported on pain relieving and functional improvement of OA. However, a meta-analysis including 10 trials showed that **c**ompared with placebo, glucosamine does not reduce joint pain or has an impact on narrowing of joint space (8). Anumber of guidelines recommended these products for the management of knee OA, while some others do not recommend that or recommend only under specific conditions. Recently, nutraceuticals were introduced as a good options to modify the OA

symptoms (9). Therefore, we attempt to compare ginger powder (from nutraceuticals drugs group) and glucosamine sulfate versus placebo in patients with the knee osteoarthritis who referred to rheumatology clinic of Rasool Akram general Hospital.

Methods

Study design and patients' eligibility

This Double-Blind Randomized Clinical trial was performed on patients referred to rheumatology clinic of Rasool Akram general Hospital, a referral university hospital, Tehran, Iran. The study was accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and study protocol was approved by the institutional review board of Iran University of medical sciences. Written informed consent was obtained from each patient. Consecutive patients with knee OA based on ACR (American College of Rheumatology) criteria were eligible to participate in the study. Age, sex, BMI, patient's complaint, knee physical examination and radiologic findings were recorded for each patient. Only patients aged between 48 to 75 years who had 19< BMI < 35 were enrolled in the study. In addition, all patients were assessed about pain by VAS-100mm criteria and function by WOMAC functional class.

Intervention and comparison

Patients were randomly assigned (1:1:1) using permuted randomized blocks to the ginger 250 mg, glucosamine sulfate 500 mg or placebo groups. All three groups received a same shape of capsule applied by MODAVA pharmaceutical company (ginger prepared by GOLDARU company in capsules of Zintoma and would be made up of same shape with glucosamine).

All patients were recommended quadriceps muscle exercise and maximum use of acetaminophen in 2g /day. Patients were evaluated at time zero, 6 weeks and 12 weeks. In addition, they were asked to avoid other medications, physiotherapy or injection into joints during the study period. If patient was intolerance or experienced any complications, they should consult as necessary.

Study outcomes

The primary end point was pain relief measured b VAC.

Statistical analysis

Data was analyzed by SPSS vs18 software, variation of function by chi-square, pain & BMI by repeat measure test was analyzed.

Results

These studies of 225 patients with knee osteoarthritis were divided in three groups of 75. The mean age of patients was 63.7± 7.12 (range 48–75) years. There were 187 females (83.2%) and 38 males (16.8%). Table 1 shows a brief description on the average BMI in three groups. BMI of three groups did not have significant difference at baseline and during the treatment.

Of 225 patients, 35 were excluded from the study due to side effects of medications. 12 of them were from ginger, 16 from glucosamine and 7 from placebo group. In addition, 25 patients did not return for follow up evaluations.

At first evaluation, there were no significant differences in the groups on rest pain score. After 6 weeks treatment, all three groups improved about rest pain severity and the differences were statistically significant in all groups. Although, this pain reduction continued to 12 weeks in all groups, the difference in placebo group was not significant (Table 2). At rest pain reduction in both glucosamine and ginger groups was significantly more than placebo patients (P value = .001 and 0.002 respectively), but the difference between glucosamine and ginger was not significant (P value = 0.2).

Regarding to the activity pain, there was no significant difference between three groups at the first admission. But after 6 and 12 weeks therapy, activity pain relieved in all three groups significantly. (Table 3)

With regard to pain score during the night, after 6 and 12 weeks from treatment, significant improvement was occurred in ginger and glucosamine group. But in placebo group, the difference was not significant. The difference of pain reduction between each of glucosamine and ginger with placebo group were significant (P value = 0.02 for

Table 1. Average BMI in three groups

BMI	Admission	6 weeks	12 weeks	P value
Ginger	29.66.3±	28.7±5.9	28.5±6.8	0.5
glucosamine	28.75.9±	28.25.3±	28.16.1±	0.5
placebo	29.56.8±	29.15.1±	28.75.9±	0.7
P value	P=0.8	P=0.5	P=0.6	

Table 2. At rest pain on the VAS scale

VAS	Admission	6 weeks	12 weeks	P value (0-6)	P value (6-12)
Ginger	32.2511.2±	28.3811.8±	22.912.3±	0.001	0.001
glucosamine	28.89.8±	22.311.1±	18.38.7±	0.001	0.001
placebo	26.38.7±	23.29.1±	22.59.2±	0.001	0.4
P value	0.13				

Table 3. Activity pain on the VAS scale

VAS	Admission	6 weeks	12 weeks	P value
Ginger	63.715.6±	57.112.5±	47.417.1±	0.001
glucosamine	60.117.2±	47.414.1±	38.0314.2±	0.0001
placebo	58.416.07±	53.217.8±	22.52.9±	0.001
P value	0.42			

Table 4. Night pain on the VAS scale

VAS	Admission	6 weeks	12 weeks	P value
Ginger	22.612.4±	20.413.2±	14.611.5±	0.01
glucosamine	23.521.2±	15.915.8±	12.067.57±	0.001
placebo	19.812.9±	16.211.3±	16.810.6±	0.2

Table 5. Distribution of patients in treatment groups based on function class

Group	improved Function Class		Non-improved Function Class		total	
	frequency	percent	frequency	percent	frequency	percent
Ginger	45	66	18	34	63	100
glucosamine	33	56	26	44	59	100
placebo	22	32	46	68	68	100
P value = 0.023						

both groups). The difference between glucosamine and ginger was not significant (p = 0.1). Table 4

45 patients in the ginger group, 33 in glucosamine and 22 of placebo group develop better function class. Statistically significant differences between performance improvement and treatment group was seen (p = 0.02). Table 5

Discussion

Various medications have been tried in the management of knee osteoarthritis. This randomized clinical trial compared the effect of two drugs as glucosamine and ginger with placebo about relieving the symptoms of OA. The both drugs were

efficacious comparing the placebo on relieving pain and improving the function class. Similar favorable response rates were observed in both treatment groups for the primary efficacy endpoint. The both drugs were generally well tolerated.

Nonpharmacologic agents are a cornerstone of OA treatment. There were evidences that glucosamine sulphate has clinical effectiveness in the treatment of the knee OA. The Osteoarthritis Research Society International (OARSI) analyzed 19 RCTs (16 of them with glucosamine sulfate and 3 with GlcN-HCl) and reported an effect size for pain of 0.46 (0.23–0.69). In addition, effect size of glucosamine sulfate for pain was more decreased with regard to high quality clinical trials (0.29 (0.003–0.57)) (10). By a meta-analysis on Six studies involving 1,502 cases, consisting only two studies on glucosamine sulfate and four studies on chondroitin sulfate, Young et al (11) reported a small to moderate significant protective effect on minimum joint space narrowing after 2 years. After a mean 8-years observation, Bruyere et al. (12) reported that treatment of knee OA with glucosamine sulphate for at least 12 months may prevent total joint replacement. However, its efficacy is constantly under debate. By a meta-analysis of 10 large-scale randomized controlled trials (3,803 patients) in patients with OA, Wandel et al. (8) reported that neither glucosamine nor chondroitin sulfate, neither alone nor combined, could significantly improve pain nor reduction of articular space when compared to placebo. This meta-analysis had numerous limitations and the analysis was not adequately supported by their data (13). However, by another meta-analysis at 2013, Wu et al. reported that glucosamine sulfate may have function-modifying effects in patients with knee OA when administered for more than 6 months. But, it has no pain-reduction benefits after 6 months of therapy (14). In contrast, we found beneficial effects of glucosamine on pain reduction and function improvement in a period of 6 and 12 weeks therapy. Although, this improvement was seen also in placebo group, which may be the result of NSAIDs and exercise, this trend was not significant in placebo group after 12 weeks.

Ginger is a most frequently used natural product which reduces the inflammatory symptoms of osteoarthritis. Ginger inhibits the COX-1, COX-2 and LOX, and also several genes encoding cytokines, chemokines and inducible enzyme COX-2, and modulates the biochemical pathway which is activated in chronic inflammation (15-18). In a randomized, placebo-controlled, cross-over study of ginger extracts and Ibuprofen, Bliddal et al. (19) reported a statistically significant effect of ginger extract in the first period of treatment before cross-over, while a significant difference was not found in the study as a whole. In another study, Alman e al. (20) found a statistically significant moderate effect of ginger extract on reducing symptoms of OA of the knee with a good safety profile. In a randomized open label study on 60 patients, Gill P et al. (21) reported numerically superior improvement in patients who received both ginger and diclofenac. In addition, Ginger powder had add-on effect on reducing the symptoms of OA of the knee with acceptable safety profile. In similar, our patients who received both ginger and acetaminophen showed statistically significant improvement with time. Haghighi et a l. (22) compared the effects of ginger extract as an alternative to NSAIDs. They found significantly more efficacy for Ginger extract and ibuprofen against the placebo in the symptomatic treatment of OA, while they did not found significant difference between the ginger extract and ibuprofen groups. In another study, they introduced Ginger as effective as indomethacin which can be substituted in relieving symptoms of osteoarthritis in patients with intolerance to indomethacin (23).

We did not found any literature comparing ginger and glucosamine. Although in the current study, both glucosamine and ginger were better than placebo, but no significant difference was seen between them.

Conclusions

Due to the effectiveness of herbal medicines on knee osteoarthritis and less adverse effects, we recommend more attention should be given to these medications. Therefore, prospective studies with more cases over ginger and other herbs are needed. It seems future studies over herbal medicines is considerable about joint space narrowing.

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

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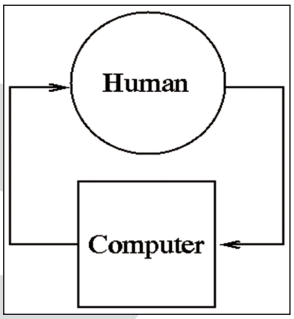

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

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