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Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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Case Report

Rare combination of autoimmune disorders Hashimoto's disease (HD), grave's disease (GD), vogt-koyanagi-harada: A case report

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Vogt-Koyanagi-Harada (VKH) syndrome is a rare entity characterized by depigmentation of the skin and eye lashes, chronic granulomatous iridocyclitis and exudative retinal detachment, as well as aseptic meningitis and encephalopathy. We describe a 33 year old female patient suffering from this syndrome, associated with Graves' disease (GD), Hashimoto's disease (HD), and Graves' ophthalmopathy (GO). The association with these various diseases respresent an extremely rare combination of autoimmune disorders.

Key words: Vogt-Koyanagi-Harada disease, Graves' ophthalmopathy, retinal detachment.

INTRODUCTION

Vogt-koyanagi-HARADA syndrome (VKH) is a bilateral granulomatous panuveitis associated with cutaneous (poliosis, alopecia, and vitiligo), neurological (aseptic meningitis) and auditory (dysacusis, tinnitus, vertigo) manifestations related to a cell-mediated autoimmune process against melanocytes (Moorthy et al., 1995). Typically, VKH syndrome consists of three phases: a meningoencephalitis phase, an ophthalmic-auditory phase, and a convalescent phase. In the ophthalmic-auditory phase, symptoms such as blurry vision, pain, and eye irritation due to inflammation of the iris (iridocyclitis) and uvea (uveitis) occur.

Graves' disease (GD) is an autoimmune disease. It most commonly affects the thyroid, frequently causing it to enlarge to twice its size or more (goiter), become overactive, with related hyperthyroid symptoms such as increased heartbeat, muscle weakness, disturbed sleep, and irritability. It can also affect the eyes, causing bulging eyes (exophthalmos). It affects other systems of the body, including the skin, heart, circulation and nervous system. While most of the visual symptoms in patient with Graves' disease considered to be caused by the hyper-thyroid state which is known as Graves' ophthalmopathy (GO), we report a rare case of VKH syndrome in a patient with Hashimoto's disease (HD), GD, GO, and vitiligo, to increase awareness about other possible retinal involve-ment in patients with multiple autoimmune disorders, especially in patients with Graves' disease with visual symptoms. Our patient has GD, HD, vitiligo and VKH syndrome, an extremely rare combination of autoimmune disorders.

CASE

A 33 year old female patient from Taiwan was diagnosed with Graves' disease in 2006, who also had positive thyroperoxidase antibodies (HD) and vitiligo. Her hyperthyroidism was mild and controlled with antithyroid medications. Patient also was found to have upper eyelid retraction, redness and bulging in both eyes and was diagnoses to have Graves' ophthalmopathy (GO), which required plastic corrective surgery. She recently developed diplopia and partial visual loss. The patient denied ever having any form of generalized muscle weakness, headache, hemiparesis or hemiplegia, joint pain, fever, or weight loss. Patient also reported negative family history of any medical importance. Magnetic

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resonance imaging (MRI) of the orbit was done which revealed findings suspicious for retinal detachment. She was seen by a neuro-ophthalmologist who diagnosed a retinal inflammatory disorder, consistent with VKH syndrome, which was confirmed later with a positive HLA-DR4 testing. She was started on oral steroids, on which she noticed improvement in vision.

DISCUSSION

VKH syndrome is a T-cell–mediated autoimmune inflammatory response against melanocytes in the eyes, ears, central nervous system, and skin (Qutub and Halder, 2012). It often affects patients with darker skin especially in Asia and America and females. Typically, VKH syndrome consists of three phases: a meningoencephalitis phase, an ophthalmic-auditory phase, and a convalescent phase. In the meningoencephalitis phase, symptoms such as generalized muscle weakness, headache, loss of muscle use on one side of the body (hemiparesis or hemiplegia), joint pain (dysarthria), and difficulty speaking or understanding language (aphasia) occur.

In the ophthalmic-auditory phase, symptoms such as blurry vision, pain, and eye irritation due to inflammation of the iris (iridocyclitis) and uvea (uveitis) occur. Auditory symptoms may include difficulty in hearing, ringing in the ear (tinnitus), or dizziness. In the convalescent phase, skins symptoms such as light or white patches of color in the hair, eyebrows, or eyelashes (poliosis), light or white patches of skin (vitiligo), and hair loss (alopecia) appear. The skin symptoms usually begin several weeks or months after the vision and hearing symptoms start. Between the ages of 20 to 50 years, the exact etiology of the disease is unknown, but genetic predisposition and association with certain HLA subtypes, namely, HLA-DRB1, are presumed to play a role. It presents with acute granulomatous uveitis that progresses to chronic uveitis, accompanied by extraocular organ involvement such as centralnervous system, auditory system, and integumentary system. Extraocular organ involvement manifesttations are included but are not limited to headache due to cerebrospinal fluid pleocytosis, dysacousia, poliosis, alopecia, or vitiligo (Setiabudiawan et al., 2011).

With steroid treatment, in VKH syndrome, two-thirds of patients maintain visual acuity (VA) of 20/40 or better, and only a minority of patients (11%) have poor VA (20/200 or better). Better outcomes are associated with good VA at 1 month after onset, younger age at onset of disease, and early treatment with corticosteroids (Setiabudiawan et al., 2011). The association of VKH syndrome with an autoimmune disease of organs, especially of the thyroid is rare, isolated cases have been reported in the literature. This was mainly Hashimoto's disease (Wiesli et al., 1999; Jaggarao et al., 1989; Kluger et al., 2008; Chi et al., 1994; Paroli et al., 2003), more rarely with Graves' disease (Seo et al., 2009) or polyglandular syndrome (Jovic et al., 1996). The pathogenesis of Hashimoto's thyroiditis appears to involve humoral immunity as evidenced by the presence of anti-thyroid, but also cellular immunity. Indeed, they are clones of T CD4+ cells specific for certain antigens (thyroglobulin and microsomal antigen major or TPO) that appear to play an important role in the destruction of thyroid epithelial cells. Abnormalities of immune regulation have also been observed with a decrease in circulating T CD8 + cells with suppressive function (Duron et al., 2004). A genetic susceptibility characterized by different alleles, including HLA-DR3 in particular, has been associated with Hashimoto's disease. Pathophysiological similarities exist therefore between these two autoimmune diseases; their association in the same patient would not be a coincidence.

CONCLUSION

VKH is a rare autoimmune syndrome which can be associated with other endocrine autoimmune disorders such as autoimmune polyglandular syndrome, autoimmune thyroid disorders and diabetes mellitus. In patients with GD who develop ophthalmic manifestations, in addition to GO as the usual differential diagnosis (etiology), it is prudent to keep VKH syndrome in mind, as a rare autoimmune manifestation, in view of potential vision loss.

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Full Length Research Paper

The prevalence of obesity and hypertension among first-year students at Trnava University in Slovakia

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The prevalence of cardiovascular disease (CVD) is considered to be high in Slovaks and it could affect young adult University students. The aim of this study was to estimate anthropometric CVD risk predictors associated with environmental conditions among university students. The study population consisted of 122 first-year students (18% males and 82% females, 19.82 ± 0.5 years) attending Trnava university faculties that include the following: 65.5% of young adults from Faculty of Education and 34.5% of other faculties (33.3% from the Faculty of Law, 33.3% from the Faculty of Philosophy and Arts and 33.3% from the Faculty of Health Care and Social Work). Anthropometric measurements, including weight, height [for body mass index (BMI)] and percentage of body fat (BF) were evaluated. Measured blood pressure (BP) was used to classify for prehypertension, and I and II degree hypertension. A total 22.1% of university students were found to be prehypertensive and 18% hypertensive. Compared with future teachers who showed higher means of body fat (24.20 ± 5.14%) and frequent sedentarism, we noted higher means of all other anthropometric parameters and a higher prevalence of hypertension, being overweight and obesity among students of other faculties. By faculty grouping, the highest prevalence of being overweight and hypertension, the highest BMI and cigarette smoking were determined among the students from the Faculty of Philosophy and Arts. Students from other faculties differed in prevalence of obesity and body fat distribution ($p < 0.001$), hypertension (0.03), leisure-time physical activity and cigarette smoking ($p < 0.05$). We found significant association between hypertension and obesity, body fat, cigarette smoking and also body fat/physical inactivity ($p < 0.001$) among students. Our results indicate the need for implementing effective preventive reduction to the major CVD factors related to an unfavorable exogenous condition among young adult Slovaks with their specific educational trend.

Key words: Obesity, hypertension, life style, gender, university students, Trnava University.

INTRODUCTION

Recent data concluded cardiovascular disease (CVD) has become a leading cause of mortality in Slovakia resulting in 54.5% of deaths a year; statistics now show that CVD related deaths is 48.1% among Slovak males and 62% among Slovak females (Main demographic aspects of development in Slovakia, 2009). Respectively, of the 508 deaths recorded last year, per 100,000 inhabitants, 28,265 of those were from CVD (Fábryová, 2010). CVD combined with lower lifespans (about 10 years lower for the average male and female Slovak than for the average in the Eurozone (Baráková, 2009) reflect the consequences of long-term overvalues of key risk biopredictors associated with unhealthy life style habits. Due to education, an important determinant of health, populations with a basic education were found to be less healthier and more disadvantaged that well-educated populations (Jarrah et al., 2011). However, numerous epidemiological studies have previously reported relatively high prevalence of obesity and hypertension among highly educated university students in Scotland

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(Smith et al., 2001), Sweden (Margareta et al., 2005), Germany (Stock et al., 2001), Hungary (Antal et al., 2006), Greece-Crete (Bertsias et al., 2003), Libya (Greiw et al., 2010; Tayem et al., 2012), Nigeria (Adams-Campbell et al., 1988; Familoni and Familoni, 2011), United Arab Emirates (Amine and Samy, 1996), Saudi Arabia (Sabra et al., 2007), Iran (Mahmoudi et al., 2004), USA (Huang et al., 2003), Brazil (Costa Silva Zemdegs et al., 2011; Rabelo et al., 1999), Chille (Chiang-Salgado et al., 1999), Malaysia (Lee et al., 2010) and Colombia (Feliciano-Alfonso et al., 2010; Hernández-Escolar et al., 2010) as well as other parts of the world (Haase et al., 2004).

Overall, university students are often being exposed to environmental condition such as cigarette smoking, inefficient nutrition from cheap energetic diets, sedentary life style, etc. which can lead to an increase of CVD risk prevalence (Girotto et al., 1996). By Steptoe and Wardle (2001), East European university students from the German Democratic Republic, Hungary and Poland compared with West Europeans from Austria, Belgium, the Federal Republic of Germany, the Netherlands and Switzerland, have shown less healthy lifestyles.

The prevalence of sedentarism varied with cultural and economic developmental factors, averages 23% North-Western Europe and the United States, 30% in Central and Eastern Europe, 39% in the Mediterranean, 42% of Pacific Asia, and 44% in developing countries. In addition, cigarette smoking varied widely, being the highest in case studies from Southern European countries and the lowest in developing countries such as Thailand, South Africa, South America. The pattern of tobacco smoking in well-educated young adults appears to conform with the wider international patterns of tobacco use (Steptoe et al., 2002).

Although, multiple health risk behaviors have been identified as a problem in young university students, only a few reports have dealt with medical Slovakian university students. By gender,16% of males and 2% of females had body mass index (BMI) of over 25 kg/m^2 (Baska et al., 2001). In a study done a year later, mild obesity was determined in 22.2% of males and 8.7% of females. 4.8% of males and 5.4% of females showed an android type of obesity. 45.6% of them recorded higher fat composition than recommended value (Janušová et al., 2002). Though medical students are known to have less CVD risk than other students.

The purpose of the present study is to characterize anthropometric risk factors of CVD, which is to investigate body fat (BF), body mass index (BMI), and blood pressure (BP) to evaluate hypertension and various degrees of obesity, and exogenous interaction (physical inactivity and cigarette smoking) in both the male and female genders within different faculties include the Faculty of Education, the Faculty of Law, the Faculty of Philosophy and Arts and the Facutly of Social Health, within the University of Trnava. CVD among varying Trnava University faculties has never been analyzed, to date. The research recorded may provide more up-to-date information on health status, and clarify possible effects of specific educational (faculty) differences in order to possibly lower potential risks.

METHODOLOGY

Study population

This research study was completed in 2012 at Trnava University in the south-west of Slovakia. The randomly selected population consisted of 122 first year students: 18% male and 82% of female between the ages of 18 and 22 (averaging 19.82 ± 0.5 years). Healthy anonymous participants (each volunteers) were randomly contacted and recruited in proportion to the full population of Trnava University students (65.6% of all 399 full-time students from Faculty of Education and 34.4% from other faculties such the Faculty of Law, the Faculty of Philosophy and Arts and the Faculty of Health Care and Social Work). The underwent physical examinations of each student (measurements regularly used in anthropometric practices) took place at the Trnava university and at its hall of residence.

Data included information on lifestyle obtained from a questionnaire which was designed to evaluate how daily physical activities and cigarette smoking can affect CVD. Smokers were classified by smoking more than one cigarette per day for at least three months. Non-smokers were defined as those who had not been smoking for the last three months and had never smoked. This study has been approved by the ethical committeee of the University of Trnava.

Only non-lipid laboratory measures of CVD indicators were studied: BF, BMI, systolic and diastolic BP. All anthropometric data was measured at two separate times and the average values recorded. Body fat was measured by bioelectrical impedance (evaluated BF > 30% for female and BF > 20% for male). Anthropometric measurements (height and weight) were taken using a stadiometer. Height was measured barefoot in standing position to the nearest 0.5 cm using a secured metal ruler, and weight was measured in light clothing using calibrated scales. BMI used for the assessment of the prevalence of being overweight (BMI > 25 kg/m^2; overweight group is considered having a BMI between 25.0 kg/m^2 and 29.99 kg/m^2), the prevalence of obesity (BMI > 30 kg/m^2) and the prevalence of being morbidly obese (BMI > 35 kg/m^2), was calculated as a quotient of weight and squared height in meters (kg m^{-2}). Blood pressure was measured from the right arm using a standard mercury sphygmomanometer with the subject in sitting position (both values were taken after 5 min rest). Over-values of tension was defined as sBP >130 mmHg, and diastolic blood pressure as > 85 mmHg, hypertension I as sBP > 140 mmHg dBP > 90 mmHg and hypertension II as sBP > 160 mmHg dBP > 100 mmHg.

The results are expressed as mean ± standard deviation (SD). A normality test was performed with the Kolmogorov-Smirnov test. When the variables were normally distributed, we used the Anova test and evaluated the variables' distribution in gender and faculties related groups. Descriptive statistics for all studied variables and chi-square test were used. A "p" value of < 0.05 was considered to indicate statistical significance. Pearson correlation coefficient (r) tested association between anthropometric parameters. Statistical analyses were performed using the statistical package for social sciences (SPSS) System software package (2010).

RESULTS

The anthropometric parameters in the examined

Table 1. Characteristics of examined University students (Trnava University).

Parameter	All (N=122)	Males (N=22)	Females (N=100)	Faculty of Education (N=80)	Other faculties (N=42)
Age (years)	19.82±0.5	19.91±0.68	19.47±0.72	19.36±0.77	19.90±0.48
sBP (mmHg)	133.63±16.99	134.05±18.79	125.06±16.27	122.86±15.8	133.95±17.09
dBP (mmHg)	81.18±12.53	82.86±13.06	78.56±10.64	77.94±10.00	82±12.83
Pre/hypertension I (%)	22.1/13.9	40.9/25.7	18/12	23.8/7.5	19/26.2
Hypertension II (%)	4.1	4.5	4	3.8	4.8
BMI (kg/m^2)	20.38±17.41	23.74±3.6	21.20±3.16	21.44±3,24	22.08±3.62
Overweight/obesity (%)	17.,2/0.8	40	12/1	16.3/1.3	19
BF(%)	21.76±5.55	15.4±6.5	20.9±7.12	19.37±8.06	20.94±5.5
Overvalues of BF (%)	10.7	9.1	11	13.8	4.8
Physical inactivity (%)	38.5	18.2	43	40	35.7
Cigarette smoking (%)	22.1	36.4	19	16.3	33.3

Table 2. Anthropometric values and life style of students of other faculties, attending the University of Trnava.

Parameter	Faculty of Law (N=14)	Faculty of Philosophy and Arts (N=14)	F. of Health Care and Social Work (N=14)
Age (years)	19.86±0.36	19.93±0.27	19.93±0.73
sBP (mmHg)	135.15±18,91	133.07±14.67	133.64±18.59
dBP (mmHg)	84,43±14,55	81.57±11.18	80±13.1
Pre/hypertension I (%)	35.7/14.3	14.3/35.7	7.1/28.6
Hypertension II (%)	14.3	-	-
Pulsation	74.57±13.43	84±17.31	83.71±22.45
BMI (kg/m^2)	21.66±3.4	23.82±4.34	20.76±2.34
Overweight (%)	14.3	35.7	7.1
BF (%)	20.09±6.61	21.65±5.99	21.08±3.82
Overvalues of BF (%)	7.1	7.1	0
Physical inactivity (%)	42.9	28.6	35.7
Cigarette smoking (%)	28.6	57.1	14.3
Consumers of fast food %	7.1	14.3	14.3

university students (N = 122) divided according to different sex and faculty groupings are given in Table 1. Overall, we found that 22.1% of university students were prehypertensive, 13.9% of them showed hypertension I and 4.1% hypertension II. 17.2% of the students were overweight, 10.7% of participants had overvalues of BF, respectively.

The means of BMI, sBP and dBP in the males were higher in comparison with the females (who showed higher body fat, higher percentage of body fat overvalues, physical inactivity than males). Generally, the means of sBP were above the acceptable range in the males (mainly from the Faculty of Law). Males were more hypertensive and overweight than the females; although, women showed relatively high prevalence of body fat composition.

In comparison within the faculties, students from the Faculty of Education had higher values of BF (and overvalues of BF) and were more physically inactive (40%) than participants from other faculties. On the contrary, the students from other faculties showed excessive values of sBP, a higher prevalence of hypertension (31%), being overweight (19%) and cigarette smoking (33%) and a sedentary life style (35.7%)

In other faculties (Table 2), the highest means of BMI were interestingly recorded in the students from the Faculty of Philosophy and Arts, and excessive values of sBP in the students from the Faculty of Law. The highest-risk values of dBP were again measured in the sudents of Faculty of Law (and hypertension II). The students from the Faculty Philosophy and Arts showed the highest hypertension I prevalence. More than half of the students from the Faculty of Philosophy and Arts demonstrated the greatest presence of cigarette smoking. The students from the Faculty of Law had the lowest level of leisure time physical activity (after the participants from the Faculty of Education).

Table 3. Pair sample test (ANOVA) - relation of CVD risk predictors in the Trnava university students.

Pair	p
Gender-sBP	0.000
Gender-obesity	0.000
Gender-body fat	0.000
Gender-physical inactivity	0.000
Gender-cigarette smoking	0.000
Faculty grouping-hypertension	0.001
Faculty grouping-obesity	0.007
Faculty grouping-body fat	0.000
Hypertension-obesity	0.000
Hypertension-body fat	0.000
Hypertension-physical inactivity	0.011
Hypertension-cigarette smoking	0.000
Obesity-body fat	0.032
Obesity-physical inactivity	0.001
Body fat-physical inactivity	0.000
Body fat-cigarette smoking	0.019

Statistical significance $p < 0.05$.

Table 4. Pearson correlation of some parameters in the examined university students.

Pearson correlations (r)	dBP	BF	BMI
Age	0.816**	0.210*	0.313**
sBP	-	-	0.263**
dBP	-	0.508**	-

Statistical significance * <0.05 and **<0.01.

Statistically significant differences of $p < 0.001$ were detected in the values of BF, BMI, sBP and dBP in university students just as strongly for males and females. By gender, significant differences were found in obesity ($p = 0.005$), hypertension ($p = 0.025$) and physical inactivity ($p = 0.03$). The students from other faculties (with the exception of the Faculty of Education) significantly differed in being hypertensive, physically inactive and cigarette smoking ($p < 0.05$). x^2 test also confirmed that three faculty subgroups were statistically different in presence of cigarette smoking ($p = 0.028$). By faculty grouping, surprisingly significant differences in prevalence of hypertension ($p = 0.03$), obesity and excessive BF ($p < 0.001$) were evaluated among the students. By a pair samples test, significant association was also analyzed in hypertension/obesity, hypertension/body fat, cigarette smoking/hypertension, physical inactivity/body fat ($p < 0.001$) (Table 3). The variables presented in Table 4 showed the middle strong Pearsons correlation BMI and BF ($r = 0.508$**), mild relation sBP and BMI ($r = 0.313$**) and BF and sBP ($r = 0.210$*).

DISCUSSION

We can state that the presence of some cardiovascular risk factors is rather high among first-year Trnava University students. 22.1% of university students were prehypertensive, 13.9% of them showed hypertension I and 4.1% hypertension II. 17.2% of the participants were overweight, 10.7% of participants showed overvalues of BF. The males (in comparison with the females) had higher excessive means of sBP (134.05 ± 18.79 mmHg). They showed a higher prevalence of prehypertension (40.9%), hypertension I (22.7%) and hypertension II (4.5%), being overweight (40.9%) and cigarette smoking (36.4%). We counted the higher means of BF (22.85 ± 5.39%) and physical inactivity (43.2%) in females. Significant gender differences were analyzed in all parameters ($p < 0.001$). We found correlations between gender-obesity (0.005), gender- hypertension (0.025), gender-physical inactivity (0.03). The excessive means of sBP (133.95 ± 17.09 mmHg) were determined in the students from other faculties.

Although the participants from the Faculty of Education showed higher means of body fat (24.20 ± 5.14%) and sedentarism (40%), we compared higher means of all other anthropometric parameters and a higher prevalence of hypertension, being overweight and obesity in the students from other faculties. By faculty grouping, the highest prevalence of being overweight, hypertension I and the highest BMI was determined among the students from the Faculty of Philosophy and Arts. The students from other faculties (with the exception of the Faculty of Education) were significantly different in being physical inactive, hypertensive and cigarette smoking ($p < 0.05$). x^2 test showed statistically significant association of faculty grouping and cigarette smoking ($p = 0.028$). Students from the varying faculties differed in the prevalence of hypertension (0.03), obesity and excessive BF ($p < 0.001$).

By a pair samples test, significant association was also analyzed in hypertension/obesity, hypertension/body fat, hypertension/cigarette smoking, and body fat/physical inactivity ($p < 0.001$). The middle strong relation BMI and BF ($r = 0.508$**), mild relation sBP and BMI ($r = 0.313$**) and BF and sBP ($r = 0.210$*) were proved. A significant correlation between being overweight and BP ($p < 0.001$) was also determined.

Regarding to CVD risk variables, our findings are in agreement with several studies performed on young adults (Keller et al., 2008). We strengthened previous reports with a relevant conclusion indicating significant relation between hypertension/obesity, hypertension/body fat, hypertension/cigarette smoking, and body fat/physical inactivity ($p < 0.001$).

It has been proved several times that gender is the

factor to which CVD factors are associated (Garaibeh et al., 2012), as well as our results confirmed by statistically significant gender variations in obesity, hypertension and leisure-time physical activity ($p < 0.05$). Similar to Jordan and Hungarian students, our examined males were more likely to be overweight and have higher BMI than females, who additionaly recorded higher physical inactivity similar to Portuguase female university students.

A relatively high proportion of students from other faculties (compared with Faculty of Education) and males (than females) were predictably determined in a higher risk of obesity and hypertension. Consistently, several reports demonstrated a higher occurrence of elevated blood pressure, and a higher percentile distribution of being overweight, abdominal obesity according to the male group generally less interested in health enhancing activities and being more physically inactive (Pihl et al., 2002; Gupta and Kopor, 2010). Lower rates of hypertension among women may be affected by protective estrogen and non-smoking (Faraijan et al., 2008). 38.5% of all participants (43% of females) do not practice any type of physical exercise, which can be regarded as a warning sign. Accordingly the often inadequate diet (caloric intake at meals and fast foods) which can result in poor nutritional habits (Irazusta et al., 2007), CVD family history and childhood obesity could be the other important contributors to the development of obesity among students of both genders over time.

Generally, all population of university students showed a high risk of sedentarism (38.5%). Our study noted prehypertension in almost a quarter of the population from the Faculty of Education, which may be affected by the 43% of sedentarism, especially among females that predominated in the sample. 42.9% of students from the Faculty of Law also prefered a sedentary life style which can certainly be associated with a higher prevalence of hypertension (compared with other faculties), too. The beneficial effects of leisure time physical exercises should change an inactive lifestyle leading to reduction of obesity and hypertension among colleges. 22% of Trnava University students admitted being smokers, somewhat lower than the population of university students from Chile (39.8%) (Palomo et al., 2006). Surprisingly, a rather high frequency of cigarette smoking was determined among students from other faculties (33.3%) which significantly varied in those faculties ($p < 0.05$). Alarmingly, a high proportion from the Faculty of Philosophy and Arts recorded 57.1% of cigarette smoking which may be the main cause of a high prevalence of hypertension I (35.7%). Relating cigarette smoking to the analysed risk predictors of CVD among our young university students was strong. Northeless, it could reach epidemic proportions and may worsen the health status among well-educated Slovak inhabitants in the future.

Our examined students from the various faculties significantly differed in being physically inactive, hypertensive and the prevalence of cigarette smoking ($p < 0.05$, the latter being detected by x^2 test) and in a prevalence of obesity and excessive BF ($p < 0.001$). Keeping this in mind, some variation in CVD predictors between faculty subgroups in university students may especially be explained by their specific educational trend (Abdullahi and Amzat, 2011). Some discrepancies hypothetically exist between individual level of the knowledge CVD risks and the practice of lifestyle. As expected, the lowest prevalence of CVD risk factors was assessed among participants from Faculty of Health Care and Social Work. The research oriented towards and tested on medical students, who naturally deal with more issues regarding health information, proved that the level of education has a positive impact on the knowledge and prevalence of CVD factors and complications (Almas et al., 2008). However, there are some studies that report notably high cardiovascular risks in young medical students (Brandao et al., 2011). Furthermore, the high representation of university education is located in Slovak cities (Trnava is the nearest city to the capital city of Slovakia). Urbanization with its negative changes can also force high-educated Slovaks to adopt an unfavorable life style. Due to the phenomenon that has existed among Eastern European university students, the same students had placed a higher value on their health contrasting with their less favourable lifestyles, which has equally contributed to poor health status among Slovak University students despite their assumed higher level of the education and knowledge about health.

The present study is limited by a relatively small number of population. In view of that fact, we can regard representative sample from the Faculty of Education and the study that provides gender differences. Furthermore, current study has shown a predisposition to obesity and hypertension related to environmental university condition among young Slovaks. Despite some limitation, there has not been any Slovak study undertaken on University students to monitor faculty variation in the prevalence of CVD risk predictors.

Further limitation of this pilot study is an absence of assessment of different socioeconomic (Johnson et al., 1994) and ocupantional stressors. We also could not investigate all cardiovascular factors (biochemical markers) because of lacks of financial funds. The next research might be able to explore and longitudinaly study the other risk predictors and negative effect of CVD on college students. By some striking findings, the following recommendations (not only for our examined students) are summarized:

1. A sedentary life style certainly related to higher composition of body fat. It is especially recommended that the females from the Faculty of Education should participate in more leisure-time physical exercises. University students from the Faculty of Law should also modify physical inactivity. As previously mentioned, the

physical activity emphasizes the importance of enhancing positive attitudes for healthier lifestyles (Abdel-Megeid et al., 2011).
2. The need to eliminate the risk of high body fat not only in female students, but also on a reduction and normalization of weight and hypertension among males. A high prevalence of cigarette smoking among males has been linked to the prevalence of hypertension.
3. Other recommendation for students from the Facutly of Philosophy and Arts is to reduce BMI and cigarette smoking. Its related complications should be improved by a better quality of life (Oncel et al., 2011; Martínez et al., 2012). Students from the Faculty of Law should mediate blood pressure overvalues with high presence of hypertension II and sedentarism.

The alarming list of health disadvantages could continue to grow among first year university students who adapt to the common, mostly sedentary and unhealthy life style that can be found at universities. The possible roles of lifestyles, knowledge about important roles of some CVD predictors, health and behaviour awareness should be integrated into specific educational programs for first-year Slovak University students in Trnava.

Conclusion

These findings accent a CVD risk among young university students, which a lot of surveys of different nations have just highlighted. Evidently, the negligible percentage of first-year university students showed some degree of obesity, hypertension, cigarette smoking and a sedentary lifestyle which should be modified. Our results indicate the need for implementing effective preventive reduction to the major CVD factors related to an unfavorable exogenous condition among young adult Slovaks with their specific educational trend .

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ABBREVIATIONS

CVD, Cardiovascular disease; **BF,** body fat percentage; **BMI,** body mass index; **BP,** blood pressure; **sBP,** systolic blood pressure; **dBP,** diastolic blood pressure; **RF,** risk factors.

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A study of the antioxidative potentials of acetone and aqueous extracts of *Parkia biglobosa* and *Tetracarpidium conophorum* stem barks *in vitro*

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A study of *in vitro* antioxidative potentials of acetone and aqueous extracts of *Parkia biglobosa* and *Tetracarpidium conophorum* stem barks were investigated. At all concentrations investigated (50 to 250 µg/ml), the acetone extract of *P. biglobosa* and *T. conophorum* stem barks failed to scavenge hydroxyl radical *in vitro*. Polyphenol extract of *T. conophorum* stem bark failed to exhibit antioxidant activity *in vitro* at all concentrations except at 250 µg/ml, where it exhibited weak antioxidant activity (19.6%). *P. biglobosa* extract showed concentration dependent increase in antioxidant activity with increase extraction time (10 to 30 min). The maximum antioxidant activity of the aqueous stem bark extracts of *P. biglobosa* and *T. conophorum* were 87.68% at 50 min and 76.16% at 10 min, respectively.

Key words: Bioassay, clinical medicine, natural products, active principle and bioactive compound.

INTRODUCTION

Free radicals are known as major contributors to several clinical disorders, such as diabetes, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism (Parr and Bowell, 2000). Natural products have the potential to be developed into new drugs for the treatment of various diseases (Chen et al., 2009). Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics (Lobo et al., 2010). It is believed that medicinal plants are a potential source of reactive oxygen species scavenger molecules (Anandjiwala et al., 2008). Plant extract could be utilized as a source of nutritional phenolics (Kuate et al., 2011). There is a growing interest in natural anti-oxidants present in medicinal and food plants that might attenuate oxidative stress (Silva et al., 2007).

Parkia biglobosa is popularly known as the African locust bean (Osundina, 1995). It is a perennial tree of legume, belonging to the family Leguminosae (Campbell-Platt, 1980). The seeds of the plant are embedded in a yellowish, mealy, sweet tasting edible pulp (Aliero et al., 2001). It is a plant recognized to be very rich in phenolic compounds (Millogo-Kono et al., 2008). The bark of the plant contained epigallocatechin, epicatechin 3-*O*-gallate and epigallocatechin 3-*O*-gallate (Alabi et al., 2005). The leaf extract contains cardiac and saponin glycosides (Ajaiyeoba, 2002). The fruit pulp and seeds are rich in proteins and lactose (Alabi et al., 2005). The seeds

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contain antinutritional factors (oxalate, hydrogen cyanide, tannin and phytate) (Mohan and Jonardhaman, 1995).The *P. biglobosa* extract possesses antibacterial (Millogo-Kone et al., 2008), antidiabetic (Odetola et al., 2006), antifungal (Fawole and Abioye, 2002), anti-inflammatory (Kouadio et al., 2000), anti-diarrhoeal (Agunu et al., 2005) and anti-hypertensive (Mohammed et al., 2005) properties. The leaves, barks and root are used in treating leprosy, eye sores, tooth ache and fever (Ajaiyeoba, 2002). The leaves, barks and pods are used to treat new and old wounds (Inngjerdingen et al., 2004). The bark is chewed by men for lack of virility (Irvine, 1961). The seeds are used as food seasoning (Ajaiyeoba, 2002). The leaves of *P. biglobosa* contained more flavonoid than the bark (Miollogo-Kone et al., 2009). Also, the total phenolics content of the leaves of the plant varied significantly year round (Miollogo-Kone et al., 2009).

Tetracarpidium conophorum belongs to the family Euphorbiaceae, and is known as African walnut (Dalziel, 1937). The plant is known as adala in Yoruba and ukpo in Ibo. It is used as a male fertility agent and the leaves of the plant are used for the treatment of dysentery (Ajaiyeoba and Fadare, 2006). *T. conophorum* extract possesses cardioprotective (Landbo and Meyer, 2001) and antioxidative (Fukuda et al., 2004) and antimicrobial properties (Ajaiyeoba and Fadare, 2006). The plant is used in hair dye (D'Amelio, 1999). The shell flour is used as a carrier for insecticides (Ensminger, 1993). It is a very low glycemic food (Liu et al., 2000). *T. conophorum* contained oxalates, phytates, tannin as well as proteins, fibres, oil and carbohydrates (Enujuigha, 2003). The seed extract of *T. conophorum* possesses agglutin I and agglutin II (Animashaun et al., 1998). The seed flour oil is used as a vulcanized oil for rubber (Oke and Fafunso, 1975). The aqueous extract of the nut is used to relieve pain, increase sperm count and enhance sexual performance (Alade and Umukoro, 2011).

The *in vitro* antioxidant activity, chelating ability and total phenolic content of the aqueous seed extract of *T. conophorum* have been reported (Olabinri et al., 2010a). The *in vitro* antioxidant activity of the stem bark of *P. biglobosa* has been studied (Molliogo-Kone et al., 2009). To the best of our knowledge, the influence of extraction time on antioxidant activity of both plant parts has not been addressed. Also, there is no scientific paper which investigates the *in vitro* hydroxyl radical scavenging potentials of the acetone fractions and aqueous extracts from the stem barks of *P. biglobosa* and *T. conophorum*. Therefore, the study was carried out to assess: (i) *in vitro* hydroxyl radicals scavenging potentials, (ii) the influence of extraction time on the total phenolics concentration of the aqueous extracts of both stem barks of the two plants, and (iii) the relationship between the total phenolics concentration and the antioxidant activity of the aqueous extracts of the stem barks of the two plants.

MATERIALS AND METHOD

Reagents

Tannic acid, iron (ii) sulphate and 1,10-phenanthroline were products of British Drug House (BDH), UK. 2,2-diphenyl-1-picrylhyrazyl was purchased from Sigma-Aldrich, USA. Folin-Ciocalteu was a product of Merck, Germany. Hydrogen peroxide used was a product of Sigma-Aldrich, Switzerland.

Preparation of extracts

Collection of plant materials and preparation of aqueous extracts

The stem barks of *P. biglobosa* and *T. conophorum* were obtained in November, 2009 from Ogbomoso North Local Government area of Oyo State, Nigeria. The stem barks were washed with distilled water and dried at room temperature for four days and pounded using pestle and mortar into powder. Aqueous extracts of the plants were prepared by adding 5 ml of distilled water to 0.05 g (0.1% w/v) of the powder and centrifuged (5,000 rpm) at different time intervals (10, 20, 30, 40 and 50) min for each 5 replicates.

Extraction of polyphenol from P. biglobosa and T. conophorum stem barks

The acetone extracts of *P. biglobosa* and *T. conophorum* stem barks were prepared by soaking 25 g of the *P. biglobosa* stem bark and 25 g of *T. conophorum* stem bark powder in 75 and 100 ml of acetone, respectively for 24 h and then filtered. The filterates were allowed to evaporate and the final residues were the acetone extracts of the two plants. They were weighed and found to be 1.5 g for *P. biglobosa* and 1.2 g for *T. conophorum*. Therefore, the yield was 6 and 4.8% respectively. A quantity (0.2 g) of the acetone extracts were weighed and mixed with 20 ml of 70% ethanol for each. One milliliter (1 ml) of these stocks were taken, mixed with 9 ml of 70% ethanol to obtain 1000 µg/ml stock from which different concentrations (50 to 250 µg/ml) were made for the two acetone extracts with 5 replicates for each concentration.

In vitro assays

Total phenolic concentration estimation

The total phenolic concentration of the samples was estimated according to the method of Hung et al. (2002). The phenolic group present in plant extract interacts with Folin-Ciocalteu reagent in alkaline medium using Na_2CO_3 solution, giving a blue colour which has maximum absorption at 765 nm. The extracts reduce Folin-Ciocalteu reagent (yellow solution of polyphosphotungstate and molbydate) in mild base medium to form deep blue colour. Briefly, five hundred microlitre of Folin-Ciocalteu reagent (10% w/v, aqueous) was added to 0.1 ml of samples of different concentrations (50 to 250 µg/ml) and of 0.1% (w/v) concentration followed by the addition of 0.4 ml of aqueous Na_2CO_3 (7.5%, w/v). The mixture was allowed to stand in the dark for 30 min.The absorbance of the blue colour solution was read at 765 nm on a

spectrophotometer (Genesy 10vis, Thermoelectronic Incorportion, USA) against blank (distilled water). Total phenolic concentration (mg/ml) of the sample was extrapolated from a standard curve constructed using tannic acid as a standard.

Antioxidant potential estimation assay

The antioxidant activity of the aqueous and acetone extracts was determined according to the method of Blois (1958). This is based on the ability of the extracts to inhibit stable diphenyl picryl hyhdrazyl radical (DPPH). In the presence of an antioxidant, DPPH radical obtains one or more electrons and the absorbance decreases (Koleva et al., 2002). Five hundred microlitre (0.5 ml) of 0.1 mM 70% methanolic DPPH solution was added to 50 µl of the samples of different concentrations (50 to 250 µg/ml) and of 0.1% (w/v) concentration in a test tube. The mixtures were allowed to stand in the dark at room temperature for 30 min. The absorbance of the yellow color solution was read at 517 nm on a UV/visible spectrophotometer after 30 min against blank (distilled water). Antioxidant activity was expressed in terms of inhibition of DPPH free radical:

$$\text{Antioxidant activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ = Absorbance of the methanol DPPH solution (0.1mM) in the absence of sample. A_{sample} = Absorbance of the reaction mixture in the presence of sample.

Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity of samples of different concentrations was determined according to the method of Yu et al. (2004). Hydroxyl radical is generated *in vitro* by mixing $FeSO_4$ which generates ferrous ion (Fe^{2+}) with H_2O_2 and phenanthroline. The 1,10–phenanthroline was used since phenathroline-Fe^{2+} is a commonly used indicator of redox reaction. The H_2O_2/Fe^{2+} system produces hydroxyl radical through the Fenton reaction with the phenanthroline-Fe^{2+} complex oxidized to Fe^{3+}. The hydroxyl radical produced was then determined due to change in absorbance at 560 nm. 60µl of aqueous $FeSO_4.7H_2O$ (1 mM) was added to 90 µl of aqueous 1,10 phenanthroline, after which 2.4 ml of 0.2 M Na_2HPO_4 (pH 7.8) was added to the mixture, followed by the addition of 150 µl of H_2O_2 (0.17 M), and 1.5 ml of different concentrations of different concentrations (50 to 250 µg/ml), and 0.1% (w/v) concentration. The mixture was incubated for 5 min at room temperature. The absorbance of the mixture was read at 560 nm on a UV/visible spectrophotometer with distilled water as blank.

$$\text{Hydroxyl radical scavenging ability (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ = Absorbance of the control reaction mixture in the absence of the extract. A_{sample} = absorbance of sample in the presence of the other reagents in the reaction mixture.

RESULTS AND DISCUSSION

Our past research revealed that the aqueous seed extract of *T. conophorum* exhibited antioxidative potential *in vitro* (Olabinri et al., 2010a), while the aqueous extract of *T. conophorum* stem bark in the present study also displayed antioxidative capability *in vitro*. Regrettably, the acetone extract of *T. conophorum* stem bark failed to exhibit antioxidative potential *in vitro* at all concentrations investigated, except at 250 µg/ml with weak *in vitro* antioxidant activity (19.63%).

The aqueous extract of *P. biglobosa* stem bark showed increasing antioxidant activity with increasing extraction time except at 40 min extraction time. The order of increasing antioxidant activity with extraction time for the extract of the plant part was 10 < 20 < 40 < 30 < 50 min. On the other hand, antioxidant activity of the aqueous extract of *T. conophorum* showed increased antioxidant decreasing order with increasing extraction time except at 30 and 50 min. The aqueous extract of *T. conophorum* stem bark displayed maximum *in vitro* antioxidant activity (76.2%) at 10 min extraction time. The minimum *in vitro* antioxidant activity of the extract was 70.6% at 40 min extraction time. Moreover, at 50 min extraction time, the maximum total phenolic (26.78 mg/ml) of the aqueous extract of *T. conophorum* stem bark extract did not correspond to the maximum antioxidant activity (Table 1). Also, the maximum total phenolic of the aqueous extract of *P. biglobosa* (29.58 mg/ml) did not correspond to its maximum antioxidant activity (Table 1).

The order of increasing antioxidant activity with time of aqueous *T. conophorum* stem bark extract was 40 < 20 < 30 < 50 <10 min. The antioxidant activity values were 70.64 ± 0.03, 73.75 ± 0.04, 73.75 ± 0.04, 75.42 ± 0.05, 78.36 ± 0.01%. The aqueous extract of *T. conophorum* stem bark had the highest antioxidant activity at 10 min with 78.36 ± 0.01% and the lowest antioxidant activity at 40 min with 70.64 ± 0.03%.

The acetone stem barks extracts of *P. biglobosa* and *T. conphorum* did not exhibit any hydroxyl radical scavenging activity at all concentrations (Table 2). We have demonstrated clearly in our past research work that the polyphenolic extract of mango (*Mangifera indica*) leaves failed to scavenge hydroxyl radical *in vitro* (Olabinri et al., 2010b) at all concentrations (50 to 250 µg/ml), which was consistent with the results obtained in the present study for the acetone extracts of *T. conophorum* and *P. biglobosa* stem bark extracts. The acetone stem bark extract of *P. biglobosa* showed concentration dependent decrease in antioxidant activity between 50 to 150 µg/ml, while at 200 and 250 µg/ml it failed to exhibit antioxidant activity. At all concentrations, the acetone stem bark extract of *T. conophorum* did not exhibit any antioxidant activity, except at 250 µg/ml.

In our present research work, we observed moderate positive non significant correlation between total phenolic content and antioxidant activity for the aqueous extract of *P. biglobosa* stem bark at 30 min extraction time (r = 0.5;

Table 1. Changes in the levels of antioxidant activities and total phenolic contents of aqueous extracts of *P. biglobosa* and *T. conophorum* stem barks.

Extraction time (min)	*P. biglobosa* stem bark		*T. conophorum* stem bark	
	Antioxidant activity (%)	Total phenolic (mg/ml)	Antioxidant activity (%)	Total phenolic (mg/ml)
10	81.49±0.05	17.46±0.83	76.16±0.01	23.90±2.63
20	84.67±0.02	16.64±1.80	73.75±0.04	25.68±3.14
30	86.52±0.81	26.52±2.61	73.99±0.04	25.68±3.14
40	84.71±1.68	29.58±6.63	70.64±0.03	22.34±2.70
50	87.68±1.77	21.82±3.33	75.42±0.05	26.78±6.65

Values are mean ± SD of 5 analyses.

Table 2. Changes in the levels of hydroxyl radical scavenging and antioxidant activities of acetone extracts (50 to 250 µg/ml) of *P. biglobosa* and *T. conophorum* stem barks.

Concentration (µg/ml)	*P. biglobosa* stem bark		*T. conophorum* stem bark	
	Hydroxyl radical scavenging (%)	Antioxidant activity (%)	Hydroxyl radical scavenging (%)	Antioxidant activity (%)
50	-9.96±5.84	55.26±1.30	-24.11±3.36	-5.67±1.47
100	-1.65±5.29	49.88±1.72	-21.07±2.26	-14.89±1.77
150	-10.12±5.43	27.18±6.66	-16.96±1.24	-9.31±2.21
200	-19.50±8.35	-5.87±1.61	-26.17±4.15	-26.98±9.63
250	-29.60±7.95	-11.06±2.03	-26.66±6.37	19.63±1.73

Values are mean ± SD of 5 analyses.

P = 0.1, 0.05, 0.01, 0.001). The total phenolic content showed non-significant negative correlation with antioxidant activity in the aqueous extract of *P. biglobosa* at 10 min extraction time (r = -0.15; P = 0.05, 0.01, 0.001). At 50 min extraction time, a weak non-significant positive correlation was observed between total phenolic content and antioxidant activity for the aqueous extract of *P. biglobosa* stem bark (0.14; P = 0.05, 0.01, 0.001).

Moreover, non-significant positive correlations were observed between these two parameters at 30 to 50 min.

In our past research work (Olabinri et al., 2010a), we have shown weak negative non-significant correlation between total phenolics and antioxidant activity for the aqueous seed extract of *Tetracarpidium conophorum* at all concentrations. However, weak positive non-significant correlations were observed between total phenolic content and *in vitro* antioxidant activity for the aqueous extract of *T. conophorum* stem bark in the present study between extraction time intervals (20 to 50 min) (r = 0.31, 0.61, 0.35 and 0.49, respectively; P = 0.05, 0.01, 0.001). At 10 min extract time, a moderate negative non-significant correlation was observed between these two parameters in aqueous extract of *T. conophorum* stem bark.

A strong relationship was observed between total phenolic content and antioxidant activity in selected plants extracts by some researchers (Javanmardi et al., 2003; Velioglu et al., 1998). In addition, some reports revealed that there was no relationship between the total phenolic content and the antioxidant activity (Hinneburg et al., 2006; Motalleb et al., 2005) even though it was demonstrated that the phenolic substances are responsible for the antioxidant activity of plant materials (Rice-Evans et al., 1996).

Plant natural antioxidant sources are primarily plant phenolics which may occur in virtually all plants (Kahkonen et al, 1999; Pratt and Hudson, 1992). Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical scavenging, lipid peroxidation and chelating of metal ions (Shahidi et al., 1997). *P. biglobosa* and *T. conophorum* are plant recognized to be very rich in phenolic compounds (Kouadio et al., 2000; Tringali et al., 2000; De and Ifeoma, 2002; Enujiugha, 2003). The phenolic composition and content depends on different factors such as infusion time, the water to-part of plants

ratio (leaf, stem bark) and the amount of agitation (Astill et al., 2001). The molecular conformation of phenolic compounds could be one of the factors affecting their antioxidant activity which is intrinsically related to DPPH (Silva et al., 2007).

Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology (Wang et al., 2006).

Conclusion

Both polyphenol extracts of *P. biglobosa* and *T. conophorum* stem barks failed to scavenge hydroxyl radical *in vitro* while the maximum antioxidant activity of both aqueous and polyphenol extract of *P. biglobosa* stem barks was significantly higher than that of the aqueous and polyphenol extract of *T. conophorum* ($P < 0.05$). The present study revealed that *P. biglobosa* is a better source of natural antioxidants than *T. conophorum.*

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Biochemical factors relevant to kidney functions among Jordanian children with beta-thalassemia major treated with deferoxamine

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Thalassemia is one of the systematical diseases that occur worldwide and is the commonest form of hemoglobinopathy in Jordan. The most important cause of mortality and morbidity in these patients with thalassemia is organ failure related with the shortened red cell life span, rapid iron turnover and tissue deposition of excess iron. These are the major factors responsible for functional and physiological abnormalities found in various forms of thalassemia. This study aied to examine the biochemical factors related to kidney functions such as glucose, urea, creatinine, sodium and potassium levels among Jordanian children with β-thalassemia major treated with deferoxamine. Forty two patients (aged 12 to 28 years) with β-thalassemia major (20 males and 22 females) that undergo periodical blood transfusion and they are on deferoxamine (DFO) as chelating agent were involved in this study. All patients were free from hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). The diagnoses of β-thalassemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles for the patients. Haemoglobin (Hb) electrophoresis for the father and mother and genetic study of the b globins genes in some disputable cases were also done. Forty controls of matched age and gender (20 males and 20 females) were also included in this study. Results showed that the significant differences ($p < 0.05$) appeared between the experimental and control groups over all the measured physiological variables (urea, creatinine, uric acid, sodium and potassium) except for blood glucose and chloride. It is concluded that the functional abnormalities of the kidney in patients with β-thalassemic patients can be attributed to chronic anemia, iron overload as well as to DFO toxicity and enhancement of the oxidative stress induced by excess iron deposits. These functional abnormalities would have any long-term effects on the patients.

Key words: β-Thalassemia major, renal function, desferrioxamine, iron overload.

INTRODUCTION

Beta-thalassemia major is one of the most common hereditary hematologic disorders characterized by severely impaired β-globulin synthesis. Beta thalassemia major (BTM) is a common health problem in the Middle East, Africa, the Indian subcontinent, and Southeast Asia. BTM is a hereditary severe anemia resulting from defects in beta-globin synthesis **(**Modell et al., 2001; Rund and Rachmilewitz, 2005). Beta thalassaemia major is the most prevalent type of thalassaemia as it is common in certain populations. It produces severe anemia in its

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homozygous state (Widad et al., 2003). About 190 million people throughout the world have genetic mutations associated with different hemoglobinopathies, and more than 90 million of them carry defective genes leading to thalassaemia (Ambekar et al., 2001; Das et al., 2004).

According to Yesilipek (2007), there are over 200,000 beta thalassemia patients in the Mediterranean area alone. According to the Jordan Ministry of Health (MOH), there are about 1400 registered thalassemic patients in Jordan till this time and noteworthy that 4 to 6% of Jordan's population has the characteristic of the disease but are not infected, of whom health care has a large burden on health budget and family economics and social complains. The disease is associated with hemolysis in the peripheral circulation, and deposition of excess iron in the tissues, are some of the causes of clinical manifestations (Yesilipek, 2007).

Iron deposition in the heart, liver, and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. Profound anemia and excess iron deposition leads to dysfunction of cardiovascular, reticuloendothelial, and other organ systems (Muncie and Campbell, 2009). However, the most serious complication of iron overload is life-threatening cardiotoxicity. Cardiac events due to iron overload are still the primary cause of death (Papanikolaou et al., 2005). Iron overload causes most of the mortality and morbidity associated with thalassemia. Iron-chelating therapy is largely responsible for doubling the life expectancy of patients with thalassemia major: it has been proven to prevent liver and heart damage, allow for normal growth and sexual development in children with thalassemia, and increase life span (Rund and Rachmilewitz, 2005). There are many reports on complications of β-thalassemia in different organs (Low 2005; Al-Rimawi et al., 2005; Angelopoulos et al., 2006; Cetin et al., 2003; Asma et al., 2003).

In the absence of chelating therapy, the accumulation of iron results in progressive dysfunction of the heart, liver, and endocrine glands. In the last 30 years, conventional treatment of β-thalassaemia major, based primarily on regular blood transfusions and iron chelation therapy with desferrioxamine (DFO) and know a day with desferizirox (exjade) has markedly improved the prognosis of the disease. Adequate administration of parenteral DFO reduces or prevents iron accumulation and iron-mediated organ damage, resulting in a consistent decrease of morbidity and mortality (Wong and Richardson 2003). There are little information available about renal involvement in this disease. In recent years, ther are evidences of aminoaciduria, hypercalciuria, phosphaturia, magnesiuria, hyperuricosuria, low urine osmolality, and excess urinary secretion of markers of tubular damage such as N-acetyl-D-glucosaminidase in patients with beta-thalassemia major (Aldudak et al., 2000; Sumboonnanonda et al., 1998; Sadeghi et al., 2008).

Renal failure is a terminal event in thalassemia major and is usually secondary to heart failure and/or hepatic failure. Acute renal failure following deferoxamine overdose or hemolysis has been reported (Prasannan et al., 2003). There are limited studies on renal involvement in β-thalassemia, mainly involving patients on deferoxamine, reporting both glomerular and tubular dysfunction. In the present study, our aim was to evaluate the renal manifestations in patients with thalassemia major. In order to evaluate the effect of DFO and iron overload on the renal functions among Jordanian thalassemic children treated with DFO, we examined, for the first time, a number of biochemical variables such as glucose, urea, creatinine, uric acid and electrolytes as Na, K, and Cl.

MATERIALS and METHODS

Study patients

Forty two patients (aged 12 to 28 years) with β-thalassemia major (20 males and 22 females) that underwent periodical blood transfusion and they were on DFO as chelating agent were involved in this study. The diagnoses of BTM were made based on the clinical, hematological and hemoglobin electrophoresis profiles and the results of β-goblin chain synthesis at Thalassaemia Unit at Princess Rahma Educational Hospital, Irbid, Jordan. In addition, forty healthy individuals of matched age and gender were also included as controls. Furthermore, approval permission was obtained from the patients and the control persons and their parents. This study was conducted, ethical approval was obtained by the Institutional Review Board of Princess Rahma Educational Hospital. Medical histories such as clinical and transfused records of all 42 BTM patients were obtained from the hospital files. Informed consent was provided for each patient and healthy control and their parents', who participated in this study. All patients and controls were interviewed and filled out standardized questionnaires during this study. In addition, all patients and controls were tested and found free from hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). None of the studied patients had undergone splenectomy or other supplement treatment. All patients received regular blood transfusion after the age of one year old, usually given regularly every 2 to 4 weeks, to maintain a pre-transfusion hemoglobin level above 10 g dl^{-1}. None of the subjects was treated with vitamin E and/or vitamin C supplementations before the study. All patients were also started on subcutaneous infusion of DFO as chelating agent (45 mg kg^{-1} day^{-1}, for 8 to 10 each week) at age of two or three years old prior to presentation to us.

Blood collection

Five (5 ml) of venous blood sample was drawn into heparin from each BTM patient before the transfusion and from each healthy control. Three (3 ml) were centrifuged at 3,000 rpm for 10 min at room temperature. The serum samples were stored at 4°C until needed for analysis of urea, creatinine, uric acid, sodium, potassium, chloride, ferritin and blood glucose level. The remaining 2 ml were used for studying some hematological parameters such as hematocrit, hemoglobin levels and leukocyte counts.

Experimentals

Serum urea, creatinine, glucose and uric acid were examined using

Table 1. Hematological and biochemical data of s-thalassemia major patients.

Parameter	Male (n=20) Control	Male (n=20) Patients	Female (n=20) Control	Female (n=20) Patients	P-values
Hematocrit (%)	36±3.8	29±2%*	35±2.6	27±4.2*	0.000*
Feritin (µg/l)	78±21	2564±762*	62 ±18	2389±684*	0.001*
Hemoglobin (g/dl)	12.9±0.7	9.2±1.6*	11.1±0.9	8.4±2.2*	0.001*
Leukocytes × 10^6/L	10.56±2.31	10.42±3.26	9.44±2.67	10.2±2.46	0.463
AST (IU/L)	37.27±5.18	39.14±3.42	36.9±6.60	41.13±5.37	0.001*
ALT (IU/L)	33.66±8.05	36.62±5.53	34.85±6.20	37.87±6.40	0.001*

*The mean difference is significant in comparison with control untreated group (P < 0.05).

Table 2. Laboratory characteristics and significance testing in the thalassemic and control groups.

Parameter	Experimental N=42	Control N=32	P-value t-test	P-value Mann-Whitney U-test
Glucose (mg/dl)	113.36±52.52	98.75±13.54	0.13	0.432
Urea	33.5±5.50	29.05±7.30	0.005*	0.009*
Creatinine	0.352±0.113	0.296±0.0533	0.012*	0.012*
Uric acid	4.42±1.083	3.57±0.473	0.009	0.008*
Sodium (mmol/L)	143.02±2.50	137.66±5.15	0.000*	0.000*
Potassium (mmol/L)	4.670±0.331	4.194±0.345	0.000*	0.000*
Chloride (mmol/L)	103.24±3.24	103.16±4.10	0.924	0.887

*Result is significant at the 5 % level.

commercial analytical kits from Sigma (St. Louis, Mo, USA). Sodium, potassium and chloride were measured using the ion selective electrod (ISE).

Statistical analyses

Analysis was conducted using Statistical Package for Social Science for Windows version 11.0 (SPSS, Chicago, IL, USA). Means and standard deviations were calculated and student's t-test was used to compare the two groups. P-values less than 0.05 were considered statistically significant**.**

RESULTS

The clinical utility of biochemical screening using multiple parameters has often been used to assess the functions of many organs in the body. The aim of the present study was to investigate the biochemical factors relevant to kidney functions among Jordanian children with β-thalassemia such as urea, creatinine, Cl, Na and K. The abnormality of these factors is known to have dangerous impact on the health of the thalassemic patients. Table 1 shows some hematological and biochemical results of the examined patients and the control group. It is clear from the results that a significant decrease of hemoglobin concentration was noticed in both males and females in comparison with controls. On the other hand, ferritin concentration was significantly higher in both males and females (2564 ± 762, 2389 ± 684, respectively) in comparison with controls (78 ± 21, 62 ± 18, respectively).

Table 2 shows the means and standard deviations for the studied variables relevant to kidney functions in the two groups. The results revealed that significant differences ($p < 0.05$) appeared between the thalassemic and the control group over all the measured physiological variables urea, creatinine, uric acid, Na and K, except for glucose, and chloride. Our results revealed a significant increase in serum urea level in experimental group compared to control group, even though it is within normal range (33.5 ± 5.50, 29.05 ± 7.30 mg/dl, respectively). The concentration of serum creatinine is the most widely used and commonly accepted measure of renal function in clinical medicine. Our results showed significant increase in creatinine concentration in the experimental group compared to the control group even though it is within normal range (79.8 and 60.5 mmol/L, respectively). Comparison of the results obtained from male and female patients showed no significant differences between them for all the variables studied. This indicates that there are no gender differences among thalassemic Jordanian patients in the studied group.

DISSCUSSION

Patients with beta-thalassaemia major are prone to metabolic complications, including different organ

dysfunction which can occur as single or multiple involvements. Although the actual mechanism is not definitive, the most likely explanation is related to anaemia and iron overload, in addition to lipid peroxidation, oxidative stress and free radical release (Walter et al., 2008).

In patients with beta-thalassaemia major, the most important cause of mortality and morbidity is organ failure due to deposits of iron. In our study, we investigated the kidney functions test in patients with beta-thalassaemia major. The determination of biochemical indices of renal function might help in the prevention of serious kidney damage. A rise in iron indices observed in our beta-thalassemia patients may be due to erythrocyte hyperhemolysis and to chronic blood transfusion. Similar results were found in the study of Asma K et al. (2003), the significant increase of serum ferritin in the patients indicated an existing iron overload. The acute iron overload found in beta-thalassemia can lead to an iron intestinal hyperabsorption and to an abnormal molecular iron form (non-transferrin-bound: NTBI) accumulation. NTBI has hepato and cardio-cytotoxic properties. Furthermore, NTBI contributes to the formation of free radicals and increases hemolytic process (Borgna–Pignatti et al., 2004). The released iron could play a central role in the oxidation of membrane cells and senescent cell antigen formation, one of the major pathways for erythrocyte removal. We revealed no significant difference of blood glucose in thalassemic patients compared to controls (113.36 ± 52.52, 98.75 ± 13.54, respectively). We suggest that the duration of iron chelating therapy can prevent the pancreatic hemosiderin deposition and the damage to β cells leads to diabetes, and intensive combined chelation therapy may have a positive effect on glucose metabolism. More studies (Brittenha et al., 1994; Brittenham, 1992) have indicated that adequate iron-chelation therapy can prevent complications, including diabetes. Serum levels of urea and creatinine as waste products formed during the digestion of proteins and in urine as the vehicle for ridding the body of nitrogen is used as indicators for renal function.

Our results revealed a significant increase in serum urea level in experimental group compared to control group (33.5 ± 5.50, 29.05 ± 7.30 mg/dl, respectively) even Though it is within normal range. The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine (Perrone et al., 1992). Our results showed significant increase in creatinine concentration in the experimental group compared to the control group (0.352 ± 0.113 and 0.296 ± 0.0533, respectively). The increasing level of urea and creatinine in thalassemic patients possibly due to higher iron deposition in their kidneys, shortened red cell lifespan and excess iron which causes functional and physiological abnormalities in various organ systems in thalassaemia patients.

β-Thalassaemia patients have a high prevalence of renal tubular abnormalities such as the kidney, suggesting that the damage might be caused by the anemia and increased oxidation induced by excess iron deposits (Oktenli and Bulucu, 2002). Iron overload, usually observed, generates oxygen-free radicals and peroxidative tissue injury as renal tubular (Kassab-Chekir et al., 2003). Some studies showed that the plasmatic urea and creatinine were significantly decreased in beta-thalassemia compared to controls (Kalman et al., 2005). Oktenli and Bulucu (2002) did not find any marked difference concerning blood urea and creatinine in the patient population and found that a urinary and suggested that the severity of renal abnormalities was correlated with anemia degree.

The least severe abnormalities were found in patients under hypertransfusion and desferrioxamine therapy. Renal involvement may occur by 3 mechanisms: deferoxamine side effects, deposition of iron in renal tissue, and vascular thrombosis and renal infarction due to increased platelet aggregation and decreased serum level of protein S and antithrombin III, and deferoxamine-induced kidney injury is more probable, because iron deposition may result in death of cardiac involvement, before kidney failure appears (Eldor et al., 1993). In one study of 19 patients treated with deferoxamine, tubular damage (by measurement of β2-microglobulin) was observed in 13 patients (Cianciculli et al., 1994). Acute kidney failure related to deferoxamine is usually nonoliguric and reversible with discontinuation of the drug (Koren et al., 1989). Based on autopsy reports of patients with thalassemia major, the most common glomerular findings are mesangial cell proliferation, mesangial matrix expansion, and hemosiderin deposition in glomerular and tubular cells. Iron deposition may result in tubulointerstitial fibrosis and atrophy (Buhl et al., 1993). Glomerular diseases may also develop; immunoglobulin A nephropathy was reported in a patient with thalassemia major (Harada et al., 1994).

Electrolyte levels are tightly controlled by several hormones and by the kidneys, which are primarily responsible for retaining and removing electrolytes when necessary and keeping them in a constant state of balance. An electrolyte imbalance can lead to serious health issues, including eventual death if not corrected. The most common imbalances occur with sodium and potassium. Such physiological variables related to glomerular filtration of the kidney as Na and K as the major cations of the extracellular and intracellular fluid were also studied. Our findings showed that there is significant increase in the serum Na and K in the patient group (143.02 ± 2.50, 4.670 ± 0.331, respectively) compared to the control (137.66 ± 5.15, 4.194 ± 0.345, respectively).

Disturbances in monovalent cation transport are manifested by osmotic swelling or shrinkage and this can be a consequence of rare genetic defects in cation

transport. Enhanced permeability of cations in thalassemia has been described previously (Wilairat et al., 1992). Increased serum level of potassium in β-thalassemia major was attributed to the rapid erythrocyte turnover (Cetin et al., 2003). There is also a relationship between abnormal K leak and hemoglobin precipitation on the membrane (Nathan and Gunn, 1966). Oxidative damage is responsible for the K-loss in β-thalassemia by increasing the activity of K-Cl cotransport (Wikramasinghe et al., 1984). Unchanged serum K concentration in both male and female thalassemic patients did not seem to be in agreement with earlier studies. The hypernatraemia in patients is associated with increased plasma osmolality, in contrasts with previously reported normal concentration. Abnormal membrane function plays a relevant role in the alteration of membrane cation transport as observed in thalassemic red blood cell counts (RBCs). The defective sodium, potassium transport in red cell and serum is associated with disturbed Na-K-ATPase (membrane bound) activity. Changes in the levels of serum sodium, potassium, calcium reflects the defective membranal transport of the cations in the red cell membrane of thalassemia. These results provide a confirmation that abnormal cation homeostasis may contribute to the pathogenesis of thalassemia.

Our results revealed that there is significant increase in uric acid in the patient group compared to the control group (Mann-Whitney U-test). Hyperuricemia is caused either by accelerated generation of uric acid through purine metabolism or by impaired excretion in the kidney, or by high levels of fructose in the diet (Chizyński and Rózycka, 2005; Nakagawa et al., 2006). We found significantly higher levels of uric acid in thalassemic group, which was predictable due to the higher cellular turnover secondary to the use of hydroxyurea (Becker et al., 2005).

Conclusion

Renal disorders are not rare in patients with beta-thalassemia major and they may increase in terms of frequency with age, increased duration of transfusion and deferoxamine usage.

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UPCOMING CONFERENCES

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