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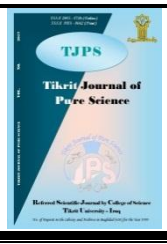

EFFECT OF OXIDATIVE STRESS ON LIPID PROFILE AND BLOOD PARAMETERS TO A SAMPLE OF STUDENTS AT UNIVERSITY OF ZAKHO DURING EXAMES

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Abstract

Worry, anxiety through Study and preparative to exam may lead to mental stress. Mental stress may contribute to oxidative stress in the body which is the change in balance between oxidants and antioxidants.
In this study a concentration of Malondialdehyde (MDA) as a lipid oxidation biomarker, lipid profile and some blood parameters like Minmum Inhibitory dilution (MID) were estimated in twenty chemistry and biology students at the day of exam as Stress Condition (SC) compared with the same parameters which obtained from the same students during the university vacation after a month of doing the latest exam as non-Stress Condition (nSC).
A significant increase in MDA ($p=0.0019$), Total Cholesterol ($p<0.05$), Triglyceride ($p<0.05$), Low Density Lipoprotein LDL-Cholesterol ($p<0.05$), Very Low density lipoprotein VLDL-cholesterol ($p<0.05$) and A significant decrease in High - Density Lipoprotein HDL - Cholesterol ($p<0.05$), HDL-Cholesterol to total cholesterol ratio($p=0.0012$) in stress condition were observed when compared with "non-stress" conditions. Also there were high significant decreases in lymphocyte and minimum inhibitory dilution (MID) ($p=0.0001$, $p<0.0001$ respectively) and significant increases in granulocyte and platelets (PLT) ($P=0.0113$, $P=0.0031$ respectively) in stress condition compared to non-stress condition. Whereas no significant changes in total WBCs and RBCs in stress and non-stress conditions.
These results suggested that during university examinations when students are under mental stress or psychiatric disorder may put them at risk of developing inflammatory disorders like atherosclerosis and/or coronary arteries disorders.

Introduction

Stress through exam always has been used in many stress research since it is being predictable, standardized, and discrete examples of real- life stressors. It is associated with increasing anxiety, change to negative mood and immune function disorder as results of changes in the mental and physical health statuses [1].
Many researchers were established the relationship between mental stress in daily life and also during university examination contribute to increase oxidative stress [2] [3].
Oxidative stress is an imbalance between oxidants which forms as free radicals like reactive oxygen species (ROS) and antioxidants defense systems [4][5], this imbalance may cause a real damage to biomolecules, such as lipids, proteins and nucleic acids but lipids are probably the most susceptible [6]. One of the initial events in the formation and development of atherosclerosis is the accumulation of foam cells that containing excess lipids within the arterial wall. In addition, increased intracellular generation of free radicals like reactive oxygen species (ROS) has been demonstrated to play an important role in chronic inflammatory responses [7].
The oxidation process which destructs lipids (lipid peroxidation) is a noxious, self-perpetuating chain

reaction, releasing Malondialdehyde (MDA) as the end product[8] which consider as One of the important biomarkers that frequently used as an indicator of the overall lipid peroxidation level as well as a free radical damage indication which result from oxidative stress [9].

Evidence suggests that mental status may effect on the lipid profile levels [10]. The biochemical features reflecting the stress levels in serum cholesterol and hypercholesteremia is consider as a major risk factor in coronary artery disease [11].

Stress situations also affect the ratio of blood cell numbers. The action of stress makes leukocytes refuge to defense sites in the body like skin, lung, gut epithelium and lymph nodes. This migrations in some blood cells associate to stress condition, psychiatric and action of individuals during scientific examinations [12][13][14].

Our study focuses on estimating the concentration of these variables MDA, lipid profile and blood cells ratios to determine how these parameters can altered with the level of examination stress.

Material and methods

1. Sample collection:

5 ml of Blood samples were collected from twenty chemistry and biology volunteers students their age range (20-22 years) when they were doing the final examine as a stress cases. Blood sample were collected again from the same students during the university vacation after a month from doing the latest examine as a non-stress cases. In the two cases 1 ml of each blood sample was added in a labeled EDTA tubes for blood account measurements and the remaining (4 ml) was left in clean and dry plain tubes for 30 minutes at 25 °C before separating the serum by using centrifuge at 3000 rpm then serum of each cases was divided by using a labeled Eppendorf tubes for MDL and Lipid profile measurements then stored in the refrigerator at -20 °C until they used .

All student were males, fasting and don't have any disease or taken any type of drugs or vitamins these information have been declared by the students themselves via questionnaire.

2. Serum Lipid Peroxidation Product (MDA) Assay:

The level of serum MDA was determined by a modified procedure described by [15].

3. Serum Lipid Profile Assay:

Total cholesterol concentration, high density lipoprotein HDL-Cholesterol concentration and triglyceride concentration were estimated by using an automatic analyzer with a tungsten lamp (Prestige 24i, Tokyo Boeki Medical System which used cormay kits) at Newroz medical Laboratory in Zakho city, Kurdistna region/Iraq. Concentrations of VLDL-cholesterol and LDL-cholesterol were calculated using the Friedewald equation [16].

VLDL = T.G/5; LDL = TC- (VLDL +HDL)

4. Blood account assay:

The blood samples of volunteers collected in anticoagulant tubes and immediately taken to Bedary hospital in Zakho city, Kurdistan region/Iraq, for blood profile estimation using Automatic hematologic Swelab Alfa Standard analyzer.

Statistical analysis

Statistical analyses were done by using paired t-test which performed using SPSS version 21.0 and all parametric data are expressed as the mean and standard error of mean, p- value ≤ 0.05 consider significant.

Results and discussion

As shown in Table (1), serum MDA, serum Total Cholesterol (T-C), serum Triglyceride (TG), serum High density lipoprotein Cholesterol (HDL-C), serum Low density lipoprotein Cholesterol (HDL-C) and very low density lipoprotein cholesterol (VLDL-C) were estimated on two occasions, during examination and after a month from doing the latest exam during university vacation. There was a significant increase in levels of MDA in stress condition at (p=0.0019) compare with non-stress condition and there were a significant increase in total cholesterol, triglyceride, Low density lipoprotein and Very low density lipoprotein at (p=0.0145, p=0.0165, p=0.0386, p=0.0165) respectively. HDL cholesterol levels showed a significant decrease in response to stress (p=0.0111). The ratio of HDL/T-C was significantly decreased in stress (p=0.0012) compared with non-stress.

Our study demonstrated elevated concentrations of MDA in stress condition which results as end product of lipid peroxidation. this is in agreement with the results of other researchers, such as Pérez-Rodríguez which proved that lipid peroxidation marker levels (plasma levels of malondialdehyde and hydroperoxidase) covary with circulating lipid levels (circulating levels of triglycerides and cholesterol) [17]. Increased lipid peroxidation thought to be a consequence of oxidative stress which occurs when peroxidants overcome antioxidants mechanism and the dynamic balance is impaired [18].

Also Surapon found that MDA level was significant correlated with serum lipid profile and other conventional cardiovascular risk factor in fifty type 2 diabetic patients compare with healthy control [9].

Many studies focused on changing the level of serum lipid profile during stress to find relations of atherosclerosis and coronary heart disease cases with mental stress. Agrawal found a significant increase in triglyceride and cholesterol levels due to examination stress in a study conducted on twelve medical students [11]. Bijlani have been shown a significant increase in T-C, LDL-C and HDL-C near exams [19]. HDL may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL [20]. Ahaneku found that during final exams, serum HDL-C and HDL to T-C ratio were altered as a risk

for coronary artery disease in a medicine students [21].

In similar results, Bhakti showed that serum T.G level increased significantly in 30 medical students exposed to examination stress and HDL to T-C ratio was significantly decreased during stress, also found non-significant increase in T-C and LDL-C in stress condition [22].

A cording to the results that are shown in Table (1), we can conclude that the mental stress may alter the level of lipid profile as a risk of coronary artery disease.

Studies showed that overexertion, anxiety, and stress increased the hormonal stress level in the blood [23].

Stress hormones include compounds released by both neurons and endocrine glands in response to stressful stimuli to provide fuel for a potential fight-or-flight situation. These neuroendocrine factors bind with receptors that induce the changes necessary to make the body ready for intense activity as liberation of energy compounds from storage [24].

When this energy left without used, it may gradually accumulates as fat tissue. In addition, high glucose level which occurs in stress condition is left unused and finally converted into triglycerides or other fatty acids; this may explain the change in lipid profile during exams.

Also Mental stress initiates the release of cortisol by activating corticotropin-releasing factor and arginine vasopressin neurons in the paraventricular nucleus of the hypothalamus [25].

Because of similarity between cortisol (cyclopentanoperhydrophenanthrene) as steroid and lipid in primary structure it is possible for this change (releasing of cortisol) to make alteration in the level of lipids concentration which consider the most cardiometabolic risk factors that may effect on the students' health during exams.

Table (1): Statistical analysis (paired t-test) for Means and SEM of MAD,T-C,TG,HDL-C,LDL-C,VLDL-C and HDL/T.C ratio using SPSS analysis

parameters	During-Exam		After-Exam		
	Mean	SEM	Mean	SEM	P-value
MAD µmol/L	9.33	0.2759	8.499	0.265	0.0019
T-C mg/dl	182.8	5.6	175.9	6.0	0.0145
TG mg/dl	170.20	7.6	157.75	8.16	0.0165
HDL-C mg/dl	41.85	0.74	42.85	0.69	0.0111
LDL-C mg/dl	106.91	6.191	101.5	6.39	0.0386
VLDL-C mg/dl	34.04	1.519	31.55	1.633	0.0165
HDL/T.C	0.2325	0.0083	0.2485	0.403	0.0012

Table (2): Statistical analysis (paired t-test) for Means and SEM of leukocyte, erythrocyte and platelets using SPSS analysis

parameters	During-Exam		After-Exam		
	Mean	SEM	Mean	SEM	P-value
WBC	7.575	0.459	7.93	0.418	0.151
LYM	2.0	0.11	2.73	0.143	0.0001
MID	0.51	0.03	0.67	0.032	<0.0001
GRAN	5.04	0.384	4.59	0.326	0.0113
PLT	115.6	10.23	87.75	6.59	0.0031
RBC	5.097	0.0902	5.1725	0.0947	0.1433

The histological tests of blood profile (Table 2) of students were showed that there were gentle decreases of total leukocytes and erythrocyte with no significant differences between SC and nSC. These results come Consistent with what many researchers conducted to [26][12][14].

Valéria showed that the physical exercises induce significant increases of circulating monocytes and small increases of lymphocytes [13]. While the current results showed decrease of MID in the SC that included eosinophil, basophil, monocyte and blastocytes with high significant differences ($p < 0.0001$) compared to nSC as shown in Table (2), Whereas granulocytes recorded significant increases ($p=0.0113$) in SC compared to nSC, these results come consistent to what [12][27][14] conducted to, and this is because neutrophil ratio is very high (60-80% of leukocytes) compare to eosinophil and basophil so any increases in neutrophil account make granulocyte ratio high in general without any effect to eosinophil and basophil ratios [28][29].

Many researchers found that the stress is responsible for activation hypothalamic–pituitary–adrenal axis (HPA) to keep effect on releasing glucocorticoids hormones that induce blood leukocytes (eosinophil, basophil, monocytes and lymphocytes) to take position at skin, mucosal lining of gastrointestinal and urogenital tract, lung, liver, spleen and lymph nodes [28][29]. Similar results have been recorded by using Adrenocorticotropic hormone (ACTH) which stimulates secretion of glucocorticoids induced alternation in the trafficking or redistribution of blood lymphocytes to other body compartments [27][30] [31].

McEwen revealed that increasable mortality and morbidity with heart coronary diseases among men who have repetitive and unstable jobs (stress conditions) attributed to high concentration of plasma fibrinogen [32]. Also, Koudouovoh-Tripp and Sperner-Unterweger mentioned that platelet activation and reactivation increased during mental stress as a result of activation of immune system [33]. Nowadays results show high significant level of platelets in SC during-test ($p= 0.0031$) compare to nSC post-test (Table 2).

Conclusion

It is concluded that scientific examinations for students showed that students are stressful enough to make alternations in some inflammatory parameters and affect psychiatric status that association to mental stress.

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تاثير الاجهاد التاكسدي على مرتسم الدهون وبعض المتغيرات الدميه لنموذج من الطلاب في جامعة زاخو اثناء الامتحانات

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الملخص

القلق والضغط النفسي خلال المذاكرة والاستعداد للامتحان قد يؤديان الى أجهاد ذهني والذي ربما يساهم بإحداث كرب تأكسدي في الجسم ناتج عن تغيير في التوازن ما بين المؤكسدات ومضادات الاكسدة.

في هذه الدراسة تم تقدير تركيز المالون داي الديهايد (MDA) كمؤشر حيوي لأكسدة الدهون وايضا مرتسم الدهون وبعض قياسات الدم مثل التخفيف المثبط الادنى (MID) لعشرين طالب من قسم الكيمياء وقسم علوم الحياة في يوم الامتحان كحالة اجهاد (SC) بالمقارنة مع نفس القياسات لنفس الطلاب خلال العطلة الجامعية بعد شهر من ادائهم اخر امتحان كحالة عدم اجهاد (nSC).

تمت ملاحظه زياده معنوية في المالونيل داي الديهايد (MDA) (P=0.0019) والكولسترول الكلي (P<0.05) و الدهون الثلاثية (P<0.05) والبروتين الدهني واطئ الكثافة (P<0.05) والبروتين الدهني واطئ الكثافة جدا (P<0.05) وانخفاض معنوي في البروتين الدهني عالي الكثافة الكوليسترول (P<0.05) وفي نسبة البروتين الدهني عالي الكثافة الى الكوليسترول الكلي(HDL-cholesterol/T-cholesterol) (P=0.0012) لدى الطلاب في حالة الاجهاد مقارنة بحالة عدم الاجهاد بالإضافة الى انخفاض معنوي عالي في الخلايا اللمفاوية والتخفيف المثبط الادنى (MID)(P<0.0001 ، P=0.0001 بالتعاقب) وزيادة معنوية بالخلايا الحبيبية والصفائح الدموية (P=0.0113 ، p=0.0031 بالتعاقب) في حالة الاجهاد مقارنة بحالة عدم الاجهاد. بينما لم يوجد اي اختلاف معنوي في كريات الدم البيضاء والحمراء في حالة الاجهاد مقارنة بحالة عدم الاجهاد.

تقترح الدراسة انه خلال الامتحانات الجامعية يكون الطلاب تحت تاثير اجهاد ذهني ونفسي ربما يؤدي الى تطور امراض التهابية كتصلب الشرايين او اعتلال الشرايين التاجية.