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Impact of Exam Stress on Blood Levels of: Cortisol, Glucose and Cholesterol in Pharmacy Students

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ABSTRACT

Stress is a sum of physical, mental and emotional tensions on a person. Examination is a stress factor for students, that may bring about blood parameters changes. Thus, this study investigates the change in Cortisol, Glucose and Cholesterol levels in blood under the effect of examination. A blood sample was used to measure the levels of glucose, cholesterol and cortisol among 30 pharmacy students at Al-Wataniya Private University. The study was conducted 2 weeks prior to the exams (pre-exam) and during exams. Glucose, cortisol, and cholesterol were increased significantly ($P<0.05$) during-exam in comparison with pre-exam period for all students and both sexes. Cortisol level increased significantly among females during exams compared to pre-exam while the increase was non-significant among males, which indicates that males react to stress in a more controlled manner than females. These findings suggest that the stress that the students are exposed to leads to blood parameters changes which predispose students to metabolic diseases.

KEYWORDS: Stress, blood parameters, Sex, Pharmacy Students.

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1. INTRODUCTION

Stress is a sum of physical, mental and emotional tensions on a person. Exposure to psychological stressors can modulate the primary antibody response and increased permanent stress levels can lead to pathological organ changes, psychological alterations as well as psychosomatic diseases [1] [2].

When an individual is threatened or faced by challenging situation, he can be subject to the activation of sympathetic adrenal medullary (SAM) system so that secretion of epinephrine and norepinephrine increases. Activation of SAM system prepares the individual to face the challenges or threats [3]. Prolonged stress is associated with the activation of hypothalamus pituitary adrenal (HPA). Activation of HPA releases adrenocorticotropic hormone (ACTH) and cortisol [3]. A study in 2018 showed that, extreme stress resulted in a nine-fold increase in cortisol compared with in relaxed periods [4].

The activation of HPA and SAM increases the arousal level, therefore energy is mobilized and aids the body in the fight or flight reaction. Energy is mobilized to vital organs for the purpose of proper functioning. The energy mobilization leads to an elevated blood concentration of glucose and lipids, and anabolism is downregulated [4] [5]. The reduced anabolism associated with prolonged stress is likely to be of great importance for stress-related disease. Almost all tissues in the body have glucocorticoid receptors. So, cortisol can affect nearly every organ system in the body, including: Nervous system, Immune system, Cardiovascular system, Respiratory system, Reproductive system, Musculoskeletal system, Integumentary system (skin, hair, nails, gland and nerve) [5].

Glucocorticoid is a functional antagonist of insulin, thus it decreases insulin sensitivity and increases blood glucose via a number of pathways such as upregulating enzymes of gluconeogenesis such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase. The increase in proteolysis and lipolysis, leads to increasing amino acids and glycerol levels which act as substrates for gluconeogenesis, decreasing insulin secretion from pancreas, and inhibiting glucose transporter-4, which is involved in the uptake of glucose into peripheral tissues. Catecholamines can also increase blood glucose concentrations by stimulating the secretion of cortisol and glucagon, as well as by enhancing metabolic rate, glycogenolysis, and gluconeogenesis [6]. Many Studies have shown changes in levels of glucose, cortisol and lipids in response to various types of stress. Mental stress such as examination stress can affect the lipid profile, it may even imply lipids elevations [7] [8] [9].

Research importance: Many research teams have investigated the impact of exams stress on students from different academic disciplines and from different communities. This study has never been done in Syria and this is the first study on a Syrian pharmacy student in Al Wataniya private university.

Research aim: Measuring blood levels of glucose, cortisol and cholesterol among pharmacy students in two periods: pre-exam and during exam to determine whether the psychological stress of the exam has an impact on these parameters.

2. Methodology

The study was conducted in the department of biochemistry laboratories of the faculty of pharmacy in Al-Wataniya private university. The study included 30 healthy pharmacy undergraduate students (18 females, 12 males) aged 21 -23 years. Exclusion criteria were hypertension, family history of diabetes, smoking, alcohol, neurological disease, psychiatric disorder, cardiovascular disease.

Fasting blood samples were drawn in the morning between 8 and 10 am from the right/left cubital vein. Blood was collected on anticoagulant (lithium heparin) tube and then it was centrifuged to obtain plasma.

The first sample was collected 2 weeks before the examination(pre-exam) when the students were not under academic stress. The second sample was drawn on the day of exam (during exam). Glucose and cholesterol were analyzed using the enzymatic method and Cortisol was analyzed using ELISA method. Normal values were: cholesterol 130-200 mg/dl, Glucose 60-110 mg/dl, Cortisol 6.02 – 18.4 µg/dl (morning hours 6-10 am). The mean of difference of values obtained at both periods: pre-exam and during exam were calculated and statistically analyzed using paired 't' test and Pearson coefficient, P-value < 0.05 was considered significant.

3. Results and discussion

3.1. Measuring the blood levels parameters in students during the rest period (pre-exams) and during exams

Plasma levels of cholesterol, glucose, and cortisol were measured 2 weeks before the exam period and compared with their levels during the exam period using Paired T-test. Plasma levels were within the normal range but significant differences were observed between the mean levels of the three blood parameters in the period pre-exams and during exams, as the levels were significantly higher ($P<0.005$), during exams (Table 1 and Fig.1). This indicates that the psychological stress experienced by students during the exam has a clear impact on increasing the levels of the three parameters. It was also noted that, Cholesterol and cortisol were correlated positively before and during the exam period as illustrated by Pearson's correlation coefficient ($r>0$, $P<0.05$), while Glucose showed weak and insignificant correlations between the two periods. (Table 2).

Table (1): Comparison of Mean values of parameters in students' blood samples in the periods pre-exams and during exams

Blood Parameters	Pre- exams (mean ±SD) n= 30	During exams (mean ±SD) n= 30	P-Value
Cholesterol (mg/dL)	132.1 ± 35.9	154.6 ±43.7	0.002
Glucose (mg/dL)	81.2 ± 21.5	93.9 ± 27.4	0.049
Cortisol (µg/dL)	10.8 ± 2.9	12.9 ± 3.4	0.003

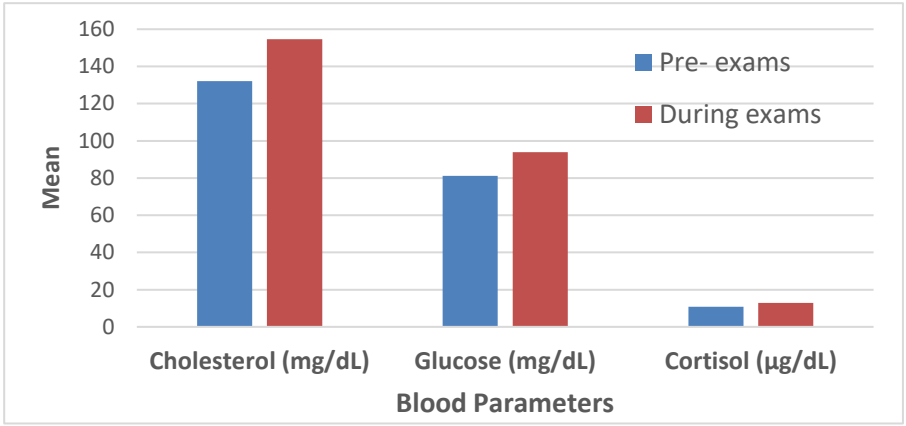


FIGURE (1): COMPARISON OF MEAN VALUES OF PARAMETERS IN STUDENTS' BLOOD SAMPLES IN THE PERIODS PRE-EXAMS AND DURING EXAMS

Table (2) : Correlation coefficients between blood samples parameters in the periods pre-exams and during exams

Blood Parameters	Pearson's Correlation (r)	P-Value
Cholesterol (mg/dL)	0.589	0.001
Glucose (mg/dL)	0.058	0.762
Cortisol (µg/dL)	0.409	0.025

Concerning Glucose, the result of this study is in agreement with a study conducted in 2016 by Jena and his comrades [6], Glucocorticoid is a functional antagonist of insulin. Thus it decreases insulin sensitivity and increases blood glucose via decreasing insulin secretion from pancreas, and inhibiting glucose transporter-4, which is involved in the uptake of glucose into peripheral tissues. Catecholamines can also increase blood glucose concentrations by stimulating the secretion of cortisol and glucagon. Thus, glucocorticoid and catecholamine induced raise in blood glucose that would help meet the increased energy demands of the stressor [6]. The activation of hypothalamus pituitary adrenal (HPA) axis and sympathetic adrenal medullary system by stress leads to an elevated blood concentration of glucose and lipids [4] [5].

Concerning Cortisol, many studies have demonstrated that, medical university students exhibit higher level of stress during exams than prior to exams which may have an effect on the students' performance [10] [11]. The result of this study is in agreement with many studies, where the students did not undergo examination stress they showed normal levels of the cortisol hormones. However, when exposed to exam conditions, the students underwent stress and consequently their HPA axis was excited, resulting in increased release of cortisol levels among the students [11] [12] [13].

The result of this study concerning Cholesterol is in agreement with a study by Singh and his comrades [14] , which showed an increase in total cholesterol during examination period due to the effect of sympathetic activation [14] . And this study is in line with Maduka and his comrades which showed statistically significant

increase in serum cortisol, adrenaline, Total cholesterol, HDL-cholesterol and LDL-cholesterol levels in students under examination stress compared to the non-examination period [15].

3.2. Investigating the effect of the sex factor on the blood levels parameters among students in the pre-exams rest period and during exams

Male and female data were sorted out and T-test was done to compare the mean levels of the studies parameters between the two groups. Pearson's correlation coefficient was applied as well to determine any relation between the parameters in the pre-exams and during exams periods among the male and female groups.

Results showed that plasma levels of **cholesterol** were higher non-significantly (P>0.05) in females compared to males in both of the study periods, which indicates that there is no effect of sex on cholesterol levels in pre-exams and during exams (Table 3).

Table (3): Comparison of Mean values of Cholesterol (mg /dL) between males and females in the periods of pre-exams and Comparison of Mean values of Cholesterol between males and females during exams.

Period of the study	Males n=12	Females n=18	P-Value
Pre- exams (mean ±SD)	113.7±24.1	139±49.5	0.113
During exams (mean ±SD)	157.4±89.2	161.3±40.9	0.870

Moreover, Cholesterol levels were higher in the during-exams period for both males and females compared to the pre-exams period but the difference was insignificant (P>0.05). Pearson's correlation coefficient showed weak and insignificant relations between the mean levels of cholesterol (Fig 2, Table 4).

Table (4): Comparison of Mean values of Cholesterol (mg /dL) and Correlation coefficients in the periods pre-exams and during exams depending on sex.

Sex	N of student	Pre- exams (mean ±SD)	During exams (mean ±SD)	t-test P-Value	Pearson's Correlation (r)	P-Value
Male	n=12	113.7±24.1	157.4±89.2	0.126	0.041	0.898
Female	n=18	139±49.5	161.3±40.9	0.081	0.376	0.124

Regarding **glucose** levels, the means were insignificantly (P>0.05) higher in males compared to females in both periods of the study, which indicates that there is no effect of sex on glucose levels in pre-exams and during exams (Table 5).

Table (5): Comparison of Mean values of Glucose (mg /dL) between males and females in the periods of pre-exams and Comparison of Mean values of Glucose between males and females during exams.

Period of the study	Males (n=12)	Females (n=18)	P-Value
Pre- exams (mean ±SD)	85.9±15.1	77.7±24.5	0.310
During exams (mean ±SD)	90.4±24	95.3 ±30.5	0.645

Furthermore, results showed an insignificant increase (P>0.05) in glucose levels for both sex in during-exam period compared to pre-exam. Pearson's correlation coefficient showed weak and insignificant relation between mean levels of glucose in the two periods of the study (Table 6, Fig. 2).

Table 6: Comparison of Mean values of Glucose (mg /dL) and Correlation coefficients in the periods of pre-exams and during exams depending on sex.

Sex	N of student	Pre- exams (mean ±SD)	During exams (mean ±SD)	t-test P-Value	Pearson's Correlation (r)	P-Value
Male	n=12	85.9±15.0	90.4±24	0.452	0.549	0.064
Female	n= 18	77.7±24.5	95.3 ±30.5	0.098	-0.196	0.435

Concerning **cortisol** levels, the mean values were insignificantly higher (P>0.05) in males compared to females in both periods of the study, which indicates that there is no effect of sex on cortisol levels in pre-exams and during exams (Table 7).

Table (7): Comparison of Mean values of Cortisol (µg/dL) between males and females in the periods of pre-exams and Comparison of Mean values of Cortisol between males and females during exams.

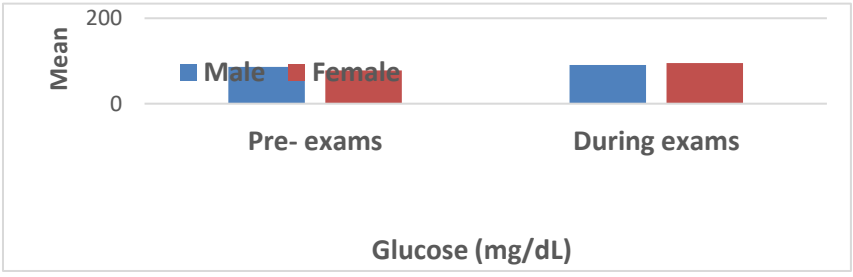
Period of the study	Males (n=12)	Females (n=18)	P-Value
Pre- exams (mean ±SD)	11.2±4	10.1±3.3	0.401
During exams (mean ±SD)	13.2 ± 4.1	12.6±3.5	0.671

Furthermore, there was an insignificant increase (P>0.05) of cortisol levels in males during exam period compared to pre-exam period with positive and significant correlation of cortisol levels between the two periods (Table.8). On the other hand, cortisol levels were higher significantly (P<0.05) in female during exams compared to pre-exams, which might explain why the results obtained in table 5 which is that glucose levels were higher in females compared to male in during exams period, and that is due to their cortisol levels being higher. Cortisol is known to increase blood glucose level through several mechanisms. Pearsons' coefficient showed no significant correlation between cortisol levels in females between the two study periods (Table 8, Fig. 2).

Table 8: Comparison of Mean values of Cortisol (µg/dL)and Correlation coefficients in the periods of pre-exams and during exams depending on sex.

Sex	N of student	Pre- exams (mean ±SD)	During exams (mean ±SD)	t-test P-Value	Pearson's Correlation (r)	P-Value
Male	n=12	11.2±4	13.2 ± 4.1	0.056	0.690	0.013
Female	n= 18	10.1±3.3	12.6±3.5	0.020	0.242	0.333

This study is in agreement with several studies which found varying differences in female and male students, females were more likely to feel as though they experienced higher levels of stress [16], Female students experience significantly higher level of test anxiety, Emotionality, and Examination stress [17] Gender differences in the role of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) in stress, The role of GRs and MRs in the HPA axis could be different between males and females during stress [18].



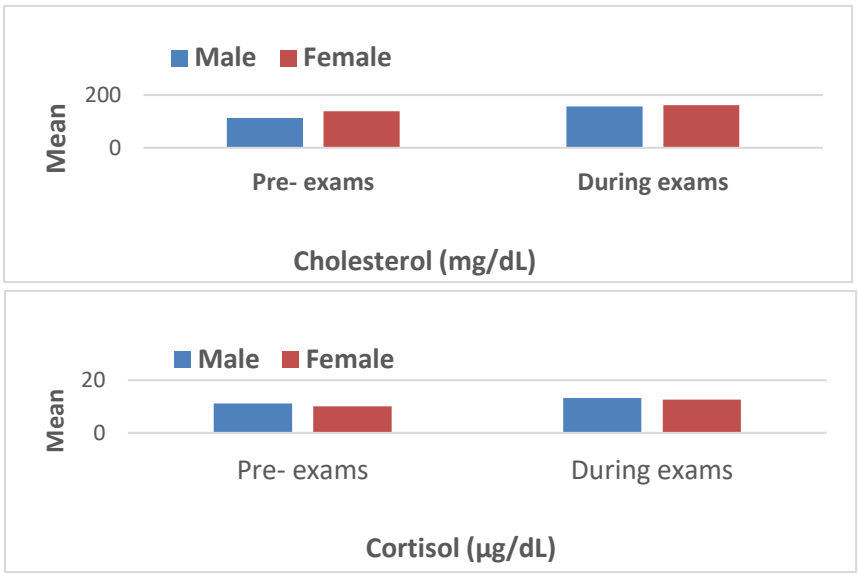


FIG.2.COMPARISON OF MEAN VALUES OF BLOOD PARAMETERS IN THE PERIODS PRE-EXAMS AND DURING EXAMS DEPENDING ON SEX.

4. Conclusion

This study concludes that examination is a stressful condition which affects the students' mental and emotional status as well as their whole body. Increased blood level of cortisol, glucose, and cholesterol can be considered as an indicators of stress. Cortisol level increased significantly among females during exams compared to pre-exam while the increase was non-significant among males, which indicates that males react to stress in a more controlled manner than females. Thus estimation of these parameters will be helpful to make the students aware of the bad consequences of stress. It well also counsels them to handle stress. This study also recommends the education system to develop better assessments methods which are less stressful for students.

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