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# Evaluation of Selected Biochemical Parameters in Sera of Iraqi Academic Students

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## ABSTRACT

**Background** Examination stress is a common and widespread phenomenon for students, so the main aim of this study was to check the effect of temporarily examination stress on some biochemical parameters in sera of Iraqi academic students. **Materials and Methods : :** A total of 55 apparently healthy undergraduate students were enrolled in this study. A blood sample was collected in two times from each undergraduate student, in exam day (E-day) and in the normal day (N-day) , the results compared between the two different days. The serum cortisol, total serum protein (T.S.P), globulin, albumin, fasting serum glucose (F.S.G), and amylase activity were measured by spectrophotometry, while total serum zinc ion was measured by atomic absorption technique. **Results:** Non-significant differences were found in serum cortisol, amylase activity, F.S.G, and zinc, while T.S.P, globulin showed significant decreases comparing E-day with N-day in all samples and depending on gender.. Also, F.S.G was increased significantly in female students. **Conclusion**: Serum cortisol levels were not affected in E-day comparing with N-day that may be due to the adaptation of Iraqi individual to traumatic events**,** that lead to the fear of exam becomes not stressful. F.S.G was significantly increased in the female group in E-day, and that may be because of the females were more stressed at examination situation.

**KEYWORDS:** Examination Stress; Cortisol; Serum glucose; Serum amylase; Proteins; and Zinc

## INTRODUCTION

Stress is the demands it possesses on an organism and how the organism attempts to adapt or cope with the specific demands, it is a necessary and unavoidable concomitant of daily living, necessary because without stress human would be a listed and apathetic creature, and unavoidable because it relates to any external event, be it pleasurable or anxiety producing [1]. A stressor is any real physical, social, or psychological event or stimulus that causes human bodies to react or respond [2], it may promote physiological or behavioral disturbances or both, meanwhile a person's response towards stress depends on whether an event is appraised as a challenge or threat [3]. The pathway to response to stress is that the corticotropin-releasing hormone (CRH) is released into hypophysial portal vessels that access the anterior pituitary gland. Binding of CRH to its receptor on pituitary corticotropes induces the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. The principal target for circulating ACTH is the adrenal cortex, where it stimulates glucocorticoid synthesis and secretion from the zona fasciculata; glucocorticoids are the downstream effectors of the HPA axis and regulate physiological changes through ubiquitously distributed intracellular receptors [4,5]. Cortisol (a glucocorticoid hormone) exerts widespread physiological effects throughout the body, acting in concert with other chemical messengers to help direct oxygen and nutrients to the stressed body site and suppress the immune response, while influencing certain functions, such as appetite and satiety; arousal, vigilance, and attention; and

**Advances in Environmental Biology**, 10(6) June 2016, Pages: 103-110

mood [6]. Several factors play a fundamental role in determining the nature and consequences of the stress response; these factors include inherent features of a given type of stressor as well as the conditions under which the stressor is encountered [6].

Examination stress refers to the pressure or stresses that are experienced by students to perform well in final school or undergraduate examinations and competitive college entrance examinations [7]. Having examination stress is a common and widespread phenomenon for students [8]. Up to 30%-50% of student's has test-induced anxiety problems [9]. Cortisol is known as the stress hormone because it is released in response to significant stresses. It promotes the conservation of glucose as an energy source in several mechanisms such as induces and maintains the activity of all of the gluconeogenic enzymes in the liver and inhibit glucose utilization in tissue, skeletal muscle, and adipose tissue by inhibiting glycolysis and promoting the use of fatty acid. Also, cortisol plays a role in preventing proliferation by weakening the activity of the immune system, lowers bone formation, increase blood pressure, allows the kidneys to produce hypotonic urine and increase the effectiveness of catecholamine's [10]. The brain uses a large amount of energy and is dependent on blood glucose as its source of energy [11], glucose taken up by the brain is completely oxidized whereas that taken up by the kidney, blood cells, splanchnic tissues, and muscle mainly undergoes glycolysis [12]. Because the brain cannot store glucose, it requires a continuous supply of glucose to function properly, any shortage in this availability of glucose to the brain has adverse consequences for its functioning [13]. α-Amylase (1,4-α-D glucanohydrolase) is a small heterogeneous enzyme, it is an essential enzyme in the physiologic digestion of starches, [14,15]. α-amylase it exists in different isoenzyme forms, salivary type (S-type) and pancreatic type (P-type) [16]. Total serum protein (T.S.P) is made up of albumin and globulin, the globulin, in turn, is made up of α1, α2, β, and γ globulins. These fractions can be quantitated using protein electrophoresis, but the total protein test is a faster and cheaper test that estimates the total of all fractions together [17].

## MATERIALS AND METHODS

A total of 55 apparently healthy undergraduate students (30 male, 25 female) from University of Baghdad College of Science, specialty from mathematic, physics, geology, and biotechnology departments and also from Al-Esraa college, specialty from department of medical analyzes were participated in this study. The age of these students ranged from 19 to 24 years (20.4 ±1.32), and BMI mean values was with (24.20 ± 2.6 kg/m$^2$). All undergraduate students were subjected to a personal interview using specially designed questionnaire format including full history with detailed information. The menstruation day was recorded for females, and also females during and before one week of menstrual cycle were excluded. Blood samples were obtained from the undergraduate students in two days, the first day was a normal day (N-day) and the second day was after 7-15 days, in the morning of written exam (E-day). These students were sitting in a relatively quiet laboratory place, and they were rested for (5-15) minutes before blood sample taken, then (5-6 ml) of venous blood sample was collected at (7:15 – 8:30 am)by using disposable syringes, and the blood type was detected immediately after blood collecting. The collected sample was placed into plain plastic tubes, centrifuged at (3000 r.p.m.) for 15 minutes after clotting, and the clear serum was separated and stored at (-20 ºc) in three eppendorf tubes until the time of assays. In normal day, the collecting of samples from undergraduate students with abnormal feeling was postponed to another day, also pains during blood sample collection were avoided as much as possible.

*Determination of biochemical parameters:*

Serum cortisol was quantitatively determined using Enzyme Linked Immune-Sorbent Assay (ELISA). Serum glucose was measured according to Dennis's method [18]. Serum amylase activity was determined by Caraway-Somogyi starch-iodine amylase method [19]. Modified biuret method was used to determine T.S.P [20], while albumin concentration was measured using a method based on albumin ability of binding bromocresol green (BCG) [21]. Serum zinc was determined using digestion method with flame atomic absorption technique [22].

*Statistical analysis:*

The data were analyzed by Duncan's multiple range tests at $p$ <0.05 accepted as statistically significant, using the SPSS version 21.0 with depending t-test and correlation analysis.

## RESULTS AND DISCUSSION

In the present study, serum cortisol, F.S.G, amylase activity with its specific activity, serum proteins, and zinc were measured in the sera of all undergraduate students and according to gender comparing E-day with N-day as listed in **Table 1.** Non -significant differences in all biochemical parameters (except T.S.P, globulin, and A/G ratio) were observed in E-day comparing with N-day in all undergraduate students. Depending on gender, T.S.P, globulin, and A/G ratio were appeared significant differences comparing E-day with N-day, while F.S.G

was indicated significant increase only for female group; other parameters were showed non-significant differences.

**Table 1:** Mean values and ±SD of studied biochemical parameters comparing E-day with N-day, in all samples and depending on gender.

| parameters | pairs | All (N=55) | *p*-value | Male (N=30) | *p*-value | Female (N=25) | *p*-value |
|---|---|---|---|---|---|---|---|
| Cortisol (ng/ml) | E-day | 12.09±2.00 | N.S | 12.46±1.13 | N.S | 11.72±2.61 | N.S |
| | N-day | 12.46±1.03 | | 12.84±0.92 | | 12.06±1.00 | |
| F.S.G (g/dL) | E-day | 101.8±15.54 | N.S | 101.6±18.31 | N.S | 104.9±13.9 | *S* |
| | N-day | 96.5±15.79 | | 101.1±19.57 | | 93.83±2.52 | |
| Amylase activity (U/L) | E-day | 184.3±47.0 | N.S | 201.0±42.36 | N.S | 174.3±47.6 | N.S |
| | N-day | 180.5±66.4 | | 221.1±63.91 | | 156.2±56.2 | |
| Amylase Sp.ac. (U/g) | E-day | 22.66±6.80 | N.S | 26.19±4.31 | N.S | 20.55±7.24 | N.S |
| | N-day | 22.68±8.11 | | 25.95±7.83 | | 20.73±7.77 | |
| T.S.P (g/dL) | E-day | 7.93± 0.98 | S | 7.62±0.74 | *S* | 7.75±1.12 | S |
| | N-day | 8.35±1.111 | | 8.60±0.86 | | 8.58±0.775 | |
| Alubmin(g/dL) | E-day | 3.46±0.283 | N.S | 3.53±0.35 | N.S | 3.39±0.402 | N.S |
| | N-day | 3.41±0.441 | | 3.62±0.32 | | 3.46±0.278 | |
| Globulin(g/dL) | E-day | 4.55±1.118 | S | 4.08±0.88 | *S* | 4.18±0.970 | S |
| | N-day | 4.94±1.156 | | 4.94±0.80 | | 5.29±1.34 | |
| A/G ratio | E-day | 0.81± 0.23 | S | 0.909±0.23 | S | 0.881±0.35 | S |
| | N-day | 0.746±0.30 | | 0.748±0.14 | | 0.711±0.25 | |
| Zn(mg/L) | E-day | 0.155±0.03 | N.S | 0.153±0.04 | N.S | 0.145±0.24 | N.S |
| | N-day | 0.144±0.05 | | 0.143±0.02 | | 0.143±0.06 | |

(S) Significant value, (N.S) non-significant value

Table 2 indicates non –significant differences of cortisol, F.S.G, and albumin levels in both days (E-day& N-day), while there were significant differences in serum amylase activity with its specific activity in favor to mals students in both days. T.S.P and globulin showed significant differences in E-day groups while N-day group showed non-significant differences. Total serum zinc showed a significant decrease in E-day group in favor of males while N-day group showed non-significant differences.

It is clear that cortisol has significant effects on emotion-related information processing [23], it is an important hormone associated with psychological, physiological, and physical health functioning [5], so it was expected that cortisol level will be increased in E-day comparing with N-day, but the results of the present study showed non-significant differences in its level in E-day comparing with N-day, which means that stress was not significant enough to activate the HPA (Hypothalamic–pituitary–adrenal ) axis that promoted by releasing corticotropin-releasing hormone ( CRH ) that causes to cortisol released from adrenal cortex and elevate in serum in response to the stress [5], these results may be explained by two main reasons: adaptation of students with stress , in which the Iraqi individuals live in situations that included of explosions, poor security, and traumatic events [24], so adaptation to threaten life may be lead to the fear of the exam becomes not stressful enough to promote HPA axis. Another reason may be due to the time of exam which is not a fateful exam (a mid-course exam) therefore, there is no concern stimulates that which lead to significant stress. This result of the present study agrees with previous studies , who found the level of serum cortisol is not significantly affected when E-day compared with N-day [25,26] , while disagrees with other studies which reported that the level of serum cortisol increased significantly comparing E-day with N-day [27-30] .

**Table 2:** Mean values and ±SD of studied biochemical parameters in E-day and N-day groups.

| parameters | E-day Male | E-day female | *p*-value | N-day Male | N-day female | *p*-value |
|---|---|---|---|---|---|---|
| Cortisol (ng/ml) | 12.46±1.13 | 11.72±2.61 | N.S | 12.84±0.92 | 12.06±1.00 | N.S |
| F.S.G (g/dL) | 101.6±18.3 | 104.97±13.96 | N.S | 101.1±19.5 | 93.83±2.52 | N.S |
| Amylase activity (U/L) | 201.0±42.3 | 174.35±47.68 | S | 221.0±63.9 | 156.2±56.23 | S |
| Amylase Sp.ac. (U/g) | 26.19±4.31 | 20.55±7.24 | S | 25.95±7.8 | 20.73±7.77 | S |
| T.S.P(g/dL) | 7.62±0.740 | 8.58±0.775 | S | 8.60 ±.868 | 7.75±1.125 | N.S |
| Alb.(g/dL) | 3.53±0.352 | 3.39±0.402 | N.S | 3.62 ±0.329 | 3.46±0.278 | N.S |
| Glo(g/dL) | 4.08±0.884 | 4.18±0.970 | S | 4.94 ±0.801 | 5.29±1.34 | N.S |
| A\G ratio | 0.909±0.230 | 0.881±0.352 | N.S | 0.748 ±0.143 | 0.711±0.257 | S |
| Zn(mg/L) | 0.153±0.04 | 0.145±0.24 | *S* | 0.143±0.023 | 0.143±0.066 | N.S |

(S) Significant value, (N.S) non-significant value

As shown in table 1 the levels of F.S.G were increased slightly but not significant in all undergraduate students in E-day comparing with N-day. These results agree with the only recent study that reported that F.S.G slightly but not significantly increased comparing before and after an exam [31].The results that listed in the same table indicate a significant increase in F.S.G in female students, there were previous studies that refer to increasing of F.S.G as a result of stress [32-35], that may be because of increasing of mental activity which is associated with increased glucose metabolisms [36,37], also, higher levels of blood glucose are associated with higher levels of glucose in the brain [38-40].

Previous studies were demonstrated that improving the memory performance , in which the number of words recalled correlated significantly with blood glucose levels from a 50-gram glucose versus a placebo(non-glucose drink ) drink in young healthy adult participants and the low blood glucose within the normal range , can negatively affect memory performance [41-42]. As mentioned above it can say that the increasing serum glucose is response to increasing its metabolism by brain due to increasing mental activity that causes by exam (thinking about the exam and try to remember some information that related to the exam ), that may be the reason of our result. Another reason maybe support the increasing serum glucose in females due to stress and high mental activity is the scientific education ability in favor to male according to [43-45]. Also [46] noted that the females students were more stressed than the males at examination situation. This two recent reasons may be contributed to increasing mental activity that lead to brain consume more glucose. As shown in table 2 there was the non-significant difference in serum glucose between males and females undergraduate students in E-day and N-day groups.

Table 1 was appeared non-significant differences in serum amylase activity with its specific in E-day comparing with N-day. Differences in our results comparing with previous studies may be due to the differences in sources of synthesis and secretion of serum and salivary amylase , in which salivary amylase synthesis in salivary gland specifically in parotid gland [47] while serum amylase synthesis and secreted mainly in pancreatic acinar cells [48] , also 80% of amylase that synthesized in pancreatic acinar cells stored in salivary gland [49].The fact that the parasympathetic activation increases salivary flow has been named as a major confounder of studies trying to establish salivary α-amylase (sAA) as a marker for sympathetic activation [50,51]. Stress-induced stimulation of the sympathetic branch is thought to inhibit the parasympathetic branch. As parasympathetic input stimulates saliva flow rate, stress-induced inhibition would, therefore, decrease salivary flow. Decreased saliva flow together with unchanged protein secretion from acinar cells would therefore theoretically lead to a higher protein concentration in saliva without increased protein secretion by the acinar cells [52], in addition, there is no study refer to the response of the pancreatic acinar cell to the psychological stress.

As shown in Table (2) there was significant difference in serum amylase activity between males and females students in E-day and N-day groups in favor of males. This finding is match with recently studies [53-55], they found that the normal serum amylase activity is higher in males comparing with females.

As listed in Table (1) there were significant decreases in T.S.P comparing E-day with N-day in all undergraduate students and separated gender. Our results may be in line with a previous study , who found that T.S.P was significantly lower in depression [59]. On the other hand, the results disagrees with Nazan *et al* who found that the T.S.P not significantly decrease in E-day comparing with N–day, in this study 100 undergraduate students participate in E-day, and only 20 subjects in N-day and there was no follow-up on to those 100 participants[56], also another study found non-significantly differences in T.S.P concentration during presentation[57]. Bosch and coworkers reported increasing in total salivary protein at the academic

examination[51], also Al-nahri found that the T.S.P increase significantly (8.01±0.099) in E-day comparing to N-day (7.88 ±0.1) [58]. The same table was showed that there were non-significant differences in albumin in E-day comparing with N-day, this result is similar with that of Nazan and coworkers , who mentioned that the serum albumin not significantly change at examination state comparing to a normal day [56]. At the same time our results disagree with Al-nahrib ( 2009) who found that the serum albumin increased significantly in E-day[58]. Unchanged of serum albumin in the current study can be described from ,that the stress before exam was not major enough to activate the response of albumin that can play as an antioxidant [60] to scavenge the oxidant molecules that may produce during stress [61,62] or may be other proteins have priority to dealing with such stress (examination stress).

Also, significantly decreases in serum globulin were shown, Table (1) , in E-day comparing with N-day, the results are match with previous studies, they were noticed that , salivary γ-globulin [11.5 – 18.8 % of total protein] was significantly decreased in undergraduate students before written exam comparing with 14 days after exam[63-65], also a study reported that the serum globulin is lower in individual with major depression than in control[64]. Meanwhile this study disagrees with previous studies ,they found that the serum globulin significantly increases before examination stress comparing with control day [58,66,67]. The decreases of globulin in current study can be explained by the following: the $\alpha_2$-globulin contain ceruloplasmin which acts as an antioxidant enzyme , and it increase in response to the stress that causes by exam [70] . Table (1) indicates significant decreases in T.S.P and globulin depending on gender. Also, Table (2) was revealed significant differences in T.S.P and globulin comparing males with females in E-day in favor to males, while in N-day there were non-significant differences in T.S.P, globulin, and albumin between males and females.

Table (1) was appeared non-significant differences in serum zinc in all undergraduate students comparing E-day with N-day, also non-significant differences in serum zinc were revealed depending on gender as shown in the same table. These results disagree with only a study that measured serum zinc in examination stress, which found that serum zinc was significantly decreased comparing exam day to control [56] , the same reference was found that the cortisol level increased a when E-ay compared with N-day in undergraduate students, also a recent study was reported that the serum zinc decreased, and cortisol increased after exposure to psychological stress for 7 to 14 days in a rat model [71]. Additionally it was proved that inhibitory effect of increasing serum zinc on cortisol secretion [72]. In the current study, the level of serum cortisol and zinc is not affected significantly, this can attribute to the level of stress that was not enough to promote significantly change in serum cortisol and hence zinc.

*Conclusion:*

Serum cortisol levels were not affected in E-day comparing with N-day that may be due to adaptation of Iraqi individual to traumatic events that lead to the fear of exam becomes not stressful. F.S.G was significantly increased in female group in E-day comparing with N-day and that may be because of the females were more stressed at examination situation. T.S.P and globulin were significant decreases in both genders comparing E-day with N-day, these both parameters decreased in females more than males.

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