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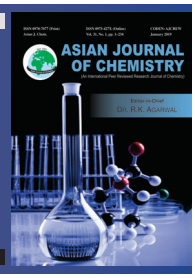

Alteration in Serum & Saliva α-Amylase Activity & Levels of Some Hormones Associated with Exposure to Chemicals: A Case Study on Iraqi Undergraduate Chemistry Students

HATHAMA RAZOOKI HASAN* and THIKRA HASAN MATHKOR

Department of Chemistry, University of Baghdad, Baghdad, Iraq

*Corresponding author: E-mail: hathamahasan@scbaghdad.com; hathama2012@gmail.com

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Numerous studies have pointed a link between exposure to environmental chemicals and disturbance of hormones. The present study consisted of 22 chemistry undergraduate final year students (11 males and 11 females) of Baghdad University, Iraq. Samples (serum and saliva) were collected in the middle of September, 2017 (before exposure group) and after 3 months of their practical study at chemistry teaching laboratory (after exposure group). All studied hormones (TSH, testosterone and cortisol) were found to increase in both male and female subgroups. The increase was statistically significant for serum TSH and testosterone ($p < 0.05$) and insignificant for cortisol. Insignificant variations were reported for salivary hormones (testosterone and cortisol). Serum and salivary α-amylase activity was found to be insignificantly decreased and increased respectively, in the exposure groups.

Keywords: Chemical exposure, Saliva's hormones, α-Amylase, Iraqi undergraduate students.

INTRODUCTION

Biological monitoring of chemical exposure in work location has particular significance in health risk evaluation as an essential part of the occupational health and safety strategy. The association between certain chemical exposure and health consequences was the major goal of environmental health sciences [1]. It have been suggested that in men, environmental factors (*e.g.* persistent organic pollutants) are strongly correlated to the increased incidence of various reproductive diseases *viz.*, testicular cancer, cryptorchidism, hypospadias, and subfertility [2,3].

Chemical pollutants which linked to dysfunction of the reproductive system are referred to endocrine disruptors. Significant influences of endocrine disruptors on the immune system have been reported, particularly in stress responses. Stress modifies the immune system through hypothalamic pituitary adrenal (HPA) and sympathetic nervous system (SNS) activation, resulted in inhibition of antitumor immunity [4,5]. Salivary steroidal hormones (*e.g.* estradiol, progesterone and testosterone) determination has got much attention because of its multiple applications in clinical medicine and other scientific research fields [6-12]. Salivary and serum steroid levels also suggested to have significant correlation [13-16].

Cortisol is an important steroid that play an important role in controlling stress response controlling, exists in two forms *viz.*, free (active) and protein-bound in the blood stream, in saliva only a free form exists [17]. It is more valid to measure cortisol in saliva [18,19], due to its easy collection, repeatable at short period, non-invasive and more economic compared with other biological fluids (*e.g.* blood). The stress-free specimen collection makes salivary cortisol a reliable biomarker for evaluation of stress [20].

On the other hand, testosterone deems a key anabolic hormone, hence it plays a main role on numerous physiological functions (*e.g.* growth and adaptation) as bone, muscle, *etc.* and red blood cells sustenance [21]. Testosterone and cortisol are secreted by hypothalamic pituitary gonadal (HPG) axis and hypothalamic pituitary adrenal (HPA) axis. They have been reported to control the bio-equilibrium of psychological and physical stress responses in human [22].

Generally, sex and stress hormones relationship have been widely examined in sports medicine [23], psychopathologies [24,25], ischemic disease [26] and ovarian tumors [27] by evalu-

ating the ratio of testosterone to cortisol (T/C) as an index to body activities of anabolism and catabolism [28].

Thyroid hormones are indispensable factors in neurodevelopment, growth and metabolism processes. Environmental agents have been recorded as disruptors of thyroid function at different levels, including synthesis, metabolism, excretion and action of thyroid hormone [29-32]. Several chemicals have been assessed as thyroid signaling disruptors [33,34]. The changing pattern of thyroid function is investigated by measuring the changes in serum T3, T4 and TSH, where serum TSH level is used as a sensitive test for identifying thyroid dysfunction [35].

α-Amylases (EC 3.2.1.1), a family of glycoside hydrolases enzyme, largely secreted by the salivary glands and pancreas. It have obvious function in starch hydrolyzing by cleaving on 1,4-α-glycosidic linkages [36] and also display antibacterial effects [37,38]. α-Amylase activity is utilized clinically as a biomarker for diagnostic and follow-up of diseases (*e.g*. inflammation and tumors) [39-41]. Moreover, α-amylase was identified as promising stress marker which rapidly elevates at traumatic and pressure conditions (*i.e*. stress), as reflective for sympathoadrenergic system action [42-44].

Chemical exposure has been stated as a key factor in developing environmentally related diseases (*e.g*. cancer) which could be arisen from the association of the exposure with genes, sex, nutrition and lifestyle [45]. The aim of environmental related health studies is to assess the risks resulted from chemicals, find the relationship between chemicals and certain diseases and reduce the consequences of these chemicals which may result in any of the five (Ds): discomfort, dysfunction, disability, disease or death [45]. Exposure biomarkers include any measurable molecular changes at different biochemical, physiological, cytological and immunological levels in body fluids or compartments [46-51].

In the present study, the authors investigated the personal health risk of a group of undergraduate final year students by determining serum and salivary thyroid stimulating hormones (TSH), cortisol (C) and testosterone (T) levels and α-amylase activity. The inter-relationship between diurnal fluctuation of testosterone with cortisol as hormonal markers and cortisol with α-amylase activity as human stress system marker were also investigated.

EXPERIMENTAL

Study subjects: This longitudinal study is complementary to previous study conducted in our laboratories [52]. This studies consist of 22 students (11 males and 11 females) of undergraduate chemistry students of Baghdad University. The students were aware with the study's purpose, procedures and agreed to participate in this project. Informed consent was obtained for all participates and ethical committee of University of Baghdad reviewed and approved the study proposal.

Questionnaire criteria: General informations (*e.g*., age, smoking, current and past diseases, place of residence and after-university work, if present) were recorded for each participant. Students who have been, or may have used steroid drug, or any kind supplement which may interfere with, or effect the studied hormones levels have been excluded. The mean age of the students was 22 years (range 22-24).

Sample collection: All samples collection were performed simultaneously for serum and saliva between 8 am to 10 am.

Saliva: The students were asked to keep fasting, not to brush their teeth, or smoke about 30 min before saliva collection. Rinsed the mouths with saline then started the unstimulated saliva collection directly into a polyethylene cup. The samples were centrifuged at 2600× g for 10 min and the supernatants were kept at -20 ºC.

Blood: Venous blood samples were collected in test tubes, stored upright for clotting for 10-15 min and centrifuged at 2600× g for 5 min. Non-hemolyzed serum was kept in coded plain tube and used for biochemical analysis.

Biochemical analyses: Analyses of cortisol, total testosterone and thyroid stimulating hormone were performed using commercially available kits of radioimmunoassay method (RIA) from Roche Diagnostics (Germany) with cobas e 411 analyzer, all the tests were performed according to clinical guidelines. α-Amylase activity was determined by colorimetric assay [53], where unit of amylase is defined as the amount of amylase per 100 mL of serum, or saliva that hydrolyzes 5 mg of starch in 15 min at 37 ºC. The factor 1.85 is used to convert to unit (U/L). To calculate the specific activity of α-amylase, protein content in serum and saliva samples was determined by Lowry's method [54] where bovine serum albumin was used as a standard protein.

Statistical analysis: The software used for calculation of the statistical data was SPSS (Chicago, USA) version 17.0. For two related samples from a continuous field, Wilcoxon non-parametric signed rank test was used to perform matched pair analysis. Rescaling Wilcoxon test to measure the effect size, which must be calculated manually, using the formula: $r = z/\sqrt{n}$ (where r = effect size; z = the test statistic output by SPSS; n = the total number of observations).

To test the association between serum and saliva parameters, Pearson's (r) correlation coefficient test was performed. At $p < 0.05$, the variation for all parameters was significance.

RESULTS AND DISCUSSION

The results of Wilcoxon signed-rank test performed on an individual basis are presented in Tables 1 and 2. When exposed and non-exposed groups were analyzed together in the statistical evaluation, the concentration of hormones (TSH, testosterone and cortisol) in serum were found to increase significantly ($p < 0.05$) in both male and female except for cortisol where the increase was non-significant. For TSH in male (average = 1.5 to 2.3 mIU/L), Z = -2.223 ($p < 0.026$) with a medium effect size (r = 0.473) was observed, while in female (average = 1.2 to 2.3 mIU/L), Z = -2.491 ($p < 0.013$) with a large effect size (r = 0.531).

Significant increases ($p < 0.05$) in testosterone levels in male (average = 5.5 to 11.0 ng/mL), Z = -2.134 ($p < 0.033$) with a medium effect size (r = 0.455), while in female (average = 0.6 to 1.9 ng/mL), Z = -2.313 ($p < 0.021$) with a medium effect size (r = 0.493). For cortisol level (average = 290.1 to 403.2 and 233.8 to 338.2 ng/mL) for male and female, respectively, but this level did not reach statistical significances.

Using Wilcoxon signed rank tests, insignificant increase between the two (exposed and non-exposed) groups in male testosterone level (average = 0.34 to 0.46 ng/mL) was noticed,

TABLE-1
α-AMYLASE ACTIVITY/SPECIFIC ACTIVITY AND HORMONE LEVELS (THYROID STIMULATING HORMONES, CORTISOL AND TESTOSTERONE) IN SERUM OF BEFORE AND AFTER EXPOSURE GROUPS

Gender	Parameters (n = 11)		Before exposure median (range)	After exposure median (range)	p Value	Effect size (r)	Z
M	Amylase activity (U/L)	↓	400.0 (142.80-888.80)	387.0 (216.22-594.59)	0.286	0.228	-1.068
F		↓	363.6 (181.80-857.10)	315.7 (157.89-666.67)	0.657	0.095	-0.445
M	Amylase sp. activity (U/mg)	↓	59.34 (25.82-130.32)	45.22 (31.34-102.87)	0.286	0.227	-1.067
F		↓	65.86 (24.63-137.89)	41.36 (22.88-116.02)	0.248	0.246	-1.156
M	Thyroid stimulating hormones (mIU/L)	↑	1.50 (0.40-2.70)	2.30 (0.50-5.10)	0.026*	0.473	-2.223
F		↑	1.20 (0.80-1.90)	2.30 (1.00-6.70)	0.013*	0.531	-2.491
M	Testosterone (ng/mL)	↑	5.50 (0.70-18.90)	11.00 (1.10-26.0)	0.033*	0.455	-2.134
F		↑	0.600 (0.20-3.10)	1.90 (0.80-12.0)	0.021*	0.493	-2.313
M	Cortisol (ng/mL)	↑	290.10 (112.30-435.50)	403.20 (230.00-553.20)	0.062	0.398	-1.867
F		↑	233.80 (176.10-403.70)	338.20 (159.60-487.09)	0.182	0.284	-1.334

*Statistically significant $p < 0.05$

TABLE-2
α-AMYLASE ACTIVITY/SPECIFIC ACTIVITY AND HORMONE LEVELS (CORTISOL AND TESTOSTERONE) IN SALIVA OF BEFORE AND AFTER EXPOSURE GROUPS

Gender	Parameters (n = 11)		Before exposure median (range)	After exposure median (range)	p Value	Effect size (r)	Z
M	Amylase activity (U/L)	↑	1111.11 (666.67-1428.57)	1151.51 (1000-1388)	0.859	0.038	-0.178
F		↑	1081.08 (727.27-1400.00)	1135.130 (944-1500)	0.534	0.133	-0.622
M	Amylase sp. activity (U/mg)	↓	613.87 (289.5-2351.6)	490.27 (268.87-1448.02)	0.424	0.171	-0.800
F		↓	854.70 (367.04-1234.98)	500.71 (262.25-1074.66)	0.110	0.341	-1.600
M	Testosterone (ng/mL)	↑	0.340 (0.10-0.70)	0.460 (0.14-1.10)	0.075	0.380	-1.782
F		↑	0.042 (0.029-0.081)	0.078 (0.03-0.14)	0.041*	0.436	-2.045
M	Cortisol (ng/mL)	↓	8.37 (5.40-12.07)	7.96 (3.68-18.10)	0.594	0.114	-0.533
F		↓	10.04 (4.04-13.54)	5.13 (3.57-17.41)	0.131	0.322	-1.511

*Statistically significant $p < 0.05$

while significant raise was found in female (average = 0.042 to 0.078 ng/mL), Z = -2.045 ($p < 0.05$) with a medium effect size (r = 0.436) was noticed. The level of cortisol was insignificantly decreased in exposed students (average = 8.37 to 7.96 and 10.04 to 5.13 ng/mL) for male and female respectively (Table-2).

Mostly, environmental exposures are latent, not noticeable, accumulating for a long period of time, therefore the connection of specific exposure to certain diseases is complicated issue [55]. Exposure of individuals to various environmental toxicants and the combination of these toxicants may result in considerable health deleterious effect [56]. Several studies have showed that the cause of different diseases (*e.g.* cardiovascular disease, leukemia and neurodevelopmental disorders) are related to chemical environmental exposure [57-59].

The chemicals which are familiar in the chemistry laboratories (*e.g.* phthalates, ethers, aromatic hydrocarbons and their derivatives, *etc.*) demonstrated to elicit a number of biochemical responses. Most of them can persist in the body for more than 30 years which may accumulate along time to significant amount leading to poisonous concentrations [60,61]. The omnipresent of these chemicals (*i.e.* in air, water, or food), make them act as environmental contaminants. The bioaccumulation of these chemicals in the body might be responsible of cardiovascular disease and other environmental diseases [62]. Akosy [63] and Hayes *et al.* [64] reported that the exposure to certain chemicals would increase the incidents of acute myeloid leukemias, acute lymphoid leukemias and non-Hodgkin's lymphomas.

Persisting stress leads to increase cortisol levels and consequently activation of sympathetic nervous system (SNS) might lead to decreases cell-mediated immunity [65,66]. Nakane *et al.* [67] provided some evidence that cortisol may enhance the higher vulnerability to infection by SNS which induced immunity implicated in cancer cell growth. Present results (Table-1) pointed an increase in serum cortisol levels and a decrease in salivary cortisol levels. Under normal conditions, cortisol levels increased in the morning and decreased in the evening. While during acute stress, levels of cortisol is elevated and the elevation is remained along the day. However, the HPA system become exhausted under chronic stress, resulting in a blunting of the diurnal cortisol rhythm [68]. Increasing risk of diseases injury through stress is currently incomprehensible [69]. It is known that up to 90 % of cortisol circulates bound to binding protein (CBG) [70], and the biologically active hormone is restricted to the free fraction [71]. While only unbound cortisol is present in saliva, but measures salivary cortisol faced another limitations that the existence of high concentrations of interfering steroids [20].

Several studies [30,31] have reported that production, metabolism and function of thyroid hormone might be influenced with specific toxic environment agents. Adequate concentrations of these toxic agents could result in disturbance of thyroid function. Increasing serum TSH and decreasing serum

thyroxine (T4) or (T3) might reflect the thyroid disruption [56]. In present study, we evaluated serum TSH levels and found exposed group exhibited significant higher TSH levels compared to non-exposed group. Present results recorded an elevation in serum TSH levels in exposed group (both male and female). It have been suggested that such elevation could be linked to risk of subclinical hypothyroidism, which is a condition with normal free T4 levels and elevated TSH levels [72]. In spite of the elevation in serum TSH is statstatistically significant, but have no clinical concern (TSH levels of ∝ 4.5 mIU/L are considered as risk factor) [56].

Involvement of sex hormones (testosterone and cortisol) in various pathologies have been documented [73,74]. Individuals with imbalance of these two steroid hormones levels might be under homeostasis disintegration risk [28]. Serum and salivary testosterone levels were significantly increased in student exposed group, whereas serum cortisol levels increased and simultaneously decreased in saliva sample. Testosterone to cortisol ratio has been used as symptomatic of adaptability and/or stress indices and also reported as a useful proximal marker for cardiovascular disease [69]. Testosterone to cortisol ratio were found to increase in serum and saliva samples after chemical exposure. The anabolic-catabolic imbalance might be ascribed to hypocortisolism at the morning; the resultant flattened response to a dysregulation of the HPA axis [75]. Li *et al*. [76] reported that the secretion of cortisol and testosterone are interconnected. When HPA axis is activated, adrenal corticosteroids secretions is increased concurrently decreasing in gonadotrophin secretion, thus result in testosterone level reduction.

The results (Table-1) showed the presence of insignificant decrease in serum α-amylase activity in both genders. Where serum α-amylase (average = 400.0 to 387.0 and 363.6 to 15.7 U/L) for male and female, respectively. Furthermore, using α-amylase specific activity did not elucidate significant decrease in the studied groups (average = 59.34 to 45.22 U/L) for male and (average= 65.86 to 41.36 U/L) for female.

Meanwhile, salivary α-amylase showed insignificant increase (average = 1111.11 to 1151.51 U/L) for male and (average = 1081.08 to 1135.13 U/L) for female. However, salivary α-amylase specific activity was observed to increase insignificantly in exposed group (average = 613.87 to 490.27 and 854.70 to 500.71 U/l) for male and female respectively (Table-2).

Stress/anxiety indices represent the ratio between cortisol levels to α-amylase activity, or specific activity in serum and saliva samples. Non-statistically significant increase in serum samples were observed between (exposed and non-exposed) groups for stress/anxiety indices (Table-3). On the other hand, insignificant differences were observed in salivary stress/anxiety indices. According to gender, male students showed an increase in stress/anxiety indices, while a decrease in female. Such variations will be confirmed when the indices was expressed using specific activity rather than activity (Table-4).

Testosterone to cortisol levels was calculated to represent anabolic/catabolic indices in serum samples. As shown in Tables 3 and 4, increase in anabolic/catabolic indices in serum and saliva samples was recorded, the increases were insignificant except for female saliva sample (average = 0.0059 to 0.0114, Z = -2.312, $p < 0.021$). It have been recorded that assessing salivary immune biomarkers (α-amylase and cortisol) associated with psychological stress, trauma, toxicant exposure that could be used for clinical diagnosis [68]. As a response to stress, SNS is activated and leading to release α-amylase [77]. The present study's results indicated that serum α-amylase tended to decrease in exposed group, although the decreasing was not statistically significantly different. Increased α-amylase activity was reported to be linked to stress variations that induces releasing of norepinephrine as adrenergic regulation system for stress rising [77-79]. Previous study has suggested that the stress and anxiety resulted in α-amylase releasing as response to SNS activation in women with endometrial cancer [68].

Higher levels of cortisol were found to be associated with lower α-amylase activity (Tables 1 and 2). These findings are

TABLE-3
TESTOSTERONE/CORTISOL AND AMYLASE/CORTISOL RATIOS IN SERUM OF BEFORE AND AFTER EXPOSURE GROUPS

Gender	Parameters (n = 11)		Before exposure median (range)	After exposure median (range)	p Value	Effect size (r)	Z
M	Cortisol/amylase (activity)	↑	0.552 (0.29-2.60)	0.930 (0.64-2.35)	0.750	0.379	-1.778
F		↑	0.644 (0.00-1.24)	1.217 (0.28-1.77)	0.110	0.341	-1.600
M	Cortisol/amylase (sp. activity)	↑	3.728 (1.90-14.36)	7.023 (5.06-16.10)	0.131	0.322	-1.511
F		↑	3.98 (1.38-9.11)	8.936 (1.38-12.29)	0.050*	0.417	-1.956
M	Testosterone/cortisol	↑	0.0229 (0.0021-0.118)	0.0272 (0.002-0.051)	0.657	0.094	-0.445
F		↑	0.0026 (0.0005-.0133)	0.0068 (0.003- 0.043)	0.050*	0.417	-1.956

*Statistically significant $p < 0.05$

TABLE-4
TESTOSTERONE/CORTISOL AND AMYLASE/CORTISOL RATIOS IN SERUM OF BEFORE AND AFTER EXPOSURE GROUPS

Gender	Parameters (n = 11)		Before exposure median (range)	After exposure median (range)	p Value	Effect size (r)	Z
M	Cortisol/amylase (activity)	↑	0.0066 (0.004-0.016)	0.0074 (0.003-0.015)	1.000	0.000	0.00
F		↓	0.007 (0.003-0.018)	0.004 (0.003-0.016)	0.131	0.322	-1.511
M	Cortisol/amylase (sp. activity)	↓	0.0157 (0.003-0.024)	0.0154 (0.003-0.065)	0.534	0.133	-0.622
F		↑	0.0095 (0.006-0.031)	0.0111 (0.003-0.053)	0.790	0.057	-0.267
M	Testosterone/cortisol	↑	0.037 (0.008-0.129)	0.046 (0.008-0.198)	0.374	0.190	-0.889
F		↑	0.0059 (0.0029-0.0166)	0.0114 (0.0036-0.0218)	0.021*	0.493	-2.312

*Statistically significant $p < 0.05$

consistent with other published data [68]. The relationships between stress/anxiety revealed that individuals have high anxiety-tension showed less α-amylase activities values. While, more cortisol levels were associated with great stress and anxiety-tension. These findings may indicate that high levels of stress and anxiety result in a blunted, more abnormal cortisol response [68]. The relationships between α-amylase and measured hormones levels in serum and saliva samples (both for male and female) subgroups were examined using bivariate correlations. Pearson's correlations coefficient (r) was calculated for all studied parameters in serum and saliva. Findings with significant correlation were discussed and displayed in Figs. 1 and 2. In male, serum α-amylase shows negative correlation (r = -0.687, p = 0.019) while (r = -0.623, p = 0.041) emerged between salivary cortisol/α-amylase activity and salivary cortisol/α-amylase specific activity, respectively as shown in Fig. 1.

Serum and saliva stress/anxiety indices indicates a positive correlation (r = 0.680, p = 0.021), even when expressed as cortisol level to α-amylase specific activity (r = 0.703, p = 0.016). Saliva cortisol represents a positive association with serum stress/anxiety indices when α-amylase is expressed as activity (r = 0.715, p = 0.013) and specific activity (r = 0.655, p = 0.029). In female, a significant interaction emerges between serum and saliva α-amylase activity (r = -0.685, p = 0.020) and its specific activity (r = -0.623, p = 0.041). In exposed group, salivary anabolic/catabolic indices was negatively associated with serum cortisol (r = -0.767, p = 0.006), as shown in Fig. 2.

Saliva samples may have many preferences over other biological fluids (*e.g.* blood, urine, *etc.*), which are readily accessible and collectible. Serum and salivary α-amylase activity showed high correlation in male and female subgroup. A strong positive relationship between stress/anxiety indices was recorded in present study. Based on the obtained results, it could be suggested that saliva could be used as a potential sample for detection the effect of chemical exposure on the present studied parameters.

Conclusion

In conclusion, it is found that three months exposure to different chemicals in chemical laboratories of different univer-

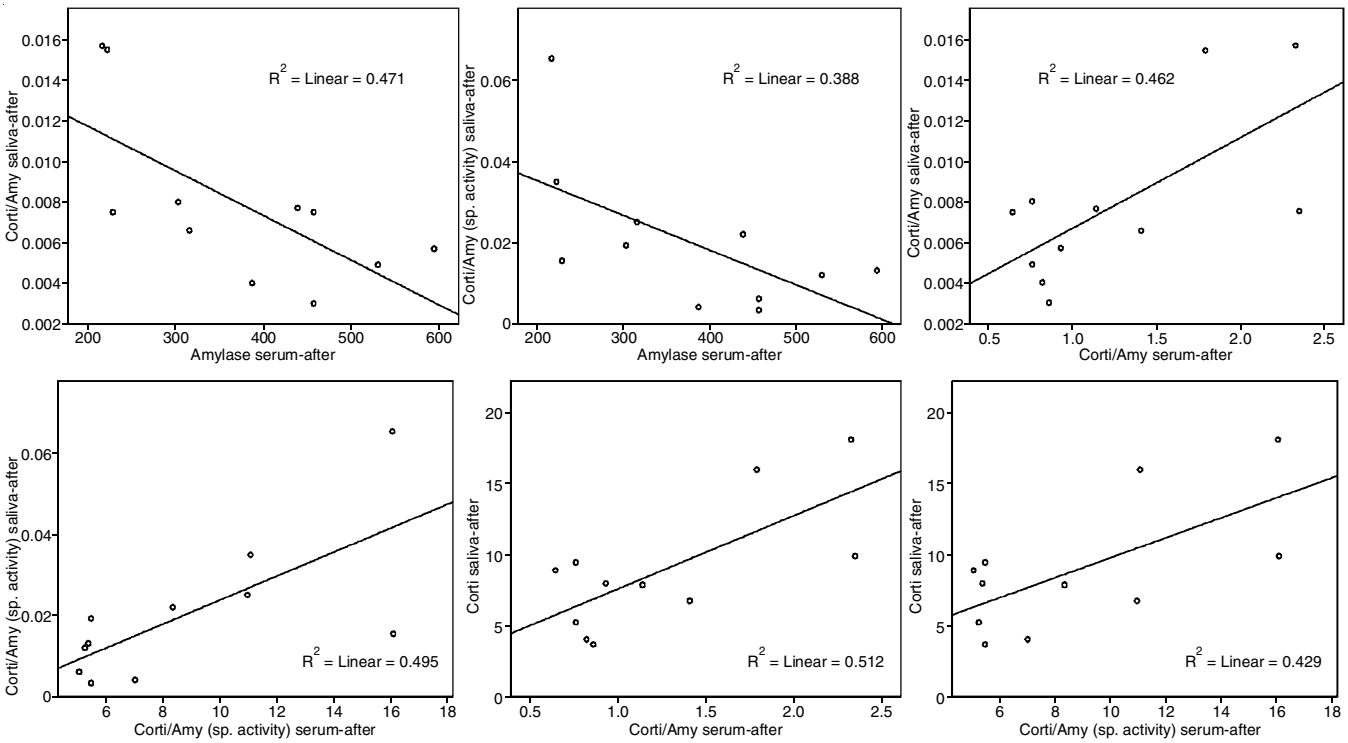

Fig. 1. Correlation of certain variables between serum and saliva in male group

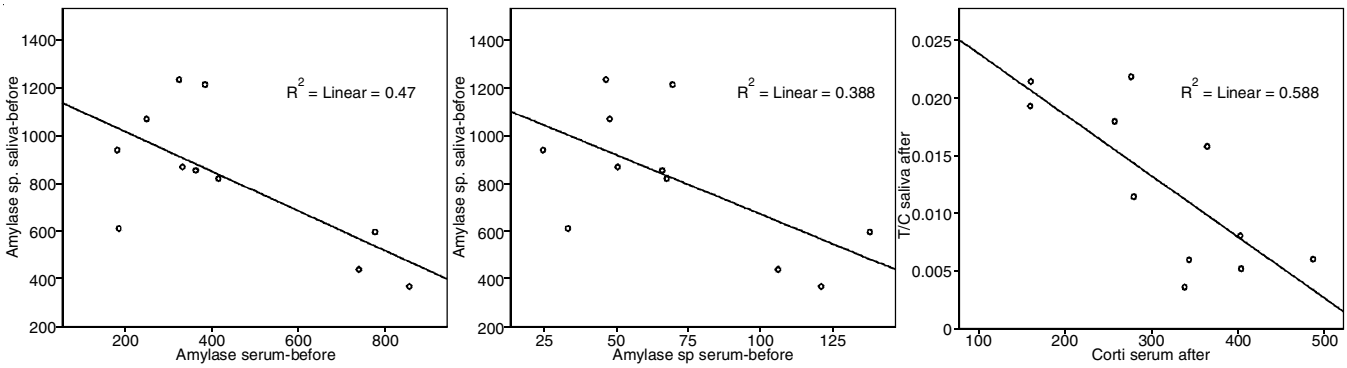

Fig. 2. Correlation of certain variables between serum and saliva in female group

sities was associated with hormonal levels variations among volunteered students at University of Baghdad. Such results suggested that the examined group of students might be under risk of endocrine-related health effects. Even though some biomarkers were statistically elevated, but they were generally in range with the upper limit of the reference values.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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