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Academic examination stress: Effects on salivary cortisol, neuropeptide Y and interleukin-1β

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ABSTRACT

Saliva is one of the preferred non-invasive body fluids for biomarker studies. This study aimed to investigate the possible alteration of stress biomarkers of the students before and after the examinations via salivary cortisol, neuropeptide Y (NPY), and Interleukin-1β (IL-1β) levels. Forty-four adults were included in the study and divided into groups of pre-examination (Group I) and post-examination (Group II). Salivary samples were collected between 8 and 9 a.m. before and after the exam, which ended at 5 p.m. by SARSTEDT saliva collection tubes. Participants were asked to soak the swab with saliva and take it out after 1 min. Swabs were kept at room temperature for 15–30 min and centrifuged for 10–15 min at 1500 g. Salivary cortisol (ng/mL), NPY (ng/mL), and IL-1β (pg/mL) levels were analyzed by Enzyme-linked immunosorbent assay (ELISA). The salivary cortisol, NPY and IL-1 β levels were significantly increased in Group II compared to Group I (9.65 ± 4.53, 6.37 ± 4.14, $p < 0.019$; 32.12 ± 4.69, 27.10 ± 4.71 $p < 0.001$; 11.69 ± 3.61, 7.20 ± 3.49, $p < 0.0003$ respectively). The IL-1β levels were positively and significantly correlated with salivary cortisol and NPY levels in Group II ($r = 0.642$, $p = 0.03$; $r = 0.589$, $p = 0.004$, respectively). Also, IL-1β levels were positively and significantly correlated with salivary NPY levels in Group I ($r = 0.430$, $p = 0.04$). These data indicated that acute stress can alter the inflammatory response and increase NPY release, which is positively associated with cortisol.

1. Introduction

It has been known that for many years, the exposure to stress and psychological state of the individuals affect the course of the diseases. So many studies were done to explore the link between psychological stress and the immune system, and scientists revealed critical physiological pathways that connect the brain and immune system (Roberto et al., 2020; Smith et al., 2007; Hirsch and Zukowska, 2012).

Stress can be defined as the body's response to changes that require physical, mental, or physiological adjustment. In addition, an uncontrollable, undesirable situation when a person has a more significant workload than they can handle is known as stress. Exams are vital in dental students' education as they assess their abilities and achievements. It is known that dentistry education, which has a comprehensive curriculum, is stressful and burdens students when forced to work.

Psychoneuroimmunology is a field of inquiry that connects the bidirectional interactions between the nervous system and immunological network (Schore, 2003; Pades and Homar, 2006). The mediators of these interactions consist of hormones, neuropeptides, neurotransmitters, and cytokines. From this point of view, the direct effects of acute and chronic stress and physical and psychological stresses can induce a wide range of immunological functions in the mediated cell.

Although the basic neurochemistry of the stress response is known, there is much to learn about how the components of this system interact with each other in the brain and throughout the body. Exposure to psychological stressors can alter the primary antibody response, and increased persistent stress levels can lead to pathological organ changes, psychological changes, as well as psychosomatic illnesses (Keil, 2004.; Moraska et al., 2002).

Psychological stress for undergraduate students is often associated with the pressure for academic success, which probably reaches its peak during tests (Omigbodun et al., 2006; Loft et al., 2007). Academic stress

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can be considered an excellent naturalistic stress model in humans. Therefore, academic exams are frequently used in stress research. They are linked to changes in mental and physical health, increased anxiety, and changes in the immune system. A predominance of either hypothalamic-pituitary-adrenal (HPA) axis activation, sympathetic nervous system activation, or a balance of both can be hypothesized. The brain awakens physiological responses to this type of stress that ultimately result in the activation of the HPA system and the release of the stress hormone cortisol from the adrenal cortex (Owens and Nemeroff, 1991; Kageyama et al., 2021). Excessive cortisol release post-examination stress has been associated with changes in immune functioning (Connor and Leonard, 1998).

Research on the relationship of neuropeptide Y (NPY) with stress, especially its effects on stress resilience, has been the focus of attention in the last fifteen years. NPY is a 36-amino acid neuropeptide in peripheral sympathetic nerves hypothesized to regulate immune response during stress. It is recognized as one of the neurochemicals that play an essential role in the etiopathogenesis, treatment, and increase of stress resilience in stress-related disorders, especially post-traumatic stress disorder (Holzer et al., 2012; Sajdyk et al., 2004).

The study aimed to investigate the alterations of the salivary levels of stress-related hormones and cytokines such as cortisol, neuropeptide Y, and interleukin-1β to understand the neuroimmunological differences better.

2. Materials and methods

2.1. Participants

Fourty-four students (18–25 years) enrolled in Prosthodontics at Cyprus Health and Social Sciences University, Term I, were recruited for this study. Exclusion criteria for the study were any chronic or acute disease, self-reported psychiatric disorders, use of psychotropic medication, pregnancy, use of oral contraceptives or estrogens, and current dental or orthodontic treatments. Physical activity, cigarette, and alcohol consumption were recorded for each subject. The exam was held between 9 a.m. and 5 p..m.. Our study was performed with the approval of the ethics review board of the Gazi University Faculty of Medicine Ethics Committee, and all the participants accepted and signed the written informed consent. The number of ethical approvals is 831–12.11.2018.

2.2. Sample collection & processing

To keep the standardization related to circadian rhythms, saliva samples were collected between 08:00 and 09:00 (pre-stress) and after 5 p.m. as a post-stress of unaware exam hour. Within 60 min before the test, individuals were not allowed to smoke, eat, drink liquids, or brush their teeth. They were asked to soak the swab with saliva for 1–2 min until saturated thoroughly. The collection tubes were waited 30 min at room temperature and then were centrifuged for 10–15 min at approximately 3200 g. After centrifugation, the swab and small insert were thrown away, and the large outer tube was stored at −80 °C until analysis.

2.3. Analytical method

The NPY (ng/mL), cortisol (ng/mL), and interleukin-1β (pg/mL) levels were analyzed by ELISA method according to the manufacturer's instructions. The commercial kit used for NPY was MyBioSource (Catalog#MBS3801243 and Lot#08/202), and the assay sensitivity was one ng/mL. Cortisol levels were detected by the Diametra-Immunodiagnostic System (LOT:4817A). The assay range was 0.5–100 ng/mL, and sensitivity was 0,12 ng/mL. IL-1β was determined by Ray Biotech, Inc kits (LOT:134,018), and the minimum detectable amount was 0.3 pg/mL, and intra-assay CV% was <10% and inter-assay CV% was <12%.

2.4. Statistical analysis

Statistical analysis was performed with SPSS 18.0 computer software, and data was analyzed using students' t-test at a 95 percent confidence limit with $p < 0.05$ considered as significant. The results were presented as mean and standard deviation. The relationship between cortisol and other parameters was determined using Spearman correlation analysis.

3. Results

Thirty participants (68.2%) were female, and 14 (31.8%) were male. The mean ages of the female and male participants were not significantly different (mean age: 19.71 ± 1.07, females: 20.2 ± 1.75, $p = 0.344$).

During the analysis, we compared the hormonal and inflammation variables of the study group in response to pre-stress and post-stress. Since gender significantly impacted cortisol values, we analyzed the data for males and females separately. However, the mean cortisol of the female and male participants was not significantly different (mean cortisol: 7.29 ± 3.58, females: 8.43 ± 5.11, $p = 0.187$).

Table 1 shows the salivary cortisol, NPY, and IL-1β concentrations of post-examination and pre-examinations. Cortisol ($p = 0.019$), NPY ($p = 0.001$), and IL-1β concentrations ($p = 0.0003$) were significantly increased during post-examination (Table 1). Salivary cortisol, IL-1β, and NPY concentrations were quite different between the two groups (pre-stress and post-stress) of students. There were no gender effects.

In terms of cortisol, NPY, and IL-1β, there was a significant difference between the pre-stress and post-stress. The correlation coefficients of parameters of pre-stress and post-stress are summarized in Table 2.

Spearman's rho correlation analysis tested the relationship between salivary NPY and IL-1β concentration. There was a positive correlation between salivary NPY, and IL-1β levels, this relationship was significant ($r = 0.430^*$, $p = 0.04$, $r = 0.589^{**}$, $p = 0.004$, respectively) (Fig. 1).

Analyses of the cortisol and IL-1β variables relationship showed that the differences in cortisol concentration from the baseline (pre-examination) to post-examination positively correlated to IL-1β ($r = 0.642^{**}$, $p = 0.003$). The change in cortisol concentrations from pre-examination to post-examination about the shift in interleukin-1 beta concentration was shown in Fig. 2.

Besides, the correlation of cortisol to NPY during pre-stress and post-stress is shown in Fig. 3. No statistically significant differences were observed between cortisol and NPY in the means of pre-stress and post-stress ($p = 0.713$, $p = 0.171$, respectively).

4. Discussion

The current study included physiological measures to depict the relationships among academic stress, cortisol secretion, and immune functioning. Results indicated that salivary cortisol concentration increased significantly in the post-exam group compared to the pre-exam group.

Table 1
Mean values of salivary cortisol, neuropeptide Y, and interleukin-1 beta concentration pre-examination and post-examination among undergraduate students.

Academic stress measures	Pre-examination Mean ± SD	Post-examination Mean ± SD	*p*-value
Cortisol (ng/mL)	6.37 ± 4.14	9.65 ± 4.53	0.019*
NeuropeptideY (ng/mL)	27.10 ± 4.71	32.13 ± 4.69	0.001**
Interleukin-1 beta (pg/mL)	7.20 ± 3.49	11.69 ± 3.61	0.0003**

*The statistical significance is marked with asterisks $p < 0.001^{**}$; $p = 0.05^*$.

Table 2
Correlation between the cortisol, neuropeptide Y, and interleukin- 1β concentration pre-examination and post-examination among undergraduate students.

	Pre-examination			Post-examination		
	NPY	IL-1β	Cortisol	NPY	IL-1β	Cortisol
Cortisol (ng/mL)	0.09	−0.265	–	0.327	0.642**	–
	0.713	0.273		0.171	0.003	
Neuropeptide Y (ng/mL)	–	0.430*	0.09	–	0.589**	0.327
		0.04	0.713		0.004	0.171
Interleukin-1β (pg/mL)	0.430*	–	−0.265	0.589**	–	0.642**
	0.04		0.273	0.004		0.003

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

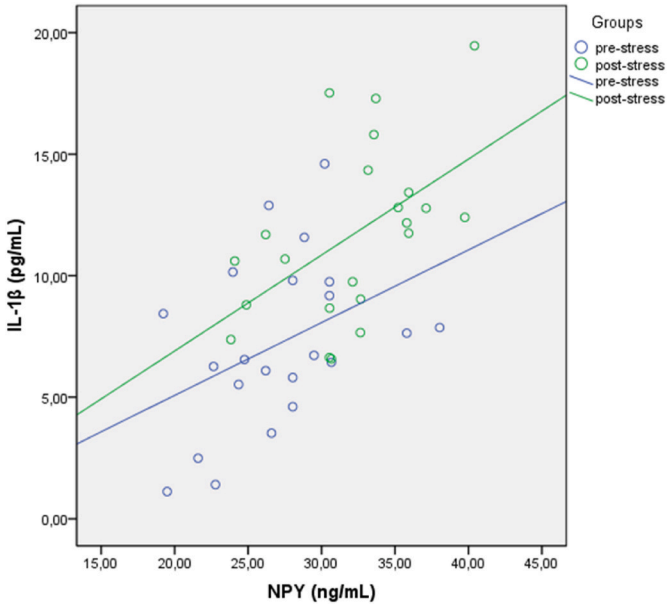

Fig. 1. Relationship between NPY and IL-1β pre and post-examination. Stress-induced increases in saliva IL-1β and salivary NPY were significantly and positively related.

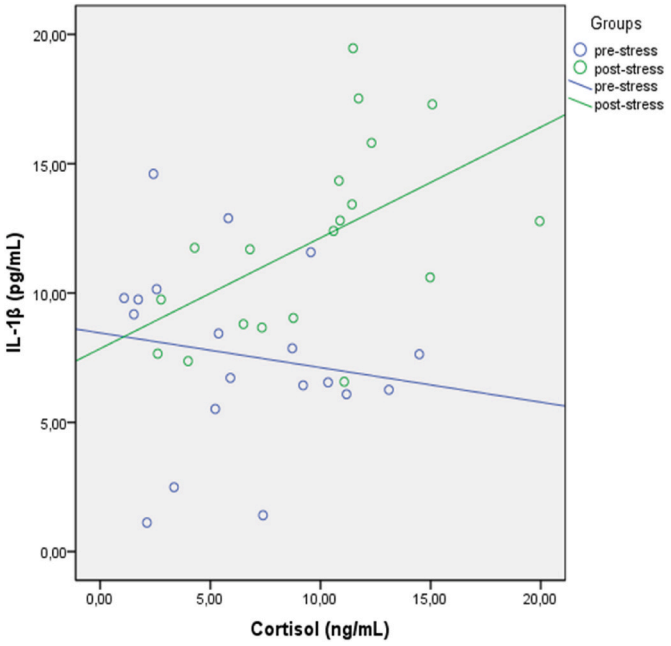

Fig. 2. Relationship between Cortisol and IL-1β post-examination. Stress-induced increases in saliva IL-1β and salivary cortisol were significantly and positively related.

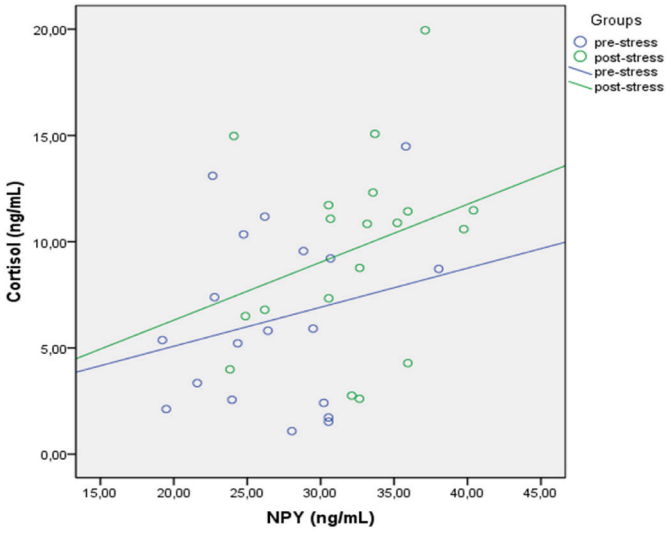

Fig. 3. Relationship between NPY and cortisol pre and post-examination.

Stress has long been hypothesized to stimulate specific neuroendocrine activity with elevated cortisol secretion. Different studies have reported increased cortisol levels during academic examinations (Romero-Romero et al.; Ng et al., 2003a; Ng et al., 2003b; Krahwinkel et al., 2004). Our undergraduate students' higher salivary cortisol level at post-exam indicates activation of the HPA axis.

The lack of a significant positive correlation between elevations in cortisol and elevations in psychological stress post-exam was in agreement with the study by Weekes et al. who had reported no significant correlations in cortisol and elevations in psychological stress measures (Weekes et al., 2006).

The increased cortisol levels observed here and the potential simultaneous increase in secretion of NPY and IL-1β may directly affect immune function. Immune cells have receptor sites for many neurotransmitters and hormones associated with stress responses. Binding to these receptors can alter cytokine production, lymphocyte proliferation, and antibody secretion (Cohen et al., 2001). Also, a complex relationship exists between stress measures and immune functions during exam stress (Krahwinkel et al., 2004).

In this study, we also evaluated the saliva levels of IL-1β, the inflammation-related cytokine. IL-1β levels were found to be significantly higher during the exam period compared to before the exam. Slavish et al. said that saliva may be a good use for detecting an inflammatory change from onset, for example, in response to acute stressors (SSlavish et al., 2015). In this context, it has been reported that

saliva levels of classical proinflammatory cytokines such as IL-1β consistently increase in response to acute stress.

Some studies have shown that proinflammatory cytokines and interleukin-1 beta levels are increased in individuals exposed to stress. Paik et al. (2000) reported that stress caused by academic exams significantly increased interleukin levels in 42 university students. Also, it is known that IL-1β stimulates HPA axis activity, and activation of the immune system is associated with the inflammatory response (Paik et al., 2000).

We also tested the hypothesis that there might be a positive correlation between the levels of NPY and IL-1β during pre and post-examination stress. This shows that undergraduate students are exposed to stress.

These results and those related to immune reactivity show that at least two mechanisms underlie modifications in response to stress conditions. The stress reaction activates the HPA axis and the autonomic nervous system. Salivary NPY levels have increased during stress (Laila Y Al-Ayadhi Laila Y., 2005; Reichmann and Holzer, 2016).

Another characteristic of the present results that can be accepted as a limitation of this study is that smoking was not used as an exclusion criterion. Although these variables may affect biochemical measurements, all participants were smokers.

Saliva is an easy and non-invasive alternative body fluid. Many researchers working in different fields use stress measurement in saliva samples. It needs an optimal method when evaluating stress-related parameters. At the same time, saliva samples should be collected and administered simultaneously (Şemsi et al., 2022). Although the kits are compatible with all body fluids, method validation should not be performed for saliva samples since their preanalytical properties are unknown. All those topics are the future aspects of our study.

5. Conclusion

The study aimed to combine the psychological stress evaluation with the analysis of salivary biomarkers of undergraduate students. In conclusion, academic stress increased the amount of IL-1β, NPY, and cortisol in saliva. Further studies with larger student populations of both genders are required to explore the relationship between biochemical mediators of stress and immune function changes.

Statement of ethics

The procedures involved in this research had previous approval of the ethics review board of the Gazi University Faculty of Medicine Ethics Committee, and all the participants had accepted and signed the written informed consent. The number of ethical approvals is 831–12.11.2018.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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