

SHORT COMMUNICATION

Effects of examination stress on salivary biomarkers in Medical Laboratory Technology students

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Abstract:

Salivary secretion is regulated by the sympathetic and parasympathetic nervous system which made it possible as useful biomarker of stress. This study aimed to determine the effects of academic examination stress on salivary cortisol, salivary immunoglobulin A (sIgA), and salivary alpha-amylase (sAA) in Medical Laboratory Technology (MLT) students. Five minutes unstimulated saliva samples were collected from thirty-nine students before and after a 1.5 hours written test. The concentration of selected salivary biomarkers were determined by enzyme-linked immunosorbent assay. Higher salivary cortisol levels were observed before the exam while for the sIgA, the levels were higher after the exam ($P < 0.05$). No significant differences were observed for alpha-amylase concentration before and after the exam. These findings suggest that the examination stress is associated with salivary cortisol and sIgA which could be useful as an acute stress biomarker.

Keywords: Alpha-amylase, cortisol, examination stress, immunoglobulin A, salivary biomarkers

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1. INTRODUCTION

Academic examination or assessment play an important role in student's life as it helps in evaluating students learning outcome and their mastery of specific subject. According to Lacey et al., academic examination could be considered as one of the most acute stress experienced by students due to its significant consequences on one's future development [1].

Studies have demonstrated that acute stress could increase the hypothalamus-pituitary-adrenal (HPA) axis activity of the nervous system [2]. Cortisol is the end-product of HPA of the endocrine system which will be increased in response to stress. A strong correlation between salivary and serum cortisol makes salivary cortisol a suitable marker to reflect the HPA activity [2]. Changes in catecholamine levels under stressful condition could be predicted by salivary alpha-amylase concentration. Thus, salivary alpha-amylase have also been suggested as a potential indirect marker for sympathoadrenal-medullary (SAM) of the sympathetic nervous system [3]. In addition, mucosal immunity of the immune system is also affected by psychological stress. In the state of chronic stress, the suppression on immune system lead to the decrease of immunoglobulin A secretion. However, this immunological stress marker was increased immediately after a short-term stressor [4].

Despite the various investigation on the potential of these salivary secretions as stress biomarkers, the findings of the academic examination effect on salivary biomarkers were not consistent. Therefore, this present study investigated the association of salivary cortisol, sIgA and alpha-amylase (sAA) with examination stress.

2. MATERIALS AND METHODS

This present study was carried out with MLT final year students from Faculty of Health Sciences, UiTM Puncak Alam Campus. Ethical clearance to conduct the study was granted from UiTM Research Ethics Committee. Thirty-nine healthy students which include thirty-two female and seven male were recruited and gave informed consent to participation. The students were requested in advance not to eat, drink, and smoke at least one hour before saliva collection to minimize possible food debris and stimulation of saliva. The participants involved were also examined to confirm that they were not wearing any dental apparatus such as braces, medication-free, not suffering from major depression, and have any endocrine, cardiovascular or other chronic diseases.

The participants underwent an hour and thirty minutes written test on the subject of Stress Management. This mid-term test is part of the continuous assessment process that constitutes a small proportion of the student's final examination marks. The whole saliva sample was collected prior to exam and immediately after the exam. The sample was collected in a sterile container within five minutes time and the saliva production is not stimulated by any sorts of material. The volume of saliva sample was recorded right after the collection process and divided by five minutes to acquire the salivary flow rate (mL/min). Saliva samples were then stored at -20°C until further analysis.

The concentrations of saliva cortisol ($\mu\text{g/dL}$), sIgA ($\mu\text{g/mL}$) and sAA (U/mL) were determined using enzyme-linked immunosorbent assay kits which were manufactured by Salimetrics® LLC (PA, USA). The secretion rate of salivary IgA ($\mu\text{g/min}$) was obtained by multiplying the sIgA concentration with salivary flow rate [5]. Data analysis was performed using GraphPad Prism Version 5.04 software for Windows (GraphPad Software Inc., San Diego, CA, U.S.A). Paired sample t-test were performed and a significance level of $P < 0.05$ was used.

3. RESULT

The salivary cortisol concentration before the examination was $0.230 \pm 0.031 \mu\text{g/dL}$, and that immediately after the examination was $0.086 \pm 0.011 \mu\text{g/dL}$ (Fig. 1). There was a statistical significance in the mean of salivary cortisol level between before and after the examination ($P < 0.05$). As shown in Fig. 2, the secretion rate of salivary IgA after the examination ($143.70 \pm 26.92 \mu\text{g/min}$) increased from that before the examination ($80.32 \pm 13.47 \mu\text{g/min}$). There was a statistical significance in the mean of salivary IgA secretion rate between before and after the examination ($P < 0.05$). The alpha-amylase concentration before the examination was $111.4 \pm 13.46 \text{ U/mL}$, and that immediately after the examination was $138.2 \pm 15.38 \text{ U/mL}$ (Fig. 3). However, the differences in sAA level between before and after the examination were not statistically significant.

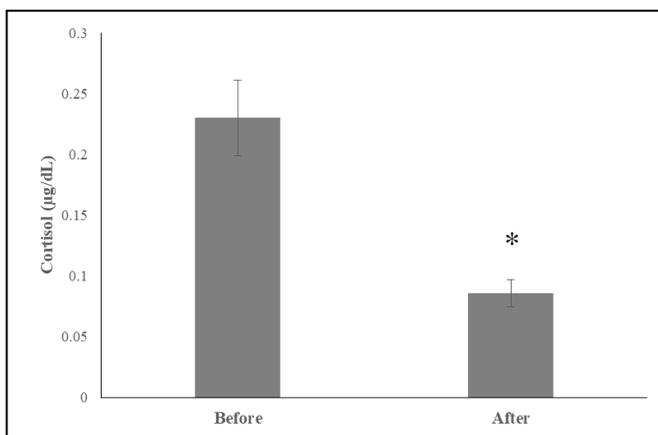


Figure 1: Mean values (with S.E.M) of salivary cortisol before and after the examination. *indicates statistically significant difference ($P < 0.05$).

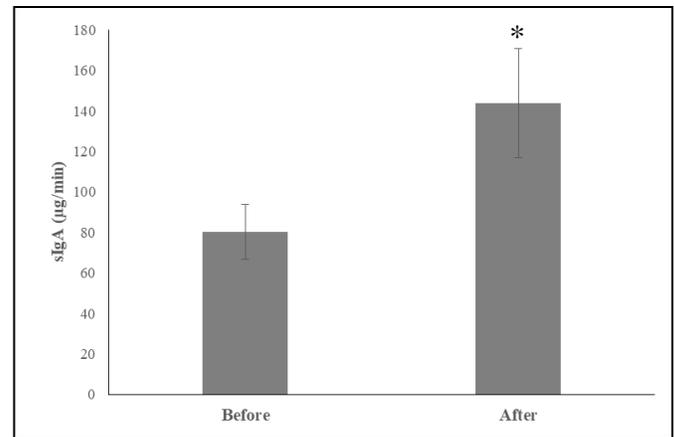


Figure 2: Mean values (with S.E.M) of salivary IgA before and after the examination. *indicates statistically significant difference ($P < 0.05$).

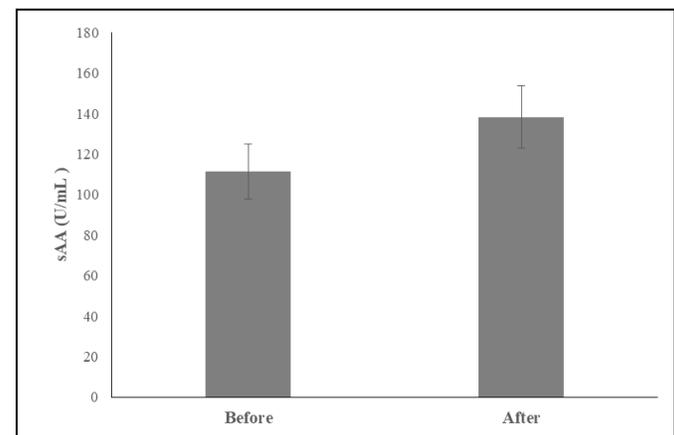


Figure 3: Mean values (with S.E.M) of salivary AA before and after the examination.

4. DISCUSSION

Cortisol is the major glucocorticoid release by adrenal cortex in HPA system. In response to psychological stress, cortisol is the biomarker that is largely influenced. Salivary cortisol accurately reflects cortisol in the serum [2]. In this present study, the salivary cortisol concentrations were noted to be higher before the examination. This may be caused by the stressor or specifically called anticipatory stress. Anticipation to perform the written examination well may cause the students to feel stress and burdened. Previous studies have also shown the same result with higher levels of cortisol during anticipation of the stressful task such as oral academic examination, stressful video viewing and public speaking [1, 6-7]. The findings that cortisol levels were elevated in anticipation of psychological stressors such as above are consistent with reports that acute stressors stimulate adrenal glucocorticoid secretion [2]. However, there are studies which reported that the difference of salivary cortisol levels before and after the stressors in their studies are not statistically significant [8-9]. These various

and contradicting findings may result from different types of acute psychological stressors which induce different impact on the participants and consequently to the cortisol secretion.

The secretory IgA in saliva is a good indicator of the status of immune system. It has been hypothesized that stress affects the immune system through its impact on the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system. Chronic stress has been reported to down-regulate the sIgA, whereas an acute psychological stress induces mobilization [10]. From this study, the salivary IgA has been found to be higher after the examination. In agreement, other study have also shown similar result with increase IgA level after the exam when the sample is collected immediately after the examination [11]. Both sIgA and norepinephrine concentration increased in forty-six students immediately after an examination [12]. According to Tsujita & Morimoto, 'immediate stress effect' caused rise in IgA level when determine immediately after the stress event [4].

Saliva consists of 99.5% water and 0.5% electrolytes, glycoproteins, enzymes, and secretory antibodies [13]. Alpha-amylase is one of the major protein components of saliva. This salivary enzyme has been suggested as a potential indirect marker for sympathoadreno-medullary (SAM) axis as its levels changes in the event psychological stress. Previous studies on acute stressors such as stressful video viewing [6], arithmetic task [8], and public speech [14] showed significant differences in salivary alpha-amylase before and after performing the task. These findings can be explained by the feeling of anticipation from the stressors or the stressful task. However, no significant difference in alpha-amylase concentration before and after the examination was observed in this study. According to Ng et al., different intensities of stress induce different changes in salivary biomarkers, suggesting the high sensitivity of alpha amylase to the intensity of the stressor [15].

It is known that the marks for this test only constitute a small portion of the total marks for the subject. Hence, it may be considered as a relatively low stressor to compare with the final examination. Final examination would be a much better stressor, however due to time constriction of this study, it is unlikely to be used as the stressor.

5. CONCLUSION

Salivary cortisol showed a higher concentration before the examination and secretory IgA demonstrate a higher level after examination. However there were no significant changes of sAA concentration before and after the exam. These findings may suggest salivary cortisol and IgA could be used as potential indicators for assessing acute stress induced by academic assessment.

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