The effects of examination stress on salivary cortisol, immunoglobulin A, and chromogranin A in nursing students

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ABSTRACT

This study aimed to assess the effects of examination stress on salivary cortisol, immunoglobulin A (IgA), and chromogranin A (CgA) in nursing students. Saliva samples were collected from 15 healthy females before and immediately after the one-hour examination, and two hours after the examination. Salivary cortisol, IgA, and CgA concentrations were determined by enzyme-linked immunosorbent assay. Both IgA and CgA concentrations statistically increased immediately after the examination (P < 0.05) and decreased two hours after the examination. No significant differences were observed between before and after the examination in the salivary cortisol concentration. These findings suggest that the acute stress due to the examination is associated with raised salivary IgA and CgA, but not cortisol.

Students repeatedly take a variety of tests and examinations in the university. As passing or failing examinations generally has major consequences for future development, academic examinations have been considered as one of the most acute stresses experienced by students. Responses to stressful situations can be observed on the physiological level. Acute stress has been reported to increase the activity of the hypothalamus-pituitary adrenal (HPA) axis with subsequent rise in salivary cortisol level (7). Salivary immunoglobulin A (IgA) became a focus of interest in psychoimmunological research since it has been shown to be sensitive to variations in subjective and objective stress levels (17). Salivary chromogranin A (CgA) may be a sensitive and quantitative index of the activity of the sympathetic nervous system (5, 10). These salivary biomarkers have made it possible to scientifically investigate the effect of stresses. However, there is an inconsistency in reports on the academic examination effect. Some studies have shown that academic examinations, as acute stress, increase cortisol level (1, 8), whereas other studies reported a decrease of the salivary cortisol level after the examinations (9, 11). Therefore, in the present study, we investigated the effect of examinations on salivary cortisol, IgA, and CgA in nursing students.

The present study was carried out with students in their fourth year at the School of Nursing, Osaka Prefecture University. Subjects were 15 healthy female students aged 21–26 years old, and they were recruited to take part in this study. All subjects gave informed consent to participation. None were receiving any medication and they did not have any disease. As salivary cortisol, IgA and CgA showed a circadian rhythm (3, 14), the experiment was performed between 14:00 h and 18:00 h. Subjects took 100 multiple-choice questions of anatomy and physiology for one hour, and then commentary on the contents of the examination by the instructor for two hours. Anatomy and physiology are one of most stressful subjects for nursing students. The students must prepare for the National Examination includ-
ing anatomy and physiology four months later. As shown in Fig. 1, saliva samples were collected 10 min before the examination, immediately after the one-hour examination, and two hours after finishing the examination. Saliva samples were collected using the Salivette system (Sarstedt Co. Ltd., Germany) and a cotton wad was placed under the tongue for 1 min in each subject. Saliva samples were extracted from cotton wads by centrifuging at 1,000 rpm for 2 min. The saliva was stored frozen in capped test tubes at −30°C until assay. The concentrations of salivary cortisol (μg/dL) and IgA (μg/mL) were determined using enzyme-linked immunoassay kits, which were manufactured by Salimetrics LLC (PA, USA). The concentration of CgA (pmol/mL) was measured using a CgA (Human) enzyme immunoassay kit (YK070; Yanaihara Institute Inc., Shizuoka, Japan). Total protein concentration in saliva was determined using Bio-Rad protein assay kit with human serum albumin as a standard. CgA concentration was corrected by protein concentration and expressed as pmol/mg protein. The reaction product was quantified spectrophotometrically at 450 nm or 492 nm with a microplate reader (Beckman Coulter, DX800 multimode detector).

Data analysis was performed using Statview for Windows. Paired t-tests were performed and the level of statistical significance was set at \( P < 0.05 \).

The cortisol concentration before the examination was 0.148 ± 0.024 μg/dL, that immediately after the examination was 0.156 ± 0.037 μg/dL, and that of two hours after the examination was 0.102 ± 0.034 μg/dL (Fig. 2). The differences among them were not statistically significant. As shown in Fig. 3, IgA concentration after the examination (mean ± S.D. = 85.822 ± 16.293 μg/mL) increased from that before the examination (mean ± S.D. = 54.035 ± 14.297 μg/mL), and IgA concentration subsequently decreased two hours after the examination (66.466 ± 13.927 μg/mL). There was a statistical significance in the mean IgA level between before and after the examination (\( P < 0.05 \)). As shown in Fig. 4, CgA concentration after the examination (mean ± S.D. = 23.500 ± 6.257 pmol/mg) increased from that before the examination (mean ± S.D. = 11.948 ± 3.359 pmol/mg), and CgA concentration subsequently decreased two hours after the examination (12.701 ± 3.250 pmol/mg). There was a statistical significance in the mean CgA level between before and after the examination (\( P < 0.05 \)).

Total protein concentration in saliva before the examination was 0.314 ± 0.083 mg/mL, that immediately after the examination was 0.356 ± 0.110 mg/mL, and that of two hours after the examination was 0.293 ± 0.066 mg/mL. The differences among them were not statistically significant.

Stress influences the secretions of IgA in mucosal immunity, CgA in the sympathetic nervous system, and cortisol in HPA axis. The present study revealed that acute stress such as an academic examination increased concentrations of salivary IgA and CgA, while such stress did not affect the concentration of salivary cortisol. The increase of IgA and CgA was not due to that of total protein. Therefore, the nursing students may perceive a higher level of stress after than before the examination. It was reported that the concentrations of IgA and CgA were elevated during mental arithmetic tasks or oral presentations in previous studies (4, 10). However, these studies have not dealt with the association of IgA, CgA, and cortisol with stress before and after written examinations. Furthermore, Ng et al. did not find the increase of IgA and CgA before and after the written examination among dental students (11). In the present study, it appears that three biomarkers might be useful as putative markers of stress, although cortisol might reflect a different underlying psychological process from IgA and CgA.

The changes in the concentrations of IgA and CgA affected by examination stress showed a similar pattern in the present study. It was reported that the concentration and secretion rate of IgA were elevated by acute psychological stressors (5). Nakane et al. (10) observed a significant elevation in salivary CgA immediately before oral presentations, and then CgA concentration decreased immediately afterwards. Salivary IgA is derived from plasma cells in the interstitium of salivary glands and CgA is an acidic glycoprotein that is stored and co-released by exocytosis with catecholamines from the adrenal medulla and sympathetic nerve endings (12, 15, 16). Recently, salivary CgA was shown to be produced by the human submandibular gland and secreted into saliva (13). The concentration and se-
IgA and CgA in stress response reflects adrenocortical activity. Activation of the HPA axis and subsequent release of cortisol are major components of the physiological stress response. However, we could detect no differences in cortisol levels before and after the examination. This is contrary to investigations reporting increased cortisol secretion in response to academic examinations (1, 8). Other studies reported decreased cortisol levels in oral academic examinations or written examinations (9, 11, 18). Therefore, the data from this and other studies suggest that salivary cortisol may not be sensitive to the examination stressor.

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REFERENCES


