Perturbation of Secretory Ig A in Saliva and Its Daily Variation by Academic Stress

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Abstract

Objectives: Several studies have reported that the secretory immunoglobulin A (S-IgA) concentration in saliva is an indicator of psychological stress. The aim of this study was to clarify the relationship between S-IgA and the stress from academic examinations.

Methods: S-IgA levels in 10 medical student volunteers from the second year course between May 4 and July 13, 2000 were examined using the ELISA method.

Results: There was a tendency for S-IgA in saliva to be higher on the day before academic examinations and during them, and lower on the days between these examinations.

Conclusions: It may be possible to use this measurement to monitor psychological stress in students and workers.

Key words: saliva, secretory Ig A, academic stress, ELISA, medical student

Introduction

Secretory immunoglobulin (Ig) A (S-IgA) measured in saliva, which is an index of mucosal immunity, has been reported to be sensitive to psychological variables^{1–10}. Several studies have demonstrated that chronic stress down-regulates S-IgA, whereas an acute psychological challenge induces mobilization^{3,4,8,9}. Among psychological stresses, academic examinations may be one of the most typical ones for students. Although there have been several studies of the relation between academic examinations and S-IgA concentration^{1,4,7,10}, it is not clear how S-IgA concentration including daily variation is altered during a months-ranging period due to academic examinations.

At Kawasaki Medical School, there is a term-end examination period at the close of each of the school's three terms, with sporadic midterm examinations being held in some courses. In the present study, we analyzed the S-IgA concentration in the saliva of second year medical students during the first term of the year 2000 and considered any changes in it during the period of academic examinations to determine if there was any relation.

Subjects and Methods

Individual student volunteers from the second year medical course

Fifteen individuals were selected from among the volunteers in this study. However, five of them were finally withdrawn due to the small number of collected samples. Therefore, analyses were performed using samples of saliva derived from 10 students (8 males and 2 females). All were Japanese and ranged in age from 19 to 27 years old (average age, 20.7±0.8).

Collection of saliva

Saliva was collected three times (on rising, at forenoon, and at bedtime) every Thursday between May 4 and July 13, using SalivetteTM (Sarsted, Aktiengesellschaft & Co., Nümbrecht, Germany) according to the manufacturer's instructions. Briefly, each volunteer kept the cotton part of the Salivette in his/her mouth and chewed for two minutes. Then, the cotton was frozen at -20°C until centrifugal collection of the saliva.

Measurement of S-IgA by enzyme-linked immunoabsorbent assay (ELISA)

Measurement of S-IgA in the collected saliva was performed using the human Ig A ELISA quantitation kit (Bethyl Laboratories, Inc., Montgomery, TX, USA) according to the manufacturer's instructions. Each sample was measured three times. In addition, the S-IgA flow-rate (µg/min) was calculated as the

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Table 1 Secretory Ig A concentration ($\mu\text{g/ml}$) in saliva from volunteer #D.

Date	5/04	5/11	5/18	5/25	6/01	6/08	6/15	6/22	6/29	7/06	7/13	Average
On rising												
Measurement 1	381.0	353.0	266.5	27.0	314.0	252.0	49.0	148.5	353.0	317.5	371.0	
Measurement 2	356.0	389.5	278.5	35.0	329.5	252.0	52.0	163.5	358.5	329.5	404.0	
Measurement 3	381.0	431.5	273.0	41.5	329.0	273.5	55.5	177.5	355.0	377.5	396.0	
Average	372.37	391.33	272.67	34.50	324.17	259.17	52.17	163.17	355.50	341.50	390.83	268.83
SD	14.43	39.28	6.01	7.26	8.81	12.41	3.25	14.50	2.78	31.75	17.21	
At forenoon												
Measurement 1	288.0	88.5	183.5	189.5	164.0	176.5	21.0	161.5	478.0	80.5	93.0	
Measurement 2	287.5	100.5	215.5	195.0	160.0	176.5	35.5	152.5	474.0	69.0	85.0	
Measurement 3	338.5	107.0	225.5	173.5	193.0	184.5	39.0	168.0	524.0	77.0	88.0	
Average	304.67	98.67	208.17	186.00	172.33	179.17	31.83	160.67	492.00	75.50	88.67	181.61
SD	29.30	9.39	21.94	11.17	18.01	4.62	9.54	7.78	27.78	5.89	4.04	
At bedtime												
Measurement 1	57.50	49.50	87.00	39.50	50.50	65.00	60.00	100.00	21.50	26.50	6.00	
Measurement 2	64.50	55.00	79.00	52.00	43.50	78.00	16.00	103.50	20.50	38.00	15.00	
Measurement 3	62.00	68.50	104.00	57.50	49.00	86.50	12.50	133.00	20.50	37.00	29.00	
Average	61.33	57.67	90.00	49.67	47.67	76.50	29.50	112.17	20.83	33.83	16.67	54.17
SD	3.55	9.78	12.77	9.22	3.69	10.83	26.47	18.13	0.58	6.37	11.59	

Table 2 Secretory Ig A flow rate ($\mu\text{g/min}$) in saliva from volunteer #D

Date	5/04	5/11	5/18	5/25	6/01	6/08	6/15	6/22	6/29	7/06	7/13	Average	SD
On rising	430.4	144.8	1130.9	8.1	246.4	199.6	12.8	40.0	85.3	76.8	111.2	135.12	122.66
At forenoon	137.1	41.4	114.5	99.5	68.1	114.7	10.3	37.8	113.2	17.7	29.3	71.23	45.83
At bedtime	35.9	47.9	45.9	17.6	28.6	36.7	12.1	33.7	5.2	9.1	3.7	25.12	16.19

concentration of S-IgA ($\mu\text{g/ml}$) times the collected volume of saliva (ml) divided by the collection time (2 min).

Relative index of S-IgA concentration and flow-rate at each sampling time-point in each volunteer

Since there was a wide range in the actual values of the S-IgA concentration and flow-rate among all volunteers, we determined a relative index of the S-IgA concentration and flow-rate in each volunteer to generalize any tendency of increase or decrease during the assayed period. The relative index was calculated by dividing the average of the data collected at each sampling time-point in each volunteer. The relative index of the average was 1.0 in each sampling time-point in each volunteer.

Statistics

In each volunteer, the average concentration and flow-rate of S-IgA among the three sampling time-points were compared. Then, a comparison of the average concentrations and flow-rates of S-IgA among the three time-points for all volunteers was carried out. In addition, we compared the average relative indices of the S-IgA concentration and flow-rate at the on rising sampling time points for all volunteers studied on all the sampling dates. For these statistics, the Student's t test was used.

Results

Measurement of S-IgA in saliva

Tables 1 and 2 respectively show the S-IgA concentration and flow-rate in volunteer #D. From these findings, it appears that the average concentration and flow-rate of S-IgA at the on rising sampling time-point were higher than those at the other two time-points.

Next, we calculated the average concentration and flow-rate of S-IgA at each sampling time-point for all 10 volunteers. As shown in Fig. 1-A, the concentration at the on rising time-point ($372.49 \pm 195.38 \mu\text{g/ml}$) was significantly higher than at the other

two sampling time-points (forenoon: 156.24 ± 83.53 , $p=0.0013$, bedtime: 140.31 ± 98.22 , $p=0.0007$). This significance was also demonstrated in the flow-rate of S-IgA as shown in Fig. 1-B.

As shown in Fig. 2-A, this tendency was significant in volun-

Fig. 1 The average S-IgA concentration (A) and flow-rate (B) at each sampling time-point (on rising, at forenoon, and at bedtime) in all 10 volunteers.

Fig. 2 The average and standard deviation of the S-IgA concentration (A) and flow-rate (B) at three sampling time-points in individual volunteers #A to J. Statistical significance of $p < 0.05$ is indicated by the black lines and of $0.05 \leq p < 0.1$ is by the dotted lines.

teers #D, F, G, and I in terms of the concentration of S-IgA. In volunteers #A, C, E, and J, a similar tendency was observed but it was not significant. However, volunteers #B and H showed no tendency in the concentration of S-IgA among the three sampling time-points. The flow-rate of S-IgA in each volunteer demonstrated a similar tendency to that found in its concentration (Fig. 2-B). These results may suggest that the S-IgA concentration and flow-rate in saliva are usually higher in the early morning, but they vary from individual to individual.

S-IgA concentration and flow-rate in saliva throughout the whole assayed period

Fig 3-A and 3-B respectively demonstrate the actual S-IgA concentration and flow-rate in saliva in volunteer #D between May 4 and July 13. These findings suggest that the concentration and flow-rate of S-IgA were low on May 25, June 15, and June 22. This tendency was more distinct at the on rising sampling time-point. There were two midterm examinations, as shown in Fig. 3. The first date (May 25) when S-IgA showed a low concentration and flow-rate was sandwiched between these two examinations. In addition, at the end of the sampling period, there were term-end examinations for two weeks and during these days, the S-IgA concentrations and flow-rates remained high in this volunteer.

To clarify this tendency in all the volunteers studied, we defined the relative index of the concentration and flow-rate in each individual as described in Subjects and Methods. As shown in Fig. 4-A (concentration) and 4-B (flow-rate), the average of the relative indices in the volunteers showed a similar tendency to that

Fig. 3 The S-IgA concentrations (A) and flow-rate (B) in volunteer #D at each sampling time-point between May 4 and July 13, 2000. The hatched boxes in the upper part of the figure indicate two midterm examinations and term-end academic examinations, respectively.

found in volunteer #D as shown in Fig. 3, particularly at the on rising time-point.

Changes in the average of the relative indices (concentration and flow-rate) at the on rising time-point are shown in Fig. 5 (A and B, respectively) with the standard deviation. These findings suggest that the S-IgA concentration and flow-rate in saliva were the lowest on May 25 and June 15, and higher values were detected immediately before the two midterm academic examinations and at the end of the term-end examination period.

Perturbation of daily variation

As shown in Fig 4-B, the daily variation of the average relative indices of S-IgA flow-rate was perturbed on June 1 and June 29. Since these dates appeared to be the most stressful sampling time-points, academic stress may influence the daily variation of S-IgA secretion.

Discussion

Several studies have shown that S-IgA in saliva may be an indicator of psychological stresses, such as academic examination stress^{1,4,7,10} and work-related stress^{2,3,6}, and that it may be influenced by personality characteristics⁷⁻¹⁰. Chronic stress has been reported to down-regulate S-IgA, whereas an acute psychological challenge induces mobilization. In addition, upper respiratory

Fig. 4 The average relative indices of the S-IgA concentration (A) and flow-rate (B) on each sampling date during the whole sampling period. The hatched boxes are the same as those for Fig. 3.

infections may affect the S-IgA concentration in mucosal immunity^{11,12}.

In the present study, we focused on S-IgA in saliva as a marker of psychological stress in medical students and tried to determine if there was any long-term alteration during the first academic term by measuring the concentration and flow-rate of S-IgA every week. It was suggested that if academic examinations were the most stressful event for them, there might be a significant perturbation of S-IgA in saliva related to the examinations. The results showed that there was a tendency for S-IgA in saliva to be higher on the days before and during academic examinations and lower on the days between these examinations. In addition, it appears that the daily variation was also perturbed by academic stress. However, it was assumed from the large standard deviations shown in Fig. 5 that the concentration and flow-rate of S-IgA in some volunteers were not influenced by the academic examinations alone. Although S-IgA in saliva could possibly be a reliable indicator of the degree of psychological stress, there may have been other forms of stress related to personal relationship, sports, hobbies and family for these medical students that could have

Fig. 5 The average relative indices of the S-IgA concentration (A) and flow-rate (B) and the standard deviation at the on rising sampling time-point during the whole sampling period. The hatched boxes are the same as those for Fig. 3. Statistical significance of $p < 0.05$ is indicated by the black lines and of $0.05 \leq p < 0.1$ is by the dotted lines. The wide dotted horizontal line indicates the relative index (1.0).

influenced S-IgA during this study.

We were unable to examine short-time alterations in S-IgA values, such as in hour-based samplings before and after academic examination or other immunological markers, such as natural killer cell activity, which has been reported as one of the indicators of psychological stress¹³⁻¹⁶, in this study. However, the present results suggested that S-IgA measurement may be a possible indicator of psychological stress if examined over a month to year-long observation period. In addition, the methods for sampling and measurement are not complicated. Therefore, it may be possible to use this measurement to monitor psychological stress in students and workers.

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