

# A Feeling of Interest was Associated with a Transient Increase in Salivary Immunoglobulin A Secretion in Students Attending a Lecture

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## Abstract

**Relations between feelings and salivary IgA secretion were assessed in co-medical students attending a lecture.**

**The assessments were performed twice in two different classes held in December, 1999 and October, 2000 in the same manner, then the data of the two trials were combined and analyzed. On the basis of the impression of the lecture, students were divided into two groups: a group who felt interested and another group who felt indifferent or bored. Saliva samples were taken three times, at the beginning of, at the end of, and 15 minutes after the class, then the secretory IgA in saliva was determined by ELISA.**

**At the end of the class, an increase in salivary IgA secretion was seen in the group who felt interested, while not in the group who felt indifferent or bored. The increasing change in salivary IgA secretion was, however, moderate and transient, namely it increased only by about +30% on average, and returned to the initial level after 15 minutes.**

**Key words:** immunoglobulin A, saliva, students, lecture, feelings

## Introduction

Secretory immunoglobulin A (sIgA), which serves as a first line of immunological defense at mucous membranes against invasion of microorganisms<sup>1,2)</sup> has been shown to be sensitive to various psychosocial factors<sup>3–8)</sup>.

Stress factors including academic stress<sup>9–13)</sup>, life events<sup>8,14)</sup>, work demands<sup>15)</sup>, daily hassles<sup>16)</sup> and so on, were reported to affect sIgA levels in saliva. In general, chronic stress would have long term depressing effects on the sIgA secretion in saliva, while acute stress would have short term increasing effects on it<sup>7,17)</sup>.

On the other hand, relaxing factors including humorous movies<sup>18,19)</sup>, relaxation<sup>5,6,8,20–27)</sup>, massage<sup>28)</sup>, self-hypnosis with specific suggestions<sup>29)</sup>, human support<sup>5,10,30)</sup>, and enjoyable events for old persons<sup>31)</sup>, have been reported to have increasing effects on the sIgA secretion in saliva.

In addition to those psychosocial factors, the mental states of subjects including anxiety, depression, and personality were shown to affect the sIgA secretion in saliva.

From these findings, it is suggested that feelings or emotions, aroused by various kinds of psychosocial factors and modulated by mental states and personality, would be essential to short time

variations in the sIgA level in saliva.

The aim of this study was to examine the relationship between feelings and sIgA levels in saliva in co-medical students attending a lecture. Attending a lecture aroused not only a feeling of interest or positive feelings in some students, but also a feeling of indifference or boredom in others. So, by comparing the sIgA levels in saliva between those students, we were able to assess the effect of feelings on sIgA secretion.

## Method

### *Outline of procedures and subjects*

This study was conducted twice in two serial years, 1999 and 2000. In each trial, co-medical students in their final year were recruited. The numbers and gender of the students were as follows: 22 students (3 males and 19 females) at the first trial on 3rd December, 1999, and 24 students (7 males and 17 females) at the second trial on 20th October, 2000. However, we omitted four subjects for the following reasons: in the first trial, one female (22 years of age) did not give sufficient saliva for determination of IgA, and one male (32 years of age) was ten years older than the average age; in the second trial, one female (21 years of age) did not give sufficient saliva for determination of IgA, and a male (24 years of age) did not answer his impression of the class. Therefore, 42 students (8 males and 34 females, mean age 22.00±0.73 (SD) years old) participated in this study.

Both trials were performed in the same manner between 1:00 p.m. to 2:45 p.m. Before the lecture, all participants were informed about the aim and details of the trial, and asked to partic-

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ipate in it. During the experimental period, saliva samples were collected three times: at the beginning of, at the end of, and 15 minutes after the class. After the last saliva sampling, the students were asked to fill the questionnaires.

Since there was no significant difference in age and gender of the students between the two classes, the two trials were combined and analyzed.

#### Collection of saliva samples

Unstimulated whole saliva was collected with a tool "Salivette" (SARSTED AG & Co.) which consisted of a piece of cotton swab in a plastic centrifugation tube. At saliva sampling, the cotton swab was held in the mouth for 2 minutes for saliva absorption, then it was returned to the tube. Within 3 hours after sampling, the saliva samples were weighed and separated from the cotton swabs by centrifugation at 2,500 rpm for 20 minutes, then frozen and stored at  $-70^{\circ}\text{C}$  until analysis.

#### Determination of salivary IgA

Total IgA concentration in the saliva was determined by a double antibody sandwich ELISA procedure. Briefly, disposable polystyrene microtiter plates (Falcon) were coated with capture antibody by adding 100  $\mu\text{l}$  of goat antihuman IgA ( $\alpha$ -chain-specific, Sigma), diluted 2.5  $\mu\text{g}/\text{ml}$  in phosphate-buffered saline (PBS, pH 7.6) which is suitable for dissolving immunoglobulins because of the markedly less protein denaturation than a high-pH carbonate buffer (pH 9.6)<sup>32</sup>. After overnight incubation at  $4^{\circ}\text{C}$ , the plates were saturated at residual sites by adding 100  $\mu\text{l}$  per well of 2% bovine serum albumin (BSA, Sigma)-PBS solution for 2 hours at room temperature (ca.  $25^{\circ}\text{C}$ ). Then the plates were washed thrice by adding 200  $\mu\text{l}$  per well of PBS containing 0.02% Tween-20 (PBS-TW). Then, 100  $\mu\text{l}$  per well of the saliva sample, diluted to 1:1,000 by 2% BSA solution, was added to wells in duplicate. Simultaneously, 100  $\mu\text{l}$  per well of the secretory IgA standard (human colostrum IgA, Sigma) solution, diluted serially from 0.02  $\mu\text{g}/\text{ml}$  to 1  $\mu\text{g}/\text{ml}$ , was also added to wells in duplicate. After incubation overnight at  $4^{\circ}\text{C}$ , the plates were washed with PBS-TW as before and then 100  $\mu\text{l}$  per well of alkaline phosphatase-conjugated antihuman IgA ( $\alpha$ -chain-specific, Sigma), diluted to 1:1,000 by 2% BSA solution, was added to the wells and incubated at room temperature for 60 minutes. After washing, 100  $\mu\text{l}$  per well of the substrate solution of p-nitrophenyl phosphate (Sigma) was added to the wells and incubated at room temperature for about half an hour until an adequate optical density (ca 1.0 at 1  $\mu\text{g}/\text{ml}$  of IgA) was obtained. The reaction was stopped by adding 50  $\mu\text{l}$  per well of 3 N NaOH. Controls were run in each assay to check for nonspecific binding of the reagents to the plastic surface. Absorbance at 405 nm was measured with a microplate reader (Sejia auto-reader Model ER-8000, Sanko Junyaku Co. LTD, Japan). The IgA concentration of samples was estimated from the standard absolute values obtained for each plate by semilogarithmic linear regression analysis. In the range of 0.02 to 0.5  $\mu\text{g}/\text{ml}$  of IgA, the standard curve was linear and coefficient of variance was less than 10%.

To control the effect of the saliva flow rate, these concentrations of IgA in saliva were multiplied by the saliva flow rate to determine the salivary IgA secretion rate in  $\mu\text{g}/\text{minute}$ .

#### Questionnaires

For assessing the feelings caused by the lecture, students

were asked to rate the impression of the lecture in three levels: 1: interesting, 2: indifferent, 3: boring. In the first trial, however, no student answered their class was "boring", and only one student did so in the second trial. So, to make the following analysis simple and clear, all of the students were divided into two groups on the basis of their impression of their class: firstly the group who felt interested, and secondly the group who felt indifferent/bored.

#### Statistics

Statistical analyses were performed using commercial software (SPSS ver 9.0, SPSS Inc., IL, USA).

The chi-square test was used to examine the independence of the factor "Impression" from other factors, namely "Date", "Gender" and "Age".

The paired-samples T test was used to examine the difference of sIgA levels within groups, and the independent-samples T test was used to examine the difference between groups.

Analysis of variance using GLM (general linear model) for repeated measure was also used for assessing the effects of feelings, trial date and gender on the changes of sIgA secretion in saliva within subjects.

Differences with p values less than 0.05 were considered significant.

## Results

Characteristics of the subjects are summarized in a cross table (Table 1). By the chi-square test of independence, it was shown that the "Impression" of the class was independent of trial "Date", "Gender" and "Age" of the subjects.

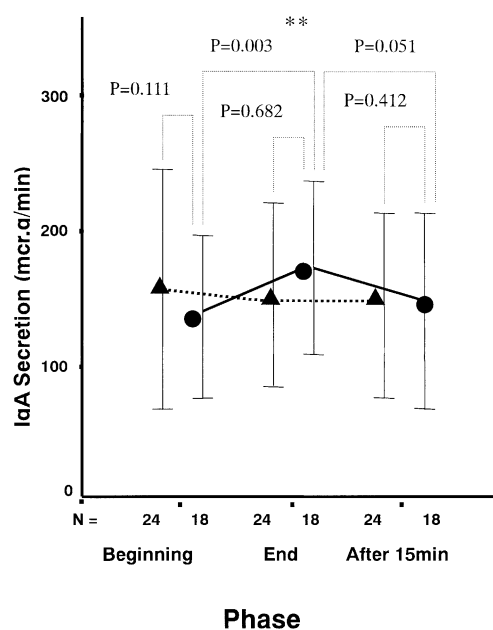
The group who felt interested showed a significant increase in salivary IgA secretion at the end of the class, while the group who felt indifferent or bored did not (Fig. 1). The increasing change in IgA secretion decreased to the initial level 15 minutes after the class (Fig. 1). In addition, the difference between the two groups was so small that it was not significant at any phase (Fig. 1).

To assess the effects of three factors, "Impression", "Date" and "Gender", on sIgA secretion in saliva, an analysis of variance using GLM analysis for repeated measures was performed. The salivary IgA secretions at the beginning and at the end of the class were assigned to "Within-Subjects Factors" as the factor "Phase". In addition the three factors, "Impression", "Gender" and trial "Date", were assigned to "Between-subjects Factors". As shown

**Table 1** Characteristics of subjects

		Impression		
		Interested	Indifferent/Bored	Total
Date	3 <sup>rd</sup> Dec., 1999	12	8	20
	20 <sup>th</sup> Oct., 2000	6	16	22
Total		18	24	42
Gender	Male	2	6	8
	Female	16	18	34
Total		18	24	42
Age	21 years old	6	4	10
	22	9	14	23
	23	2	6	8
	24	1		1
Total		18	24	42

Counts of subjects are shown in a cross table: impression against date, gender, or age.



**Fig. 1** Means of IgA secretion rate at each phase. Closed triangles and dotted line: the group who felt indifferent/bored. Closed circles and solid line: the group who felt interested. Vertical bars: SD, \*\*:  $p < 0.01$ .

in Table 2, PHASE\*IMPRESSION was significant ( $p < 0.05$ ) but other sources were insignificant. These results suggest that the difference in salivary IgA secretion between the two phases, the beginning and the end of the class, depended upon the “Impression” of the class, as shown in Fig. 1, meanwhile they were not affected by “Gender” and trial “Date”.

**Table 2** Results of analysis of variance

**A. Tests of within-subjects contrasts**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
PHASE <sup>(1)</sup>	1,322.794	1	1,322.794	.675	.417
PHASE*IMPRESSION	9,852.485	1	9,852.485	5.028	0.031*
PHASE*GENDER	818.912	1	818.912	0.418	0.522
PHASE*DATE	1,789.229	1	1,789.229	0.913	0.346
PHASE*IMPRESSION*GENDER	2,775.613	1	2,775.613	1.416	0.242
PHASE*IMPRESSION*DATE	0.729	1	.729	0.000	0.985
PHASE*GENDER*DATE	552.684	1	552.684	0.282	0.599
Error (PHASE)	68,585.188	35	1,959.577		

(1) Within-subjects	PHASE	Dependent Variable
Factor	1	IgA secretion at the beginning
	2	IgA secretion at the end

**B. Tests of between-subjects**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	969,900.371	1	969,900.371	106.864	0.000**
IMPRESSION	4.547	1	4.547	0.001	0.982
GENDER	44.896	1	44.896	0.005	0.944
DATE	11,712.942	1	11,712.942	1,291	0.264
IMPRESSION*GENDER	875.884	1	875.884	0.097	0.758
IMPRESSION*DATE	9,128.555	1	9,128.555	1.006	0.323
GENDER*DATE	14,106.026	1	14,106.026	1.554	0.221
Error	317,659.535	35	9,075.987		

GLM for repeated measures was used. Results of tests of within-subjects contrasts (Table A) and tests of between-subjects effects (Table B) are shown separately. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

## Discussion

### Salivary IgA concentration and salivary flow

It is recommended to use “salivary IgA secretion rate” (derived from multiplying salivary IgA concentrations by salivary flow) for studying the change in salivary IgA levels, because salivary IgA concentration misleadingly correlates negatively with salivary flow. In this study, there was surely a negative correlation between the salivary IgA concentration and the salivary flow (Pearson Correlation coefficient =  $-0.277$ ,  $p < 0.01$ ). So, we chose the salivary IgA secretion rate as an index of mucosal immunity.

However, the negative correlation was rather weak ( $R$  square =  $0.077$ ) in this study, and so we were able to obtain a similar conclusion from both the salivary IgA secretion rate and the salivary IgA concentration (data not shown). So, using the salivary IgA concentration was not so misleading, at least in this study.

### Variation in salivary IgA secretion

Feeling interested, a kind of positive feeling, was associated with an increase in salivary IgA secretion in this study. Namely, the students who were interested in the lecture showed a transient increase in the salivary IgA secretion, meanwhile the other students who felt indifferent or bored did not (Fig. 1). This result was supported by the analysis of variance using GLM analysis for repeated measures (Table 2). The results of GLM analysis showed none of the variances except for that of the source “PHASE\*IMPRESSION” was significant. This suggests that the change in sIgA secretion at the end of the class depended upon only “Impression” (or feelings) and not upon “Gender” or trial “Date”. Namely, sIgA secretion increased at the end of the class in the

group who felt interested, irrespective of gender and date.

However, no between-subjects factor was significant. This was because the variance of error in sIgA secretion was large, while the increasing change in sIgA was small, only about +30%. Unknown factors that may cause the large variance of error in sIgA secretion include personality and lifestyle.

It is also unclear if the sIgA secretion change at the end of the class was relevant to circadian rhythm. It was reported that salivary IgA levels showed a circadian rhythm, that is salivary sIgA showed a similar diurnal cycle to cortisol in a study on eight healthy young adults. An early morning acrophase was followed by a decline to a stable base some 6 h after awakening<sup>33</sup>). In addition, such circadian rhythm could be seen in young people, but not in old people<sup>31</sup>). Meanwhile a short-term within-subject variation

in sIgA level in the saliva was reported during daytime<sup>34</sup>). The change in sIgA secretion in this study was seen during a short time between 1:00–2:45 p.m., and disappeared quickly after only 15 minutes. So, it might be a short-term within-subject variation and hardly a part of circadian rhythm.

After all, these results imply that the short time variation in sIgA secretion in saliva will depend, at least partly, on the feelings aroused under a certain circumstance.

In addition, the feelings aroused by a minor event like attending a lecture may depend upon personality or mental state of the subject. So, it appears interesting to clarify what kind of personality, mental state or lifestyle the subjects who showed a positive feeling and sIgA increase after the lecture had. We are currently preparing studies to solve those problems.

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