

《Original Articles》

## Biomarkers in saliva reflecting positive and negative mental states

Takashi Okochi, Kotoyo Fujiki, Katsumi Yamanaka

### Abstract

The purpose of this study was to establish the biomarkers in saliva that reflect mental state. In this study we evaluated the level of motivation, active emotion in positive (happiness) and negative (stress) mental states. Subjects were 24 students (6 men, 18 women) about to take the National Board Examination for Registered Dietitians. We measured the subjects' mental state and salivary biomarkers before and after the examination. Subjective happiness significantly increased after the examination compared to before the examination ( $p<0.01$ ). Stress and motivation significantly decreased after the examination ( $p<0.01$ ). Levels of salivary amylase, a known stress marker, were significantly higher before the examination than after it ( $p<0.01$ ). This tendency was high in subjects who had high stress and high motivation. Levels of salivary testosterone, a known motivation marker, were significantly lower before the examination than after it ( $p<0.01$ ) and were slightly higher in subjects who had low stress and high motivation. After the examination, levels of salivary chromogranin A significantly increased ( $p<0.01$ ) and those of interleukin-6 was declining. However, salivary levels of both markers were not correlated with any of the psychological indices.

**Keywords:** Salivary biomarkers, Mental health, QOL: Quality of life

### Introduction

Quality of life (QOL) evaluation is important not only to prevent illness but also to actively improve physical well-being. However, objective biomarkers that can be used to evaluate QOL have not yet been established, and a non-invasive determination method is needed for no painful stress.

Biomarkers that reflect mental state have been identified in saliva. These biomarkers, amylase, chromogranin A, and cytokines (1–3), are known stress markers, but there are few reports demonstrating their relation with a positive mental state. In fact,

whether it is even possible to measure mental relaxation through analysis of  $\alpha$ -amylase, cortisol, or chromogranin A has been questioned (4). In recent years, the salivary biomarker testosterone has been drawing attention as an indicator of motivation in men (5–10), although no evidence of a salivary biomarker has been found in women. Many cytokines have been reported as stress markers; however, only IL-6 has been suggested as a biomarker for psychological well-being as well as stress (11). Since the purpose of our study was to establish biomarkers for positive as well as negative mental states, we

focused on IL-6.

The purpose of this study was to establish a painless, non-invasive method where salivary biomarkers reflect a broader range of mental states, particularly positive mental states, in young men and women.

## **Material and Methods**

### **Subjects**

Twenty-five university students (6 men, 19 women) aged 21–22 years were randomly recruited to participate in this study. We have excluded the data for one woman infected with influenza. All subjects were studying for the National Board Examination for Registered Dietitians. Students could roughly estimate their results on the evening of the examination, and about 90% of the university's students pass the examination each year.

### **Biomarker measurement**

Concentrations of amylase, testosterone, and IL-6 were measured by the enzymatic method, enzyme-linked immuno-sorbent assay (ELISA), and chemiluminescence enzyme immunoassay, respectively (SRL Inc., Nagoya, Japan). Concentrations of chromogranin A were measured with enzyme immunoassay (Yanaihara Institute Inc., Shizuoka, Japan). Concentrations of total protein were measured with the Bradford protein assay.

### **Saliva sample collection**

Between 13:00 and 14:00 on the day around 3 weeks before and on the day after the examination, we collected saliva samples by asking subjects to chew a swab (Salimetrics Oral Swab, Salimetrics Co., PA, USA) for 1 min. Samples were kept at  $-80^{\circ}\text{C}$  until measurement. Because the levels of the biomarkers are affected by saliva flow rate during rest or after stimulation, the values were calculated as contents per mg protein in saliva.

### **Self-reported questionnaire**

To assess subjective mental state at the time of saliva collection, we asked subjects to complete a

questionnaire (Appendix 1) regarding subjective happiness (10 levels), stress (4 levels), and motivation (4 levels).

### **Statistical analysis**

We performed a paired t-test to analyze subject's levels of subjective happiness, stress, and motivation before and after the national examination. For comparison of salivary levels of amylase, chromogranin A, testosterone, and IL-6 we used a paired t-test (two parameters) or ANOVA (four parameters). Significance level was set at  $p < 0.05$ .

### **Ethical considerations**

This study was approved by the Research Ethics Committee of Nagoya University of Arts and Sciences. Written informed consent was obtained from all subjects prior to their participation.

## **Results**

### **Changes in subjective mental states**

Subjective happiness significantly increased after the examination compared to before it ( $p < 0.01$ , Figure 1), with 19 subjects reporting an increase in subjective happiness, 2 reporting no change, and 3 reporting a decrease. Stress significantly decreased after the examination ( $p < 0.01$ , Figure 2); none of the subjects reported an increase in stress, 9 reported no change, and 15 reported a decrease. Likewise, motivation significantly decreased after the examination ( $p < 0.01$ , Figure 3), with an increase in 3 subjects, no change in 8, and a decrease in 13.

### **Changes in salivary biomarker levels**

Salivary amylase levels before the examination ( $2.6 \times 10^5 \pm 1.1 \times 10^5$  U/mg protein) were significantly higher than those after the examination ( $2.2 \times 10^5 \pm 0.8 \times 10^5$  U/mg protein,  $p < 0.05$ ). Salivary amylase levels before the examination were slightly higher in subjects with high stress and high motivation (Figure 4).

Chromogranin A significantly increased from  $3.0 \pm 2.1$  pmol/mg protein before the examina-

## Appendix 1

### Questionnaire on mental state

1) Please rate your current level of happiness, from 1–10.

Happiness:

Unhappy

Happy

1      2      3      4      5      6      7      8      9      10

2) Please rate your current level of stress, from 1–4.

Stress:

None

Low

Moderate

High

1

2

3

4

3) Please rate your current level of motivation, from 1–4.

Motivation:

None

Low

Moderate

High

1

2

3

4

tion to  $5.5 \pm 4.3$  pmol/mg protein after it ( $p < 0.01$ , Figure 5). There was no correlation with any of the psychological indices.

Testosterone significantly increased from  $0.38 \pm 0.14$  ng/mg protein before the examination to  $0.50 \pm 0.19$  ng/mg protein after it ( $p < 0.01$ ). Levels of salivary testosterone before the examination were higher in subjects with low stress and high motivation (Figure 6). Testosterone levels also showed a sex difference. Levels in women significantly increased from  $0.38 \pm 0.03$  ng/mg protein before the examination to  $0.53 \pm 0.04$  ng/mg protein after it ( $p < 0.01$ ,  $n = 18$ ). However, levels in men ( $n = 6$ ) before ( $0.37 \pm 0.07$  ng/mg protein) and after ( $0.43 \pm 0.07$  ng/mg protein) the examination showed no significant change.

IL-6 tended to decrease from  $3.3 \pm 0.9$  pg/mg protein before the examination to  $1.8 \pm 0.3$  pg/mg protein after it ( $p = 0.112$ , Figure 7). There was no correlation with any of the psychological indices.

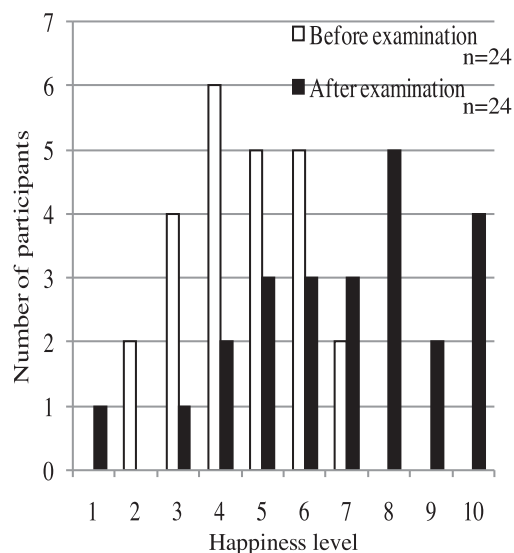
The summary of these data were shown in Table 1.

## Discussion

In this study, all subjects were placed under common stress load and release conditions as they were

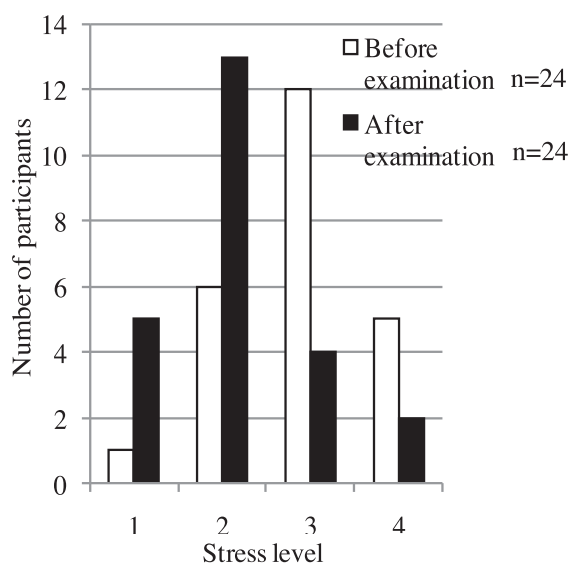
all in the same situation of preparing for and having taken the National Board Examination for Registered Dietitians. Examinees could estimate their results on the evening following the examination and approximately 90% of the students in the university pass the examination each year. We therefore felt that the board exam itself was the stressor, and that stress would be reduced after the examination. The conditions can be described as “high motivation, high stress” before the examination and “low stress, low motivation, high happiness” after the examination. In all subjects, salivary amylase was high before the examination and chromogranin A and testosterone were high after it.

Salivary amylase has been previously used as a stress marker (1, 3, 12), including in simulated outer space (3). Stress-induced amylase secretion from the salivary gland increases when stress stimulates the sympathetic nervous system and enhances norepinephrine secretion from the adrenal medulla and nerve function (12). Salivary amylase level reflects both pleasant and unpleasant feelings and changes more quickly than cortisol in response to stress (1). In the present study, levels of salivary amylase



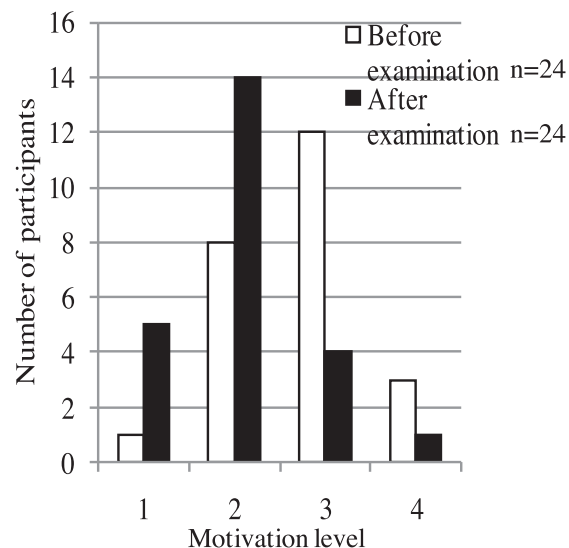
**Figure 1**  
**Change in happiness level before and after the National Examination**

Subjective happiness significantly increased after the examination compared to before it ( $p < 0.01$ ), with 19 subjects reporting an increase in subjective happiness, 2 reporting no change, and 3 reporting a decrease.



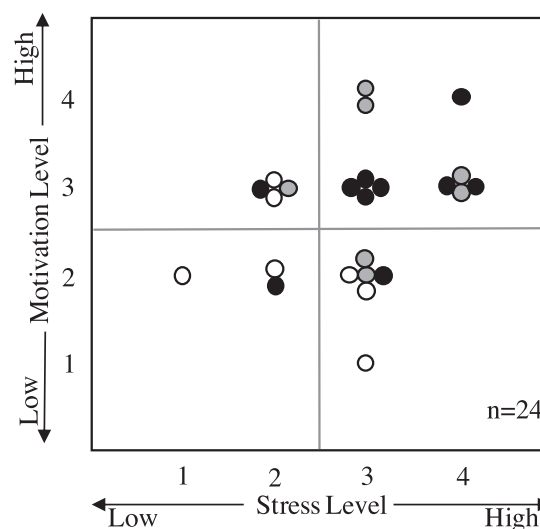
**Figure 2**  
**Change in stress level before and after the National Examination**

Stress significantly decreased after the examination ( $p < 0.01$ ); none of the subjects reported an increase in stress, 9 reported no change, and 15 reported a decrease.



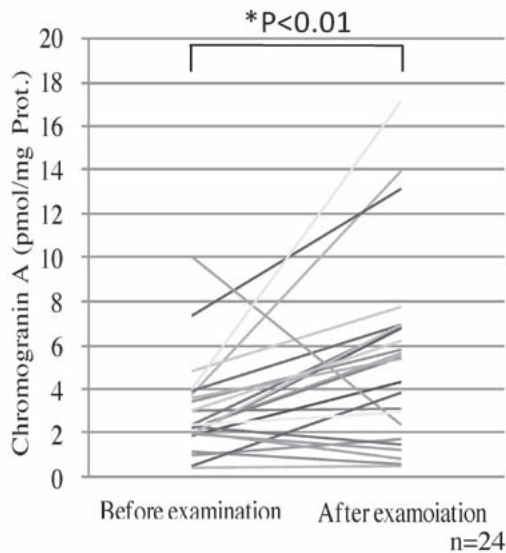
**Figure 3**  
**Change in motivation level before and after the National Examination**

Motivation significantly decreased after the examination ( $p < 0.01$ ), with an increase in 3 subjects, no change in 8, and a decrease in 13.



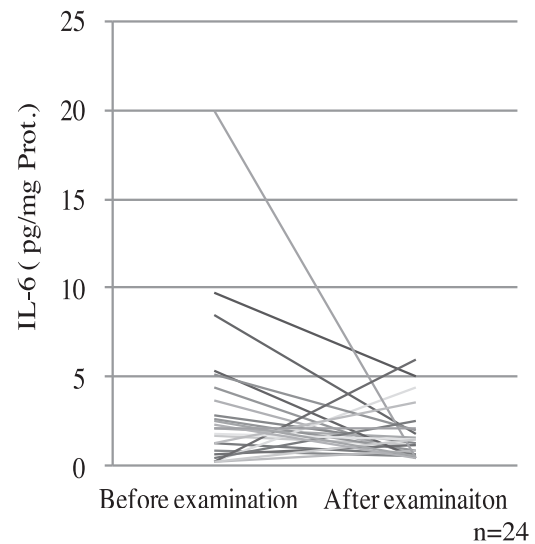
**Figure 4**  
**Salivary amylase level and mental state**

Salivary amylase levels before the examination tended to be higher in subjects with high stress and high motivation. ○;  $< 2.0 \times 10^5$  U/mg protein, ◐;  $2.0 \leq 3.0 < 4.0$  U/mg protein, ●;  $4.0 \leq$  U/mg protein.



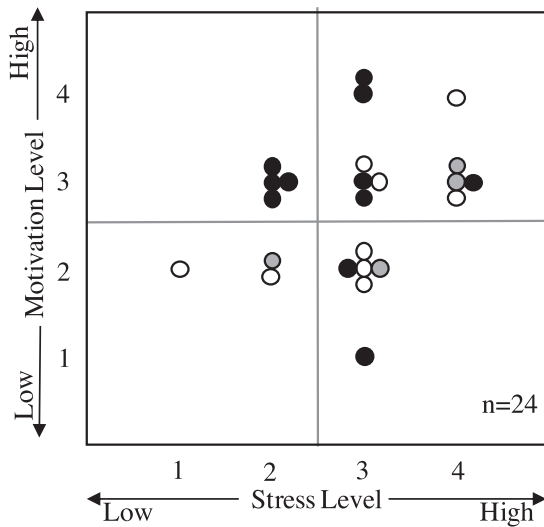
**Figure 5**  
**Change in Chromogranin A level before and after the National Examination**

Chromogranin A significantly increased from  $3.0 \pm 2.1$  pmol/mg protein before the examination to  $5.5 \pm 4.3$  pmol/mg protein after it ( $p < 0.01$ ).



**Figure 7**  
**Change in IL-6 level before and after the National Examination**

IL-6 tended to decrease from  $3.3 \pm 0.9$  pg/mg protein before the examination to  $1.8 \pm 0.3$  pg/mg protein after it ( $p = 0.112$ , Figure 7).



**Figure 6**  
**Salivary testosterone level and mental state**

Levels of salivary testosterone before the examination tended to be higher in subjects with low stress and high motivation. ○;  $< 0.30$  ng/mg protein, ◐;  $0.30 \leq 0.40$  ng/mg protein, ●;  $0.40 < \text{ng/mg protein}$ .

increased under the condition of high motivation, high stress before the examination, indicating that amylase may be a suitable biomarker for not only elevated stress, but also elevated motivation.

Chromogranin A is an acidic glycoprotein that exists in the secretory granules of many endocrine organs such as the chromaffin cells of the adrenal medulla and sympathetic nerve terminals (13). It is a known stress marker and has been shown to increase with the stress of taking an examination or being in a noisy environment (14, 15); however, it also increases when taking a bath or watching comedy programs, indicating that it might also reflect a positive mental state (16, 17). In the present study, levels of salivary chromogranin A significantly increased after the examination, supporting the possibility that it might increase in response to a positive mental state. However, chromogranin A showed no correlation with any of the psychological indices. Additionally, there was no correlation between testosterone and chromogranin A, similarly to testosterone and chromogranin A levels.

Salivary testosterone was higher after the examination. Testosterone is secreted by the adrenal gland in small amounts, but is mainly secreted by the testes

Table 1 The summary of changes in subjective mental states and salivary biomarker levels

ID	Gender	Before the exam						After the exam							
		Happines s Level	Stress Level	Motivation Level	Amylase × 10 <sup>5</sup> U/mg Prot.	CgA pmol/mg Prot.	Testosterone ng/mg Prot.	IL-6 pg/mg Prot.	Happiness Level	Stress Level	Motivation Level	Amylase × 10 <sup>5</sup> U/mg Prot.	CgA pmol/mg Prot.	Testosterone ng/mg Prot.	IL-6 pg/mg Prot.
1	F	6	3	3	3.9	3.8	0.40	3.71	5	2	3	3.4	13.9	0.44	0.65
2	F	3	4	3	2.6	1.0	0.33	4.45	1	4	2	2.7	1.7	0.55	0.59
3	F	5	3	3	3.5	3.1	0.24	2.82	7	2	2	3.3	3.2	0.29	1.33
4	F	6	3	3	3.1	2.3	0.44	1.31	8	2	1	2.0	6.8	0.44	0.63
5	F	2	4	3	3.0	7.4	0.32	8.44	5	2	1	2.1	13.2	0.53	1.85
6	F	3	3	4	2.8	1.9	0.44	9.71	4	3	1	2.3	4.4	0.40	5.02
7	F	7	3	2	3.9	4.8	0.28	1.73	8	2	2	3.0	7.7	0.50	1.16
8	F	4	2	3	2.7	2.2	0.46	2.11	7	2	2	1.9	5.6	0.42	1.63
9	F	5	2	3	1.5	3.4	0.57	2.64	6	1	1	0.8	5.8	0.48	0.84
10	F	6	4	3	4.0	2.2	0.22	0.89	6	4	2	2.6	5.5	0.42	0.60
11	F	4	4	3	2.8	3.9	0.42	0.71	4	3	2	2.4	6.9	0.41	1.20
12	F	2	3	2	2.8	2.0	0.28	5.31	5	2	2	2.1	6.8	0.36	0.46
13	M	3	3	1	1.9	0.4	0.49	0.23	8	2	2	3.7	0.5	0.44	4.45
14	M	5	3	2	0.9	10.1	0.20	0.22	8	2	2	2.4	2.4	0.19	0.88
15	F	5	3	2	2.3	1.1	0.31	19.92	3	3	3	1.8	0.6	0.58	0.51
16	F	6	1	2	1.0	2.3	0.26	5.17	10	1	2	1.1	1.5	0.72	2.02
17	M	3	4	4	3.0	0.5	0.21	0.42	6	3	3	2.8	3.9	0.41	2.56
18	F	5	2	3	1.9	4.0	0.66	0.27	9	2	2	2.1	17.2	1.03	5.96
19	F	4	2	2	4.2	3.0	0.23	1.79	9	2	2	2.1	6.2	0.50	1.50
20	F	6	2	2	1.7	2.0	0.35	0.21	10	2	1	2.2	7.0	0.40	1.55
21	F	4	3	2	0.1	3.6	0.59	1.33	10	1	2	0.2	5.4	0.91	3.57
22	M	4	3	4	2.8	2.1	0.61	2.39	8	2	2	1.6	0.9	0.66	0.44
23	M	4	2	3	3.1	2.0	0.44	2.50	7	1	3	1.7	1.3	0.30	0.52
24	M	7	3	3	3.8	2.4	0.28	2.09	10	1	4	2.3	3.0	0.61	2.15



in male mammals and by the ovaries in female mammals. Testosterone acts on muscle growth, encouraging anabolic functions in protein and an increase in body hair. It also stimulates motivation and feelings of pleasure and has therefore attracted attention in the United States as a treatment for depression (7). Salivary testosterone has also attracted the attention as an indicator for motivation. For instance, testosterone increases when a favorite candidate wins an election (8), when a favorite sumo wrestler wins a match (6), and when motivation increases during a competitive computer game (9, 10). One study found that testosterone increased when playing Shogi (Japanese chess) regardless of the match's outcome (5). In the present study, salivary testosterone also increased, albeit slightly, in subjects with high motivation and low stress before the examination, suggesting that testosterone might be a good biomarker for not only high motivation but also low stress.

Testosterone was also rather high after the examination for those students showing low motivation, which was influenced by their low stress and high happiness levels. However, because the conditions were "high motivation, high stress" before the examination and "low motivation, high happiness" after it, the condition of "high motivation, low stress" is outside the scope of this study and its relation to mental state should be investigated further. Additionally, this study failed to clarify whether testosterone is a good biomarker for motivation in women. Although estrogen also may be a good biomarker for mental state, it fluctuates in concentration according to biorhythm in women.

Cytokines are inflammatory markers produced by T cells and other cells such as macrophages. Cytokines such as IL-2, IL-8, IL-10, and TNF- $\alpha$  are known to fluctuate depending on mental state (2), although it is unclear whether they are influenced by positive psychological well-being. There are reports that recreational drumming showed some relation with the constancy of natural killer cell activity (18), and plasma levels of IL-6 were found to be lower in elderly women with positive well-being (11). In

the present study, cytokine levels tended to be lower after the examination. It is possible that salivary cytokines may be influenced by the secretion of polymorphonuclear neutrophil leukocytes in saliva; therefore, the salivary level does not necessarily reflect the circulating level. Minetto reported the lack of significant correlation between salivary and serum IL-6 in resting and post-exercise condition (19).

In this study, we simplified the quantification of mental state, but we plan to evaluate mental state in more detail in future studies by using psychological tests such as the Profile of Mood States.

Sampling saliva had some beneficial features. Since collection is painless, we can avoid exerting additional stress on the subjects and also collect many samples frequently. A precaution to take when collecting samples is to minimize variations in the amount of saliva and salivary proteins due to daily fluctuations and sampling methods used. In this study, we tried to do this by collecting saliva samples at the same time before and after the examination and for the same duration of 1 min each time (20).

As the saying goes, "all illness comes from the mind," and we can say that mental state greatly influences health. To improve QOL, it is generally recommended to rest and enjoy leisure activities. However, objective methods to evaluate the results of doing this have not yet been established, we evaluated the level of motivation, active emotion in positive (happiness) and negative (stress) mental states. We hope that the biomarkers reported here will assist in efforts to establish objective biomarkers that can help people to monitor maintenance and improvement of physical and mental health.

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《原著》

## ポジティブあるいはネガティブな心理状態を反映する 唾液中バイオマーカーの検討

大河内貴士    藤木理代    山中克己

### 要旨

ポジティブ（幸福）あるいはネガティブ（ストレス）な心理にモチベーションという動的感情を加え、多様な心理状態を反映する唾液中バイオマーカーを調べた。

管理栄養士の国家試験を控えた24人の大学生（男性6人、女性18人）を対象に、試験前後の主観的幸福度（10段階）、ストレス度（4段階）、モチベーション度（4段階）をアンケート調査し、唾液中アミラーゼ値、テストステロン値、クロモグラニンA値、インターロイキン6（IL-6）値との関連を調べた。

その結果、国家試験終了後、主観的幸福度は有意（ $p<0.01$ ）に上昇し、ストレス度とモチベーション度は有意（ $p<0.01$ ）に減少した。ストレスマーカーとして知られている唾液中アミラーゼは、試験前に有意（ $p<0.01$ ）に高く、とくにストレス度とモチベーション度の両者が高い者で高値の傾向にあった。モチベーションのマーカーとして知られている唾液中テストステロンは、試験前に有意（ $p<0.01$ ）に低かったけれども、とくにストレス度が低くモチベーション度が高い者で高値の傾向にあった。試験後クロモグラニンA値は有意（ $p<0.01$ ）に上昇し、IL-6は減少傾向にあったが、心理状態との有意な相関は認められなかった。

**キーワード：**唾液中生体指標、メンタルヘルス、QOL：生活の質