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ORIGINAL ARTICLE

# Changes in salivary chromogranin A levels in adults with atopic dermatitis are correlated with changes in their condition

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## ABSTRACT

**Stress-induced scratching is an issue in patients with adult atopic dermatitis (AD). Symptoms of stress-induced AD are common in clinical practise. Salivary chromogranin A (CgA) level has research value as a possible index related to a patient's psychological stress. Using saliva, which is easily collectable, we compared two assessments of the severities of AD and stress with the levels of stress proteins in the saliva of 30 patients with AD in the Department of Dermatology of Shimane University between April 2015 and May 2017. The severities of AD and stress were assessed using the Scoring Atopic Dermatitis (SCORAD) score and State–Trait Anxiety Inventory score, respectively. Additionally, the assessments included those of personality using the Tokyo University Egogram (TEG)-II score and quality of life using the Dermatology Life Quality Index score. Simultaneously, we measured their salivary CgA levels. The change in salivary CgA per protein in patients with AD was correlated with their changes in SCORAD score (correlation coefficient, $r = 0.596$, $P = 0.001$) and objective SCORAD ($r = 0.608$, $P < 0.001$). The changes in CgA per protein correlated with those in TEG-II A ($r = 0.370$, $P = 0.022$), while the changes in SCORAD score correlated with those in DLQI ($r = 0.309$, $P = 0.048$). Our results suggest that changes in a patient's condition are reflective of the changes in the patient's stress. The changes in salivary CgA level in patients with AD correlated with the changes in their condition.**

**Key words:** atopic dermatitis, chromogranin A, disease severity, saliva, stress marker.

## INTRODUCTION

Atopic dermatitis (AD), one of the most common chronic allergic skin inflammatory diseases, is increasing in prevalence. In recent years, with the socioeconomic developments and changes in the environment and diet, the incidence of AD has been increasing.[1] The clinical manifestations of AD are diverse and the most typical features are dry skin, chronic eczema-like dermatitis and severe itching. AD not only produces physical symptoms such as itching, skin discomfort and sleep disorders (including difficulty falling asleep, reduced sleep time, difficulty in awakening, fatigue during the day and irritability), but it can also cause emotional disturbances such as depression, loneliness, frustration, and differences in self-esteem and self-image. The incidence of AD is closely related to genetic and environmental factors.[2] A history of allergic disorders in parents and other family members increases the risk of AD because genetic factors affect the skin barrier function and immune balance. Environmental factors leading to AD include environmental changes, lifestyle changes, excessive washing, infections and allergens. Additionally, psychological factors such as mental stress, anxiety and depression also play a role in the pathogenesis of AD.[3] Most assessments of stress are subjective; therefore, an objective marker of stress is needed. Saliva samples can be collected non-invasively, making them useful in diagnosing specific oral pathologies,[4,5] as well as other systemic illnesses.[6,7]

Chromogranin A (CgA) is an acidic glycoprotein dissociated from chromaffin granules in the adrenal medulla, reflects the secretion of catecholamines in the blood, and acts as a marker of the sympathetic adrenomedullary system activity. CgA is secreted in the saliva through the ducts of submandibular glands following stimulation of the autonomic nervous system.[8] A previous study demonstrated that saliva CgA levels are associated with psychological stress.[9] Another study reported the use of salivary CgA levels to assess the differences in psychological stress between the elderly and young adults during gastrointestinal endoscopy.[10] Salivary CgA is attracting attention as a new indicator of psychological stress. We previously reported that the CgA per protein in saliva correlates with the severity of AD.[11]

In this study, we focused on observing the changes in the salivary stress marker at different points of time in the course

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of the disease in order to study the correlation between them and quantify the stress associated with Scoring Atopic Dermatitis (SCORAD) scores for the purpose of identifying the threshold levels of this objective biomarker.

## METHODS

### Subjects

The study included 30 patients with AD (19 men, 11 women; mean age, 32.71 years; range, 17–57) who were examined in the Department of Dermatology, Shimane University Hospital, between April 2015 and May 2017, and consented to participate in this study. The patients who participated in this experiment met the diagnostic criteria of AD proposed by the Ministry of Health, Labor and Welfare Research Group.[11] Patient data was anonymized prior to the analyses. The changes in the indicators were observed by performing different tests in the same patient at different points of time and analyzing the relationship between these changes. Saliva samples were collected at the time of the consultation. Evaluations were performed in an outpatient setup and care was taken to maintain uniform durations and same collection time between the assessments in all patients. The average period between the two assessments was 533 ± 210 days (mean ± standard deviation, SD). This study was approved by the ethics committee of Shimane University and the Dean of the Faculty of Medicine (approval no. 1773).

### Evaluation of severity of AD, anxiety, personality and life quality

We used the SCORAD and objective SCORAD scores to objectively evaluate the severity of AD. The patients were asked to complete the following questionnaires during their examinations: State–Trait Anxiety Inventory (STAI), the new Tokyo University Egogram (TEG)-II and the Dermatology Life Quality Index (DLQI) survey as detailed in our previous report.[11]

### Measurements of stress markers in saliva

The protocol for collection of saliva samples and measurement of the levels of CgA are described in our previous report.[11] In brief, each individual was issued a saliva collection kit. When these conditions were met, the subjects rinsed out their mouths three times with water, rested for 10 min and waited for saliva formation in the mouth, leaned forward to allow it to flow into a container, and repeated this process until at least 1 mL of saliva was collected. The collected saliva was centrifuged at 905 *g* for 15 min, transferred to a polypropylene tube and frozen at 20°C until measurement.[11] Protein in the saliva were measured using a DC™ Protein Assay (Bio-Rad, Hercules, CA, USA). CgA concentrations in the saliva were measured using a YK070 Human Chromogranin A EIA Kit (Yanaihara Institute, Shizuoka, Japan).

### Statistical analyses

The results are presented as mean ± SD. IBM SPSS Statistics version 22 (IBM, Armonk, NY, USA) was used for statistical analyses. The median number and 25–75 percentiles of anxiety levels were calculated. Linear regression correlation was used to identify the relationships with probability values of less than 5% being considered significant.

## RESULTS

The results of the changes in salivary CgA levels and SCORAD, objective SCORAD, STAI, TEG-II and DLQI scores are summarized in Tables 1–4. The correlation data for all subject parameters with the levels of salivary stress markers are summarized in Table 5.

### Correlations between changes in SCORAD score and changes in salivary CgA levels

The changes in salivary CgA levels per protein were significantly correlated with the changes in a patient's SCORAD score and objective SCORAD score (Fig. 1). However, there

**Table 1.** Subject characteristics

| Collect data for the first time | | | Collect data for the second time | | |
|---|---|---|---|---|---|
| Parameters | *n* | Mean ± SD | Parameters | *n* | Mean ± SD |
| SCORAD | 30 | 34.04 ± 14.33 | SCORAD | 30 | 28.03 ± 14.11 |
| Objective SCORAD | 30 | 28.22 ± 13.05 | Objective SCORAD | 30 | 24.06 ± 13.23 |
| STAI state | 30 | 44.87 ± 9.48 | STAI state | 30 | 44.90 ± 11.99 |
| STAI trait | 30 | 48.80 ± 8.62 | STAI trait | 30 | 48.46 ± 9.23 |
| TEG-II CP | 30 | 9.36 ± 4.57 | TEG-II CP | 30 | 9.93 ± 4.83 |
| TEG-II NP | 30 | 13.10 ± 4.50 | TEG-II NP | 30 | 13.13 ± 4.13 |
| TEG-II A | 30 | 10.93 ± 5.68 | TEG-II A | 30 | 11.73 ± 5.18 |
| TEG-II FC | 30 | 9.73 ± 5.17 | TEG-II FC | 30 | 10.00 ± 4.92 |
| TEG-II AC | 30 | 13.93 ± 5.28 | TEG-II AC | 30 | 14.06 ± 5.12 |
| DLQI | 30 | 6.60 ± 6.37 | DLQI | 30 | 5.40 ± 4.56 |
| Salivary CgA (pmol/mL) | 30 | 17.93 ± 17.07 | Salivary CgA (pmol/mL) | 30 | 12.18 ± 9.42 |
| Salivary CgA/protein (pmol/mL) | 30 | 10.64 ± 8.41 | Salivary CgA/protein (pmol/mL) | 30 | 6.29 ± 4.42 |

A, adult; AC, adapted child; CgA, chromogranin A; CP, critical parent; DLQI, Dermatological Life Quality Index; FC, free child; IgE, immunoglobulin E; LDH, lactate dehydrogenase; NP, nurturing parent; SCORAD, Scoring Atopic Dermatitis; SD, standard deviation; STAI, State–Trait Anxiety Inventory; TARC, thymus and activation-regulated chemokine; TEG, Tokyo University Egogram.

**Table 2.** Correlations between the patient change of disease severity markers and salivary stress markers

| | SCORAD | Objective SCORAD |
|---|---|---|
| Salivary CgA (pmol/mL) | $r = 0.191$ | $r = 0.160$ |
| | $P = 0.156$ | $P = 0.200$ |
| Salivary CgA/protein (pmol/mL) | $r = 0.596$ | $r = 0.608$ |
| | $P = 0.001$ | $P < 0.001$ |

Underlining indicates significant data. CgA, chromogranin A; SCORAD, Scoring Atopic Dermatitis.

**Table 3.** Correlations between the patient change of AD patient's parameters and salivary stress marker

| | Salivary CgA (pmol/mL) | Salivary CgA/protein (pmol/mL) |
|---|---|---|
| DLQI | $r = 0.059$ | $r = 0.008$ |
| | $P = 0.378$ | $P = 0.483$ |
| STAI state | $r = 0.101$ | $r = 0.135$ |
| | $P = 0.298$ | $P = 0.238$ |
| STAI trait | $r = 0.056$ | $r = 0.026$ |
| | $P = 0.384$ | $P = 0.446$ |

Underlining indicates significant data. AD, atopic dermatitis; CgA, chromogranin A; DLQI, Dermatological Life Quality Index; STAI, State–Trait Anxiety Inventory.

**Table 4.** Correlations between the patient change of AD patient's parameters and salivary stress marker

| | Salivary CgA (pmol/mL) | Salivary CgA/protein (pmol/mL) |
|---|---|---|
| TEG-II CP | $r = 0.028$ | $r = 0.246$ |
| | $P = 0.442$ | $P = 0.095$ |
| TEG-II NP | $r = 0.235$ | $r = 0.206$ |
| | $P = 0.106$ | $P = 0.137$ |
| TEG-II A | $r = 0.090$ | $r = 0.370$ |
| | $P = 0.318$ | $P = 0.022$ |
| TEG-II FC | $r = 0.144$ | $r = 0.103$ |
| | $P = 0.223$ | $P = 0.294$ |
| TEG-II AC | $r = 0.217$ | $r = 0.187$ |
| | $P = 0.125$ | $P = 0.161$ |

Underlining indicates significant data. A, adult; AC, adapted child; AD, atopic dermatitis; CgA, chromogranin A; CP, critical parent; FC, free child; NP, nurturing parent; TEG, Tokyo University Egogram.

was no correlation between the changes in salivary CgA levels and changes in SCORAD or objective SCORAD scores (Table 2).

### Correlation between the changes in salivary CgA and anxiety scale scores (STAI, TEG-II and DLQI)

The changes in salivary CgA levels per protein were significantly correlated with TEG-II A scores (Fig. 2). However, there was no correlation between changes in STAI scores and salivary CgA, DLQI or TEG-II (Tables 3,4).

### Correlation between the changes in SCORAD score and anxiety scale scores (STAI, TEG-II and DLQI)

The changes in SCORAD and objective SCORAD scores were significantly correlated with DLQI scores. The changes in DLQI score and STAI state were correlated with those in TEG-II CP; the changes in STAI state and STAI trait were correlated with those in TEG-II NP; and the changes in TEG-II AC were correlated with SCORAD scores (Fig. 3). However, the remaining items were not significantly related (Table 5).

## DISCUSSION

Atopic dermatitis is a chronic inflammatory disease of the skin that results from an interplay of environmental and physical stressors. Patients with AD are not only disturbed by the disease itself, but also by the associated sleep disorders, mood disturbances and stress resulting from the disease, which collectively seriously affect their quality of life. AD can lead to psychological stress due to stigmatization, social isolation and discrimination. Considerable evidence has emerged that psychosocial stress can affect a variety of immune functions through neuroendocrine processes such as activation of the hypothalamus–pituitary–adrenal axis or the adrenergic system.[12,13] It has been reported that the stress after the Hanshin Awaji earthquake lead to exacerbation of AD in many patients.[14] With the shift of medicine from a purely biomedical model to a comprehensive medical model, the importance of social, psychological and medical treatment and the quality of life of patients have been widely recognized. Therefore, it is important for patients with AD and their families to investigate the relationship between the patient's stress and the resulting changes in the severity of the disease and quality of life.

Saliva has various physiological functions. Wound licking, in which saliva is applied to a wound, promotes wound healing.[15] Saliva prevents the onset of allergic diseases.[16] We feel thirsty when we get nervous and saliva can be used as a stress marker because its secretion decreases in times of stress. We focused on saliva, which is easy to collect, to investigate whether factors such as AD SCORAD score and patients' stress levels are reflected in the levels of salivary stress proteins. Our previous study showed that saliva CgA levels are positively correlated with SCORAD scores in patients with AD,[11] and no correlation was observed between SCORAD score and salivary cortisol or amylase levels. A previous study found that patients undergoing initial evaluation for AD have significantly higher levels of salivary CgA compared with patients without AD undergoing an initial examination as well as patients with AD undergoing repeat examinations.[17] Our study showed that CgA levels in saliva were correlated with a change in SCORAD scores in patients at different stages of the disease, suggesting that the level of CgA in the saliva of patients with AD may be an objective marker of change in the severity of the disease to some extent. In routine clinical practise, tests are performed at almost every examination and CgA may be more useful for objectively investigating the levels of stress.

**Table 5.** Patient change of correlations of subject parameter

| | SCORAD | Objective SCORAD | | | |
|---|---|---|---|---|---|
| DLQI | $r = 0.309$ | $r = 0.330$ | DLQI | | |
| | $P = 0.048$ | $P = 0.037$ | | | |
| STAI state | $r = 0.029$ | $r = 0.020$ | $r = 0.036$ | STAI state | |
| | $P = 0.440$ | $P = 0.458$ | $P = 0.426$ | | |
| STAI trait | $r = 0.038$ | $r = 0.010$ | $r = 0.210$ | $r = 0.222$ | STAI trait |
| | $P = 0.420$ | $P = 0.478$ | $P = 0.133$ | $P = 0.119$ | |
| TEG-II CP | $r = 0.267$ | $r = 0.262$ | $r = 0.320$ | $r = 0.490$ | $r = 0.072$ |
| | $P = 0.077$ | $P = 0.081$ | $P = 0.042$ | $P = 0.003$ | $P = 0.353$ |
| TEG-II NP | $r = 0.210$ | $r = 0.194$ | $r = 0.065$ | $r = 0.416$ | $r = 0.322$ |
| | $P = 0.133$ | $P = 0.152$ | $P = 0.367$ | $P = 0.011$ | $P = 0.041$ |
| TEG-II A | $r = 0.025$ | $r = 0.053$ | $r = 0.063$ | $r = 0.217$ | $r = 0.097$ |
| | $P = 0.448$ | $P = 0.391$ | $P = 0.370$ | $P = 0.125$ | $P = 0.305$ |
| TEG-II FC | $r = 0.064$ | $r = 0.080$ | $r = 0.233$ | $r = 0.020$ | $r = 0.134$ |
| | $P = 0.369$ | $P = 0.337$ | $P = 0.107$ | $P = 0.457$ | $P = 0.241$ |
| TEG-II AC | $r = 0.312$ | $r = 0.391$ | $r = 0.006$ | $r = 0.105$ | $r = 0.185$ |
| | $P = 0.047$ | $P = 0.059$ | $P = 0.487$ | $P = 0.290$ | $P = 0.163$ |

Underlining indicates significant data. A, adult; AC, adapted child; CP, critical parent; DLQI, Dermatological Life Quality Index; FC, free child; NP, nurturing parent; SCORAD, Scoring Atopic Dermatitis; STAI, State–Trait Anxiety Inventory; TEG, Tokyo University Egogram, TEG, Tokyo University Egogram.

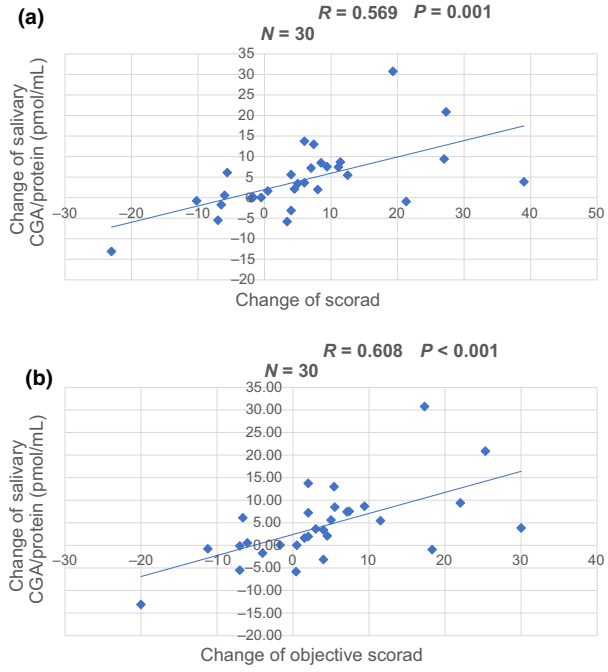


**Figure 1.** Correlation between the change of Scoring Atopic Dermatitis (SCORAD) scores and salivary chromogranin A (CgA). (a) There was significant correlation between the change of SCORAD score and the change in salivary CgA levels per protein ($r = 0.569$, $P = 0.001$). (b) There was significant correlation between the change of SCORAD objective score and salivary CgA levels per protein ($r = 0.608$, $P < 0.001$).

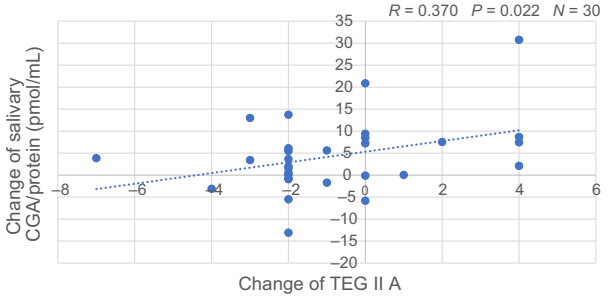


**Figure 2.** Correlation between the change of salivary chromogranin A (CgA) and anxiety scales. There was significant correlation between change of Tokyo University Egogram (TEG)-II A and salivary CgA levels per protein ($r = 0.370$, $P = 0.022$).

In this study, we provided detailed feedback on the results of the initial questionnaire survey to patients and explained the results to them. We examined the results of regular dermatological examination and offered treatment to the patients. However, we did not provide services like professional psychotherapy. We also used questionnaires to evaluate the level of anxiety and type of personality in patients with AD and found that changes in salivary CgA levels were correlated with changes in TEG-II A scores at different stages of the disease. TEG indicates the ego status of a person, which can be adult self-state or child's ego state.[18] TEG-II A represents the adult self-state with features such as objective and theoretical understanding of things based on facts. In other words, with increasing adult ego, adherence to treatment may improve and the severity of the disease may decrease. The changes in DLQI score were correlated with the changes in SCORAD score in patients with AD, suggesting that changes in the severity of the disease are related to the quality of life. In our previous study, we demonstrated that there was no correlation between serum markers of the severity of AD (thymus and activation-regulated chemokine, lactate dehydrogenase and total immunoglobulin E) and salivary CgA levels.[11]

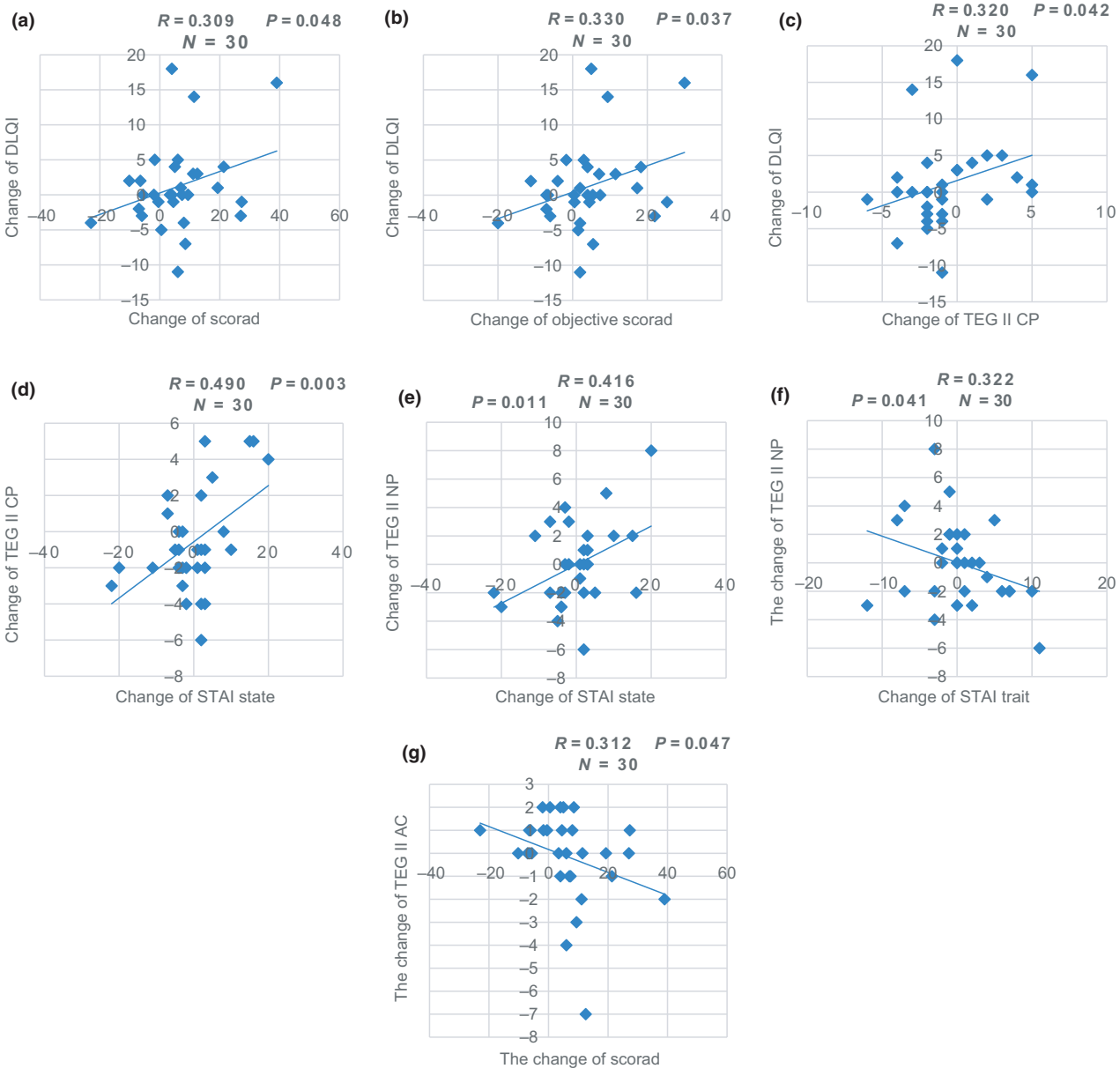


**Figure 3.** Correlation among anxiety scale scores (State–Trait Anxiety Inventory [STAI], Tokyo University Egogram [TEG]-II and Dermatological Life Quality Index [DLQI]). (a) There was significant correlation between the change of DLQI and Scoring Atopic Dermatitis (SCORAD) ($r = 0.309$, $P = 0.048$). (b) There was significant correlation between the change of DLQI and objective SCORAD ($r = 0.330$, $P = 0.037$). (c) There was significant correlation between the change of DLQI and TEG-II CP ($r = 0.320$, $P = 0.042$). (d) There was significant correlation between the change of STAI state and TEG-II CP ($r = 0.490$, $P = 0.003$). (e) There was significant correlation between the change of STAI state and TEG-II NP ($r = 0.416$, $P = 0.011$). (f) There was significant correlation between the change of STAI trait and TEG-II NP ($r = 0.322$, $P = 0.041$). (g) There was significant correlation between the change of SCORAD and TEG-II AC ($r = 0.312$, $P = 0.047$).

The present study further demonstrates that salivary CgA levels in patients with AD can objectively reflect the changes in the severity of the disease to a certain extent. When the degree of dermatitis in patients with AD is maintained in a relatively severe case, the level of CgA in the saliva is usually maintained at a higher level. In contrast, when dermatitis symptoms are relieved, the CgA level in the patient's saliva is usually significantly lower than before. However, our previous study showed that there was no correlation between serum markers of the severity of AD and salivary CgA levels. CgA

may represent more stress-sensitive elements among the elements that constitute severity. Therefore, because the serum markers are more responsive to changes in the patient's dermatitis symptoms, a clear correlation between the change of CgA level in the saliva and the change of serum markers in patients with AD could not be observed.

This finding has potential clinical applications because obtaining samples of saliva is easy and painless. Patients are more likely to take this test. CgA is a good marker of psychological stress response because it selectively reflects psychological stress and not physiological stress,[19,20] which is important in AD because AD has a strong relationship with psychological stress. As an indicator that can be accurately measured, CgA is a more objective biomarker than the conventional tools such as STAI, TEG-II and DLQI, and there is a possibility that the changes in psychological stress of the patient can be reflected. In clinical management, we should pay more attention to the changes in a patient's psychological stress, and find ways to reduce the stress, which can result in better treatment outcomes in AD. The use of saliva as an objective stress marker may become a useful tool in stress management and stress coping in the near future.

In conclusion, CgA in saliva was found to be related to stress and the severity of AD. Additionally, changes in the subjective severity scores correlated with changes in salivary CgA. Therefore, measurement of CgA in saliva is a simple objective marker that can evaluate both the severity and level of stress in AD.

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**CONFLICT OF INTEREST:** None declared.

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