Stress evaluation in adult patients with atopic dermatitis

using salivary cortisol

唾液コルチゾールを用いた成人アトピー性皮膚炎患者のストレス評価

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using salivary cortisol

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Contents

1. Abbreviations:

AD: atopic dermatitis

BDI: Beck Depression Inventory

CAs: catecholamines

CgA: chromogranin A

CRH: corticotropin-releasing hormone

DC: Dendritic cells

HPA: hypothalamic-pituitary-adrenal

Ig: immunoglobulin

IL: interleukin

LC: Langerhans cells

LDH: lactate dehydrogenase

QOL: quality of the life

SAM: sympathetic-adreno medullary

SAS: Self-rating Anxiety Scale

SCORAD: scoring atopic dermatitis

SNS: sympathetic nervous system

SP: substance P

TARC: thymus and activation-regulated chemokine

TH: T-helper

2.   Summary

The symptoms of atopic dermatitis (AD) are often aggravated by stress, and AD can also lead to psychological stress due to social isolation and discrimination. The salivary cortisol level reflects psychological stress and it is considered to be a good index to assess chronic stress. In this study, we measured the salivary cortisol levels in patients with AD (n = 30) and compared them with those in healthy control subjects (n = 42). AD patients were also evaluated for general disease severity using the Scoring Atopic Dermatitis index (SCORAD). The serum levels of TARC, total immunoglobulin (Ig) E, lactate dehydrogenase (LDH) and peripheral blood eosinophil counts were measured by laboratory tests. The Skindex-16 was used as a skin disease-specific, quality of life measure, instrument. The results showed that the saliva cortisol level was significantly higher in AD patients compared to healthy subjects ($p < 0.01$). The salivary cortisol level was significantly correlated with the SCORAD index (r = 0.42, $p < 0.05$), while the serum TARC and LDH levels were positively correlated with the SCORAD index. However, no statistically significant correlations were observed between the salivary cortisol level and Skindex-16. These results suggest that the saliva cortisol level is therefore a useful biomarker to evaluate the stress in AD patients.

3. Introduction

3.1. Atopic dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by inflammatory infiltration, extensive pruritus and a clinical course defined by symptomatic flares and remissions. Firm criteria to define the disease were first created by Hanifin and Rajka in 1980 [1], and included almost 30 signs, symptoms, and laboratory abnormalities. More recently, the Japanese Dermatological Association developed a more straightforward criteria include pruritus, typical morphology and distribution, and chronic or chronically relapsing course [2].

The pathogenesis of the disease is becoming better understood, and important clues about the pathogenesis of the disease have been discovered, including genetic factors, skin barrier dysfunction and immune dysregulation [3]. New insights into its etiology include filaggrin mutations [4,5]. Filaggrin is involved in the forming cornified cell envelope in epidermis and is critical for maintaining epidermal barrier function. A defective epidermal barrier allows the penetration of environmental allergens through the skin, facilitating the interaction of these allergens with the local antigen-presenting cells and immune effector cells. Langerhans cells

(LC) take up and present these allergens to T-helper (TH) cells and recruit cluster of CD4+ T

cells. Activated DC and IL-4, expressed by CD4+ T cells, promote TH1 to TH2 switching

with the subsequent release of pro-inflammatory cytokines and elevation of IgE levels [6].

TH2-type responses, characterized by substantial production of interleukin (IL) -4, IL-5,

IL-10 and IL-13 play a pivotal role in the maintenance and exacerbation of AD symptoms [7].

AD may be influenced by many triggering factors such as stress factors, food, irritants,

climatologic factors, and illness **(Figure 1)**. In the last decade, there has been growing

evidence indicating that psychological factors such as personality and stress may play an

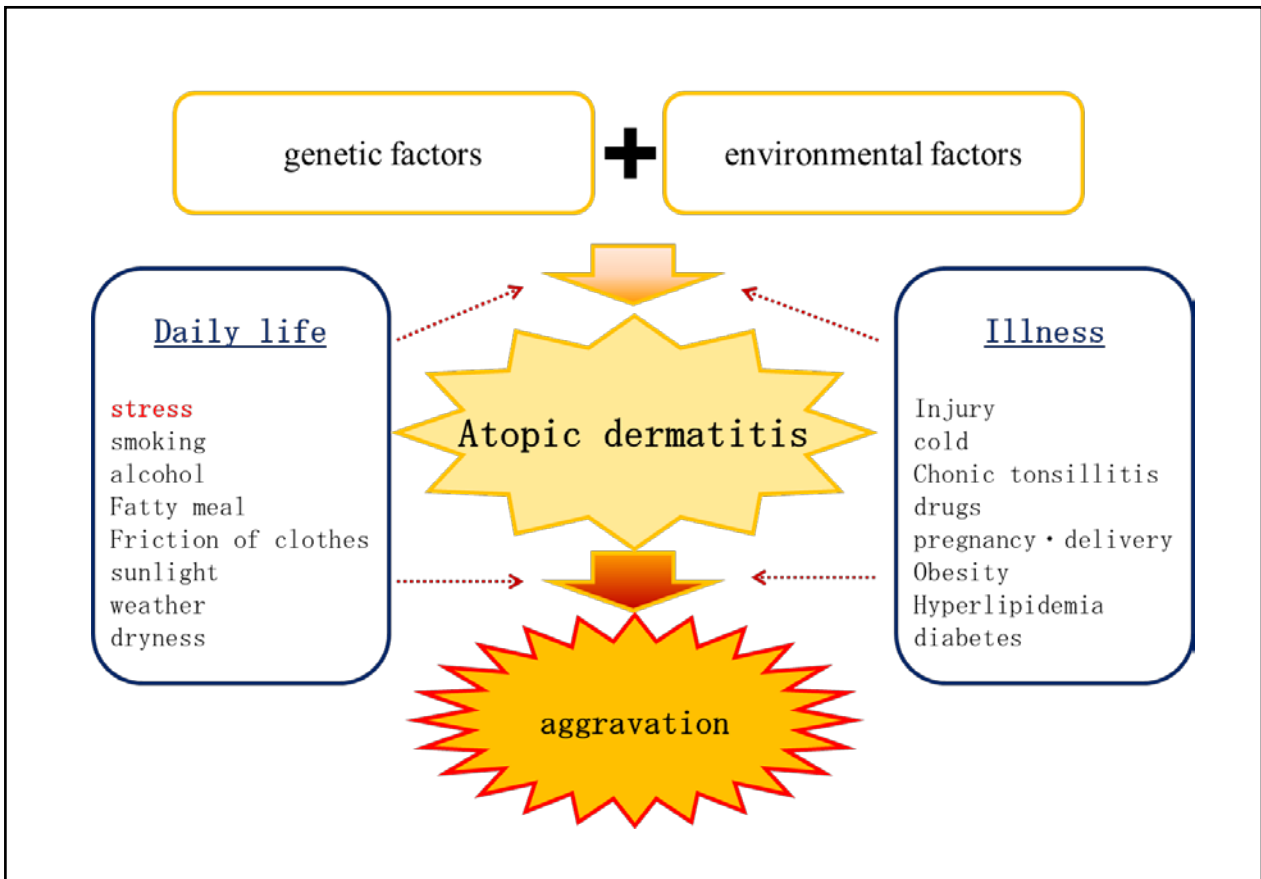important role in the pathogenesis of AD.

Figure 1. Triggering factors of AD.

3.2. Atopic dermatitis and stress

AD can lead to psychological stress, due to stigmatization, social isolation, and discrimination. In contrast, it has been reported that the stress after the HanShin Awaji earthquake disaster in Japan influenced the symptoms of AD, suggesting that natural disasters affect AD symptoms [8]. This observation suggests that influence of stress might exacerbate the symptoms in AD patients.

Similar, in a study by Arima *et al.*, the Beck Depression Inventory (BDI) as a scale for

depression and Self-rating Anxiety Scale (SAS) as a scale for anxiety SAS scores were high

in the severe AD group. In the patients with AD, the BDI scores were significantly higher than

those in the healthy controls [9]. In a cross-sectional study in Korea by Kwon *et al.*, degree of

stress is positively correlated with likelihood of being diagnosed with this condition and

increasing the severity [10]. Furthermore, Linnet *et al.* reported that AD patients with a higher

anxiety level are more likely to improve their psychologic and dermatologic condition after

psychotherapy, but are more vulnerable to nonadherence when no adequate psychologic

treatment is offered [11].

According to these reports, we consider that proper psychologic assessment and treatment

is important for AD patient in addition to dermatologic treatment.

3.3. Salivary cortisol and stress

The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS)

constitute the main effector pathways of the stress system. SNS is stimulated by adrenergic

receptors leading to secretion of catecholamines (adrenalin/noradrenalin) (CAs) and

chromogranin A (CgA) by adrenal medulla and sensory nerve fibres [12]. When the brain

identifies an external perceived stressor, corticotropin-releasing hormone (CRH) is secreted

from the hypothalamus, transported through the portal circulation to the pituitary, and induces

adrenocorticotropic hormone release from the anterior pituitary into the general circulation.

Adrenocorticotrophic hormone acts on the adrenal cortex and increases production and release

of cortisol [13]. Cortisol level increases not only due to acute stress, but also due to chronic

stress, such as results from stigmatization, social isolation, and discrimination. Cortisol is

secreted in diurnal cycle. Saliva is considered to be a good material for evaluating stress

conditions, especially in a depressive state, and the use of salivary biomarkers to evaluate

stress in humans has received much attention in the last 30 years or so [14, 15] (Figure 2).

Among the salivary biomakers incruding cortisol, α- amylase, CgA, and IgA, salivary cortisol

level was recently reported to be an excellent index of the free cortisol level, and thus, it has

been utilized in the field of stress hormone research as a index of the activation of the HPA

axis, particularly in the setting of psychological stress [9]. Correlations between salivary and

plasma cortisol levels have been reported at about 0.70 in adults [16-18] and at about 0.67 in

preterm infants [19]. The highest level of this hormone in the blood is observed at about 8 a.m.

and falls down during the day. Taking into account that in a healthy adult only 1 % of the

cortisol is excreted with urine and saliva, the highest cortisol level in saliva occurs between 9

and 10 a.m [20]. Accordingly, in the present study, we evaluated the salivary cortisol to access
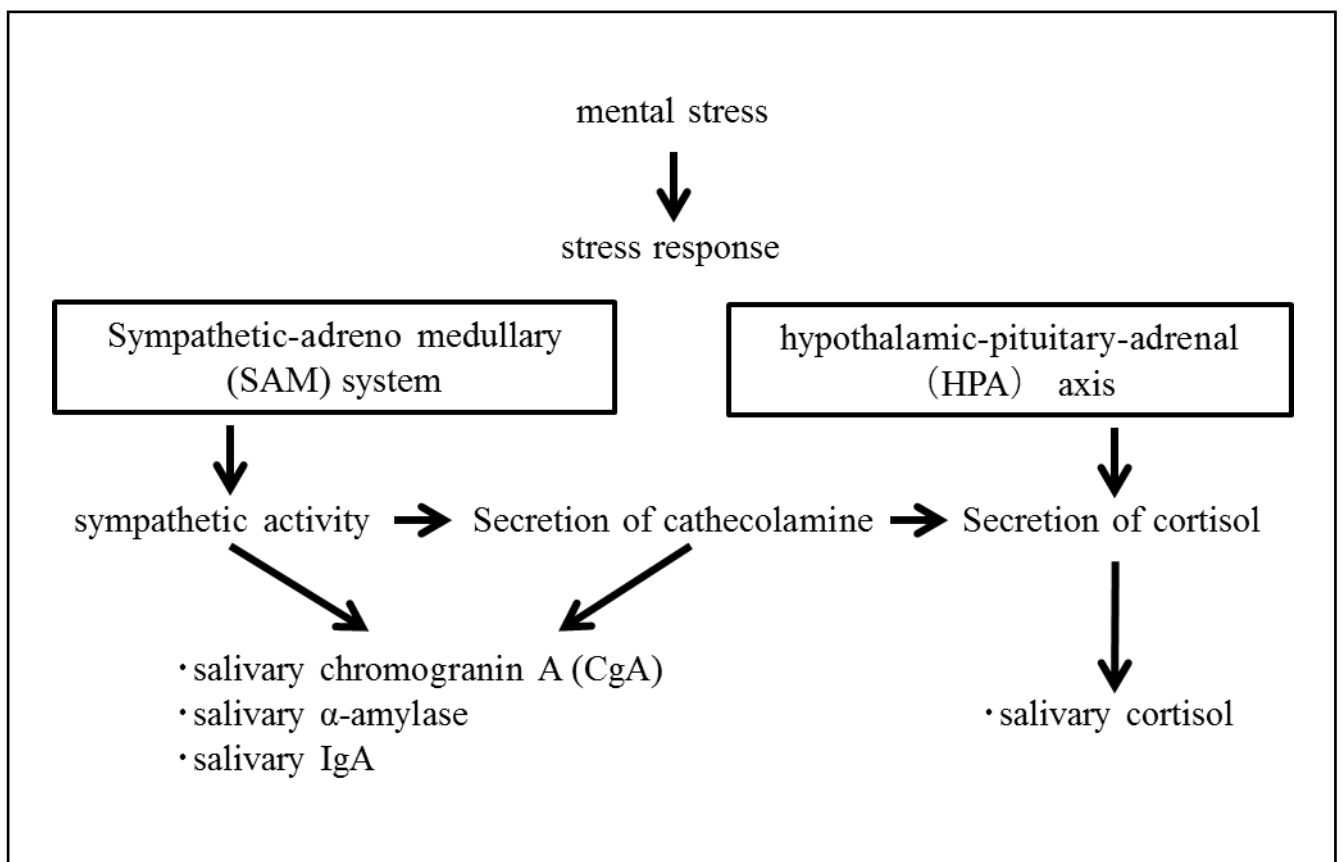
the stress of AD patients.

Figure 2. Mental stress response and salivary mental stress proteins.

4. Purpose

The evaluation of stress biomarker would be helpful to control skin inflammation by allowing for a more proactive management of AD patients. However, so far, there are no reliable biomarkers are available to assess the level of stress in AD patients. The salivary cortisol level is known to be a psychological stressor and is a useful index to assess chronic stress [21]. Additionally, saliva sampling has the advantage of being non-invasive, making multiple sampling easy and stress free.

In this study, we examined the salivary cortisol levels in patients with AD and compared them with those of healthy control subjects.

5. Materials and Methods

5.1. Subjects

The study subjects included thirty AD patients (15 males and 15 females; age, 15-62 years; mean age, 29.6 years). The diagnosis of AD was based on the Rajka and Hanifin criteria, and patients had no other concomitant diseases. Fourty-two normal healthy control subjects (27males and 15 females; age, 31-54years; mean age, 39.4 years) were also enrolled in this study. The study was approved by the Ethics Committee of the University of Toyama and Iwate University.

5.2. Collection of saliva

Experimental sessions were limited to the hours between 9:00 (Am) and 11:00 to minimize time of day effects. Prior to the experiment, the subjects rinsed their mouth in order to clean the oral cavity and then waited five minutes. A braided cotton dental rope was then placed in their mouth and left in place for five minutes to collect saliva (**Figure 3**). Saliva samples were collected from AD patients and normal healthy controls. Then, the saliva samples were centrifuged and the supernatant was stored at -80°C until it was analyzed.
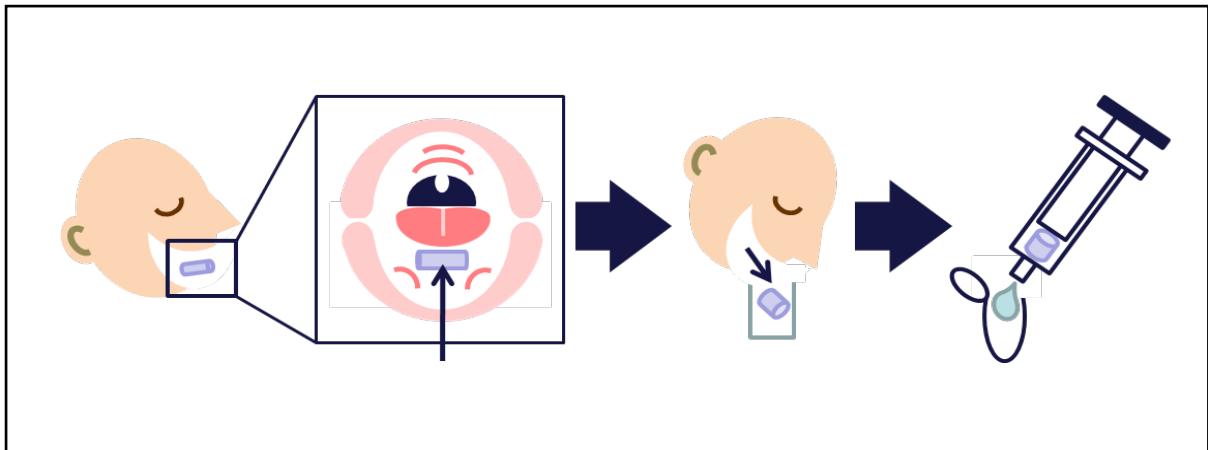
Figure 3. Methods of collection of saliva. AD patient and healthy control subjects rinsed their mouth in order to clean the oral cavity and then waited five minutes. Braided cotton dental rope was placed in their mouth for five minutes to collect saliva.

5.3. Salivary cortisol analysis

The salivary cortisol levels were measured using commercial-linked immunosorbent assay kits (1-3002; Salimetrics LLC, State College, PA) and a plate reader (450 nm measurement wavelength; ARVO MX; Perkin Elmer Life Science, Boston, MA). The intra- and inter-assay variations were 3.35–3.36 and 3.75–6.41%, respectively. The cortisol concentration was expressed in nanomoles per liter.

5.4. Assessment of clinical severity

AD patients were also evaluated for the general disease severity using the Scoring Atopic Dermatitis index (SCORAD). The serum thymus and activation-regulated chemokine (TARC) level, serum total IgE level, serum lactate dehydrogenase (LDH) level, and peripheral blood eosinophil count were measured by standard laboratory tests.

5.5. Skin disease-specific Quality of Life (QOL) assessment

The Japanese version of the Skindex-16, consisting of 16 items in three scales (symptoms, emotions, and functioning), was used as a skin disease-specific instrument. In the present study, the subjects described the degree of anguish caused by the disease by assessing each item on a scale of 0 (never) to 6 (all of the time). Each of the 16 items was quantified using a scale from 0 to 100, and the average was calculated.

5.6. Statistical analysis

Data are presented as the mean values and standard errors of the means (± S.E). Mann-Whitney's U test was used to assess the salivary parameters between the test and

control groups. The Pearson's correlation coefficient (r) was determined for the relationship

between the SCORAD index and levels of salivary cortisol. The correlation between levels of

salivary cortisol and AD-related clinical severity markers and Skindex-16 were performed

using Pearson's correlation coefficient, while IgE and LDH, were analyzed by Spearman's

rank correlation coefficient. Statistical significance value was accepted at $p < 0.05$.

6. Results

6.1. Clinical characteristics

The distribution of the SCORAD index in the enrolled AD patients ranged from 9.9 to 80.3 (46.7 ± 3.2, mean ± SE). The results of laboratory tests are summarized in **Table 1**.

	range	mean±SE
SCORAD index	9.9 - 80.3	46.7±3.2
TARC (pg/ml)	443 - 17160	3093.5±767.7
IgE (IU/ml)	105 - 36009	5180.6±1404.3
LDH (IU/l)	153 - 519	268.4±18.4
EOS (%)	2.2 - 23.4	9.95±1.2
Skindex-16		
Global	7.3 - 94.8	66.3±3.8
Symptoms	8.3 - 100	63.2±5.0
Emotions	11.9 - 100	81.4±4.0
Functioning	0 - 99.3	47.5±5.0

EOS: peripheral blood eosinophils.

Table.1 Clinical chTable.1 Clinical characteristics and laboratory tests

The SCORAD indices were positively correlated with both the serum TARC levels (r = 0.57, p = 0.003) and the serum LDH levels (r = 0.46, p = 0.016) (**Figure 4**). However, no statistically significant correlation was observed between the SCORAD indices and serum IgE levels (r = 0.30, p = 0.12), or the number of peripheral blood eosinophils (r = 0.27, p = 0.16). The Skindex-16 was measured as an assessment of skin disease-specific quality of the life (QOL). The emotional domain was the most affected domain, followed by the symptoms and functioning domains. However, no statistically significant correlations were observed between the SCORAD indices and the Skindex-16 (r = 0.19, p = 0.32).
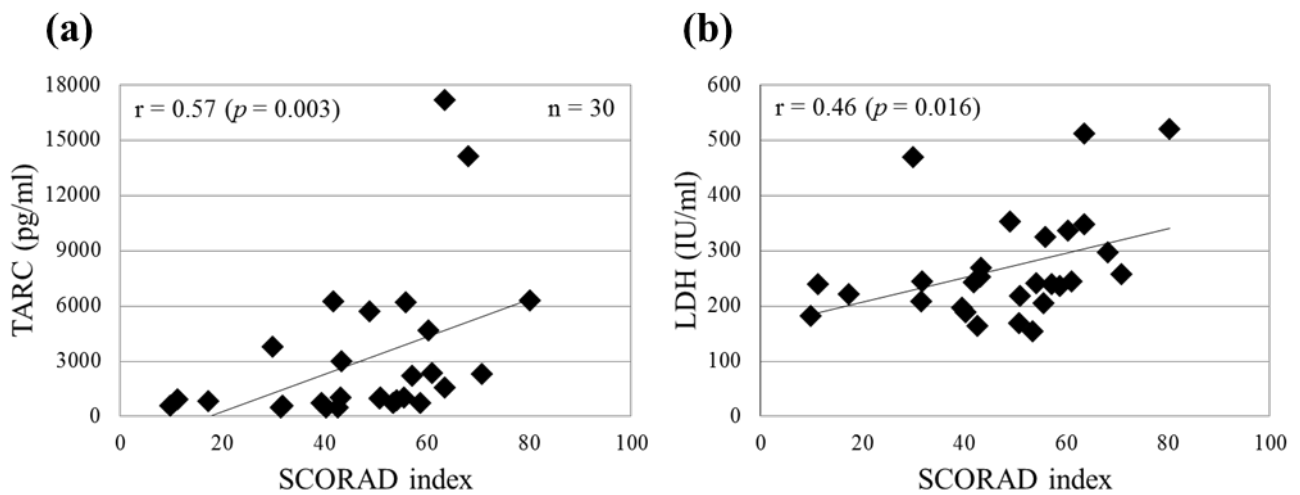
(a) **(b)**

Figure 4. Correlation between the SCORAD index, the TARC and LDH in AD patients.
(a) Significant correlation between the SCORAD index and the serum level of TARC (pg/ml) (r = 0.57, p = 0.003). **(b)** Significant correlation between the SCORAD index and the serum level of LDH (IU/ml) (r = 0.46, p = 0.016).

6.2. Salivary cortisol levels

We examined the salivary cortisol levels in patients with AD and compared them with those of healthy control subjects. The salivary cortisol level in AD patients ranged from 0.47 to 5.18 ng/ml (1.97 ± 0.22 ng/ml; mean $\pm$ SE), which was significantly higher compared to that of the healthy controls (from 0.028 to 0.334 ng/ml; 0.11 ± 0.01 ng/ml; $p < 0.001$) (**Figure 5**).
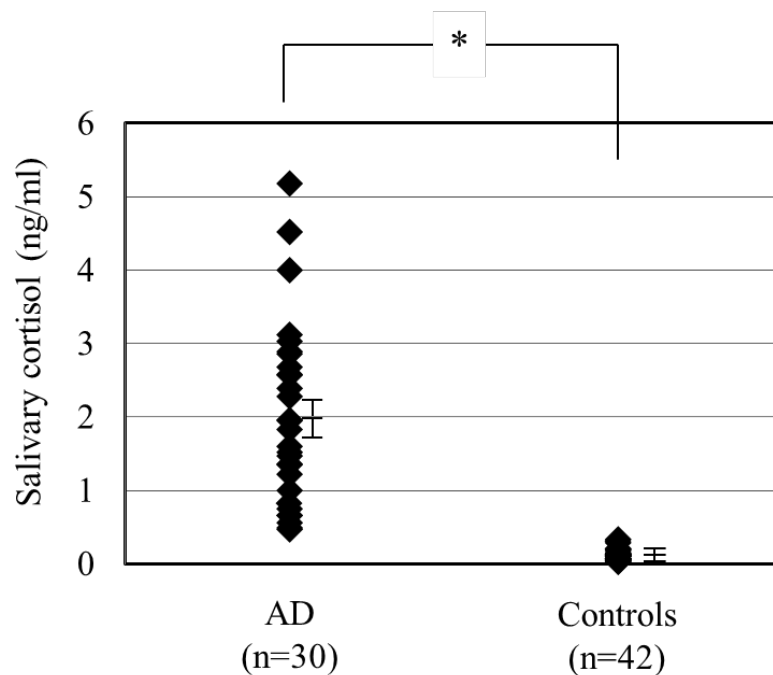

Figure 5. Comparison of the salivary cortisol levels in AD patients and normal healthy controls. The salivary cortisol levels (ng/ml) in AD patients (n = 30) were compared with those in controls (n = 42). *$p < 0.001$.

We next analyzed the relationship between the salivary cortisol levels and other clinical

severity markers and the Skindex-16. The levels of salivary cortisol were significantly

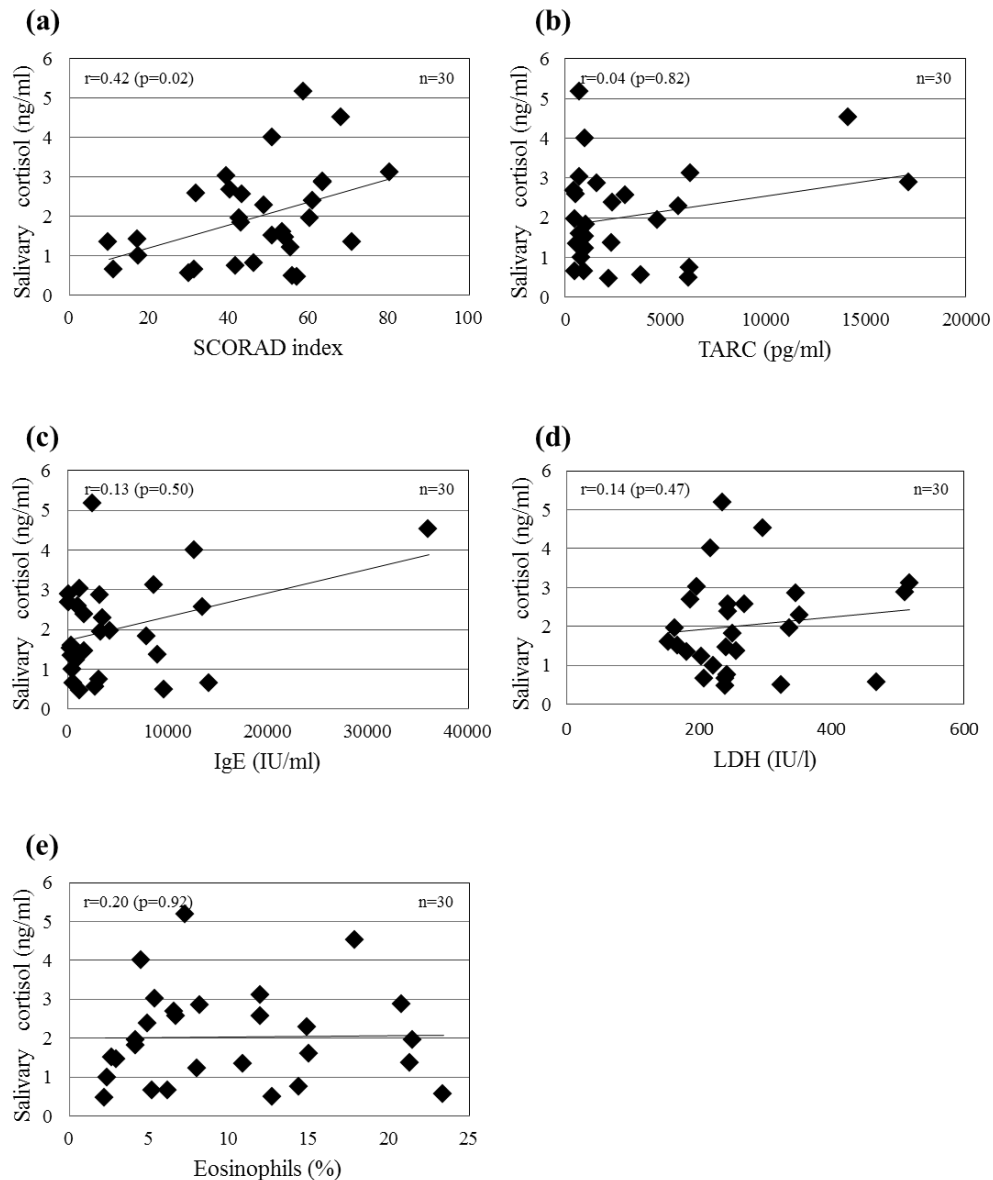correlated with the SCORAD index (r = 0.42, p = 0.02) (**Figure 6(a)**).

Figure 6. Correlation between the salivary cortisol levels, the SCORAD index and other clinical severity markers in AD patients. (a) Significant correlation between the SCORAD index and salivary cortisol levels (ng/ml) (r = 0.42, p = 0.02). **(b-e)** No significant correlations were observed between the salivary cortisol level and the serum levels of TARC, IgE, LDH or the number of peripheral blood eosinophils.

However, the serum levels of TARC, IgE, LDH or the number of peripheral blood eosinophils did not show statistically significant correlations with the salivary cortisol level (TARC; $r = 0.04$, $p = 0.82$, IgE; $r = 0.13$, $p = 0.50$, LDH; $r = 0.14$, $p = 0.47$, eosinophils; $r = 0.02$, $p = 0.92$) (**Figure 6(b-e)**). The correlation between salivary cortisol levels and skindex-16 was also found not statistically significant (global; $r = -0.07$, $p = 0.70$, symptoms; $r = 0.09$, $p = 0.62$, emotions; $r = -0.38$, $p = 0.39$, functioning; $r = -0.20$, $p = 0.30$). (**Figure 7**)
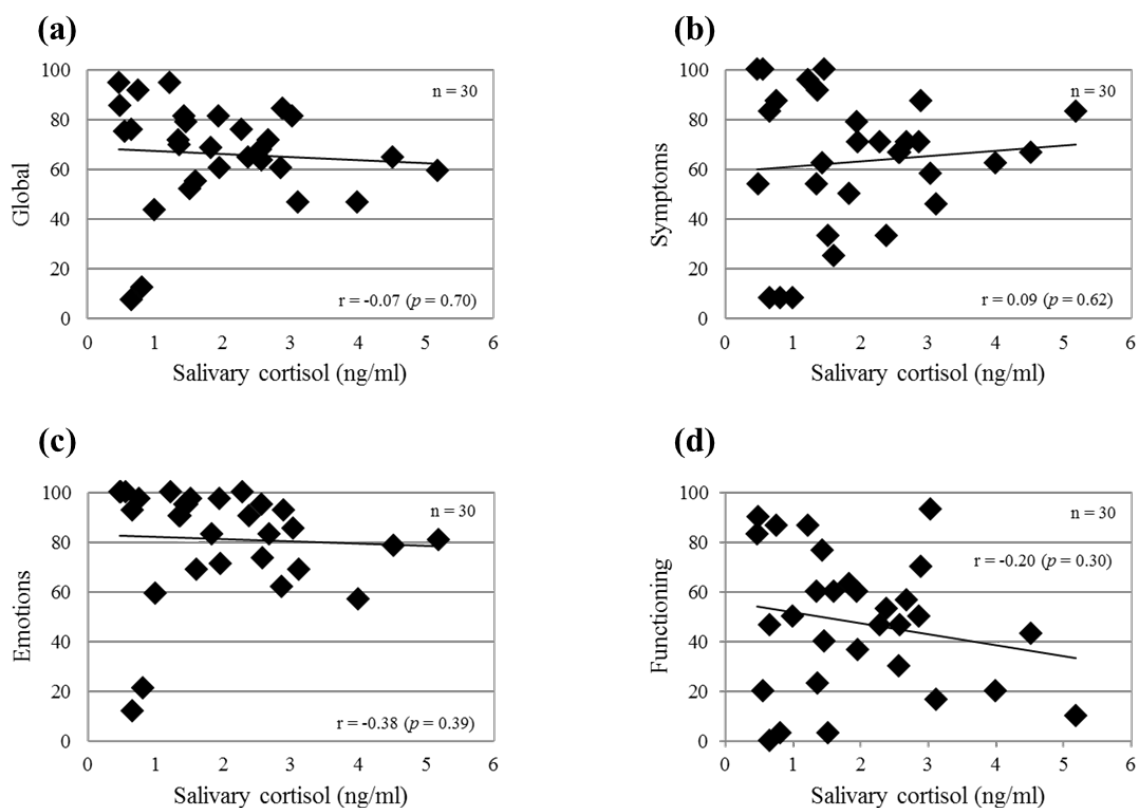

Figure 7. Correlation between the salivary cortisol levels and the Skindex-16 in AD patients. (a-d) No statistically significant correlations were observed between the salivary cortisol levels and Skindex-16 (global, symptoms, emotions or functioning).

7.   Discussion

AD is a stress-prone disorder that involves the autonomic nervous system. Several triggers of AD have been identified such as food and airborne allergens, contact allergens, skin microorganisms, irritants and psychological stress. The strength of the stress depends on the individual perception, subjective rating and the extent of the stressful event. However, the actual effect of stress on AD is poorly understood due to the lack of a method to objectively quantify stress.

In this study, it was observed that the salivary cortisol level was significantly increased in AD patients in comparison to healthy subjects ($p < 0.01$). This suggests that AD patients might be suffering from chronic stress. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system constitute the main effector pathways of the stress system. Cortisol is secreted from the adrenal cortex in the HPA axis, and its level increases not only due to acute stress, but also due to chronic stress, such as that resulting from stigmatization, social isolation, and discrimination [22-24]. Rai *et al*. found that stress and a salivary stress marker, cortisol, were significantly correlated with the clinical parameters of periodontal disease, and suggested that stress might be associated with the activity of periodontal diseases

as a result of physiological and behavioral mechanisms [25]. In a study by Koray *et al.*, the

salivary cortisol level was significantly increased in oral lichen planus patients, who often

related the onset and aggravation of oral symptoms to increased levels of stress [26]. Based on

these findings, the level of salivary cortisol is considered to be a useful index of chronic

stress.

The present study showed that the levels of salivary cortisol were significantly

correlated with the SCORAD index, which is the useful marker to determine the clinical

severity of AD. Therefore, we speculated that severe AD might be associated with more stress

than mild and moderate AD. Furthermore, it was considered that stress may interact with an

immune pathway by acting on the central nervous system and thereby affecting the endocrine

system [27]. Stress causes a decrease in the serum dehydroepiandrosterone level, affecting the

Th1-type cytokine responses, thereby facilitating a shift to a Th2-type cytokine profile and

exacerbating the AD symptoms. In addition, stress induces the release of substance P (SP)

from C-fibers. SP activates both keratinocytes [28] and mast cells, and these activated cells

synthesize and secrete more than 50 biologically active molecules, including cytokines, nerve

growth factor, and histamine, which are mediators of neurogenic inflammation [29]. In

contrast, no significant correlations were observed between the salivary cortisol level and other serum biomarkers, including the levels of TARC, IgE, LDH and eosinophils. This difference might be caused by the change in the serum marker levels due to treatment with either topical corticosteroid or anti-histamine. Assessment of the QOL by the Skindex-16 showed that AD patients were especially impacted with regard to the emotional domain. This might induce psychological stress in AD patients; however, no statistically significant correlations were observed between the salivary cortisol level and Skindex-16.

It was reported that the serum cortisol levels in inpatients with severe AD were significantly suppressed compared to those in outpatients with mild and moderate AD [30, 31]. This result is different from our present result. Recently, Fukuda *et al.* [31] described that approximately 88% of severe AD patients with low serum cortisol levels had sleep disorders, which is suggested to induce suppression of the endocrine system. Therefore, the levels of serum cortisol might be suppressed in severe AD patients in the previous study. In contrast, all of the patients we examined in this study were outpatients and they did not have any sleep disorders. We speculate that an existence of sleep disorders may have led to the discrepancy in the cortisol levels of severe AD patients, although other factors may also be related to the

cortisol level.

In conclusion, our study suggests that AD patients might be under chronic stress, and the severity of AD may be correlated with the intensity of the stress. Saliva sampling has the advantage of being non-invasive, making multiple sampling easy and stress free. Therefore, these results suggest that the saliva cortisol level is a useful biomarker to evaluate the stress in AD patients, and to help physicians in order to plan more effective treatment strategies for these patients.

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9. Acknowledgements

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