

Salivary Cortisol Response to Stress in Young Children with Atopic Dermatitis

Reiji Kojima, M.D.,*,†,‡,¶,|| Akio Matsuda, Ph.D.,† Ichiro Nomura, M.D., Ph.D.,*
Osamu Matsubara, M.D., Ph.D.,‡ Shigeaki Nonoyama, M.D., Ph.D.,¶ Yukihiro Ohya, M.D.,
Ph.D.,* Hirohisa Saito, M.D., Ph.D.,† and Kenji Matsumoto, M.D., Ph.D.†

**Division of Allergy, †Department of Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo, Japan, Departments of ‡Basic Pathology and ¶Pediatrics, National Defense Medical College, Saitama, Japan*

Abstract: Poor responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis under stress may be one explanation for stress-induced exacerbation of atopic dermatitis (AD) symptoms. In previous studies, children and adults with AD showed attenuated salivary cortisol responses to psychosocial stress, suggesting hyporesponsiveness of the HPA axis, but few studies have been conducted in young children, who are vulnerable to systemic side effects of topical corticosteroid (TCS) therapy. We evaluated whether salivary cortisol responses to the stress of venipuncture in young children with AD were related to the severity of AD or performance of TCS therapy. We studied 38 young children with AD (median age 16.5 mos, range 3–66 mos) being treated at our outpatient unit. Patients were divided into three groups according to the scoring of atopic dermatitis index: mild ($n = 12$), moderate ($n = 14$), and severe ($n = 12$). To evaluate the responsiveness of the HPA axis to stress, salivary cortisol was determined before and after venipuncture. Salivary cortisol responsiveness to stress correlated negatively with severity of AD ($p = 0.048$) but not with previous use of TCS ($p = 0.43$) in young children with AD. Our findings suggest that the disease activity of AD, rather than TCS use, is responsible for HPA axis dysfunction in children with AD.

Clinical observations and experimental findings have emphasized that exacerbation of atopic dermatitis (AD) symptoms is closely related to psychosocial stress (1,2). Stress itself can reportedly cause epidermal barrier dysfunction and mast cell activation through release of neuropeptides, which in turn facilitates exacerbation of allergic inflammation (reviewed in reference 1). Stress

also activates the hypothalamic–pituitary–adrenal (HPA) axis to release cortisol, a potent attenuator of inflammatory reactions in general, although previous studies indicated that children and adults with AD showed attenuated salivary cortisol responses to psychosocial stress, suggesting hyporesponsiveness of the HPA axis (3,4). Therefore, poor responsiveness of

Address correspondence to Reiji Kojima, M.D., Division of Allergy, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan, or e-mail: rkojima@nch.go.jp.

the HPA axis under stress may be one explanation of stress-induced exacerbation of AD symptoms. Similar endocrine irregularities of the HPA axis have also been reported after pharmacologic challenge in patients with AD (5,6). It may be argued that hyporesponsiveness of the HPA axis in patients with AD is simply a side effect of topical corticosteroid (TCS) therapy, which may lead to steroid phobia (fear of TCS use) in patients with AD and even in health care professionals (7). Previous reports have investigated the influence of TCS on the HPA axis in children with AD, with contradictory results (8–10). Other studies have suggested that AD disease activity, rather than the use of TCS, is responsible for changes in the HPA axis in patients with severe AD (5,11). Young children are believed to be at special risk for systemic effects of TCS because of high percutaneous absorption of TCS due to their relatively large body surface area per weight. Therefore, it is important to investigate whether HPA dysfunction is observed in young children with AD and is related to TCS therapy.

Blood cortisol concentration increases in response to unpredictable, uncontrollable, and novel situations (12,13). Salivary cortisol levels correlate with plasma unbound cortisol levels (14). Moreover, because saliva samples can be obtained without stress, salivary cortisol assessment is a reliable tool for investigating HPA axis function, especially in young children (13). Previous studies reported a significantly attenuated cortisol response to standardized laboratory psychological stress, which consisted mainly of public speaking and mental arithmetic tasks in front of an audience, in children and adults with AD (3,4), but those procedures are difficult to implement in children younger than 6 years of age. Moreover, venipuncture is an unpredictable, uncontrollable, novel situation for young children, so it itself might be a significant stressor resulting in activation of the HPA axis in young children, detected as high cortisol levels. We used venipuncture as the acute stressor to investigate changes in the level of salivary cortisol.

We evaluated whether impairment of the salivary cortisol response to the stress of venipuncture in young children with AD was related to the severity of the AD symptoms and whether TCS use affected the response.

METHODS

Subjects

We studied 38 young children (24 boys and 14 girls) with AD (median age 16.5 months, range 3–66 months) being treated at our outpatient unit. Patients who had received regular TCS therapy were included, but those who had received inhaled or oral corticosteroids in the preceding 6 months were excluded. All patients were Japanese whose parents were of middle socioeconomic status. An allergist made the diagnosis of AD in accordance with the clinical criteria defined by the Japanese Dermatological Association (15). The severity of AD in each patient was assessed using the scoring of atopic dermatitis (SCORAD) index (16). The severity of AD was defined according to the SCORAD index (mild < 25, moderate 25–50, severe ≥ 50).

Previous use of TCS was assessed by a modification of the previously defined score, the treatment score (Table 1) (10), which was based on the potency of the preparation used (TCS preparations were grouped according to clinical potency, as described in the Japanese Therapeutic Guideline for AD (17)), percentage of body surface area to which it was applied, the duration of treatment in the last month. The hospital ethics committee of the National Center for Child Health and Development approved this study. Informed consent was obtained from all caregivers.

Procedures

To evaluate the responsiveness of the HPA axis to stress, salivary cortisol concentrations were determined before and after venipuncture. Venipuncture for clinical purposes and saliva sampling were performed between 10:00 A.M. and 3:00 P.M. to avoid any possible inhibitory effect of a high morning basal cortisol level on further cortisol release (18). To determine free cortisol concentration, saliva samples were collected 5 minutes before and 15 to 20 minutes after the venipuncture was completed, because that is when salivary cortisol level peaks (13).

Cortisol Measurement

Saliva was obtained using a Sorbette sampling device (Salimetrics, State College, PA), which consists of a

TABLE 1. Score for Topical Corticosteroid Treatment (Ref. 10)

Score (total 10)	0	1	2	3	4
Potency of preparation (0 to 4)	None	Mild	Strong	Very strong	Strongest
Body surface area treated, % (0 to 3)		< 9		> 36	
Treatment duration* (0 to 3)		< 7	≥ 7	Continuous	

*Days in the past month.

TABLE 2. *Clinical Characteristics*

Characteristic	Mild <i>n</i> = 12	Moderate <i>n</i> = 14	Severe <i>n</i> = 12	p-value*
Sex, male/female, <i>n</i>	6/6	9/5	9/3	0.44
Age, months, median (range)	19 (4–66)	21 (3–60)	10.5 (3–57)	0.41
SCORAD, median (range)	16 (8–25)	40 (26–48)	64.5 (51–86)	< 0.001
Score for TCS treatment, median (range)	4.5 (4–7)	5 (0–7)	0 (0–7)	0.38
Non-TCS users, <i>n</i>	0	2	7	0.002
Number of venipunctures, median (range)	2 (1–3)	1 (1–4)	1 (1–3)	0.10
Total immunoglobulin E, IU/mL, median (range)	163 (5.2–4,562)	212 (12.9–9,500)	1,093.5 (55.6–4,161)	0.12

TCS, topical corticosteroid.

*Kruskal–Wallis test or chi-square test.

microsponge with a short plastic shaft as a handle. After the microsponge had been put under the tongue for 1 minute, the Sorbette was placed in a plastic tube and centrifuged for 15 minutes at 1,800 *g*, resulting in a clear, watery supernatant. The samples were stored at -30°C until analysis. For cortisol determination, 50 μL of saliva was used for duplicate analysis with a salivary cortisol enzyme-linked immunosorbent assay kit (Salimetrics), according to the manufacturer's protocol.

Statistical Analysis

Salivary cortisol concentrations did not show a normal distribution and were therefore log-transformed for analysis. Group variables were compared using the Mann–Whitney *U* test, the Kruskal–Wallis test, or the chi-square test. The paired *t* test was used to compare salivary cortisol response to the stressor (venipuncture). Spearman rank correlation was used to investigate the relationship between the parameters of the salivary cortisol response to the stressor and the variables (disease severity and TCS treatment). Data were analyzed using STATA software (Windows version 8.0, Stata Corp., College Station, TX). $P < 0.05$ was considered to indicate statistical significance in all comparisons.

RESULTS

Clinical Characteristics

Clinical characteristics of the patients are summarized in Table 2. The patients were grouped according to severity of AD (mild [12 patients], moderate [14 patients], and severe [12 patients]). There were no significant differences between the AD severity groups in terms of the age, sex, score for TCS treatment, number of venipunctures, or total immunoglobulin (Ig)E level (IU/mL), but there was a statistically significant difference in the number of patients not treated with TCS ($p = 0.002$).

Salivary Cortisol Response to Venipuncture

First, we evaluated whether venipuncture could be a stressor that induced significantly higher salivary cortisol levels in young children with AD. The number of venipunctures and the change in salivary cortisol level were investigated for possible association in patients in whom—for technical reasons—several venipunctures were needed to achieve blood sampling. The change in salivary cortisol level was calculated by dividing the postvenipuncture cortisol concentration by the pre-venipuncture cortisol concentration, yielding a ratio. When subjects were stratified according to number of venipunctures, the increase in salivary cortisol level depended on the number of venipunctures ($p = 0.04$; Kruskal–Wallis test, Fig. 1). This result suggested that venipuncture was a sufficient stressor to induce a change in cortisol level in children younger than 6 years of age.

There were no significant differences in pre-venipuncture and postvenipuncture salivary cortisol levels between the three groups, but the salivary cortisol level increased significantly after venipuncture in all groups ($p < 0.05$; paired *t* test, Fig. 2).

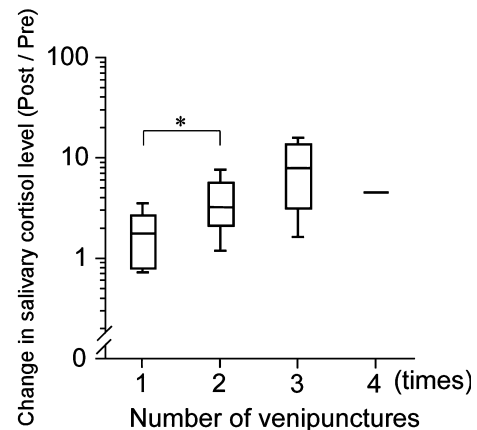


Figure 1. Change in salivary cortisol level (post/pre) versus number of venipunctures (* $p = 0.02$; Mann–Whitney test).

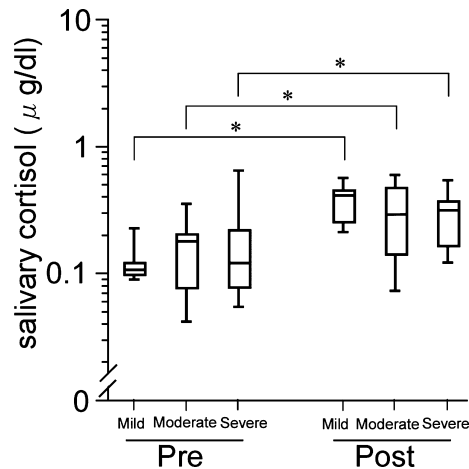


Figure 2. Salivary cortisol level in response to venipuncture in individuals with atopic dermatitis (* $p < 0.05$; Paired t test).

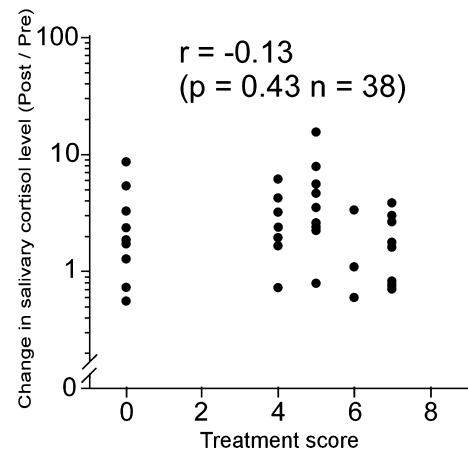


Figure 4. Correlation between change in salivary cortisol level (post/pre) and treatment score ($n = 38$, $r = -0.13$, $p = 0.43$).

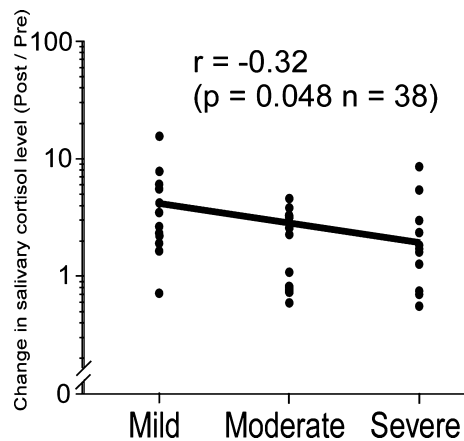


Figure 3. Correlation between change in salivary cortisol level (post/pre) and severity of atopic dermatitis ($n = 38$, $r = -0.32$, $p = 0.048$).

Correlation Between Salivary Cortisol Response and Severity of AD or TCS Treatment

The change in salivary cortisol level correlated significantly with the pre- and postvenipuncture salivary cortisol levels ($r = -0.50$, $p = 0.01$; $r = 0.61$, $p < 0.001$, respectively; data not shown). Conversely, it correlated negatively with severity of AD ($r = -0.32$, $p = 0.048$; Fig. 3). No significant correlation was found between change in salivary cortisol level and score for TCS treatment ($p = 0.43$; Fig. 4), age, sex, or total IgE (data not shown).

DISCUSSION

In previous studies, children and adults with AD showed attenuated salivary cortisol responses to psychosocial

stressors, suggesting hyporesponsiveness of the HPA axis, which may be one explanation for stress-induced exacerbation of AD symptoms (3,4), but few studies have been performed in young children, who are vulnerable to systemic side effects of TCS therapy. In this study, we found that salivary cortisol response to the stress of venipuncture in young children with AD correlated negatively with the severity of symptoms.

We first evaluated whether venipuncture could be a stressor in children younger than 6 years of age. Although previous studies reported that changes in cortisol level after venipuncture were not significant in children aged 6 and older (19,20), we found that venipuncture is a stressor in children younger than 6 years of age. This difference might be because children have more occasions to undergo venipuncture and become used to it as they get older. Although venipuncture is difficult to standardize, and the individual characteristics of the children would influence its role as a stressor, salivary cortisol level rose dependent on the number of venipunctures. These results suggest that venipuncture is a sufficient stressor to induce a change in cortisol level in children younger than 6 years of age. The number of venipunctures did not differ significantly between the three groups of patients with different AD severity (mild 1.75 ± 0.72 , moderate 1.29 ± 0.80 , severe 1.42 ± 0.64 , mean $\pm$ SD; $p = 0.10$), suggesting that patients in all three groups received virtually the same level of stress.

The salivary cortisol response to venipuncture as a stressor correlated negatively with the severity of AD. This result is in line with previous studies that found a weaker cortisol response to stressors in adults and children with AD (3,4). Meanwhile, there were no significant

differences in pre-venipuncture cortisol levels between the groups with various AD severity, which is also in line with previous studies (3,4). Dysfunction of the HPA axis in individuals with AD may become apparent only when a stress stimulus is present.

According to a previous study (21), the normal salivary cortisol level in young children was approximately 0.16 to 0.36 $\mu\text{g}/\text{dL}$ at baseline. The cortisol level in each group of subjects before venipuncture was compatible with that range, and there were no significant differences between the groups. Therefore, we think that our time points for performing venipuncture (10:00 A.M. and 3:00 P.M.) were sufficient for detecting any elevation in cortisol level, regardless of whether severe pruritus might have somewhat altered the circadian rhythm of cortisol release.

The underlying psychobiologic mechanisms of hyporeactivity of the HPA axis in patients with AD are not fully understood. Historically, these changes have been interpreted as a consequence of an ongoing chronic allergic inflammatory process, which releases pro-inflammatory cytokines (22). Some studies reported that an atopic disposition in neonates is associated with greater responsiveness of the HPA axis to stressors, which may promote the development of AD in later life (21,23). It remains to be determined whether these changes in HPA function precede or follow the onset of AD.

Most individuals with AD have high serum concentrations of total and allergen-specific IgE, and the severity of AD is known to be weakly associated with serum IgE levels and degree of Th2-type immune predisposition (24). In the present study, we also found that serum IgE levels were higher in those with more severe AD, but the differences were not statistically significant, presumably because of limited power or the fact that we studied very young infants, but further study is needed to elucidate whether hyporeactivity of the HPA axis is simply a consequence of the chronic inflammatory process or is specific to Th2-type immune responses.

The effects of TCS on HPA function in children with AD have been studied using various methods but with contradictory results (8–10). Our present study found no significant correlation between salivary cortisol level and previous TCS treatment, suggesting that there are other factors related to the disease. Our results are in line with previous findings showing that patients with AD and not treated with TCS had a weaker cortisol response than control subjects (3,4). Moreover, some studies showed that a significant decrease in the disease activity of AD after intensive treatment with large amounts of a potent TCS during hospitalization was associated with normalization of the basal serum cortisol level compared with levels at admission (5,11). These results suggest that the disease activity of AD, rather than TCS use, is

responsible for dysfunction of the HPA axis in patients with severe AD.

In children with AD, percutaneous absorption of TCS was proven to be significantly lower in the convalescent phase of the disease than in the acute phase, probably because of the restoration of the skin barrier (25). Although percutaneous absorption of a potent TCS is likely to occur, especially during the acute phase of severe AD, the positive effect of adequate disease control seems to clinically outweigh the suppressive effect on adrenal gland function. Therefore, early restoration of the skin barrier by appropriate TCS therapy might contribute to reducing any undesirable effect of TCS on the HPA axis in individuals with AD in the long term. Good control of AD would improve HPA axis function, which might reduce stress-induced exacerbation of AD symptoms.

In addition, to clarify the effect of TCS on the HPA axis in patients with AD, we would like to compare the cortisol responses of patients with similar severity of AD but different TCS usage. We believe that appropriate use of TCS may improve the skin condition and quality of life of patients and in turn improve the HPA axis response. Suppression of the HPA axis can be seen only in patients with extremely severe AD or inappropriate administration of TCS. Further large-scale, longitudinal studies should be undertaken to elucidate the underlying mechanisms of HPA axis hyporeactivity in patients with AD. Limitations of this study are that the number of subjects was not sufficiently large and the sleep patterns of the patients were not closely determined, but a strength of the study is that we can evaluate HPA axis function to stress non-invasively in young children with AD.

Salivary cortisol responsiveness to the stress of venipuncture correlated negatively with the severity of AD but showed no correlation with previous use of TCS in young children with AD. These findings have major implications for daily practice when treating young patients with moderate to severe AD and steroid phobia.

ACKNOWLEDGMENTS

The authors would like to express their sincere thanks to Drs. Kyoko Satsuka, Koichi Yoshida, Yuki Tsumura, Kenta Horimukai, Masaki Futamura, and Masami Narita (Division of Allergy, National Center for Child Health and Development) for collecting data. This study was supported by a grant from the Japanese Ministry of Health, Labour and Welfare.

REFERENCES

1. Arndt J, Smith N, Tausk F. Stress and atopic dermatitis. *Curr Allergy Asthma Rep* 2008;8:312–317.

2. King RM, Wilson GV. Use of a diary technique to investigate psychosomatic relations in atopic dermatitis. *J Psychosom Res* 1991;35:697–706.
3. Buske-Kirschbaum A, Geiben A, Hollig H et al. Altered responsiveness of the hypothalamus-pituitary-adrenal axis and the sympathetic adrenomedullary system to stress in patients with atopic dermatitis. *J Clin Endocrinol Metab* 2002;87:4245–4251.
4. Buske-Kirschbaum A, Jobst S, Wustmans A et al. Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosom Med* 1997; 59:419–426.
5. Matsuda K, Katsunuma T, Iikura Y et al. Adrenocortical function in patients with severe atopic dermatitis. *Ann Allergy Asthma Immunol* 2000;85:35–39.
6. Rupprecht M, Hornstein OP, Schluter D et al. Cortisol, corticotropin, and beta-endorphin responses to corticotropin-releasing hormone in patients with atopic eczema. *Psychoneuroendocrinology* 1995;20:543–551.
7. Charman C, Williams H. The use of corticosteroids and corticosteroid phobia in atopic dermatitis. *Clin Dermatol* 2003;21:193–200.
8. Ellison JA, Patel L, Ray DW et al. Hypothalamic-pituitary-adrenal function and glucocorticoid sensitivity in atopic dermatitis. *Pediatrics* 2000;105:794–799.
9. Feiweil M, James VH, Barnett ES. Effect of potent topical steroids on plasma-cortisol levels of infants and children with eczema. *Lancet* 1969;1:485–487.
10. Patel L, Clayton PE, Addison GM et al. Adrenal function following topical steroid treatment in children with atopic dermatitis. *Br J Dermatol* 1995;132:950–955.
11. Haeck IM, Timmer-de Mik L, Lentjes EG et al. Low basal serum cortisol in patients with severe atopic dermatitis: potent topical corticosteroids wrongfully accused. *Br J Dermatol* 2007;156:979–985.
12. Gunnar MR. Reactivity of the hypothalamic-pituitary-adrenocortical system to stressors in normal infants and children. *Pediatrics* 1992;90:491–497.
13. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 1989;22:150–169.
14. Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 2009;34:163–171.
15. Tagami H. Japanese Dermatological Association criteria for the diagnosis of atopic dermatitis. *J Dermatol* 1995;22:966–967.
16. Stalder JF, Taieb A. Severity scoring of atopic dermatitis: the SCORAD index. Consensus report of the European task force on atopic dermatitis. *Dermatology* 1993;186:23–31.
17. Furue M. [Summary of therapeutic guideline for atopic dermatitis]. *Arerugi* 2000;49:324–326.
18. Kudielka BM, Hellhammer DH, Wust S. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 2009;34:2–18.
19. Conte PM, Walco GA, Kimura Y. Temperament and stress response in children with juvenile primary fibromyalgia syndrome. *Arthritis Rheum* 2003;48:2923–2930.
20. Lee T, Shimizu T, Iijima M et al. Evaluation of psychosomatic stress in children by measuring salivary chromogranin A. *Acta Paediatr* 2006;95:935–939.
21. Ball TM, Anderson D, Minto J et al. Cortisol circadian rhythms and stress responses in infants at risk of allergic disease. *J Allergy Clin Immunol* 2006;117:306–311.
22. Chrousos GP. Stress, chronic inflammation, and emotional and physical well-being: concurrent effects and chronic sequelae. *J Allergy Clin Immunol* 2000;106:S275–S291.
23. Buske-Kirschbaum A, Fischbach S, Rauh W et al. Increased responsiveness of the hypothalamus-pituitary-adrenal (HPA) axis to stress in newborns with atopic disposition. *Psychoneuroendocrinology* 2004;29:705–711.
24. Akdis CA, Akdis M, Bieber T et al. Diagnosis and treatment of atopic dermatitis in children and adults: European academy of allergology and clinical immunology/American academy of allergy, asthma and immunology/PRACTALL consensus report. *Allergy* 2006;61:969–987.
25. Turpeinen M, Lehtokoski-Lehtiniemi E, Leisti S et al. Percutaneous absorption of hydrocortisone during and after the acute phase of dermatitis in children. *Pediatr Dermatol* 1988;5:276–279.