

Transepidermal water loss, serum IgE and β -endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis

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Summary

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Conflicts of interest

None declared.

Background Although itch is the predominant symptom of atopic dermatitis (AD), it is poorly characterized and subjective. The objective assessment of itch intensity is important for treatment and follow-up in patients with AD.

Objectives To determine what objective clinical parameter(s) could be used as bio-marker(s) for itch intensity in patients with AD.

Methods This is a retrospective and cross-sectional study. Seventy-five patients, aged 7 months–49 years with equal sex ratio, were enrolled in 2000 according to criteria proposed by Hanifin and Rajka. Thirty-five age- and sex-matched subjects who visited the dermatological clinic but were otherwise healthy served as controls. Subjective itch intensity was divided into four grades of severity. Disease severity was measured by SCORAD index, which also includes itch intensity as part of the measurement. Transepidermal water loss (TEWL) and skin surface pH were measured by noninvasive methods in clinically normal skin on the forearm. Serum β -endorphin and vasoactive intestinal peptide (VIP) were determined by radioimmunoassay. Ordinal logistic regression was used to assess the trend of the subjective itch intensity and SCORAD index by serum IgE, β -endorphin, VIP, TEWL and skin pH.

Results There were significant trends for itch intensity with IgE, β -endorphin and TEWL. After adjustment for sex, age and other variables, the odds ratio (OR) for itch intensity by log IgE, β -endorphin and TEWL was 2.103 [95% confidence interval (CI) 1.222–3.618], 1.100 (95% CI 1.005–1.203) and 1.081 (95% CI 1.009–1.158), respectively. The OR for disease severity by log IgE, β -endorphin and TEWL was 2.250 (95% CI 1.149–4.407), 1.156 (95% CI 1.086–1.231) and 1.071 (95% CI 0.971–1.182), respectively. In contrast, there was no association between serum VIP concentration and itch intensity.

Conclusions β -endorphin and IgE are both useful biomarkers for itch and disease severity in patients with AD, while TEWL is a good biomarker for itch intensity. These biomarkers provide a way to assess the itch intensity in patients with AD.

Itch has been defined as an unpleasant sensation that provokes the desire to scratch.¹ Atopic dermatitis (AD) is a common inflammatory skin disease characterized by severe pruritus, a chronically relapsing course, and a personal or family history of atopic diseases. Itch is the most important symptom in patients with AD and it constitutes a major diagnostic criterion. The itch threshold in AD skin is lower than that in nor-

mal skin and AD skin is inherently itchy.² It interferes in many aspects of the atopic patient's life and impairs quality of life in numerous ways.^{3,4} By interfering with sleep, it affects physical growth, mental development, emotional equanimity, and performance at school and work.^{5,6} Therefore accurate assessment and management of pruritus is very important in the care of patients with AD. Although there have been many

attempts to determine and characterize this sensory quality, the pathophysiology of itch in AD is ill-understood. Recent experimental studies using improved methods have demonstrated which of the suspected chemical itch mediators such as histamine, neuropeptides, prostaglandins, serotonin, acetylcholine or bradykinin act pruritogenically on cutaneous nerve endings.⁷

Itch perception is transmitted through the free nerve endings of nonmyelinated C-fibres in the epidermis and at the dermoepidermal junction. The impulse travels through the dorsal root ganglion to the dorsal horn of the spinal column, through the contralateral spinothalamic tract, finally radiating to the postcentral gyrus. Many mediators have been proposed to be involved in itch in AD, including neuropeptides, neurotransmitters, histamines, proteinases, cytokines, eosinophils, basophils, and some triggering factors modulating pruritus perception.⁸ In clinical practice, eosinophil count, eosinophil cationic protein,⁹ IgE^{10,11} and lactate dehydrogenase isoenzyme 5¹² are all useful clinical markers for assessment of the severity and clinical course of AD. However, how these markers are related to the generation of pruritus is poorly understood. IgE causes pruritus mainly through liberation of mediators including histamine from mast cells when IgE binds mast cells.

Numerous neuropeptides have been identified by immunochemistry in human skin, in both primary sensory C-fibres and small myelinated nerve A- δ endings.^{13,14} Tachykinins including the neuropeptide substance P, calcitonin gene-related peptide and vasoactive intestinal peptide (VIP) are found in the cutaneous free nerve endings of unmyelinated nociceptor neurones.¹⁵ They are considered as peripheral mediators of itch in skin diseases.¹⁶ Neuropeptides may also serve as a modulatory factor underlying the pathogenesis of some inflammatory dermatoses, as stress exacerbates some skin diseases.¹⁷ β -endorphin is one of the most important mediators of stress,^{18,19} and is known to be generated upon stimulation of the pituitary-adrenal axis.²⁰

Apart from the role of serum IgE and neuropeptides in AD, the lack of ability to maintain hydration in atopic skin is one of the fundamental defects in AD pathophysiology.²¹ Transepidermal water loss (TEWL) is increased and water content is decreased in AD skin. A decrease of skin hydration below 10% seems to be crucial for induction of itch and scratching.²² The impaired skin barrier function facilitates the entry of irritants and itch-causing agents.²³ Furthermore, skin surface pH has been correlated with the severity of experimentally induced irritant dermatitis,²⁴ and many forms of dermatitis show an increase in skin pH.²⁵ In this study, we try to define important biomarkers in assessment and monitoring of pruritus intensity in AD. We measured IgE, β -endorphin and VIP in the blood, together with TEWL and pH in the skin, from patients with AD. The association of these biomarkers with subjective itch intensity in AD was clarified by ordinal logistic regression, which has the advantage of enabling adjustment for confounders in the assessment.

Materials and methods

Patient selection

Subjects were recruited in 2000 from a dermatological clinic in a tertiary centre. Patients with AD were selected according to the diagnostic criteria proposed by Hanifin and Rajka.²⁶ The patients had to meet three major criteria plus at least one minor criterion or two major criteria plus at least three minor criteria. They had not received any topical treatments for at least 2 weeks or systemic treatment for 2 months prior to the study. Seventy-five patients with AD (40 males and 35 females; mean $\pm$ SD age 16.2 ± 7.4 years, range 7 months–49 years) were collected. Thirty-five age- and sex-matched controls (19 males and 16 females; mean $\pm$ SD age 17.3 ± 9.1 years, range 2–53) who visited the dermatological clinic in the same hospital, but without a history of atopic diseases, served as the normal control group. Most of the control group visited the dermatology clinic for removal of small benign skin lesions, without other remarkable skin symptoms and signs. A dermatologist took the medical history and inspected the whole body surface of every person in the normal control group. Normal controls with symptoms of itching, physical findings, or past history of atopic diseases, including allergic asthma, allergic rhinitis and allergic conjunctivitis, were excluded. The selection bias was minimal because age and sex were comparable, subjects with pruritus or other atopic diseases were excluded, and most people of the control group were healthy. Venous peripheral blood was collected from all patients and controls. After centrifugation, the serum was stored at -70°C until assay for β -endorphin and VIP. The study was approved by the Institutional Review Board of the Hospital.

Determination of clinical severity and itch intensity

The clinical severity score were assessed by the SCORAD index (from 0 to 103 in 1-unit intervals) proposed by the European Task Force on AD.²⁷ The SCORAD index includes measurements of disease extent, erythema, oedema, oozing, excoriation, lichenification as well as pruritus and insomnia. Thus, the assessment of itch intensity was also included and contributed to part of the SCORAD index for disease severity (20%).²⁷ Subjective itch intensity was measured using a 1–4 scale modified from the categorization according to Atherton *et al.*:²⁸ 1, no pruritus at all; 2, mild pruritus that does not interfere with sleep; 3, moderate pruritus that makes patients awaken easily; 4, severe pruritus that makes patients unable to fall asleep.

Serum total IgE measurement

Ten millilitres of venous blood was allowed to clot at room temperature. Serum was collected within 2 h and was stored at -70°C until used. Serum concentration of total IgE was measured by IMMULITE[®] Total IgE kit (DPC, Los Angeles, CA,

U.S.A.) according to the manufacturer's instructions. IMMULITE® Total IgE kit is a solid-phase, two-site chemiluminescent enzyme immunoassay designed for the quantitative measurement of total IgE in serum. The intra-assay and interassay coefficients of variance were 5.3% and 5.9%, respectively. The cut-off value for a positive result in this kit was set at 100 IU mL⁻¹.

Determination of serum β -endorphin and vasoactive intestinal peptide

The concentrations of β -endorphin and VIP in sera were determined by radioimmunoassay (RIA) with the use of the ¹²⁵I-RIA kit (Phoenix Pharmaceuticals, Belmont, CA, U.S.A.). The serum samples were incubated with rabbit β -endorphin/VIP antibodies for 20 h at 4 °C, and the ¹²⁵I- β -endorphin/¹²⁵I-VIP was added for a further 20 h. After addition of the second antibody and incubation for 90 min, free and bound β -endorphin/VIP was separated by centrifugation at 1700 g for 20 min at 4 °C. After aspirating off the supernatant, the precipitate was counted in an automatic gamma counter. The results were read from a standard curve, which was plotted on the basis of analysis of control sera containing known concentrations of β -endorphin/VIP ranging from 1 to 128 pg mL⁻¹.

Skin transepidermal water loss and pH measurement

Measurements were carried out at a clinically normal area of the extensor forearm. All measurements were carried out after a 30-min acclimatization period in reclining subjects at room temperature (20–22 °C) and 35–50% humidity. TEWL was measured first, followed by pH. TEWL was measured by Evaporimeter EP01 (Servomed AB, Stockholm, Sweden), which is based on vapour pressure gradient estimation. An insulating glove for holding the probe and the protection cover without the screen and grid was employed during measurements. The damping filters smooth the fluctuations in TEWL, in order to record a TEWL value during a 30-s period after stabilization. Low intrainstrumental variability and high interinstrumental variability are noted in the Evaporimeter EP01.²⁹

Skin surface pH at the test site was measured by a skin pH meter (PH 900, Courage + Khazaka, Cologne, Germany) based on an electrochemical method. The intraclass and interclass coefficient of variance is <10%.

Statistical methods

The data were analysed using SAS ver. 8.01 (SAS Inc., Cary, NC, U.S.A.). Student's t-test was used to compare the continuous variables between patients with AD and normal controls. We used Pearson's correlation test to assess the associations between severity score, log IgE, β -endorphin, VIP, TEWL and pH in order to perform subsequent regression analysis. Ordinal logistic regression was performed to explore the relationship of subjective itch intensity or severity index (SCORAD

index) with different biological markers including log IgE, β -endorphin, VIP, TEWL and pH with adjustment for gender and age under a proportional odds model, for which itch intensity was classified into three ordered categories (itch score 2, 3, 4) and severity (SCORAD index) was classified into three ordered categories of severity (mild, SCORAD index 1–31; moderate, SCORAD index 32–62; severe, SCORAD index 63–103). The significance level (α -value) was set at 0.05, and two-tailed tests were used.

Results

Transepidermal water loss, pH, β -endorphin and vasoactive intestinal peptide in patients with atopic dermatitis and normal controls

Mean $\pm$ SD TEWL was significantly elevated ($P < 0.01$) in patients with AD (22.8 ± 12.9 g m⁻² h⁻¹) compared with normal controls (7.5 ± 4.1 g m⁻² h⁻¹) (Fig. 1a). Mean $\pm$ SD skin pH was less acidic ($P < 0.01$) in patients with AD (5.58 ± 0.63) compared with normal controls (4.92 ± 0.48) (Fig. 1b). The mean $\pm$ SD serum concentration of β -endorphin in patients with AD (12.40 ± 5.66 pg mL⁻¹) was increased ($P < 0.01$) compared with normal controls (6.96 ± 3.65 pg mL⁻¹) (Fig. 1c). However, the mean $\pm$ SD serum concentration of VIP in patients with AD (8.55 ± 3.39 pg mL⁻¹) was not significantly different ($P = 0.16$) from that in normal controls (7.67 ± 2.23 pg mL⁻¹) (Fig. 1d).

SCORAD index, IgE, transepidermal water loss, pH, β -endorphin and vasoactive intestinal peptide in patients with atopic dermatitis by itch intensity

The mean $\pm$ SD SCORAD index of patients with AD was 35.53 ± 17.01 , 42.17 ± 17.01 and 52.17 ± 24.40 in itch score categories 2, 3 and 4, respectively (Fig. 2a). Mean $\pm$ SD IgE after logarithmic transformation (log IgE) was 2.40 ± 0.72 , 2.79 ± 0.67 and 2.92 ± 0.77 in itch score categories 2, 3 and 4, respectively (Fig. 2b). Mean $\pm$ SD TEWL was 17.3 ± 5.4 , 19.7 ± 9.9 and 29.6 ± 18.2 g m⁻² h⁻¹ in itch score categories 2, 3 and 4, respectively (Fig. 2c). Mean $\pm$ SD skin pH was 5.12 ± 0.62 , 5.33 ± 0.72 and 5.56 ± 0.46 in itch score categories 2, 3 and 4, respectively (Fig. 2d). The mean $\pm$ SD serum level of β -endorphin was 10.27 ± 1.85 , 10.36 ± 3.99 and 13.44 ± 5.58 pg mL⁻¹ in itch score categories 2, 3 and 4, respectively (Fig. 2e). The mean $\pm$ SD serum level of VIP was 8.54 ± 2.24 , 11.67 ± 3.90 and 9.63 ± 4.19 pg mL⁻¹ in itch score categories 2, 3 and 4, respectively (Fig. 2f). To investigate the trend for different variables by itch intensity, we performed ordinal logistic regression analysis using only one independent variable with itch intensity as the outcome. The odds ratio (OR) for log IgE, TEWL, β -endorphin, pH and VIP was 2.22 [95% confidence interval (CI) 1.33–3.7, $P < 0.01$], 1.07 (95% CI 1.01–1.14, $P < 0.05$), 1.13 (95% CI 1.04–1.23, $P < 0.05$), 1.001

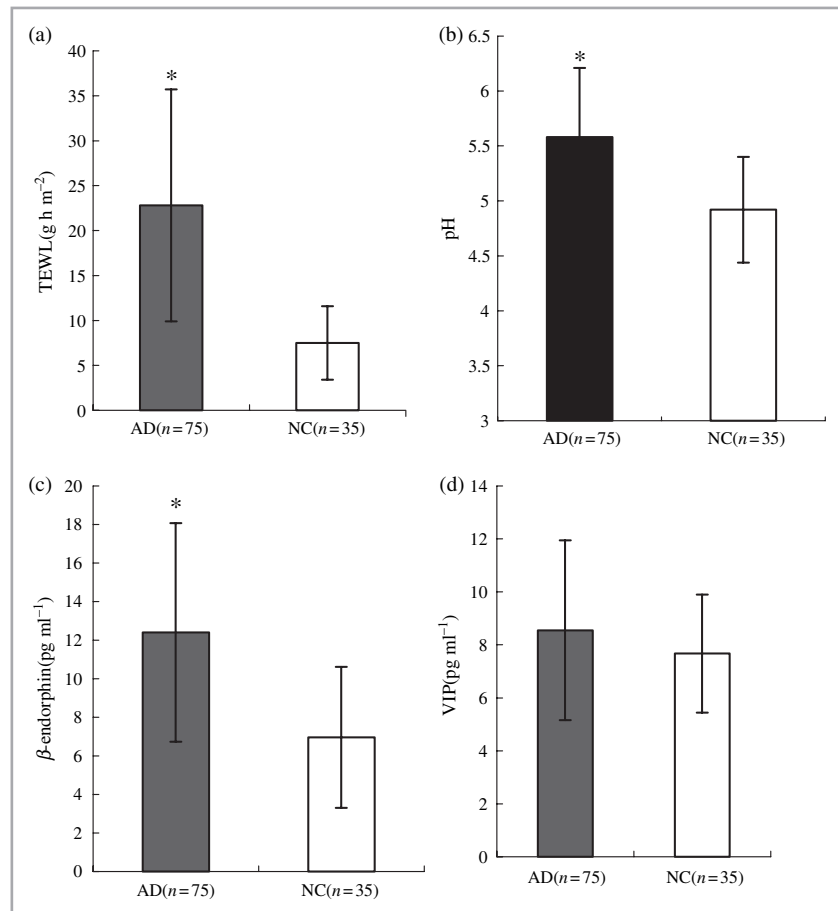


Fig 1. (a) Transepidermal water loss (TEWL) and (b) pH at clinically normal forearm skin of patients with atopic dermatitis (AD) and normal controls (NC). Both TEWL and pH were elevated in AD compared with NC. (c) Serum levels of β -endorphin were elevated in AD compared with NC. (d) Serum levels of vasoactive intestinal peptide (VIP) were similar in AD and NC. Results are given as mean $\pm$ SD. * $P < 0.05$, Student's *t*-test.

(95% CI 1.0003–1.002, $P < 0.05$) and 0.99 (95% CI 0.96–1.03, $P = 0.71$) for itch intensity, respectively. Those data mean that there are trends in values of log IgE, TEWL, β -endorphin and pH in regard to the itch intensity, while there is no significant trend in VIP level by itch intensity. For example, for log IgE, the OR is 2.22, which means 1 unit increase of log IgE increases the likelihood of patients with AD to develop the next level of itch by 2.22-fold. However, the present test did not take other variables into consideration in the regression analysis simultaneously. The way to solve this problem is to perform an ordinal logistic regression by multiple covariates that could adjust for effects from other variables at the same time. To do this, we have to perform the correlation analysis first, as we could only put one of those variables in the multivariate ordinal logistic regression if those variables were closely correlated.

Correlation of SCORAD index, IgE, vasoactive intestinal peptide, β -endorphin, transepidermal water loss and skin pH

To demonstrate associations between the SCORAD index, IgE, VIP, β -endorphin, TEWL and skin pH, we used the Pearson correlation test to study the association of these skin and blood biomarkers (Table 1). We found that pH and TEWL

were highly correlated ($P < 0.01$), as were SCORAD index and serum IgE ($P < 0.001$). However, there were no correlations between other pairs of biomarkers.

Ordinal logistic regression of itch intensity by multiple covariates

Instead of trend analysis, we used ordinal logistic regression to study the relationship between itch intensity and the other variables. In brief, we used as the outcome the three levels of itch intensity, modelled as ordered categories, and the independent variables log IgE, TEWL, β -endorphin, age and sex. Thus we estimated the OR between groups for each increase of 1 in the three ordered categories. The relative importance of covariates on itch intensity was examined by ordinal logistic regression analysis (Table 2). Because the SCORAD index is so closely correlated to serum IgE, we used serum IgE instead of SCORAD index as a covariate. Similarly, TEWL was used instead of skin pH because they were so closely correlated. We found significant trends for itch intensity with log IgE (OR 2.103, 95% CI 1.222–3.618, $P < 0.01$), TEWL (OR 1.081, 95% CI 1.009–1.158, $P < 0.05$) and β -endorphin (OR 1.100, 95% CI 1.005–1.203, $P < 0.05$). The data mean, for example, that each unit increase of β -endorphin increases the likelihood of patients with AD to develop the next level of itch

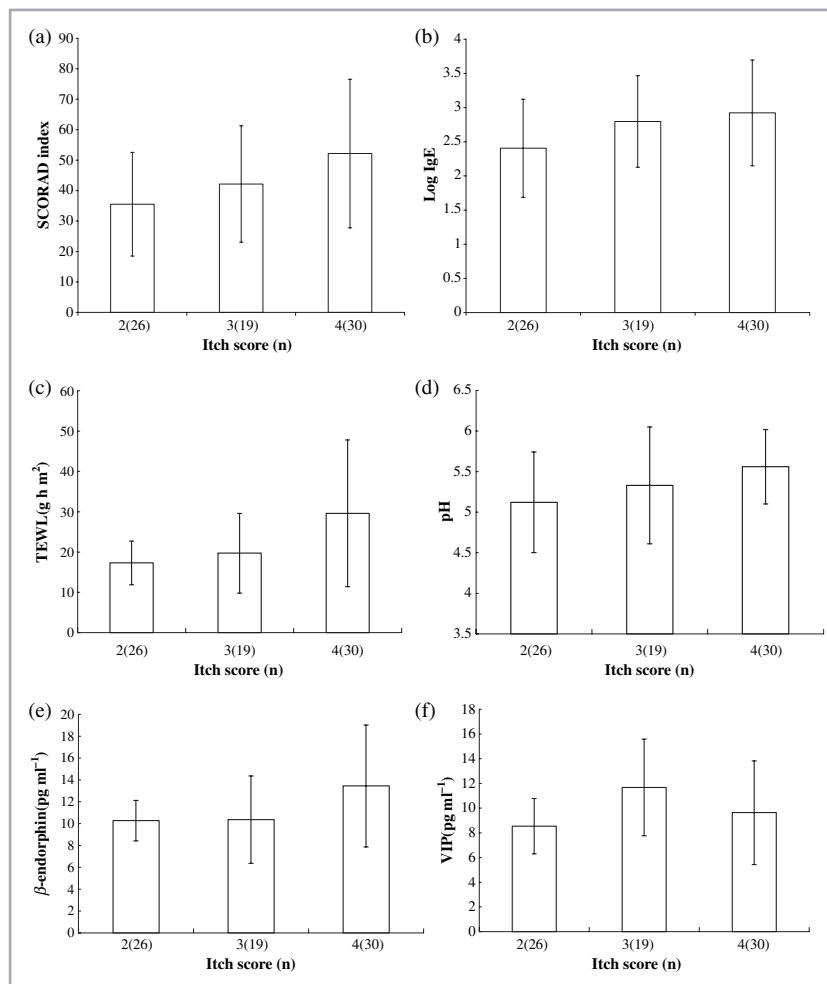


Fig 2. (a) SCORAD index, (b) log IgE, (c) transepidermal water loss (TEWL), (d) pH, (e) serum β -endorphin and (f) vasoactive intestinal peptide (VIP) in patients with atopic dermatitis (AD) by itch intensity. Results are given as mean $\pm$ SD. Significant trends were found for SCORAD index, log IgE, TEWL, pH and serum β -endorphin level in patients with AD by itch intensity ($P < 0.05$) using ordinal logistic regression for each single independent variable listed.

	Severity	Log IgE	TEWL	pH	β -endorphin	VIP
Severity						
Correlation	1	0.431	-0.041	0.355	-0.023	0.141
P-value	—	<0.001	0.782	0.172	0.072	0.069
Log IgE						
Correlation		1	0.03	0.079	0.116	0.091
P-value		—	0.724	0.477	0.438	0.809
TEWL						
Correlation			1	0.923	0.081	0.155
P-value			—	<0.01	0.622	0.091
pH						
Correlation				1	0.155	0.23
P-value				—	0.477	0.375
β -endorphin						
Correlation					1	0.116
P-value					—	0.443
VIP						
Correlation						1
P-value						—

We found severity index and log IgE to be highly correlated. TEWL and pH were also significantly correlated. However, no statistical correlation could be demonstrated between other parameters.

Table 1 Correlation of disease severity (SCORAD index), log IgE, transepidermal water loss (TEWL), pH, β -endorphin and vasoactive intestinal peptide (VIP) in patients with atopic dermatitis

Table 2 Ordinal logistic regression of itch score (categories 2–4) and clinical severity by log IgE, transepidermal water loss (TEWL) and β -endorphin after adjustment for age and sex. Odds ratios for log IgE, TEWL and β -endorphin by itch score were 2.103 ($P < 0.01$), 1.081 ($P < 0.05$) and 1.100 ($P < 0.05$), respectively. TEWL, log IgE and β -endorphin are independent biomarkers for itch intensity in atopic dermatitis. Odds ratios for log IgE, TEWL and β -endorphin by disease severity were 2.250 ($P < 0.05$), 1.071 ($P > 0.05$) and 1.156 ($P < 0.01$), respectively. Thus, there are significant trends for itch intensity and clinical severity to both log IgE and β -endorphin, whereas there is a significant trend for itch intensity only but not clinical severity to TEWL

	Estimated	SEM	Odds ratio	95% CI
Itch score				
Intercept 1	2.278	1.326		
Intercept 2	0.79	0.443		
Log IgE	0.743	0.277	2.103	1.222–3.618
TEWL	0.078	0.035	1.081	1.009–1.158
β -endorphin	0.095	0.046	1.100	1.005–1.203
Age	0.002	0.023	1.002	0.958–1.048
Sex	-1.300	0.703	0.272	0.069–1.081
Clinical severity				
Intercept 1	2.399	1.572		
Intercept 2	0.992	0.568		
Log IgE	0.811	0.343	2.250	1.149–4.407
TEWL	0.069	0.05	1.071	0.971–1.182
β -endorphin	0.145	0.032	1.156	1.086–1.231
Age	0.03	0.045	1.030	0.943–1.125
Sex	-1.4	0.877	0.247	0.044–1.376

CI, confidence interval.

by 1.100-fold after adjustment for other variables including serum IgE, TEWL, age and sex, as the OR for β -endorphin by itch intensity is 1.100. Next, we wanted to see if those variables are also correlated with clinical severity. Therefore we classified patients into three groups by clinical severity based on SCORAD index (mild, moderate and severe). If we put the ordered clinical severity index into the ordinal logistic regression as a dependent variable, log IgE (OR 2.250, 95% CI 1.149–4.407, $P < 0.05$) and β -endorphin (OR 1.156, 95% CI 1.086–1.231, $P < 0.01$) were both significantly associated with disease severity (Table 2). However, TEWL was not significantly associated with disease severity (OR 1.071, 95% CI 0.971–1.182, $P > 0.05$).

Discussion

Itch is defined subjectively as a poorly localized, nonadapting, usually unpleasant sensation which elicits a desire to scratch.¹⁵ Four factors may be of importance hypothetically in the pathomechanism of itch in AD: (i) peripheral mediators of itch; (ii) central mediators of itch; (iii) itch susceptibility; and (iv) allokinesis.³⁰ In this study, we showed that serum IgE, β -endorphin and skin TEWL serve as independent biomarkers for the subjective itch intensity. In addition, we also showed that serum

IgE and β -endorphin were significantly related to disease severity of the patients with AD. It was not surprising that both IgE and β -endorphin were independent biomarkers for subjective itch intensity and disease severity as one of the assessments of AD disease severity by SCORAD score included measurement of itch intensity. One could argue that IgE and β -endorphin might lead to increased itch intensity through elevated disease severity or vice versa. However, at least in patients with AD, the elevated IgE and β -endorphin both predict the tendency of these patients either to develop more intense pruritus or to have higher disease severity. In addition, in this regression analysis, TEWL was significantly related to itch intensity but not to disease severity. Thus, TEWL is an independent biomarker for itch intensity but not disease severity in patients with AD in this model of logistic regression. TEWL is closely related to itch intensity, probably not via disease severity. Thus, TEWL is useful for clinical evaluation of the itch intensity rather than disease severity in patients with AD.

Xerosis of the skin, as reflected by reduced skin hydration, reflects a disturbed epidermal barrier and is a well-known activator of pruritus in patients of all ages with AD. In AD, lipid constituents, especially ceramides, which act as molecular rivets to consolidate the stratum corneum, are depleted.³¹ Basically, elevated TEWL provides more chances for noxious substances to enter defective skin in AD. Easy ingress of allergens through the epidermis due to loss of epidermal barrier function leads to stimulation of active T cells that contribute to more inflammation, and hence more itch, of the skin.³² In AD we found an important and independent association between itch intensity and skin barrier abnormality as shown by increased TEWL. Increased TEWL together with increased likelihood of developing pruritus implicates the defective skin barrier in relation to itch generation.

A significant increase in pH towards alkalinity is noticed in the forearm skin of the patients with AD. This emphasizes the subtle change in skin pH even in clinically normal skin of atopic patients. The significance of altered skin pH in pruritus of AD is that pH changes within skin may activate itch receptors.³³ Because of the close correlation between skin pH and TEWL measurements, we did not use skin pH in the further regression test to determine the influence of pH in itch perception. How and why skin surface pH is correlated to TEWL and induction of pruritus requires further clarification. Similarly to TEWL, skin pH may contribute to pruritus by a mechanism that is not yet fully understood.

This study showed that serum total IgE reflects the itch status in AD after adjusting for age and sex. IgE reflects not only inflammation but also subjective itch intensity. The serum total IgE level is elevated in 43–82% of patients with AD.¹⁰ Elevation of serum IgE has several impacts on skin inflammation in AD. IgE induces acute- and late-phase skin reactions. Activation of IgE-bearing macrophages contributes to skin inflammation and the release of cytokines, which in turn leads to pruritus.³⁴ Mononuclear cells from patients with food allergy produced an IgE-dependent histamine-releasing factor

in vitro that provoked histamine release from basophils of other food-sensitive patients but not from normal control patients. Although direct evidence of the involvement of IgE in pruritus is obscure, the multiple aspects of inflammation induced by IgE may lead to inflammation in AD and subsequently to pruritus. This study demonstrated that IgE level is related independently to subjective itching intensity. Local levels of IgE in atopic diseases may determine the involvement of individual organs.³⁵

Our study demonstrated that serum β -endorphin also serves as an independent and important factor influencing pruritus in AD. Opioid peptides and their μ -specific receptors modulate the function of calcium channels specifically on unmyelinated C-fibres of the central nervous system, and thus they are probably involved in the central itch-regulatory mechanism.³⁶ There have been reports to suggest that endogenous opioid peptides may be involved as central mediators of itch.^{37,38} β -endorphin, belonging to the endogenous opiate family, is generated upon stimulation of the pituitary-adrenal axis after stress.^{19,20} In contrast to VIP, the concentration of β -endorphin in sera was markedly increased in AD patients with a high itch score in our study. This result supports the hypothesis that endorphinergic neurones are probably involved in central itch processing.³⁹

It is known that itch in AD is exacerbated by emotional stress, scratching and sweating.⁴⁰ Neuropeptides were hypothesized to be involved in the pathogenesis of AD and generation of pruritus.⁴¹ VIP levels, assessed by RIA, were markedly increased in lesional AD skin compared with controls.⁴² Furthermore, VIP is known to control sweating,⁴³ and thus, it may play a role in the abnormal sweating phenomenon that is commonly observed in AD.⁴⁴ In our results, serum VIP levels were not significantly higher in patients with AD than in controls. VIP concentration in patients with AD showed no correlation with itch score. The role of VIP in the immune system is suggested to be anti-inflammatory.⁴⁵ Thus, it is conceivable that elevation of serum VIP levels in patients with AD might be associated with inflammation rather than with itch.

We used an ordinal logistic regression model in this study. There are several advantages of this model. One advantage is that trend analysis could be carried out easily to explore the relationship between ordered category variables, i.e. itch intensity or disease severity, and several continuous variables, including log IgE, TEWL, β -endorphin and VIP. The other advantage, which is more important, is that one can exclude the contribution of other confounding variables simultaneously. After adjustment for sex and age, serum IgE, β -endorphin and TEWL represent independent biomarkers for pruritus in patients with AD. The higher the level of IgE, β -endorphin or TEWL, the more likelihood there is of the patient developing more intense pruritus. The cause of itching is multifactorial. Besides peripheral mediators in diseased skin, dysregulation of the central nervous system, as evidenced by increased serum β -endorphin, might play a role in some patients with severe pruritus. The role of physiological

changes, as demonstrated by increased TEWL, may also relate to pruritus. Serum IgE causes itch both through the well-known pathway of mast cell histamine liberation and through other proinflammatory pathways. Serum IgE and β -endorphin not only reflect the disease severity in AD but also reflect pruritus intensity. Skin TEWL in clinically normal skin in patients with AD provides a useful independent objective measurement of itch intensity in patients with AD.

In conclusion, we found that serum β -endorphin, but not VIP, is closely related to itch perception and disease severity. This indicates the importance of β -endorphin in modulating the itch generation in central itch transmission and in tuning peripheral immune response in AD. TEWL, a measurement of skin barrier function, was related to pruritus intensity but not to disease severity. Serum IgE level represents not only disease severity but also subjective itch intensity. β -endorphin, IgE and TEWL are all useful biomarkers for the itch intensity in AD. More insights into the neurophysiological and molecular pathways in AD may represent a new approach to effective therapy.

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