

Pathophysiology of nocturnal scratching in childhood atopic dermatitis: the role of brain-derived neurotrophic factor and substance P

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Summary

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Key words

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Conflicts of interest

None declared.

Background Childhood atopic dermatitis (AD) is a distressing disease associated with pruritus and sleep disturbance. The pathophysiology of pruritus is complex and various neuropeptides may be involved.

Objective To evaluate whether or not brain-derived neurotrophic factor (BDNF) and substance P are associated with disease severity, quality of life and nocturnal scratching in AD.

Methods Patients with AD aged under 18 years were recruited. Disease severity was assessed with the SCORing Atopic Dermatitis (SCORAD) index, and quality of life with the Children's Dermatology Life Quality Index (CDLQI). Concentrations of plasma BDNF, substance P, AD-associated chemokines [cutaneous T-cell attracting cytokine (CTACK), thymus and activation regulated chemokine (TARC)], serum total IgE and eosinophil counts were measured in these patients. All children were instructed to wear the DigiTrac monitor on their dominant wrist while sleeping. The monitor was programmed to record limb motion between 22.00 and 08.00 h the following morning.

Results Twenty-eight children with AD [mean (SD) age 11.1 (3.3) years] were recruited. The mean (SD) SCORAD was 48.1 (21.5) and CDLQI was 8.7 (5.4) in the patients with AD. Their mean (SD) plasma concentrations of BDNF, substance P, CTACK and TARC were 1798 (935), 94 (42), 1424 (719) and 824 (1000) pg mL⁻¹, respectively. BDNF was significantly correlated with SCORAD ($r = 0.478$, $P = 0.010$) and CDLQI ($r = 0.522$, $P = 0.004$), whereas substance P showed significant correlation only with CDLQI ($r = 0.441$, $P = 0.019$). BDNF and substance P were also significantly correlated with the average ($r = 0.905$, $P < 0.001$ and $r = 0.925$, $P < 0.001$) and frequency-specific ($r = 0.826$, $P < 0.001$ and $r = 0.870$, $P < 0.001$) nocturnal wrist activities measured by DigiTrac. However, there was no correlation between BDNF or substance P and the subjective symptoms of pruritus or sleep-loss scores as reported by the parents in the SCORAD. In contrast, serum total IgE levels showed significant correlations with the subjective symptoms of pruritus ($r = 0.576$, $P = 0.001$) and sleep loss ($r = 0.419$, $P = 0.027$).

Conclusions Serum levels of BDNF and substance P correlate with the clinical score and quality of life score in patients with AD. The strong correlations with nocturnal wrist movements suggest that they may be the pathogenic factors of the annoying symptoms of scratching.

Atopic dermatitis (AD) is a distressing disease associated with pruritus and sleep disturbance. Scratching due to pruritus is an important mechanism in the exacerbation of AD. However, little is known about the pathophysiology of nocturnal itching, the inflammatory mediators that cause itching, the neurologi-

cal processes involved in either the detection of an itching stimulus or the induction of the scratching.¹ Studies have suggested that neuroimmune mediators such as brain-derived neurotrophic factor (BDNF) and substance P have a role in AD and are possibly involved in the pathogenesis of the itching

sensation. BDNF is a growth factor initially found in the nervous system.² Increased blood levels of BDNF have been documented in patients with AD and correlated with the disease severity.^{3,4} BDNF has also been found to be produced, stored and released by human circulating eosinophils.⁵ In addition, eosinophil apoptosis was inhibited by BDNF.³ Substance P is another neuropeptide probably involved in the pathogenesis of AD via the neuroimmunocutaneous mechanism, and significantly correlated with disease activity.⁶

The severity of itch is traditionally assessed by questionnaires or visual analogue scales; both rely on the assumption that the subject or the caregiver is able to relate the perceived experiences accurately.^{7,8} We demonstrated that nocturnal wrist activities, nonintrusively measured at home, are closely correlated with the objective clinical scores and levels of peripheral blood chemokine markers for AD, but not necessarily with symptoms of pruritus or sleep loss. Therefore, these activities are good indicators of AD severity in children and may substitute for the pruritus and sleep-loss components of the SCORing Atopic Dermatitis (SCORAD) index.⁹ The AD-associated chemokines have been extensively studied in both adults and children, and include the cutaneous T-cell attracting cytokine (CTACK) and thymus and activation regulated chemokine (TARC).^{10–12} The purpose of this study was to evaluate whether BDNF and substance P are associated with disease severity, quality of life, nocturnal wrist activities and laboratory markers of disease activity in AD.

Patients and methods

Patients with AD under 18 years of age were recruited from the paediatric dermatology clinic of a university teaching hospital. AD was diagnosed according to criteria proposed by Hanifin and Rajka,¹³ and severity and symptomatology including pruritus and sleep loss (in the preceding 3 days) in the patients with AD was evaluated using the SCORAD index.^{14,15} Subjects were excluded if information regarding pruritus or sleep loss could not be recalled by patients and caregivers, or if obvious parasomnias were evident from history. Thereafter, all enrolled children were instructed to wear the DigiTrac monitor (IM Systems, Baltimore, MD, U.S.A.) on their dominant wrist while sleeping. In our pilot testing on 17 patients with AD,¹⁶ the DigiTrac was found to provide essential data on a wide spectrum of frequencies of wrist movements and quantity of movements in terms of acceleration or *g* values. The monitor was programmed to record limb motion between 22.00 and 08.00 h the following morning. The data were then downloaded to a computer using DigiTrac 3.9 (IM Systems). Wrist activities were expressed in units of average value of acceleration ($g \text{ min}^{-1}$). We previously found that wrist activities between 1 and 3 Hz for the first 3 h of sleep were good indicators of AD severity in children.⁹ Consequently, two DigiTrac datasets were used for analyses of correlations, namely the average activities over the first 3 h of sleep ($\bar{A}$) and frequency-specific activities between 0 and 3 Hz over the first 3 h (f).

One set of peripheral blood was collected from the patients with AD, centrifuged at 4 °C and 2041 *g* for 10 min, and then stored at –70 °C until analysis. Plasma concentrations of BDNF as well as AD-associated chemokines (CTACK and TARC)^{10–12} were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, U.S.A.). The lower limits of detection for these biomarkers were 20, 1.6 and 7.0 pg mL^{-1} , respectively. Plasma substance P concentration was measured by enzyme immunoassay (Sigma-Aldrich, St Louis, MO, U.S.A.), and its detectable limit was 8.0 pg mL^{-1} . Serum concentration of total IgE was measured by microparticle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL, U.S.A.), and results were log-transformed before analysis. Eosinophil counts were determined using a Coulter STKS counter (Beckman-Coulter, Miami, FL, U.S.A.). Informed written consent was obtained from parents, and the Clinical Research Ethics Committee of the University approved the study.

Data are expressed as proportion or mean and SD unless otherwise stated. χ^2 was used to compare proportions, and the Student's *t*-test was used to analyse numerical variables. The correlations between different SCORAD components, inflammatory markers and DigiTrac data were analysed by Pearson correlation coefficients. All comparisons were made two-tailed, and $P < 0.05$ was considered to be statistically significant.

Results

Twenty-eight children with AD (13 boys and 15 girls) were recruited. The mean (SD) age for patients was 11.1 (3.3) years. The mean (SD) SCORAD and Children's Dermatology Life Quality Index (CDLQI) scores in the patients with AD were 48.1 (21.5) and 8.7 (5.4), respectively; and 25 (89%) patients had moderate-to-severe AD judged by their objective SCORAD scores.¹⁵ Their mean (SD) plasma concentrations of BDNF, substance P, CTACK and TARC were 1798 (935), 94 (42), 1424 (719) and 824 (1000) pg mL^{-1} , respectively. The mean (SD) log-transformed serum total IgE level and peripheral blood eosinophil percentage were 3.29 (0.73) kIU L^{-1} and 8.3% (5.2).

BDNF was correlated with SCORAD ($r = 0.478$, $P = 0.010$) and CDLQI ($r = 0.522$, $P = 0.004$), whereas substance P showed significant correlation only with the CDLQI ($r = 0.441$, $P = 0.019$). In addition, BDNF and substance P were highly significantly correlated with average ($r = 0.905$, $P < 0.001$ and $r = 0.925$, $P < 0.001$; Fig. 1) and frequency-specific ($r = 0.826$, $P < 0.001$ and $r = 0.870$, $P < 0.001$; Fig. 2) wrist activities measured by the DigiTrac. However, there was no correlation between BDNF or substance P and pruritus or sleep-loss scores reported by the parents in the SCORAD (Table 1). Conversely, serum total IgE levels showed significant correlations with the perceived symptoms of pruritus ($r = 0.576$, $P = 0.001$) and sleep loss ($r = 0.419$, $P = 0.027$).

The plasma levels of the two neuropeptides, BDNF and substance P, were closely correlated ($r = 0.867$, $P < 0.0001$),

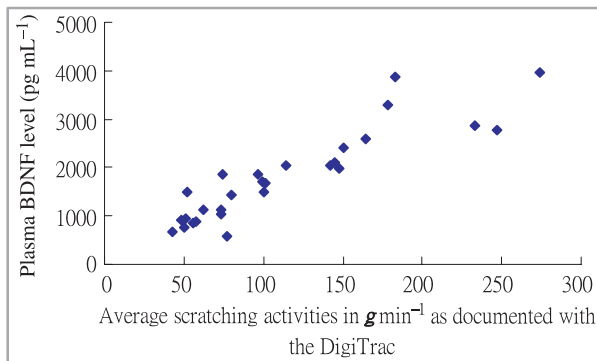


Fig 1. Correlation between scratching and plasma brain-derived neurotrophic factor (BDNF) levels ($r = 0.905$, $P < 0.001$).

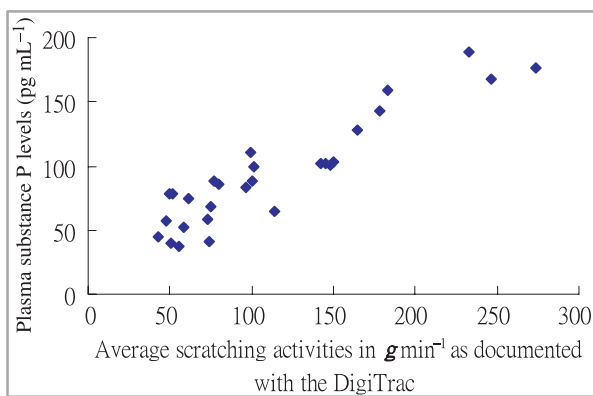


Fig 2. Correlation between scratching and plasma substance P levels ($r = 0.925$, $P < 0.001$).

whereas these two neuropeptides showed a weaker correlation with plasma TARC level ($r = 0.461$, $P = 0.014$ and $r = 0.426$, $P = 0.024$, respectively) and no significant association with

plasma CTACK level, serum total IgE or circulating eosinophil percentage ($P > 0.15$ for all comparisons).

Discussion

Itching and scratching are important clinical parameters for assessing the degree of disability or impairment to quality of life associated with AD, and to gauge therapeutic efficacy of its treatment. As far as we are aware, this is the first report to demonstrate that BDNF and substance P are neuropeptides that are significantly correlated with disease activity, quality of life, as well as the objective DigiTrac-documented sign of scratching. In contrast, the chemokines (CTACK and TARC) and laboratory markers (serum total IgE and eosinophil counts) are less well or even poorly correlated with scratching.

Intradermal injection of substance P elicits the itch sensation in human subjects and an itch-associated response in mice. Andoh *et al.* demonstrated that substance P induces an arachidonate cascade to produce leukotriene B_4 by keratinocytes which in turn trigger the itch-scratch response in mice.¹⁷ This substance P-induced itch-associated response is not inhibited by antihistamine. We confirmed that substance P plays an important role in the scratch response which is independent of the perceived sensation of itch in humans with AD. Our findings also support further trials on leukotriene B_4 receptor antagonist and 5-lipoxygenase inhibitor as novel antipruritic drugs. In addition, BDNF has a similar and possibly more pivotal role in AD pathophysiology in that plasma BDNF levels also significantly correlate with disease severity, extent, intensity and quality of life. Raap *et al.* reported a significant correlation between BDNF and SCORAD score only in patients with the intrinsic type of AD.⁴ As 90% of our patients have the extrinsic type of AD, as evidenced by the elevation of serum IgE levels, we speculate that a significant correlation between BDNF and SCORAD score also exists in the extrinsic type of AD. In recent studies, interleukin (IL)-31 was also suggested to be

Table 1 Pearson correlation coefficients between clinical parameters and laboratory markers in patients with atopic dermatitis ($n = 28$)

Clinical parameters	Laboratory markers					
	BDNF	SP	CTACK	TARC	IgE _{log}	Eos
Total SCORAD index	0.478*	0.329	0.238	0.573†	0.617	0.432*
SCORAD components						
Extent	0.449*	0.343	0.242	0.565†	0.643‡	0.436*
Intensity	0.468*	0.316	0.299	0.591†	0.560†	0.379
Pruritus	0.317	0.160	0.021	0.314	0.576†	0.429*
Sleep loss	0.353	0.259	-0.072	0.283	0.419*	0.364
CDLQI	0.522†	0.441*	-0.081	0.377*	0.323	0.239
Average wrist activity $\bar{A}$	0.905‡	0.925‡	0.210	0.506**	-0.075	0.091
Frequency-specific activity f	0.826‡	0.870‡	0.200	0.396*	-0.126	0.068

BDNF, brain-derived neurotrophic factor; SP, substance P; CTACK, cutaneous T-cell attracting cytokine; TARC, thymus and activation regulated chemokine; IgE_{log}, log-transformed serum total IgE level; Eos, peripheral blood eosinophil percentage; SCORAD, SCORing Atopic Dermatitis index; CDLQI, Children's Dermatology Life Quality Index. Correlation coefficients between SCORAD and CDLQI = 0.599 ($P = 0.001$); average wrist activity and CDLQI = 0.443 ($P = 0.018$); frequency-specific activity and CDLQI = 0.289 ($P = 0.136$).

* $P < 0.05$; ** $P < 0.01$; † $P < 0.005$; ‡ $P < 0.001$.

important in pruritus induction in AD.^{18,19} We did not save any RNA samples from our patients for quantifying IL-31 RNA. It would be interesting to include IL-31 levels by quantitative polymerase chain reaction in future research.

There has been no good evidence to support the use of various antihistamines to relieve itch in AD. Anecdotally, sedating antihistamines have sometimes been useful by virtue of their soporific effect and bedtime use may be warranted. There is also no evidence to support the effectiveness of expensive nonsedating agents.²⁰ The majority of trials on antihistamines are flawed in terms of the sample size or study design.²⁰ Specifically, crude visual analogue scales for pruritus were often used and precise documentation of scratching had not been performed in these trials. We previously found that pruritus and sleep loss (as reported in SCORAD by the parents or patients) were only weakly correlated with the objective SCORAD^{7,8,15} and chemokine markers of eczema severity,^{11,12,21} and concluded that the lack of a good correlation was probably because pruritus and sleep loss were subjective and imprecise symptoms perceived by parents or patients.⁸ Such imprecise perception was problematic and could invalidate the outcomes of clinical trials. Recently, we applied the wrist motion monitor in clinical studies and found its objectivity offers additional advantage to complement clinical scores in AD research.^{22,23}

In conclusion, serum levels of BDNF and substance P correlate with SCORAD index and quality of life scores in patients with AD. The strong correlations with nocturnal wrist movements suggest that they may be the pathogenic factors of the annoying symptoms of scratching. Our findings also suggest that the perceived symptom of itch and the objective sign of scratching should be separately evaluated in clinical trials.

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