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ORIGINAL ARTICLE

Urinary Eosinophil-derived Neurotoxin Concentrations in Patients with Atopic Dermatitis: A Useful Clinical Marker for Disease Activity

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ABSTRACT

Background: It has been reported that measurements of eosinophil-derived neurotoxin (EDN) may be useful for identifying eosinophil activities in allergic diseases including atopic dermatitis.

Methods: EDN concentrations in the urine were measured by enzyme-linked immunosorbent assay, and the number of eosinophils in the peripheral blood was counted in 30 patients with atopic dermatitis. The severity of atopic dermatitis was graded on the criteria proposed by Rajka and Langeland. The disease activity was assessed by each patient on a visual analogue scale (VAS).

Results: Urinary concentrations of EDN in patients with atopic dermatitis showed a significant positive correlation with disease severity. Urine EDN concentrations also correlated with VAS scores for itching, skin condition, overall skin symptoms and total VAS score, but not with the VAS score for skin dryness. Urinary EDN concentrations did not correlate with the number of eosinophils in the peripheral blood.

Conclusions: The urinary EDN concentration in patients with atopic dermatitis is a useful clinical marker for monitoring disease activity.

KEY WORDS

atopic dermatitis, eosinophil, eosinophil-derived neurotoxin, visual analogue scale

INTRODUCTION

Eosinophils are important effector cells in allergic diseases. It is therefore arguable that proper disease monitoring should include an evaluation of the degree of eosinophilic inflammation[1] in addition to symptom recordings and function measurements. One of the most extensively investigated specific markers of eosinophils has been the serum level of eosinophil cationic protein (ECP), but the clinical usefulness of serum ECP levels has been questioned because of problems in sample processing and the great variability of measured values.[2] Eosinophil-derived neurotoxin (EDN), also called eosinophil protein X (EPX), is the basic eosinophil granule protein that can be accurately measured only in urine,[3] and it has been suggested that urinary EDN may be a valid alternative to measurements of eosinophil proteins in serum.[4]

We reported an efficient enzyme-linked immunosorbent assay (ELISA) method for measuring specifically human blood and urinary EDN.[5] In addition, we showed that the method may be useful for identifying the possible roles of eosinophils in ongoing asthma[6] and allergic diseases.[7] We have expanded these studies and, in this paper, demonstrated that the urinary concentration of EDN in patients with atopic dermatitis is a useful clinical marker for monitoring disease activity.

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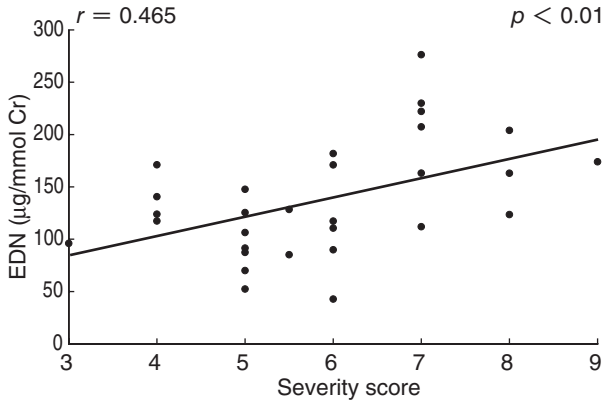

Fig. 1 Significant relationships between urinary EDN concentrations and the disease severity in patients with atopic dermatitis. The correlation between urinary EDN concentrations and severity score was $r = 0.465$, $p < 0.01$.

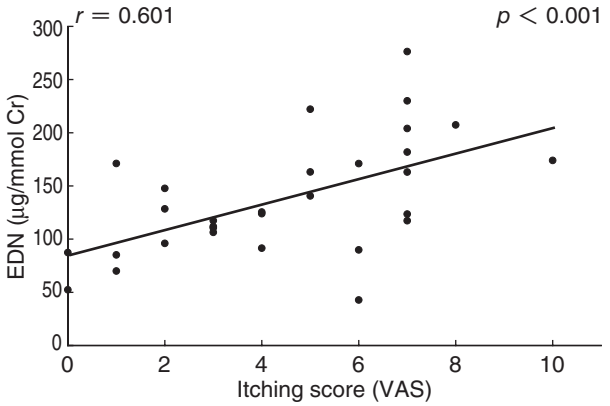

Fig. 2 Significant relationships between urinary EDN concentrations and VAS score for itching in patients with atopic dermatitis. The correlation between urinary EDN concentrations and the VAS score was $r = 0.601$, $p < 0.001$.

METHODS

SUBJECTS

Thirty patients with atopic dermatitis (18 males and 12 females), median age 29.2 years with ages ranging from 16 to 35 years, underwent an examination at Shin-Ohra Hospital, Gunma Institute for Allergy and Asthma, between August and December, 2005. The diagnosis of atopic dermatitis was made according to the clinical and morphological criteria defined by Hanifin and Rajka.[8] The severity of atopic dermatitis was graded according to the criteria proposed by Rajka and Langeland.[9] All subjects were on their first visit to our hospital, and had been treated with topical corticosteroids, anti-allergic agents, antihistamines, and so on. Each patient assessed the grade of nine items (general physical condition, fatigue, mental stress, sleep, excessive eating or drinking, excessive work or study, and the dermatological signs itching, skin dryness and skin condition) on a 10-cm visual analogue scale (VAS). Samples of blood and urine were obtained from the patients.

The Institutional Ethics Committee of Gunma Institute for Allergy and Asthma at Shin-Ohra Hospital approved the study, and written informed consent was obtained from each individual before the study commenced.

URINE SAMPLING PROCEDURES

Because of the reports showing circadian variations in urinary EDN,[10-12] samples of blood and urine were obtained in the morning (9.00 to 11.00). Urine was collected from each individual as a spot sample. After 30 minutes of collection, urine samples were centrifuged at $1350 \times g$ for 10 minutes at 4℃, and then transferred to new plastic tubes. Each sample was aliquoted into a new plastic tube and stored at −20℃ until the assay below.

SANDWICH ELISA FOR EDN

We developed an efficient ELISA method for EDN,[5] which has already been introduced to the market by Medical and Biological Laboratories Co., Ltd., Nagoya, Japan. All assays were performed in duplicate for each sample, and the mean values were reported. The concentrations of urinary creatinine (Cr) were determined with an L-type creatinine-F assay system (Wako Pure Chemical, Osaka, Japan). Urinary EDN concentrations were expressed as μg per millimole of Cr (μg/mmol Cr) to correct them for urine volume.

COUNTING THE NUMBER OF EOSINOPHILS

The eosinophils in the peripheral blood were counted automatically by the Beckman Coulter counter (Beckman Coulter, Fullerton, CA, USA) and the MAXM A/L system (Beckman Coulter).

STATISTICAL ANALYSIS

Correlation coefficients were obtained using the Spearman's rank correlation procedure. Probabilities less than 5% were considered statistically significant.

RESULTS

RELATIONSHIP BETWEEN URINARY EDN CONCENTRATION IN ATOPIC DERMATITIS AND DISEASE SEVERITY

EDN concentrations in urine from patients with atopic dermatitis correlated significantly with the disease severity, as shown in Figure 1 ($r = 0.465$, $p < 0.01$).

RELATIONSHIP BETWEEN URINARY EDN CONCENTRATION IN ATOPIC DERMATITIS AND VAS ASSESSMENTS

As Figures 2, 4, 5 show, urinary EDN concentrations significantly correlated with each VAS score for itching, skin condition and overall skin symptoms, which

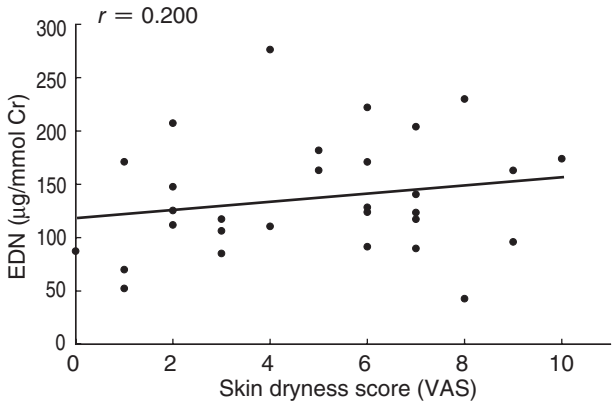

Fig. 3 Relationships between urinary EDN concentrations and VAS score for skin dryness in patients with atopic dermatitis. There was no significant correlation between urinary EDN concentrations and the VAS score ($r = 0.200$).

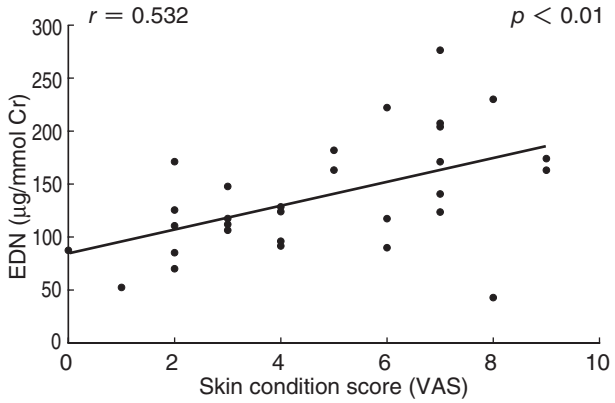

Fig. 4 Significant relationships between urinary EDN concentrations and VAS score for skin condition in patients with atopic dermatitis. The correlation between urinary EDN concentrations and the VAS score was $r = 0.532$, $p < 0.01$.

was the sum of three individual skin aspects (itching, skin dryness, skin condition) ($r = 0.601$, $p < 0.001$, $r = 0.532$, $p < 0.01$ and $r = 0.445$, $p < 0.05$, respectively). However, there was no significant correlation between EDN concentration in urine and VAS score for skin dryness ($r = 0.200$) (Fig. 3). There was a significant correlation between urinary EDN concentrations and the total VAS score, which was the sum of the scores of the nine items ($r = 0.446$, $p < 0.05$) (Fig. 6). However, there was no significant correlation between urinary EDN concentration and individual VAS scores such as those for general physical condition, fatigue, mental stress, sleep, excessive eating or drinking, and excessive work or study.

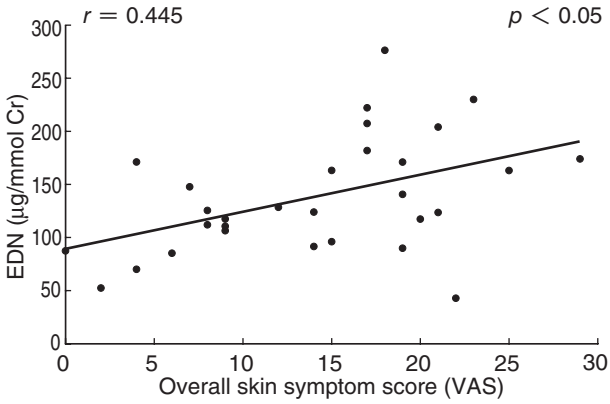

Fig. 5 Significant relationships between urinary EDN concentrations and VAS score for overall skin symptoms, which was the sum of three individual skin aspects (itching, skin dryness, skin condition), in patients with atopic dermatitis. The correlation between urinary EDN concentrations and the VAS score was $r = 0.445$, $p < 0.05$.

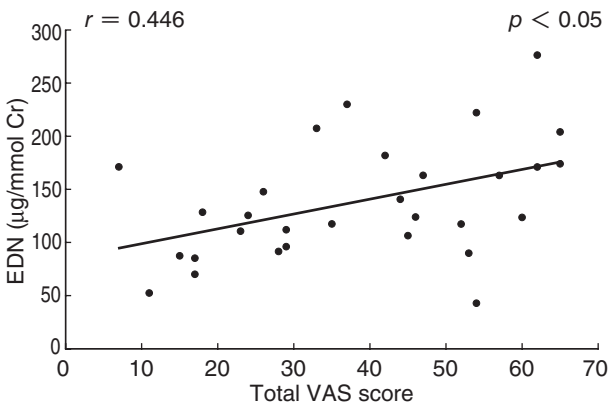

Fig. 6 Significant relationships between urinary EDN concentrations and total VAS score which was the sum of the scores of nine items in patients with atopic dermatitis. The correlation between urinary EDN concentrations and the VAS score was $r = 0.446$, $p < 0.05$.

NUMBER OF EOSINOPHILS IN PERIPHERAL BLOOD FROM PATIENTS WITH ATOPIC DERMATITIS AND URINARY EDN CONCENTRATIONS IN THE PATIENTS

As Figure 7 shows, no significant relationships were observed between urinary concentrations of EDN and the number of eosinophils in peripheral blood from the patients with atopic dermatitis ($r = -0.208$).

DISCUSSION

It has been suggested that measurements of eosinophil-derived proteins in serum, especially assessment of serum ECP, may be used to reflect the inflammatory activities of eosinophils in allergic diseases. However, ECP is a very sticky and extremely

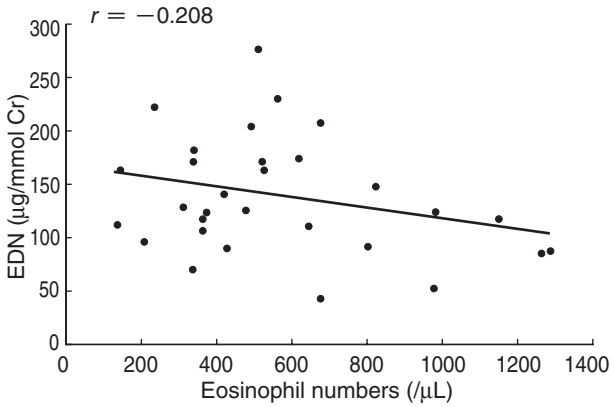

Fig. 7 Relationships between urinary EDN concentrations and the number of peripheral blood eosinophils in patients with atopic dermatitis. There was no significant correlation between urinary EDN concentrations and the number ($r = -0.208$).

charged protein,[13] and the clinical usefulness of ECP has been questioned because of problems in sample processing and the great variability of measured values.[2]

EDN, also called EPX, is the basic eosinophil granule protein which can be accurately measured only in urine,[3] and it has been reported that urinary EDN may be a valid alternative to the measurements of eosinophil proteins in the serum.[4] Recent studies have indicated the usefulness of measuring the concentrations of urinary EPX in monitoring childhood bronchial asthma,[14,15] and atopic dermatitis in children and adults.[16-18] However, we are aware of no report indicating the relationship between urinary EDN concentrations from patients with allergic diseases and the disease severity, or of a linear analogue scale questionnaire for evaluating patient-reported clinical activity.

We reported an efficient ELISA method specifically for measuring human blood and urinary EDN.[5] In addition, we showed that the method may be useful for indicating the possible roles of eosinophils in ongoing asthma[6] and allergic diseases.[7] We have expanded these studies and, in this paper, urinary concentrations of EDN in patients with atopic dermatitis showed a significant positive correlation with the severity of the disease, supporting the previous data that urinary EPX is an *in vitro* parameter of inflammation in atopic dermatitis.[18]

Some scoring systems for evaluating clinical itch have been described in the literature.[19] The European Task Force on Atopic Dermatitis has developed a composite severity index based on a broad consensus by dermatologists. The resulting SCORAD index[20] (scoring of atopic dermatitis) combines objective symptoms (extent, intensity) and subjective criteria (daytime pruritus and sleep loss). It has been extensively tested in trials, although it seems complicated for a routine clinical setting. Sprikkelman *et al*.[21] showed poor agreement between the SCORAD index and other scoring systems in assessing the severity of atopic dermatitis. Darsow *et al*.[22] presented the English version of a multidimensional itch questionnaire (Eppendorf Itch Questionnaire) which was developed in Germany in a cooperative effort between dermatologists and neurophysiologists as a modified analogon to the McGill index. Pucci *et al*.[23] have reported that infants and young children with atopic dermatitis have distinctive intensity items of the SCORAD index that probably depend on a distinctive immunopathogenesis. However, they also reported that the SCORAD index still remains the gold standard for grading the severity of atopic dermatitis in everyday practice and clinical trials.

Recently, simple questionnaires using a linear analogue scale have been developed to measure and quantify disturbances in the health-related quality of life of patients with allergic diseases such as atopic dermatitis[24] and bronchial asthma.[25] It has been demonstrated that a simple VAS score of patient assessment of disease severity showed the highest and most significant correlations with most of the health-related quality of life in patients with atopic dermatitis,[24] and a linear analogue scale questionnaire was useful for assessing health-related quality of life in elderly patients.[26] In this study, we used a 10 cm VAS consisting of nine items with "none" on the left side of the scale and "severe" on the right. As a result, urinary EDN concentrations in patients with atopic dermatitis were found to correlate with VAS scores for itching, skin condition, overall skin symptoms and total VAS score, indicating that measurements of urinary EDN concentrations are useful for monitoring disease activity in atopic dermatitis.

Patients with atopic dermatitis commonly have dry skin, particularly on the extensor surfaces of the extremities, and impairment of the water permeability barrier function in the skin has been suggested.[27] However, it has been shown that atopic dry skin revealed normal barrier structures, and dry skin disorders were not always accompanied by an impairment of the water permeability barrier.[28] In fact, it has been demonstrated that an insufficiency of ceramides in the stratum corneum was an etiologic factor in atopic dry and barrier-disrupted skin,[29] and sphingomyelin metabolism was altered in skin affected by atopic dermatitis.[30] Interestingly, in the present study, urinary EDN concentrations in patients with atopic dermatitis did not correlate with VAS scores for skin dryness, indicating less involvement of eosinophilic inflammation in the formation of atopic dry skin. Further studies are required.

Urinary EDN concentrations in patients with atopic dermatitis did not correlate with the number of peripheral blood eosinophils, and these data support the

findings of our previous report.[7] It has been reported that urinary EPX may reflect disease activity, and measurements of urinary EPX may reflect the inflammatory activity of eosinophils in atopic dermatitis in children and adults.[16-18] Considering our results, counting the eosinophils in peripheral blood may not be useful in assessing the disease activity of atopic dermatitis.

In this study, urine was collected from each patient who came on a first visit to our hospital, and EDN concentrations in urine were measured. However, the preliminary study from our laboratory[7] has already shown that EDN concentrations in the urine from patients with atopic dermatitis were higher when their skin symptoms were active than when they were not. So, the question remains as to whether serial urinary EDN concentrations in patients with atopic dermatitis correlate with VAS scores in parallel with their clinical symptoms such as itching and skin condition. Very recently, Fujisawa *et al*.[31] reported that serum measurement of thymus and activation-regulated chemokine/CCL17 represented accurately the disease activity of atopic dermatitis. Further studies are required to ascertain whether urinary EDN concentration can serve as an adequate clinical marker of atopic dermatitis.

In conclusion, our results in this paper demonstrate that measurement of the urinary EDN concentration is useful for monitoring disease activity in atopic dermatitis.

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