

Plasma α -defensins are elevated during exacerbation of atopic dermatitis

N. N. Tsybikov,¹ I. V. Petrisheva,¹ E. V. Fefelova,¹ B. I. Kuznik² and E. Magen³

¹Pathophysiology Department and ²Normal Physiology Department, Chita Medical Academy, Chita, Russia; and ³Clinical Immunology and Allergy Unit, Medicine B Department, Barzilai Medical Center, Ben Gurion University of the Negev, Ashkelon, Israel

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Summary

Background. There is no currently no information regarding the role of α -defensins in the pathophysiology of atopic dermatitis (AD).

Aim. To investigate levels of plasma α -defensins at exacerbation and remission of AD, and to assess their association with clinical severity of AD.

Methods. Patients with AD during exacerbation were recruited from the Dermatology department at the Chita Medical Academy (Chita, Russian Federation). SCORAD (SCORing Atopic Dermatitis), itch intensity, plasma concentrations of α -defensins, and serum total IgE, interleukin (IL)-1 β , IL-8 and IL-10 levels were measured at study entry and after 4 months.

Results. In total, 82 patients with AD [35 (45.1%) female and 47 (54.9%) male patients, mean $\pm$ SD age 42.2 ± 11.5 years, range 15–40] and 30 healthy controls (HCs) were included. Mean objective SCORAD score was 48.8 ± 19.4 and 16.1 ± 8.3 during AD exacerbation and remission, respectively ($P < 0.01$). Plasma α -defensin levels in patients with AD (1.41 ± 0.32 μ g/L) were significantly higher than in HCs (0.91 ± 0.34 μ g/L) ($P < 0.001$). Significant correlations were found between plasma α -defensin levels with objective SCORAD ($r = 0.55$, $P < 0.001$), itch severity ($r = 0.25$, $P < 0.05$) and serum IgE levels ($r = 0.38$, $P < 0.001$) during AD exacerbation. Serum concentrations of IL-1 β , IL-4 and IL-8 were significantly higher and levels of IL-10 were lower at AD exacerbation in patients than in HCs, with a weak negative correlation between plasma α -defensin and serum IL-10 levels ($r = -0.22$; $P < 0.05$).

Conclusion. During AD exacerbation, plasma α -defensin levels are elevated, and are positively correlated with AD clinical severity, itch intensity and serum IgE levels.

Introduction

Atopic dermatitis (AD) is a chronic or chronically relapsing, pruritic inflammatory skin disease.¹ Recent evidence also suggests the involvement of innate immunity in the pathogenesis of AD.² Defensins are

small, arginine-rich, cationic proteins, 3–5 kDa in size, which can be divided into α -, β - and θ -defensin families, depending on the position of the cysteine residues that participate in disulphide linkages.³ Mammalian defensins are significant players in the innate immune system, and exhibit antimicrobial activity against enveloped viruses, bacteria and fungi. α -Defensins include human neutrophil peptide (HNP) 1–4, expressed mainly by human neutrophils, and intestinal human defensin (HD)-5 and HD6 produced by Paneth cells.⁴ In addition to their antimicrobial effects, α -defensins display chemotactic activity and induce proinflammatory cytokines, participating in

Correspondence: Dr Eli Magen, MD, Clinical Immunology and Allergy Unit, Medicine B Department, Ben Gurion University of Negev, Zionut 21/33, Ashdod 77456, Israel
E-mail: allergologycom@gmail.com

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the regulation of immunological processes in allergic diseases.⁵

To date, most research has focused on the role of β -defensins in AD, which have been shown to be either constitutively expressed or upregulated in AD,⁶ and have a significant impact on allergic skin inflammation.⁷ Little is known about the involvement of α -defensins in this disease.

In this observational study, we investigated plasma α -defensin levels during exacerbation and remission of AD, and compared them with clinical data reflecting the disease activity.

Methods

The study was approved by the ethics committee at the Chita Medical Academy, (Chita, Russia) and all participants gave informed consent.

Study participants

The subjects were recruited during 2011–2014 from the Dermatology Dispensary (Chita Medical Academy, Chita, Russian federation). Patients with AD who were aged between 15 and 40 years were enrolled in this study. In total, 82 patients with AD [35 (45.1%) male and 47 (54.9%) female patients, mean $\pm$ SD age 42.2 ± 11.5 years] were enrolled, along with a group of 30 healthy controls (HCs), matched for age and sex. The clinical characteristics of the participants are presented in Table 1.

AD was diagnosed by the criteria of Hanifin and Rajika, with a duration of ≥ 1 year and by the SCORing Atopic Dermatitis (SCORAD) index. The objective SCORAD has a range of 0–83 (plus an additional 10 points for severe disfiguring eczema on the face and hands). Objective SCORAD of < 15 was classified as mild, 15–40 as moderate and > 40 as severe disease. Subjective itch intensity was measured on a visual analogue scale (VAS), ranging from 0 to 10, by the same dermatologist.

Exclusion criteria were: pregnancy and lactation, presence of any other chronic disease or acute infection, oral intake of any medications or use of ultraviolet therapy conducted < 2 months before study entry. Before enrolment, each patient was evaluated clinically to exclude clinical signs (appearance of widespread crusting and oozing patches, pustular eruptions, impetigo, erysipelas or cellulitis) of skin infection.

There were two main reasons for choosing AD subjects aged 15–40 years old: (i) in our region, the prevalence of asthma in children with moderate to

Table 1 Demographic and laboratory data of the study population.

	Patients with AD (n = 82)	HCs (n = 30)	P
Age, years	24.5 $\pm$ 7.9	24.9 $\pm$ 6.2	0.80
Female sex, n (%)	37 (45.1)	14 (46.7)	1
Mean duration of AD, years	17.1 $\pm$ 11.2	0	< 0.001
Body mass index, kg/m ²	25.2 $\pm$ 2.2	25.4 $\pm$ 1.9	0.68
Objective SCORAD			
Exacerbation (baseline)	48.8 $\pm$ 19.4	0	< 0.001
4 months post-exacerbation	16.1 $\pm$ 8.3*	0	< 0.001
Serum IgE, kU/L†			
Exacerbation (baseline)	355.6 $\pm$ 331.2	49.2 $\pm$ 27.9	< 0.001
4 months post-exacerbation	281.5 $\pm$ 194.7	45.7 $\pm$ 32.1	< 0.001
Neutrophils, cells/mm ³			
Exacerbation (baseline)	8.4 $\pm$ 2.7	8.1 $\pm$ 2.9	0.61
4 months post-exacerbation	8.2 $\pm$ 3.1	8.3 $\pm$ 2.5	0.87

AD, atopic dermatitis; HCs, healthy controls; SCORAD, SCORing Atopic Dermatitis. Data are mean $\pm$ sd unless otherwise stated. * $P < 0.001$ (exacerbation vs. 4 months post-exacerbation); †normal range 2–87 kU/L.

severe AD ranges from 25% to 50%, so all our patients had AD without any other chronic disease; and (ii) children with AD have increased rates of infections, especially during the cold seasons in Siberia, therefore it seemed likely that it would be easier to find subjects with AD without comorbidities in those aged 15–40 years, to eliminate the potential influence of such comorbidities on laboratory investigations. A group of age- and sex-matched HCs formed the control group.

The patients with AD were enrolled during exacerbation, manifested by flares of eczema at ≥ 3 sites, or generalized skin lesions. Every recruited patient was assessed at two time points: during the exacerbation (study entry) and at 4 months post-exacerbation, after using emollients, antihistamines and topical steroids. None of the patients used oral corticosteroids and/or immunomodulatory medications during the study.

Laboratory analysis

Blood samples were collected from the cubital vein into tubes using sterile citrate plasma tubes. Plasma was obtained by centrifugation at 3000 **g** for 15 min within 30 min of blood collection, and then stored at

–70 °C until required for analysis. Sampling was performed at the same time of day for each patient.

Plasma concentrations of α -defensins (HNP1–3) were assessed according to the manufacturer's protocol (Hycult Biotechnology, Uden, the Netherlands). The measurable concentration range of α -defensins was 0.156–10 $\mu\text{g/L}$.

Serum IL-1 β , IL-4, IL-8 and IL-10 were measured using ELISA according to the manufacturer's instructions (VECTOR-BEST, Novosibirsk, Russia).

Serum samples were analysed for total IgE levels using an immunometric assay (Immulite 2000; Diagnostic Products Corporation, Global Siemens Healthcare, Erlangen, Germany).

Statistical analyses

Statistical tests were performed using Statistica software (v6; StatSoft, Inc. Tulsa, OK, USA). Continuous variables were presented as means $\pm$ SD and compared by independent samples *t*-test. Variables without normal distribution were compared by the Mann–Whitney *U*-test. Fisher exact test was used to compare the categorical variable (sex) between the patients with AD and the HCs. Correlations between variables were analysed by Pearson correlation and Spearman rank correlation, respectively, depending on the distribution of the data. Statistical significance was defined as a two-sided *P*-value of < 0.05 .

Results

The study was approved by the ethics committee at the Chita Medical Academy, (Chita, Russia) and all participants gave written informed consent.

Severity of atopic dermatitis

Mean objective SCORAD score was 48.8 ± 19.4 and 16.1 ± 8.3 during exacerbation and remission of AD, respectively ($P < 0.01$), while mean itch score was 6.9 ± 1.9 and 2.6 ± 0.7 during exacerbation and remission, respectively ($P < 0.01$).

Levels of α -defensins

Plasma levels of α -defensins in patients affected by AD ($1.41 \pm 0.32 \mu\text{g/L}$) were significantly higher than those of HCs ($0.91 \pm 0.34 \mu\text{g/L}$, $P < 0.001$) (Fig. 1). No significant age- or sex-dependent influence on plasma α -defensin levels was observed in either the patient or the HC group.

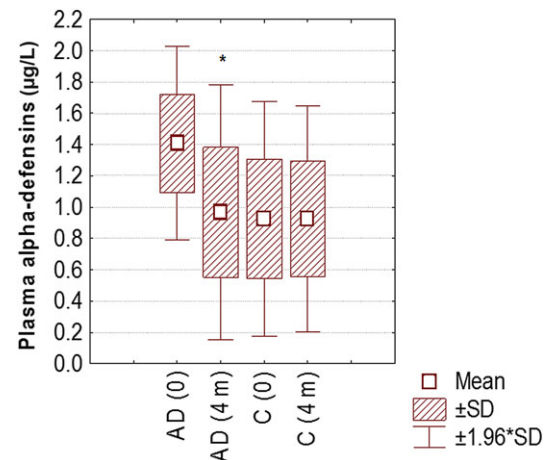


Figure 1 Plasma concentrations of α -defensins in (a,b) patients with atopic dermatitis at (a) exacerbation and (b) 4 months postexacerbation, and in (c,d) healthy controls at (a) baseline and (b) 4 months later. * $P < 0.001$.

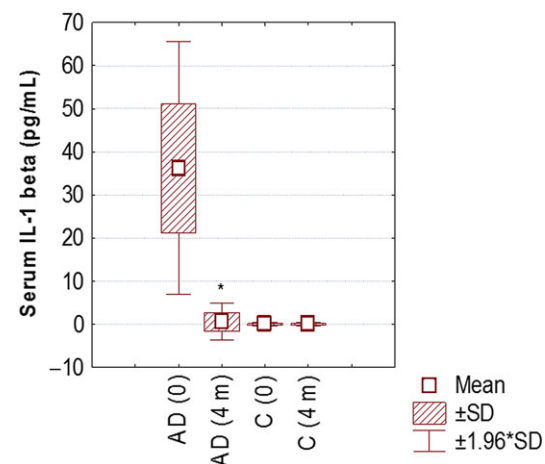


Figure 2 Serum concentrations of interleukin (IL)-1 β in (a,b) patients with atopic dermatitis at (a) exacerbation and (b) 4 months postexacerbation, and in (c,d) healthy controls at (a) baseline and (b) 4 months later. * $P < 0.001$.

Serum concentrations of IL-1 β ($36.3 \pm 14.8 \text{ pg/mL}$) (Fig. 2), IL-4 ($7.2 \pm 3.5 \text{ pg/mL}$) (Fig. 3) and IL-8 ($229.6 \pm 169.1 \text{ pg/mL}$) (Fig. 4) in patients with AD were significantly higher at exacerbation than at 4 months postexacerbation (IL-1 β 0 pg/mL , $P < 0.001$; IL-4 $0.2 \pm 0.4 \text{ pg/mL}$, $P < 0.001$; and IL-8 $13.7 \pm 5.1 \text{ pg/mL}$, $P < 0.001$). Serum concentrations of IL-10 in AD at exacerbation ($0.6 \pm 0.3 \text{ pg/mL}$) were significantly lower than at 4 months postexacerbation ($1.4 \pm 0.9 \text{ pg/mL}$) ($P < 0.001$) (Fig. 5).

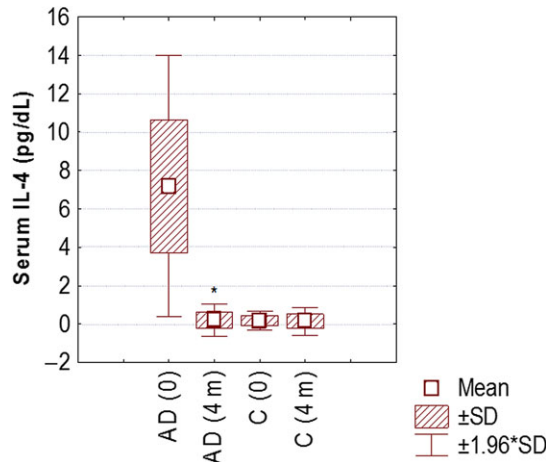


Figure 3 Serum concentrations of interleukin (IL)-4 in (a,b) patients with atopic dermatitis at (a) exacerbation and (b) 4 months postexacerbation, and in (c,d) healthy controls at (a) baseline and (b) 4 months later. * $P < 0.001$.

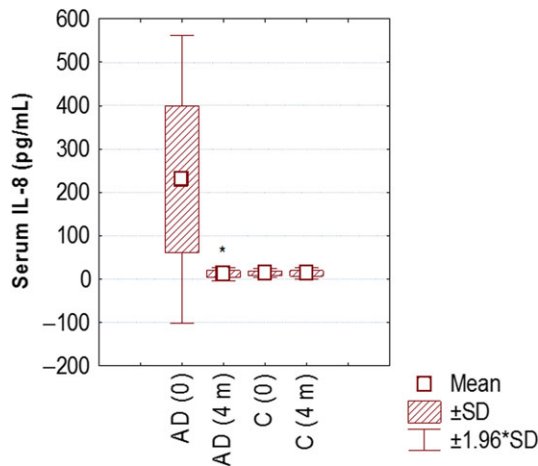


Figure 4 Serum concentrations of interleukin (IL)-8 in (a,b) patients with atopic dermatitis at (a) exacerbation and (b) 4 months postexacerbation, and in (c,d) healthy controls at (a) baseline and (b) 4 months later. * $P < 0.001$.

Correlation between α -defensins and other parameters

Correlation analysis between blood levels of plasma α -defensins, objective SCORAD, itch severity, and serum IL-1 β , IL-8, IL-10 and IgE during AD exacerbation is presented in Table 2. Significant correlations were found between plasma α -defensin levels and objective SCORAD ($r = 0.54$, $P < 0.001$) (Fig. 5), Itch severity ($r = 0.25$, $P < 0.05$), serum IgE ($r = 0.379$, $P < 0.001$), serum IL-8 ($r = 0.48$, $P < 0.001$) and serum IL-10 ($r = -0.22$, $P < 0.05$) levels at the exacerbation time point in patients with AD. No signifi-

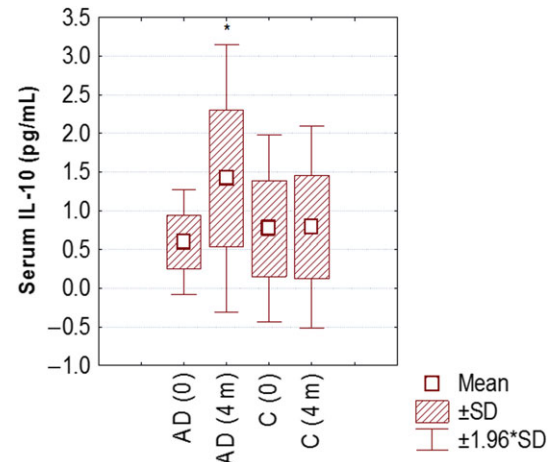


Figure 5 Serum concentrations of interleukin (IL)-10 in (a,b) patients with atopic dermatitis at (a) exacerbation and (b) 4 months postexacerbation, and in (c,d) healthy controls at (a) baseline and (b) 4 months later. * $P < 0.001$.

Table 2 Correlation analysis between blood levels of α -defensins, objective SCORAD, interleukins and IgE during AD exacerbation.

Parameter	α -defensins, $\mu\text{g/mL}$	
	r	P
Objective SCORAD	0.55	< 0.001
Itch severity	0.25	< 0.03
IL-1 β , pg/mL	0.23	0.84
IL-4, pg/mL	0.21	0.06
IL-8, pg/mL	0.48	< 0.001
IL-10, pg/mL	-0.22	< 0.05
IgE, kU/L	0.38	< 0.001

AD, atopic dermatitis; IL, interleukin; SCORAD, SCORing Atopic Dermatitis.

cant correlations between these parameters were observed at the remission time point in patients with AD or in the control group.

There were no significant correlations between the number and percentage of blood neutrophils and clinical or laboratory parameters (objective SCORAD, itch intensity, plasma α -defensins, and serum IL-1 β , IL-4, IL-8, IL-10 and IgE levels).

Discussion

In the study, we determined plasma α -defensin levels in patients with AD and healthy controls. The study clearly demonstrates that during exacerbation of AD, plasma α -defensin levels are elevated, then they return to normal levels during the 4-month post-exacerbation

period. In addition, during exacerbation, plasma levels of α -defensins are positively correlated with AD clinical severity and serum IL-8 and IgE levels, and negatively correlated with serum IL-10 levels.

All our patients with AD exhibited moderate to severe disease as measured by objective SCORAD (mean 48.8 ± 19.4). Such patients are characterized by severely reduced skin levels of antimicrobial molecules, deficient production of inducible nitric oxide synthase, and lack of an adequate inducible innate immune response.² A remarkable finding in lesional biopsies from patients with AD is the absence of neutrophils, even in the setting of intense scratching or *Staphylococcus aureus* colonization and/or infection.⁸ Several studies have pointed to a chemotactic defect of neutrophils in AD, and such defects were found to correlate with serum IgE levels.⁹ Numerous additional functional alterations were observed in the neutrophils of patients with AD.¹⁰ The absence of neutrophils in AD skin was also proposed to be due to reduced production of the chemoattractant cathelicidin (LL-37), or to reduced expression of IL-8.¹¹

Recently, Choy *et al.*¹² observed a correlation between the number of neutrophils and the presence of crusts on AD lesions. Presence of crusts and excoriations is a characteristic and diagnostic feature of AD, and they are probably associated with scratching due to severe itching.¹³ Additionally, a raised blood level of neutrophils was reported to be correlated with AD severity as assessed by SCORAD.¹⁴

In the current study, we found that AD exacerbations were characterized by high levels of plasma α -defensins. The concentration of α -defensins correlates with the number of neutrophils in the blood of patients with inflammatory diseases,¹⁵ but we did not observe any difference in blood neutrophil count between patients with AD and HCs, and no difference in this parameter was detected during exacerbation or remission of AD. Unlike in the IgE-mediated late phase reaction, neutrophils do not appear to play a role in established AD lesions,¹⁶ and it is unclear whether α -defensins contribute to the skin inflammatory processes in AD. Nevertheless, it is well recognized that patients with AD are more likely to develop skin infections. α -Defensins display microbicidal activity against a wide spectrum of bacteria, fungi and viruses.³ They also link the innate and adaptive immune systems, depending on their concentration range;¹⁷ at high doses they downregulate CD86 and human leucocyte antigen (HLA)-DR expression and tumour necrosis factor- α , IL-1 β and IL-10 secretion, while strongly upregulating IL-8.¹⁸ Our finding of a positive correla-

tion between α -defensins and IL-8 levels in patients with AD fits well with previous reports of the association between α -defensins and IL-8 in various types of inflammatory diseases.¹⁹ Initially, α -defensins were identified as T-cell chemoattractants released by IL-8-induced neutrophil degranulation, and IL-8 is a potent neutrophil attractant that can induce the release of α -defensins from neutrophils.²⁰ Although neutrophils produce the highest levels of α -defensins, these molecules can also be found in natural killer cells, B cells, $\gamma\delta$ T cells, monocytes/macrophages and immature dendritic cells.⁴ Although IL-8 and LL-37 are the main regulators of α -defensin release from neutrophils,²¹ α -defensins can also be released by chemokines, via FC γ receptor cross-linking, or by activation of Toll-like receptors.⁴ In addition, many cells can absorb and internalize α -defensins intracellularly, and if neutrophil degranulation does not occur in AD,¹⁶ this finding emphasizes the complexity of defining true α -defensin-producing cells in this disease.

In the study we observed increased total IgE levels and a positive correlation of these with plasma α -defensins in AD. Human neutrophils can be activated by IgE through Fc ϵ RI and galectin-3 receptors, and IL-8 is produced by IgE-activated neutrophils in atopic patients via stimulation of the Ca²⁺-calmodulin-calcineurin axis and of nuclear factor κ B.²² Neutrophils from patients with AD have elevated leucotriene A4 hydrolase activity and release leucotriene (LT)B4 (in response to IgE).²³ LTB4 can in turn trigger the release of α -defensins and IL-8 from activated neutrophils²⁴ and enhance IL-4-induced IgE production.²⁵

We observed significantly higher serum IL-1 β and IL-4 and lower IL-10 levels in AD, which, along with their relationship with AD severity, is in agreement with previous studies that evaluated cytokine profiles in AD. The observed weak, but statistically significant negative correlation between IL-10 and α -defensins at AD exacerbation might be explained by possible downregulation of IL-10 mRNA expression by α -defensins.²⁶

Our finding that plasma concentrations of α -defensins reflect the clinical course in AD suggests that plasma α -defensins might be a useful marker of disease activity.

Several limitations of our study warrant consideration. First, plasma levels of α -defensins may not accurately reflect the pathophysiological situation at the site of skin inflammation. Second, although we observed a significant association between plasma α -defensin levels and clinical findings, their biology is unclear at this stage, and the reasons for the

apparent elevation in α -defensins and their contribution to the pathophysiology of AD need further investigation.

Conclusion

We found that during AD exacerbation, plasma α -defensin levels are elevated, and are positively correlated with AD clinical severity, itch intensity and serum IgE levels. The results of our study demonstrate that, during exacerbation of AD, plasma α -defensin levels are elevated, and are positively correlated with AD clinical severity, serum IL-8 and IgE levels. Our findings suggest that plasma α -defensins might be a useful marker of disease activity. Further studies that include skin biopsy for investigation of α -defensins' contribution to the pathophysiology of AD are warranted.

What's already known about this topic?

- β -defensins have been shown to be either constitutively expressed or upregulated in AD and have a significant impact on allergic skin inflammation.
- Little is known about the involvement of α -defensins in AD.

What does this study add?

- We found that levels of α -defensins in plasma are elevated in AD.
- Our finding that concentrations of α -defensins reflect the clinical course of AD suggests that they might be a useful marker of disease activity.

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