

SHORT REPORT

Circulating LL-37 is a biomarker for eczema severity in children

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Abstract

Background Eczema lesions are characterized by impaired expression of antimicrobial peptides such as cathelicidin, which play crucial roles in the innate immune defence against cutaneous infections. LL-37 corresponds to amino acids 134–170 of human cathelicidin and is a multifunctional host defence molecule essential for normal immune responses to infection and tissue injury.

Objectives The aim of this study was to investigate the relationship between childhood eczema and circulating LL-37 levels.

Methods One hundred and forty-four eczema children and 36 controls were recruited. Eczema severity was assessed by SCORing Atopic Dermatitis (SCORAD) and serum LL-37 concentration measured using enzyme immunoassay. Patients' skin hydration and transepidermal water loss at forearms were measured using Corneometer and Tewameter.

Results Patients' mean SCORAD was 49.2 and their disease was classified as mild ($n = 28$; 12.8%), moderate ($n = 95$; 43.6%) and severe ($n = 95$; 43.6%). Serum LL-37 concentrations did not differ between eczema patients and controls (mean: 832 pg/mL vs. 952 pg/mL, $P = 0.471$). However, serum LL-37 concentrations increased with increasing eczema severity among the patients ($P = 0.005$ for trend). This biomarker shows weakly positive correlation with patients' objective SCORAD ($r = 0.207$, $P = 0.013$) and age ($r = 0.170$, $P = 0.041$), but not skin hydration or transepidermal water loss ($P > 0.09$). Linear regression confirmed significant association between objective SCORAD and serum LL-37 when adjusted for age and gender as covariates ($\beta = 0.171$, $P = 0.038$). On the other hand, serum LL-37 did not differ between patients with and without heavy growth of staphylococci ($P = 0.151$).

Conclusions Circulating LL-37 may be a biomarker for severity of childhood eczema, which supports the importance of innate immunity in eczema pathogenesis.

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Conflict of interest

None declared.

Funding source

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Introduction

Eczema is a chronic inflammatory skin disease associated with cutaneous hyperreactivity to environmental stimuli that are otherwise tolerant among normal subjects.¹ Apart from adaptive immunity that involved type-2 T-helper lymphocyte (Th2)-derived cytokines, dysregulation of the innate immune system plays indispensable roles in eczema pathogenesis.² The innate system quickly mobilizes an inflexible, standardized first-line response against different pathogens. Acid mammalian chitinase, being an integral component of the innate immune system, degraded chitins found

ubiquitously in exoskeleton of many microbes.³ The gene encoding this chitinase (*CHIA*) was associated with asthma,⁴ eczema and *Staphylococcus aureus* carriage.⁵ Antimicrobial peptides (AMPs) confer another facet of cutaneous innate immunity. Cathelicidin is expressed in leucocytes and epithelial cells. LL-37 corresponds to amino acids 134–170 of human cathelicidin (hCAP-18/LL-37) and is a multifunctional AMP essential for normal immune responses to infection and tissue injury. A deficiency of LL-37 increased risk of eczema herpeticum.⁶ Nonetheless, all studies on eczema and cathelicidin analysed the histological expression of this AMP in skin lesions. It is unknown whether circulating

LL-37 is associated with eczema diagnosis or severity. We hypothesized that the histopathological alteration of cathelicidin expression in eczema lesions was associated with changes in circulating LL-37 levels. In this study, we investigated the clinical usefulness of serum LL-37 measurement in childhood eczema assessment.

Methods

Subjects

Children with eczema diagnosed according to widely accepted criteria⁷ were recruited from our dermatology clinic. Eczema severity was assessed by SCORing Atopic Dermatitis (SCORAD). Patients were classified into mild, moderate and severe eczema by objective SCORAD.⁸ They underwent skin prick tests (SPTs) as described below. Skin swabs were collected from patients' most severely affected areas for bacterial cultures. Controls consisted of secondary schoolchildren without eczema, asthma and rhinitis who joined our community study on childhood obesity.⁹ They were negative for specific IgE to house dust mite, cat and cockroach, and free from any chronic illness or regular drug treatment. All subjects were free from respiratory tract infection for ≥ 4 weeks before study. The Clinical Research Ethics Committee of our University approved this study. Subjects' parents gave written consent prior to study.

Allergen skin prick testing

Eczema patients underwent SPTs using an allergen panel of locally prevalent inhalants and foods including dust mites, cockroach, cat, dog, alternaria, *Aspergillus fumigatus*, Bermuda grass, egg white, egg yolk, cow's milk, peanut, salmon, beef, shrimp, crab and lobster (ALK Abelló, Round Rock, TX, USA).¹⁰ Positive (histamine 10 mg/mL) and negative (diluent) controls were also performed. Atopy was defined as having at least one SPT with ≥ 3 mm weal.

Assessment of skin hydration and transepidermal water loss

Eczema patients sat comfortably in an air-conditioned room for 20–30 min. After acclimatization, skin hydration (SH) and transepidermal water loss (TEWL) were measured in duplicate at right antecubital fossa, 2 cm distal to right antecubital flexure and right mid-forearm using Corneometer[®] CM 825 and Tewameter[®] TM 210 (Courage + Khazaka electronic GmbH, Cologne, Germany).¹¹

Serum LL-37 measurement

Serum LL-37 concentration was measured using enzyme immunoassay (Bachem, San Carlos, CA, USA). Samples were diluted 90-fold prior to measurement. The sensitivity of this assay was 1 ng/mL.

Statistical analysis

Patient characteristics were compared using Student's *t*-test or ANOVA for numerical data and chi-square or Fisher's exact test for

categorical variables. Serum LL-37 concentrations were logarithm-transformed before analysis. The correlations between LL-37 and objective SCORAD and other patient factors were analysed using Pearson coefficient and confirmed using linear regression. All comparisons were made two-tailed using SPSS v.17 (SPSS Inc., Chicago, IL, USA), with level of significance set at 5%.

Results

Subjects

One hundred and forty-four eczema children and 36 controls were recruited. Table 1 summarizes clinical and laboratory characteristics of these subjects. Patients' eczema was classified as mild ($n = 28$; 12.8%), moderate ($n = 95$; 43.6%) and severe ($n = 95$; 43.6%). Coexisting allergic rhinitis and asthma were present in 97 (67.4%) and 53 (36.8%) patients respectively. One hundred and three eczema patients underwent SPTs and those with and without SPT were matched for age, gender and eczema severity ($P > 0.1$). Ninety six (93.2%) of them were atopic (Table 2).

Eczema and serum LL-37 concentrations

The mean (SD) serum LL-37 concentrations in pg/mL of patients and controls were 832 (1117) and 952 (1353) respectively ($P = 0.471$). Among children with eczema, however, serum LL-37 concentrations increased with increasing eczema severity (Fig. 1). This marker correlated positively with patients' total ($r = 0.181$, $P = 0.030$) and objective ($r = 0.207$, $P = 0.013$) SCORAD scores. Serum LL-37 showed weak correlation with age of patients ($r = 0.170$, $P = 0.041$) but not controls ($r = 0.134$, $P = 0.437$). Atopic patients had higher serum LL-37 concentrations than non-atopic patients (mean [SD] $\log_{10}$ LL-37: 2.64 [0.52] vs. 2.16 [0.90], $P = 0.027$). Linear regression confirmed association between objective SCORAD and serum LL-37 in patients when adjusted for age and gender as covariates ($\beta = 0.171$, $P = 0.038$). Skin swabs were obtained from 117 patients and 30 (25.6%) of them had heavy growth of staphylococci. The mean (SD) $\log_{10}$ LL-37 levels in these patients and others with none, scanty and moderate growth of staphylococci were 2.75 (0.49) and 2.59 (0.55) respectively ($P = 0.151$).

LL-37, skin hydration and transepidermal water loss

Table 3 summarizes the correlations of SCORAD and serum LL-37 with patients' SH and TEWL. Objective SCORAD correlated inversely with SH and positively with TEWL ($P \leq 0.001$), which were confirmed using linear regression ($\beta = -0.349$ and 0.396 respectively, $P < 0.001$ for both). On the other hand, serum LL-37 was not correlated with SH or TEWL.

Discussion

In this case-control study, we show for the first time that circulating LL-37 is a biomarker for eczema severity but not susceptibility. Serum LL-37 concentrations were higher in patients with

Table 1 Clinical and laboratory characteristics of our subjects

Characteristic	Eczema (n = 144)	Controls (n = 36)
Age, year	10.8 ± 4.5*	15.0 ± 1.6
Male, n (%)	82 (56.9)	23 (63.9)
Log-transformed serum total IgE level	3.28 ± 0.76*	1.65 ± 0.34
Circulating eosinophil count, 10 ⁹ /L	0.79 ± 0.55	NA
Circulating eosinophil percentage, %	9.7 ± 5.4	NA
Total SCORAD	49.2 ± 19.3	NA
Objective SCORAD	37.7 ± 16.9	NA
Skin hydration, arbitrary unit†		
Right antecubital fossa	28.4 ± 14.4	NA
2-cm distal to right antecubital flexure	25.8 ± 12.1	NA
Right mid-forearm	25.5 ± 11.3	NA
Transepidermal water loss, g/m ² /h†		
Right antecubital fossa	13.4 ± 4.9	NA
2-cm distal to right antecubital flexure	9.9 ± 4.0	NA
Right mid-forearm	8.5 ± 3.7	NA

* $P < 0.001$ between patients and controls. Results expressed in mean ± SD unless stated otherwise.

†Measured in 111 eczema patients.

NA, not applicable or available.

Table 2 Pattern of allergen sensitization in 103 eczema patients

Allergen	Number (%) positive
Inhalant allergens	
<i>Dermatophagoides pteronyssinus</i>	93 (90.3)
<i>Dermatophagoides farinae</i>	94 (91.3)
Cat dander	35 (34.0)
Dog dander	16 (15.5)
Cockroach	43 (41.7)
<i>Alternaria</i> †	23 (24.2)
<i>Aspergillus fumigatus</i>	15 (14.6)
Bermuda grass	15 (14.6)
Food allergens	
Shrimp†	35 (36.8)
Lobster†	35 (36.8)
Crab†	30 (31.6)
Peanut	34 (33.0)
Egg white	26 (25.2)
Egg yolk	26 (25.2)
Salmon	17 (16.5)
Cow's milk	10 (9.7)
Beef	10 (9.7)

†Only performed in 95 patients.

moderate-severe eczema when compared with mild disease. This biomarker also correlated weakly and positively with objective SCORAD. However, serum LL-37 was not associated with staphylococcal growth on patients' skin. These findings suggest circulating LL-37 to be associated with severity of childhood eczema.

Epidermal expression of psoriasin that preferentially kills *Escherichia coli* was increased in eczema lesions and further induced by

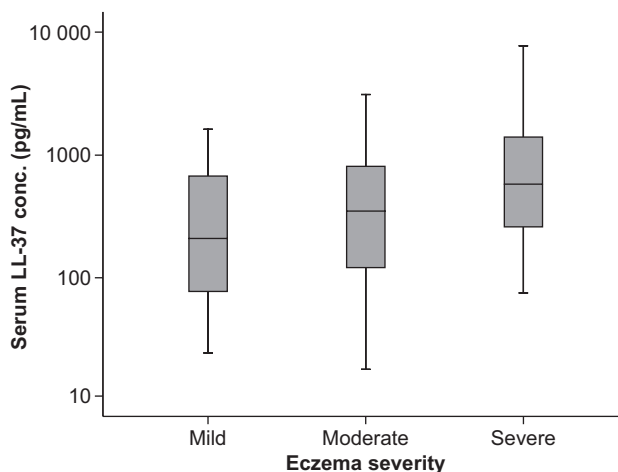


Figure 1 Distribution of serum LL-37 concentrations in patients with different degree of eczema severity (y-axis in logarithmic scale; $P = 0.005$ for trend).

skin barrier disruption,¹² which explained why eczema patients do not have skin infections with this bacterium. Antimicrobial response in eczema would thus depend on the type of AMPs produced by the skin. Gambichler *et al.* also reported enhanced lesional expression of AMPs such as beta-defensins and psoriasin.¹³ In contrary, LL-37 expression was lower in eczema. Together with similar circulating LL-37 between our patients and controls, these findings questions whether serum LL-37 in eczema patients is derived from their skin lesions and whether LL-37 production is decreased in eczema despite epidermal barrier defects and cutaneous inflammation.

Table 3 Correlation between objective SCORAD, serum LL-37 and clinical and laboratory parameters in 144 eczema patients

Characteristic	Serum LL-37†		Objective SCORAD	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Serum total IgE†	0.086	0.305	0.514	<0.001
Circulating eosinophil count	−0.052	0.539	0.322	<0.001
Circulating eosinophil percentage	0.008	0.926	0.270	0.001
Skin hydration				
Right antecubital fossa	−0.081	0.397	−0.313	0.001
2-cm distal to right antecubital flexure	−0.111	0.244	−0.349	<0.001
Right mid-forearm	−0.158	0.097	−0.343	<0.001
Transepidermal water loss				
Right antecubital fossa	0.115	0.231	0.310	0.001
2-cm distal to right antecubital flexure	0.084	0.381	0.391	<0.001
Right mid-forearm	−0.032	0.738	0.356	<0.001

†Log-transformed before analysis.

Defective epidermal barrier also increases protein allergen penetration through the epidermis and predisposes to secondary skin infections. Type 1 interferon-producing plasmacytoid dendritic cells were decreased in eczema skin, which predisposed patients to viral skin infections.¹⁴ Eczema is associated with eczema vaccinatum, a disseminated viral skin infection that follows inoculation with vaccinia virus. Howell *et al.* showed that skin from eczema patients exhibited increased vaccinia virus replication and decreased cathelicidin expression compared with normal or psoriasis skin. Besides, allergy-driving cytokines IL-4 and IL-13 enhanced replication of this virus and suppressed cathelicidins in keratinocytes. Cathelicidin-deficient mice exhibited reduced ability to control vaccinia virus replication in the skin.¹⁵ Despite these observations, there has not been any study of circulating cathelicidin in different types of skin allergy.

Cathelicidin is an effector of innate immunity and is expressed in epithelial cells and many circulating leucocytes such as monocytes and neutrophils. LL-37 corresponds to amino acids 134–170 of human cathelicidin (hCAP-18/LL-37) and is released extracellularly from cathelicidin by proteolytic processing. It has broad-spectrum antimicrobial activity, being potent against bacterial wall molecules such as lipopolysaccharide. LL-37 participates at the interface of innate and adaptive immunity by possessing chemo-attractant function, inhibiting neutrophil apoptosis, stimulating angiogenesis, tissue regeneration and cytokine release and directing macrophage differentiation to a more proinflammatory state.^{16,17} Deficiency of LL-37 and β -defensins predisposed eczema patients to develop eczema herpeticum.^{6,18} Our results support that circulating LL-37 is useful in assessing eczema severity.

This study may be underpowered to detect some outcomes. Given our sample size, this study had 10% power to detect a 120 pg/mL difference in mean LL-37 between cases and controls with 95% confidence (STATMATE v.2.00, GraphPad, La Jolla, CA, USA). Therefore, we are not confident to comment whether circulating LL-37 is associated with eczema susceptibility. Larger studies

should be performed to address this issue. Besides, our controls from local secondary schools were older than eczema patients (see Table 1). Because most eczema patients manifest their disease in early childhood,^{19,20} these non-allergic adolescent controls should represent a 'clean' group for comparison of study outcomes.

In conclusion, serum LL-37 shows weakly positive correlation with eczema severity, although it is not associated with eczema diagnosis and patients' SH and TEWL. Circulating LL-37 may be a biomarker for eczema severity in children.

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