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Original Research Article

Eosinophilia triggers changes in IL-5, eotaxin and IL-17, and acts as a prognostic biomarker for atopic dermatitis

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Abstract

Purpose: *To investigate the implication of eosinophilia in atopic dermatitis (AD).*
Methods: *A total of 139 AD patients from The First Affiliated Hospital of Guangxi University of Chinese Medicine between February 2013 and May 2015, were involved in this study. Scoring atopic dermatitis (SCORAD) index was used to evaluate the skin lesions. The levels of IL-4, IL-5, IL-13, IL-17, INF-gamma, IP-10, eotaxin and Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), were determined with commercial enzyme-linked immunosorbent assay (ELISA) kits. Eosinophil counts were carried out by granulocyte count method. Correlation between SCORAD scores and levels of cytokines was analyzed using the Spearman correlation method.*
Results: *SCORAD scores significantly increased in the eosinophil-positive group when compared to eosinophil-negative group ($p < 0.05$). Eosinophil counts correlated with SCORAD scores in the eosinophil-positive group ($p < 0.05$). INF-γ, IP-10 and RANTES levels were significantly higher in the eosinophil-positive group than in eosinophil- negative group, while IL-5, eotaxin and IL-17 levels significantly decreased in eosinophil-positive group ($p < 0.05$). In the eosinophil-positive group, IL-5, eotaxin and IL-17 levels positively correlated with SCORAD scores.*
Conclusion: *Eosinophilia triggers IL-5, eotaxin and IL-17 changes and acts as a prognostic biomarker for atopic dermatitis. These findings may give further insights into the pathogenesis of AD.*

Keywords: *Atopic dermatitis, Eosinophilia, SCORAD score, Biomarker, Cytokines*

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INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory and relapsing skin disease, which is always associated with food allergies and asthma [1]. Previous studies showed that industrialization and urbanization influence the rates of atopic disease [2,3]. Recent studies revealed that the prevalence of AD decreases from in central and rural areas of Shanghai, China [4]. The International Study of Asthma and Allergies in Childhood (ISAAC) showed that the incidence and prevalence of AD are on the increase all over the world, especially in the developing countries [5]. AD places a heavy economic burden on the society and is a source of huge financial costs to health systems [6].

Many studies have been reported that AD may be induced when the skin is stimulated by allergens [7,8]. Recent studies also reported that some of environmental allergens could produce eczematous reactions on the skin [7,8]. However the pathogenesis of AD and its immune responses are still not clearly understood. It has been shown that chronic immune activation

contributes to the pathophysiology of this common skin disease, and that eosinophils are frequently observed in AD patients [8].

Elevated blood eosinophil count is an important characteristic of AD patients [9]. However, the reasons for the elevation in blood eosinophil count have not been fully elucidated [10]. It has been speculated that the eosinophils may play protective roles in AD [9,10]. Previous studies have demonstrated that eosinophils are activated pro-inflammatory cells which could cause some of the allergic inflammation symptoms.

Recent studies on activities of eosinophils revealed that they contain potent toxic proteins with the potential to mediate tissue damage [8-10]. Furthermore, immuno-fluorescent localization of eosinophil granule proteins has shown that the toxic granule proteins were deposited in tissues when the eosinophils become disrupted [10]. The deposition of granule proteins in several diseases is vastly out of proportion with the number of identifiable cells [9]. Specifically, the deposition of eosinophil granule proteins outside of eosinophils has been observed in lichenified eczematous disorders with elevated serum levels of immunoglobulin E; in urticarial and angioedematous disorders, and in bullous diseases [10].

The present study was carried out to investigate the prognostic biomarker potential of eosinophils in AD patients.

EXPERIMENTAL

Patients

The survey was performed at the First Affiliated Hospital of Guangxi University of Chinese Medicine, China. A total of 139 children aged 2 to 12 years, who were clinically diagnosed with AD, were recruited. This study was approved by the Ethical Committee of The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, China (approval no. [2013]-K002). Informed consents were obtained from all of the participants. All of the patients have been approved this study. The duration of disease in every patient was more than six months, and the remission was less than 3 months in a year (Figure 1). In accordance with the Guidelines of the World Medical Association Declaration of Helsinki [11], patients were treated with loratadine granules *per os* and desonide for external use.

All the AD patients underwent 2 year-long follow-up and 3 months of regular visits. Blood samples were collected for determination of eosinophil counts and related cytokines, including IL-4, IL-5, IL-13, IL-17, INF-gamma, IP-10, eosinophil chemotactic factor eotaxin and RANTES. The study subjects were grouped into eosinophil-positive group (58 cases) and eosinophil-negative group (61 cases).

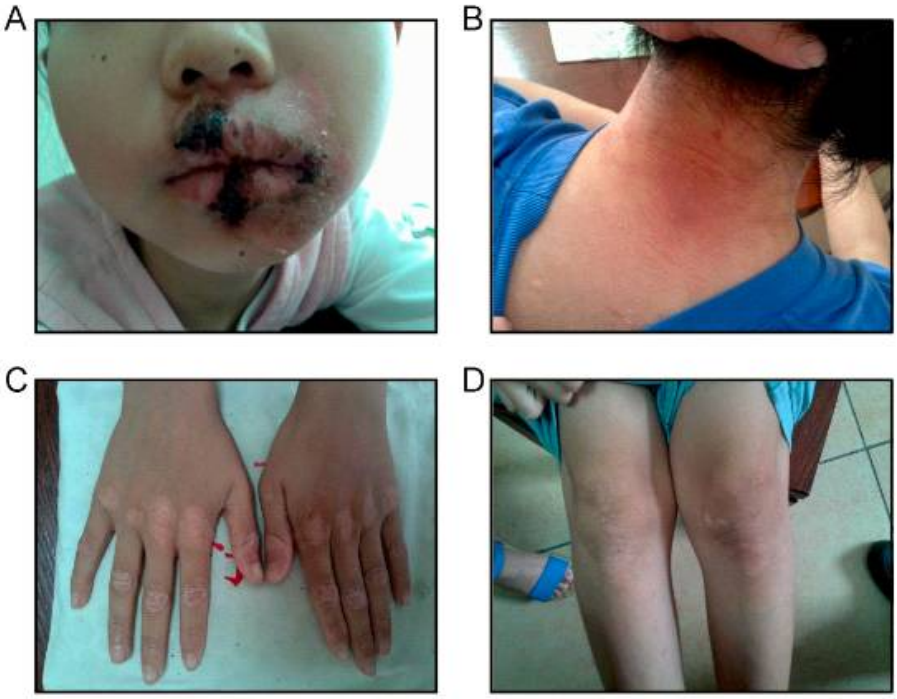

Figure 1: Patients clinically diagnosed with AD illustrating different syndromes. A. Dermatitis at oral lips. B. Dermatitis at nape. C. Dermatitis at hand. D. Dermatitis at leg

ELISA and eosinophil counts assay

Blood levels of IL-4, IL-5, IL-13, IL-17, INF-gamma, IP-10, eotaxin and RANTES were determined using ELISA commercial kits. Eosinophil counts were carried out using granulocyte count method.

European AD score (SCORAD)

Scoring atopic dermatitis index (SCORAD) was used as AD standard to assess the extent of skin lesions (A), the severity of skin lesions (B) and pruritus and sleep (C). The score guide was as follows:

A (1) *for adults:* 9 % for head, neck and arm; 13.5 % for each side of trunk, and 22.5 % for the lower extremities. A (2) *for children under 14 years of age:* 9% for head and neck, and 18% for arm, trunk and lower limbs.

B represented scores (0 - 3) in respect skin lesions such as erythema, papules, edema, skin exfoliation, cracking, chapping and exudation, scab, moss or dry skin. C, which represented scores (0 - 3) for pruritis and extent of effect on sleep, was divided into four grades: severe (3 points), moderate (2 points), mild (1 point) and nil (0 point).

Statistical analysis

Data analyses were performed using SPSS 20.0 software. Student's *t* test was used to evaluate significant differences between groups. $P < 0.05$ was considered statistically significant. Correlation between SCORAD scores and levels of cytokines were analyzed using Spearman correlation method.

RESULTS

SCORAD scores

The results indicated that eosinophil counts in the eosinophil-positive group were significantly higher when compared to corresponding values in the eosinophil-negative group, irrespective of AD duration ($p < 0.05$, Table 1). SCORAD scores were also significantly higher in the eosinophil-positive group than in the eosinophil-negative group in "Half year", "One year", "One-and-half year" and "Two years" categories ($p < 0.05$, Table 1). However, eosinophil counts and SCORAD scores were significantly lowered from "Half year" to "Two years" in the eosinophil-negative group ($p < 0.05$, Table 1). SCORAD scores were correlated with eosinophil counts in both groups. The results also revealed that eosinophil counts were positively correlated with SCORAD scores in the eosinophil-positive group (Figure 2, $p < 0.05$).

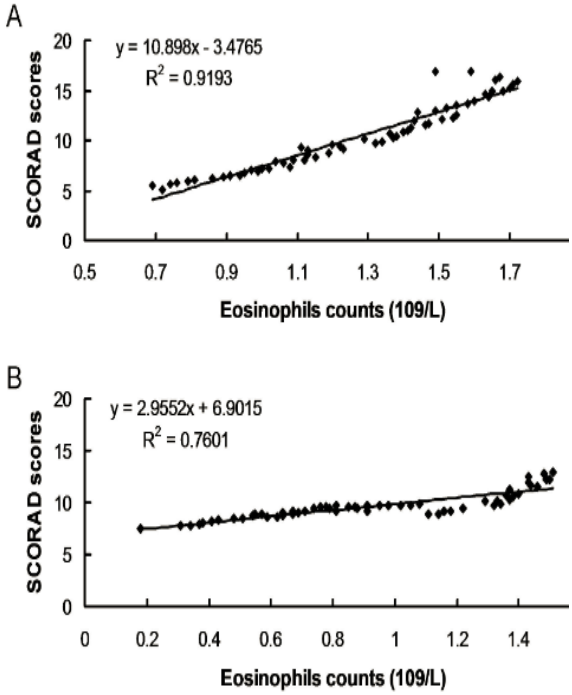

Figure 2: Correlation analysis between SCORAD scores and the eosinophils counts in eosinophils positive group (A) and negative group (B)

INF-γ, IP-10 and RANTES levels

The results showed that INF-γ, IP-10 and RANTES levels were significantly higher in "One year" compared to "Half year"; and in "One-and-half year" AD when compared to "One year" AD (post-diagnosis) in the eosinophil-positive group ($p < 0.05$, Table 2). For the INF-γ and RANTES,

Table 1: Eosinophil counts and SCORAD scores in eosinophils positive and negative groups at half year, one year, one and half year and two years after diagnosis of AD

Time	Eosinophils positive group		Eosinophils negative group	
	Counts (109／L)	SCORAD	Counts (109／L)	SCORAD
Half year	0.97±0.28	6.28±1.77	1.32±0.29	10.05±3.12
One year	1.05±0.33	9.41±2.85	1.06±0.25	6.91±1.83
One and half year	1.53±0.41	14.03±4.26	1.06±0.25	9.24±3.07
Two year	1.46±0.37	15.74±5.14	0.47±0.08	8.32±2.75

Table 2: Observation for the INF-γ, IP-10 and RANTES expression in the eosinophils positive group at half year, one year, one and half year and two years after diagnosis of AD

Time	INF-γ (pg/ml)	IP-10 (pg/ml)	RANTES (pg/mL)
Half-year	29.563±3.815	70.324±43.501	54.813±12.068
One year	47.06±5.493	116.550±58.947	93.054±30.245
One and half year	324.612±76.524	209.548±97.513	912.356±105.436
Two years	291.64±64.307	241.538±122.095	876.590±94.021

Table 3: Examination of the IL-5, extaxin and IL-17 expression in the eosinophils positive group at half year, one year, one and half year and two years after diagnosis of AD

Time	IL-5 (pg/mL)	Eotaxin (pg/mL)	IL-17 (pg/mL)
Half year	84.786±10.257	54.910±20.136	92.744±17.391
One year	75.042±9.035	34.756±15.382	30.927±3.895
One and half year	66.793±8.578	49.651±18.523	75.782±8.653
Two year	36.245±5.749	41.207±16.113	58.237±6.507

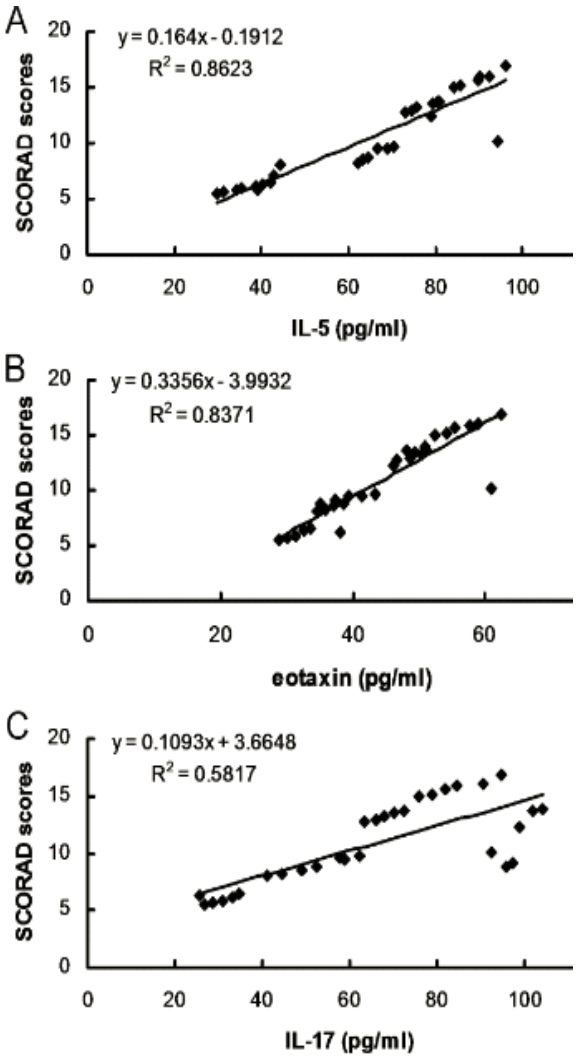

Figure 3: Correlation analysis between SOCRAD scores and the INF-γ (A), IP-10 (B) and RANTES (C) levels in the ensinophils positive group

the levels were significantly lower in the "Two years" compared to "One-and-half year" AD post-diagnosis (Table 2).

Results from analysis of the correlation between SCORAD scores and INF-γ, IP-10 and RANTES levels indicated that there were no significant correlations between INF-γ and SCORAD scores (Figure 3A, $p > 0.05$); or between IP-10 and SCORAD scores (Figure 3B,) or between RANTES and SCORAD scores (Plate 3C, $p > 0.05$).

IL-5, eotaxin and IL-17 levels

The results obtained showed that the levels of IL-5, eotaxin and IL-17 were significantly lower in "One year", "One-and-half year" and "Two-year" post AD diagnosis categories when compared to the "Half year" post-diagnosis group (Table 3, $p < 0.05$).

Results from cytokine assays showed that IL-5, extaxin and IL-17 levels were positively correlated with SCORAD scores (Figure 4, $p<0.05$).

Furthermore, IL-4 and IL-13 levels were significantly lower in "One year", "One-and-half year" and "Two-year" when compared to "Half year" post-diagnosis (data not shown). However, IL-4 and IL-13 were not correlated with SCORAD scores in the eosinophil-positive patients (data not shown).

negative groups. The results indicated that SCORAD scores were significantly increased in the eosinophil-positive group. Furthermore, eosinophil counts correlated positively with SCORAD scores in eosinophil-positive group. These results suggest that eosinophil levels were associated with the SCORAD scores in the eosinophil-positive patients, indicating that eosinophil levels could reflect the level of skin injury in AD patients.

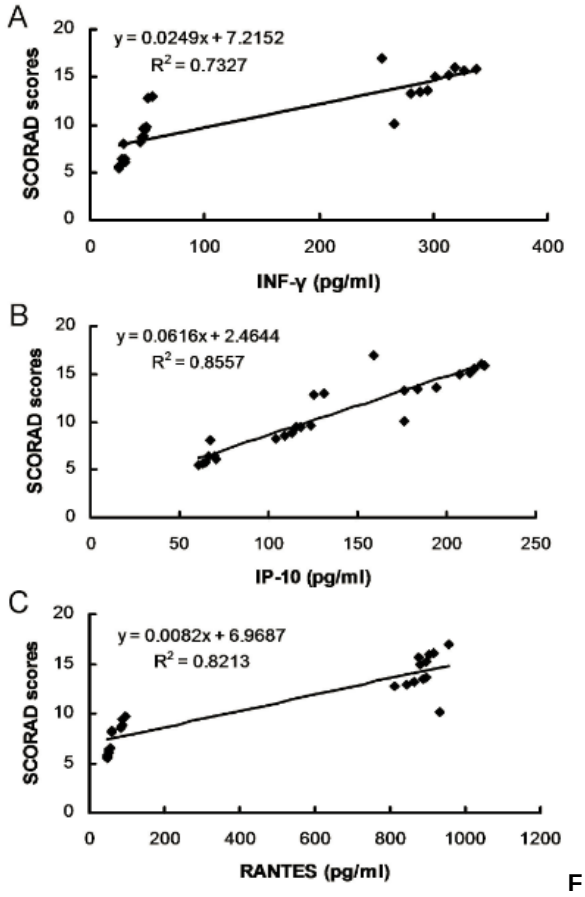

Figure 4: Correlation analysis between SOCRAD scores and the IL-5 (A), eotaxin (B) and IL-17 (C) levels in the eosinophils positive group

Although INF-γ, IP-10 and RANTES levels were significantly increased in the eosinophil-positive group, no correlations were established between levels of INF-γ, IP-10 and RANTES, and SCORAD scores in the eosinophil-positive patients. These results suggest that the INF-γ, IP-10 and RANTES cannot be used to evaluate the severity of AD. However, IL-5, eotaxin and IL-17 levels were correlated with SCORAD scores in eosinophil-positive patients, indicating that the IL-5, eotaxin and IL-17 can be used to evaluate the severity of AD. Studies by Valirlis *et al* [17] also found that some of the cytokine were associated with SCORAD scores in acute AD patients. However, not much was known about the involvement of INF-γ, IP-10, RANTES, IL-5, eotaxin and IL-17. The present study is the first report to demonstrate that cytokines, IL-5, eotaxin and IL-17 levels correlated with SCORAN scores and skin injury in eosinophil-positive patients. However, IL-4 and IL-13 were not of predictive significance for the prognosis of AD.

DISCUSSION

AD is a chronic, relapsing and highly pruritic dermatitis which always develops in early childhood. It has a characteristic age-dependent distribution. About 10 to 20 % children suffer from AD in the developing countries [12]. AD is characterized by sino-pulmonary infections, dermatitis, cutaneous viral infections, altered eosinophil levels, elevated serum IgE, squamous cell carcinomas, and a high incidence of food allergies [12-14]. Aarkawa *et al* reported that different cytokines are present in the peripheral blood mononuclear cells of patients with atopic dermatitis [15]. Therefore, in this study, we investigated the prognostic value of eosinophil and related cytokines in atopic dermatitis.

Previous studies reported that the peripheral blood eosinophil could act as a diagnostic parameter in differentiating allergic AD from non-allergic AD [16]. The present study also showed that eosinophil counts in AD patients changed significantly, which is consistent with the previous study [16]. Furthermore, the AD patients were divided into eosinophil-positive and eosinophil-

CONCLUSION

The results obtained in this study strongly suggest that eosinophils act as prognostic biomarkers for AD by triggering changes in IL-5, eotaxin and IL-17. The findings of this study may give further insights into the pathogenesis of AD.

DECLARATIONS

Acknowledgement

None declared

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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