

Serum concentration of macrophage-derived chemokine may be a useful inflammatory marker for assessing severity of atopic dermatitis in infants and young children

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Chemokines are responsible for the trafficking of leukocytes to sites of inflammation. Serum chemokine levels were previously shown to be increased in adult patients with atopic dermatitis (AD). We tested whether serum concentrations of chemokines, including macrophage-derived chemokine (MDC), thymus and activation-regulated chemokine (TARC), eotaxin (EOX), interferon gamma inducible protein 10 (IP-10) and monocyte chemoattractant protein 1 (MCP-1), are useful inflammatory markers for assessing AD severity in infants and young children. To investigate this, we assessed the severity of AD clinically using the SCORing Atopic Dermatitis (SCORAD) index system. Serum chemokine concentrations were determined by sandwich enzyme immunoassay. Twenty AD patients with a median age of 2.1 years [interquartile range (IQR): 0.6–4.2] were recruited. Their SCORAD score was 23.5 (12.5–33.5). Serum concentrations of MDC, TARC, EOX, IP-10 and MCP-1 were 2551 (1978–3935), 1469 (1125–3070), 68 (57–85), 126 (101–226) and 518 (419–614) pg/ml, respectively. Serum MDC levels correlated with SCORAD ($r = 0.608$, $p = 0.004$) and its extent ($r = 0.629$, $p = 0.003$) and intensity ($r = 0.557$, $p = 0.011$) components. Serum TARC concentration showed weaker correlation with extent ($r = 0.474$, $p = 0.035$) and intensity ($r = 0.465$, $p = 0.039$) of skin involvement but not SCORAD. The median serum levels of MDC (3131 vs. 2394 pg/ml; $p = 0.031$) and EOX (80 vs. 61 pg/ml; $p = 0.046$) were also higher in children with moderate as compared with mild AD. The other chemokines did not correlate with AD severity. In conclusion, our results suggest that serum MDC concentration may be a useful inflammatory marker for assessing AD severity in infants and young children.

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Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease commonly associated with inhalant allergy (1). The International Study of Asthma and Allergies in Childhood found that nearly 20% of Hong Kong school-children suffered from AD (2). The pathogenesis of AD is mediated by CD4⁺ T lymphocytes that produced a Th2 cytokine profile (3–5). Besides, B cells from these patients synthesized high

levels of IgE (4). In this study, we included five chemokines that play crucial roles in regulating the balance between Th1 and Th2 immunity. Thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) are Th2 chemokines that specifically act on chemokine receptor CCR4 (6) to attract Th2 cells into sites of allergic inflammation (6–9). Th2 cytokines including IL-4 and IL-13

have been shown to be potent stimulators of monocyte MDC production (10, 11). Serum levels of TARC and MDC were significantly higher in Japanese adults with AD than those of healthy controls (12, 13). In that study, serum TARC level also correlated with SCORAD that is a widely accepted clinical tool for assessing AD severity (14). Eotaxin (EOX) is another chemokine involved specifically in Th2-type immune responses (15). Plasma EOX levels were increased in children with AD compared with controls (16). Monocyte chemotactic protein 1 (MCP-1) played a role both in Th1 and Th2 antigen-induced granuloma formation (17), whereas interferon gamma inducible protein 10 (IP-10) was chemotactic for neutrophils and activated T lymphocytes (18). Expression of IP-10 is up-regulated in many Th1-type inflammatory diseases, such as tuberculosis and sarcoidosis, in adults (19). The aim of this study was to investigate the utility of serum concentrations of the above chemokines in evaluating the disease severity of AD in infants and young children.

Subjects and methods

Study population

This study recruited children suffering from AD who were younger than 6 years old and managed in the Prince of Wales Hospital, Hong Kong. The diagnosis of AD was made according to criteria proposed by Hanifin (20). The clinical severity of AD was assessed by the same investigator using the SCORing Atopic Dermatitis (SCORAD) index system (range 0–103) (14). The disease activity of AD was further divided into three groups according to the objective (extent and intensity of the lesions) SCORAD criteria. In brief, 'mild' is the group with objective SCORAD <15 points; 'moderate' is defined as 15–40 points; 'severe' is defined as >40 points (21). All recruited subjects were free from intercurrent illness for 4 weeks prior to study. Each subject underwent skin prick tests (SPT), and blood samples were collected for inflammatory markers. The Clinical Research Ethics Committee of the University approved this study. Written informed consent was obtained from parents before investigations.

Skin prick testing for allergen sensitization

All patients underwent SPT by the same investigator, according to standard procedures and using purified allergen extracts (Hollister-Stier

Laboratories, Spokane, WA, USA). The panel of common food allergens consisted of whole cow's milk, whole egg, wheat, soybean, mixed codfish and mixed peanut. Purified *Dermatophagoides pteronyssinus* extract was also included. Positive and negative controls were performed using histamine (10 mg/ml) and normal saline. Reactions were considered to be positive if skin induration was 3 mm or more in diameter larger than the negative control.

Serum total IgE and chemokine concentrations

Serum samples were obtained from clotted blood following centrifugation at 4°C and 2041 *g* for 10 min, and then stored at –70°C until analyzed for chemokine concentrations in batches. Serum concentration of total IgE was measured by microparticle immunoassay (IMx analyzer, Abbott Laboratories, Abbott Park, IL, USA). Serum levels of EOX (Biosource International, Camarillo, CA, USA) as well as TARC, MDC, MCP-1 and IP-10 (R & D Systems, Minneapolis, MN, USA) were measured on neat samples in duplicates using the sandwich enzyme immunoassay technique according to manufacturers' instructions. The 96-well polystyrene microplates used were coated separately with murine monoclonal antibodies against human chemokines. The sensitivities of the kits in detecting the above chemokines were 2.2, 7.0, 62.5, 5.0 and 1.67 pg/ml, respectively.

Statistical analysis

Numerical data were expressed as medians (interquartile ranges, IQR) and compared between groups using the Mann–Whitney *U*-test. Correlation between numerical variables was determined using Spearman's rank correlation test. All comparisons were made two-sided using SPSS 10.1 for Windows (SAS Institute Inc., Chicago, IL, USA). *p*-values less than 0.05 were considered significant.

Results

Study population

Twenty Chinese AD patients aged 2.1 (0.6–4.2) years were recruited. The demographic data are summarized in Table 1. Thirteen (65%) patients were skin test-positive to foods or *D. pteronyssinus*. The median (IQR) total SCORAD was 23.5 (12.5–33.5) whereas eczematous lesions affected 18.5 (10.8–26.5) % of body surface area of our patients. The median (IQR)

Table 1. The demographic and laboratory data of our 20 patients

Characteristics*	Results
Background Information	
Age at evaluation (years)	2.1 (0.6–4.2)
Sex (male/female)	11:9
Serum logarithmic total IgE levels (kIU/l)	2.12 (1.36–2.89)
Food allergen sensitization, n (%)	
Whole cow's milk	1 (5)
Whole egg	11 (55)
Wheat	3 (15)
Soybean	1 (5)
Mixed codfish	1 (5)
Mixed peanut	4 (20)
Skin test positivity to <i>Dermatophagoides pteronyssinus</i> , n (%)	11 (55)

*Expressed as median (interquartile range) unless stated otherwise.

Table 2. Summary of correlation between AD severity in our patients as indicated by SCORAD and inflammatory markers in their blood samples

Markers	Spearman's Rho correlation				
	Total SCORAD	Extent of skin involvement	Intensity	Pruritus	Sleep loss
Serum MDC	0.608†	0.629†	0.557*	0.235	0.289
Serum TARC	0.393	0.474*	0.465*	0.052	0.011
Serum EOX	0.256	0.456*	0.215	−0.001	−0.090
Serum IP-10	0.237	0.159	0.178	0.267	0.255
Serum MCP-1	0.197	−0.140	−0.029	0.188	0.208

* $p < 0.05$.

† $p < 0.005$.

serum concentrations of MDC, TARC, EOX, IP-10 and MCP-1 were 2551 (1978–3935), 1469 (1125–3070), 68 (57–85), 126 (101–226) and 518 (419–614) pg/ml, respectively. Among the five chemokines studied, only TARC and MDC levels significantly correlated with each other ($r = 0.724$, $p = 0.0003$).

Correlation between AD severity and serum chemokine levels

The relationships between serum chemokine levels and AD severity as evaluated by SCORAD are summarized in Table 2. Serum MDC concentration significantly correlated with the total SCORAD as well as the components of extent and intensity of skin involvement. As classified by objective SCORAD, 11 AD patients were mild cases, 9 patients suffered from moderate AD and none of them had severe disease. Children with moderate AD had higher serum levels of MDC (Fig. 1; $p = 0.031$) and EOX (Fig. 2; $p = 0.046$) compared with those with mild disease. However, serum chemokine levels did not differ between subjects with and without allergen sensitization on SPT (results not shown).

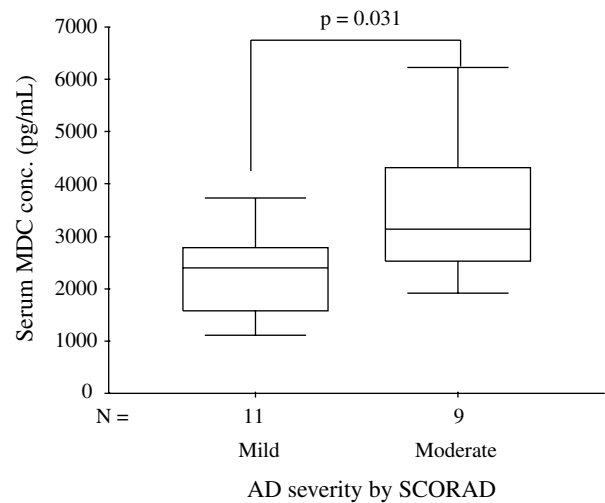


Fig. 1. Serum MDC concentrations in children with different severities of atopic dermatitis as defined by objective SCORAD. The median (IQR) MDC levels for patients with mild and moderate AD were 2394 (1277–2983) pg/ml and 3131 (2463–5237) pg/ml, respectively.

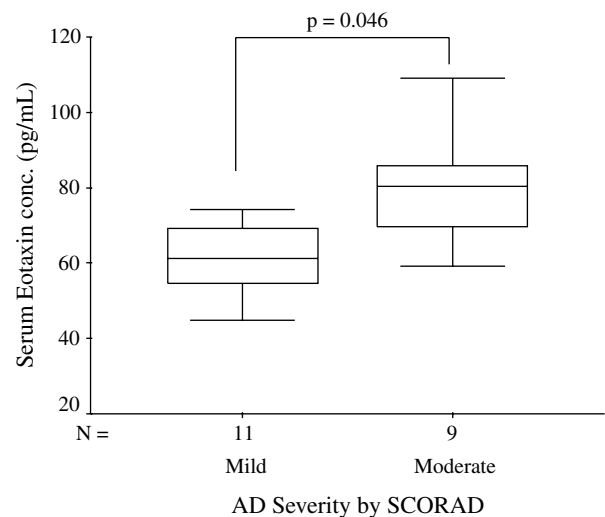


Fig. 2. The relationship between serum EOX concentration and AD severity as assessed by objective SCORAD. The median (IQR) EOX level for patients with mild AD was 61 (53–74) pg/ml and that for moderate disease was 80 (68–97) pg/ml.

Discussion

The pathophysiology of AD is characterized by a predominant infiltration of Th2 cells, increased secretion of Th2-type cytokines in the acute phase of lesional skin, and high responsiveness of peripheral blood mononuclear cells for IL-4 (3–5). There is local infiltration of allergen-specific Th2 cells (3, 4) and B cells from patients with AD synthesize high levels of IgE (4). Imbalance in Th1/Th2 cytokine secretion is thus

believed to play an important role in the pathogenesis of AD. TARC and MDC are potent chemoattractants to Th2 cells (6–9, 22, 23). In AD patients, TARC could be detected in keratinocytes and to a lesser extent in endothelial cells and dermal infiltrating cells including CD3 lymphocytes and CD1a dendritic cells (12, 24, 25). Elevated TARC concentrations were found in the serum of adult patients with AD compared with normal controls (12, 26). Similar results were recently reported for serum MDC levels (13). Serum TARC and MDC levels also correlated with the clinical and laboratory markers for AD severity in adults (12, 13, 26). These chemokines appear to be useful inflammatory markers for assessing severity of AD in adults. However, the same results have not been replicated in children with AD. The present study is the first to suggest that serum MDC, and to a lesser extent TARC, levels may also be useful in evaluating the disease severity of AD in the pediatric population. In addition, serum MDC levels were higher among patients with moderate AD compared with those with mild disease. Further larger studies should address the issue whether serum MDC levels may be used to monitor longitudinal changes in AD severity with therapeutic interventions. The consideration of changes in circulating chemokines may also reveal the mechanisms involved in successful therapeutic intervention that can be the focus of further in depth study.

EOX is specifically involved in Th2-type immune responses. The expression of EOX was found to be stimulated by IL-4 (15). Miotto et al. found that EOX expression was specifically associated with asthma but not tuberculosis, sarcoidosis or chronic bronchitis in adults (19). Increased EOX expression has been detected after allergen challenge in allergic disease (27). Plasma EOX levels increased in children with AD compared with controls (16). The present study found increased serum EOX levels in children with moderate AD compared with those with mild disease. Nonetheless, there was only a weak correlation between serum EOX and extent of skin involvement but not total SCORAD or disease intensity in AD patients. Thus, this inflammatory marker might not be as useful as MDC in assessing and monitoring the disease severity of childhood AD.

MCP-1 played a role both in Th1 and Th2 antigen-induced granuloma formation (17). IP-10, a C-X-C chemokine secreted from cells stimulated with interferons and lipopolysaccharides, is chemotactic for neutrophils and

activated T lymphocytes (18). IP-10 production was inhibited by the Th2 cytokine IL-4. Expression of IP-10 is up-regulated in many Th1-type inflammatory diseases in adults, where it is thought to play an important role in recruiting activated T cells into sites of tissue inflammation (19). An IP-10-deficient mouse exhibited an reduced recruitment of activated CD4⁺ and CD8⁺ lymphocytes, impaired contact hypersensitivity response and decreased ability to control viral infections (28). Nonetheless, neither MCP-1 nor IP-10 was found to correlate significantly with AD severity in infants and young children.

Similar to AD, the pathogenesis of asthma is mediated by T lymphocytes that produced a Th2 cytokine profile (12). Th chemokines also play important roles in regulating this disease. Asthmatic patients exposed to a relevant allergen released large quantities of TARC in bronchoalveolar lavage fluid (29). Our group recently found an increase in plasma TARC but not MDC level in steroid-naïve children with intermittent asthma when compared with control subjects (30). Sekiya et al. also reported that TARC but not MDC concentrations were increased in serum and induced sputum from adult asthmatics (31). These observations were substantiated by *in vitro* experiments indicating a differential regulation of TARC and MDC expression in bronchial epithelium from asthmatics (32). In contrast, the present study found that MDC might be a better inflammatory marker for AD than TARC. These findings suggest some degree of tissue specificity in the biological effects that are mediated by the Th2 chemokines TARC and MDC. Although MDC, TARC and EOX were associated with various parameters of SCORAD in our patients, the present study suggests that the serum MDC level was more superior to other Th chemokines as an inflammatory marker for AD because this chemokine showed much stronger correlations with total SCORAD as well as its extent and intensity components.

In conclusion, serum MDC concentration, and to a lesser extent TARC and EOX, were associated with the disease severity of AD in our infants and young children. Serum MDC levels appear to be a useful inflammatory marker for evaluating AD severity in the pediatric population. Larger studies are necessary to investigate whether this marker can reflect longitudinal changes in AD severity with treatment, and to define the possible roles of serum MDC in the management of childhood AD.

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