

Serum Fractalkine (CX3CL1) Concentration Correlates with Clinical Severity in Pediatric Atopic Dermatitis Patients

Shubin Chong¹, Haimei Lan², Kang Zeng², and Xuefeng Zhao³

¹Department of Dermatology, Nan Hai Hospital affiliated to Southern Medical University, ²Department of Dermatology, Nanfang Hospital affiliated to Southern Medical University, and ³Department of Laboratory Medicine, Nan Hai Hospital affiliated to Southern Medical University, China

Abstract. Background. Atopic dermatitis (AD) is a common inflammatory allergic disease presenting pruritic skin lesions mainly in early childhood. Elevated fractalkine (FKN) expression is detected in AD patients. **Objective.** We aimed to investigate the levels of FKN in serum and their association with disease severity in pediatric AD patients. **Methods.** Serum samples were obtained from 57 AD children and 53 healthy children. Disease severity was assessed according to the following scoring systems: Leicester sign score (LSS), simple scoring system of costa (SSS), SCORing atopic dermatitis (SCORAD) index, and objective SCORAD. Serum FKN levels were measured using a sandwich enzyme-linked immunosorbent assay. Serum levels of immunoglobulin (Ig) E and interleukin (IL)-31 and the association of serum FKN levels with the scoring systems as well as IgE and IL-31 levels were evaluated. The FKN levels were re-assessed after symptomatic relief. **Results.** Serum FKN levels in AD children during both flare and quiescence episodes were significantly higher than in the healthy individuals. Additionally, these correlated positively with all disease severity related scoring systems and serum IgE and IL-31 levels. **Conclusion.** FKN may serve as a reliable biomarker for evaluating disease severity in AD patients. Therapeutic interventions for AD, targeting FKN, deserve further study.

Key words: Fractalkine, atopic dermatitis, clinical severity.

Introduction

Atopic dermatitis (AD) is one of the most common chronic inflammatory and immunological skin disorders affecting 10-20% children and 1-3% adults [1]. Clinically, it is characterized by eczematous skin lesions and inflammatory infiltrate that is mainly comprised of CD4+ and cutaneous lymphocyte-associated antigen-positive memory T cells [2]. Although insights into the pathogenesis of AD have evolved over the past several years, the etiology remains elusive. Recently, the progression of cutaneous lesions in patients with AD has been closely associated with the gathering of leukocytes migrating from the blood and into the affected skin via the endothelium. This process is highly related to the expression of various cytokines and inflammatory mediators [3].

Chemokines represent a superfamily of small cytokine-like proteins, which mediate directional migration *in vitro* and control leukocyte trafficking *in vivo* [4,5]. Recent studies have shown that chemokines play an important role in the elucidation of innate and adaptive immune responses in many diseases, including skin disorders [6,7]. Chemokines also induce the migration of inflammatory cells towards the skin and facilitate their retention on the inflammatory sites [8,9]. Together with their receptors, they play a crucial role in orchestrating the initiation as well as exacerbation of inflammation in response to allergens [10,11], with higher expressions in the skin lesions than in the uninvolved skin area of the same patient [12].

Fractalkine (FKN), also known as CX3CL1, is a CX3C chemokine that is expressed on the activated vascular endothelial cells that have been stimulated with various pro-inflammatory cytokines [13]. FKN is secreted or expressed in various organs and tissues [14] and interacts with its unique receptor CX3CR1, which is widely expressed on natural

Address correspondence to Shubin Chong, Department of Dermatology, Nan Hai Hospital affiliated to Southern Medical University, Fo Ping Road No.40, Fo Shan, Guang Dong Province, 528200, China; phone: +86 0757 86337457; fax: +86-0757 86337457; e mail: csb198198@126.com

killer (NK) cells, monocytes, and some T cells [15]. It is believed to play a significant role in the pathophysiology of AD. FKN and CX3CR1 expressed on T cells play a crucial role in the development of AD by retaining effector T cells in the inflamed skin [16]. CX3CR1 mRNA expression and concentration are elevated in the skin of patients with AD and are significantly increased in patients with moderate to severe AD [17]. In addition, elevated serum FKN levels have been detected in patients with AD compared with healthy individuals [18]. Furthermore, CX3CR1 single nucleotide polymorphisms have been related to atopy in German children [19].

All these data suggest that FKN may be involved in the pathogenesis and progression of AD. However, no investigation has focused on the association between FKN levels and the clinical severity of AD. Therefore, the aim of the present study was to determine the association between serum FKN levels and disease severity in children with AD.

Materials and Methods

Subjects. We recruited 57 children with AD (29 boys and 28 girls; mean age 5.41 ± 2.17 years; mean $\pm$ SD) who visited the Department of Dermatology of the Nan Hai Hospital (an affiliate of the Southern Medical University) from March 2014 to February 2014. AD was diagnosed according to the criteria established by Hanifin and Rajka [20]. Patients were excluded if they had current or family history of other atopic or allergic diseases and other dermatological illnesses, as well as a history of corticosteroid and anti-histamine administration in the past 2 weeks. Fifty-three sex and age-matched healthy children undergoing physical examination in the outpatient department who did not have past, current, or family history of allergic disorders were enrolled as controls. Written informed consent was obtained from the children's parents before the study was initiated. The ethical committee of the Nan Hai Hospital, which is affiliated with the Southern Medical University, approved this study.

Study design. While presenting with an AD flare, the children were initially assessed clinically at our hospital by two blinded experts for disease severity before samples for the FKN assay were collected. Patients were followed-up and were administered symptomatic therapy (systemic and topical corticosteroids, immunosuppressive therapy, and anti-histamines) until disease quiescence. All interventions were discontinued for at least 2 weeks before serum samples were collected and assayed

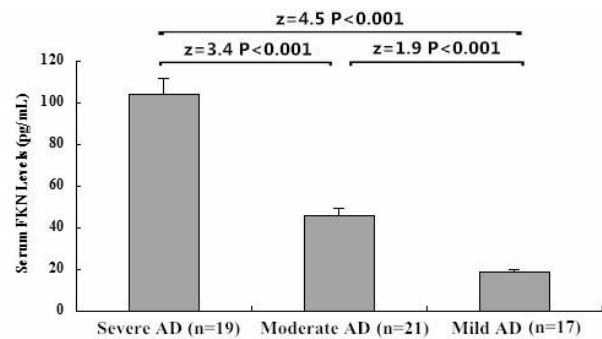


Figure 1. Summary of serum FKN (pg/mL) levels among the pediatric AD subjects according to disease severity. The Mann-Whitney test was used to compare the groups. Data are presented as the median value.

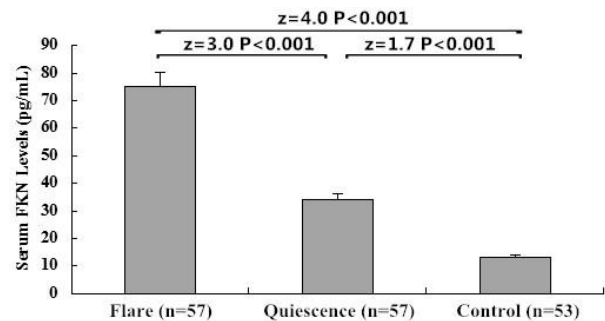


Figure 2. Comparison of serum FKN (pg/mL) levels between the control and pediatric AD subjects, as classified according to the objective SCORAD index of severity during flares. The Mann-Whitney test was used to compare the groups. Data are presented as the median value.

again. Quiescence and response to treatment were checked according to the Physician's Global Assessment (PGA) score [21]. We found an insignificant rate of patient drop-out throughout the study process.

Laboratory Methods. Serum was collected from the children with AD and healthy controls, centrifuged at 3000 rpm, and then stored at -80°C before evaluation. The serum samples were diluted 10 times, and were subsequently analyzed for FKN and IL-31 using commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations and expressed as pg/mL. Serum total immunoglobulin (Ig) E was assessed in all participants using a quantitative enzyme immunoassay (Euroimmun, Lübeck, Germany) and the results were expressed in IU/mL. Serum levels of FKN were re-examined in the patients after symptomatic remission.

Definition of Disease Severity. AD severity was evaluated on the basis of four different scoring systems: the simple scoring system of costa (SSS), Leicester sign score (LSS), SCORing atopic dermatitis (SCORAD) index,

Table 1. Comparison between the baseline data of study participants.

	AD children (n=57)	Healthy children (n=53)	P value
Age (Y)	5.41±2.17	5.25±2.54	0.63
Sex (F/M)	28/29	25/28	0.32
SSS scores	40 (12-60)	/	
LSS scores	49 (22-82)	/	
SCORAD scores	39 (13-65)	/	
Objective SCORAD scores	8 (10-58)	/	
Serum Total IgE Levels (IU/mL)	143 (37-405)	31 (10-57)	<0.001
Serum FKN Levels (pg/mL)	75 (10-518)	15 (6-52)	<0.001
Serum IL-31 Levels (pg/mL)	1656 (786-2814)	178 (112-247)	<0.001

SSS: Simple scoring system of costa; LSS: Leicester sign score; SCORAD: Scoring atopic dermatitis; Ob SCORAD: Objective scoring atopic dermatitis. Basic values are expressed as the mean value ± SD. The scores, serum FKN, IgE, and IL-31 levels are expressed as median (interquartile range).

and objective SCORAD. These scales are widely used for evaluating AD severity, and their feasibility and validity have been well established among patients with AD.

The simple scoring system (Costa's score) sums up the intensity of 10 signs and symptoms in 10 different skin regions. Factors such as erythema, edema, vesicles, crusts, excoriation, scales, lichenification, pigmentation or depigmentation, pruritus, and loss of sleep were included in the intensity criteria assessment [22]. In the LSS, the assessment for severity consists of six items (erythema, purulence, excoriation/crusting, dryness/scaling, cracking/fissuring, and lichenification), which are graded at six different body sites with scores ranging from 0 (none) to 3 (severe) for each site, for a total score ranging from 0 to 108 points [23]. The SCORAD was utilized to assess the severity of eczema by integrating two objective (degree and intensity of eczema) and subjective symptoms (pruritus and sleep loss) with a total score, which ranged from 0 to 103 points [24]. The objective SCORAD is a modification of the SCORAD that includes only questions on the objective items and are eventually graded according to the total score (<15: mild AD; 15-40: moderate AD, and >40: severe AD)[25].

Statistical Analysis. All statistical data were analyzed using the SPSS (Statistical Package for the Social Science) software version 18 for Windows system (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD or median. Student's t-test and Mann-Whitney U-test were used for comparing parametric data and non-parametric comparison of serum FKN among different groups, respectively. Correlation between various parameters was determined using the Pearson's or Spearman's correlation coefficient (r) test, when appropriate. All comparisons were two-tailed, and $P < 0.05$ was considered significant.

Results

Serum FKN concentrations between the two study groups. Serum FKN levels during AD flare ranged from 10-518 pg/mL (median=75 pg/mL). After resolution of the acute attacks, a significant decrease in these levels [34 (10-106) pg/mL] ($Z = .0$; $P < 0.001$) was observed. The healthy children had much lower serum levels [13(6-52) pg/mL] than the patients with AD, both during flare ($Z = 4.0$; $P < 0.001$) and quiescence ($Z = 1.7$; $P < 0.001$) (Figure 1) episodes.

Correlation of serum FKN levels with other clinical and laboratory variables. According to the SCORAD index of severity, 27(47%) and 30(53%) children with AD had a moderate and severe disease, respectively, whereas based on the objective SCORAD index, 17(30%), 21(37%), and 19(33%) had mild, moderate, and severe disease, respectively. The difference between the two groups was statistically significant for the total serum IgE and IL-31 levels ($P < 0.001$) (Table 1).

According to the objective SCORAD index of severity, the median serum FKN levels in the severe AD group [104 (26-518) pg/mL] was significantly higher than in the moderate group [46 (16-149) pg/mL] ($Z = 3.4$, $P < 0.001$). The FKN concentration in the moderate group was significantly higher than the corresponding values in the mild group [19 (10-57) pg/mL] ($Z = 1.9$, $P < 0.001$) (Figure 2).

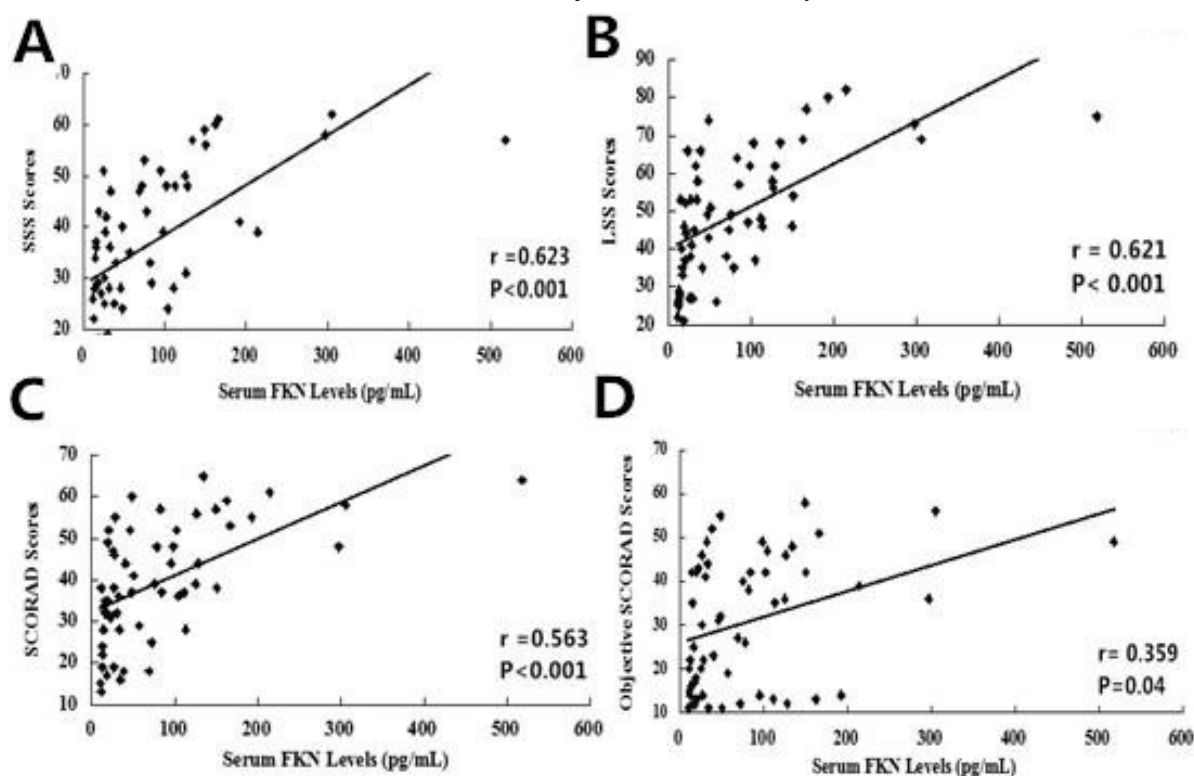


Figure 3. Correlation between serum FKN levels (pg/mL) with SSS, LSS, SCORAD, and objective SCORAD scores during disease flares in pediatric patients with AD.

Significant positive associations were observed between the serum FKN levels and each of the indices, SSS ($r=0.623$, $P<0.001$) (**Figure 3A**), LSS (**Figure 3B**) ($r=0.623$, $P<0.001$), SCORAD ($r=0.563$, $P<0.001$) (**Figure 3C**), and objective SCORAD ($r=0.359$, $P=0.04$) (**Figure 3D**), indicating that FKN expression increased with disease severity. Statistically significant differences were also observed in the serum FKN, IgE ($r=0.613$, $P<0.001$) (**Figure 4**), and IL-31 ($r=0.489$, $P=0.001$) (**Figure 5**) levels.

Discussion

The current study assessed the association between serum FKN levels and disease severity in pediatric patients with AD. To our knowledge, this was the first study to demonstrate that increased serum FKN levels were independently and positively associated with AD clinical severity by using different grading scores as well as serum levels of IgE and IL-31. These results indicate that serum FKN level may serve as a potential biomarker for clinical severity in patients with AD.

Atopic dermatitis (AD) is a highly pruritic chronic relapsing skin disease that is typically characterized by skin lesions with multifactorial pathophysiological aspects. The series gathering of memory or effector T cells represents a pivotal immunological role in both the initiation and amplification of AD lesions. The role of memory T cells with a Th2 phenotype in infiltration of the skin at the sites having atopic inflammation in the early phases of AD has been proven. However, chronic lichenified AD lesions have highly dominant and skin-infiltrating Th1 cells [26].

Chemokines are crucial mediators and regulators of leukocyte trafficking during immunosurveillance and inflammation [27,28]. Their involvement in the development and progression of inflammatory diseases has been the subject of intense investigations. The expression studies of distinct chemokine and adhesion receptor combinations have been useful in functionally defining the various leukocyte subsets, as best exemplified by distinct classes of T cells, including those involved in skin diseases [29,30]. FKN is the only chemokine in the CX3C

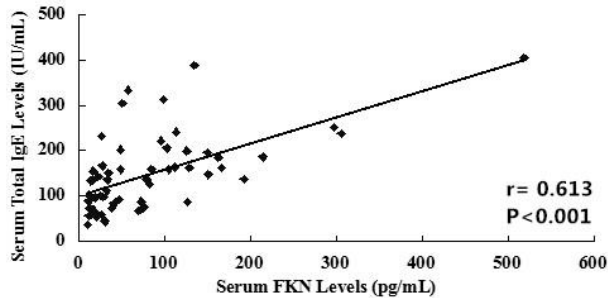


Figure 4. Correlation of serum FKN levels (pg/mL) with serum Total IgE levels (IU/mL) during disease flares in pediatric patients with AD.

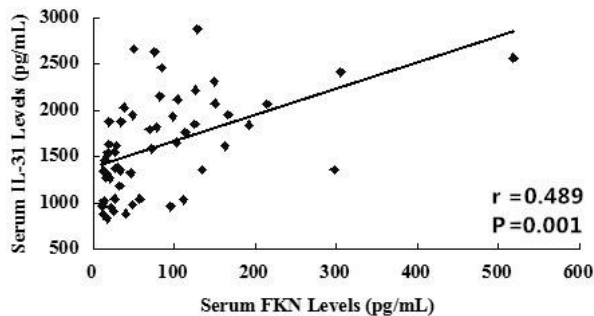


Figure 5. Correlation between serum FKN (pg/mL) and IL-31 levels (pg/mL) during disease flares in pediatric patients with AD.

class of chemokines and is unique in its capacity to be either membrane-tethered or released as a secreted protein [31]. Soluble FKN is secreted mainly by endothelial cells and is a chemoattractant for monocytes/macrophages, natural killer cells, T lymphocytes, and vascular smooth muscle cells, all of which express its receptor (CX3CR1) [32].

Unlike the unique Th2 pathology in allergic asthma, recent studies have shown that the involvement of FKN/CX3CR1 in the pathogenesis of AD is believed to probably result from its combined effect on both Th1 and Th2 cells [33]. This could be proven by using the mild inflammation model guided by epicutaneous sensitization in the absence of adjuvants instead of merely using models of highly polarized Th2 inflammation with adjuvants [16]. A previous study also demonstrated that CX3CR1-deficient and -proficient mice exhibited similar responses in a model of contact hypersensitivity/DTH to oxazolone [34]. Fraticelli and his colleagues have identified FKN and CX3CR1 as part of the chemokine-based amplification of polarized Th1 responses [35]. These may explain the earlier failure to detect FKN [35] in skin, and reinforce the high potential of FKN as therapeutic targets for controlling chronic allergic diseases rather than acute flares.

In the present study, we utilized the simple scoring system of costa (SSS), Leicester sign score (LSS), scoring atopic dermatitis (SCORAD) index, and objective SCORAD in order to assess the clinical severity of AD. Dermatologists have used these tools for the objective measurement of clinical factors measures during clinical trials and for selecting treatment strategies, [22-25].

We also examined serum IgE and IL-31 levels in this study. IgE-mediated hypersensitivity is one of the most important factors in the pathogenesis of AD [36]. Increased total and allergen-specific serum IgE levels are detected in more than 80% of patients with AD [37]. IL-31 is a novel T cell-derived cytokine that induces severe pruritus and dermatitis [38], and functions as a biomarker of severity in AD [39-40].

Similar to previous studies, the present study demonstrated significantly higher FKN levels among AD children than among the control group [18]. We also demonstrated the positive correlation between serum FKN levels and the SSS, LSS, and SCORAD indices as well as the objective SCORAD and total WOMAC scores. These results indicate that higher serum FKN concentrations were independently related to clinical severity in patients with AD.

Several limitations should be taken into account in this study. First, the sample size was not large enough for definitive conclusions. Second, we only focused our investigation on serum FKN levels, and examination of other chemokines may be more powerful and meaningful for illustrating their roles in AD. Lastly, we did not explore and discuss the FKN levels in adult AD despite the lower morbidity in children.

Collectively, this study revealed a significant elevation in the serum FKN levels of pediatric patients with AD compared to healthy controls and illustrated a significant positive correlation between serum FKN levels and the extent of clinical severity in pediatric patients with AD. To our knowledge, this study is the first to demonstrate the potential utilization of serum FKN as a novel biomarker for reflecting the disease severity of AD. Further investigations are needed in order to reveal the mechanisms underlying this correlation with the aim of developing effective pharmacological drugs to delay the progression of AD.

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