Differences in cytokine levels between Extrinsic and Intrinsic Atopic Dermatitis

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Abstract:

Atopic dermatitis (AD) is a common inflammatory skin disease characterized by several clinical , immunological and biochemical alterations . Comparing the patients with Extrinsic and Intrinsic forms of AD, we investigate the role of immunological markers in the pathogenesis of AD. Serum concentrations of IL-12, IL-13, IL-18 and IgE were measured using ELISA method and severity was detected by a special scoring system (SCORAD Index). Serum IgE and IL-13 concentrations were both elevated in the EAD group compared to IAD and control group, whereas, serum IL-12 and IL-18 concentrations were decreased in EAD group compared to IAD and control group, this may suggest a specific cytokine profile involved in each form of AD which need further investigation.

Introduction :

Atopic dermatitis is the commonest childhood skin disease. It is a chronic relapsing cutaneous inflammatory disorder associated with asthma, allergic rhinitis, increased IgE and eosinophilia (1). Atopic dermatitis usually starts at early age and is subjected to strong genetic predisposition, interlaping with the immunological and environmental factors (2).

Two forms of AD have been delineated, including an extrinsic (EAD) or allergic AD form, associated with high total serum IgE levels and the prescence of specific IgE for environmental and food allergens. The other form is the intrinsic (IAD) or non-allergic AD form, exhibits normal IgE values and absence of specific IgE (3). The clinical features of IAD include relative late onset, milder severity and Dennie-Morgan folds, but no ichthyosis vulgaris or palmer hyperlinearity. The skin barrier is perturbed in the EAD, but not IAD form (4).

Atopic dermatitis is well known as a Th_2-polarized disease. However,there have been reported some differences in systemic cytokine polarization between the two forms of AD. The EAD form mount a Th_2 skewing action showing high levels of IL-4, IL-5 and IL-13 while IAD form shows less Th_2 skewing stste linked with much lower levels of IL-4 and IL-13 or relative overproduction of

Th_1 cytokine IFN-α (5). Along with the elevation of IL-5 (6), eosinophil counts (7) and eosinophil cataionic protein levels (8) are increased in the EAD form .

Material and Methods

Study population :

This study was carried out at the consulting dermatologic clinic of Al-Kazymia teaching hospital-Baghdad. A total of 54 children patients with AD, 33 with EAD form (serum IgE > 100IU/ml), 21 patients with IAD form (serum IgE < 37 IU/ml), their ages ranged between (3 months-12 years). The clinical diagnosis of AD patients was confirmed by a dermatologist in the dermatologic clinic referred above . Only patients who fulfilled the criteria of Hanifan and Rajka, 1980 (9) and who had not used topical or systemic antimicrobial; drugs, corticosteroids or antihistamin for at least 2 weeks befor investigation, were included in the study. Healthy group consisted of 18 apparently healthy individuals with no symptoms or history of atopic dermatitis or other allergic or skin disease.

Clinical scoring system for severity of disease :

The SCORAD index was used, which included the assessment of objective signs (extent and severity) and subjective symptoms (pruritis and sleeploss), the last value complied on an analogue scale by the parents. Intensity items were: erythma, edema/population, oozing/crusts, excoriation, lichenification and dryness of uninvolved skin (0-3)points for each item. The final score was then calculated by using the following equation : A/5+7B/2+C (A= extent, B=intensity, C=subjective symptoms). A SCORAD index lies between 0-83 (10).

Measurement of serum total IgE, IL-12, IL-13 and IL-18 levels :

Venous blood was obtained from both patients and control groups. Blood samples (5 ml) were collected from each subject by vein puncture using disposable syringes in sterile tubes. Blood was allowed to clot at room temperature for 30 min. , and was then centrifuged for 10 min. at 4000 rpm, sera was separated and divided into several aliquots, frozen at -20C°, and thawed immediately prior to analysis of total IgE an interlukines. Total IgE levels were measured by direct enzume linked immonosorbent assay technique (ELISA) (Human,Germany). IL-12 p40 kit (Biosource,Belgium), IL-13 (Immunotech,France) and IL-18(MBL,Germany) levels were measured by ELISA method , the minimal detection levels of cytokines using this method were 2.0 pg/ml for IL-12, 1.5 pg/ml for IL-13, and 12.5 pg/ml for IL-18. Cytokine levels below the detection limits is considered as zero.

Statistical analysis :

Data on circulating IL-12,IL-13,IL-18 and IgE levels are presented as mean ± SEM. A paired t-test was used to compare the serum level of the previous

parameters between EAD and IAD . Statistical analysis was assumed for P value less than 0.05.

Results :

From the total AD patients, 62% were categorized as EAD patients, while 38% were IAD patients. The mean age of EAD group was 6.7 years with male : female ratio 17:16 , while in IAD was 3.1 years with male : female ratio 13:8 ,Table (1).

Table (1): Percentage, gender and mean age of study groups.

	Extrinsic AD (EAD)	Intrinsic AD (IAD)	Total AD	Control
No. %	33 (62%)	21 (38%)	54 (100)	18 (100)
M/F	17/16	13/8	30/24	10/8
Mean age	6.7	3.1	4.6	6.0

The mean SCORAD index for the EAD group was 32, and for the IAD group 27 showing milder severity in this group. The mean ± serum concentration of total IgE in EAD were increased 350 ± 23.5 IU/ml compared with IAD 21 ± 1.84 IU/ml and controls 35.2 ± 2.8 IU/ml ($p<0.01$), while serum IL-12 concentration were increased 248.7 ± 22.47 pg/ml in IAD compared to 216 ± 18.44 pg/ml in EAD and 146.4 ± 13.51 pg/ml in control group. IL-13 serum concentration was elevated in both EAD 18.4 ± 1.9 pg/ml and IAD 16.7 ± 1.07 pg/ml compared to 5.5 ± 0.62 pg/ml healthy control group, they were also significantly increased in EAD compared to IAD ($P<0.05$). The serum concentration of IL-18 was increased in IAD 184 ± 16.74 pg/ml compared to EAD 121 ± 14.58 pg/ml and healthy control 47.8 ± 3.16 pg/ml , they were also significantly increased in IAD compared to EAD ($p< 0.01$) , Fig (1).

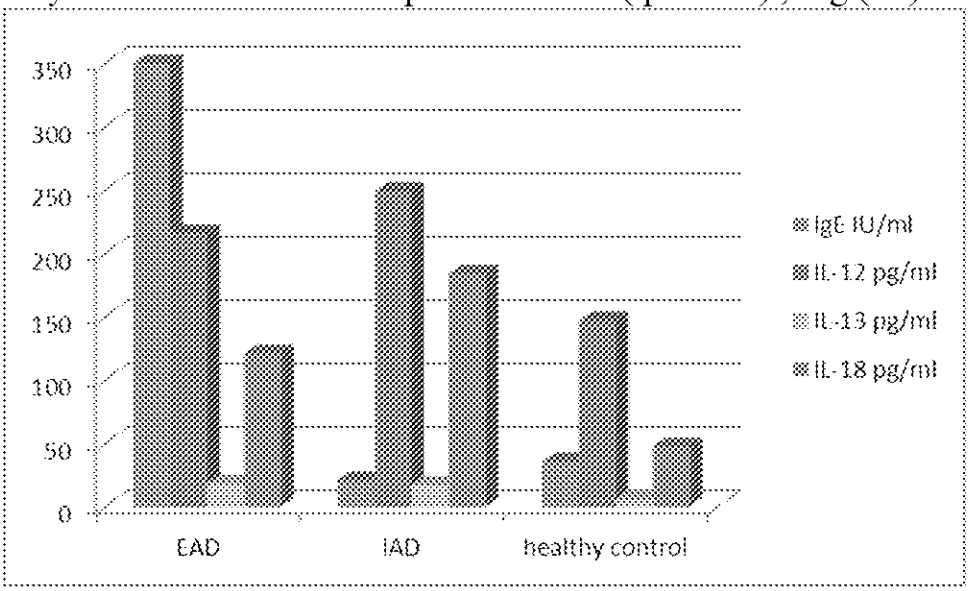

Figure (1) : Distribution of IgE,IL-12, IL-13, and IL-18 levels among EAD, IAD and control groups.

Discussion :

A majority of AD patients (70%-80%) have elevated serum IgE levels known as extrinsic AD, whereas (20%-30%) have low or normal IgE levels known as intrinsic AD (11). The results of this study comes to close percentage of 62% for EAD and 38% for IAD. It was found in hospital based studies the percentage of children with EAD ranged between 47%-75% in contrast, in population based studies the percentage of children with EAD ranged from 74%-78% (12) .

It was shown in this study that the majority of AD patients in the IAD group had a mean age of 3.1 years which suggest a more prevalence of infants and young children compared to the EAD group, mean age 6.7 years elderly children . Several reports on the prevalence may provide an implication that the IAD type is seen in higher frequencies in infancy (7). A study by a German group demonstrated that one third of child AD was the extrinsic one, and more common in females (13).

Although AD might be a syndrome caused by multiple etiologies (i.e., involving different combinations of multiple sets of cell types or multiple signaling pathways) , it is also possible that the presence of IAD might suggest that IgE is not essential for the development of human AD , at least for the initiation of the disease (14).

Further studies identified variants of the IL-13 encoding region , functional mutations of the promoter region of chemokine RNATES (17q11) and gain-of function polymorphisms in the α-sub unit of the IL-4 receptor (16q12) this could be linked to the incidence of IAD which occur without any IgE sensitization (15; 16) .

In this study , serum IL-12, IL-13, and IL-18 levels in EAD and IAD patients were evaluated among other main T-helper cell cytokines .To the best of our knowledge , this comparison was not previously investigated in human children in vivo.

The results of the current study show that serum concentration of total IgE was highly elevated in the EAD group , that is because this group depends mainly on the IgE – mediated mechanism in the disease, as it is documented by other studies (17 ; 11).

The current study showed nearly close levels of serum IL-13 concentration in both EAD and IAD patients . Differences in the capacity to produce IL-13 by T-cells might be responsible for the variation of IgE production , differences of IgE regulation may be explained by different genetic background in EAD and IAD patients, but also by varying exposure to environmental stimuli (18).

Although the EAD group had high serum IgE concentration and IAD group had low serum IgE concentration , IL-13 level in both groups revealed a high

level compared to control group , as it was supposed to be low in the IAD group corresponding the low IgE level. In addition ,there was a significant positive correlation between IgE levels and both SCORAD Index and IL-13 levels in EAD group ,Appendix VI-(1 and 7) compared to IAD group which was found to be not significant, Appendix VI-(2 and 8) . There can be multiple mechanisms for the development of IAD, the over expression of IL-31 , a cytokine produced by activated T-cells which may be involved in the mechanism of IAD , as it elevate the expression of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) (19).

Animal studies on transgenic mice showed that the inability of CD_4^+ T-cells to up-regulate CD40L expression upon stimulation might account for their inability to up-regulate the IgE level, which may be another mechanism of IAD (20). In comparison with previous phenotypic studies , CLA+ T-cells were enriched for expression of CD45RO, HLA-DR, CD40L and CD25, which are activated in EAD and regulate IgE by an IL-13 dominant cytokine pattern (21).

It has been postulated that the IAD could be a pure transitional form of AD in which genetic and environmental basis are essential for primary IAD, involving the skin barrier role in protecting against environmental stimuli (22).

The dysbalance between Th_1 and Th_2 immune response in AD may be elucidated by the detections of polymorphisms of the IL-18 gene resulting in Th_2 predominance (15 ; 23).

The present study found a statistical significant difference between the mean values of serum IL-18 and IL-12 in EAD and IAD patients . This implies the involvement of serum IL-18 pathogenesis of both EAD and IAD which was described in previous studies (24).

Studies suggested that AD development depends on IL-18 , develop dermatitis even in the STAT6-deficient background as cedar pollen dermatitis does not require STAT6 or IgE and skin lesions induced by Derf/SEB are normal in B-cell deficient mice (25). In the presence of IL-3 , IL-18 can directly stimulates basophiles and mast cells to produce their mediators in an IgE-independent manner (26), which may explain the pathogenesis of AD in the case of IAD.

Conclusion :

Both forms of AD exhibit a unique cytokine profile which can support the differentiation between them beside IgE levels , as well as their role in pathogenesis and initiation of disease symptoms.

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