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Gehan Fathi
Departments of Child Health
and Medical Biochemistry,
National Research Center,
Dokki, Cairo, Egypt

Increased Urinary Leukotriene E4 and its Correlation to Severity and Laboratory markers of Atopic Dermatitis in Children

Gehan Fathi, Khalid Saber, Fatma Shaaban and Daooud Fakhry

This study was meant to evaluate the importance of LTS in Atopic Dermatitis (AD) and to study the correlation of urinary LTE4 with disease severity and some commonly altered parameters in AD. The study included 30 children and adolescents diagnosed to have atopic dermatitis. Ten age and sex matched healthy children and adolescents were enrolled for comparison. They were subjected to clinical evaluation and measurement of urinary LTE4, absolute eosinophilic count, serum IgE, IL-4 and IL-5 in Peripheral Blood Mononuclear Cell Culture (PBMC) supernatant. The patients were categorized into mild (n = 5), moderate (n = 16) and severe (n = 9) AD subgroups. The study revealed a significant increase in absolute eosinophilic count, Urinary LTE4, serum IgE and IL-4 and IL-5 in PBMC culture supernatant in the patients as compared to controls. Moreover, urinary LTE4 levels were significantly increased in moderate and severe cases of AD as compared to the control group, whereas mild cases had levels that were comparable to controls. Urinary LTE4 levels were higher in severe (p<0.01) and moderate cases (p<0.05) when compared to mild cases. Significant positive correlations could be elicited between urinary LTE4 and PBMC IL-4, disease severity scale, absolute eosinophilic count and serum total IgE. However, urinary LTE4 could not be correlated statistically with PBMC IL-5. Elevation in urinary LTE4 excretion in AD patients was demonstrated reflecting increase production of cysteinyl LTs. Urinary LTE4 was correlated to clinical and laboratory markers of severity suggesting that it could be an easy, non invasive and objective prognostic test in AD. Trials of 5-lipoxygenase inhibitors and LT receptor antagonists as additional lines of therapy in AD could thus be suggested.

Key words: Atopy, atopic dermatitis, children, leukotrienes, urinary LTE4, IgE, IL, 4, IL, 5, Eosinophilia

INTRODUCTION

There is increasing evidence that the frequency of atopic diseases including atopic dermatitis has increased over the last few decades with prevalence rates ranging between 10-20%, (Leung and Bieber, 2003; Ben-Gashir *et al.*, 2004; Baron *et al.*, 2006). Atopic Dermatitis (AD) is a chronic relapsing inflammatory skin disease seen in patients with a personal or family history of atopy. Understanding the mechanisms that underlie AD has important clinical implications for the development of new management protocols for this illness (Hishinuma *et al.*, 2001).

Leukotrienes (LTs) are phospholipids derived from arachidonic acid in a pathway involving phospholipase 5- lipoygenase and 5-lipoygenase-activating proteins. The cysteinyl LTs (LTC₄, LTD₄ and LTE₄) are inflammatory mediators which are thought to play a role in the pathogenesis of Atopic Dermatitis (AD) although their exact cellular source is not specified (possibly eosinophils, basophils and mast cells, (Kagi, 2001). Urinary leukotriene E₄ (U-LTE₄) is a metabolite of LTC₄ and LTD₄ and is a marker of whole body cysteinyl-leukotriene production (Hon *et al.*, 2004; Øymar and Aksnes, 2005).

IgE-mediated mechanisms play a multifactorial role in the pathogenesis of AD. Serum IgE levels are elevated in 80-85% of patients and are considered an important hallmark of the disease (Cooper and Stevens, 2001). Eosinophilia is another important finding in the peripheral blood and plays an important role in the pathogenesis of AD as the eosinophils migrate from the blood into lesional skin and release cytotoxic mediators, (Leung, 1999). Th2 cytokines as IL-4 and IL-5 are usually elevated in peripheral blood of AD patients. Their elevation contributes to the elevated peripheral blood IgE level (Tang *et al.*, 1993; Baanfield *et al.*, 2001; Farrel *et al.*, 2001).

With this as a background we thought to investigate the role of urinary LTE₄ in AD and anticipate its possible correlation with clinical severity and other laboratory parameters as absolute eosinophilic count, serum IgE and IL-4 and IL-5 levels in PBMC culture supernatant.

MATERIALS AND METHODS

This study was conducted on 30 children and adolescents diagnosed to have atopic dermatitis according to the UK refinement of Hanifin and Rajka's diagnostic criteria for AD (Williams *et al.*, 1994). They were recruited consecutively from the outpatient clinics of the Pediatric Department of Cairo University Hospitals

during the period January to June 2005. They were 14 males and 16 females and their ages ranged between 3.7 and 11.2 years with a mean age of 7.4 ± 2.6 years. Atopic dermatitis was graded into mild, moderate and severe according to the grading system of Rajka and Langeland (1989). Accordingly 5 patients were considered to have mild, 16 had moderate and 9 had severe AD.

Inclusion criteria: Patients stopped systemic and topical treatments before the study for at least one month and two weeks, respectively. Antihistaminic were also stopped one week before the study and only topical emollients were used. Patients who concomitantly had any chronic systemic illness or dermatologic diseases apart from atopic dermatitis were excluded from the study.

Controls: Ten apparently healthy age and sex matched children and adolescents were included as controls. They were 5 males and 5 females and their ages ranged between 3.8 and 11.7 years with a mean age of 6.9 ± 2.8 years. They were enrolled after exclusion of a personal or family history of atopy or chronic systemic or dermatologic diseases.

An informed consent was obtained from parents of each subject.

Patients were subjected to the following:

- History taking.
- Clinical examination.
- Laboratory investigations:
 - Estimation of urinary Leukotriene E₄: measured by direct enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) and expressed in Picograms (pg) per milligram of creatinine. Urinary creatinine was determined using a creatinine test kit.
 - Estimation of serum levels of IgE was done using the ELISA technique, (Pathozme IgE, Omega, Diagnostic Limited, UK) (Clendenning *et al.*, 1973).
 - Measurement of levels of IL-4 and IL-5 in PBMC culture supernates: Five milliliter of venous blood were drawn aseptically into a disposable syringe containing heparin-free preservative to give a final concentration of 10 IU mL^{-1} of blood. In a sterile centrifuge tube, 5 milliliter blood were carefully layered over 3 mL sterile ficoll hypaque mixture and centrifuged for 15 min, followed by collection of mononuclear cells being washed twice in sterile complete RPMI 1640 (Gibco, Grand Island, NY, USA). It contained L- Glutamine, penicillin, streptomycin

and N-2Hydroxyethyl piperazine and was centrifuged for 10 min. The final cell pellet was gently tapped and re-suspended in a known volume of complete RPMI 1640 medium to which 200 µL of PHA was added as mitogen and incubated for 72 h at 37°C in 5% CO₂. The suspension was taken in a test tube, centrifuged and kept at 20°C till time of assay. Estimation of culture supernatant IL-4 and IL-5 was done by sandwich enzyme immunoassay technique (R and D Systems, Inc., McKinley Place NE, Minneapolis, USA) (Custer and Lotze, 1990).

- Determination of Eosinophilic count.

Statistical analysis: The data were analyzed statistically using SPSS statistical package version II using student t-test for independent samples as well as ANOVA (Analysis of Variance), post hoc and Pearson's correlation coefficient (r) tests. p<0.05 were considered significant.

RESULTS

The AD patients were found to have significantly higher levels of absolute eosinophilic count, urinary LTE4, serum IgE and IL-4 in PBMC culture supernatant fluid (p<0.001) as well as PB IL-5 (p<0.05) compared to the controls, Table 1.

Table 2 shows that urinary LTE4 levels were significantly higher in moderate as well as severe cases of AD than controls, whereas mild cases were comparable to controls. Urinary LTE4 mean level was also higher in severe (p<0.01) and moderate cases (p<0.05) as compared to mild cases. However, no significant difference could be

elicited between moderate and severe cases, (Fig. 1). The absolute eosinophilic count was significantly elevated in moderate (p<0.05) and severe (p<0.01) cases of AD as compared to controls. It was also significantly higher in severe compared to mild and moderate cases, while no statistically significant difference was found between mild and moderate cases or between mild cases and controls. The serum total IgE levels were significantly higher in moderate (p<0.05) and severe (p<0.01) cases of AD than the control group and were significantly increased in severe as compared to moderate and mild cases of AD. On the other hand, no statistically significant difference was detected between mild and moderate cases or between mild cases and controls. A highly significant elevation was found in the mean level of PBMC-IL-4 in moderate

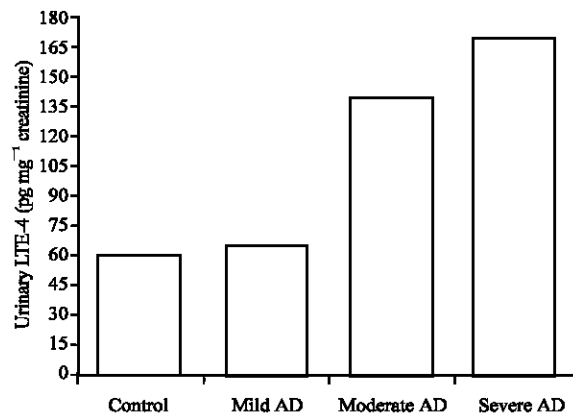


Fig. 1: Variation of urinary LTE-4 of enrolled subjects according to AD severity in comparison to control. F value by ANOVA test = 12.009 (p<0.01), AD: Atopic dermatitis, LTE4: Leukotriene E4

Table 1: Laboratory parameters of the studied sample

Laboratory parameters	AD patients (n = 30)	Controls (n = 10)	t-value	p-value
Urinary LTE4 (pg mg ⁻¹ creatinine)	136.13±62.18	57.10±12.46	6.58	<0.001
Absolute eosinophilic count (10 ⁹ L ⁻¹)	0.64±0.49	0.18±0.13	4.71	<0.001
Serum total IgE (µ mL ⁻¹)	1019.27±937.70	63.41±29.67	5.04	<0.001
PBMC-IL-4 (µ mL ⁻¹)	90.99±68.80	0.53±0.45	7.20	<0.001
PBMC-IL-5 (µ mL ⁻¹)	17.74±14.03	0.39±0.34	4.45	<0.05

AD: Atopic Dermatitis, IgE: Immunoglobulin E, IL-4: Interleukin-4, IL-5: Interleukin-5, PBMC: Peripheral Blood Mononuclear Cells

Table 2: Variation of laboratory parameters of enrolled subjects according to AD severity in comparison to controls

Laboratory parameters	Controls (A) (n = 10)	Mild AD (B) (n = 5)	Moderate AD © (n = 16)	Severe AD (D) (n = 9)	BvsA	BvsC	BvsD	CvsA	Cvs D	DvsA
Urinary LTE4 (pg mg ⁻¹ creatinine)	57.1±12.46	67.4±44.88	139.25±46.9	168.78±68.65	>0.05	<0.05	<0.01	<0.01	>0.05	<0.01
Absolute eosinophilic count (10 ⁹ L ⁻¹)	0.18±0.13	0.28±0.17	0.44±0.26	1.2±0.46	>0.05	>0.05	<0.01	<0.05	<0.01	<0.01
Serum total IgE (µ mL ⁻¹)	63.41±29.67	283.2±214.1	778.81±550.15	1855.67±1430.16	>0.05	>0.05	<0.05	<0.05	<0.05	<0.01
PBMC-IL-4 (µ mL ⁻¹)	0.53±0.45	23.7±12.3	63.73±35.82	176.83±45.52	>0.05	<0.05	<0.01	<0.01	<0.01	<0.01
PBMC-IL-5 (µ mL ⁻¹)	0.34±0.31	3.72±2.31	6.09±5.48	29.79±11.45	>0.05	>0.05	<0.01	>0.05	<0.01	<0.01

AD: Atopic Dermatitis, IgE: Immunoglobulin E, IL-4: Interleukin-4, IL-5: Interleukin-5, PBMC: Peripheral Blood Mononuclear Cells

and severe cases of AD as compared to controls and in severe cases as compared to mild and moderate ones. It was also significantly increased in moderate as compared to mild cases of AD. On the contrary, its level in mild cases was comparable to that of controls. The mean level of PBMC-IL-5 was significantly higher in severe cases as compared to controls and to mild and moderate cases of AD. However, moderate cases, mild cases and controls were comparable in their PBMC-IL-5 mean levels.

Urinary LTE4 could be positively correlated to disease severity scores ($r = 0.69$, $p < 0.001$), absolute eosinophilic count ($r = 0.41$, $p < 0.001$), serum total IgE and PBMC-IL-4. ON the other hand, urinary LTE4 did not correlate significantly to PBCM-IL-5 ($r = 0.3$, $p > 0.05$).

DISCUSSION

The mean level of urinary LTE4 was significantly increased in atopic dermatitis patients compared to the control group. The same observation was reported by Adamek-Guzik *et al.* (2002); Hon *et al.* (2004) and Øymar and Aksnes (2005). The enhanced urinary excretion of cysteinyl LTs can result from either enhanced synthesis or reduced hepatic elimination (Fauler *et al.*, 1993). Since all our patients suffered no hepatic problems, so the increase in urinary LTE4 levels actually reflects an enhanced synthesis of these metabolites. This supports the role of cysteinyl LTs in the pathogenesis of AD which is probably mediated through increased vascular permeability and dilating the skin blood vessels thus contributing to the inflammatory reaction in AD (Brain and Williams, 1990).

In contrast to the present findings Sansom *et al.* (1997), found no significant difference in urinary LT levels in AD patients compared to the control group. This might be due to sample to sample variability within an individual which thus necessitates evaluation of multiple samples or longer urine collection rather than a single sample collected during a short period (Asano *et al.*, 1995). This was taken into consideration in the present study where urine sample was taken after at least 6 h without voiding.

Regarding the severity of the disease it was found that urinary LTE4 was significantly higher in moderate and severe cases compared to the mild ones. Similarly, Miyoshi *et al.* (1999) and Hon *et al.* (2004) reported a higher urinary LTE4 concentration in patients with moderate- to- severe disease compared to mild cases with AD. On the other hand Øymar and Aksnes (2005) found that U-LTE4 was higher in children with only severe AD than in controls, whereas the levels in moderate and mild disease were similar to controls.

In the present study a significant positive correlation was found between the ULTE4 and disease severity

scores in AD. This finding was in accordance to many other investigators (Adamek-Guzik *et al.*, 2002; Miyoshi *et al.*, 1999; Hon *et al.*, 2004). On the contrary, Yamamoto *et al.* (1996) found that there was no correlation between the increased ULTE4 and disease severity in AD patients.

The current study revealed that the absolute eosinophilic count, total serum IgE, PBMC- IL4 and PBMC-IL5 were significantly increased in patients having AD than controls. These results are in agreement with other studies as those of Tang *et al.* (1993) and Mochizuki *et al.* (1998). We also observed that the aforementioned parameters were in general significantly related to grades of clinical severity which comes in accordance with some studies as those of Tang *et al.* (1993) and Mochizuki *et al.* (1998). However Kaminishi *et al.* (2002) stated that elevated levels of IL-4 in peripheral T-cells of patients with AD are not correlated with disease severity.

The above data confirm the importance of eosinophilia, increased serum IgE, IL-4 and to a lesser extent IL-5 in the pathogenesis of AD. Moreover, these data support the immunoregulatory abnormalities seen in patients with AD that lead to increased expression of Th2 cytokines as IL-4 and IL-5 which in turn stimulate the IgE synthesis by B cells and are chemotactic to eosinophils with a concomitant decreased of IFN- γ (Leung, 1999). The eosinophils infiltrate the affected skin in response to IL-4 and IL-5 and they show enhanced response to these cytokines especially IL-4. The number of eosinophils and the quantity of their products as cationic proteins and major basic proteins are found in previous studies to be increased in the peripheral blood of patients with AD and this increase was proved to be correlated with the clinical severity of the skin disease (Monta *et al.*, 1995). IL-4 and IL-5 in the AD lesions, by attracting the inflammatory cells such as eosinophils and macrophages to the area, they prolongs inflammation by preventing their apoptosis. IL-4 can also lead to steroid resistance by inducing abnormalities in glucocorticoid receptor binding affinity (Nimmagadda *et al.*, 1997). Previous studies proved that about 85% of patients with AD have increased amount of total IgE. Meanwhile, the mean levels of IgE were proved to increase in amounts with increasing severity and extent of dermatitis and patients with high levels were proved to have poorer prognosis (Uehara, 1989; Sampson, 1997).

In the present study, a significant positive correlation was found between urinary LTE4 and the serum total IgE. This result is in accordance with that of Hishinuma *et al.* (2001) while Adamek- Guzik *et al.* (2002) did not found such correlation. Moreover, we reported the presence of a significant positive correlation between urinary LTE4 and both the absolute eosinophilic count and the PBMC-IL-4, while Adamek-Guzik *et al.* (2002) reported

this correlation with eosinophilic count only. These findings suggest that the increased levels of urinary LTE4 in AD might be due to increased total cellular response to stimulation with IgE which is in turn triggered by IL-4 and also suggest that eosinophils might be the source of increased cysteinyl LTs in AD and such increase in eosinophils could be due to stimulation by IL-4 (Yamamoto *et al.*, 1996). On the other hand, PBMC IL-5 showed no significant correlation with urinary LTE4.

CONCLUSION

This study demonstrated an increase in urinary LTE4 excretion in AD patients reflecting increased whole body production of cysteinyl LTs. Moreover, urinary LTE4 in AD correlated positively with disease severity making it a simple, non invasive, objective prognostic test in AD patients. Its correlation with eosinophilic count, IgE and IL-4 suggests an IgE dependent mechanism for its release and that eosinophils might be its possible cellular source. We thus recommended clinical trials on the effect of 5-lipoxygenase inhibitors and LT receptor antagonists as additional or alternative lines of therapy in AD.

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