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Original Article

Increased lipoxin B4 levels in children with atopic dermatitis

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Abstract

Background Atopic dermatitis (AD) is the most common chronic inflammatory skin disease in the pediatric population. The pathophysiology of AD is complex and not clearly understood. The role of lipoxin B4 (LXB4), an anti-inflammatory mediator, has not been sufficiently investigated in children with AD to our knowledge.

Objective To compare the levels of serum LXB4 between children with AD and healthy controls.

Methods Three groups of children were enrolled in this study: a SPT-Pos group (skin prick test positive 21 subjects with AD), a SPT-Neg group (skin prick test negative 22 subjects with AD), and a control group (23 healthy subjects). Subjects' serum LXB4 levels of were measured with an ELISA technique. Also, eosinophil counts and total immunoglobulin E (IgE) levels were compared among all groups.

Results We observed significantly higher LXB4 levels in AD patients than in controls. Also, LXB4 levels were significantly higher in the SPT-Pos group than in the SPT-Neg group and control group. However, no significant difference was observed between the SPT-Neg and control groups.

Conclusion The LXB4 may have an anti-inflammatory mediator role in the pathogenesis of AD in children. The LXB4-associated pathways may be considered in the development of novel therapeutic approaches for the treatment of patients with AD. [**Paediatr Indones. 2019;59:271-5; doi: http://dx.doi.org/10.14238/pi59.5.2019.271-5**].

Keywords: *lipoxin B4; atopic dermatitis; LXB4; skin prick test*

Atopic dermatitis is one of the most common chronic inflammatory skin diseases affecting 15-20% of children worldwide.[1] The pathogenesis of AD is not clearly understood. However, complex interactions between environmental exposure and genetic factors are important in the pathogenesis of AD.[2] The role of eosinophils in the pathogenesis of AD also has not been clearly understood. It seems possible that eosinophils contribute to host defense against invading microbes through the defective skin barrier by generating eosinophil extracellular DNA traps which regulate immune responses.

Approximately 80 % of AD patients have increased total and specific IgE to allergens, especially food allergens. Atopic dermatitis is the first manifestation of allergic march preceding respiratory allergic diseases, such as asthma and allergic rhinitis in most children.[3] Recently, a study that focused on

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resolution of inflammatory processes gave rise to the identification of anti-inflammatory mediators such as lipoxins (LXs, i.e., LXA4 and LXB4).[4] The LXs are produced by membrane phospholipids during cell–cell interactions via 15-lipoxygenase and 5-lipoxygenase enzymes. As an anti-inflammatory mediator, LXs have been associated with some chronic inflammatory diseases, such as rheumatoid arthritis, sarcoidosis, and asthma.[5] In allergic diseases, the presence of LXs have been demonstrated in the upper and lower respiratory tracts in nasal lavage fluid and in bronchoalveolar lavage fluid, respectively.[6,7] In an animal study, LXB4 significantly decreased airway inflammation, nasal mucosal leukocytes as well as decreased IgE-mediated mast cell and eosinophil degranulation. Also, LXB4 decreased eotaxin-dependent eosinophil chemotaxis and expression of type 2 cytokine receptors which have some different roles in the pathogenesis of AD.4 A role for LXB4 has not been investigated in patients with AD, to our knowledge.

The aim of this study was to investigate serum LXB4 levels among SPT-Pos, SPT-Neg, and healthy controls.

Methods

This prospective study was performed at the Maternity and Children's Hospital, Batman, Turkey. Atopic dermatitis was diagnosed based on standard Hanifin and Rajika criteria's in this study.[8] A total of 66 children aged 3 months - 3 years were enrolled in the study. Forty-three patients with AD were divided into either the SPT-Pos (21 subjects) or the SPT-Neg group (22 subjects). The control group comprised 23 healthy children. Children with infectious diseases such as upper or lower airway infections within the four weeks prior to the study were excluded. At presentation, children underwent skin prick testing (SPT) on the upper back. The SPT kit (*Allergopharma*, Reinbek, Germany) tested 10 antigens including two aero-allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cow's milk, egg white, egg yolk, soy, nut, peanut, wheat, and tuna), with positive and negative controls, histamine and serum physiologic, respectively. Reactions were considered to be positive if an induration >3 mm was observed, compared to the negative control. Total IgE levels were measured with a nephelometric method. Eosinophil counts were determined from Coulter counter leukocyte measurements. Serum LXB4 levels were measured using an LXB4 ELISA kit (*E2264Ge, EIAab Company*, United Kingdom), according to the manufacturer's instructions

Data were analyzed using *SPSS version 22* software (*IBM*, USA). Shapiro-Wilk test was carried out to determine the normality of data distribution and revealed abnormal data distributions for LXB4 levels, total IgE, and eosinophil counts ($P<0.05$). Hence, median values and minimum-maximum ranges were determined, and all groups were compared by Kruskal-Wallis test. We also used a post-hoc Bonferroni modified Mann-Whitney U test for binary comparison. Correlation analysis was performed with Spearman's correlation test. Results with P values <0.05 were considered to be statistically significant.

This study was approved by the ethic comity of Batman District State Hospital in Batman, Turkey. Written informed consent was acquired from the parents of all participating children

Results

Patients' characteristics and median total IgE, eosinophil counts, and LXB4 values are shown in **Table 1**. Of 43 patients with AD, 21 in the SPT-Pos group had positive SPT, median age 8 months (range 3-24 months), with 9 males and 12 females. Of the SPT-Pos patients, 10 had egg white allergy, 9 had cow's milk allergy, and 2 had multiple food allergy (one had cow's milk and egg white; one had egg white and wheat). The remaining 22 with AD in the SPT-Neg group had negative SPT, median age 11 months (range 3-24 months), with 10 males and 12 females. The control group had 23 healthy children, median age 12 months (range 3-34 months), with 13 males and 10 females. Median IgE levels were significantly increased in AD patients (SPT-Pos + SPT-Neg groups) compared to the control group [210 (range 50-843) IU/mL vs. 140 (range 3-450) IU/mL, respectively; ($P=0.015$)]. However, no significant difference in IgE levels was found between the SPT-Pos and SPT-Neg groups [230 (range 50-710) IU/mL vs. 204 (range 58-843) IU/mL, respectively; ($P=0.86$)]. Median eosinophil counts were also significantly

Table 1. Patient characteristics and median values of age, total IgE, eosinophil count, and LXB4

Characteristics	SPT-Pos group n=21	SPT-Neg group n=22	Control group n=23
Gender			
Female	12	12	10
Male	9	10	13
Median age (range) months	8 (3-28)	11 (3-24)	12 (3-24)
Median total IgE (range), IU/mL	230 (50-710)	204 (58-843)	140 (3-450)
Median eosinophil (range), n/mL	420 (120-1030)	280 (60-710)	176 (4-450)
Median LXB4 (range), pg/mL	1.55 (0.96-3.49)	1.11 (0.46-1.80)	0.80 (0.28-1.99)

higher in the AD groups than the control group [370 (range 60-130) /mL) *vs.* 176 (range 4-450) / mL), respectively; (P=0.001)]. As expected, the median eosinophil count in the SPT-Pos group was higher than in the SPT-Neg group [420 (range 120-1030)/mL *vs.* 280 (range 60-710)/mL, respectively; (P=0.021)]. Furthermore, median serum LXB4 level was significantly higher in the AD patients (SPT-Pos + SPT-Neg groups) than in the control group [1.43 (range 0.46-3.49) pg/mL *vs.* 0.80 (range 0.28-1.99) pg/mL, respectively; (P=0.004)]. In binary comparisons among the groups, serum LXB4 level was higher in the SPT-Pos group than in the SPT-Neg group [1.55 (range 0.96-3.49) pg/mL *vs.* 1.11 (range 0.46–1.80) pg/mL, respectively; (P=0.002)]. Also, the LXB4 level in the SPT-Pos group was significantly higher than in the control group [1.55 (range 0.96-3.49) pg/mL *vs.* 0.80 (range 0.28-1.99) pg/mL, respectively; (P=0.0001)]. No significant difference in the median LXB4 was observed between SPT-Neg and control groups (P=0.26) Also, a positive correlation was observed between LXB4 levels and eosinophil counts in all groups (r=0.316, P=0.010) (**Figures 1** and **2**).

Discussion

Atopic dermatitis, allergic rhinitis, and asthma are common allergic conditions with high morbidity. To our knowledge, no one has investigated a possible role of serum LXB4 in the pathogenesis of AD in children. In our study, serum LXB4 levels were significantly higher in AD patients than in controls, especially in the SPT-Pos group. Also, serum LXB4 levels were positively correlated with eosinophil counts. Chronic inflammation is one of the most

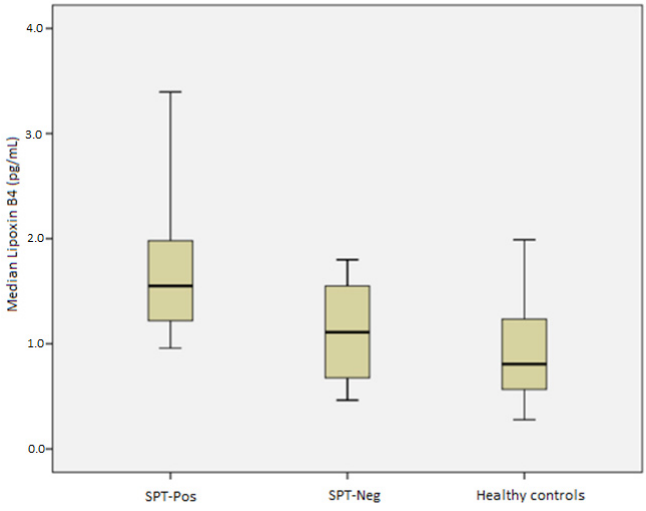

Figure 1. Increased LXB4 in children with atopic dermatitis

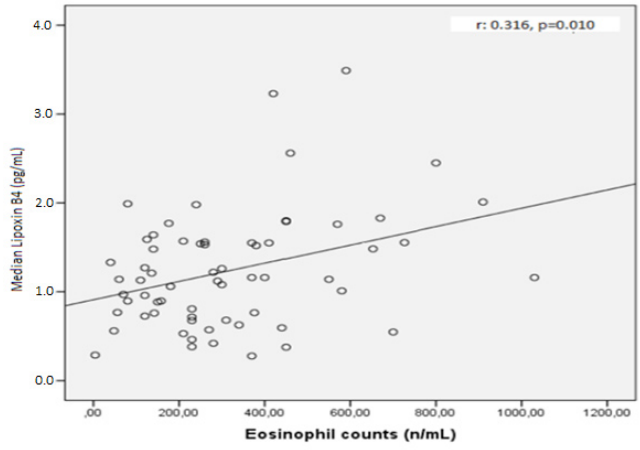

Figure 2. Positive correlation between LXB4 levels and eosinophil counts

important characteristics of patients with AD. At sites of inflammation, LXs are synthesized from membrane phospholipids and have anti-inflammatory effects.[9] The LXA4 blocks histamine release from mast cells and decreases the degranulation of azurophilic granules from neutrophils.[10-11] The LXB4 is also a product of endogenous arachidonic acid metabolism which has anti-inflammatory effects.[12] In a recently reported animal study on allergic airway diseases, LXB4 reduced leukocyte infiltration and mucus secretion in the nasal mucosa. Also, LXB4 decreased degranulation of mast cells and eosinophils, in addition to reducing serum specific IgE levels, eosinophil chemotaxis, and cytokine release from mast cells following IgE-mediated activation. Consequently, the authors concluded that LXB4 regulates allergic inflammation as an anti-inflammatory mediator.[4] In our study, serum LXB4 levels were higher in AD patients than in controls, which suggests a potential role of LXB4 in the pathogenesis of AD. In allergic diseases, different results have been reported with regards to LXs, especially LXA4. A previous study reported that serum LXA4 levels were lower in wheezy infants than in controls.[13] In contrast, increased LXA4 levels were reported in exhaled breath condensate in asthmatic children, which was in parallel with our results.[14] Also, higher LXA4 concentrations were found in asthmatic patients than in control subjects in induced sputum.[9] In an animal model of asthma and allergic rhinitis, LXB4 had an anti-inflammatory effect on allergic mucosal inflammation.[4] Moreover, in a previously published human study, local LXA4 application improved the severity eczema scale score, eczema area and severity index, and dermatologic quality of life index for infants, with effects similar to local corticosteroid (mometasone) treatment of children with AD. In that study, AD could be temporarily controlled and effectively treated with an LXA4 analog.[15]

In conclusion, the higher levels of LXB4 seem to reflect response to chronic inflammatory processes in patients with AD. There is no curative therapy for chronic allergic inflammation in patients with AD. For this purpose, new therapeutic approaches are needed to treat allergic diseases. The LXB4 and related compounds may provide novel therapeutic approaches for the topical treatment of human skin diseases such as AD.

Conflict of interest

None declared.

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