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ARAŞTIRMA RESEARCH ARTICLE

Evaluation of serum RANTES levels in childhood asthma

Çocukluk çağı astımında serum RANTES düzeylerinin değerlendirilmesi

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ABSTRACT

Objective: RANTES is considered to play an important role in various allergic disorders since it is a potent chemoattractant for inflammatory cells such as eosinophils, memory T cells and monocytes. The aim of this study is to determine serum RANTES levels and to assess whether or not it varies in acute attack in the children with asthma.

Materials and Methods: Serum RANTES levels were measured in 16 asthmatic children (2 to 14 years of age, median 4.5 years) longitudinally during asthma attacks and two weeks later by ELISA technique. Ten healthy age-matched children without any atopic and infectious disease served as controls.

Results: There was no significant difference in serum levels of RANTES between asthmatic patients with acute attacks and controls. Two weeks following the acute attack, the serum RANTES levels were higher than the controls, however the difference was insignificant. When we compared the serum RANTES levels of asthmatic patients during acute attack and two weeks following the acute attack, the serum RANTES levels were elevated in 11 asth-

ÖZ

Giriş: İnflamatuvar hücreler için kemoatraktan bir molekül olan RANTES, değişik allerjik hastalıklarda rol oynar. Bu çalışmanın amacı, çocukluk çağı astımında serum RANTES düzeylerini değerlendirmek ve akut astım atağındaki değişimini incelemektir.

Gereç ve Yöntem: Serum RANTES düzeyleri yaşları 2-14 yıl arasında olan 16 astımlı çocukta (median yaş: 4.5 yıl) akut astım atağı sırasında ve astım atağından iki hafta sonra ELISA yöntemiyle ölçüldü. Yaş grubu benzer 10 sağlıklı çocuk da kontrol grubunu oluşturdu.

Bulgular: Astımlı çocukların atak sırasında ölçülen serum RANTES düzeyleri kontrol grubu ile benzerken, atak sonrası ikinci haftada kontrol grubundan yüksek bulundu (p> 0.05). Astımlı hastaların 11'inde atak sonrası serum RANTES düzeyleri yükselirken, dördünde düşme saptandı, birinde ise değişiklik gözlenmedi. Astımlı hastalarda gözlenen bu değişiklik istatistiksel olarak anlamlı bulundu.

Sonuç: Akut astım atağı sonrası ikinci haftada gözlenen serum düzeylerindeki yükselme eğilimi, RAN-

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matic patients, decreased in four, and remained unchanged in one. This variation occurring among asthmatic acute attack versus stable asthmatic children was statistically significant ($p< 0.05$).

Conclusion: The up-regulation in the production of RANTES detected in stable asthmatic children two weeks after the acute attack suggests that RANTES is a mediator in the pathogenesis of childhood asthma.

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Key words: Asthma, RANTES, childhood

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TES'in çocukluk çağı astımının patogenezinde rol oynayabilen bir molekül olabileceği savını desteklemektedir.

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Anahtar kelimeler: Astım, RANTES, çocukluk

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INTRODUCTION

During recent years, a number of chemokines have been identified in asthmatic inflammation and reactive airway responses[1-3]. RANTES (regulated on activation, normal T-cell expressed and secreted) was the first chemokine identified as a potent chemoattractant and RANTES might be directly involved in the late asthmatic reaction[2]. RANTES is a Cys-Cys (CC) chemokine shown to be a potent chemoattractant for memory T cells, eosinophils, basophils, monocyte/macrophages and mast cells, and considered to play an important role in various immune and allergic disorders[1-3]. Since asthma is characterized by eosinophilic airway inflammation, it is possible that RANTES may be involved in the recruitment of eosinophils to the airways[4,5]. Indeed, increased RANTES levels have been demonstrated in bronchoalveolar lavage fluid, sputum, and plasma from adult asthmatic patients[2,4,6,7]. It has been measured in various body fluids and tisues[4,6-9]. Among the few studies measuring soluble (plasma,serum) RANTES levels in the literature, only four were on asthmatic children[4,8-11]. However, only one measuring the serum RANTES levels in asthmatic children with acute asthma attack was found.

Although a few studies evaluated the soluble (plasma, serum) RANTES levels in asthmatic children, it is controversial that serum RANTES levels are increased or not in asthmatic children with acute attack and also during stable period of the disease[8-11]. The aim of this study is to determine serum RANTES levels and to assess whether or not it varies in acute attack in the patients with asthma.

MATERIALS and METHODS

Study Population

Sixteen asthmatic children, under follow-up with mild/moderate persistent asthma who have been admitted for treatment of acute asthma attack were included in the study. The characteristics of the study group are summarized in Table 1. The diagnosis of asthma was based on the criteria of International Pediatric Asthma Consensus Group Reports[12]. The asthmatic patients had neither an asthma attack nor any infection for at least four weeks, and had not been on systemic corticosteroid (CS) therapy or immunotherapy until the onset of the disease. None of the subjects were taking long-acting beta-agonist, ipratropium bromid, antihistamines, or non-steroidal anti-inflammatory drugs. They also have not been taking systemic steroids and/or immunotherapy during the study.

Acute asthma diagnosis was made in the presence of tachypnea, ronchi, and prolonged expiration and wheezing during physical examination. After the treatment for asthma attack, the patients were treated with inhaled corticosteroids during the study.

The atopic status of these patients was defined by positive skin-prick tests to extracts of

Table 1. Demographic characteristics of asthmatic children and the control group

	Asthmatic children	Control group	p
Age (year) (mean ± SD, median)	6 ± 3.7 (4.5)	6 ± 2.75 (5)	> 0.05
Age distribution	2-14	3-11	> 0.05
Gender (male/female)	9/7	5/5	> 0.05
Atopic/non-atopic	8/8	None	
Inhaler corticosteroid use (n)	7/16	None	

common aeroallergens and/or serum specific immunoglobulin (Ig) E by CAP System (Pharmacia, Sweden).

Ten healthy age-matched children without having any atopic disease and infectious disease during the last four weeks served as controls. All subjects in this study provided written informed consent using documents approved by local ethical committee.

Study Measurements

5 mL of peripheral venous blood was collected from asthmatic patients in the acute and the stable phases, after two weeks of treatment, and also from the control subjects. Blood samples were obtained from the antecubital vein and the serum was separated from the cells within two hours and stored at (-20°C) until the time of the assay. Serum RANTES levels were measured by enzyme-linked immunosorbent assay (ELISA) (Biosource International Inc., Catalog no: KHC1032/ KHC1031, California, USA). The minimum detectable level of RANTES is 3 pg/mL. Intra-assay cost variance percent (CV%) of the kit is given as 2.4-4.7 and inter-assay CV% is given as 3.6-8.2 respectively.

Statistical Analysis

SPSS 9.0 statistical pocket program was used for evaluation. Characteristics of the patients and control group were compared by Mann-Whitney U test, Fisher's Exact test and Kruskall-Wallis one way Anova test, and t test were used for within-group comparisons. p-values of less than 0.05 were considered statistically significant.

RESULTS

Characteristics of asthmatic children and the control group enrolled in this study are summarized in Table 1. The serum RANTES levels of two groups are shown in Table 2. There was no significant difference in serum RANTES levels between asthmatic children at acute attack (range: 19750-42250 pg/mL) and controls (range: 16750-44750 pg/mL). The serum RANTES levels of the asthmatic children two weeks following the acute attack (range: 23750-70250 pg/mL) were higher than during the acute attack and those of the control subjects. However, the difference between clinical settings (acute attack versus stable) was not found to be significant. When the serum RANTES levels of

Table 2. The serum RANTES levels of two groups

	Asthmatic patients (acute attack) (n= 16)	Asthmatic patients (two weeks following the acute attack) (n= 16)	Control group (n= 10)
Serum RANTES levels (pg/mL) (X ± SD)	30.000 ± 7.655	37.047 ± 13.428	29.230 ± 9.171
(median)	28.625	33.250	27.375

asthmatic patients during acute attack and two weeks following acute attack were compared with paired t test within-group comparison, they were elevated in 11 asthmatic children, decreased in four, and remained unchanged in one (Figure 1). This variation occurring in asthmatic children was statistically significant ($p< 0.05$).

The serum RANTES levels were not found to be related to the age and gender of the controls. Similarly, no correlation was found between serum RANTES levels and the age and gender of the subjects with acute attack and the ones with stable asthma, two weeks after the acute attack. Seven out of 16 patients were receiving inhaler corticosteroid (ICS) treatment (200-400 µg, budesonide). There were no differences in serum RANTES levels between the patients on or off ICS treatment, both in the acute and stable periods of the disease. Also, the serum RANTES levels was not correlated with atopy ($p> 0.05$).

DISCUSSION

During recent years, a number of chemokines (eotaxin, MCP, MIP, etc.) have been identified in human asthma. Their production appears to be related to the asthmatic inflammation and reactive airway responses[2,3,11,13-16]. RANTES was the first chemokine identified as a potent eosinophil attractant and the levels of RANTES correlated with eosinophil numbers in BAL fluid, suggesting that this chemokine might be directly involved in the recruitment of eosinophils that characterizes the late asthmatic reaction[2]. It has been measured in various body fluids and tisuues[4,6-9,13,17-19]. Among the few studies measuring soluble (plasma, serum) RANTES levels in the literature, only four were on asthmatic children[4-11,20]. However, only one measuring the serum RANTES levels in asthmatic children with acute asthma attack was found. The results of the present study were compared with these on adult asthmatic patients and stable asthmatic children[4,8-10,18]. This study documented that the serum RANTES levels were elevated in asthmatic children during the stable period. The results have also shown an up-regulation in the production of serum RANTES in asymptomatic asthmatic children. This variation was not associated with the age, gender, atopy and inhaler corticosteriod use. All of these findings are consistent with an association between RANTES production and different clinical settings of pediatric asthma which allowed us to accept that serum RANTES is a mediator of asthmatic responses in childhood[8-10].

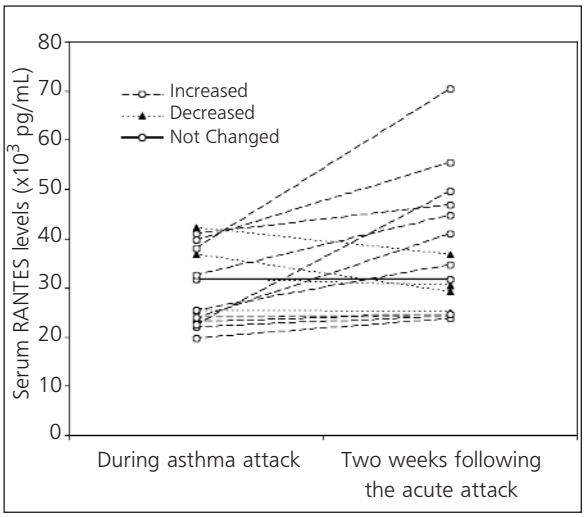

Figure 1. The up-regulation of RANTES levels in asthmatic children.

Hsieh et al. conducted the first study measuring soluble RANTES levels in children[10]. They investigated the plasma RANTES levels in 25 patients newly diagnosed asthma, 25 asthmatic patients with good response to immunotherapy, 25 asthmatic patients with no response to immunotherapy, 25 asthmatic patients with acute asthma attack, and 13 healthy children. They reported that successful immunotherapy decreased the production of chemokines after stimulation with phytohemagglutinin and mite allergen in vitro. In accordance with the results of our study, they found no statistical significance in plasma RANTES levels between asthmatic children with acute attack and healthy controls.

Chihara et al. evaluated the plasma RANTES levels of 12 adult asthmatic patients and 15 healthy controls[4]. They reported significantly higher plasma RANTES levels in subjects with acute asthma as opposed to controls while plasma RANTES levels were similar in asymptomatic asthmatic patients and controls. Plasma RANTES levels of 7 atopic and 5 non-atopic patients with acute attack were not found to be statistically different. Similarly, our findings pointed out to insignificant levels of serum RANTES between asymptomatic asthmatic children, asthmatic children with acute attack and controls, and no correlation with atopy existed.

In a similar study measuring the serum RANTES levels in pediatric asthmatic children was performed by Boznanski et al., between the serum RANTES level and exercise induced stimulation which can reveal acute attack in asthmatic patients[8]. They observed decreased serum RANTES levels in children with positive or negative results of the test. These findings suggest that serum RANTES levels decreased in the acute asthmatic attack just like in the exercise induced test. The results of our study are a mirror image of the study performed by Boznanski et al.[8].

Kokuludag et al. showed no statistical significance in the serum RANTES levels between adult asthmatic patients during or after acute attack and the controls[18]. In addition, the serum RANTES levels were found to be higher after, than during the acute attack and the control levels in this study[18]. In another study, serum RANTES levels were found to be significantly higher in asymptomatic children with asthma and recurrent wheezing than in healthy controls[9]. The higher RANTES levels in asymptomatic asthmatic patients found in such studies and our study suggest that increased serum RANTES levels in stable asthma may be an indicator of an asthmatic inflammation[9,18].

It has been shown that eosinophil-rich bronchial inflammation was a prominent feature of all asthmatics even in the mild form of the disease[9,17,20]. The role of RANTES in this inflammation is likely to be important at the level of the local tissue microenvironment rather than the systemic[1]. In addition to the binding of many receptors such as CCR1, CCR5, CCR3, CCR4, RANTES also binds the Duffy antigen receptor for chemokine (DARC) on the endothelial cells[21,22]. Consequently, it becomes rapidly cleared from circulation and immobilized thus increasing the local concentration of RANTES in the inflammation area[21,22]. Based on our findings, we thought that higher RANTES levels detected in asymptomatic asthmatic children after an acute attack might be an indicator of an asthmatic inflammation. Inversely, the RANTES levels in asthmatic children with acute attack, which was found to be lower than in stable asthmatic children may be due to recruitment of RANTES into the asthmatic airways. The elevated RANTES levels after the treatment of acute asthma attack support these speculations.

The elevated RANTES levels found in stable asthmatic children in the present study suggest a possible role of RANTES in the pathogenesis of childhood asthma. However, this also calls attention to the necessity of performing further studies in which both the soluble and tissue RANTES levels are measured to establish the role of RANTES in asthmatic inflammation.

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