

The prognostic importance of neuropeptides and indices of the immune system in children with allergic asthma

Allerjik astımlı çocuklarda nöropeptidlerin prognostik önemi ve immün sistemin indeksleri

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ABSTRACT

Objective: It has been revealed that substance P and vasoactive intestinal peptides (VIP) have important roles in the neural control of bronchial tone. In this study, we aimed to investigate the levels of substance P and VIP as well as of some cytokines, complements and immunoglobulins in children with moderate to persistent allergic asthma in comparison with values in healthy children.

Materials and Methods: The study consisted of 80 children with allergic asthma and 15 healthy age-matched controls. A peripheral blood sample was obtained from each group. Serum levels of substance P and VIP as well as interleukin (IL)-2, IL-12, IgA, M, G and E, NBT-test, and C3-C4 were determined by solid phase ELISA.

Results: Substance P level increased and VIP levels decreased with increasing severity of asthma. A dysregulation in cytokines, immunoglobulins and complement levels was also detected.

Conclusion: Differences in the levels of neuropeptides, cytokines and immune system parameters increased as the severity of asthma increased. Demonstration of dysregulation in the immune system might also predict allergic asthma in children with asthma.

ÖZET

Giriş: Substans P ve vazoaaktif intestinal peptid (VIP)'in bronş tonusunun nöral kontrolünde önemli roller bulunmaktadır. Bu çalışmada, sağlıklı çocuklarla karşılaştırmalı olarak orta-ağır persistan astımı olan allerjik astımlı çocuklarda serum substans P, VIP düzeyleri ile bazı sitokin, kompleman ve immünglobulin düzeylerinin araştırılması hedeflenmiştir.

Gereç ve Yöntem: Çalışma allerjik astımı olan 80 çocuk ve yaş uyumu olan 15 sağlıklı kontrolü kapsamıştır. Her bir çalışma grubundan periferik kan örneği alınmıştır. Serum interlökin (IL)-2, IL-12, IgA, M, G, E, NST-testi, C3 ve C4 düzeyleri değerlendirilmiştir.

Bulgular: Astım şiddeti arttıkça substans P düzeyi daha çok artmış ve VIP düzeyi daha çok azalmıştır. Sitokin, immünglobulin ve kompleman düzeylerinde bir regülasyon bozukluğu saptanmıştır.

Sonuç: Hastalığın şiddeti arttıkça, nöropeptid, sitokin düzeyleri ve immün sistem parametrelerindeki farkların da arttığı gösterilmiştir. İmmün disregülasyonun gösterilmesi de allerjik astım gelişeceğini ön görebilir.

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INTRODUCTION

In recent decades, there has been a steady increase in the incidence of asthma worldwide^[1-3]. The main pathogenetic mechanism of asthma is chronic inflammation, which leads to the development of pathological syndromes such as bronchial hyperactivity, bronchoconstriction, plasma exudation, mucus hypersecretion, activation of sensitive nerves, and remodelling.

Chronic inflammation is associated with various violations of the autonomous control of bronchial tone, in which there is a close interaction between the nervous system and inflammatory cells^[4]. Autonomous neurological control of the respiratory tract is based on classical cholinergic and adrenergic mechanisms, as well as non-adrenergic or non-cholinergic nerves and various neuropeptides in the respiratory tract^[5-7]. These neuropeptides can modulate the reactivity of certain groups of neurons, stimulate or inhibit the release of hormones, and regulate tissue metabolism^[8,9]. In the case of asthma, neuropeptides are also involved in regulation of non-cytotoxic degranulation of mast cells and basophils^[3,10]. It has been revealed that substance P and vasoactive intestinal peptides (VIP) have important roles in neural control of bronchial tone. In asthma, deviation of T lymphocytes towards Th2 type leads to activation of IgE and results in an imbalance of subpopulations of helpers. Recent research has focused on defining the cytokines synthesized by T cells in addition to studying the regulation of Th1 and Th2 cells^[9,11].

The main purpose of our work was to define some cytokines along with neuropeptides to re-

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Anahtar kelimeler: Astım, immün sistem, nöropeptid, sitokin, substans P, vazoaktif intestinal peptid

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veal the relation between allergic and neurogenic inflammation in children with asthma.

MATERIALS and METHODS

Study Group

The study included 80 consecutive children with asthma aged 3 to 17 years attending the Allergy Department of the Children's Clinical Hospital, No: 6, Baku and the 2nd Department of Pediatrics of Azerbaijan University, Faculty of Medicine. The group with moderate persistent asthma included 44 children while the group with severe persistent asthma included 36 children. The control group included 15 age-matched healthy children. Asthma was diagnosed according to Global Initiative for Asthma (GINA) criteria^[12]. All study subjects gave informed consent prior to the study.

Study Parameters

A peripheral blood sample was drawn from both children with asthma and healthy controls. The study quantified the level of substance P and VIP. Interleukin (IL)-2, IL-12, immunoglobulin classes of A, M, G and total Ig E, nitroblue tetrazolium (NBT) test, and C3-C4 complement fractions were also determined in the study groups. Quantification of substance P, VIP (Peninsula Laboratories Inc., USA), IL-2, IL-12 as well as of all classes of immunoglobulins (BioSource International Inc.) and complement fractions was carried out by the method of solid-phase ELISA analysis using appropriate test kits, according to the manufacturer's instructions. To assess the activity of the oxygen-dependent phagocyte system, functional and metabolic status of neutrophils in NBT test were studied. Heavy flow was applied to non-uni-

form sequential procedure based on the previously described probabilistic Bayesian method^[2,13].

Statistics

The statistical analyses were carried out using the SPSS program (SPSS, Version 6.0; Chicago, IL). Nominal values are described as n (percentages), whereas numeric values are described as mean and range (min-max). Nonparametric tests (Mann-Whitney U, Wilcoxon) were used in statistical analysis. A p value less than 0.05 was considered statistically significant. Prognostic coefficients (PCs) for each factor were calculated using the formula: $PC = 10 \lg P1/P2$, where P1 refers to frequency of a factor in the main group and P2 to the frequency of occurrence of the same factor in the control group. Identified risk factors were used for creating predictive tables.

RESULTS

Substance P and VIP Values

The mean levels of substance P (4.36 pg/mL) were higher in patients with severe persistent disease compared to patients with moderate persistent asthma and the control group ($p < 0.001$). On the contrary, VIP levels (0.132 pg/mL) were lower in patients with severe persistent disease compared to patients with moderate persistent asthma and the control group ($p < 0.001$). The patients with moderate persistent allergic asthma also had higher substance P and lower VIP levels compared to healthy control subjects ($p < 0.001$) (Table 1).

Cytokines, Immunoglobulins and Complement Levels

Neuropeptide levels of the patients were aligned with the dynamics of cytokines in serum (Table 2). Among the patient group, current allergic asthma was characterized by increased levels of cytokine IL-12 ($p < 0.001$) and lower mean level of IL-2 ($p < 0.001$) when compared to controls. In patients with severe disease, there was an even greater increase in the mean level of IL-12 ($p < 0.001$, compared with control), while the level of IL-2 decreased ($p < 0.001$, compared to controls).

Analysis of the humoral immune system in children with moderate and severe persistent asthma revealed a statistically significant decrease in the synthesis of immunoglobulin classes A, M, and G and increase in the synthesis of IgE ($p < 0.001$ compared to control group for each) (Table 2).

Study parameters investigating oxygen-dependent metabolism in neutrophils revealed a statistically significant decrease in indices of spontaneous recovery NBT in all patients compared to the control group ($p < 0.001$).

Complementary activity of patients with moderate persistent asthma showed decreased C3 ($p < 0.001$) and C4 complement fractions ($p < 0.001$) compared to controls. This difference was more pronounced in severe persistent asthmatics compared to controls. This difference was also significant between moderate and severe persistent asthmatic children. C3 complement fraction was reduced 2.2 times and C4

Table 1. Determination of substance P and VIP in study groups [variables are given as mean (min-max)]

Variable	Children with allergic asthma (n= 80)		Control group (n= 15)
	Moderate persistent (n= 44)	Severe persistent (n= 36)	
Substance P (pg/mL)	3.07 (1.6-4.6)*	4.36 (2.2-9.4)*,**	1.28 (0.3-2.1)
VIP (pg/mL)	0.209 (0.1-0.66)*	0.132 (0.03-0.2)*,**	0.987 (0.31-1.60)

* $p < 0.001$ compared to controls.
** $p < 0.001$ compared to moderate persistent asthma.

Table 2. Indicators of the immune system in study groups [variables are given as mean (min-max)]

Indices	Children with allergic asthma (n= 56)		Control group (n= 15)
	Moderate persistent (n= 33)	Severe persistent (n= 23)	
IL-2 (pg/mL)	2.00 (0.3-5.2)*	0.94 (0.0-2.6)*	5.39 (333.0-922.0)
IL-12 (pg/mL)	157.0 (86.6-267.2)*	228.5 (152.0-357.0)*,**	74.9 (45.7-137.0)
IgA (g/L)	1.68 (0.7-3.0)*	0.96 (0.2-1.7)*,**	2.17 (1.40-3.10)
IgM (g/L)	0.88 (0.28-1.49)*	0.65 (0.26-0.88)*,**	1.25 (0.90-1.50)
IgG (g/L)	9.04 (4.7-14.1)*	5.41 (1.90-8.1)*,**	11.9 (8.2-15.5)
IgE (g/L)	346.6 (333.5-255.5)*	437.5 (25.0-75.0)*	213.8 (202.6-225.0)
C3 component	108.9 (62.0-169.0)*	48.3 (25.0-75.0)*,**	137.5 (75.0-190.0)
C4 component	28.3 (14.0-49.0)*	18.4 (14.0-23.0)*,**	36.5 (22.0-50.0)
NBT-test unit	62.3 (16.0-81.0)*	56.9 (44.0-70.0)*,**	76.2 (68.0-85.0)

* p< 0.001 compared to the control group.
 ** p< 0.01 compared to moderate asthma.

Table 3. The prognostic coefficient indices for predicting severe asthma

Indices	Prognostic coefficient (PC)*
P substance level > 4.36 pg/mL	34.1
VIP level < 0.132 pg/mL	1.3
IL-2 level < 0.9 pg/mL	1.6
IL-12 level > 228.5 pg/mL	30.5
IgA level < 0.96 g/L	4.4
IgM level < 0.64 g/L	5.1
IgG level < 5.41 g/L	4.5
Total IgE level > 437.5 BV/L	20.4
C3 complement fraction level < 48.3 V	3.5
C4 complement fraction level < 48.3 V	5.0
Spontaneous NBT-test level < 56.9 units	7.4

* A prognostic factor of more than 5.0 was defined as important or essential for the development of severe asthma in children.

fraction 1.5 times in children with severe persistent asthma compared to those with moderate persistent asthma.

Depending on the degree of significance, predictive coefficients were classified as weakly or significantly influencing the development of allergic asthma. While a prognostic factor of 5.0 or less was defined as weakly associated with allergic asthma, those with a prognostic factor of more than 5.0 were defined as important or essential for the development of allergic asthma in children (Table 3).

DISCUSSION

In this study, we identified that changes in neuro-immune regulation in patients with allergic asthma might contribute to and maintain a chronic persistent course of the disease. We also evaluated the prognostic significance of individual neuropeptides and immunological factors.

The complex pathogenesis of bronchial asthma is not limited to inflammation, which develops as a result of allergic reactions, but also includes neurogenic inflammation involving neuropeptides^[5-9]. In our study, the level of substance P and VIP in the blood plasma of children with allergic asthma showed a multi-directional operation, i.e. increase in substance P and decrease in VIP value. Thus, our results might indicate the involvement of substance P and VIP in neurogenic inflammation in bronchial asthma, in accordance with previous studies^[7-9]. It appears that the role of the studied neuropeptides in the pathogenesis of asthma is total reduction in the accumulation of VIP and an increase in substance P in plasma levels, which may lead to smooth muscle spasm, mucosal edema, and hypersecretion of mucus, all totally resulting in an increased chronic inflammation in bronchial asthma^[12].

Production of cytokines or other immune cells largely determines the nature and strength of immune responses, which might consequently influence the severity of the disease. In our other study parameters, we showed a cytokine imbalance response—a delay in production of IL-2 and rapid synthesis of IL-12, parallel to increased severity of asthma.

Our study allows us to highlight the most significant prognostic factors for the development of allergic asthma. It was revealed that a substance P level > 4.36 pg/mL, IL-12 level > 228.5 pg/mL and total IgE level of 437.5 IU/L in the peripheral blood are the most important factors predicting allergic disease. On the other hand, VIP levels < 0.132 pg/mL, IL-2 levels < 0.9 pg/mL, IgA level < 0.96 g/L, IgG levels < 5.41 g/L, and levels of C3 complement fraction < 48.3 units and of C4 complement fraction < 18.4 units were found to be weakly associated with allergic asthma. Thus, our research may help to predict the course of the disease in the early stages and to implement an appropriate treatment and also provides a clue for immunodiagnostic methods for asthma.

In conclusion, our results indicated that neuropeptides and neurogenic inflammation can

accompany and aggravate ongoing allergic inflammation. Our analysis also showed that differences in neuropeptides and cytokine levels might play an important role in strengthening airway inflammation, and the level of dysregulation in immune system parameters might predict allergic asthma in children.

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