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BRONCHIAL ASTHMA IN CHILDREN AND SERUM LEVELS OF STROMELYSIN 1 (MMP-3)

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ABSTRACT

Bronchial asthma (BA) among children is an important health issue worldwide, including Bulgaria. The prevalence is increasing although the reasons are poorly understood. The common characteristics of asthma include bronchial spasm, airway remodeling with variable narrowing, bronchial hyperresponsiveness, and airway inflammation. One of the key pathogenetic mechanisms is the disturbance of the protease/antiprotease balance, as the isoenzymes of matrix metalloproteinases (MMPs) family are shown to participate in the airway wall remodeling in BA. The MMP-3 (Stromelysyn 1) is found to be secreted by variety of inflammatory (monocytes/macrophages) and non-inflammatory cells (airway epithelial cells and lung fibroblasts).

The aim of this study was to evaluate the serum concentrations of MMP-3 in children with BA, to compare it with those of control individuals and to elucidate its possible role as a biomarker in BA in children. The levels of MMP-3 were measured by ELISA in the serum of 23 healthy controls (20 adults and 3 children under 17 years of age) and 24 asthmatic children (6 -17 years of age).

There was no difference in MMP-3 levels of the children with BA (mean of 3.05±3.96ng/ml) compared with adult controls (4.99±3.82ng/ml, p=0.307), or with healthy children (2.47±1.71ng/ml, p=0.880). When studied the asthmatic children, we observed a tendency for lower level of MMP-3 in younger children with BA (up to 12 years) than those above 12 years of age (2.06±1.95ng/ml vs. 5.47±6.34ng/ml, p=0.052) and in those with lower IgE levels (1.67±1.12 ng/ml vs. 4.88±6.10 ng/ml, p=0.077) .

According to our results we suggest that the serum MMP-3 levels could not be used as biomarker for BA in children. We suppose that the levels of MMP-3 and possibly the airway remodeling in asthmatic children might be affected by the age, as it was reported in a murine acute asthma model.

Key words: Bronchial asthma, children, MMP-3, IgE

INTRODUCTION

Bronchial asthma (BA) is chronic inflammatory disease of the airways characterized by variable recurring symptoms of airflow obstruction and bronchospasm (a result of epithelial desquamation, goblet cell hyperplasia, mucus hypersecretion and thickening of submucosa) (1, 2). The worldwide prevalence of the disease is increasing especially among children (3). Similar trend is observed also in Bulgaria (4).

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One of the major hypotheses for the pathogenesis of the disease is related to chronic inflammation and protease/antiprotease imbalance. The family of matrix metalloproteinases (MMPs) are secreted by inflammatory and non-inflammatory cells. MMPs are zinc-dependent proteinases which have an important role in many physiological and pathological conditions (wound healing, senescence, cancer, fibrosis and inflammation) due to their ability to degrade extracellular matrix (ECM), proteolytic modulation of biologically active proteins, and cell migration (5-7). MMPs are synthesized in non-active forms, zymogens. The activation can be done in several ways: stepwise, intracellular and cell surface-mediated mechanism. MMP activity is

tightly regulated on the level of transcription, activation and inhibition by the tissue inhibitors of MMPs (TIMPs: TIMP-1, TIMP-2, TIMP-3, and TIMP-4) and α2-macroglobulin. (6, 8-11).

On the basis of substrate specificity, sequence similarity, and domain organization, MMPs can be divided into six groups: collagenases (MMP-l, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), membrane-type MMP (MMP-14 to MMP-25), matrilysin (MMP-7, -26), and macrophage metalloelastase (MMP-12) (10-12). MMP3, or Sromelysisn 1, cleaves collagens type III, IV, IX, X; gelatin; aggrecan; versican; perlecan; fibronectin; laminin; elastin; casein; fibrinogen, α-macroglobulin; ovostatin; pro-TNF; and activates pro-MMP-1, 7, 8, 9, 13 (11, 13).

Different type of cells (neurtophils, eosinophils, fibroblasts, mast cells, airway epithelial cells) and mediators participate in the development of the pathological changes in the lungs. In addition to the inflammatory response, there are structural changes, often due to airway remodeling (synthesis and degradation of ECM). For example, fibroblasts secrete not only ECM products but also MMPs and therefore are involved in the degradation of ECM (3, 6, 12). Mast cells and eosinophils are the key effector cells in the asthmatic inflammatory response and it has been shown immunohistochemically that these cells, together with neutrophils, are the main secreting MMP-3 cell types. Mast cells also release histamine, prostaglandins, thromboxanes and leukotrienes, all of them leading to bronchoconstriction, vasodilation and mucus secretion. Activated MMP-3 by cleaving the ECM leads to release of the stored growth factors, such as basic fibroblast growth factor (bFGF, FGF2), transforming growth factor α (TGFα) platelet derived growth factor (PDGF) and insulin like growth factors (IGF), which additionally may influence the pathogenetic mechanisms of Bronchial asthma (2, 3, 14, 15).

The aim of the current study was to evaluate the serum concentrations of MMP-3 in children with Bronchial asthma and to elucidate its possible role as a biomarker in BA in children.

PATIENTS AND METHODS

The levels of MMP-3 were measured in the serum of 20 adult controls (9 men and 11 women, 45-80 years of age), 3 healthy children under 17 years of age and 24 asthmatic children (14 boys and 10 girls, 6 -17 years of age). The age at the diagnosis of the disease varied between 1 and 14 years of age (median of 7 years) and the duration of the disease was between 0 (5 newly diagnosed patients) to 10 years (median of 3 years). The total serum IgE was measured in 21 of the asthmatic children and it varied between 7.47 U/l to 2500 U/l (median of 140.40 U/l).

The concentration of MMP-3 was measured from venous blood which was centrifuged immediately and serum samples were stored at -20˚C until the assay. The MMP-3 was measured by ELISA (Invitrogen, USA). The results were measured as optical density (OD) at 450 nm and calculated in ng/ml according to the OD of the standard solutions .

A standard blood characteristics and differential blood count of the asthmatic children were evaluated. In eight of the children nasal and throaty secret was investigated for eosinophils. Only three of the children had eosinophilia. In the other patients the levels of eosinophils were in normal range. In one child the throaty secret was positive for eosinophils.

Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc.). Factors with $p<0.05$ were considered statistically significant.

RESULTS

There was no difference in serum MMP-3 levels of the children with BA (mean of 3.05±3.96 ng/ml) compared with the adult controls (4.99±3.82 ng/ml, p=0.307), or with the healthy children (2.47±1.71 ng/ml, p=0.880) **(Figure 1).** In the group of asthmatic children the serum MMP-3 levels varied depending of the age and IgE levels. Children up to 12 years of age tended to have lower serum MMP-3 levels than those which were elder (2.06±1.95 ng/ml vs. 5.47±6.34 ng/ml, p=0.052) **(Figure 2).** According to the age and corresponding reference ranges of IgE, the asthmatic children were divided into two groups: 13 (62%) of the children had serum IgE values in the normal range, whereas the rest of 8 children (38%) had higher levels of IgE. Asthmatic children with higher IgE serum levels had also higher MMP-3 serum values (4.88±6.10 ng/ml vs. 1.67±1.12 ng/ml, p=0.077) **(Figure 3).**

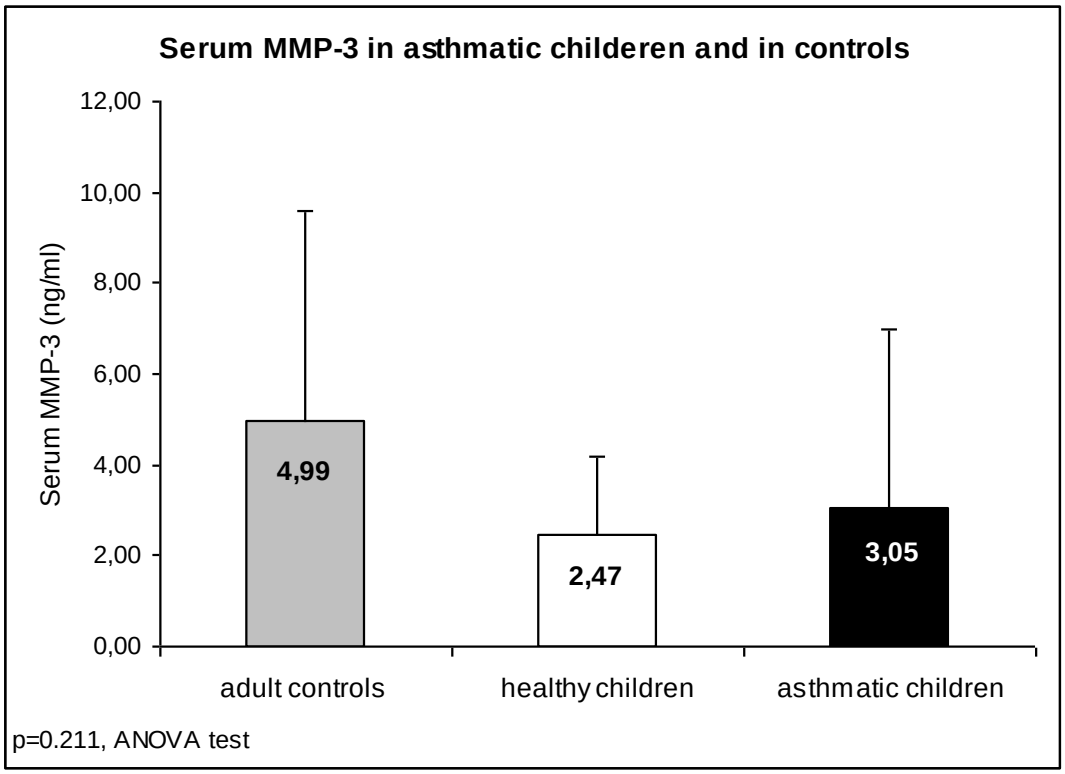

Figure 1. MMP-3 serum levels (ng/ml) in adult controls, healthy children and children with Bronchial asthma. Data are presented as mean±SD.

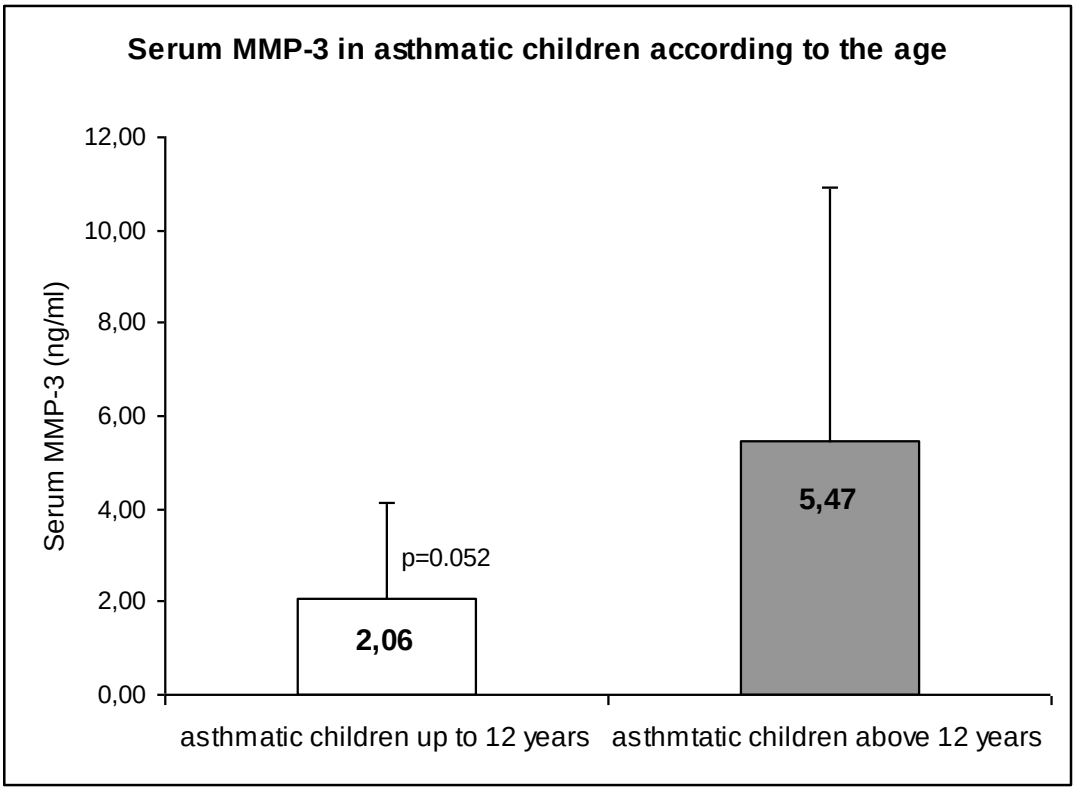

Figure 2. MMP3 serum levels (ng/ml) in children with Bronchial asthma up to 12 years and children with Bronchial asthma above 12 years. Data are presented as mean±SD.

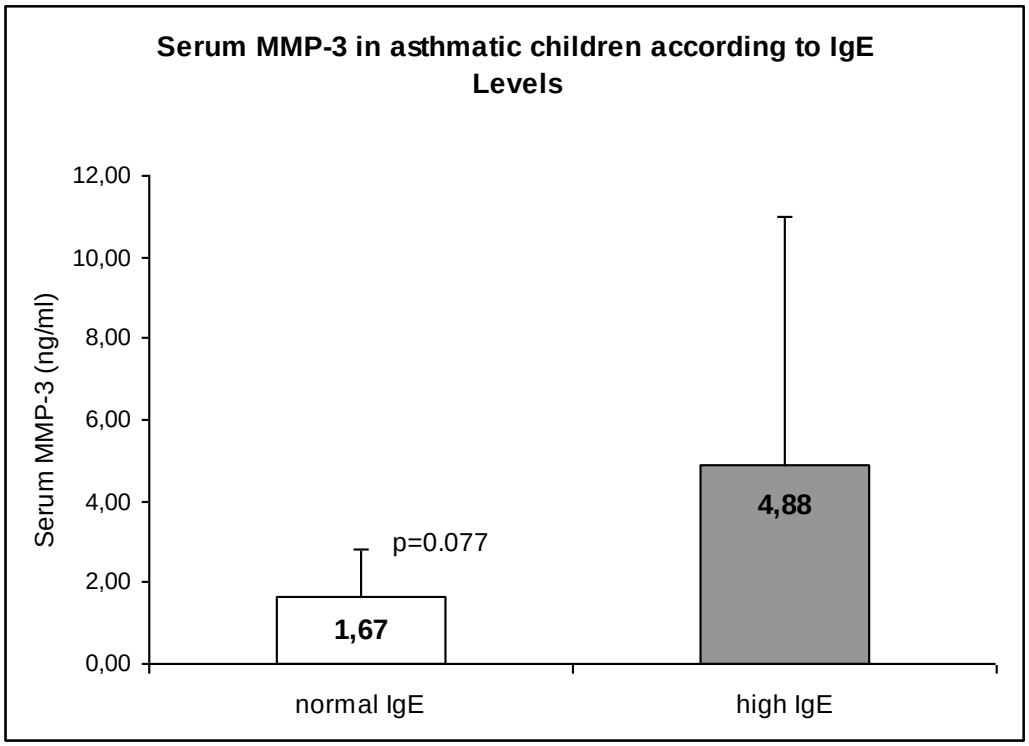

Figure 3. MMP3 serum levels (ng/ml) in children with Bronchial asthma according to the serum levels of IgE. Data are presented as mean±SD.

DISCUSSION

In Bronchial asthma increased number of activated mast cells and eosinofils are observed in the inflamed lung. In addition to the inflammatory response, there are structural changes in the airways (airway remodeling). When airway inflammation is ongoing, repair processes may contribute significantly to airway remodeling and irreversibility of lung injury. For repair and remodeling MMPs seem to play central role (3, 5, 16, 17). Up-regulation of MMPs is thought to be a part of the innate immune response and host defense system. By activating/inactivating cytokines and chemokines, MMPs may influence the recruitment and function of inflammatory cells. Earlier, it has been reported that disruption of the balance between MMP-3 and TIMPs plays a role in the pathogenesis of Bronchial asthma (18-20).

The ECM preserves the typical shape and structural integrity of various organs and tissues, but it is not only a scaffold that provides support for cells, it is further involved in cell-cell interactions, proliferation, and migration. MMPs might contribute to the breakdown of ECM, resulting in the destruction of healthy lung parenchyma. (12).

In our current study we did not find any differences in the serum levels of MMP-3 between control groups and asthmatic children. Nevertheless there was a tendency for rising the levels of the enzyme in the serum of children with higher serum IgE levels and in those above 12 years of age compared to younger ones (up to 12 years). The latter observation suggests that probably along with the increasing of the age, the inflammatory response becomes greater. Illi et al found that allergen exposure and sensitization in the first 3 years of life predisposes toward the development of chronic bronchial hyperresponsiveness and abnormal lung function by school age (21). This research suggests that aging may influence the pathogenesis of asthma. From clinical aspect adult asthma seems to be more severe, compared with pediatric asthma.

In a study on murine acute asthma model, Kang et al (22) found that aged mice have increased inflammatory cells and IL-4 in BALF and more accentuated pattern of airway remodeling in lung tissue, compared with young mice. They also found that the histological changes in the airways of the older mice were greater than in the group of young ones. A study in

symptomatic infants aged 3–26 months with reversible airflow obstruction has shown no reticular basement membrane thickening and eosinophilic inflammation even in the presence of atopy (23).

These findings correlate with our results, even more it is known that adaptive immunity in children matures over the first 6 years of life (21). As the cells secreting MMP-3 are responsible for airway remodeling and immune response, it is reasonable that MMP-3 serum levels increase with the age.

Unfortunately, due to the ethical concerns, bronchoalveolar lavage in our patients has not been studied for eosinophils and MMP-3 and we can not track their levels in the lungs.

In conclusion, according to our results we suggest that the serum MMP-3 levels could not be used as biomarker for BA in children. We suppose that the levels of MMP-3 and possibly the airway remodeling in asthmatic children might be affected by the age and severity of the allergic reaction.

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