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Serum Transforming Growth Factor-β (TGF-β), Matrix Metalloproteinase-2 (MMP-2), Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitors of Metalloproteinase (TIMP-1) Levels in Childhood Asthma*

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Aims: Airway remodelling is a characteristic feature of asthma and is a dynamic process involving extracellular matrix (ECM) production and its degradation. In this regard, the matrix metalloproteinases (MMPs) and their specific inhibitors known as tissue inhibitors of metalloproteinases (TIMPs) have been shown to be important in this process. In the present study, transforming growth factor (TGF)-β, MMP-2, MMP-9 and TIMP-1 levels in serum samples of children with stable asthma and healthy controls were evaluated to determine whether these levels show any difference between asthmatic and healthy children and whether or not these levels change with atopic status.

Materials and Methods: Thirty-one mild to moderate stable asthmatic children aged 6-16 years (16 atopic, 15 non-atopic) who were followed in our clinic and 13 age-matched healthy volunteers were enrolled in the study. Serum TGF-β, MMP-2, MMP-9 and TIMP-1 levels were measured by ELISA.

Results: There were no significant differences in serum levels of TGF-β, MMP-2 and TIMP-1 between patients and controls. However, serum MMP-9 levels of asthmatic children were found to be significantly higher than those of healthy controls. No difference was observed between atopic and non-atopic asthmatics for TGF-β, MMP-2, MMP-9 and TIMP-1 levels.

Conclusions: Results of the present study support the evidence regarding the involvement of MMPs, especially MMP-9, in the asthma pathogenesis. However, MMP-9 levels do not seem to be influenced by atopic status, which is the most significant risk factor in childhood asthma.

Key Words: Asthma, children, metalloproteinase, airway remodelling

Astımlı Çocuklarda Serum Transforming Growth Factor-β (TGF-β), Matrix Metalloproteinaz-2 (MMP-2), Matriks Metalloproteinaz-9 (MMP-9) ve Metalloproteinaz Doku İnhibitörü-1 (TIMP-1) Düzeyleri

Amaç: Hava yollarında yeniden yapılanma (remodelling) ekstraselüler matriksin yapım ve yıkımını içeren dinamik bir süreçtir ve astımın karakteristik özelliğidir. Matriks metalloproteinazları(MMP) ve metalloproteinazların doku inhibitörleri olarak bilinen spesifik inhibitörleri (TIMP) bu süreçte önemli rol oynarlar. Bu çalışmada stabil astımlı çocuklarda ve sağlıklı kontrollerde serum TGF-B,MMP-2, MMP-9 ve TIMP-1 düzeylerini ölçerek bu belirteçlerin astımlı ve sağlıklı çocuklarda farklı olup olmadığının ve atopi varlığından etkilenip etkilenmediğinin belirlenmesi amaçlandı.

Yöntem ve Gereç: Polikliniğimizde tanı alan ve izlenen yaşları 6-16 arasında değişen 31 stabil astımlı olgu (16 atopik, 15 nonatopik) ve benzer yaş grubundan 13 sağlıklı gönüllü çocuk çalışmaya alındı. Serum TGF-β,MMP-2, MMP-9 ve TIMP-1 düzeyleri ELISA yöntemi ile ölçüldü.

Bulgular: Hastalar ve kontroller arasında TGF-β,MMP-2, ve TIMP-1 düzeyleri açısından fark bulunmazken, serum MMP-9 düzeyleri astımlı çocuklarda sağlıklı çocuklara göre belirgin yüksek bulundu. Atopik ve nonatopik astımlılar arasında TGF-β, MMP-2, MMP-9 ve TIMP-1 düzeyleri açısından farklılık saptanmadı.

Sonuç: Bu çalışmanın sonuçları astım patogenezinde MMP'lerin özellikle MMP-9'un rol oynadığı yönünde kanıtları desteklemektedir. MMP-9 düzeyleri çocukluk çağı astımında en önemli risk faktörlerinden olan atopiden ise etkilememektedir.

Anahtar Sözcükler: Astım, çocuklar, metalloproteinaz, hava yolu yeniden yapılanması

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Introduction

Asthma is the most common chronic illness in childhood (1). Even if there are good guidelines for the management of childhood asthma, knowledge related to remodelling status and clinical severity is rather inadequate (1-3).

Recent studies revealed that chronic inflammation and airway remodelling are the two main factors in the asthma pathogenesis (4,5). Epithelial destruction, excessive production of profibrotic growth factors (transforming growth factor-β [TGF-β]) and changes in extracellular matrix (ECM) homeostasis were thought to be responsible for airway remodelling (6). The matrix metalloproteinases (MMPs) are probably the main physiological mediators of ECM degradation (7). Tissue matrix metalloproteinases inhibitor-1 (TIMP-1) is the natural inhibitor of this process (7).

The aim of this study was to compare serum levels of TGF-β, metalloproteinases (MMP-2, MMP-9) and their inhibitor TIMP-1 in stable asthmatic patients and healthy children and to evaluate whether these levels show any difference between asthmatic and healthy children and whether or not these levels change with atopic status.

Materials and Methods

Thirty-one mild to moderate stable asthmatic children who were diagnosed as asthma according to the international asthma diagnosis and treatment guidelines and who were being followed in our clinic and also 13 age-matched healthy children were enrolled into the study. Exclusion criterion for the patients was systemic steroid usage during the last month. Stable asthma was defined on the basis of lack of clinical symptoms such as wheezing, tachypnea, and prolonged expiration time in addition to peak expiratory flow rate greater than 80% of predicted in patients who were able to undergo an evaluation. Sixteen of the children with asthma were atopic whereas 15 were non-atopic. Atopy was defined as having at least one positive skin prick test or specific IgE to common aeroallergens.

Venous blood samples of subjects were centrifuged at 3000 rpm for 5 minutes. Obtained serums were stored at -20 °C until time of analysis. Serum TGF-β, MMP-2, MMP-9 and TIMP-1 levels were determined by ELISA (Quantikine, R&D systems, USA) method. The lowest limits of detection for the TGF-β, MMP-2, MMP-9 and TIMP-1 assays were 15.4 pg/ml, 0.4 ng/ml, 0.156 ng/ml and 0.08 ng/ml, respectively.

Demographic data was evaluated with Kruskal Wallis variance analysis; serum levels of TGF-β, metalloproteinases and TIMP-1 were compared with Mann-Whitney U test. The ethics committee at our institution approved the present study and written informed consent was obtained from parents of all participants.

Results

Some characteristics of the patients are given in Table 1. Serum TGF-β, MMP-2, MMP-9, and TIMP-1 levels are compared in Figure. There were no statistical differences in serum TGF-β and MMP-2 levels among the groups. However, serum MMP-9 levels of asthmatic children were significantly higher than those of healthy controls (P=0.03).

Although serum TIMP-1 levels of asthmatic children were higher than the control group, the difference between them was not statistically significant. No difference was observed between atopic and non-atopic asthmatics for serum TGF-β, MMP-2, MMP-9 and TIMP-1 levels, while MMP-9 levels were significantly higher than those of the healthy controls both for atopic (P = 0.01) and non-atopic (P = 0.01) asthmatics (Table 2).

Table 1. Some characteristics of the groups.

	Asthmatics n=31	Controls n=13
Median age (years) (range)	8.9 (6-16)	9.5 (3-15)
Gender (Male/Female)	17/14	8/5
Asthma severity		
Mild-persistent	23	
Moderate-persistent	8	
Inhaler steroid usage	25	-
Dosage (budesonide)	200-400 mcg	
Duration (month)	1-30	
Montelukast usage	8	
Duration	3-12	

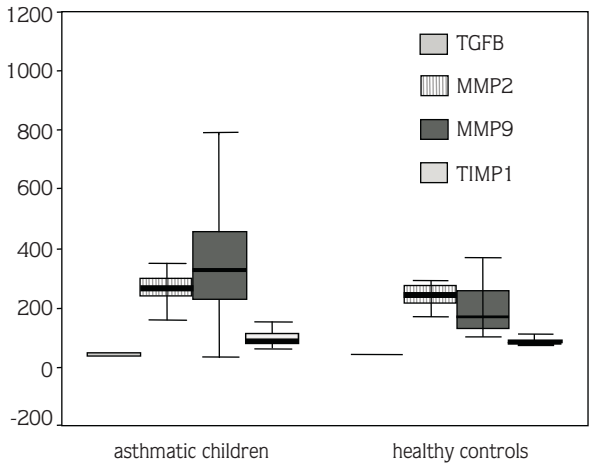

Figure. TGFb (pg/ml), MMP-2 (ng/ml), MMP-9 (ng/ml) and TIMP-1 (ng/ml).

No correlation was detected between the serum levels of TGF-β, MMP-2, MMP-9 and TIMP-1 and the dosage or duration of inhaler corticosteroid treatment.

Discussion

Deregulation of the proteolytic-antiproteolytic network and inappropriate secretion of various MMPs by stimulated structural or inflammatory cells are thought to take part in the pathogenesis of numerous lung diseases including asthma, chronic obstructive pulmonary disease (COPD), lung fibrosis, and lung cancer (8). The aim of this study was to compare serum levels of TGF-β, metalloproteinases (MMP-2, MMP-9) and their inhibitor TIMP-1 in stable asthmatic patients and healthy children and to evaluate their role in asthma. Although there are some studies evaluating metalloproteinases in bronchoalveolar fluid or sputum of asthmatic patients (7,9), we could only find the study of Belleguic et al. evaluating metalloproteinases in the blood of asthmatic adults (10). They compared the levels of MMPs, eotaxin and soluble interleukin-2 receptor in the plasma of healthy subjects, atopic but non-asthmatic and asthmatic patients. They found that the MMP-9 level was significantly enhanced in acute severe asthma compared with the others. No difference was found in TIMP-1 levels in plasma. Furthermore, they speculated that the MMP-9/TIMP-1 ratio in acute severe asthmatics may be higher than in the other groups. Moreover, they did not observe any difference in MMP-2 between the groups of patients (10). Similarly, in the present study, serum MMP-9 levels of stable asthmatic children were higher than those of healthy controls and MMP-2 levels were not different between the groups. These findings suggest that an increase in the serum MMP-9 level may indicate a defect in ECM homeostasis even in stable asthmatic children and perhaps propose MMP-9 as a noninvasive marker of inflammation and remodelling in asthma.

Table 2. Comparison of serum TGF-β, MMP-2, MMP-9 and TIMP-1 levels of atopic and non-atopic asthmatic patients.

	Atopic asthmatics n = 16	Non-atopic asthmatics n = 15	Controls n = 13
TGF-β (pg/ml) mean ± SD	38.8 ± 13.8	39.7 ± 13.7	37.7 ± 15.6
(Min-Max)	4-52	8-59	4-58
MMP-2 (ng/ml) mean ± SD	249.6 ± 52.6	266.1 ± 43.5	245.9 ± 37.0
(Min-Max)	130-220	160-350	170-290
		P = 0.01	
		P = 0.01	
MMP-9 (ng/ml) mean ± SD	373.8 ± 239.5	378.7 ± 217.8	209.6 ± 103.2
(Min-Max)	72-1000	35-800	100-445
TIMP-1 (ng/ml) mean ± SD	98.5 ± 30.5	100.6 ± 32.6	88.8 ± 11.4
(Min-Max)	62-183	40-180	71-111

TGF-β: tissue growth factor-β; MMP: matrix metalloproteinase; TIMP-1: tissue inhibitor of metalloproteinases.

Structural changes in airways characterizing remodelling may occur early in the natural history of asthma, even before diagnosis can be made based on clinical symptoms (1). A common limitation in evaluating airway changes in childhood asthma is that invasive maneuvers such as fiberoptic bronchoscopy with biopsy studies are problematic for a variety of reasons including ethical considerations. Although airway remodelling could not be demonstrated by biopsy in the present study, several recent studies in the literature provide important supportive findings of our data. Pohunek et al. evaluated markers of inflammation and tissue remodelling in bronchial biopsies from children with early respiratory symptoms before the clinical diagnosis of asthma. They found that eosinophilic inflammation and airway remodelling were present even before asthma diagnosis based on clinical symptoms (1). In another study, Barbato et al. analyzed bronchial studies of 23 asthmatic children undergoing bronchoscopy for clinical indications other than asthma. They reported that bronchial basement membrane thickening was already present in children with mild asthma (11). In their study, expression of TGF-β and its receptors was found to be similar in atopic and non-atopic asthmatic children as well as children without asthma. Likewise, in the present study, similar serum TGF-β levels were detected in both asthmatic and healthy children.

The relationship between airway remodelling, airway inflammation and symptoms of asthma has been difficult to elucidate. Both remodelling and loss of lung function in asthma seem to occur in early childhood (12,13). However, it is not known whether remodelling contributes to loss of function or is even a marker for it. In an interestingly designed study, Matsumoto et al. measured sputum levels of MMP-9 and TIMP-1 and their molar ratio in 26 asthmatic patients, and their relationship with pulmonary function and airway wall thickness was assessed by a computerized tomography technique (5). They found that sputum MMP-9 levels and MMP-9/TIMP-1 molar ratio were inversely correlated with airway wall area. Matsumoto et al. interpreted these findings as an absolute increase in TIMP-1 or its relative excess over MMP-9 and decreased MMP-9/TIMP-1 molar ratio in the asthmatic airway may be associated with airway wall thickening (5). Doherty et al. also found the ratio of MMP-9 to TIMP-1 reduced in bronchoalveolar lavage fluid in asthmatic children (9). However, they also did not evaluate serum levels of these factors. In this respect, increased serum MMP-9 levels and slightly increased TIMP-1 levels in the present study may indicate homeostasis of a repair process according to these findings.

Corticosteroids influence both inflammatory and remodelling processes in asthma, although their effects on ECM, MMPs and TIMPs are less clear. It has been reported that moderate doses of inhaled corticosteroids diminish MMP-9+ cells in the airways of patients with asthma (14). In the present study, no associations were detected between serum MMP-9 levels and duration or dosage of inhaler corticosteroid treatment. Further studies are needed to identify the effect of corticosteroids on serum MMP levels.

In conclusion, the results of the present study support the evidence about derangement of MMP/TIMP homeostasis in the asthma pathogenesis regardless of the atopic status.

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