KLİNİK ÇALIŞMA/RESEARCH ARTICLE Tuberk Toraks 2012; 60(4): 321-326

Geliş Tarihi/Received: 01/07/2012 - Kabul Ediliş Tarihi/Accepted: 06/11/2012

Inflammatory markers in exhaled breath condensate in patients with asthma and rhinitis

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ÖZET

Astım ve rinitli hastalarda yoğunlaştırılmış soluk havasında inflamatuvar belirteçler

Giriş: *Hafif astım, persistan rinit ve astım-rinit birlikteliği olan hastalarda ekshale soluk havasında malondialdehid ve total protein düzeyleri incelenerek alt hava yolu inflamasyonu değerlendirilmiştir.*

Hastalar ve Metod: *Hafif astım, en az bir yıldır semptomatik olan persistan rinitli hastalar ve sağlıklı bireyler çalışmaya dahil edildi. Astımlı ve rinitli hastalar yeni tanı almış olup, kortikosteroid tedavisi almamıştı. Hiçbir olgu sigara içmiyordu ve son bir ay içinde solunum yolu infeksiyonu öyküsü yoktu. Astım semptomları olan ve bronş provokasyonu pozitif olan rinitli hastalar persistan rinit ve astım birlikteliği olarak gruplandırıldı. Olgulardan toplanan ekshale soluk havasında malondialdehid ve total protein düzeyleri ölçüldü.*

Bulgular: *Persistan rinitli 53, hafif astımlı 12, persistan rinit ve astımlı 16 hasta ve 13 sağlıklı kontrolden oluşan dört çalışma grubunun ekshale soluk havasında malondialdehid ve total protein ölçümlerinde istatistiksel olarak anlamlı fark bulunmadı (p> 0.05). Ekshale soluk havasındaki malondialdehid ve total protein düzeyleri ile atopi ve nazal eozinofili arasında ilişki saptanmadı.*

Sonuç: *Hafif astım, rinit ve her iki hastalığın birarada bulunduğu hastalarda, alt hava yolu inflamasyonu hastalığa özgü bir durum olmadığı gibi şart da değildir.*

Anahtar Kelimeler: *Astım, ekshale soluk havası, malondialdehid, rinit, total protein.*

SUMMARY

Inflammatory markers in exhaled breath condensate in patients with asthma and rhinitis

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Introduction: *The present study investigates the lower airway inflammation using malondialdehyde and total protein measurement in exhaled breath condensate in mild asthma, persistent rhinitis, and concomitant asthma and rhinitis.*

Patients and Methods: *Asthmatics with mild disease, patients with persistent rhinitis symptomatic for at least one year and healthy controls were included. Asthmatics and rhinitis patients were all newly diagnosed and were free of corticosteroid therapy. Participants were nonsmokers, had no respiratoy tract infection within the previous month. Rhinitis patients with asthmatic symptoms and positive bronchial challenge were grouped as patients with persistent rhinitis and concomitant asthma. Malondialdehyde and total protein were measured in the exhaled breath condensate collected from the subjects.*

Results: *No statistical difference was found in the malondialdehyde and total protein levels in exhaled breath condensate between the four study groups which are; 53 patients with persistent rhinitis, 12 with mild asthma, 16 persistent rhinitis patients with concomitant asthma and 13 healthy controls ($p > 0.05$). Atopy and nasal eosinophilia were not related to malondialdehyde and total protein levels in exhaled breath condensate.*

Conclusion: *Lower airway inflammation is not a disease specific process and is not a prerequisite concerning patients with mild asthma or rhinitis or patients with coexistence of both diseases.*

Key Words: *Asthma, exhaled breath condensate, malondialdehyde, rhinitis, total protein.*

INTRODUCTION

The increasing recognition over the last 50 years that allergic rhinitis and allergic asthma frequently coexist has led to the concept of united airway disease. Asthma and rhinitis have common and interrelated inflammatory processes of the upper and lower airways (1). It is commonly accepted that oxidative stress plays an important role in the pathogenesis of asthma. Although similar mechanisms are expected to be valid for allergic or nonallergic rhinitis, the role of oxidative stress has not been well studied in these disorders upto now (2). Exhaled breath condensate (EBC) is a noninvasive method to sample the respiratory tract and to study biomarkers (3). Aldehydes (malondialdehyde and others) are lipid peroxides which reflect oxidant-induced damage (3). Malondialdehyde (MDA) levels in EBC is well-studied in asthma, levels are increased compared to healthy controls (4). Hypoxia and exacerbations in asthma are also related to high levels MDA (5-7). Total protein levels in EBC are increased in many pulmonary pathologies as well as in smoking subjects (8,9). However whether rhinitis patients have increased inflammatory markers similar to asthmatics or whether rhinitis patients with concomitant asthma have more prominent lower airway inflammation compared to pure asthmatics is not well known.

The present study is a prospective study which aims to determine lower airway inflammation status in mild asthma and persistent rhinitis as well as whether there is an additive inflammatory process in coexistence of these two diseases by measuring MDA and total protein levels in EBC. Secondly it is investigated whether the atopic status and nasal eosinophilia influences the MDA levels in EBC.

PATIENTS and METHODS

Study Population and Study Design

Study participants included three groups of patients which are;

1. Mild asthmatics,

2. Patients with persistent rhinitis without asthma and

3. Persistent rhinitis patients with concomitant asthma and healthy volunteers.

All asthmatic patients included in the study were mild asthmatics (intermittent or mild persistent) who were newly diagnosed in our allergy department based on the symptoms that patients report, pulmonary function tests revealing forced expiratory volume in 1 second (FEV_1) ≥ 80% and positive methacholine challenge test (MCT) (10). None of the asthmatics were on inhaled or systemic corticosteroid therapy previously, as expected, since they were all newly diagnosed. Patients with asthma were stable as they were recruited in the study. Patients with persistent rhinitis were symptomatic for at least one year and were newly diagnosed as well and were also free of nasal or systemic corticosteroid therapy. The rhinitis patients who were free from asthma-

tic symptoms (cough, wheezing, dyspnea, chest tightness) and had negative results with MCT were grouped in persistent rhinitis group, otherwise (those with asthmatic symptoms and with positive MCT) were grouped as patients with persistent rhinitis and concomitant asthma. All patients and controls were nonsmokers and had no upper-lower respiratoy tract infection within one month prior to the study. Atopic subjects were not included in the control group. MDA and total protein were measured in the EBC collected from the subjects. The study has been approved by Eskisehir Osmangazi University ethics committee and informed consent was obtained from the subjects prior to the study.

Spirometry and Methacholine Challenge Test

Spirometry was performed with a spirometer (Vitalograph Alpha III; Vitalograph, United Kingdom). In patients with $FEV_1 \geq 80\%$ of predicted, methacholine challenge test was performed to evaluate bronchial hyperresponsiveness. MCT was performed according to the recommendations of American Thoracic Society Guidelines (11). Briefly, methacholine was administered using a dosimeter methacholine concentrations from 0.125 to 16 mg/mL (Zan 200; Zan, Oberthulpa, Germany) with doubling doses in each step. Three maneuvers of FEV_1 were performed after each dose and the highest FEV_1 was reported. The challenge was stopped when the FEV_1 dropped by > 20% from the postphysiological saline or when the highest concentration of methacholine had been administered. Subjects were considered to have positive response if they showed a decline in FEV_1 of ≥ 20% at a concentration of methacholine < 16 mg/mL. PC_{20} value was calculated from log dose–response curve (11). At the end of a positive challenge, patients received 200 µg of salbutamol from a metered-dose inhaler to relieve airway obstruction.

Skin Prick Test

Skin prick tests were performed by using a common panel including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass, tree and weed pollens, animal dander (cat, dog), animal feathers and molds (including *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*) allergen extracts (ALK, Spain). Positive and negative controls were histamine and phenolated glycerol saline, respectively. A mean wheal diameter of 3 mm or greater than that of obtained with control solution was considered as positive.

Nasal Smear

Nasal smear was studied in all subjects to determine nasal eosinophilia. Nasal specimens for cell counts were obtained by passing a straight cotton swab along the base of the nose and drawing it under the inferior turbinate with a twisting motion and the collected secretions and cells were smeared onto a glass slide and dried in the air. The slides were stained with Wright-Giemza stain and examined under a light microscope by an experienced hematologist, who was blinded to the clinical status of the patients. At least 300 cells were counted in each slide at high power (oil immersion, x1000) excluding epithelial cells and eosinophil count was expressed as a percentage of the total cells.

Malondialdehyde and Total Protein Measurement in Exhaled Breath Condensate

EBC collection was performed from all subjects in the morning 10 a.m. It was collected with a simple homemade apparatus designed as a F 14 PVC suction catheter immersed in an ice-filled jar with the distal end placed into a polypropylene test tube. The apparatus was designed according to the model described in the review by Mutlu et al. (12). Subjects sitting comfortably in the laboratory performed repeated tidal breathing for 10-15 minutes through the suction catheter into the polypropylene test tube immersed in dry ice at -20°C. No nose clip was used during the procedure. Approximately 1-3 mL of condensate was collected from the subjects the samples were immediately stored at -80°C until the time of analysis, which was performed within at most six months after sample collection, which is an acceptable storage time for studying aldehydes according to previous studies (13). Subjects were told to rinse their mouth before the procedure and to keep the mouth dry by periodically swapping their saliva to minimize the salivary contamination.

Malondialdehyde concentrations in EBC samples were measured according to the procedure stated by Uchiyama and Mihara. Basically the EBC samples were heated with thiobarbituric acid (TBA) under acidic conditions and the pink-coloured MDA-TBA reaction as lipid peroxidation product was measured at 532 nm. To increase sensitivity, the complexes were extracted into an organic solvent butanol and measured spectrophotometrically (14).

Total protein levels were determined using a commercial kit (Bio-Clinica, Istanbul, Turkey) according to Biuret reaction spectrophotometrically.

Statistical Analysis

The numerical variables were presented as median (range). Mann-Whitney U test was used for the comparisons of numerical variables between two groups and Kruskal-Wallis test was used for more groups. Categorical variables were compared with chi-square

test. p value less than 0.05 was considered as statistically significant. SPSS for Windows (version 13.0; SPSS Inc. Chicago, IL) was used for the calculations.

RESULTS

Eighty-one patients, 53 with persistent rhinitis, 12 with mild asthma, 16 with concomitant mild asthma and persistent rhinitis and 13 healthy controls were included in the study. Age and gender were similar in between the four groups (Table 1). Atopy was significantly more common in rhinitis group and in asthma plus rhinitis patients (Table 1). The MDA and total protein levels in EBC did not differ between the groups (Table 1).

In the study population 55% of the subjects were atopic and 16% of the subjects had nasal eosinophilia (Table 2, 3). In 5 subjects nasal smear was not appropriate for evaluation (Table 3). Presence of atopy and nasal eosinophilia were not related to MDA and total protein levels in EBC (Table 2,3).

DISCUSSION

In the present study EBC samples were evaluated in patients with persistent rhinitis, allergic or nonallergic, patients with mild asthma, atopic or nonatopic, and rhinitis patients with concomitant asthma. MDA and total protein levels were compared in the EBC samples of all patient groups and healthy controls to assess the extent of inflammation ongoing in the lower airways. MDA or total protein levels were not significantly different among the groups.

In asthma, like in many other chronic airway diseases, oxidative stress is a part of inflammatory process (15). Lipid peroxidation-derived products can be used to assess and monitor the oxidative stress (16). MDA which is a by-product of lipid peroxidation reflects oxidant induced damage on unsaturated lipids in cell membranes (17). Total protein level in EBC is another indicator of airway inflammation especially found to be increased in smokers, though it is rather an unspecific marker related very much on measurement techniques (8,9).

In the present study MDA and total protein levels were not statistically different between all groups of patients and healthy controls. Possible reason for this outcome is that the asthmatic patients involved in this study had mild disease and were not in attack period. The similar levels of MDA in EBC between patients with asthma and patients with rhinitis may be related to the fact that

Table 1. Demographic and laboratory data of the study groups.

	Rhinitis (n= 53)	Asthma (n= 12)	Asthma + Rhinitis (n= 16)	Control (n= 13)	p
Age (years)	29 (40)	42 (53)	36 (38)	24 (32)	0.082
Gender (male/female) (n)	14/39	2/10	2/14	5/8	0.373
MDA EBC (nmol/mL)	4.43 (7.43)	4.65 (1.15)	4.65 (49.56)	4.87 (3.27)	0.125
Total protein EBC (g/dL)	4.88 (4.46)	4.52 (1.76)	4.52 (1.26)	4.68 (1.60)	0.126
Atopic/nonatopic (n)	41/12	1/10	10/6	0/13	< 0.001

Data are presented median (range), unless otherwise stated.

Table 2. Laboratory data of the study population according to atopic status.

	Atopics (n= 52)	Nonatopics (n= 41)	p
MDA EBC (nmol/mL)	4.47 (4.69)	4.60 (49.65)	0.448
Total protein EBC (g/dL)	4.84 (4.46)	4.60 (2.02)	0.390

Total number of subjects does not add to 94 because the atopic status was not determined in one of the asthmatic patients due to dermatographism.

Table 3. Laboratory data of the study population according to nasal eosinophilia.

	Nasal eosinophilia (+) (n= 15)	Nasal eosinophilia (-) (n= 74)	p
MDA EBC (nmol/mL)	4.43 (7.35)	4.51 (49.65)	0.822
Total protein EBC (g/dL)	4.66 (1.92)	4.80 (4.46)	0.669

Total number of subjects does not add to 94 because nasal smears prepared from 5 subjects were not appropriate for evaluation.

lipid peroxidation is not a disease specific process, instead it is related to the degree of inflammation in inflammatory diseases of airways. Although in the literature studies report higher MDA levels in EBC in asthmatics compared to healthy controls in these studies the study groups include heterogenous asthmatics concerning the disease severity and smoking status (4,13). The present study was designed to include homogeneous group of asthmatic patients with mild disease, who are non smokers and free from any form of steroids priorly and during the recruitment to the study.

In a recent study held by Bartoli et al. higher MDA levels in EBC were reported in COPD patients compared to other chronic lung diseases including asthma. MDA levels were even much higher in COPD patients with higher percentages of sputum neutrophilia (4). On the other hand asthma is characterized by eosinophilic airway inflammation except in a particular group of patients that have severe disease and frequently are smokers, those present with neutrophilic inflammation (10). However, as stated earlier, smoking subjects and severe asthmatics, who frequently present neutrophilic inflammation, were not included in the present study. Malondialdehyde in EBC is elevated in acute asthma exacerbation and levels fall with the treatment of the attack (5). Moreover hypoxia is another factor related to elevation of inflammatory markers in lung tissue (6,7). The transient hypoxia that patients experience during asthma exacerbations may be responsible for the elevated MDA levels. On the other hand, according to the design of the present study focusing a homogeneous group of patients, the asthmatic patients had mild disease and none had hypoxemia and all were in stable period.

The upper and lower airways have parallel inflammation with possible bidirectional extension of inflammation in patients with coexisting asthma and allergic rhinitis (18). Epidemiological studies suggest that rhinitis predict the development of asthma which may occur either because these disease entities are manifestations of a progressive disease, or they may reflect separate disease processes that can afflict a susceptible population (19). So the present study was designed to assess the inflammatory biomarkers in EBC in patients with rhinitis without any symptoms presenting asthma. However the results deny an ongoing lower airway inflammation process in patients with rhinitis without asthma since the MDA and total protein levels in EBC of patients with rhinitis were not statistically different from that of healthy controls.

It was previously shown that nasal eosinophilia in persistent rhinitis is related to bronchial hyperreactivity (20). Asymptomatic bronchial hyperresponsiveness is generally caused by airway inflammation and is also frequently present in allergic rhinitis (21). In the present study, markers of airway inflammation was evaluated according to the presence or absence of nasal eosinophilia. As stated previously, the MDA and total protein levels in EBC did not differ significantly in the study population according to presence of nasal eosinophilia. Neither presence of atopy was related to MDA and total protein levels in EBC.

As a result we conclude that lower airway inflammation is not a disease specific process and is not a prerequisite concerning patients with mild asthma or rhinitis or patients with coexistence of both diseases.

ACKNOWLEDGEMENTS

The contents of this manuscript have been published in part as an abstract and poster at the 30th Congress of the European Academy of Allergy and Clinical Immunology and in 18th Congress of National Allergy and Clinical Immunology.

CONFLICT of INTEREST

None declared.

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