ORIGINAL ARTICLE

Oxidative stress in the airways of children with asthma and allergic rhinitis

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Abstract

Introduction: Even though it is well known that oxidant stress plays an important role in the pathogenesis of asthma, less is known about allergic rhinitis. Moreover, it is not known whether the co-existence of the two diseases augments the level of oxidant stress within a united airway concept.
Aim: To define the level of oxidative stress in children with asthma and/or allergic rhinitis in nasal and oral exhaled breath condensates (EBC) of children.
Method: Children aged 6–18 years with asthma (n = 28), allergic rhinitis (n = 17), asthma and allergic rhinitis (n = 100), and healthy controls (n = 74) were enrolled in the study. Levels of malondialdehyde (MDA) as a marker of oxidative stress and reduced glutathione (GSH) as an antioxidant were measured by High-Performance Liquid Chromatography in the EBC.
Results: Malondialdehyde levels were higher, and GSH levels were lower in all patient groups compared to healthy controls in both nasal and oral EBC samples ($p < 0.01$) but there were no differences among the different patient groups. Interestingly, oral MDA levels were lower in patients with asthma and allergic rhinitis [17.78 nM (11.62–23.94)] compared to patients with asthma only [25.71 nM (18.81–32.61)] ($p < 0.01$).
Discussion: Both asthma and allergic rhinitis are associated with increased oxidative stress in the airways in children. However, the co-existence of the two diseases does not augment the oxidant stress further.

The respiratory system is composed of an upper and lower part and together they form a unique anatomic-functional unit (1–2). It is now generally well appreciated that most patients with asthma have rhinitis (3) which have eventually led to the well established 'one airway one disease' concept (4). However, not all patients with rhinitis have asthma and there are differences between rhinitis and asthma (4–5).

An imbalance between the oxidative forces and the antioxidant defense systems favoring an oxidative injury has been implicated in the pathogenesis of asthma (6–7). Oxidative injury leads to increased lipid peroxidation, increased airway reactivity and secretions, production of chemoattractant molecules, and increased vascular permeability (8–10), which collectively lead to an augmentation of the existing inflammation that is a hallmark of asthma. In addition to the systemic oxidative stress, there is also an increased oxidative burden locally within the airways. Inflammatory cells in the airways such as macrophages and eosinophils were shown to produce elevated amounts of reactive oxygen species (11–12). In addition, levels of oxidized glutathione was higher in the bronchoalveolar lavage, induced sputum and erythrocyte hemolysates of patients with asthma (13–14). We have recently shown that exhaled breath condensate (EBC) contained significantly higher levels of oxidant markers (malondialdehyde) and lower levels of anti-oxidants (reduced glutathione) in asthmatic children compared with healthy controls (15).

Because of the epidemiological, physiological, and immunopathological links between allergic rhinitis and asthma, there is also reason to believe that oxidant stress may play an important part in allergic rhinitis, which may contribute to the asthma–rhinitis link and augment the existing pathology in the united airways disease. However, only a few studies investigated the link between oxidative stress and allergic

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diseases, other than asthma. Moon et al. (16) have shown that the expression of NADPH oxidase (NOX)1 and NOX4, and superoxide anion was increased in the nasal mucosa of patients with allergic rhinitis and nasal polyps compared to healthy individuals. Profita et al. (17) reported higher FE_{NO} and IL-5 levels and lower pH in nasal and oral EBC of children with atopic dermatitis, allergic rhinitis, and asthma. Finally, Gratziou et al. (18) have shown that nitrate/nitrite and exhaled NO levels were elevated in patients with asthma and seasonal allergic rhinitis compared to those with seasonal allergic rhinitis only.

Even though there is evidence that the nasal mucosa can be a major source of oxidant stress, the number of studies that have investigated the role of oxidant stress in allergic rhinitis and the contribution of the nasal mucosa to the inflammatory process in the united airway disease is very limited. In fact, it is largely unknown whether the presence of allergic rhinitis together with asthma within the context of united airway disease augments the oxidants stress that is already a well-known part of the pathological process in asthma.

An important result of oxidative stress is lipid peroxidation leading to oxidative degeneration of lipids. Lipid peroxidation decreases intracellular reduced glutathione which is a major substrate for many enzymes including glutathione peroxidase. Glutathione is the key molecule in both the enzymatic and non-enzymatic antioxidant systems (13). MDA is derived from lipid peroxides, and there is a good correlation between MDA concentrations and lipid oxidation levels (20). Together, MDA and GSH represent a good coverage of oxidant and antioxidant systems. We have previously shown that asthma is associated with increased MDA and decreased GSH, and the changes are augmented with increasing disease severity (15). In this study, we aimed to determine the oxidant/antioxidant imbalance in children with asthma and/or allergic rhinitis. We measured levels of malondialdehyde (MDA) as a measure of oxidant stress and reduced glutathione (GSH) as a marker of the anti-oxidant defense in orally or nasally collected exhaled breath condensate samples obtained from children with asthma and/or allergic rhinitis and from healthy controls.

Methods

Study population

Patients aged 6–18 years were recruited from the Pediatric Allergy and Asthma Unit of Hacettepe Children's Hospital, Ankara, Turkey between February 2006 and September 2007.

Patients with asthma
All children had evidence of reversible airway obstruction as defined by at least a 12% improvement in FEV1% following bronchodilator administration, or an abnormal result in methacholine bronchoprovocation test (PC20 <8 mg/dl) (21). All patients in this group had at least one positive result on skin prick testing.

Patients with allergic rhinitis
Allergic rhinitis was diagnosed according to ARIA guidelines by the presence of two or more of the symptoms of allergic rhinitis (sneezing, watery rhinorrhea, nasal obstruction, nasal pruritus) lasting more than 1 h on most days and a concordance between a typical history of allergic symptoms and skin prick tests (4).

Patients with asthma and allergic rhinitis
Patients who fulfilled both criteria mentioned above were classified as asthma and allergic rhinitis.

Healthy controls
The healthy control group consisted of children without a history of asthma and/or allergic rhinitis or other chronic diseases with normal physical examination and lung function tests. They presented for reasons such as minor trauma or for their regular follow-up to the outpatient department of the same hospital.

Exclusion criteria
Children with severe asthma or with a systemic disorder other than asthma and those who received immunotherapy in the past were not included in the study. Children who had an asthma exacerbation or an upper or lower airway infection within the last 4 wk, those who received an antihistamine within the last week, systemic corticosteroids within the last 4 wk, or leukotriene modifiers within the last 3 days were also excluded from the study.

Study procedures

Collection of the EBC
A commercially available device, EcoScreen (Jaeger, Wurzburg, Germany), was used for the collection of EBC. In this collection system, exhaled air enters and leaves the chamber through a two-way, non-breathing valve, and saliva is filtered. Expiratory air flows through a lamellar condenser that is surrounded by a cooling cuff. The interior temperature is −15°C or cooler. Nasal and oral collections were carried out during tidal breathing for 10 min each (22). Nasal samples were collected in all children by placing a mask over the nose and keeping the mouth closed. Oral samples were collected by placing a mouth piece and keeping the nose occluded with a nose-clip. Immediately upon collection, the samples were frozen on dry ice and stored in −80°C until measurement within 6 months of collection.

Malondialdehyde and reduced glutathione
Exhaled breath condensates levels of MDA and GSH were measured by high-performance liquid chromatography as previously described(18). The lower limit of detection for MDA was 1.2×10^{-8} mM) and for GSH was 0.15 nM. The person performing the measurements was blinded to the groups of the study population. All samples were run in triplicate.

Spirometry (2130Spirometer; Sensor Medics Co., Yorba Linda, CA, USA) and IgE measurements (Uni-Cap; Pharmacia, Kalamazoo, MI, USA) were carried out with standard

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procedures (23, 24). Eosinophil counts were determined from Coulter Counter leukocyte measurements. Skin testing was performed with a battery of 38 antigens including 30 aeroallergens and eight food allergens with appropriate positive and negative controls. A panel of seven aeroallergens including house dust mites, alternaria, grass mix, tree mix, weed mix and cat dander was used as SPT in healthy controls. Reactions with an induration ≥3 mm that of the negative control wheal were considered positive and children with at least one positive test were considered atopic.

All study procedures were performed in accordance with a protocol previously approved by the Ethics Committee of Hacettepe University. All parents provided written informed consent for the study procedures.

Statistical analyses

Statistical analyses were performed with SPSS 15.0 package program (SPSS Inc, Chicago, IL, USA). Age, FEV1%, eosinophil counts, IgE levels, MDA and GSH levels showed a non-normal distribution, and data are given as medians and interquartile ranges, and statistical comparisons were performed using nonparametric Mann–Whitney *U*-test or ANOVA on ranks. For pair-wise comparisons of the non-normally distributed data, Mann–Whitney *U*-test with Bonferroni correction was used. As there was no similar study in the literature, *a priori* sample size calculation could not be carried out. However, *post hoc* analysis of the data showed that the number subjects in each group was sufficient to reach a 95% power with the observed differences among each group with respect to both MDA and GSH. A value of $p < 0.05$ was considered significant.

Results

Twenty-eight children with asthma, 17 children with allergic rhinitis, 100 children with both asthma and allergic rhinitis, and 74 healthy controls were included in the study. The characteristics of the subjects are summarized in Table 1.

MDA and GSH levels in the EBC

There was a highly significant increase in the MDA and a similar decrease in GSH in the EBC both in the orally and in nasally collected EBC samples in all groups of patients compared to controls (Figs 1 and 2) (Table 2). However, individual patient groups did not show any difference from one another.

Interestingly, patients with asthma had the highest MDA levels in the oral EBC. MDA levels in the oral EBC showed a progressive decrease from patients with asthma to patients with allergic rhinitis followed by patients with asthma + allergic rhinitis. The difference between asthma and asthma + allergic rhinitis groups reached significance ($p < 0.001$) (Fig. 2b).

There was a positive correlation between nasal and oral MDA ($r = 0.684$, $p < 0.001$) and GSH concentrations ($r = 0.684$, $p < 0.001$) (Fig. 3a,b). Negative correlations were detected between GSH and MDA levels both in nasal samples ($r = -0.573$, $p < 0.001$) and in oral samples ($r = -0.387$, $p < 0.001$)

Discussion

Generation of reactive oxygen species to a degree that overwhelm the antioxidant defenses is critical for the amplification of inflammation in allergic diseases (25). Our study shows that in addition to asthma, allergic rhinitis is also associated with oxidant stress. However, our findings suggest that oxidant stress is stronger in the airways of patients with asthma and that in contrast to our hypothesis, coexistence of asthma and allergic rhinitis does not augment the extent of oxidant stress.

Table 1 Demographic characteristics of the study groups

	Controls (n = 74)	Asthma (n = 28)	Allergic rhinitis (n = 17)	Asthma + Allergic rhinitis (n = 100)	p
Age*	9.3 (7.6–12.5)	10.6 (9–14.1)	11.4 (10.4–13.7)	10 (8.4–12.7)	>0.05†
Gender (Male)	42 (56.8%)	11 (39.3%)	9 (52.9%)	37 (37%)	>0.05‡
Asthma severity					
Mild intermittent & persistent	–	22 (78.6%)	–	78 (78%)	>0.05‡
Moderate persistent		6 (21.4%)		20 (20%)	
Allergic rhinitis severity					
Mild	–	–	6 (35.3%)	74 (74%)	
Moderate-severe			11 (64.7%)	25 (25%)	0.001‡
FEV1%*	104 (96–114)	93 (86–97)	102 (98–113)	96 (86–102)	<0.001†
Eosinophils/mm^3§	129 (85–228)	284 (197–475)	345 (170–539)	345 (193–553)	<0.05†
IgE (kU/l)§	38 (15–93)	161 (78–324)	175 (68–297)	192 (94–468)	<0.05†
Use of inhaled corticosteroid	–	14 (50%)	–	57 (57%)	>0.05‡
Use of nasal corticosteroids	–	–	8 (47.1%)	19 (19%)	0.025‡

*Mean ± s.e.m., †Kruskal Wallis anova on ranks, ‡Chi-square, §Median (Interquartile range).

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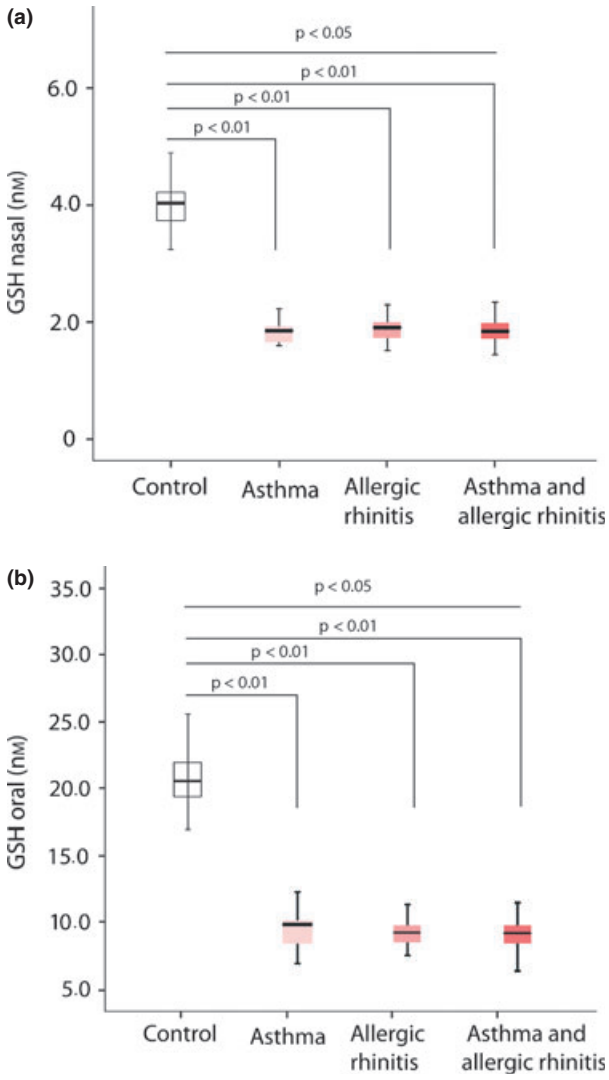


Figure 1 Glutathione concentrations in (a) nasal exhaled breath condensates (EBC) and (b) oral EBC.

We have previously shown that asthma is associated with increased oxidant burden and decreased anti-oxidant defense both in the systemic circulation and locally in the airways (15, 26, 27). This time, we aimed to determine the oxidative stress in allergic rhinitis and therefore we collected samples of air exhaled from the nose. There was no difference in the oxidative stress markers in nasally collected samples among different patient groups (asthma, rhinitis, asthma + rhinitis) but MDA levels were higher and GSH levels were lower compared to healthy controls. Our results are quite similar to those reported by Williamson et al. (28), who have shown that nasal nitric oxide levels were higher in similar patient groups compared to healthy subjects and that there was no difference among the patient groups themselves.

The diagnosis of allergic rhinitis was made if the patients had symptoms of rhinitis but did not show symptoms or signs of bronchial obstruction. This well-selected group of patients with allergic rhinitis had increased oxidative stress demonstrated by higher MDA and lower GSH levels in nasally and orally collected EBC samples. Still, the contamination from lower airways might contribute to this finding to some extent. The only difference between the patient groups was observed in the oral MDA levels in EBC. Patients with asthma had higher levels of oral MDA than patients with asthma + allergic rhinitis. This might be due to higher utilization of anti-inflammatory drugs including inhaled corticosteroids in patients with asthma and allergic rhinitis than asthma alone. Some of the patients with both asthma and allergic rhinitis were also using nasal corticosteroids which might have an additive effect on suppression of inflammation and oxidative stress markers. This explanation, however, cannot accurately account for the similarity of nasal MDA levels in EBC in patients with allergic rhinitis (47.1% using nasal corticosteroids) and patients with asthma + allergic rhinitis (19% using nasal corticosteroids). One possibility is that the use of inhaled corticosteroids may have indirectly influenced the MDA levels recovered in the nasal EBC. However, these observations require further investigation.

In this study, MDA levels and GSH levels showed positive correlations in both nasally and orally collected samples

Table 2 Reduced glutathione and malondialdehyde levels in the study population

	Control nasal (n = 68) Oral (n = 74)	Asthma nasal (n = 24) Oral (n = 27)	Allergic rhinitis nasal (n = 17) Oral (n = 17)	Asthma + allergic rhinitis nasal (n = 94) Oral (n = 99)	p	p
GSH_{nasal}	4.02 (3.73–4.2)	1.85 (1.66–1.92)	1.9 (1.71–2.05)	1.84 (1.7–1.97)	<0.05*	>0.008†
(Min–max)	1.22–6.25	1.59–2.47	1.51–2.5	1.43–2.48		
GSH_{oral}	20.49 (19.24–21.9)	9.06 (8.26–10.3)	9.12 (8.39–9.75)	9 (8.26–9.69)	<0.05*	>0.008†
(Min–max)	6.34–32.5	6.78–12.16	7.39–11.23	6.23–14.7		
MDA_{nasal}	3.84 (1.78–5.90)	7.84 (4.13–11.55)	7.25 (2.96–11.54)	5.44 (0.9–9.98)	<0.05*	>0.008†
(Min–max)	2.01–6.45	4.08–9.92	4.39–10.33	3.76–13.57		
MDA_{oral}	16.29 (7.83–24.75)	25.71 (18.81–32.61)	19.81(11.16–28.44)	17.78 (11.62–23.94)	<0.05*	<0.001‡
(Min–max)	8.74–28.06	14.3–32.54	14.97–28.86	13.29–30.67		

Data are presented as medians (interquartile ranges) (nm). *p Kruskal–Wallis anova on ranks, †p: Bonferroni correction (statistical analyses of mediator concentrations between patient groups), ‡p: Mann–Whitney *U*-test (statistical analyses between asthma and asthma + allergic rhinitis groups), GSH, glutathione; MDA, malondialdehyde.

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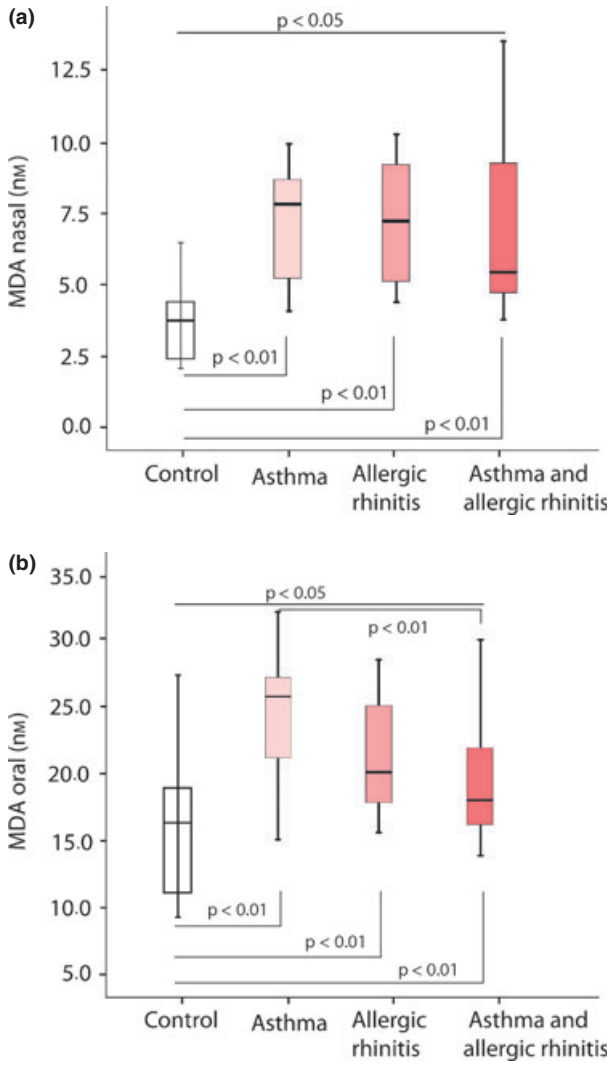


Figure 2 Malondialdehyde concentrations in (a) nasal exhaled breath condensates (EBC) and (b) oral EBC.

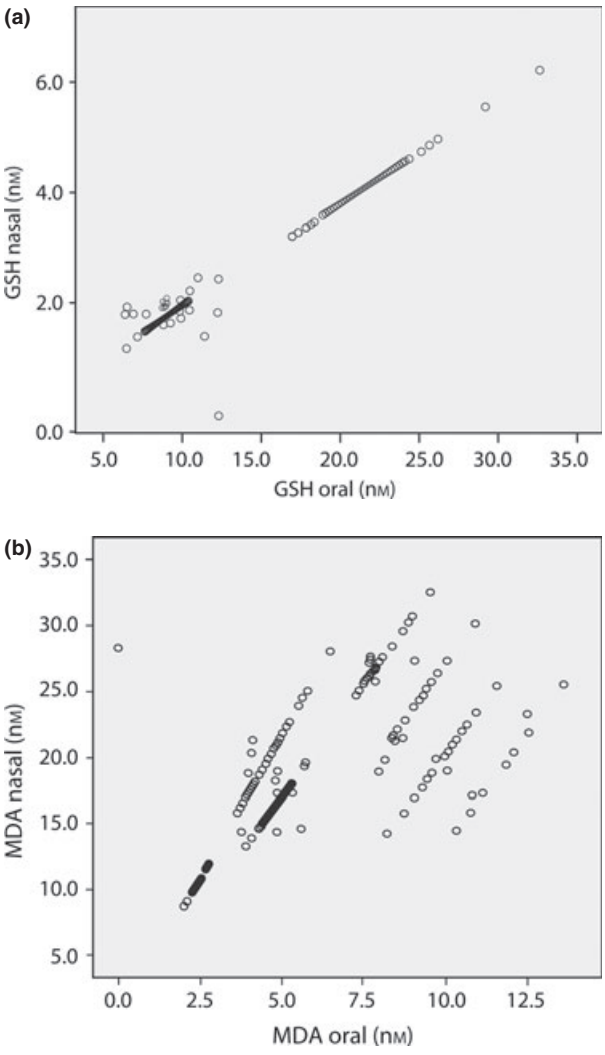


Figure 3 The correlation between concentrations of oxidative stress markers in nasal and oral exhaled breath condensates (a) glutathione (r_s = 0.684, p < 0.001) (b) malondialdehyde (r = 0.684, p < 0.001).

suggesting that the two methods of collection did not have superiority over each other. The negative correlation between MDA and GSH levels supports our previous observation in children with asthma (26) and suggests a common mechanism underlying both oxidant and antioxidant systems.

Our study has some limitations. We had small numbers of children with allergic rhinitis and asthma, but the observed power was enough to detect a difference between the groups. The technical difficulty of collection of especially nasal samples might cause salivary contamination. In EcoScreen device, exhaled air enters and leaves the chamber through a two-way valve system in which inspiratory and expiratory gas do not mix, and saliva is filtered. Although amylase measurements are carried out to test for the presence of salivary contamination in EBC, these measurements are not ideal and produce false positive and negative results (29). The use of the same method to collect EBC in all patients does not eliminate the risk of interindividual differences. Furthermore, even though we have collected EBC samples separately from the nose and lower airways, with the current approach, it is impossible to exclude the possibility of contamination from the lower airways in nasal samples.

To conclude, our study shows that there is an increased oxidative stress in children not only with asthma but also in those with allergic rhinitis compared to healthy controls lending further support to 'one airway, one disease' concept. In accordance, it also raises the possibility of treating the allergic respiratory diseases with an antioxidant approach especially as an adjunctive measure. As yet, however, no antioxidant approach has been consistently useful in treating chronic diseases. Thiol antioxidants and some dietary molecules including sulforaphane, quercetin, genistein, resveratrol are currently being investigated (30).

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