INTRODUCTION

Reactive oxygen species (ROS) serve as essential molecules for cell homeostasis at low concentration, but evoke harmful, pernicious effects if they are produced in excess (1). Numerous disorders in childhood have been linked to oxidative damage, but the role of ROS in their pathogenesis and progression is not entirely clear (1-3). One such disorder is the airway inflammation (4,5); exemplified by bronchial asthma which is characterized by reversible airflow obstruction, airway hyperresponsiveness, and activation of inflammatory cells and mediators in the airways. Environmental and genetic factors may play a role in the development of asthma; the exact mechanisms of these factors are not fully determined (6,7). Macrophages, neutrophils, and eosinophils in the airways release increased amounts of ROS in asthmatic patients (8). One of the most important targets of oxidative damage is proteins (1, 9). Proteins can go through many covalent changes after exposing to oxidants (10). Genome-wide search studies have demonstrated that many candidate regions contribute to asthma (11). The glutathione S-transferase (GST) (E.C.2.5.1.18) enzyme superfamily consists of alpha, kappa, mu, omega, pi, sigma, theta, and zeta isofoms in humans (12). \( \text{GST-T1} \) belong to the GST theta category of the enzyme. GST enzymes are critical for protecting cells from ROS, because they can utilize a wide range of products of oxidative stress as substrates. These enzymes act as antioxidant defenses in the lung through ROS metabolism, repairing damaged ROS, and they belong to the phase II detoxification system (12-14). Variants in GST confer risk to the development of asthma when the children are exposed to smoke (15). Because oxidative stress plays a role in the pathogenesis of asthma, and GST is critical for protecting cells from ROS, in the present study we hypothesized that GST gene polymorphisms could be a potential determinant of the development of asthma. We, therefore, set out to evaluate the oxidative stress and \( \text{GST-T1} \) polymorphism in children with asthma.

MATERIAL AND METHODS

Study subjects

The Ethical Committee of Jessenius Faculty of Medicine in Martin, Slovakia approved the study, and informed written consent was obtained from the parents of all tested children. The study population consisted of 225 children participants. Asthmatic children's histories were recorded using standard questionnaire categories: age, sex, exposure to tobacco smoke, and family history of asthma, wheezing and allergy. There were 201 asthmatic patients of the mean age 12 ±0.3 yr and 24 healthy age-matched children, used as controls, recruited in the Department of Pediatrics. The demographics of the study population are shown in Table 1. The asthmatic children were characterized by recurrent airways obstruction manifested by wheeze and dyspnea, relieved spontaneously or by bronchodilator therapy (as defined in GINA). 27 children (13%) were tested during an acute exacerbation (severe persisting wheezing with dyspnoea and/or coughing without sufficient response to therapy) and 174 children (87%) were examined when their disease was clinically controlled. Atopy was defined by the presence of history and positive skin prick test responses with a panel of 19 common aeroallergens. All the children with asthma, and GST is critical for protecting cells from ROS, in the present study we hypothesized that GST gene polymorphisms could be a potential determinant of the development of asthma. We, therefore, set out to evaluate the oxidative stress and \( \text{GST-T1} \) polymorphism in children with asthma.

Key words: bronchial asthma, children, glutathione S-transferase, oxidative stress, protein and lipid damage
at least one positive skin prick test to common aeroallergens were considered as atopic.

Venous blood was collected in ethylenediaminetetraacetic acid-coated tubes. Blood was used for genomic DNA preparation. Part of blood was centrifuged for 20 min, 2000 x g at 4°C and plasma was prepared.

**Determination of GST-T1 genotypes**

Genomic DNA for polymerase chain reaction (PCR) was isolated from peripheral blood leukocytes by a standard method. The presence of the GST-T1 null genotypes was detected using primers for GST-T1 and β-globin (positive internal control). The PCR products were visualized by electrophoresis on 2% agarose gel.

**Plasma protein sulfhydryl groups**

Plasma protein content of free sulfhydryl (-SH) groups were measured using Ellman’s reagent according to the method of Hu (16). Absorbance was read at 412 nm and the –SH group content was calculated using molar absorption coefficient of 13600 M⁻¹cm⁻¹, after subtraction of blank absorbance from the absorbance of a sample.

**Lipid peroxides assay**

Determination of thiobarbituric acid-reactive substances (TBARS) formation in plasma was performed according to Das (17). TBARS concentration was determined from the absorbance at 532 nm.

**Statistical analysis**

All values were presented as means ±SE. Subjects were compared with Student’s t-test and chi-square (χ²) test. Odds ratios (OR) and confidence intervals (95% CI) were used to analyse the frequencies of the GST-T1 genotypes in patients with asthma compared to the control group. A value of P<0.05 was considered to be statistically significant.

**RESULTS**

**Measures of oxidative stress**

Plasma protein modification in the asthmatic patients was demonstrated by a significant decrease in the total -SH group content by about 14.6±0.8% compared with the healthy subjects (P<0.001) (Fig. 1A). There were no appreciable differences in the -SH group content between the exacerbated and controlled asthma (Fig. 1B). However, asthmatics with atopy had a lower level of -SH group content (P<0.01) than the non-atopic ones (Fig. 1C).

Modifications in plasma lipid structure were assayed by the measurement of a level of TBARS. Oxidative stress caused an accumulation of TBARS; its levels increased by 33±3% in asthmatics (P<0.001) (Fig. 2A). TBARS concentration was significantly higher in the exacerbated than in the well controlled asthmatics (P<0.01) and in the non-atopic asthma compared to the atopic asthma (P<0.05).

**Table 1. Characteristics of the study population.**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Controls (n, %)</th>
<th>Patients (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 (100)</td>
<td>201 (100)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (50)</td>
<td>116 (58)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (50)</td>
<td>85 (42)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>14 ±0.7</td>
<td>12 ±0.3</td>
</tr>
<tr>
<td>Exacerbated AB</td>
<td>0</td>
<td>27 (13)</td>
</tr>
<tr>
<td>Controlled AB</td>
<td>0</td>
<td>174 (87)</td>
</tr>
<tr>
<td>Atopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopic</td>
<td>0</td>
<td>117 (58)*</td>
</tr>
<tr>
<td>Non-atopic</td>
<td>24 (100)</td>
<td>49 (24)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>0</td>
<td>35 (17)</td>
</tr>
</tbody>
</table>

AB – asthma bronchiiale, *P<0.001

**Table 2. Glutathion S-transferase genotype and the risk of developing asthma.**

<table>
<thead>
<tr>
<th>GST-T1</th>
<th>Controls (n=24)</th>
<th>Asthmatics (n=201)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>22 (92)</td>
<td>156 (78)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Null</td>
<td>2 (8)</td>
<td>45 (22)</td>
<td>3.17 0.72-14.01</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Glutathion S-transferase genotype and the risk of developing atopic asthma.**

<table>
<thead>
<tr>
<th>Atopic (n=111)</th>
<th>Non-atopic (n=49)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-T1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>92 (79)</td>
<td>36 (74)</td>
<td>1</td>
</tr>
<tr>
<td>Null</td>
<td>25 (21)</td>
<td>13 (27)</td>
<td>0.75 0.35-1.63</td>
</tr>
</tbody>
</table>
asthmatics (Fig. 2B) and also in atopic compared with non-atopic children (P=0.05; Fig. 2C).

GST-T1 gene polymorphism in control and asthmatic patients

Patients with asthma had a higher prevalence of the GST-T1 null genotype (22%) compared with that in the control group (8%). There was a 3.17-fold increased risk of asthma in individuals with the GST-T1 null genotype (OR=3.17; 95% CI, 0.72-14.01) (Table 2). On the other hand, the atopic asthmatics had a lower prevalence of the GST-T1 null genotype than the non-atopic ones (OR=0.75; 95% CI, 0.35-1.63) (Table 3). These differences were significant (P<0.05).

DISCUSSION

In the present study we observed increased oxidative stress in children with asthma, as evidenced by changes in protein and lipid markers of oxidative damage in the blood. We also found that total protein sulphhydrals in plasma were significantly decreased in asthmatic children and atopic asthmatic patients had a lower content of sulphhydrals than non-atopic ones. However, the content of -SH groups did not differ between the exacerbated and stable asthmatic children. In contrast, Nadeem et al. (18) have observed a significant difference in the total content of -SH groups between acute and stable adult asthmatics. It is, however, known that pediatric and adult asthma differ in several clinical characteristics.

Recent studies demonstrate that oxidative damage is enhanced not only in the lungs, but also in the blood in children and adults with asthma (18-20). Inflammation is associated with increased production of ROS and increased oxidative stress in the lungs. Increased generation of superoxide radical has been shown in asthmatic children (21). In accord with those findings, in the present study, we noted increased level of TBARS in asthmatic children and higher concentrations of TBARS were present in both astotic, compared with non-atopic asthmatic children, and during exacerbations. TBARS have been found decreased in exhaled breath condensate and in plasma in adult and children asthmatics (19, 21). Increased lipid peroxidation expressed by malondialdehyde concentration, has also been observed in peripheral blood samples (22). All these changes may result from overproduction of ROS or decreased antioxidant defense ability in children with asthma (21, 23).

In the present study, we showed that polymorphism in GST-T1 is associated with increased susceptibility to asthma in children. Asthmatic children had a higher prevalence of the null genotype than that in control subjects. This finding confirms previous data showing increased risk for asthma in people with the GST-T1 null genotype (24). However, we did not observe a difference between atopic and non-atopic asthmatic children. Several, but not all, studies had similar findings (25, 26). In some other studies, a higher prevalence of null genotype was noted in atopic children and adult asthmatics (24, 27). Due to some other studies, a higher prevalence of null genotype was noted in atopic children and adult asthmatics (24, 27). In contrast, Nadeem et al. (18) have observed a significant difference in the total content of -SH groups between acute and stable adult asthmatics.

In summary, the findings of the present study suggest that increased oxidative stress and GST-T1 genetic polymorphism are associated with childhood asthma and atopy and, therefore, may contribute to the pathogenesis of asthma.

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REFERENCES


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