Alpha-1 Antitrypsin Gene Polymorphism in the Egyptian Population: Association with Obstructive Lung Diseases

Rasha Daabis, Shaden Muawia, Amal Ahmed, Mohammed El-Shahat, Ahmad Youssef and Tarek Fekry

1Associate professor of Chest diseases, Department of Chest diseases, Faculty of Medicine, Alexandria University, Egypt
2Professor of Biochemistry and molecular biology, Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt
3Professor and head of Molecular Biology Department, Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt
4Professor of Biochemistry and molecular biology, Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt
5Professor of Chest diseases, Department of Chest diseases, Faculty of Medicine, Alexandria University, Egypt
6Assistant lecturer of Molecular Biology, Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt

Citation

ABSTRACT

Background: Given the potential adverse effects of asthma and Chronic Obstructive Pulmonary Disease (COPD), this study was undertaken to explore Alpha-1 Antitrypsin (AAT) polymorphism in the Egyptian population and its role in the development and/or progression of asthma and COPD. The identification of IL-10 as a potential modifier gene for COPD susceptibility provided insight into additional inflammatory pathways to consider in AAT deficiency.

Methods: This study was carried on 90 unrelated Egyptians; 37 asthmatics, 33 COPD patients and 20 controls. Patients were evaluated clinically and with spirometry. The frequency of AAT gene polymorphism was assessed by real-time PCR. Serum levels of AAT protein, IL-10 and IgE were estimated.

Results: The PiZ allele was found in COPD and asthma patients as well as controls. While the PiS allele was never shown up in all the groups. The prevalence of PiZ was higher in asthma and COPD than in controls (75.75%, 72.7% and 50% respectively). Serum AAT was significantly decreased in patients with asthma and COPD. Patients with the PiZ allele, despite having lower values of the serum AAT, this difference was not significant. Serum AAT was significantly correlated with severity of airflow obstruction in both asthma and COPD. There was a significant elevation of serum IgE in COPD patients carrying PiZ allele. Serum IL-10 was significantly higher in asthma and COPD patients than the controls. There was a positive significant correlation between IL-10 and IgE in COPD patients.

Conclusion: The PiZ allele frequency in the Egyptian population is higher among asthmatic and COPD patients, suggesting that it could in fact be an underlying hidden risk factor for the development of these diseases. Asthmatics carrying this deficient allele have a genetic predisposition for progressing to COPD. Genetic counselling of patients having obstructive airway diseases is very important for diagnosis, prognosis and treatment.

KEYWORDS: Alpha-1 antitrypsin deficiency; AAT gene polymorphism; Heterozygous PiMZ; Asthma; COPD; IL-10; IgE; Obstructive lung diseases.
INTRODUCTION

Alpha 1 antitrypsin (AAT) deficiency is a hereditary autosomal disorder, resulting from a variety of mutations in the alpha1-AT gene and associated with a high risk for the development of early-onset pulmonary emphysema. AAT is a highly polymorphic protein with more than 70 variants, known as Proteinase Inhibitor (PI) types. The Pi M allele and its serum subtypes are the most common of the normal alleles. The Pi Z is the commonest allele for the homozygous (PiZZ) severe deficiency that significantly increases the susceptibility to lung function loss and emphysema in smokers and non-smokers. PiMZ, the heterozygous condition, carries only a slightly higher independent risk of obstructive lung disease. The inheritance of an intermediate deficiency state such as PiSZ leads to intermediate susceptibility. AAT has a function of protecting the pulmonary parenchyma from the effects of Neutrophil Elastases (NE) which are potent destructive proteases. In case of AAT deficiency, a gradual destruction of the pulmonary tissue occurs, resulting finally into Chronic Obstructive Pulmonary Disease (COPD), emphysema and early death. Along with the enhanced susceptibility to the development of Chronic Obstructive Pulmonary Disease (COPD) there may also be an enhanced susceptibility to asthma. Asthma is the most common respiratory diagnosis in patients with AAT Deficiency (AATD) prior to the diagnosis of AATD.

Asthma and Chronic Obstructive Pulmonary Disease (COPD) are the most common obstructive lung diseases. They are both characterized by airway remodelling and chronic inflammation. Genetic factors play an important role in the development of these diseases, which has prompted much research to identify the underlying disease susceptibility genes. Given the potential adverse effects of asthma and COPD, this study was undertaken to explore AAT polymorphism in the Egyptian population and to elucidate the possible role of the Pi S and PiZ AAT alleles in the development and/or progression of asthma and chronic obstructive pulmonary disease. The identification of IL-10 as a potential modifier gene for chronic obstructive pulmonary disease susceptibility provided insight into additional inflammatory pathways to consider in alpha1-antitrypsin deficiency. Therefore, we estimated the serum level of AAT protein, IL-10 and IgE in our studied groups.

SUBJECTS AND METHODS

This study was conducted on 90 unrelated Egyptian persons, who were divided into three groups; group 1 included 37 asthmatic patients, group 2 included 33 COPD patients, and group 3 included 20 normal subjects as control.
kit (QIAGEN, HILDE, GERMANY). Real-time PCR mutation detection by allelic discrimination snpsig kit (Primer Design® Ltd), (Applied Biosystems Corporation) - California, USA. After optimizing the thermal cycling conditions, the reaction plate was loaded into the thermal cycler (Rotor–Gene Q, Applied Biosystems). The genotype of each sample is calculated by comparing the ratio of signals between the two channels (ROX and VIC).

STATISTICAL ANALYSIS

Data were collected, tabulated, then analyzed using SPSS version 13. Qualitative data were presented as numbers and percentage. Quantitative data were described using mean and standard deviation. Comparison between different groups regarding categorical variables was tested using Chi-square test. For normally distributed data, comparison between COPD, asthma and control groups were done using F-test (ANOVA) and pair wise comparisons; between each two groups was assessed using Post Hoc test (Scheffe), while for abnormally distributed data comparisons between the three groups were done using Kruskal Wallis test and pair wise comparisons was done using Mann Whitney test. Also the comparisons between mutant and wild cases in COPD or asthmatic groups was done either with Student t-test or Mann Whitney according to the normality of data. Significance test results are quoted as two-tailed probabilities. For all statistical tests, a p-value of <0.05 was considered significant.

RESULTS

Characteristics of the studied groups

The characteristics of the three studied groups are presented in table 1. There was a significant positive family history in asthmatic patients in comparison to the COPD group (p≤0.001). However, the airway obstruction was more severe in the COPD group than the asthmatic patients as measured by the FEV1/FVC (p<0.017).

<table>
<thead>
<tr>
<th></th>
<th>COPD (n=33)</th>
<th>Asthma (n=37)</th>
<th>Control (n=20)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>37</td>
<td>33</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.86 ± 13.52</td>
<td>61.27 ± 11.31</td>
<td>32.60 ± 5.66</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Sex M/F (%)</td>
<td>43/57</td>
<td>64/36</td>
<td>75/25</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Positive family history n(%)</td>
<td>16(43%)</td>
<td>1(3%)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>10(27%)</td>
<td>11(36%)</td>
<td>6(30%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>51.0 ± 14.0</td>
<td>50.06 ± 10.73</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>FVC% predicted</td>
<td>65.05 ± 13.60</td>
<td>64.58 ± 7.69</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>63.86 ± 12.30</td>
<td>57.55 ± 8.69</td>
<td>0.017</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Characteristics of the studied groups

Comparison between the three studied groups according to serum IgE, AAT and IL-10 levels (table 2)

Serum IgE was significantly elevated in the asthmatic group in comparison to the COPD and control groups (p≤0.001 for both). Also it was elevated in the COPD group in comparison to the control group (p≤0.01). (Table 5)

Serum AAT was significantly lower in the COPD and the asthmatic group in comparison to the control group at p≤0.01 and ≤0.05 respectively.

IL-10 was significantly higher in the COPD and the asthmatic group in comparison to the control group at p≤0.01 and ≤0.05 respectively.

Correlation between different parameters in COPD group (table 3)

In the COPD group, the serum AAT was positively correlated with FEV1, FVC and FEV1/FVC (P<0.001, 0.001 and 0.004 respectively). Concerning the IL-10, it was positively correlated with IgE (P<0.001).

Correlation between different parameters in asthma group (table 4)

In the Asthma group, the AAT revealed positive significant correlation with FEV1, FVC and FEV1/FVC (p=0.034, 0.031 and 0.014 respectively). Also, IL-10 showed positive significant correlation with FEV1, FVC and FEV1/FVC (P=0.020, 0.017 and 0.049 respectively).
Comparison between the three studied groups according to Z allele (PiZ) (Table 5)

- The M wild type allele was found in 9 COPD patients (27.3%) while the PiZ mutant type allele was found in 24 COPD patients (72.3%). The M wild type allele with Z primers was found in 9 asthma patients (24.3%) while the PiZ mutant type allele was found in 28 Asthma patients (75.7%).

- In the control group the percentage of wild and mutant types of PiZ allele was 50% for both types.

- This variation among the three groups was not statistically significant. The only significant difference was observed between Asthma group and control group (P=0.049) concerning wild and mutant type of PiZ (Table 5).

Comparison between the patients’ group and the control group according to PiZ (Table 6)

- When grouping the COPD and asthmatic patients in a single group and comparing them to the control group we found that the patients’ group presented more with the mutant type of PiZ than the control group (p= 0.039).

Relationship of the PiZ allele with different clinical and laboratory parameters in COPD and asthma group (Table 7)

- Patients carrying the mutant PiZ allele in the asthma group didn’t reveal any significant difference in the clinical or laboratory parameters in comparison to patients with wild type. However, in the COPD group, we found that COPD patients with the PiZ mutant allele had a significantly higher serum IgE (P = 0.015) in comparison to patients with wild type.

DISCUSSION

Alpha-1 antitrypsin deficiency (AATD) is a hereditary recessive autosomal disease caused by mutations in the AAT gene. This disease is characterized by abnormally low AAT concentrations in plasma. The clinical manifestations of AATD...
To our knowledge, there are no available data about the AAT deficiency status in our country. Also Edwin and Robert showed that, AAT deficiency remains undiagnosed in many patients, and there are often long delays between the onset of respiratory symptoms and diagnosis, and the condition is frequently not diagnosed. Therefore we sought to uncover any underlying mutations of the alpha1-AT gene in our population and their role in the predisposition to COPD or asthma.

The results of the study showed that PiZ allele was found in COPD and asthma patients as well as controls. While PiS allele was never shown up in all the groups. Moreover, the prevalence of PiZ was higher in asthma and COPD than in controls (75.75%, 72.7% and 50% respectively), suggesting that the presence of this deficiency allele could in fact be an underlying hidden factor that increased the susceptibility to the development of these diseases in our population.

In this study, the PiZ allele was detected in the heterogeneous state in Asthma and COPD patient. The role of potential deficiency genotypes other than PiZZ remains controversial in the pathogenesis of COPD. In particular, the involvement of MZ and other genotypes that do not lead to severe AAT deficiency are of interest in the susceptibility to COPD.

We have observed a decreased serum level of AAT in patients with asthma and COPD these results are in agreement with other studies carried by various workers. However, patients with the PiZ allele, despite having lower values of the serum AAT than those with the wild type, this difference was not statistically significant, which might need a larger studied population to demonstrate a statistically significant difference. The AAT plasmatic concentration may vary in the patient’s sample with other physiological or pathological states like age, asthma duration, an acute bronchial inflammation and/or the corticoid treatments.

Moreover, some reported data suggest that besides the AAT deficiency, smoking, atopic constitution and other factors may also contribute to the progression of pulmonary lesions which might explain this lack of statistical significance due to the interplay of several factors.

We have found that the serum concentration of AAT was significantly correlated with the severity of airway obstruction in both asthmatic and COPD patients. In their study, Eden et al. suggested that individuals with AATD lack a major anti protease defence against airway inflammation; they are more susceptible to allergen-mediated asthma and consequent progressive airway obstruction. Such patients may be candidates for measures aimed at reducing the impact of environmental aeroallergens.

The monitoring of these patients with wide-range lung function variations should provide an additional insight into the origin and pathogenesis of obstructive lung diseases correlated to the AAT deficiency.
Asthma and COPD have long been considered to be separate disease entities due to their different clinical phenotypes. There are, however, similarities in the types of inflammatory cells observed in the airways of patients with these diseases, and cytokines secreted by these types of cell interact as a network of inflammatory mediators.35

Considering the important role of cytokines in COPD and asthma, it is necessary to define the IL-10 as an inflammatory mediator. IL-10 has pleiotropic effects in immune regulation and inflammation. In addition, IL-10 has been known to inhibit the lymphokine production by Th1 but not Th2 clones and down regulate the Th1 cell differentiation.29-31

The results of the present study showed that the levels of IL-10 in patients of Asthma and COPD are significantly higher than the control group (P ≤ 0.001). The elevated level of IL-10 in the serum of asthma subjects indicated an increase in Type-2 activity through which the production of IL-4 and IL-13 may promote an isotype switch to IgE. Thus, a prominent shift in patient’ cytokine milieu from Type-1 to Type-2 may have resulted in the elevated levels of total IgE which was also demonstrated in our asthma and COPD patients.

There have been conflicting reports in literature on the levels of IL-10 in asthma patients. Kumar et al., showed that the levels of IL-10 in patients of asthma increased significantly (P = 0.001) in comparison to controls, he also found an increase in a big panel of cytokines (IL-1β, IL4, IL5, IL6, IL8) and he postulated that; these observations emphasized the fact that there is a complex series of inflammatory events in this disorder where eosinophils and neutrophils play interactive roles.21

However, Takaashi et al., in their study on Japanese subjects reported reduction in IL-10 level in sputum of bronchial asthma and in normal smokers as compared to healthy non-smokers.32 In a study carried out by Ceyhan et al., IL-10 in sera and induced sputum of asthma patients were found to be unaltered.33

Moreover, a study was done by Bhadoria et al. on inflammatory cytokines in Indian COPD patients, the study was undertaken for a cytokine profile including IL-10 and other cytokines (IL-1β, IL-4, IL-5, IL-6, IL-8) which showed a marked significant increase in serum concentration of IL-10 and the other cytokines in COPD patients compared to healthy controls. This pattern of serum cytokines indicates a switch of type-1 to type-2 cytokine predominance that may result in enhanced synthesis of IgE creating a systemic inflammatory response.34 This is further supported by our finding of a positive significant correlation between IL-10 and IgE levels in COPD patients (P<0.001).

Also we found that the COPD patients carrying the mutant allele PiZ had a significantly higher levels of serum IgE (p=0.015) which might indicate that asthmatic patients carrying this deficient allele might have a genetic predisposition for progressing to COPD.

The development of asthma in patients with AATD may have additive long-term effects on the development of irreversible airway obstruction and emphysema. In this regard, both an increased serum IgE titer and atopy have been associated with the development of chronic obstructive lung disease.27

An increased serum IgE level is also not specific for asthma. It is associated with cigarette smoking,35 and may be a marker of airway inflammation.27 However, it is unlikely that smoking was the causative factor in our study, since the proportion of current smokers in the COPD and the control groups did not show significant difference. In addition, there was no significant difference between smokers and non-smokers in mean total serum IgE concentrations. Therefore, airway inflammation or underlying asthma is a more likely cause of the increased mean serum IgE in COPD patients with the mutant allele PiZ.

Over the long term, asthma may have an adverse impact on lung function in persons with AATD. Chronic bronchial-wall inflammation could result in structural remodelling that leads to irreversible narrowing of airways. In this regard, Villar and co-workers reported that atopy and bronchial responsiveness in elderly, former and current smokers, predisposes to an accelerated decline in FEV1.36

A significant proportion of patients with severe AATD and advanced emphysema show clinical features of asthma, and asthma appear to be more common in patients with this condition than in those with COPD and a normal Pi phenotype. The increased serum IgE level indicates that allergic mechanisms could contribute to the development of chronic airway obstruction. It is suggested that because individuals with AATD lack a major anti protease defence against airway inflammation, they are more susceptible to allergen-mediated asthma and consequent progressive airway obstruction.37 In addition, increasing the concentration of AAT in these patient’s airways might ameliorate the effect that environmental factors have on them.37

In conclusion, this study demonstrated that the z allele frequency in the Egyptian population is higher among the asthmatic and COPD patients, suggesting that it could in fact be an underlying hidden risk factor for the development of these diseases. The early identification of this mutant allele and other polymorphisms presents predictive and therapeutic avenues in the context of obstructive airway diseases. Asthmatics carrying this deficient allele have a genetic predisposition for
progressing to COPD. Genetic counseling of patients having obstructive airway diseases is very important for diagnosis, prognosis and treatment.

DECLARATION OF INTEREST

I declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

FUNDING

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

REFERENCES


