Research

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

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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: PiZ allele was found in COPD and asthma patients as well as controls. While 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 z 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.

ABBREVIATIONS: AAT: Alpha 1 antitrypsin; PI: Proteinase Inhibitor; COPD: Chronic Obstructive Pulmonary Disease; NE: Neutrophil Elastases.

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.[1] 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.[2] 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.[3] 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.[4-5] 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.[6]

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.[7-8] 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.[9] 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; group1 included 37 asthmatic patients, group 2 included 33 COPD patients, and group 3 included 20 normal subjects as control.

Asthmatic patients were diagnosed according to the standard clinical presentation and spirometry. Asthmatic patients exhibited a positive airway reversibility test, as defined by a post bronchodilator improvement of Forced Expiratory Volume in one second (FEV_1) by more than 12% and 200ml.[10]

The diagnosis of COPD was based on the definition provided by the Global initiative for chronic Obstructive Lung Disease (GOLD), which is characterized by a post-bronchodilator Forced Expiratory Volume in one second (FEV_1) to Forced Vital Capacity (FVC) ratio of < 70% and a post bronchodilator reversibility of <12% and 200 ml.[11]

The control group included normal subjects who were recruited from the general population and had no respiratory symptoms, and no evidence of airflow obstruction. They were excluded if they had a history of atopy, an acute pulmonary infection in the 4 weeks preceding assessment for the study, or a family history of asthma or COPD. All the cases and controls were unrelated Egyptian people who were selected from the same population. The cases were recruited from the inpatient and the outpatient clinic of the chest diseases department, Alexandria main university hospital. All subjects were enrolled in the study after a written informed consent according to the protocol approved by the Ethics Committee of the Alexandria Main University Hospital.[12]

Quantitative determination of Immunoglobulin E

Measurement of Immunoglobulin E (IgE) by The Electrochemi-luminescence immunoassay (ECLIA) technique based on the sandwich principle. It was used on Elecsys and (cobas e) immunoassay analyzers (Roche Diagnostics GmbH, Mannheim, Germany). Using the mean absorbance value for each sample determine corresponding concentration of Total IgE in IU/ml from the standard curve.[13]

Quantitative determination of AAT level

Measurement of Alpha 1 antitrypsin(AAT) serum level by Radial Immune Diffusion (RID) plates (BIOCIENTIFICA S.A., Buenos Aires-Argentina).[14]

Quantitative determination of serum level of Interleukin-10

Measurement of plasma IL-10 was done by Enzyme-Linked Immunosorbent Assay (ELISA) method. Avi Bion-Human IL-10 ELISA Kit was used (Orgenium Laboratories-Vantaa, Finland).[15]

Genotyping

Genomic DNA was isolated from 300 μL of whole venous blood using QIAamp DNA Mini and Blood Mini

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 FEV_1/FVC (p=0.017).

	Asthmatic ptients	COPD pa-tients	Control subjects	P
Subjects (n)	37	33	20	
Age (years)	40.86 ± 13.52	61.27 ± 11.31	32.60 ± 5.66	<0.001*
Sex M/F (%)	43/57	64/36	75/25	NS
Positive family history n(%)	16(43%)	1(3%)		<0.001*
Current smoking	10(27%)	11(36%)	6(30%)	NS
FEV_1 % predicted	51.0 ± 14.0	50.06 ± 10.73		NS
FVC% predicted	65.05 ± 13.60	64.58 ± 7.69		NS
FEV_1/FVC	63.86 ± 12.30	57.55 ± 8.69		0.017*

M=Male F=Female n, number, NS= not significant
*: Statistically significant at p < 0.05

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.

	COPD (n=33)	Asthma (n=37)	Control (n=20)	X^2	p
IgE (Iu/ml)					
Mean ± SD.	112.18 ± 160.50	336.99 ± 370.34	29.20 ± 11.45	45.087*	<0.001*
Pairwise comp.	I-II***, I-III**, II-III***				
AAT (mg/dl)					
Mean ± SD.	94.65 ± 33.31	101.96 ± 44.75	123.82 ± 33.09	8.099*	0.017*
Pairwise comp.	I-III**, II-III*				
IL-10 (pg/ml)					
Mean ± SD.	4.25 ± 2.37	4.14 ± 2.62	2.76 ± 0.41	8.460*	0.015*
Pariwise comp.	I-III**, II-III*				

x^2: Chi square for Kruskal Wallis test
Pair wise comparison was done using Mann Whitney test
*: Statistically significant at p < 0.05
**: Statistically significant at p < 0.01
***: Statistically significant at p < 0.001

Table 2: Comparison between the three studied groups according to IgE, AAT and IL-10 serum levels

Correlation between different parameters in COPD group (table 3)

In the COPD group, the serum AAT was positively correlation with FEV_1, FVC and FEV_1/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 FEV_1, FVC and FEV_1/FVC. (p =0.034, 0.031 and 0.014 respectively). Also, IL-10 showed positive significant correlation with FEV_1, FVC and FEV_1/FVC (P =0.020, 0.017 and 0.049 respectively).

		IgE	AAT	IL-10
FEV₁	r	-0.081	0.537*	0.021
	p	0.655	0.001	0.907
FVC	r	-0.083	0.531*	-0.027
	p	0.646	0.001	0.881
FEV₁/FVC	r	-0.106	0.494*	-0.066
	p	0.558	0.004	0.714
IgE	r		0.099	0.603*
	p		0.583	<0.001
AAT	r			0.236
	p			0.186

r: Pearson or Spearman coefficient
*: Statistically significant at p < 0.05
**: Statistically significant at p < 0.01
***: Statistically significant at p < 0.001

Table 3: Correlation between different parameters in COPD group

		IgE	AAT	IL-10
FEV₁	r	0.196	0.349*	0.381*
	p	0.246	0.034	0.020
FVC	r	0.263	0.354*	0.392*
	p	0.116	0.031	0.017
FEV₁/FVC	r	0.248	0.401*	0.326*
	p	0.139	0.014	0.049
IgE	r		0.289	-0.059
	p		0.083	0.730
AAT	r			-0.104
	p			0.539

r: Pearson or Spearman coefficient
*: Statistically significant at p < 0.05
**: Statistically significant at p < 0.01
***: Statistically significant at p < 0.001

Table 4: Correlation between different parameters in asthma group

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).

	COPD (n=33)		Asthma (n=37)		Control (n=20)		χ²	p
	No.	%	No.	%	No.	%		
PiZ								
Wild type (M)	9	27.3	9	24.3	10	50.0	4.352	0.114
Mutant type (PiZ)	24	72.7	28	75.7	10	50.0		
p₁	0.140		0.049*					
p₂	0.778							

χ²: Chi square test
p1: p value for Chi square test for comparing between control and each patient group
p2: p value for Chi square test for comparing between COPD and Asthma group
*: Statistically significant at p < 0.05
**: Statistically significant at p < 0.01
***: Statistically significant at p < 0.001

Table 5: Allele frequency in the three studied groups according to PiZ

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).

	Cases (n=70)		Control (n=20)		χ²	p
	No.	%	No.	%		
PiZ						
Wild type (M)	18	25.7	10	50.0	4.281*	0.039*
Mutant type (PiZ)	52	74.3	10	50.0		

χ²: Chi square test
*: Statistically significant at p < 0.05
**: Statistically significant at p < 0.01
***: Statistically significant at p < 0.001

Table 6: Comparison between the allele frequency in the patients and control group according to PiZ

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.[16] The clinical manifestations of AATD

	COPD		Asthma	
	M Wild type allele (n = 9)	PiZ Mutant allele (n = 24)	M Wild type allele (n = 9)	PiZ Mutant allele (n = 28)
IgE				
Mean±SD.	35.63 ± 29.07	140.89 ± 179.98	269.84 ± 256.16	358.58 ± 401.80
Z (p)	2.426* (0.015*)		0.637 (0.524)	
AAT				
Mean ± SD.	104.12 ± 26.48	91.10 ± 35.38	107.69 ± 42.60	100.13 ± 46.02
Z (p)	1.497 (0.135)		0.673 (0.501)	
IL-10				
Mean ± SD.	4.13 ± 1.97	4.29 ± 2.55	3.87 ± 1.56	4.23 ± 2.90
Z (p)	0.101 (0.919)		0.071 (0.943)	

t: Student t-test
Z: Z for Mann Whitney test
*: Statistically significant at $p < 0.05$
**: Statistically significant at $p < 0.01$
***: Statistically significant at $p < 0.001$

Table 7: PiZ allele distribution according to IgE, AAT and IL-10 in each group of patients

vary widely among individuals, ranging from asymptomatic in some to fatal liver or lung disease in others. The lung manifestations of AATD include emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), bronchiectasis, and asthma.[17]

AATD has been identified in all population groups worldwide. The MM phenotype includes the individuals who are homozygotic for the normal M allele and have a normal concentration of AAT in the plasma enabling the adequate antprotease protection, while the ZZ phenotype includes the individuals homozygotic for the Z allele which is responsible for 95% of severe alpha1-AT deficiency. The role of a moderate AAT (MZ and SZ genotypes), and MM deficiency is less clear. Some authors think the role of AAT deficiency in COPD might have been overestimated.[18]

To study the AAT deficiency, we performed genotyping of the AAT gene to detect any polymorphism and assess its frequency. In addition, the serum AAT protein level was estimated.

There are extensive data in the literature on the prevalence of the two most common deficiency alleles, corresponding to PiS and PiZ variants, in countries all over the world. Prevalence of AAT deficiency varies from one country to another and knowledge about the AAT deficiency status in every country is essential. AAT deficiency is mostly prevalent in European countries with high frequencies in Spain and Portugal.[19]

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.[20] 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 heterozygous 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.[21-24] 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.[25,26]

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.[18]

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.[27]

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.[28]

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 FEV_1.[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.[27]

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

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