



Original article

Association between severity of asthma and fungal sensitization in severely asthmatic patients

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ABSTRACT

Background: Fungal sensitization is usually associated with increased asthmatic severity, morbidity and mortality, including higher rates of hospital and intensive care admission. However, the possible association between fungal airway colonization with regard to sensitization remains controversial. **Objectives:** to investigate the presence of severe asthma with fungal sensitization among asthmatic patients and to find evidence of *Aspergillus fumigatus* (*A. fumigatus*) role in severely asthmatic patients. **Methods:** this study investigated forty asthmatic patients and twenty controls. Demographic data of all subjects within the study was collected, as well as total serum IgE, *A. fumigatus* specific IgE and *A. fumigatus* galactomannan antigen ELISA test. **Results:** our results showed significant elevation of total IgE, *Aspergillus* specific IgE and *A. fumigatus* galactomannan antigen in 75% of severely asthmatic patients, compared to patients with mild asthma and negative values in control. **Conclusion:** We report a high percent of *A. fumigatus* colonization among severely asthmatic patients. This supports the hypothesis of the role of fungal elements colonization in the airways of patients with severe asthma.

Introduction

Fungal colonization has always been recognized as a predisposing factor in asthmatic conditions, involved in seasonal outbreaks of high risk asthmatic categories [1].

Fungal sensitization is associated with increased asthma severity, morbidity, and mortality, including higher rates of hospital and intensive care admission. [2]. However, the possible association between fungal airway colonization with regard to

sensitization remained controversial for a long period of time [3].

In asthmatic patients, sensitization by *Aspergillus* is stated to be more frequently associated with severe asthmatic conditions. The chief respiratory conditions triggered by these fungal elements are *Aspergillus* induced asthma (AIA), severe asthma with fungal sensitization (SAFS) and allergic broncho-pulmonary aspergillosis (ABPA) [4]. Phenotypes of asthmatic conditions with fungal sensitization were mostly recognized as ABPA that

was defined by the presence of high IgE level above 1000 IU/mL with association of airway colonization detectable in investigated patients [5]. Severe asthma with fungal sensitization sub-phenotype was recognized especially in adults [6] and interconnected to reduced respiratory functions together with increased morbid conditions [7,8]. The definition of SAFS includes severe asthma treatment at British Thoracic Society level 4 or equivalent; fungal sensitization demonstrated by a positive skin prick test (SPT) response or specific IgE to at least one of 7 fungi (ie, *Aspergillus fumigatus* (*A. fumigatus*), *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *Candida albicans*, *Trichophyton mentagrophytes*, or *Botrytis cinerea*); with no evidence of allergic bronchopulmonary aspergillosis [9].

Low count of airway fungal colonization was used to clarify the former effort in isolating colonization using the standard plate culturing procedures especially as culture-based approaches are not that sensitive [10]. This poor sensitivity of the traditional culture-based approaches has hindered investigating SAFS, although more technologically advanced methods have been recently in use. Moreover, this debate around the role of airway fungal colonization in these conditions might have led to consequent controversy about the optimum management line, especially for severe conditions as in SAFS. [4-10].

We recognize that SAFS may be associated with a variety of fungal elements. Previous reports indicated that *Aspergillus* (specifically *A. fumigatus*) is associated with most cases of fungal sensitization and airway colonization [8-11]. Therefore, in this study we hypothesized that *A. fumigatus* is associated with severity of asthma in asthmatic patients and aimed to investigate the prevalence of SAFS among asthmatic patients and to find evidence of *A. fumigatus* role in SAFS patients.

Methods

In the present cross sectional study, a total of 60 (40 asthmatic and 20 control) subjects were included during a 6 months period. They were divided into three groups, 20 healthy individuals were presented as control (group I), 20 patients with mild asthmatic conditions (group II) and 20 patients with severe asthmatic clinical conditions (group III). Patients of the age group between 20-65 years diagnosed with asthma but free of other ailments such as parasitic infection, etc., were registered for the

study. Each patient was thoroughly examined by the physician and classified as having mild or severe asthma using the guidelines of the European Respiratory Society [12]. Age and sex matched healthy subjects with no history of respiratory disorder, any atopic signs and symptoms, helminthic or parasitic infection, were considered as control subjects. Informed consents were taken from all participants before testing. Ethical approval was obtained from the Research Ethics Committee, Faculty of Medicine, Tanta University.

Demographic data of all patients within the study was collected, as well as measurement of total serum IgE (T- IgE), *A. fumigatus* specific IgE (Asp-IgE) and *A. fumigatus* galactomannan antigen ELISA test.

The registration of participants, data collection and blood samples collection were done in the Out-Patient Clinic of Chest Department, Faculty of Medicine, Tanta University. The participant patients were newly diagnosed with no history of corticosteroids intake. All laboratory investigations were performed in Medical Microbiology & Clinical Pathology Departments, Faculty of Medicine, Tanta University. The blood samples were collected by vein puncture method under complete aseptic conditions. Sera were separated and stored at -70°C until analysis.

Quantitation of total serum IgE and *Aspergillus fumigatus* specific IgE:

Peripheral blood samples were collected from the participants in plain tubes using the standard vein puncture protocol. Samples were allowed to clot at room temperature then centrifuged for 10 min at 1000 xg to separate sera. Total IgE was quantitated using AIA-900 Immunology analyzer (TOSOH BIOSCIENCE, CA, USA). Specific IgE against *A. fumigatus* was quantitated using Immulite - 2000/xpi immunoassay system (Siemens® Healthineers, GmbH, Germany). The upper accepted limits of normal T- IgE and Asp- IgE have been reported to be 150-300 UI/ml and 0.1 KU/L respectively [13].

Aspergillus fumigatus galactomannan antigen ELISA test:

Galactomannan test was carried out using Human Galactomannan, GM ELISA Kit (cat no E3948Hu, Bioassay Technology Laboratory, Shanghai, China). The kit employs the double antibody sandwich technique to quantitate the galactomannan antigen with an assay range of 0.2-60ng/ml and analytical sensitivity of 0.08ng/mL.

Statistical analysis

Quantitative data were expressed as mean and standard deviation (mean \pm SD). One-way ANOVA with Tukey's post hoc test was applied to evaluate the association between the numerical variables using SPSS, Version 26 (IBM Corp, Armonk, NY, United States, 2019).

Results

For the data analysis, blood samples were obtained from 40 asthmatic patients and similar samples from 20 controls. For a patient to be considered having SAFS [5], they should have elevated total IgE (above 100 IU/ml but not above 1000 IU/ml), elevated *A. fumigatus* specific IgE and positive results for *A. fumigatus* galactomannan antigens. The average patient age was 48 years with 68% of them females. Out of the SAFS patients, there were 67% females and 33% males.

Table 1 shows significant elevation of total IgE levels in patients with SAFS with a mean SD \pm 345.68, compared to mild elevation in patients with mild asthma (\pm 72.0) and normal values in controls (\pm 27.95). Also, significant elevation of *A. fumigatus* specific IgE levels was found in patients with SAFS with a mean SD \pm 2.18, compared to mild elevation in patients with mild asthma (\pm 0.261) and normal values in controls (\pm 0.15).

Table 2 shows significant positive results of galactomannan antigen found in 75% of severely asthmatic patients, compared to 15% of patients with mild asthma and negative values in control.

Table 3 shows persons' correlation analysis between Total IgE, *A. fumigatus* specific IgE, and Galactomannan antigens. It showed a significant positive correlation between total IgE, *A. fumigatus* specific IgE, and galactomannan antigens.

Table 1. Comparison between levels and values of total serum IgE, *Aspergillus fumigatus* specific IgE and *Aspergillus fumigatus* galactomannan antigen in the three studied groups.

Parameter	Groups			F-value
	Group (I) Control (n=20)	Group (II) Mild asthma (n=20)	Group (III) Severe asthma (n=20)	
Total IgE (U/mL)	27.95 \pm 13.23 ^c	72.0 \pm 8.03 ^c	343.55 \pm 345.68 ^{a, b}	14.640
<i>Aspergillus fumigatus</i> specific IgE (KU/L)	0.15 \pm 0.107 ^c	0.261 \pm 0.50 ^c	1.95 \pm 2.18 ^{a, b}	12.523
Galactomannan ELISA test	1.0 \pm 0.0 ^c	1.15 \pm 0.37 ^c	1.75 \pm 0.44 ^{a, b}	28.5

^{a-c} Significant difference between groups at * $p < 0.05$. ^a significance from control; ^b significance from mild asthma; ^c significance from severe asthma. The degree of freedom is (2) between groups and (57) within groups (total = 59).

Table 2. *Aspergillus fumigatus* galactomannan antigen positivity in the three studied groups.

Galactomannan ELISA test	Control (n=20)		Mild asthma (n=20)		Severe asthma (n=20)	
Positive	0	0%	3	15%	15*	75%
Negative	20	100%	17	85%	5	25%

Table 3. Correlation between total IgE, *Aspergillus fumigatus* specific IgE and galactomannan antigens.

		Total IgE (U/mL)	<i>Aspergillus fumigatus</i> specific IgE (KU/L)
<i>Aspergillus fumigatus</i> specific IgE (KU/L)	r	0.395	
	P	0.002	
Galactomannan antibodies	r	0.480	0.673
	P	<0.001*	<0.001*

*Significance at p -value <0.05

Discussion

Sensitization of the respiratory tract with fungal elements in severely asthmatic patients has impending association to the clinical and therapeutic aspects of respiratory asthmatic conditions as well as further research implications.

In this study, the average patient age was 48 years. Out of the severely asthmatic patients, there were 67% females and 33% males. Similarly, in a study by O'Driscoll et al, most of SAFS patients were females [14]. While **Castanhinha et al.** showed that most of SAFS patients were males. This can be explained by the different age group of their study, in which the study population was mainly pediatric [9].

Our study investigated the role of *A. fumigatus* in severely asthmatic and SAFS patients through measurement of total serum IgE (T- IgE), *A. fumigatus* specific IgE (Asp- IgE) and *A. fumigatus* galactomannan antigen ELISA test. Similarly, a recent study by **Moss et al.** highlighted that there should be an increased focus on *A. fumigatus* as a commonly isolated species in respiratory allergic conditions; although others may be involved, *A. fumigatus* remains the main pathogenic fungal element encountered in the vast majority of cases [15].

Our results showed that 45% of the studied asthmatic patients (from both mild and severe groups) had fungal sensitization based on elevated total IgE (above 100 IU/ml but not above 1000 IU/ml), elevated *A. fumigatus* specific IgE and positive results for *A. fumigatus* galactomannan antigens. Similar to our results, **Agrwal et al.** revealed that fungal sensitization prevalence among asthmatic patients was ranging from 15% to 48%, with a pooled prevalence of 28% [16]. Similar frequency of *Aspergillus* sensitization has been documented in following studies [17, 18]. This analysis included patients with asthma being managed in asthma or chest clinics in which most of the patients can be classified as having severe asthma.

On the other hand, another systematic review has reported lower prevalence at 12.9% [19]; while Patterson et al. estimated that fungal allergy affects only 1 to 2% of the asthmatic population [20]. Moreover, **Moghtaderi et al.** reported low prevalence of ABPA and SAFS in their study considering the high prevalence of severe asthmatic conditions in the studied area [4]. This may be

attributed to different genetic bases, geographical and atmospheric conditions between different countries.

Recent studies documented the association between fungal sensitization and asthmatic severity. **Lin et al.** reported that the connection between fungal sensitization and poorer clinical findings is evident [21]. While **Kao et al.** added that fungal sensitization not only contributes to the development of asthma but also the severity of asthmatic condition [22].

Other studies discussed that SAFS is thought to affect 20 to 25% of patients with persistent asthma, suggesting that the dynamics between asthmatic severity and sensitization of fungal elements is not well understood, and both conditions may be represented in a variety of different respiratory allergic conditions [6, 23].

Our study revealed that most of the severely asthmatic studied population had elevated total IgE levels >100 kIU/L together with elevated *Asp- specific IgE* and positive results for *A. fumigatus* galactomannan antigens. Similarly, **Hogan et al.** suggested that a highly elevated nonspecific IgE level, elevated fungal specific IgE level with clinical features of severe asthma, would be better labeled as SAFS [24]. Moreover, **Kanj et al.** confirmed that screening of SAFS and ABPA should be better performed using the *A.fumigatus specific* IgE blood test than the less sensitive *Aspergillus* skin prick test [25].

In addition, **Chishimba et al.** suggested that total IgE and *A. fumigatus* specific IgE might be associated with reduced lung function, more asthmatic severity and may be linked to disease pathogenesis [26]. Moreover, the high positivity of galactomannan (75%) reported in SAFS cases supports the theory of airway colonization in most of the severely asthmatic cases.

Conclusion

This study aimed to find evidence of *Aspergillus* role in severely asthmatic patients. Our results suggest a high prevalence of *A. fumigatus* in SAFS patients. This supports our hypothesis about the association between fungal elements colonization in the airways of asthmatic patients and the severity of their asthmatic conditions.

The reporting of new phenotypes and sub-phenotypes of fungal sensitization in asthmatic patients permits the commencement of a new era for our consideration of the role of these frequently met

fungal elements in asthmatic conditions. Further research, especially when associated with immunological and genetic based research into the disease mechanisms will allow better decision-making regarding management of the affected patients. It is also more likely that other internal and/or external cofactors as concomitant respiratory diseases, even non-respiratory chronic diseases e.g., diabetes mellitus, and environmental factors could influence these conditions presentation and management lines. Therefore, new classification of these conditions will enable increased understanding of the possible interactions between *Aspergillus* and those factors and offer us deeper insight to the attitude of *Aspergillus* as a sensitizer, colonizer and/or pathogen which will definitely have great implication on patient management and the choice of suitable therapeutic strategies.

However, our study might have several limitations, due to its “realistic” nature. The limited study population and being a single center study will require further provision by larger multi-center research and further analysis of the galactomannan positive patients in order to prove the theory proposed in this study. It might also be supported by associative evaluation of significant clinical data including respiratory physiological tests, airway inflammatory markers and microbiological testing to differentiate between various *Aspergillus* spores, hyphae and other fungal.

Conflicts of interest

No conflict of interest.

Financial disclosure : None.

Authors' Contribution

All authors contributed equally to this work.

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