International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com

Research Article

Frequency of Fungal Sensitisation in Patients with Severe Atopic Asthma

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Abstract

Background: The incidence of mould allergy ranges from 6 to 24% in the general population, up to 44% among atopics and 80% among asthmatics. Since 2006, SAFS became recognized as a new phenotype of asthma, and was conceptualized as a continuum of fungal sensitization, with asthma at one end and ABPA at the other. The paucity of data and ambiguity in diagnostic criteria currently renders SAFS more of a diagnosis of exclusion than a specific entity. **Objective:** Our aim was to identify frequency of SAFS in atopic severe asthma patients attending our tertiary care clinic. **Materials and methods:** The study was conducted on a cohort of 50 patients with severe atopic asthma. All patients were subjected to history taking for asthma symptoms, skin test with fungal antigens, total IgE level, peripheral blood eosinophilia, chest x-ray and high resolution CT chest. **Results:** We demonstrated SAFS in 38 patients (76%). Fungal allergens that demonstrated statistical significance in SAFS patients were *Alternaria alternata* in 65.8%, *Aspergillus fumigatus* in 53.3% and *Cladosporium* in 52.6%. **Conclusion**: We report high frequency of fungal sensitisation (76%). There is a need for better recognition of SAFS as it is rather under recognized.

Keywords: SAFS, fungal sensitisation, *Aspergillus fumigatus*, *Alternaria*, *Cladosporium*, severe asthma

Introduction

The immunological mechanisms underlying mould allergies are hypersensitivity reactions of type I, II, III and IV. The spectrum of allergic symptoms caused by these hypersensitivity reactions is broad, including rhinitis, asthma and atopic dermatitis. Type I allergy is induced by a large number of fungal genera. The majority of them are members of the Ascomycota or the Basidiomycota. The most important allergy-causing fungal genera belonging to the Ascomycota are *Alternaria, Aspergillus, Bipolaris, Candida, Cladosporium* and *Epicoccum* (1). Mould sensitivity has been associated with increased asthma severity and death, hospital admission and intensive care admissions in adults (2). Amongst patients with persistent asthma requiring specialist referral, 20–25% exhibit skin-test reactivity to *Aspergillus* or other fungi (3). The term severe asthma with fungal sensitization (SAFS) was first introduced by Denning and colleagues in 2006 (2). SAFS is diagnosed by the presence of severe asthma, fungal sensitization, and exclusion of allergic bronchopulmonary aspergillosis (ABPA). Our aim was to detect frequency of SAFS in patients with severe atopic asthma.

Materials and Methods

The study is a pilot study conducted on a cohort of 50 patients with atopic asthma attending the Allergy and Immunology clinic at Ain Shams University hospitals. We included patients with severe bronchial asthma, having positive skin prick test (SPT) response to one or more of the common locally encountered allergens with positive family history of atopy as evidence of the atopic status. The study was approved by Ain Shams Ethics committee, and participants gave informed consent to participating.

Exclusion criteria included patients with ABPA fulfilling at least 5 criteria out of 8 criteria for diagnosis of ABPA according to Patterson et al (4), patients with inflammatory or septic conditions, including oral sepsis, patients with associated systemic autoimmune diseases, patients with organ failure, patients on systemic glucocorticoids and/or systemic antifungals, patients on antihistamines or cytotoxic drugs and pregnant females.

All patients were subjected to the following

1- Detailed allergic history and clinical examination, with emphasis on presence of family history of asthma and/or atopic disorders, and associated allergic rhinitis and/or urticaria.

2- Pulmonary function test (spirometry) for staging of bronchial asthma by Peak expiratory flow rate measurement by a Wright Peak Flow Meter (by Fred Ferraris (Clerkenwell) Ltd, London, United Kingdom). Assessment of asthma severity was done according to according to GINA 2015 guidelines (5).

3- SPT using common environmental allergen extracts and skin testing (prick and intradermal) with fungal allergen extracts of *Aspergillus fumigatus, Alternaria alternata, Cladosporium, Candida, Epicoccum* and *Trichophyton* provided by Greer, Lenoir UNITED STATES.

4- Routine biochemical tests for exclusion of systemic diseases, examination of Leishman-stained peripheral blood smears with stress on differential leukocytic count especially for the absolute eosinophilic count using Sysmex KX-21N, USA.

5- Total serum IgE levels using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (BioCheck, Inc., Foster City, USA).

6- Radiological investigations in the form of plain chest x-ray with posteroanterior view (CXR, P/A view) and high resolution CT chest (HRCT chest).

Skin tests
SPT (puncture) method

The allergen panel included some common locally encountered allergens: Hay dust, horse hair, mixed pollens, goat hair, cat hair, mite, cotton dust, pigeon, dog hair, cockroach, straw, house dust, feather, wool, in addition to standardized allergen extract for the fungi *Aspergillus fumigatus, Alternaria alternata, Cladosporium, Penicillium, Candida* and *Trichophyton* provided by Greer, Lenoir UNITED STATES.

After sterilization of the forearm with propyl alcohol swab, a drop of each allergen extract was put on marks 1 centimeter apart. Additionally, a drop of histamine phosphate and a drop of saline 0.9% were used as a positive control and negative control respectively. Then, inside the allergen drops on the skin, a prick with a needle was performed (6). The immediate response was evaluated after 20 minutes.

Interpretation of SPT:

A skin test panel was considered valid if the difference in mean wheal diameters between the positive and negative controls was at least 1 mm (6). In most studies, a wheal diameter ≥ 3 mm greater than the negative control is usually considered to be positive (7).

Intracutaneous (intradermal) skin test method

For intradermal skin testing, sterile, disposable, l-ml plastic syringes mounted with intradermal, 26-gauge needles are used. About 0.02-0.05 ml of test solution is injected into the skin, causing a bleb of approximately 3 mm in diameter. The amount introduced does not influence the size of the reaction nearly as much as does the concentration of test solution. Wheal and erythema reactions less than 5 mm in diameter (area 20 mm^2) were regarded as negative reactions (3).

Results

The demographic, clinical and laboratory characteristics of the whole study population is displayed in table1. We demonstrated SAFS in 38 patients (76%) (Figure 1).There was no statistically significant difference between SAFS and non-SAFS subjects as regards age, gender or family history of bronchial asthma. Patients with SAFS did not have associated allergic rhinitis or urticaria more frequently than non-SAFS patients. The mean age of SAFS patients was 36.3 ± 10.5 years, with females to male ratio 11:27 (table 2).20 (52.6%) of SAFS patients had positive family history of asthma. 5 (13.2%) had associated urticaria and 16 (42.1%) had allergic

Table 1: Characteristics of the whole study population

Variable	Frequency
Age (years)	35.8 ± 9.8
Gender (male/female)	15/35
Family history	28 (56%)
Associated allergic conditions	
Urticaria	8 (16%)
Allergic rhinitis	23 (46%)
Abnormal CXR	3 (6%)
Relevant HRCT findings	
Peripheral nodules	6 (12%)
Bronchial thickening	6 (12%)
Bronchiectasis	1 (2%)
Serum IgE level (IU/ml)	153.5 (67.8 – 447)
Allergy to common allergens	
Epicoccum	3 (6%)
Aspergillus fumigatus	22 (44%)
Alternaria alternata	27 (54%)
Candida albicans	7 (14%)
Penicillium	8 (16%)
Trichophyton	9 (18%)
Cladosporium	21 (42%)
Number of allergens	2 (1 – 3)
SAFS	38 (76%)

Data are presented as mean ± SD, ratio, number (%), or median (interquartile range).

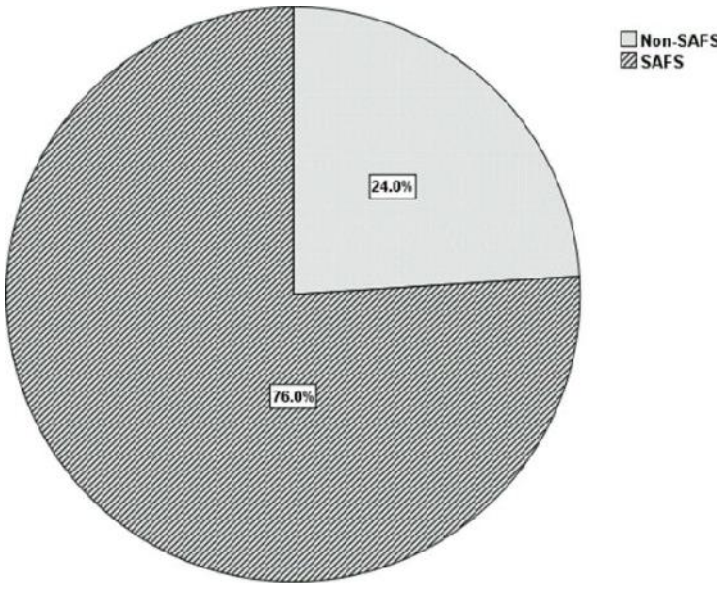

Figure 1: Prevalence of SAFS in the study population.

Table 2: Characteristics of patients with or without SAFS

Variable	Non-SAFS (n=12)	SAFS (n=38)	p-value
Age (years)	34.2 ± 7.5	36.3 ± 10.5	0.514¶
Gender (male/female)	4/8	11/27	1.000§
Family history	8 (66.7%)	20 (52.6%)	0.603¥
Associated allergic conditions			
Urticaria	3 (25.0%)	5 (13.2%)	0.379§
Allergic rhinitis	7 (58.3%)	16 (42.1%)	0.515¥

Data are presented as mean ± SD, ratio, or number (%).¶Unpaired t test, §Fisher's exact test, ¥Chi-squared test with Yates' continuity correction.

rhinitis. Positive SPT with fungal allergens that demonstrated statistical significance in SAFS patients were *Alternaria alternata* in 25 patients (65.8%), *Aspergillus fumigatus* in 21 patients (53.3%) and *Cladosporium* in 20 patients (52.6%) with p-values 0.008, 0.012 and 0.018 respectively (table 3, figure 2). Mean serum IgE level of SAFS patients was slightly statistically significantly higher than non-SAFS patients (p-value= 0.048) (table 4).

Table 3: Prevalence of allergy to common allergens in patients with or without SAFS

Variable	Non-SAFS (n=12)	SAFS (n=38)	p-value
Allergy to common allergens			
Epicoccum	0 (.0%)	3 (7.9%)	1.000¶
Aspergillusfumigatus	1 (8.3%)	21 (55.3%)	**0.012§**
Alternariaalternata	2 (16.7%)	25 (65.8%)	**0.008§**
Candida albicans	2 (16.7%)	5 (13.2%)	1.000¶
Penicillium	0 (.0%)	8 (21.1%)	0.173¶
Trichophyton	1 (8.3%)	8 (21.1%)	0.425¶
Cladosporium	1 (8.3%)	20 (52.6%)	**0.018§**
Number of co-allergens	0 (0 – 0)	2 (1 – 3)	**0.0001¥**

Data are presented as number (%) or median (interquartile range), ¶Fisher's exact test, §Chi-squared test with Yates' continuity correction, ¥Mann-Whitney test.

Table 4: Relevant radiological findings in patients with or without SAFS

Variable	Non-SAFS (n=12)	SAFS (n=38)	p-value
Abnormal CXR	1 (8.3%)	2 (5.3%)	1.000¶
Relevant HRCT findings			
Peripheral nodules	1 (8.3%)	5 (13.2%)	1.000¶
Bronchial thickening	1 (8.3%)	5 (13.2%)	1.000¶
Serum IgE level (IU/ml)	193 (134 – 756)	130 (40– 368)	**0.048¥**

Data are presented as number (%), ¶Fisher's exact test.

HRCT done detected radiological findings more frequently in SAFS rather than non- SAFS patients in the form of peripheral nodules found in 5 patients (13.2%) and bronchial wall thickening found in 5 patients (13.2%). However the difference didn't reach statistical significance (table 4).

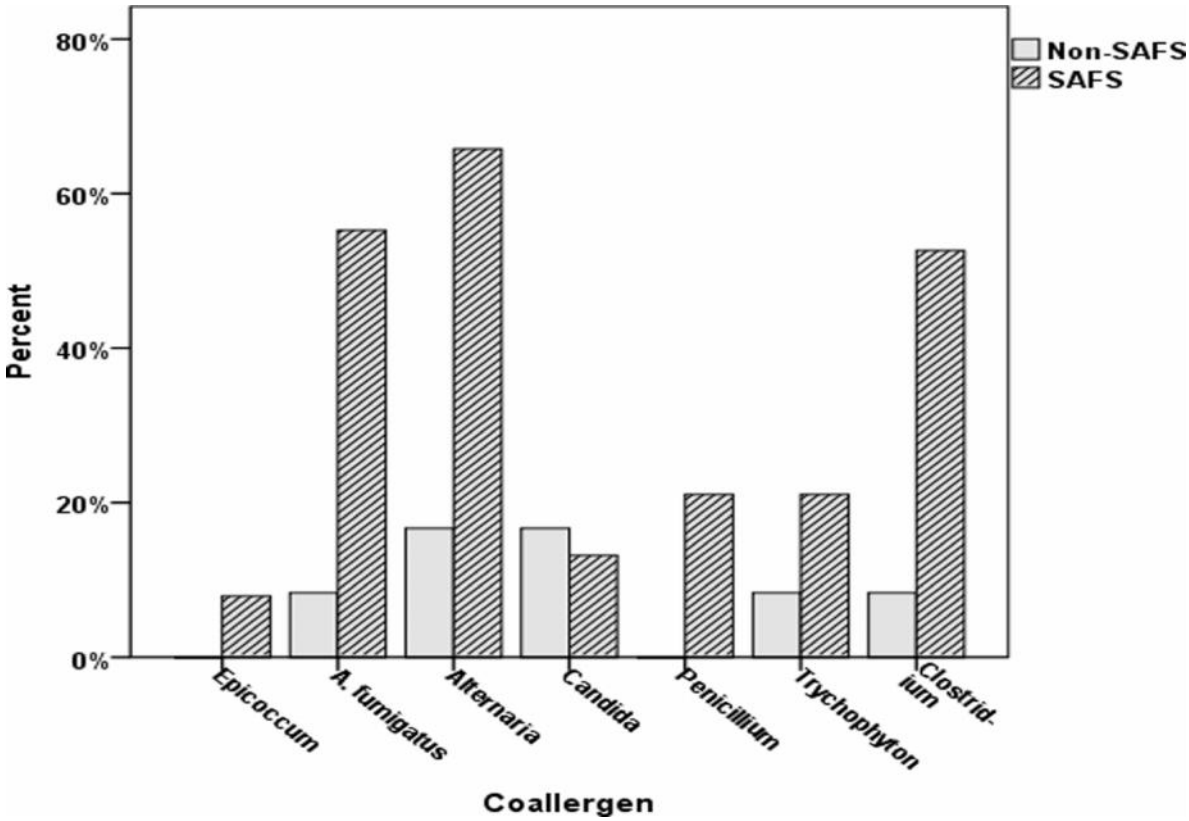

Figure 2: Prevalence of allergy to common allergens in patients with or without SAFS.

Discussion

The incidence of mould allergy ranges from 6 to 24% in the general population, up to 44% among atopics and 80% among asthmatics (1). Since 2006, SAFS became recognized as a new phenotype of asthma, and was conceptualized as a continuum of fungal sensitization, with asthma at one end and ABPA at the other. The paucity of data and ambiguity in diagnostic criteria currently renders SAFS more of a diagnosis of exclusion than a specific entity (8). Our aim was to identify frequency of SAFS in atopic severe asthma patients attending our tertiary care clinic.

Fungi produce a wide range of allergens that can be broadly classified into cell wall components such as β $(1\rightarrow3)$-glucans, secreted products such as proteinases and glycosidases, and enzymes. However, the proteinases are considered the most important allergenic agents (8). Proteinases alone were incapable of initiating asthma-like disease in mice; however, the concomitant presence of fungi and proteinases readily establishes vigorous allergic inflammation (9).Asthma was found not to be a result of hypersensitivity to fungal allergens alone but could be a byproduct of protective response against active fungal infection in an attempt to contain the fungal infection. Thus it is hypothesized that SAFS occurs due to an immune response against larger amounts of fungal proteinases and smaller quantities of fungal conidia (10).

In our study we found no statistically significant difference between SAFS and non- SAFS patients regarding age, sex and positive family history of asthma. We report a prevalence of 76% of SAFS patients among all asthma patients included in the study.

The most common fungal allergens elicited in SAFS patients were *Alternaria alternata* (65.8%), *Aspergillus fumigatus* (55.3%) and *Cladosporium*(52.6%). Sensitisation to *Penicillium* was found in 8 patients (21.1%); however it did not exhibit statistical significance. In accordance to this, 22% of asthmatic children in Taiwan showed a positive reaction in intracutaneous skin tests for *Penicillium* species (11). *Penicillium* and *Aspergillus* species are among the indoor fungi associated with

hypersensitivity reactions such as rhinitis, sinusitis or asthma (12), with *Cladosporium*, *Alternaria* and *Epicoccum* commonly causing such reactions outdoors (1). Sensitization to *Alternaria* and *Cladosporium* has been reported to be 3% to 30% in European countries (13).

Mari et al. tested 4,962 patients having respiratory symptoms; 19% of whom reacted to at least one fungal extract, whereas the incidence of sensitization to *Alternaria* was 66%, which is very similar to our results. Although the overall incidence of *Cladosporium* sensitization was 13%, within the group of patients sensitized to more than two fungal sources, the prevalence of *Cladosporium* sensitization reached 84%, concluding that monosensitization to *Cladosporium* is relatively rare within mold-allergic patients (3). On the other hand another study that investigated prevalence of respiratory allergy to fungi spores using SPT in atopic asthmatics throughout a 15-year period recorded the most frequent positive skin reaction was *to Alternaria* species observed in 13.5% of patients, in 7.4% to *Cladosporium* and 5% to *Aspergillus* (14).

Alternaria and *Cladosporium* spp spores are considered the most common airborne particles of fungal origin, and are recognized among the significant fungi responsible for allergic asthma (15). In a recent study, exposure to *Cladosporium, Alternaria, Aspergillus*, and *Penicillium* species increased the exacerbation of current asthma symptoms by 36% to 48% compared with those exposed to lower concentrations of these fungi, as shown by using random-effect estimates (16). In an earlier study by Tariq et al., (1996). *Cladosporium* together with *Alternaria* were the third most common causes of sensitization after house dust mite and grass pollen, however the study included only children age 4 years or less (17) . More recent studies demonstrated that the frequency of sensitization to *Alternaria* or *Cladosporium* spp, or both, is a potent risk factor for severe asthma in adults (18).

In our study, we demonstrated 8 patients (21.1%) showed skin test positivity to the fungus *Tricophyton*. A UK study reported a frequency of 17% in polysensitised severe asthmatics; 10% were sensitized to *Trichophyton* as a single mould sensitisation, but only 2.5% were sensitized to *Trichophyton* alone (19). A clinical entity acknowledged especially in several southern countries is the "Tricophyton Asthma". Numerous lines of evidence suggest sensitisation to *Tricophyton* proteins in asthma patients, where inhalation and/or dermal absorption of *Trichophyton* antigens are the possible routes of exposure (20).

We also demonstrate skin test positivity to *Candida albicans* in only 5 patients (13.2%), and *Epicoccum* sensitivity in 3 patients (7.9%). Although six *Candida albicans* allergens have been described so far, it is still controversial whether the inhalation of this mold is causative for its allergenicity (1).

We detected that patients in our study were sensitized to an average of 2(1-3) fungal allergens (p-value=0.0001). Earlier, O'Driscoll et al (2005) studied the relationship between asthma severity and immediate type hypersensitivity to mold (fungal) and non-mold allergens in 181 asthmatic subjects. Positivity to SPT and/or specific serum IgE was as follows*: Aspergillus fumigatus* (45%), *Candida albicans* (36%), *Penicillium notatum* (29%), *Cladosporium herbarum* (24%), *Alternaria alternata* (22%), *Trichophyton* (17%) (specific serum IgE only). 66% of patients were sensitized to one or more fungi based on SPT and/or specific serum IgE results. The reactivity of asthmatic patients to multiple mold allergens could be due to genuine sensitization to a variety of molds or it could be due to cross-reactivity between mold allergens. The paper of Hemmann and colleagues suggests that *Aspergillus* and *Candida* allergens may share IgE-binding epitopes (22). However, it is believed that multiple mold sensitization skin test reactions are usually due to sensitivity to multiple antigens rather than cross reactivity (23).

Our patients with SAFS did not have higher incidence of associated allergic conditions (urticarial and/or allergic rhinitis) than non- SAFS patients. However, a study that investigated whether natural exposure to Alternaria induces rhinoconjuctivitis symptoms in Alternaria-sensitized children concluded that exposure to Alternaria spores may be an important cause of allergic rhinoconjunctivitis (24).

In terms of treatment, important aspects in the clinical management of fungal allergy in asthma include avoidance of fungi, proper control of the inflammatory process, improvement of airway air flow through reduction of mucus and obstruction and reduction of fungal burden (25). Environmental management

established from the viewpoint of both the ecology and life cycle of the responsible fungi can enable proper control of fungus-associated asthma (26). Moulds are not dominant allergens and outdoor mold, rather than indoor ones, are the most important. To reduce the risk of developing or exacerbating allergies, mould should not be allowed to grow unchecked indoors (12). Patients should be encouraged to decrease exposure by avoiding indoor conditions that facilitate the growth of moulds—for example, by better ventilation and by decreasing dampness (27).

Moreover, clinical efficacy of specific immunotherapy with fungal extracts has been previously shown in 79 actively treated patients in four controlled trials, with only two fungal species, namely *Alternaria alternata* and *Cladosporium*. The use of recombinant fungal allergens might create new prospects in diagnosis and specific immunotherapy for fungal allergy (28).

In conclusion, we report high frequency of fungal sensitisation (76%), patients were sensitized to an average of 1-3 fungi, with the most statistically significant fungi being *Aspergillus fumigatus*, *Alternaria alternata* and *Cladosporium*. There is a need for better recognition of SAFS as it is rather under recognized; the most important step in clinical management of SAFS is the avoidance of exposure.

A major limitation is that our study did not relate the fungal sensitization to severity of asthma, and that it was performed in a single center. We recommend performance of larger longitudinal observational controlled studies of people with SAFS, including detailed imaging for a better understanding of co-morbidities and their impact on natural history of atopic asthma.

Acknowledgments

Authors report they have no conflict of interest; no grant was received for this study.

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