

Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis and non-cystic fibrosis bronchiectasisSoheila Alyasin¹, Mozhgan Moghtaderi², Shirin Farjadian³, Maryam Babaei⁴, Saeed Hosseini Teshnizi⁵¹ MD, Allergy Research Center, Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran² MD, Allergy Research Center, Allergy Clinic of Ali-asghar Hospital, Shiraz University of Medical Sciences, Shiraz, Iran³ Ph.D., Professor in Immunology, Allergy Research Center, Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran⁴ M.Sc. in Microbiology, Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran⁵ Ph.D. Candidate in Biostatistics, Clinical Research Development Center of Children Hospital, Hormozgan University of Medical Sciences, Bandar Abbas, Iran**Type of article:** Original**Abstract****Background:** Aspergillus sensitization (AS) and allergic bronchopulmonary aspergillosis (ABPA) can occur as a cause of permanent lung damage in patients with cystic fibrosis (CF) and non-CF bronchiectasis.**Objective:** The aim of this study was to determine the frequency of AS and ABPA in patients with CF and non-CF bronchiectasis in southwestern Iran.**Methods:** This cross-sectional study was conducted on 33 patients with CF and 27 patients with non-CF bronchiectasis from southwestern Iran who were referred to Namazi Hospital affiliated to Shiraz University of Medical Sciences from July 2015 to February 2016. Skin prick test to *Aspergillus fumigatus*, peripheral blood eosinophil count, total serum IgE, specific IgE and IgG against *Aspergillus fumigatus* as well as radiologic chest studies were done for each patient. Statistical analysis was done by Mann-Whitney U test, Fisher Exact test, and Kappa weighted in SPSS software version 18. Level of significance was set at $p < 0.05$.**Results:** Nine patients with CF (27.3%) and one patient with non-CF bronchiectasis (3.7%) had positive skin tests to *Aspergillus*. There was 81.2% agreement between positive skin test and specific IgE to *Aspergillus fumigatus* ($p < 0.001$). Three patients with CF (9%) met the diagnostic criteria for ABPA, whereas ABPA was not seen in patients with non-CF bronchiectasis.**Conclusion:** ABPA was low in this study, considering more frequency of AS in patients with cystic fibrosis, clinicians should keep in mind the diagnosis of ABPA for those CF patients that do not respond to usual medical therapy and have positive skin tests to *Aspergillus* allergens.**Keywords:** Aspergillus, Allergic bronchopulmonary aspergillosis, Bronchiectasis, Cystic fibrosis, Fungal antibodies**1. Introduction****1.1. Background and study logic**

Patients with cystic fibrosis (CF) are prone to chronic fungi infections due to increased mucus viscosity in the airways (1-3), and *Aspergillus fumigatus* (AF) is the most common fungi in the sputum of these patients (4,5). Colonization by AF can mainly manifest as transient carrier, *Aspergillus* sensitization (AS), *Aspergillus* colonization and allergic bronchopulmonary aspergillosis (ABPA) in these patients (6). *Aspergillus* sensitization is the indicator of hypersensitivity to AF which is defined by positive immediate skin reaction to *Aspergillus* antigens or elevated serum IgE level to AF, and is the first step in the pathogenesis of ABPA (7). The pooled prevalence of AS in

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patients with CF has been reported as 39.1% (8). ABPA is an allergic pulmonary manifestation of repeated inhalation of AF which leads to IgG and IgE formation against AF as well as rising of total IgE (9). A systematic review and meta-analysis study showed the prevalence of ABPA with a pooled prevalence of 8.9% in patients with CF (8). Diagnosis of ABPA in CF patients is challenging because of overlapping criteria with CF. In recent diagnostic criteria for ABPA proposed by the International Society for Human and Animal Mycology (ISHAM), CF was identified as predisposing conditions for ABPA. Two of the three major criteria including positive immediate skin reactivity to AF or elevated specific IgE levels against AF and elevated total serum IgE >417 kU/L (>1000 IU/mL), and two of the three minor criteria including serum precipitating antibodies to aspergillus, elevated IgG against AF and peripheral blood eosinophilia count >500 cells/ μ L as well as new or recent abnormalities on chest radiography or chest computed tomography (CT) consistent with ABPA must be present, to consider a CF patient as ABPA (10). ABPA occurs predominantly in patients with asthma and CF while it has been less frequently noted in other lung diseases such as non-CF bronchiectasis (11). Non-CF bronchiectasis is a respiratory disease with irreversible bronchial dilatation and persistent airway inflammation resulted from multiple inflammatory and infectious injuries to the conducting airways (12, 13). The best way for the diagnosis of non-CF bronchiectasis is high-resolution computed tomography (HRCT) of the chest (14).

1.2. Objectives

Due to under-diagnosis, CF is rare among Asians. Recent epidemiological studies show a severe course of the disease among the Asian population (15, 16). Unrecognized or poorly treated ABPA can lead to airway destruction and pulmonary fibrosis in patients with CF and non-CF bronchiectasis, resulting in significant morbidity and mortality. The present study was carried out in order to determine the frequency of AS and ABPA in individuals with CF and non-CF bronchiectasis in southwestern Iran.

2. Material and Methods

2.1. Research design

This cross-sectional study was carried out on all patients with CF and non-CF bronchiectasis from southwestern Iran who referred to Namazi Hospital affiliated to Shiraz University of Medical Sciences from July 2015 to February 2016.

2.2. Selection criteria

Cystic fibrosis was diagnosed based on the typical clinical features of the disease and sweat chloride concentrations more than 60 mEq/L. The diagnosis of non-CF bronchiectasis was made by the clinical features of the lung disease and result of HRCT of the chest. All volunteered patients with CF and non-CF bronchiectasis were eligible to enroll in the study. Patients were excluded if they had positive dermatographism for doing skin test. The patients were instructed not to take any anti-histamine for 5 days prior to the skin test. The diagnosis of ABPA was considered based on the criteria of the International Society for Human and Animal Mycology (ISHAM) working Group (10).

2.3. Data collection

A questionnaire was used to obtain information about age, sex and history of medications in the last 12 months. Genetic analysis of CF patients and the result of previous chest radiography were recorded from their medical charts.

2.4. Skin prick test

Skin prick tests were done for all patients with standard commercial extracts of AF, *Penicillium* and *Alternaria* (Greer, Lenoir, NC, USA). Histamine (10 mg/mL) and saline were used as positive and negative controls, respectively. The results of the skin tests were examined after 15 min and considered positive when the wheal was ≥ 3 mm greater in diameter than the negative control.

2.5. Blood eosinophil, total serum IgE level and aspergillus antibodies test

The peripheral blood eosinophil count was done by standard hematoxylin and eosin staining, and eosinophil count > 500 cell/ μ L was considered as eosinophilia. Total serum IgE level (Monobind, Lake Forest, CA, USA) and IgG to AF (IBL International, Hamburg, Germany) were determined with an enzyme-linked immunosorbent assay according to the manufacturers' instructions. Serum specific IgE to AF was done by the ImmunoCAP system (Thermo Fisher-Phadia, Uppsala, Sweden). Total IgE >200 IU/mL, specific IgG >12 U/mL and specific IgE ≥ 0.1 kU/L were considered positive.

2.6. Radiological images

A recent chest x-ray and HRCT of the chest were analyzed to identify opacities by the same radiologist.

2.7. Statistical analyses

Data analyses were performed using descriptive statistics (mean± standard deviation, frequency). Fisher Exact test was used to analyze the association between sex and age with skin test. Mann-Whitney U test was used to compare total serum IgE in sex and age groups. Kappa weighted was used to find concordance between skin test, total serum IgE and specific IgE. Analyses were conducted using SPSS version 18 (SPSS Inc., Chicago, IL, USA) and statistical significance was set at $p < 0.05$.

2.8. Research ethics

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (EC-P-92-6337). All patients or their parents in the study were completely voluntary and they read and signed the written informed consent forms prior to the study. Each of the participants could be excluded from the study at any time during the program. Also, confidentiality of all participants' information was ensured.

3. Results

Thirty-three patients with CF (14 females and 19 males, mean age 7.8 ± 6.2 years, age range 1 to 28 years) and 27 patients with non-CF bronchiectasis (14 females and 13 males, mean age 12.7 ± 4.4 years, age range 3.5 to 22 years) were included in this study. All patients were from Shiraz, southwestern Iran. The paraclinical data from these patients are summarized in Table 1. Sensitization to AF was more frequent in male than female patients with CF ($p=0.047$). Sensitization to AF in CF patients aged less than 16 years did not differ significantly from the older patients (Table 2). There was no correlation between total serum IgE level and sex or age in patients with CF (Table 3).

Table 1. Paraclinical characteristic of patients with CF and non-CF bronchiectasis

Characteristic	CF (n=33); n (%)	Non-CF bronchiectasis (n=27); n (%)
Skin test reactivity to Aspergillus	9 (27.2)	1 (3.7)
Elevated total serum IgE level >200 IU/mL	5 (15.1)	4 (14.8)
Elevated total serum IgE level >417 kU/L	7 (21.2)	1 (3.7)
Elevated specific IgE to Aspergillus	11 (33.3)	1 (3.7)
Elevated specific IgG to Aspergillus	14 (42.4)	15 (55.5)
Eosinophilia >500 cells/ μ L	6 (18.2)	0
Chest X-ray opacities	11 (33.3)	14 (51.8)
Bronchiectasis	7 (21.2)	27 (100)

Table 2. Comparison of sensitization to Aspergillus fumigatus based on sex and age in patients with cystic fibrosis

Characteristics		Skin test		Total	Fisher's Exact Test
		Positive	Negative		
Sex	Male	8 (42.1)	11 (57.9)	19 (57.6)	0.047
	Female	1 (7.1)	13 (92.9)	14 (42.4)	
Age (year)	≤16	6 (20.7)	23 (79.3)	29 (87.9)	0.052
	>16	3 (75.0)	1 (25.0)	4 (12.1)	

Table 3. Comparison of total serum IgE level based on sex and age in patients with cystic fibrosis

Variables		n	Median	IQR	Mann-Whitney U test	p-value
Sex	Male	19	142.80	268.9	124.500	0.756
	Female	14	48.35	212.3		
Age (year)	≤16	29	74.9	237.09	30.50	0.127
	>16	4	322	485.37		

Total serum IgE >200 IU/mL showed no discordance with positive skin test to AF. There was a high agreement between positive skin test and specific IgE to AF ($\text{kappa}=81.2\%$, $p < 0.001$). Also, there was a high agreement between total serum IgE >200 IU/mL and IgE specific to AF >0.1 kU in patients with CF ($\text{kappa}=84.3\%$, $p < 0.001$) but no agreement was seen between specific IgE levels and specific IgG levels to AF. In 10 patients with positive skin tests to Aspergillus, 5 patients had positive skin reaction to Penicillium extract and 1 patient had positive skin

test to *Alternaria*. Three patients with CF met the diagnostic criteria for ABPA. Chest x-rays of these patients showed opacities especially in the right upper and middle lobe. The characteristics of these patients are summarized in Table 4. There was no case of ABPA among the patients with non-CF bronchiectasis.

Table 4. Characteristics of three patients with cystic fibrosis who had diagnostic criteria for ABPA

Characteristics	Patient 1	Patient 2	Patient 3
Age (year)	16	12.5	28
Sex	Female	Male	Male
Positive skin test to <i>Aspergillus</i>	Yes	Yes	Yes
Elevated total serum IgE level	Yes	Yes	Yes
Elevated specific IgE to <i>Aspergillus</i>	Yes	Yes	Yes
Elevated specific IgG to <i>Aspergillus</i>	Yes	Yes	Yes
Eosinophilia	Yes	Yes	Yes
Central bronchiectasis	No	Yes	Yes
CFTR gene mutation	2183AA>G	R1162X	No mutation

4. Discussion

Human exposure to fungi is near universal and sensitization to fungi induces ABPA in patients with underlying diseases. The results of skin test reactivity to AF were 27.3% in patients with CF and 3.7% in patients with non-CF bronchiectasis, which were lower than that reported by Valletta et al. They found 56% sensitization to AF in 222 CF patients older than 6 years (17). Geller et al. also found 66% sensitization to AF in 51 adult patients with CF (18). Similar to our results, Carneiro et al. found 26% positive reaction to AF in 74 Brazilian patients with CF (19). A recent meta-analysis study reported the prevalence of positive skin test to AF from 20% to 65% in patients with CF, with a pooled prevalence of 39.1% (8). This variation could be explained by the differences in the AF extracts used for skin tests and the methods of skin tests, whether prick or intradermal. Fungi growth is influenced by climate humidity which is in relation to fungi sensitization. The area of our study is hot and dry and is not suitable for fungi to grow. However, there is a report of AF presence in the dust of 86% of homes in the studied area, which does not include air-borne fungi allergens (20). In our study, the higher sensitization to AF in male patients with CF can be related to high exposure to fungi in men who often go out. There was a high concordance between positive skin test and specific IgE to AF, therefore, a skin test can be reliably used in screening for AS. Similar to our results, Sharma et al. also found 85% agreement between total serum IgE >500 kU and specific IgE to AF >3.5 kU in Indian children with CF (21). In our study, only 9% of patients with CF had criteria for ABPA. The mean prevalence of ABPA among 12,447 patients with CF has been reported to be 7.8%, ranging from 2.1% in Sweden to 13.6% in Belgium (9). Maturu et al. in a meta-analysis study, observed a varying prevalence of ABPA in patients with CF from 3% to 25%, with a pooled prevalence of 8.9% (8). The lack of a uniform diagnostic criterion and standard tests for the diagnosis of ABPA can be considered the main reasons for this variation. There was no case of ABPA in our patients with non-CF bronchiectasis. Although there are a few reports of ABPA in patients with Kartagener syndrome (as one of the causes of non-CF bronchiectasis), ABPA is rare in patients with non-CF bronchiectasis (22). ABPA has been reported to be more prevalent in adult patients with CF than in children suffering from CF (8). All three patients with concurrent CF and ABPA were older than 12 years in our study. Increased exposure to AF over lifetime, more administration of antibiotics and inhaled corticosteroids have been suggested as potential factors in the increase of ABPA in older patients with CF. Although skin reactivity to AF in patients with CF is in favor of ABPA, more work ups are required for definitive diagnosis of ABPA. Nevertheless, differential diagnosis of ABPA is important because therapeutic management of ABPA and AS is different. AF is the first causative pathogen in ABPA; the disease can be caused by other members of *Aspergillus* species as well as other fungi (10). We found sensitization to *Penicillium* in three patients with CF. Elevated total serum IgE >417IU/mL is as an obligatory criterion in the diagnosis and management of ABPA. High titer of total IgE was seen in 7 (21%) of our CF patients and 1 (3.7%) patient with non-CF bronchiectasis, whereas only 3 of these patients were considered to have ABPA. Serum IgE levels may be increased in many patients with CF and non-CF bronchiectasis without ABPA, because all serum IgE is not directed to *Aspergillus* (23). It is proposed that serum IgE level >1000 IU/mL will be an important predictive factor for the development of ABPA in the future (24). Among twelve patients with elevated levels of *Aspergillus*-specific IgE, three were diagnosed as having ABPA. Increased *Aspergillus*-specific IgE levels in serum is also noted in patients with allergic fungal rhinosinusitis caused by *Aspergillus* (25). It has been suggested that quantitative measurement of *Aspergillus*-specific IgG can distinguish patients with ABPA among CF patients (26). This study found that the mean *Aspergillus*-specific IgG concentration in CF patients with ABPA was 147.5 IU/mL,

compared to 38.4 IU/mL in CF patients without ABPA. It is reported that patients with chronic lung disease often have elevated level of Aspergillus-specific IgG (27). In our study, 55% of the patients with non-CF bronchiectasis had elevated levels of Aspergillus-specific IgG. As one of the minor criteria in diagnosis of ABPA, eosinophilia was observed in all of our patients with ABPA. Bronchiectasis, one of the diagnostic criteria for ABPA, was diagnosed in 7 (21%) patients with CF but only 2 of them were diagnosed as having ABPA. The prevalence of bronchiectasis is reported as 50-70% in patients with CF (28, 29). CF patients with $\Delta F508$ mutation are reported to be more susceptible to developing ABPA (30, 31), while we detected 2183AA>G and R1162X mutations in two of the three CF patients with ABPA. Therefore, CFTR gene mutation may make CF patients susceptible to ABPA, but high frequency of $\Delta F508$ mutation among CF patients caused a stronger connection between $\Delta F508$ mutation and the ABPA. All patients were recruited from one referral center in Shiraz and therefore the sample size of our patients was limited. Multicenter studies could help to determine the exact prevalence of ABPA in patients with CF and non-CF bronchiectasis in our country. Complicated patients with ABPA will present with worsening of respiratory symptoms, and in this study, we could not consider deterioration of clinical symptoms. ABPA should be considered in patients with CF, especially those patients with positive skin tests to Aspergillus allergens.

5. Conclusions

The findings of the present study showed that the number of patients with ABPA was low among patients with CF and non-CF bronchiectasis, and indicated more frequency of AS in these patients. Therefore, it is suggested that in patients with CF and non-CF bronchiectasis that do not respond to usual medical therapy and have positive skin tests to Aspergillus allergens, treatment for ABPA should be considered. Currently, considering the better diagnosis of CF and non-CF bronchiectasis, more studies with a larger sample size are suggested.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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