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Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis patients using two different diagnostic criteria

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Key words

cystic fibrosis (CF); allergic bronchopulmonary aspergillosis (ABPA); diagnostic criteria; aspergillus sensitization; prevalence

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Summary

Objective. *There are different diagnostic criteria for the diagnosis of Allergic bronchopulmonary aspergillosis (ABPA) in CF patients. In this present study we evaluated the prevalence of ABPA in Iranian CF patients by two more usual diagnostic criteria as ISHAM working criteria (A) and CF Foundation Consensus Conference criteria (B).* **Methods.** *Eighty-six CF patients were included in the study. All CF patients underwent for Aspergillus skin prick test (AST), Aspergillus-specific IgE (sIgEAf) and Aspergillus-specific IgG (sIgGAf), total IgE. The ABPA prevalence was estimated by two diagnostic criteria, (A) and (B) and compared.* **Results.** *The frequency of positive AST, total IgE, sIgEAf and sIgGAf were 47 (54.6%), 9 (10.5%), 42 (48.8%) and 67 (77.9%), respectively. The obtained rate of ABPA prevalence (10.5%) was identical in two diagnostic criteria A and B (kappa value of 1.000).* **Conclusions.** *The applied diagnostic criteria had no significant effect on the reported rate of ABPA prevalence.*

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder resulting from mutations in the CF transmembrane conductance regulator (CFTR) protein (1). CF patients are susceptible to colonization by the various types of microorganisms including fungi due to abnormality in lung function such as defect in airway clearance (2). Pulmonary colonization by fungi, especially *Aspergillus* species, may lead the CF patients toward a different type of disease ranged from allergic reactions to life threatening invasive infections. Allergic bronchopulmonary aspergillosis (ABPA) is an immunological disorder caused by a hypersensitivity reaction to *Aspergillus* species allergens especially *A. fumigatus*. ABPA is a frequent event in patients with asthma and CF (3). The estimated ABPA prevalence in patients with CF was reported to be from 3% to 25% in adult patients, and 8% to 10% in children, with an overall prevalence of 8.9% (4,5).

The diagnosis of ABPA in CF patients is complex and remains problematic, because there is an overlapping between ABPA and CF in aspects of clinical symptoms, radiological, serological and microbiological features (4-7). In the diagnosis of ABPA, the evaluation of several different parameters including immediate skin test reaction to *Aspergillus* allergens, raised serum specific IgE against *Aspergillus fumigatus* (sIgE*Af*) and serum specific IgG against *Aspergillus fumigatus* (sIgG*Af*), elevated total IgE values, central bronchiectasis, infiltration in chest radiologic findings, raised peripheral eosinophil count, positive serum precipitins and sputum positive for *Aspergillus* culture have been considered. Since there is no consensus on the number of parameters needed for the ABPA diagnosis in patients with CF (8), several different diagnostic criteria were proposed which may have led to different reporting of ABPA prevalence (9). On the other hand, the occurrence of ABPA in patients with CF leads to impairment in pulmonary function and an undesirable pulmonary image, therefore, rapid diagnosis of ABPA and timely treatment is essential (10-12). In this present study we evaluated the ABPA prevalence in Iranian patients with CF using two different diagnostic criteria.

Material and methods

Ethics statement

The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (code: IR.MAZUMS.REC.95.2354), and a written informed consent was completed by all patients or next of kin.

Subjects

Eighty-six CF patients from different parts of Iran, admitted to the referral respiratory diseases center, Masih Daneshvari hospital (Tehran, Iran), from January 2017 to February 2018 were enrolled in this study. The diagnosis of CF was confirmed by Computed Tomography scan (CT-scan), spirometry and clinical parameters including sweat chloride test and genotyping (the analysis of CFTR gene). The inclusion criteria were subjects with confirmed cystic fibrosis, without previous ABPA diagnosis. Patients with pregnancy, tuberculosis, asthma, chronic pulmonary obstructive disease were excluded from the study. All included patients were not using antifungal in time of the study. History of clinical details and demographic characteristics were evaluated in all CF patients.

Diagnostic criteria

Two ABPA diagnostic criteria (ISHAM working group criteria (A) (13) and The Cystic Fibrosis Foundation (CFF) Consensus Conference criteria (B) (14) were applied in this study. **Table I** shows the detailed criteria. The diagnosis of ABPA was evaluated by a team work consisting of pulmonologist, allergist-immunologist, radiologist and medical mycologist. In final the results

Table I *- Criteria for diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients.*

Criteria	Evaluation parameters	
ISHAM working group (A) (13)	predisposing conditions	asthma, cystic fibrosis
	essential criteria (both must be met)	positive serum specific IgE (> 0.35 kUA/L) or immediate skin test
		serum total IgE > 1000 IU/mL
	additional criteria (at least 2 of 3)	presence of serum specific IgG
		thoracic imaging findings consistent with ABPA
		peripheral blood eosinophil count > 500 cells/mL (may be historical)
Cystic Fibrosis Foundation Consensus Conference (B) (14)	cystic fibrosis with acute or subacute clinical deterioration	
	serum total IgE concentration > 1000 IU/mL unless the patient is receiving systemic corticosteroids	
	positive serum specific IgE (> 0.35 kUA/L) or immediate skin test	
	precipitating antibodies to *A. fumigatus* or serum IgG antibody to *A. fumigatus* by an in vitro test	
	new or recent infiltrates (or mucus plugging) on chest radiography or computed tomography that do not respond to antibiotics and standard physiotherapy	

were compared for the differences in the prevalence of ABPA in these two diagnostic criteria.

Pulmonary function test

Spirometry test (Easy One NDD spirometer, Swiss) was performed for all patients and we obtained two important measurements, forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) from this test according to manufacture instructions. Values of FEV1 and FVC were recorded for study population.

Aspergillus fumigatus skin prick test

All subjects underwent *Aspergillus* skin prick test (SPT) with commercial *Aspergillus* allergens (Alk-Abelló, Lincoln Diagnostics, Dallas, Tx, USA) in the forearm as well as histamine 0.1 w/v served as positive control, and normal saline 0.9 w/v served as negative control. The appearance of a wheal 3 mm larger than negative control 15 minutes after exposure was considered as immediate-type hypersensitivity and positive SPT reaction.

Aspergillus specific IgE

We screened serum sIgE*Af* for all CF patients by using immunoCap method (an automatic immunoassay system, Phadia, Belgium) (15) according to the manufacturer's instructions. Serum sIgE*Af* level greater than 0.35 KU/L was considered as positive result.

Aspergillus specific IgG

Serum sIgG*Af* were measured by using a commercially available ELISA kit (IBL ELISA Kit, Hamburg, Germany) according to the manufacturer's instructions. sIgG*Af* value > 12 U/ml was considered as positive result.

Serum total IgE measurement

The serum total IgE (stIgE) levels was measured by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Genesis, Omega Diagnostic Group, UK) according to manufacturer's guidelines. Concentrations values were reported as IU/ml and values greater than 1000 IU/ml were considered as positive result.

Peripheral blood eosinophil count

The total count of white blood cells and the value of eosinophil in percentage were determined by an auto-analyser (Sysmex XT-1800i, USA) and was then recorded for each patient. Eosinophil count > 500 cells/µl was considered as positive result.

High Resolution Computed Tomography (HRCT) and/or chest X-Ray

All enrolled CF patients were screened by HRCT and/or chest X-Ray for evidence of bronchiectasis, centrilobular nodules/ mucoid impaction/ hyper-dense mucus. A radiologist reviewed the CT scans without knowledge of study population's clinical and paraclinical data.

Sputum processing

Sputum samples (once per patient) were collected from all patients who were enrolled in the present study. Each collected sputum sample was mixed in equal volume of pancreatin 0.5% for homogenization. After centrifugation, the sediment was then divided into two parts; one for fungal culture (Malt extract agar [MEA], QUELAB, Canada) and the other for direct microscopic examination mounted with 20% potassium hydroxide (KOH). All grown mold colonies were then identified at species level by molecular methods.
For molecular identification, genomic DNAs were extracted from all mold isolates, then universal primers including ITS1 and ITS4 as well as Bt2a and Bt2b (16) were used for identification of studied fungi to the species level. Herein, PCR reactions were prepared (17) and then the PCR products were analyzed by gel electrophoresis and visualized by UV illumination after safe staining. The amplified products of the ITS and β-tubulin fragments of isolated molds were conducted to automated DNA purification platform, and the purified amplicons were then sequenced. The resulting sequences were compared to the sequences deposited in the GenBank database and identified with 99-100% similarity to the corresponding ITS and β-tubulin sequences.

Statistical analyses

Data analyses were performed using descriptive statistics (mean ± standard deviation, frequency) by SPSS version 18 (SPSS Inc., Chicago, IL, USA). Kappa weighted test was used to find concordance between criteria A and criteria B and p-value < 0.05 was set as statistical significance.

Results

Out of 86 included patients, 42 (48.9%) were females and mean ± SD (range) of age was 16.14 ± 7.21 (0.7 - 34.0) years. The demographic, clinical and paraclinical data of study population (ABPA and non-ABPA) are presented in **table II**. *Aspergillus* SPT and sIgE*Af* were positive in 47 (54.7%) and 42 (48.8%) of CF patients, respectively. The overall prevalence of *Aspergillus* sensitization (positive result in *Aspergillus* SPT or sIgE*Af*) was 51 (59.3%) in study population. The mean ± SD (range)

Table II *- Clinical, paraclinical and demographic data of cystic fibrosis patients with allergic bronchopulmonary aspergillosis.*

		non-ABPA (n = 77)	ABPA (n = 9)	total (n = 86)
age in year	mean ± sd (range)	15.9 ± 7.1 (0.6-32.0)	17.8 ± 8.4 (5.0-34.0)	16.1 ± 7.2 (0.6-34.0)
gender n (%)	females	39 (50.6)	3 (33.3)	42 (48.8)
	males	38 (49.3)	6 (66.7)	44 (51.2)
pred-FEV1 (%) (mean ± SD)		47.9 ± 24.7	54.9 ± 22.3	48.6 ± 24.4
pred-FVC (%) (mean ± SD)		49.5 ± 22.6	56.2 ± 19.8	50.2 ± 22.3
history of CF in family n (%)		25 (32.5)	5 (55.5)	30 (34.9)
duration of CF diagnosis n (%)		13.6 ± 7.1	15.2 ± 9.7	13.8 ± 7.4
family history of respiratory disease n (%)		28 (36.4)	2 (22.2)	30 (34.9)
seasonal allergies n (%)		3 (3.9)	1 (11.1)	4 (4.6)
nasal polyps n (%)		20 (26.0)	2 (22.2)	22 (25.6)
cough n (%)		75 (97.4)	9 (100.0)	84 (97.7)
shortness of breath n (%)		58 (75.3)	8 (88.9)	66 (76.7)
hospitalisation n (%)		70 (91.0)	9 (100.0)	79 (91.9)
previous exposure to inhaled antibiotics n (%)		54 (70.1)	9 (100.0)	63 (73.2)
previous use of systemic antibiotics n (%)		73 (94.8)	9 (100.0)	82 (95.3)
previous exposure to inhaled corticosteroids n (%)		70 (91.0)	9 (100.0)	79 (91.9)
previous use of oral corticosteroids n (%)		9 (11.7)	7 (77.8)	16 (18.6)
previous use of oral antifungal n (%)		9 (11.7)	8 (88.9)	17 (19.8)
haemoptysis n (%)		13 (16.9)	0	13 (15.1)
increased volume of sputum n (%)		58 (75.3)	8 (88.9)	66 (76.7)
positive *Aspergillus* SPT (n = %)		38 (49.3)	9 (100.0)	47 (54.6)
Aspergillus-specific IgE > 0.35 KU/L	n (%)	33 (42.8)	9 (100.0)	42 (48.8)
	mean ± sd	2.8 ± 5.5	17.8 ± 13.5	4.4 ± 8.1
	median (range)	0.31 (0.1 - 22.4)	16.5 (1.07 - 44.5)	0.33 (0.1 - 44.5)
Aspergillus-specific IgG >12 U/ml	n (%)	58 (75.3)	9 (100.0)	67 (77.9)
	mean ± sd	55.8 ± 44.9	91.2 ± 50.4	59.5 ± 46.5
	median (range)	52.8 (0.0 - 197.7)	83.2 (28.1 - 162.1)	59.8 (0.0 - 197.7)
stIgE > 1000 IU/ml	n (%)	0	9 (100.0)	9 (10.5)
	mean ± sd	290.9 ± 267.8	1078.2 ± 68.2	373.3 ± 351.2
	median (range)	212.8 (2.3 - 898.7)	1054.0 (1008.6 - 1200.5)	291.5 (2.3 - 1200.5)
current or history of bronchiectasis n (%)		69 (89.6)	9 (100.0)	78 (90.7)
peripheral blood eosinophil count > 500 cell/µl n (%)		34 (44.1)	3 (33.3)	37 (43.0)

CF, cystic fibrosis; ABPA, allergic bronchopulmonary aspergillosis; SPT, skin prick test; sIgE, specific IgE; sIgG, specific IgG; stIgE, serum total IgE; FEV1, forced expiratory vol-ume in one second; FVC, forced vital capacity.

of serum sIgE*Af* was 4.3 ± 8.1 (0.1 - 44.5) KU/L. sIgG*Af* was positive in 67 (78.0%) patients with mean ± SD (range), 75.5 ± 40.1 (18 - 197.7) U/ml. stIgE value > 1000 IU/ml was reported in 9 (10.5%) of CF patients and the mean ± SD (range) of stIgE value was 373.3 ± 351.2 (2.3 - 1200.5) IU/ml.

In total, 86 sputum samples were collected from 86 CF patients of which 66 (76.7%) revealed positive fungal cultures. Two patients had more than one *Aspergillus* species isolates from the sputum samples. *Aspergillus* species (69/79, 87.3%) were the most common isolated filamentous fungi followed by *Penicil-*

lium spp. (5/79, 6.3%), *Scedosporium* species (3/79, 3.8%), *Alternaria alternata* (1/79, 1.3%) and *Fusarium fujikuroi* (1/79, 1.3%). Among the *Aspergillus* species, *A. flavus* (23/69, 33.3%) was the most common followed by *A. tubingensis* (19/69, 27.5%) and *A. fumigatus* (13/69, 18.8%).
In HRCT the bronchiectasis was observed in 78 (90.7%) of patients. Out of 86 patients with CF, 37 (43.0%) were presented peripheral eosinophil count greater than 500 cell/µl.

Prevalence of ABPA

Of 86 patients with CF, 9 (10.5%) cases were met ABPA diagnosis (classified as ABPA-central bronchiectasis), according to criteria A and B. There was no patient with diagnosed ABPA-serologic and none of them had been previously recognized as ABPA patients. Out of 9 CF patients with diagnosed ABPA, 6 (66.7%) were > 16 years old.
Concordance in positivity of SPT and sIgE*Af* was observed in all diagnosed ABPA patients as both of the criteria A and B. Baseline characteristics and the laboratory examination findings of nine diagnosed ABPA patients were summarised in **table III**. Out of 9 ABPA patients, 8 (88.9%) were positive for *Aspergillus* species growth in sputum samples of which *A. flavus* (3, 37.5%), *A. fumigatus* (2, 25%), *A. terreus* (2, 25%) and *A. tubingensis* (1, 12.5%) were identified. All patients with ABPA showed the evidence of bronchiectasis in HRCT. Three patients with ABPA (33.3%) were presented peripheral eosinophil count greater than 500 cell/µl.

Discussion

Our results on stIgE > 1000 IU/ml, sIgE*Af*, *Aspergillus* SPT and eosinophilia was comparable with some previous studies (18,19) and in contrast with Baxter et al. (20) study who reported a rate 28.8% of SPT and 15.8% of sIgE*Af*. In this present study, positivity in *Aspergillus* SPT and sIgE*Af* were concordance in all CF patients with ABPA however out of 51 CF patients with *Aspergillus* sensitization (19.6%) had discordance in positivity of *Aspergillus* SPT and sIgE*Af*. These findings in line with some previous studies show that skin test is more sensitive and

Table III - *Clinical and paraclinical data of cystic fibrosis patients with allergic bronchopulmonary aspergillosis.*

	P 1	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P 7**	**P 8**	**P 9**
age in year	17	5	20	18	12	24	10	20	34
sex	F	F	M	M	F	M	M	M	M
SDM	+	+	+	+	+	+	+	+	+
SFC	+	+	+	+	+	+	+	+	+
sputum appearance	with brown plugs	normal	normal	with blackish plugs	normal	normal	normal	with black plugs	normal
isolated *Aspergillus* species	*A. fumigatus*	*A. flavus*	*A. terreus*	*A. flavus*	*A. flavus*	*A. terreus*	-	*A. tubingensis*	*A. fumigatus*
CB	+	+	+	+	+	+	+	+	+
MI	+	+	+	+	+	+	-	+	+
Aspergillus SPT	+	+	+	+	+	+	+	+	+
sIgE*Af* > 0.35 KU/L	4.5	14.8	26.4	24.8	16.5	22	44.5	1.07	6.0
sIgG*Af* > 12 U/ml	83.9	160.3	66.6	60.8	95.6	162.1	28.1	33.2	130.4
stIgE > 1000 IU/ml	1125.6	1013.9	1010.5	1104.2	1054.0	1200.5	1142.3	1008.6	1044.7
eosinophil count > 500 cells/ml	1341	488	329	928	210	1236	375	241.3	436.8

CF, cystic fibrosis; SDM, sputum direct microscopy; SFC, sputum fungal culture; SPT, skin prick test; sIgE, specific IgE; sIgG, specific IgG; stIgE, serum total IgE; CB, central bronchiectasis; MI, mucoid impaction; A, *Aspergillus*.

less specific than sIgE*Af* test, may due to use of crude antigen in skin test (21,22). Therefore, the combination of sIgE*Af* test along with *Aspergillus* skin test is recommended to improve the diagnosis of ABPA (23). Among our 9 CF patients with ABPA, only 3 cases showed an eosinophil count of > 500 cells/µL. It is suggested that inhaled corticosteroids therapy before ABPA screening can reduce the eosinophil count (24).

In the present study, 88.9% of CF patients with ABPA and 74.2% of CF patients without ABPA were positive for *Aspergillus* in sputum samples. The different rate of culture positivity due to *Aspergillus* in CF patients was reported (20,25,26). In our recent study on CF patients, 73.3% of the cases were positive for fungal cultures in sputum samples (27). These variations in the isolation of *Aspergillus* could be explained by various factors including environmental exposure, interactions with other CF pathogens, and therapeutic interventions (28). Interestingly, in contrast to different reports from different countries, *A. flavus* had relatively more frequency than *A. fumigatus* in CF patients with ABPA may due to geographical differences (29,30). Interestingly, *A. flavus* has also been reported as the most prevalent of *Aspergillus* species in different clinical and environmental samples in Iran (27,31).

In this present study, different *Aspergillus* species were isolated from sputum samples of nine patients with ABPA, however all of these patients showed raised sIgG*Af* and sIgE*Af*. It was noted either co-sensitization or cross-sensitization between *A. flavus* or *A. fumigatus* (32), however there is no valuable data on the correlation between culture results and skin test and in vitro antibody assays in ABPA patients.

In this present study we found an overall prevalence of 10.5% of ABPA. The obtained rate of ABPA prevalence was identical in two diagnostic criteria A and B (kappa value of 1.000). According to Rosenberg and Patterson diagnostic criteria for ABPA (33) in which a stIgE > 417 IU/ml was considered as one of the criteria for diagnosis of ABPA, some of our CF patients met the diagnosis of ABPA. According to criteria A or B on the stIgE parameter, we excluded these patients for calculation of ABPA prevalence. Recently, stIgE greater than 1,000 IU/ml was recommended as an important diagnostic indicator for ABPA diagnosis (34). stIgE level may be increased by other environmental factors in many CF and non-CF patients (29). Therefore, it has been suggested that stIgE values greater than 1000 IU/mL can be a major contributor in the prediction of ABPA (30).

Due to the use of different ABPA diagnostic criteria with a distinct sensitivity, the duration of follow-up and monitoring of the patient, the number of study population and the concentration of fungal spores in the environment, different prevalence rate of ABPA is reported by different authors (4,35,36). A 3.4% and 14.9% rate of ABPA has been reported from France (37) and Greece (38), respectively. Regardless the applied criteria, our findings were in range with different report from different countries (5,25,38-41). Two studies from Iran reported the rate 33.3% (42) and 9.0% (43) of ABPA in patients with CF. On the other hand, the numerous applied diagnostic criteria and the absence of any gold standard for the diagnosis, the comparison of the prevalence of ABPA in CF patients reported form various CF centers is very difficult (6). Considering the fact that bronchiectasis as one of the main observations in CF and in ABPA (8), all patients with ABPA showed the evidence of bronchiectasis in this present study. In the present study, the majority of patients with CF and all suspected ABPA patients were positive for bronchiectasis and sIgG*Af*. The same prevalence of ABPA was reported by two applied criteria. It should be noted that the diagnosis of ABPA in patients with CF is difficult and often delayed due to the overlapping of most ABPA pulmonary symptoms with common CF symptoms, such as bronchiectasis (39).

Conclusions

According to our results, the prevalence rate of ABPA in Iranian CF patients in line with other previous studies from different countries was considerable. The applied diagnostic criteria had no significant effect on the reported rate of ABPA prevalence.

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Conflict of interests

The authors declare that they have no conflict of interests.

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