Original article

Allergic bronchopulmonary aspergillosis as a cause of bronchial asthma in children

Background: *Allergic bronchopulmonary aspergillosis (ABPA) occurs in patients with asthma and cystic fibrosis. When aspergillus fumigatus spores are inhaled they grow in bronchial mucous as hyphae. It occurs in non immunocompromised patients and belongs to the hypersensitivity disorders induced by Aspergillus.* ***Objective:*** *To diagnose cases of allergic bronchopulmonary aspergillosis among asthmatic children and define the association between the clinical and laboratory findings of aspergillus fumigatus (AF) and bronchial asthma.* ***Methods:*** *Eighty asthmatic children were recruited in this study and divided into 50 atopic and 30 non-atopic children. The following were done: skin prick test for aspergillus fumigatus and other allergens, measurement of serum total IgE, specific serum aspergillus fumigatus antibody titer IgG and IgE (AF specific IgG and IgE) and absolute eosinophilic count.* ***Results:*** *ABPA occurred only in atopic asthmatics, it was more prevalent with decreased forced expiratory volume at the first second (FEV1). Prolonged duration of asthma and steroid dependency were associated with ABPA. AF specific IgE and IgG were higher in the atopic group, they were higher in Aspergillus fumigatus skin prick test positive children than negative ones .Wheal diameter of skin prick test had a significant relation to the level of AF IgE titer. Skin prick test positive cases for aspergillus fumigatus was observed in 32% of atopic asthmatic children.* ***Conclusion:*** *ABPA occurs in 1/3 of atopic asthmatic children and is related to the duration and severity of asthma.*

Keywords: Aspergillosis, bronchial asthma, children.

Dina Shokry, Ashgan A. Alghobashy, Heba H. Gawish*, Manal M. El-Gerby*

Departments of Pediatrics and Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt.*

Correspondence:
Dina Shokry, Department of Pediatrics, Faculty of Medicine, Zagazig University, Egypt.
E-mail: *dinaa1111@yahoo.com*

INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) was first reported in England during 1952[1], since then a number of cases have been diagnosed and reported from many countries[2,3]. This pulmonary disorder arises from an allergic response to multiple antigens expressed by aspergillus fumigatus[4]. In temperate climates, aspergillus is one of the common indoor allergens responsible for asthma. Swimming pools and basements that are moist and humid for prolonged periods of time harbor these fungi. ABPA may develop in individuals with episodic obstructive lung disease such as asthma and cystic fibrosis that produce thick tenacious sputum[5]. The factors underlying the development of ABPA remain unclear. The role of genetic factors, mucous quality, preactivation of epithelial cells and the extent to which this activation facilitates the development of aspergillus spores into hyphae, bronchial penetration of aspergillus, the immune response and bronchiolar inflammation that leads to destruction are not yet fully understood[6]. The condition under which aspergillus fumigatus colonizes the respiratory tract in patients developing ABPA is a factor associated with the pathophysiology of the disease[7].

Improved diagnostic methods and awareness have led to recent reports of higher prevalence of ABPA in patients suffering from chronic asthma (1-40%) and 7-15% of cystic fibrosis (CF) patients[8,9,10]. ABPA leads to poorly controlled asthma with pulmonary exacerbations and detrimental consequences; dependence on oral-corticosteroids increases the risk for secondary infections[11]. In rare cases, ABPA disease has also been reported to complicate other lung diseases including idiopathic bronchiectasis, chronic obstructive pulmonary disease (~38%)[6,12,13]. Moreover, ABPA has also been reported in patients with pulmonary aspergilloma and chronic necrotizing pulmonary aspergillosis[14]. The prevalence of AF hypersensitivity is even higher in patients with acute severe asthma (~51%)[15]. Aspergillus fumigatus-sensitized asthmatic patients have been reported to have poor lung function[16,17].

It has been noted that proteases activate protease activated receptors (PARs) which are G-protein coupled receptors present on the airway cells and other cells such as mast cells, eosinophils, neutrophils, macrophages and lymphocytes[18]. To date, four PARs have been identified; PAR-2 is the most important in allergic airway disease owing to its increased expression on the airways of asthmatic patients[19]. Interestingly, injured airway epithelial cells also secrete trypsin, a PAR-2 agonist that further aggravates allergic inflammatory responses.

Genetic association studies have shown that polymorphisms in the SP-A and MBL gene lead to a predisposition to develop ABPA[19,20]. ABPA can occur at any age in individuals with atopy. Patients have recurrent episodes of asthma exacerbation with wheezing and chest infiltrates. They have systemic manifestations such as fever, malaise and anorexia. Sputum production is characterized by chunks of green or beige colored sputum plugs. Mucus plugging results in partial obstruction of airways leading to bronchiectasis as a complication[21].

METHODS

This study was carried out at the Pulmonology Unit, Pediatric Department and at Allergy and Immunology Unit, Microbiology Department, Zagazig University during the period from September 2008 to October 2011.

This study included 80 asthmatic children diagnosed according to National Asthma Education and Prevention Programme [22]. Their ages ranged from 3 to 14 years. The severity of asthma was classified according to GINA guidelines for asthma severity[23,24].

Asthmatic children were divided into: Group (1): Fifty atopic children according to positive family history, history of atopy to a specific allergen, positive skin prick test to one or more allergens, eosinophilia and elevated IgE level and Group (2): Thirty non- atopics diagnosed by exclusion of atopy, precipitation of asthma by exercise, cold air or hormonal changes. Informed consents were taken from the parents or care-givers of all children.

Exclusion criteria were: Children with parasitic infestations, or those taking any medication other than anti-asthma drugs and children with immunocompromised disorders.

The following were done: History taking to find if there was positive family history of asthma, assess asthma severity and differentiate between atopic and non-atopic asthmatics. Thorough clinical examination to exclude any other cause of distress as chest infection. Pulmonary function tests to prove the diagnosis and assess the severity of asthma as FEV_1 ≥80%: intermittent or mild asthma, 60-80%: moderate asthma, <60%: severe asthma[25]. The apparatus used was master screen Viasys Health Care GmBh, Germany.

Stool analysis was done to exclude parasitic infestations. Chest x-rays were obtained as needed. Skin prick test for all children, absolute eosinophilic count, serum total IgE and specific serum anti fumigatus IgE and IgG were done.

All sera were tested for AF specific antibodies using UniCAP 100 assay (CAP; Pharmacia/Upjohn, Kalamazoo, MI). *A.fumigatus* total extract and recombinant AF allergens Asp f1, f2, f3, f4, and f6 coupled ImmunoCAPs were used to detect specific IgE in the sera, according to the protocol recommended by the manufacturer. An ImmunoCAP class of 1 or more (35 mg_A/L) was considered to be positive. The same lot of ImmunoCAPs and reagents were used for all the study[26,27,28].

Allergy skin test

Reagents: Allergen 8 Coca's extracted allergens were used, saline (0.9% NaCl) was used as a negative control and histamine (histamine acid phosphate 1 mg/ml) was used as a positive control. For the intradermal skin test, 1/1000 W/V (100 µl of 1/10 W/V of diluted extract to 9900 µl saline) of diluted extract was used [29]. The allergens used were: house dust, human hair, smoke, wool, cotton, mixed fungi (Aspergillus niger, Aspergillus flavus, and Aspergillus fumigatus), mixed pollens and hay dust.

The procedure of skin intradermal test: The skin of the volar surface of the forearm was cleaned and marked, 0.1 ml of 1/1000 dilution of each extract, negative and positive controls were injected intradermally; after15 minutes the tested sites were inspected. Fully developed erythema and wheal reactions were remeasured after 30 minutes. The mean wheal size was represented as (X + x)/2 (X is the maximum diameter, and x is the perpendicular diameter). Mean wheal size of greater than or equal to 3 mm was regarded as positive[30]. No antihistamines were taken in the last 48 hours prior to the test, so as not to affect the result.

Serum Total IgE

It was measured using RIDASCREEN® Spezifisches IgE kit by EIA technique[31]**.** Analytical sensitivity and specificity of the kit were 0.24 IU/ml and 91.2% respectively.

Statistical analysis

Data were presented as mean ± standard deviation (X± SD), median and range or percentage (%). All

statistical comparisons were performed using the student's t test, X^2, Z test, Fisher's exact test and Kruskal-Wallis H test. Data were checked and analyzed using Statistical Package of Social Science (SPSS) for windows version 8. P values less than 0.05 were considered significant.

RESULTS

Both atopic and non-atopic asthmatic children were age and sex matched. Skin prick test positive cases to anti fumigatus (SPT AF) was present only in the atopic group where 16/50 of atopic cases were positive which represented (32%). Total IgE, specific IgE (AF), IgG (AF) and eosinophilic count were statistically higher in the atopic group (table 1). SPT AF positive cases were associated with prolonged duration of asthma. History of steroid dependency was higher in AF positive cases. FEV1 was lower in AF positive cases than in AF negative ones (table 2). Wheal diameter was larger with increased serum IgE (AF) titer , but no relation between wheal diameter and either IgG (AF) or total IgE (table 3).The percentage of atopic children sensitive to mixed fungi represented 40% (table 4).

Table 1. Demographic and laboratory data of atopic and non-atopic children.

	Atopic children (n =50)	Non-atopic children (n =30)	Test of significance	p
Age (yr)	7.2± 2.3	6.4 ± 2.5	t = 1.45	0.15
Sex Male Female	28 55% 22 45%	11 55% 9 45%	X^2 = 0.02	0.89
Skin prick test positive cases (n)	16	0	X^2 = 12.0	0.000**
IgE ng/ml	500 (200- 20120)	200 (100-300)	Z= 4.64	0.000**
IgE (AF) mg/L	25 (15-100)	15 (5-30)	Z= 4.0	0.000**
IgG (AF) mg/L	30 (15-80)	10 (5-35)	Z= 4.1	0.000**
Absolute eosinophilic count/µL	500 (200-1000)	100 (50-300)	Z= 6.97	0.000**

IgE : immunoglobulin E; IgG : immunoglobulin G; AF: aspergillus fumigatus; ** Highly significant; * significant.

Table 2. History and clinical evaluation of atopic cases both with positive and negative SPT for A. fumigatus.

	SPT positive for AF (n =16)	SPT negative for AF (n =34)	Test of significance	P
Duration of asthma (yr)	9.15	5.1	t = 12.9	0.000**
History of steroid dependency (n)	10/16	10/34	t = 4.96	0.02*
Severe asthma (n)	13/16	16/34	t = 5.12	0.02*
FEV1 % of predicted	57± 8.9%	75± 9.5%	t = 6.3	0.000**
Allergic rhinitis (n)	13/16	30/34	Fisher's exact	0.66
Eczema (n)	10/16	25/34	Fisher's exact	0.51
Family history of asthma (n)	14/16	32/34	Fisher's exact	0.58

FEV1 : forced expiratory volume 1st second; SPT: skin prick test; ** Highly significant; * significant.

Table 3. Wheal diameter in relation to immunoglobulin levels in SPT positive patients.

Wheal diameter	IgE ng/ml	IgE (AF) mg/L	IgG (AF) mg/L
3-5 mm (n = 6)	1500 (200-17.352)	25 (15-100)	55 (15-80)
5-7 mm (n = 6)	1430 (220- 20.120)	45 (30-80)	60 (20-75)
> 7 mm (n = 4)	1550 (300- 18.600)	200 (90-300)	70 (20-80)
Kruskal-Wallis test	0.05	5.7	0.51
P	0.97	0.05*	0.77

* Significant

Table 4. Frequency of positive skin test to the common allergens in atopic patients.

Type of allergens	Number of patients	Percentage
Mixed fungi	20	40%
Aspergillus fumigatus	16	32%
House dust	24	48%
Human hair	4	8%
Smoke	26	52%
Wool	9	18%
Cotton	13	26%
Mixed pollens	38	76%
Hay dust	29	58%

DISCUSSION

Fungi are ubiquitous and responsible for causing a broad spectrum of type I-IV hypersensitivity diseases[6]. Recent epidemiologic studies clearly outline the link between fungal sensitization and exacerbations of allergic asthma, leading to increased morbidity and mortality[7,32].

Our study was conducted on 50 atopic and 30 non-atopic asthmatics. We found that skin prick test positive cases for aspergillus fumigatus was positive in 16 out of 50 (32%) atopic children, but no positive cases were found among non-atopic group. Knusten et al[8] reported that 40% of patients suffering from chronic asthma had ABPA. This might be due to improved diagnostic methods and awareness that have led to recent reports of higher prevalence of ABPA. However another study[7] found that airway colonization by fungi, predominantly by *Aspergillus fumigatus*, had been demonstrated in airways of both subjects with and without asthma. They suspected that ABPA is a florid hypersensitivity reaction to *A. fumigatus* and the colonization in the airways was reported in up to 8% of patients with asthma and 13% with cystic fibrosis (CF). SPT for A. fumigatus was negative in all non- atopics as it was considered a hypersensitivity reaction[33]. The absolute eosinophilic count and total IgE were higher in the atopic group; many other studies reported the same results[5,6,9,23].

Specific immunoglobulin to AF (IgE and IgG AF) were higher in the atopic group as ABPA was considered a hypersensitivity disorder[33]. We found that A. fumigatus positive cases were significantly associated with prolonged duration of asthma and lower FEV1 than negative ones. It is not possible to tell whether the association between *A. fumigatus*–IgE sensitization and reduced lung function is causal; it is possible that sensitization is related to long-term colonization in the airways of asthma, which may occur preferentially in damaged airways[34]. This would be consistent with the observation that *A. fumigatus*-IgE-sensitized patients with asthma had a longer duration of asthma, as has been previously shown. Another study by Hargreave and Nair[35] reported that, there was a clear association between asthma duration and *A. fumigatus*–IgE sensitization, suggesting that the longer the duration of asthma, the more likely *A. fumigatus* sensitization would occur.

Bronchiectasis, which again could be a consequence of colonization, did not explain the association between *A. fumigatus*–IgE and reduced FEV_1. It is therefore probable that *A. fumigatus* is at

least responsible for the development of fixed airflow obstruction in asthma, either as a result of the effects of its many toxins on the bronchial mucosa, or through stimulating a vigorous and persistent inflammatory reaction[35].

As regards positive skin test to the common allergens in atopic patients we found that mixed fungi represented 40% of atopic asthmatics. Maccario et al.[36] evaluated the skin prick test responses and showed that nearly 34% of resistant asthma was due to fungal infections. Nouer et al.[37] reported that aspergillus fumigatus was the reason for nearly 29% of resistant asthmatic cases. Another study reported that over 80% of *Aspergillus*-related conditions, such as extrinsic allergic alveolitis, asthma, allergic sinusitis, chronic eosinophilic pneumonia, hypersensitivity pneumonitis, and allergic bronchopulmonary aspergillosis (ABPA) were most frequently caused by *A. fumigatus*[38].

We found that *A. fumigatus* represented 80% of fungal infections. Another study succeeded to grow AF fungi in 30% only of mixed fungal infection in atopic children[39]. The difference in the results may be due to different weather conditions; as humid air is a cofactor for AF fungi to grow[40].

In conclusion; aspergillus fumigatus can play a crucial role in inducing atopic asthma in children. Evaluation of AF sensitization especially in severely asthmatic patients may be helpful in diagnosis and for the planning of management.

REFERENCES

1. **Hinson KFW, Moon AJ, Plummer NS.** Bronchopulmonary aspergillosis a review and report of eight new cases. Thorax 1952; 7:317-33.
2. **Franket T, Gimenez A, Hidalgo A.** Imaging of opportunistic fungal infections in immunocompromised patient. Eur J Radiol 2004;51(2):130-8.
3. **Palmer LB, Greenberg HE, Schiff MJ.** Corticosteroid treatment as a risk factor for invasive aspergillosis in patients with lung disease. Thorax 1991;46(1):15-20.
4. **Pepys J.** Antigens and hypersensitivity pneumonitis. J Allergy Clin Immunol 1978;61(4):201-3.
5. **Henderson AH, English MP, Vecht RJ.** Pulmonary aspergillosis. A survey of its occurrence in patients with chronic lung disease and a discussion of the significance of diagnostic tests. Thorax 1968;23(5):513-8.
6. **Eaton T, Garrett J, Milne D, Frankel A, Wells AU.** Allergic bronchopulmonary aspergillosis in the asthma clinic. A prospective evaluation of CT in the diagnostic algorithm. Chest 2000;118(1):66-72.
7. **Kraemer R, Deloséa N, Ballinari P, Gallati S, Crameri R.** Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. Am J Respir Crit Care Med 2006 1;174(11):1211-20.
8. **Knutsen AP, Bellone C, Kauffman H.** Immunopathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis. J Cyst Fibros 2002;1(2):76-89.
9. **Agarwal R, Khan A, Garg M, Aggarwal AN, Gupta D.** Chest radiographic and computed tomographic manifestations in allergic bronchopulmonary aspergillosis. World J Radiol 2012 28;4(4):141-50**.**
10. **Stevens DA, Melikian GL.** Aspergillosis in the 'nonimmunocompromised' host. Immunol Invest 2011;40(7-8):751-66.
11. **Ganassini A, Cazzadori A.** Invasive pulmonary aspergillosis complicating allergic bronchopulmonary aspergillosis. Respir Med 1995;89(2):143-5.
12. **Krasnick J, Greenberger PA, Roberts M, Patterson R.** Allergic bronchopulmonary aspergillosis: serologic update for 1995. J Clin Lab Immunol 1995;46(3):137-42.
13. **Agarwal R, Khan A, Garg M, Aggarwal AN, Gupta D.** Pictorial essay: Allergic bronchopulmonary aspergillosis. Indian J Radiol Imaging 2011;21(4):242-52.
14. **Fairs A, Agbetile J, Hargadon B, Bourne M, Monteiro WR, Brightling CE, et al.** IgE sensitization to Aspergillus fumigatus is associated with reduced lung function in asthma. Am J Respir Crit Care Med 2010 1;182(11):1362-8.
15. **Menzies D, Holmes L, Mccumesky G, Prys-Picard C, Niven R.** Aspergillus sensitization is associated with airflow limitation and bronchiectasis in severe asthma. Allergy 2011;66(5):679-85.
16. **Reed CE, Kita H.** The role of protease activation of inflammation in allergic respiratory diseases. J Allergy Clin Immunol 2004;114(5):997-1008.
17. **Knight DA, Lim S, Scaffidi AK, Roche N, Chung KF, Stewart GA, et al.** Protease-activated receptors in human airways: upregulation of PAR-2 in respiratory epithelium from patients with asthma. J Allergy Clin Immunol 2001;108(5):797-803.

18. **Saxena S, Kumar R, Madan T, Gupta V, Muralidhar K, Sarma PU.** Association of polymorphisms in pulmonary surfactant protein A1 and A2 genes with high-altitude pulmonary edema. Chest 2005;128(3):1611-9.

19. **Kaur S, Gupta VK, Shah A, Thiel S, Sarma PU, Madan T.** Elevated levels of mannan-binding lectin [corrected] (MBL) and eosinophilia in patients of bronchial asthma with allergic rhinitis and allergic bronchopulmonary aspergillosis associate with a novel intronic polymorphism in MBL. Clin Exp Immunol 2006;143(3):414-9.

20. **Vaid M, Kaur S, Sambatakou H, Madan T, Denning DW, Sarma PU.** Distinct alleles of mannose-binding lectin (MBL) and surfactant proteins A (SP-A) in patients with chronic cavitary pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. Clin Chem Lab Med 2007;45(2):183-6.

21. **Mccarthy DS, Pepys J.** Allergic broncho-pulmonary aspergillosis. Clinical immunology. 2. Skin, nasal and bronchial tests. Clin Allergy 1971;1(4):415-32.

22. **Camargo CA Jr, Rachelefsky G, Schatz M.** Managing asthma exacerbations in the emergency department: summary of the National Asthma Education And Prevention Program Expert Panel Report 3 guidelines for the management of asthma exacerbations. Proc Am Thorac Soc 2009 1;6(4):357-66.

23. **Masoli M, Fabian D, Holt S, Beasley R;** Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004;59(5):469-78.

24. **Bousquet J.** Global initiative for asthma (GINA) and its objectives. Clin Exp Allergy 2000;30 Suppl 1:2-5.

25. **Reddel HK, Vincent SD, Civitico J.** The need for standardisation of peak flow charts Thorax 2005;60(2):164-7.

26. **Kurup VP, Banerjee B, Hemmann S, Greenberger PA, Blaser K, Crameri R.** Selected recombinant Aspergillus fumigatus allergens bind specifically to IgE in ABPA. Clin Exp Allergy 2000;30(7):988-93.

27. **Crameri R, Hemmann S, Ismail C, Cenz G, Blaser K.** Disease-specific recombinant allergens for the diagnosis of allergic bronchopulmonary aspergillosis. Int Immunol 1998;10(8):1211-6.

28. **Rodrigo MJ, Benavent MI, Cruz MJ, Rosell M, Murio C, Pascual C, et al.** Detection of specific antibodies to pigeon serum and bloom antigens by enzyme linked immunosorbent assay in pigeon breeder's disease. Occup Environ Med 2000;57(3):159-64.

29. **Vijay HM, Kurup VP.** Fungal allergens. Clin Allergy Immunol 2008;21:141-60.

30. **Llanora GV, Ming LJ, Wei LM, Van Bever HP.** House dust mite sensitization in toddlers predict persistent wheeze in children between eight to fourteen years old Asia Pac Allergy 2012;2(3):181-6.

31. **Kersten W, Stollewerk D, Von Wahl PG.** Acarex test and acarosan effect in house dust mite allergy in 2 year follow-up. Pneumologie 1992;46(1):26-31.

32. **Simon-Nobbe B, Denk U, Poll V, Rid R, Breitenbach M.** The spectrum of fungal allergy. Int Arch Allergy Immunol 2008;145(1):58–86.