# Suspected Allergic Bronchopulmonary Aspergillosis Cases in Adult Bronchial Asthma Patients Attending a Tertiary Care Clinic

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**Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to *Aspergillus* species (generally *Aspergillus fumigatus*) that occurs almost exclusively in patients with asthma or, less commonly, cystic fibrosis. Immune responses to *Aspergillus* antigens cause airway obstruction and, if untreated, bronchiectasis and pulmonary fibrosis. Our objective was to define the clinical characteristics, laboratory and radiological findings of suspected ABPA cases among a cohort of Egyptian patients with bronchial asthma. 52 moderate and severe asthma patients were recruited from the Allergy and Immunology clinic at Ain Shams University hospitals. Patients were subjected to history taking for asthma symptoms, skin test with *Aspergillus fumigatus* antigen, total IgE level, peripheral blood eosinophilia, chest x-ray and high resolution CT chest. 27 patients had positive skin prick and /or intradermal test to *Aspergillus fumigatus* antigen, and 11 (21.2%) of them fulfilled 4 of the criteria for ABPA diagnosis. Patients with suspected ABPA had significantly higher serum total IgE levels (median (IQR) = 625 IU/ml (514.9-762) with *P*-value <0.0001). Our study suggests a high frequency of suspected ABPA cases for further confirmation by appropriate diagnostic tests; there is a need for better recognition of ABPA as it is yet under recognized in Egypt. Clinicians ought to have a high index of suspicion for ABPA while managing any patient with bronchial asthma to detect ABPA prior to development of irreversible complications.**

Allergic bronchopulmonary aspergillosis (ABPA) is a complex hypersensitivity reaction, usually affecting patients with asthma or cystic fibrosis (Tillie-Leblond & Tonnel, 2005). ABPA leads to poorly controlled asthma with pulmonary exacerbations and detrimental consequences; dependence on oral corticosteroids increases the risk for secondary infections (Shokry *et al.,* 2012). After first being reported in England during 1952, a number of cases of ABPA were reported from various other countries (Kumar & Gaur, 2000). The number of reported cases has been on the rise lately, which could possibly be attributed to heightened clinician awareness in addition to good availability of immunologic tests facilitating the diagnosis of ABPA (Agarwal *et al.,* 2007). None the less, the population prevalence of ABPA complicating asthma remains unclear; it ranges from 1-3.5% based on the data reported from secondary care cohorts in various countries. The prevalence of ABPA in chest or asthma clinics is even higher, ranging from 2 to 32% (Agarwal & Chakrabarti, 2013), and delay in diagnosis and treatment of ABPA could cause irreversible lung damage in the form of bronchiectasis, lung fibrosis, and, finally, end-stage lung disease and respiratory failure (Schulman & Pohlig, 2015). The criteria for the diagnosis of ABPA include bronchial asthma, immediate skin test reactivity to *Aspergillus Fumigatus (A. fumigatus)*, elevated total and specific *A. fumigatus* serum immunoglobulin E (IgE), pulmonary opacities, central bronchiectasis, peripheral blood eosinophilia and positive serum precipitins (IgG) against *A. fumigatus* antigen. None of these are specific for ABPA,

and there is still no consensus on the number of criteria needed for diagnosis or the optimum disease specific cut-off values for the various serological tests used. Moreover, patients at different stages of ABPA may not fulfill all these criteria (Greenberger, 2002).

It is crucial to detect the disease before bronchiectasis has developed because the occurrence of bronchiectasis is associated with poorer outcomes. Our aim was to define the clinical characteristics, laboratory and radiological findings of suspected ABPA cases among a cohort of Egyptian patients with bronchial asthma.

## Materials and Methods

Our study was conducted on a cohort of 52 bronchial asthma patients recruited from the Allergy and Immunology clinic at Ain Shams University hospitals during the period from January till May 2015.We included adult patients (≥16 years) with history of moderate or severe asthma; the severity of asthma was classified according to GINA guidelines for asthma severity (GINA 2015). Exclusion criteria included patients with inflammatory or septic conditions, including oral sepsis, patients with associated systemic autoimmune diseases, patients on systemic glucocorticoids and/or systemic antifungals, patients who had received antihistaminic drugs, cytotoxic drugs, patients with organ failure and pregnant females. All patients were subjected to full detailed history with emphasis on history of wheezes, chest tightness, dyspnea, cough, factors causing increase of symptom, nocturnal symptoms and seasonal variation, family history of asthma and/or atopic disorders. Symptoms and signs of ABPA are those of asthma in addition to productive cough and, occasionally, fever and anorexia. Pulmonary function test was done for staging of bronchial asthma. ABPA was suspected if the patients met four of the following criteria: clinical diagnosis of asthma; immediate skin test reactivity to *A. fumigatus;* elevated total IgE level (≥417 IU/mL); peripheral blood eosinophilia and radiological evidence of ABPA.

### *Aspergillus* skin testing procedure

The *A. fumigatus* skin tests (prick and intradermal) were performed using *A. fumigatus* antigen provided by Stallergenes. The initial test performed was skin prick test (SPT) followed by intradermal skin test in case of negative SPT since some patients only manifest hypersensitivity with an intradermal test (Agarwal *et al.,* 2013[a]).

Technique of SPT: After sterilization of the forearm with propyl alcohol, single drop of *A. fumigatus* allergen was applied. A skin-prick test was performed within the allergen drop on the skin with a 26-gauge needle. Additionally, a drop of histamine phosphate (at a concentration of 2.7 mg/mL) and a drop of the diluent were used as a positive control and a negative control, respectively. A wheal ≥ 5 mm in size in reaction to histamine was considered adequate for the competency of the test. A wheal ≥ 3 mm in diameter (more than the negative control) read after 20 minutes was considered a positive test result for sensitization to *A. fumigatus* allergen (Koshak *et al.,* 2006). Immediate cutaneous hypersensitivity to *A. fumigatus* antigens represents *Aspergillus* sensitization and is considered the hallmark of ABPA as it denotes the presence of IgE antibodies specific to *A. fumigatus* (Agarwal *et al.,* 2013[a]).

### Intradermal skin test procedure

The skin of the volar surface of the forearm was cleaned and marked, 0.1 ml of 1/1000 dilution of *A. fumigatus* antigen, negative and positive controls were injected intradermally; after15 minutes the tested sites were inspected. Fully developed erythema and wheal reactions were re-measured 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 (Shokry *et al.,* 2012).

### Serum total IgE

3 ml of venous blood were withdrawn aseptically into a sterile disposable syringe from all patients and controls, and collected in plain tubes were clotted and centrifuged. The yielding serum was used for the detection of total serum immunoglobulin E (IgE) levels, which were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (BioCheck, Inc., Foster City, USA) according to the manufacturer's instructions. The normal limit of total IgE was 100 IU/ml. The minimum detectable amount was 5 IU/ml. A cut-off value of ≥ 417 IU/ml was considered positive criteria for suspected ABPA (Agarwal *et al.,* 2013[b]).

### Total eosinophil count

The blood count was performed using the Coulter counter (Coulter micro DIFF 18, CA, USA). The differential leukocyte counts were estimated manually from the blood film and expressed in absolute count values. Eosinophilia was considered when the absolute eosinophil counts exceeded > 1000 cells/µL.

### Radiological investigations

Chest X-ray posteroanterior view and HRCT were performed for all patients.

### HRCT Image acquisition

Image was taken using a 16-row, multiple-detector CT scanner (LightSpeed Plus; GE Medical Systems; Slough, UK) with a matrix size of 512×512. The scans were obtained from the lung apex to the base using a scan time of three seconds while the patient was in the supine position at full end-tidal inspiration. Image acquisition was contiguous, and the images (1.25 mm at 10-mm intervals) were reconstructed using a high-spatial-frequency algorithm. Main findings of ABPA in HRCT included bronchiectasis, mucoid impaction, mosaic attenuation, centrilobular nodules, tree-in-bud opacities and pleuropulmonary fibrosis. Uncommon radiological manifestations include miliary nodular opacities, perihilar opacities simulating hilar lymphadenopathy, pleural effusions and pulmonary masses (Agarwal et al., 2013[a]). The images were reviewed by an experienced radiologist (10 years' experience) and were classified into chest X-ray; normal or abnormal. The HRCT findings suggestive of ABPA diagnosis were divided into three main groups (a) nodules, (b) bronchial thickening and (c) bronchiectasis. HRCT of the chest is normal in almost 50% of the patients (Agarwal *et al.,* 2011).

### Statistical Methods

Data were analyzed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA) and MedCalc© version 14 (MedCalc© Software bvba, Ostend, Belgium). Normality of numerical data distribution was examined using the D'Agostino-Pearson test. Normally distributed numerical variables were presented as mean ± standard deviation, and inter-group differences were compared using the unpaired t test. Skewed numerical data were presented as median (interquartile range), and between-group differences were compared using the Mann-Whitney test. Categorical variables were presented as ratio or number (%), and differences between groups were compared using the chi-squared test with Yates' continuity correction or Fisher's exact test, when appropriate. P-value <0.05 was considered statistically significant.

## Results

Mean age of the study population was 36±10 years, including 14 males and 38 females. 57.7% had positive family history of asthma. We report suspected ABPA in 21.2% of patients (Figure 1).

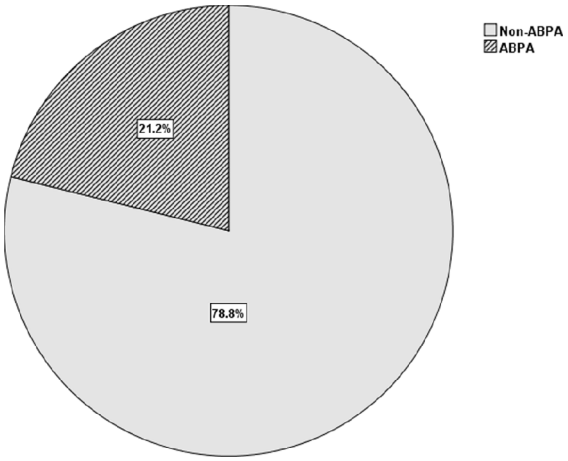


**Figure 1.** Prevalence of ABPA in the study population.

Comparison between clinical characteristics and relevant laboratory data of suspected ABPA and non-ABPA patients are demonstrated in table 1. 27 (51.9%) of included patients had positive skin test to *A.fumigatus* antigen; 18 patients had positive skin prick test response and 9 patients had positive intradermal skin test to *A.fumigatus.* Patients with suspected ABPA had statistically significantly higher serum total IgE level than non- ABPA patients. Median (IQR) of total IgE level in suspected ABPA cases versus non-ABPA was 625 IU/ml (514.9-762) and 125 IU/ml (50.2-211) respectively ($P$<0.0001) (figure 2). 5 (45%) of suspected ABPA cases had peripheral eosinophilia in contrast to 14 (34.1%) of non-ABPA patients with no statistical significance.

The X-ray findings showed abnormal X-ray in three non-ABPA patients. HRCT of suspected ABPA cases showed nodules in 4 (36.4%) and bronchial thickening in 2 (18.2%) patients, none had bronchiectasis (table 2).

Table 1. Characteristics of suspected ABPA and non-ABPA patients

| Variable | Non-ABPA (n=41) | Suspected ABPA (n=11) | *P*-value |
|---|---|---|---|
| Age (years) | 37.1 ± 10.0 | 32.9 ± 9.7 | NS¶ |
| Gender (male/female) | 11/30 | 3/8 | NS§ |
| Family history of asthma | 22 (53.7%) | 8 (72.7%) | NS¥ |
| Associated allergic conditions | | | |
| Urticaria | 7 (17.1%) | 1 (9.1%) | NS¥ |
| Allergic rhinitis | 19 (46.3%) | 5 (45.5%) | NS§ |
| Severity of asthma | | | NS¥ |
| Moderate | 29 (70.7%) | 8 (72.7%) | |
| Eosinophilia | 14 (34.1%) | 5 (45.5%) | NS¥ |
| Serum total IgE level (IU/ml) | 125 (50.2-211) | 625 (514.9-762) | <0.0001# |

Data are presented as mean ± SD, ratio, number (%), or or median (interquartile range). *P*>0.05 is not significant (NS).
¶Unpaired t test, §Chi-squared test with Yates' continuity correction, ¥Fisher's exact test, #Mann-Whitney test

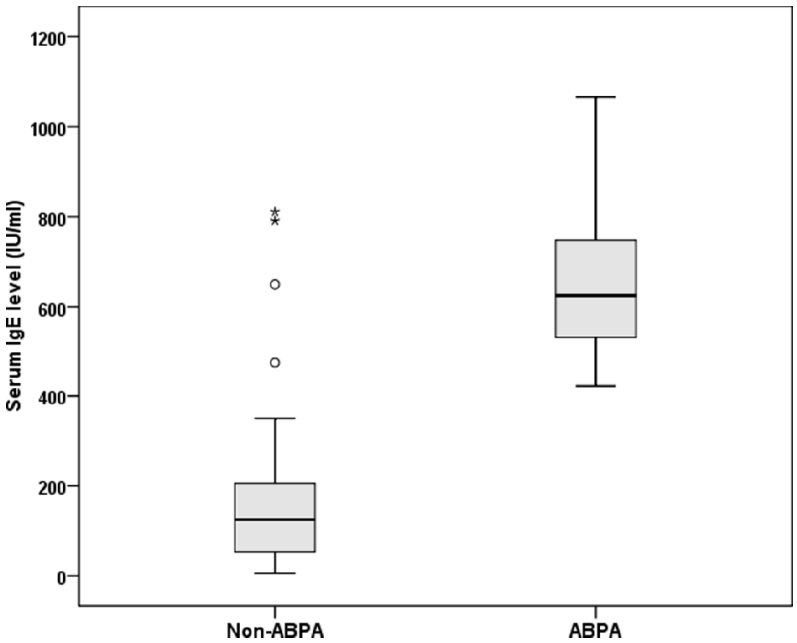


**Figure 2.** Box plot showing serum IgE level in patients with or without ABPA. Box represents the interquartile range. Horizontal line inside the box represents the median. Error bars represent the minimum and maximum values excluding outliers (rounded markers) and extreme values (asterisks).

Table 2. Relevant radiological findings in suspected ABPA and non-ABPA patients

| Variable | Non-ABPA (n=41) | Suspected ABPA (n=11) | *P*-value |
|---|---|---|---|
| Abnormal CXR | 3 (7.3%) | 0 (0.0%) | NS |
| Relevant HRCT findings | | | |
| *Nodules* | 5 (12.2%) | 4 (36.4%) | NS |
| *Bronnchial thickening* | 4 (9.8%) | 2 (18.2%) | NS |
| *Bronchiectasis* | 1 (2.4%) | 0 (0.0%) | NS |

Data are presented as number (%).

¶Fisher's exact test. *P*>0.05 is not significant (NS).

## Discussion

*Aspergillus* fungus is a ubiquitous mold that primarily affects the lungs, causing four main syndromes, namely ABPA, chronic necrotizing *Aspergillus* pneumonia, Aspergilloma and Invasive aspergillosis (Harman, 2015). Our study detected *A. fumigatus* skin test positivity in 27 patients, and 11 (21.2%) fulfilled four criteria for diagnosis of ABPA. *A. fumigatus* sensitisation was demonstrated in the current study using skin test rather than serum specific IgE. Reports from a latent class analysis reported the sensitivity and specificity of *A. fumigatus* skin test positivity was 94.7%, 79.7% , while *A.fumigatus* specific IgE levels>0.35 kUA/L had sensitivity and specificity of 100%, 69.3% respectively (Agarwal *et al.,* 2013[b]). It is documented that patients who only demonstrate positive immediate skin reactivity to *A. fumigatus* (20 to 30 % of all asthmatics) (Eaton *et al.,* 2000) without fulfilling any other criteria of ABPA are labelled "severe asthma with fungal sensitization", a term that is increasingly used to describe such patients (Akuthota & Weller, 2014).

In a meta- analysis that included studies published from 1965 to 2008 that report the prevalence of *Aspergillus* sensitization /ABPA in asthma, the calculated prevalence of *Aspergillus* sensitization /ABPA in bronchial asthma was 28% and 12.9% respectively (95%CI 7.9–18.9), which is consistent with our findings (Agarwal *et al.,* 2009). Although the population prevalence of ABPA is estimated to be approximately 1-3.5% (Al-Mobeireek *et al.,* 2001; Benatar *et al.,* 1980), higher prevalence was recorded in chest or asthma clinics (Agarwal & Chakrabati, 2013), which is the case in our clinic as it represents a tertiary care specialized allergy clinic. In our study cut off value for total IgE was 417 IU/ml. The Patterson criteria, which is still considered as the most frequently used yardstick for diagnosis of ABPA (Rosenberg *et al.,* 1977; Patterson *et al.,* 1986), uses a minimum concentration for total IgE of 1000 IU/ml. However, a recent latent class analysis that evaluated the diagnostic performance of various tests employed in ABPA diagnosis reported that sensitivity and specificity of employing a cut-off value of 417 IU/ml for total IgE is 95.8% and 23.7% respectively (Agarwal *et al.,* 2013[b]). Nonetheless, although IgE level demonstrates low specificity in diagnosing ABPA, it is by far the most reliable test used for follow up of patients with ABPA (Agarwal *et al.,* 2010), and a 50% increase in IgE levels may signify an impending exacerbation (Agarwal &

Chakrabati, 2013). Our study demonstrates a significantly higher level of total IgE in suspected ABPA patients in comparison to non-ABPA asthmatics. Exposure of atopic individuals to fungal spores or mycelial fragments (allergen) seems to evoke a vigorous IgE and IgG mediated immune responses superimposed on the asthmatic milieu. However, the fungus seems to overcome this response and virtually colonize the asthmatic airway and evoke recurrent symptoms (Akuthota & Weller, 2014). Eventually, Th2 –mediated eosinophilic inflammation and IL-8-mediated neutrophilic inflammation result in airway damage and central bronchiectasis (Gibson *et al.,* 2003). ABPA is frequently raised as a diagnostic possibility in patients with asthma, especially in patients demonstrating positive skin prick to *Aspergillus* extract, and ABPA has been documented in up to 32 % of patients with asthma and skin test reactivity to *Aspergillus* in various studies (Akuthota & Weller, 2014). ABPA is an advanced stage of *Aspergillus* sensitization, with *Aspergillus* sensitization being the first pathogenetic step in development of ABPA (Agarwal *et al.,* 2013). In conclusion, our study suggests a high frequency of suspected ABPA in subjects with moderate and severe bronchial asthma, and ABPA should be confirmed by measuring serum specific IgE and serum precipitins (IgG) against the *A. fumigatus* antigen. There is a need for better recognition of ABPA which is yet under recognised in Egypt. Clinicians ought to have a high index of suspicion for ABPA while managing any patient with bronchial asthma to detect ABPA prior to development of irreversible complications. The limitation of our study is the small sample size and the conduct of the study at a single center. Studies with similar design need to be conducted at different centers with a larger sample size employing both in-vitro and in-vivo tests to demonstrate *A. fumigatus* related diseases.

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