Allergic bronchopulmonary mycosis in patients with asthma: period prevalence at a university hospital in Saudi Arabia

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Allergic bronchopulmonary mycosis (ABPM) is a known complication of asthma and can result in progressive lung damage, respiratory failure and death. Asthma is a common disease in Saudi Arabia and until now the prevalence of ABPM has not been investigated. The aim of this study was to estimate the period prevalence of ABPM due to Aspergillus and Candida in patients with asthma. The setting was an outpatient pulmonary clinic at a university hospital in the central region of Saudi Arabia. Two hundred and sixty-four consecutive patients with asthma (150 or 57% females) were evaluated. All patients were screened for ABPM with skin prick test (SPT) using a panel of fungal antigens. Those with positive skin reactions had further clinical, immunological, respiratory and radiological assessment. ABPM was diagnosed by the presence of a minimum of five of the major criteria suggested by Rosenberg in 1977. Of the 264 patients, 62 (23%) had a positive SPT for at least one fungal allergen, of whom 44 (71%) were females ($P = 0.01$). Seven patients (six females) were diagnosed with ABPM due to Aspergillus and (or) Candida species. Therefore, we estimate the period prevalence of ABPM to be 2.7% (95% confidence interval 1.3–5.4%). A. niger was the commonest fungal species isolated in our group. In conclusion, ABPM is not uncommon in Saudi Arabia and females seem to be more at risk. Because asthma is common, physicians need to have high index of suspicion for this disease and pursue the diagnosis with the appropriate tests.

Key words: asthma; Aspergillus; Candida; allergy.
approved by the Ethical Committee of the College of Medicine Research Council. The diagnosis of asthma was confirmed clinically by the treating pulmonologist and by the demonstration of airway obstruction and significant response to bronchodilator on testing with spirometry. For the minority of patients who could not perform spirometry, peak expiratory flow rate was used. Skin prick test (SPT) was used for screening patients for ABPM, as it is believed to be highly sensitive (5). Pregnant women and patients taking continuous oral steroids were excluded from the study, since these conditions may interfere with the results of some of the tests. Patients on anti-histamines were re-tested after stopping them for 4–6 weeks. All patients with positive SPT underwent further immunological, microbiological and radiological assessment as detailed below.

ABPM was defined by the presence of a minimum of five of the following seven major criteria originally proposed by Rosenberg (4), with the addition of specific IgE (5):

1. Asthma
2. Peripheral blood eosinophilia
3. Immediate skin test reactivity for Aspergillus or Candida antigens
4. Elevated total serum immunoglobulin E (IgE) (above 1000 kU l\(^{-1}\)) and (or) specific IgE
5. Positive serum precipitins (IgG) against Aspergillus or Candida antigens
6. Fleeting or fixed pulmonary shadows
7. Central bronchiectasis.

IMMUNOLOGICAL INVESTIGATIONS

Skin prick test (SPT)

The skin prick test was performed in all patients using 17 fungal antigens. The panel included: Aspergillus fumigatus, A. niger, A. versicolor, A. clavatus, A. repens, Alternaria, Cladosporium, Rhizopus, Penicillium, Mucor, Trichophyton, Candida, Herbarum, Phoma, Fusarium, mould I and mould II (a mixture of fungal allergens), histamine (positive control), and negative control. The technique for SPT was followed as described by Pepys (13). Skin test extracts ‘Soluprick’ were purchased from ALK Laboratories, Horsholm, Denmark. Immediate reactions were read after 10–15 min and were interpreted as positive only if wheal and flare measured 3 mm or more with a positive histamine control.

Total, specific IgE measurement and serum precipitins

Total IgE measurement. Blood was obtained by venipuncture from all patients. The sample was allowed to clot and the serum separated by centrifugation. Specimens were kept at -20°C until assayed. The Pharmacia CAP System IgE fluoroenzyme immunoassay (FEIA) method was used. This is an in-vitro test system for the quantitative measurement of circulating total IgE in human blood samples. The results were recorded in kU l\(^{-1}\).

Specific IgE measurement. The Pharmacia CAP System RAST FEIA method was used. This is an in-vitro test, which measures the concentration of circulating allergen-specific IgE in human blood samples. All serum samples were tested against a standard panel of fungal allergen which included: Aspergillus fumigatus, Alternaria, Cladosporium, Rhizopus, Penicillium, Mucor, Candida, Fusarium and Phoma. Values more than 0-70 kU l\(^{-1}\) were considered positive. The test for measuring total and specific IgE was performed according to the manufacturer’s procedural guidelines.

Serum precipitins. Sera of patients with positive skin reactions were tested for presence of precipitating antibodies against Aspergillus and Candida antigens using immunodiffusion and counter-immunoelectrophoresis. The antigens used (by Immunomycologics, Norman, Oklahoma, U.S.A.) were Aspergillus polyvalent antigen (containing antigens of Aspergillus fumigatus, A. flavus and A. niger), monovalent antigens of A.terrei, A. nidulans, and Candida spp. antigen.

Eosinophil count. Eosinophil count of equal or more than 400 µl\(^{-1}\) was used to indicate significant eosinophilia.

MICROBIOLOGICAL STUDIES

Direct microscopy and culture

Sputa were collected of patients with positive SPT who were able to expectorate specimens. Smears were made and stained with Giemsa stain, then examined microscopically for fungal elements. The specimen was cultured on each of Sabouraud’s dextrose agar and Sabouraud’s dextrose agar with chloramphenicol and incubated at room temperature (26±1°C) for up to 4 weeks before reporting as negative if no growth occurred. Cultures were examined periodically to detect any growth, and when growth was found, they were subcultured onto Sabouraud’s dextrose agar slopes and identified using standard mycological methods.

PULMONARY FUNCTION TESTS

Spirometry was performed according to the American Thoracic Society (ATS) recommendations (14). Measurements included the forced expiratory volume in 1 sec (FEV\(_1\)) and forced vital capacity (FVC), and were expressed as percentage of predicted values.

RADIOLOGICAL STUDIES

Chest radiographs (CXR) were performed for all patients with positive SPT. A high resolution computed tomography (HRCT) scan of the chest was done for patients with suspicious findings on CXR (e.g. mild bronchial dilatation or thickening). HRCT was done from the apex to the base.
(with around 18–20 slice cuts depending on the size of the thorax of the patients) or limited (with 1·5 × 20 mm cuts from the aortic arch to the diaphragm and six cuts on expiration). All radiological material was reviewed by one pulmonary radiologist who was not aware of the results of the other investigations. The diagnostic findings for ABPM included central bronchiectasis (CB) and fleeting pulmonary shadows (FS) that are not explained by other causes such as infection, infarction or autoimmune diseases.

**Results**

During the study period 273 consecutive patients with asthma were evaluated. Nine patients were excluded (six pregnant women and three on chronic steroid therapy), leaving 264 patients for further evaluation.

**CLINICAL CHARACTERISTICS**

A total of 264 patients were screened with the skin prick test (SPT). The age of the patients ranged between 7 and 93 years, mean age and standard deviations were 38 ± 17 years. One hundred and fifty (56.8%) were females and 114 (43·2%) were males. Two hundred and two patients (76·5%) had negative SPT and 62 (23·5%) had positive SPT to one or more fungal allergen. In the group with positive SPT 44 (71%) were females, as opposed to 106 (52·5%) in the group with negative SPT (P = 0·01, Table 1).

Before assessment, 50 (81%) of patients with positive SPT and 101 (50%) of patients with negative SPT were taking consistently inhaled corticosteroids (P < 0.001). There were few smokers (none in patients with positive SPT and four in patients with negative SPT). Ex-smokers were one and two in the two groups, respectively.

The clinical features of patients according to the skin test result are shown in Table 1. Cough was the only symptom showing a statistically significant difference. History of atopy (allergic rhinitis, conjunctivitis or dermatitis) was slightly higher in patients with positive SPT but the difference was not statistically significant.

**IMMUNOLOGICAL INVESTIGATIONS**

**Skin prick test**

Sixty-two (23%) out of 264 patients showed positive SPT to one or more of the allergens tested. Details are available for 53 patients (Table 2) and were as follows: 16 patients (30%) reacted to the mould I extract; 16 patients (30%) to Candida; 15 patients (28%) to Aspergillus niger; 14 patients (26%) to Alternaria; 12 patients (23%) to Aspergillus fumigatus; 12 patients (23%) to mould II; seven patients (13%) each to A. versicolor, A. clavatus, A. repens, Cladosporium, Penicillium; two patients (4%) to Trichophyton; and one (2%) to Mucor. There were no reactions to Herbarum, Phoma and Fusarium. Details of the remaining nine patients could not be located, although it was known for certain that they had positive SPT.

**Total, specific IgE measurement and serum precipitins**

**Total IgE.** Total IgE ranged from 2·88 to > 1000 kU l⁻¹, mean 368 ± so 338. Fourteen patients had values above 1000 kU l⁻¹.

**Specific IgE.** Details are available for 54 patients (Table 2). Twenty patients (37%) had a positive specific IgE test to at least one fungal allergen. Candida and Alternaria were the most prominent allergens showing positive reactions. The frequency of specific IgE antibodies to different allergens is as follows: Candida, 15 patients (28%); Alternaria, 14 patients (26%); A. fumigatus, 12 patients (22%); Cladosporium, 11 patients (20%); Rhizopus and Fusarium, 10 patients (19%) each; and Penicillium, Phoma and Mucor, nine patients (17%) each. Unfortunately, specimens from the remaining eight patients were not received by the laboratory. However, even if these patients were assumed to have positive results, none of them would have had sufficient other positive criteria for the diagnosis of ABPM.

**Table 1. Clinical characteristics of the patients**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with positive skin prick test (%)</th>
<th>Patients with negative skin prick test (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>71</td>
<td>52·5</td>
<td>0·01</td>
</tr>
<tr>
<td>SOB*</td>
<td>84</td>
<td>89</td>
<td>0·3</td>
</tr>
<tr>
<td>Cough</td>
<td>98</td>
<td>86</td>
<td>0·0002</td>
</tr>
<tr>
<td>Sputum</td>
<td>70</td>
<td>60</td>
<td>0·1</td>
</tr>
<tr>
<td>Sputum casts</td>
<td>16</td>
<td>16</td>
<td>0·9</td>
</tr>
<tr>
<td>Wheeze</td>
<td>93</td>
<td>86</td>
<td>0·1</td>
</tr>
<tr>
<td>Atopy*</td>
<td>66</td>
<td>56</td>
<td>0·2</td>
</tr>
<tr>
<td>On inhaled steroids</td>
<td>81</td>
<td>50</td>
<td>&lt;0·001</td>
</tr>
</tbody>
</table>

*Shortness of breath at rest or on exertion.

Atopy: history of allergic rhinitis, conjunctivitis or dermatitis.
Serum precipitins. Of 62 patients with positive SPT, eight patients (13%) had positive serum precipitins for *Aspergillus* species-specific antigen and two patients (3%) had positive results for *Candida* antigen. One patient had positive serum precipitins for both *Candida* and *Aspergillus* antigens.

Eosinophil count

The range of the eosinophil counts was 10–2580 \( \mu l^{-1} \). Seventeen patients (27%) had more than 400 eosinophils \( \mu l^{-1} \) in their peripheral blood.

MICROBIOLOGICAL STUDIES

Of the group of patients with positive SPT, 35 patients managed to provide sputum for fungal studies (Table 2). Sputum smears for fungi showed hyphae in 16, yeast in two and were negative in the remaining specimens. Culture results for *Aspergillus* were as follows: five *A. niger*, and one each of *A. fumigatus*, *A. flavus*, *A. terreus* and *Aspergillus* spp. in patients with ABPA (Table 2). *A. niger* was isolated from four patients and *A. terreus* from one patient. *A. fumigatus* was not isolated from any patient with ABPA.

*C. albicans* was cultured from 13 patients, *C. glabrata* and *C. parapsilosis* from one patient each. *C. albicans* was associated with *Aspergillus* in two patients with a positive specific IgE (Table 3, patient nos 1 and 2). *C. albicans* was also isolated solely from one patient but with a negative specific IgE and SPT, which indicates that a pathogenic role is unlikely.

PULMONARY FUNCTION TESTS

Two hundred and eleven patients had spirometry (46 with positive SPT and 165 with negative SPT). Patients with positive SPT had lower FEV\(_1\) and FVC than patients with negative SPT (\(P\)-value for FEV\(_1\) = 0.0016 and for FVC = 0.0037) as shown in Table 4.

RADIOLOGICAL STUDIES

Chest radiographs of the 62 patients with positive SPT showed the following: 42 were normal, 17 showed non-diagnostic abnormalities, three showed fleeting shadows and one showed CB. Patients with abnormal CXR went on for HRCT scan, which showed CB in four patients.

PATIENTS WITH ABPM

Out of the 264 asthmatic patients screened with SPT, 62 (23%) had positive skin test reactions. Seven patients fulfilled five or more of the major criteria for the diagnosis of ABPM (Table 3), i.e. a period prevalence of 2.7%, 95% confidence interval 1.3–5.4% (15). Of those patients six were females and one only was male. Patients 1, 2 and 7 were referred from coastal cities in the south-west of Saudi Arabia, and the remainder were from central Arabia.

Discussion

To our knowledge, this is the first survey of ABPM in patients with asthma in the Middle East. Studies have shown that fungal allergens are prevalent both in the indoor and outdoor environment in Riyadh (2), as well as positive skin reactions to fungi in asthmatic patients (3). Despite this, only one series of 10 patients with ABPA was reported in a retrospective study in Saudi Arabia from 1984 to 1994 (8). It was thought that the low natural humidity in central Arabia (<40%), which can inhibit the growth of fungi, might have contributed to the rarity of this syndrome (8). This study shows period prevalence close to 3% for ABPM due to *Aspergillus* and *Candida*, suggesting that the disease is not uncommon but more likely overlooked among asthma patients attending our outpatient clinics.

In Western countries the reported frequencies ranged between 1 and 28% depending on the patient population and methods of the study. For example, in the U.K. an earlier study found a prevalence of 11% (9). The criteria used to define ABPA in that study included: asthma, eosinophilia, a positive skin reaction to *A. fumigatus* and a
positive sputum fungal culture. In Ireland, Donnelly et al. (10) found the period prevalence of ABPM due to *Aspergillus* and *Candida* to be just over 1% in an outpatient clinic between 1985 and 1988. They screened patients with asthma and eosinophilia or unexplained pulmonary infiltrates requiring a minimum of four criteria that included a positive serology to *A. fumigatus* or *C. albicans*.

In the U.S.A. two surveys of ABPA were done on asthmatic patients with a positive skin reaction i.e. following methodology similar to the one used in our study. More strict criteria were used that included specific serological assays to *Aspergillus* and the radiological finding of central

## Table 3. Clinical profile and criteria of the patients with allergic bronchopulmonary mycosis (ABPM)

<table>
<thead>
<tr>
<th>Initials</th>
<th>FL</th>
<th>FA</th>
<th>MSH</th>
<th>HM</th>
<th>HO</th>
<th>NSH</th>
<th>BGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>28</td>
<td>50</td>
<td>15</td>
<td>27</td>
<td>15</td>
<td>58</td>
<td>15</td>
</tr>
<tr>
<td>Presenting picture</td>
<td>Chronic mild to moderate asthma since childhood, <em>Aspergillus</em> sinusitis</td>
<td>Sputum casts, cough, recurrent atelectasis, recent asthma</td>
<td>Mild asthma since childhood, urticaria, dermatitis, rhinitis</td>
<td>Cough, wheeze, sputum, recent asthma</td>
<td>Mild asthma for 10 years, rhinitis</td>
<td>Severe asthma for 22 years, rhinitis</td>
<td>Mild to moderate asthma for 6 years</td>
</tr>
<tr>
<td>Residence</td>
<td>Jizan</td>
<td>Jizan</td>
<td>Najd</td>
<td>Najd</td>
<td>Najd</td>
<td>Najd</td>
<td>Sebia</td>
</tr>
<tr>
<td>Eosinophil count ($\mu l^{-1}$)</td>
<td>690</td>
<td>390</td>
<td>610</td>
<td>640</td>
<td>500</td>
<td>830</td>
<td>640</td>
</tr>
<tr>
<td>Chest radiograph</td>
<td>FS</td>
<td>FS</td>
<td>FS</td>
<td>FS</td>
<td>NS</td>
<td>NS</td>
<td>CB</td>
</tr>
<tr>
<td>CT scan of chest</td>
<td>CB, MI</td>
<td>NS</td>
<td>NS</td>
<td>CB, MI</td>
<td>NS</td>
<td>CB, MI</td>
<td>CB, MI</td>
</tr>
<tr>
<td>Immediate skin test</td>
<td><em>A. fumigatus</em></td>
<td>Mould II</td>
<td>Mould I</td>
<td>Mould I</td>
<td><em>A. fumigatus</em></td>
<td><em>A. fumigatus</em></td>
<td><em>A. fumigatus</em></td>
</tr>
<tr>
<td></td>
<td><em>A. niger</em></td>
<td><em>Mucor</em></td>
<td><em>Alternaria</em></td>
<td><em>Penicillium</em></td>
<td><em>A. niger</em></td>
<td><em>A. niger</em></td>
<td><em>A. niger</em></td>
</tr>
<tr>
<td>Total IgE (kU l$^{-1}$)</td>
<td>&gt; 2000</td>
<td>&gt; 2000</td>
<td>&gt; 2000</td>
<td>327</td>
<td>1132</td>
<td>&gt; 2000</td>
<td>1020</td>
</tr>
<tr>
<td>Specific IgE (kU l$^{-1}$)</td>
<td>All</td>
<td>+ for all except <em>Mucor</em>, <em>Penicillium</em></td>
<td>+ for all except <em>Mucor</em></td>
<td>Neg.</td>
<td><em>Rhizopus, mucor</em></td>
<td>+ for all</td>
<td><em>A. fumigatus</em></td>
</tr>
<tr>
<td>Smear/hyphae Culture</td>
<td><em>A. niger</em></td>
<td><em>A. terreus</em></td>
<td><em>A. fumigatus</em></td>
<td>Neg.</td>
<td><em>A. niger</em></td>
<td><em>C. albicans</em></td>
<td><em>A. niger</em></td>
</tr>
<tr>
<td></td>
<td><em>C. albicans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as percentage of predicted value.

Jizan and Sebia: south-west costal Saudi Arabia; Najd: central Arabia.

## Table 4. Spirometric results

<table>
<thead>
<tr>
<th>Spirometry</th>
<th>Skin prick test parameter*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ %</td>
<td>Positive (n = 46)</td>
</tr>
<tr>
<td>72.4</td>
<td>79</td>
</tr>
<tr>
<td>FVC %</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as percentage of predicted value.
bronchiectasis. In the first, Greenberger and Patterson reported a prevalence of 6% (11). Surprisingly, a much higher rate of 28% was found by Schwartz and Greenberger in the second survey in Cleveland, Ohio, U.S.A. (12). They attributed this to the more rigorous and diligent testing they followed. These compare to 11% in our survey (i.e. seven out of 62 of our patients with positive skin tests).

Differences in methodology and definitions make comparison between these studies difficult. Recent surveys have taken advantage of the more specific and sensitive serological and radiological testing allowing more accuracy in the diagnosis. The results of our survey, which adopted methodology similar to the U.S. surveys, suggest that the disease is not uncommon in central Arabia. It is important, therefore, to promote physicians' awareness to ABPM.

More obstacles follow when trying to establish the diagnosis. A number of criteria are required and these may not be all present at the point of the assessment during a 'quiescent' phase (5). Moreover, ABPM activity may not be evident symptomatically (5). Therefore we think that the true period prevalence rate is underestimated. Vigilance is needed when following patients especially those known to have some positive criteria (three or four). Another difficulty is the acquisition of the specific serological tests and the proper antigen for skin testing. Most of the available serological tests and skin extracts are specified for *A. fumigatus*, the species implicated in the classical descriptions of the disease. By contrast, *A. niger* was more frequently isolated in our study. Misdiagnosis may occur if the proper antigens are not used, even though some cross-reactivity exists. We used extracts made from species that were shown to be prevalent locally. Physicians in remote and less privileged hospitals may experience difficulties in obtaining resources and expertise to facilitate the diagnosis. Pitfalls that can lead to misdiagnosis were discussed in detail by Schwartz and Greenberger (12).

It is interesting to notice that far more females were sensitized to fungal allergens, and also more had ABPM. Women in the reproductive years are thought to have higher rates of skin test reactivity than men (16). This age-specific sex difference is evident in this study as most of the patients fell within this age range. Previous studies have shown conflicting results. ABPM was more frequent in females in the series reported by Donnelly *et al.* from Ireland (10) and from a study of cystic fibrosis patients in U.S.A. (17). However, one retrospective British series had more males (18). Another noteworthy observation is that patients with positive skin tests had poorer lung function than those with negative tests, confirming the results of the Cleveland–London study (1). There were significantly more patients with positive skin test on inhaled steroids, which also supports the observation that this group had more severe asthma. Smoking was too uncommon among our patients to account for such a difference.

As mentioned above, although *A. fumigatus* received more emphasis in previous studies as having a more pathogenic role, *A. niger* was more predominant in this study. This may be explained by the higher prevalence of this fungus in the warm and arid climate. Isolation of this fungus in some of the cases may be merely the result of contamination. In our series *Candida* spp. is probably implicated in two patients. Some cases may be due to other fungi, as evident by the high frequency of positive skin tests and specific IgE to the panel of fungal allergens tested. ABPM due to these fungi could not be proven because the specific serological tests were not available commercially. Physicians should be alert to this possibility when the usual tests (such as eosinophilia, elevated serum IgE, and radiological signs) are positive but tests to *Aspergillus* or *Candida* are negative.

In conclusion, ABPM is not uncommon in this study from a university hospital in the central part of Saudi Arabia. Since asthma is a common disease, clinicians need to maintain a high index of suspicion for ABPM and keep in mind its diverse manifestations. Advances in the more specific serological and radiological investigations should facilitate the diagnosis, but such tests should include a wide range of the locally prevalent fungal species. Studies on the prevalence in other areas of Arabia would be interesting, particularly the south-western coastal areas.

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