Indoor Aeroallergens in Asthmatic Pediatric Population in Annaba (Algeria)

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ABSTRACT

The aim of the present study is to highlight the kind of indoor aeroallergens and assess their role in asthma severity in children from Annaba city (Algeria). Our study concerned 68 asthmatic children and 50 healthy children. All children's blood samples were obtained to determine biological parameters, followed by home visit to these children to assess meteorological parameters and fungal exposure.

It was found that the asthma severity was associated with eosinophilia ($\chi^2$, $p = 0.01$), rate of total IgE ($\chi^2$, $p < 0.001$) and sensitization to indoor aeroallergens. This association between the severity of the disease and sensitization was strengthened with the presence of mites ($\chi^2$, $p < 0.01$) and pets, namely cats ($p < 0.001$). Moreover, all houses of asthmatic children contain fungal spores, which were also associated with the sensitization to fungi ($\chi^2$, $p < 0.001$). The average indoor temperature and humidity in the houses of asthmatic children represent favorable conditions for the growth of mold and development of mites, whose presence, especially Cladosporium and Alternaria, was associated with sensitization to fungi and severity of asthma. The strongest sensitization was found with dust mites (Dermatophagoides pteronyssinus, Der p1 (d1) and Dermatophagoides farinae, Der f2 (d2)) which is closely associated with asthma severity.

Keywords: Annaba (Algeria); Asthma; Indoor aeroallergens; Children; Sensitization.

INTRODUCTION

Asthma is a chronic respiratory disease affecting around 5–16% people worldwide (Akinbami et al., 2012), and its prevalence is in perpetual growth, especially in children. This percentage is rising due to demographic growth and lifestyle changes. It is well known that genetic factors as well as the presence of allergens in the environment are the main causes for triggering asthma in children. Nowadays, we spend 80–95% of our lives in indoor environments and breathe approximately 10 m$^3$ of air per day (Dacarro et al., 2003) containing a large number of fungal spores. So, the indoor air quality plays a key role in the development of several diseases of the airway tract especially asthma. Literature reviews suggest that children living in damp houses, houses with mold growth, or both are more likely to experience lower respiratory tract symptoms of cough and wheeze than children who do not (Fisk et al., 2007).

In Algeria, asthma prevalence rate is 3.4% (Bourdin et al., 2009) representing the most frequent chronic illness encountered in children (Anane and Boukari, 2001). In fact, a recent study conducted in the city of Annaba on 286 (asthmatic and non-asthmatic) schoolchildren aged 10–12 years has demonstrated that the characteristics of a high bronchial hyperresponsiveness could be facilitated by the polluted environment of the city (Benarab-Boucherit et al., 2011). Furthermore, in the clinical-biological study published by Boumendjel in 2012, exposure to airborne allergens was approached via the data of a questionnaire, without the visit and exploration of the patient's home environment. Despite these findings, the association of asthma symptoms and exposure to indoor fungi is less clearly established in Algeria.

The purpose of this study is to identify and count the genera of fungi present in the houses of asthmatic pediatric population in Annaba city (Algeria). Firstly, we conducted a general description of the study population through a questionnaire, then evaluated the total and specific IgE and eosinophilia to find the association with clinical parameters.
Secondly, we examined the relation between the exposure to indoor fungi in air and dust with asthma severity.

**MATERIALS AND METHODS**

**Study Population**

The study was conducted in Annaba city (extreme East of Algeria). Participants were recruited between March 2011 and January 2012. All patients who presented themselves to pediatric service were potentially recruited. A letter of convocation was given to them in reporting to medical analysis laboratory. However, in the end, only patients who came to the blood sampling were retained. Therefore, our study concerned 68 asthmatic children aged 2 to 15 years old (sex ratio M/F 1.72). A total of 50 healthy children with no history of asthma aged 1 to 15 years (sex ratio M/F 1.5) were also included in the study. During the consultation with their doctor (pediatricians and lung specialists), each child’s parent answered a questionnaire regarding health outcomes, history of the disease, home characteristics, environmental exposure, presence of animals and plants at home, and characteristics of the bedroom of the child. Permission to collect blood and home visit to collect indoor air and dust simples was obtained from the children’s parents who provided written informed consent.

This study protocol was supported through a national research project whose submission was approved by the ethics committee of the Agency of Thematic Research Sciences Health (ATRSS) of the Algerian Ministry of Higher Education and Scientific Research.

**Determination of Clinical Scores**

Asthma was defined and classified according to the Global Initiative for asthma (GINA) guidelines. Global Initiative for Asthma (www.ginasthma.org), which takes into consideration asthma severity with values ranging from 1 to 4. These classifications were obtained from the questionnaires.

**Blood Sampling and Immunobiological Analysis**

Blood samples were obtained from children on an empty stomach, and each blood sample was collected in two tubes: EDTA tube to determine leukocyte formula and heparin tube to analyze the total and specific IgE. All samples were centrifuged at 1200 g for 5 minutes.

**Determination of the Total IgE Concentration**

The serum levels of the total IgE were determined by the electrochemiluminescence method (Elecsys, 2010). The analysis is based on the sandwich technique and enzyme labeling of the antigen-antibody complex. The values considered as normal are the following: 0.01–60 U l\(^{-1}\) (from 1 to 5 years old); 0.01–90 U l\(^{-1}\) (from 6 to 9 years old); 0.01–200 U l\(^{-1}\) (from 10 to 15 years old); 0.01–100 U l\(^{-1}\) (over 15 years old).

**Determination of Specific IgE Concentration**

We carried out a semi quantitative test using Euroline kit (Euroline, 2011) to detect the serum concentration of specific IgE against the most aeroallergens common in the Mediterranean countries and involved in allergies. The kit contains 16 strips, on which the allergens are coated in a parallel manner are: mites (Dermatophagoides pteronyssinus, Der p1 (d1); Dermatophagoides farinae, Der f2 (d2) and Acarus siro), animal hair (cat: e1 and dog: e2) and molds (Cladosporium herbarum: m2 and Alternaria alternata: m6). Firstly, these strips were humidified and incubated with the patient’s serum. During the first incubation the specific IgE present in the serum binds with the allergens. A second incubation was carried out thanks to a human antibody anti-IgE coupled with an enzyme, catalyzing a colored solution in which the substrate is transformed into a chromogen product. The IgEs values greater than 0.35 kU L\(^{-1}\) were regarded as positive.

**Determination of Eosinophilia**

The blood samples which were collected on EDTA tubes were used to determine the leukocyte formula. A smear test had to be prepared and fixed with methanol for 2 to 3 minutes then was air-dried and stained with May–Grunwald-Giemsa for the differential count of macrophages, lymphocytes, eosinophils, monocytes and neutrophiles. Eosinophilia is expressed as an absolute value (number of elements per µL), after carrying out a globular enumeration using a counter. We consider 500 elements µL\(^{-1}\) as the threshold value (Gotlib, 2011).

**Home Visit and Sampling of Air and Dust**

Home visit was conducted between May and October 2012. However, we were not able to get into all the houses to collect dust samples because the latter was assimilated by the participants in the dirty places, which created a non-collaborative behavior. Therefore, among 118 children who had the blood sampling, 44 of them accepted home visit for the sampling of air and dust.

The air samples were obtained by using a calibrated DUO SAS 360 Air Sampler (Surface Air System, International PBI, Milan, Italy). The instrument includes two heads that can work independently or together, in our study we used one head. Air was aspirated through a 219-hole sieve head plate and over a 90 mm Petri dish containing a culture medium. Aspiration was at a flow rate of 180 L min\(^{-1}\). The sampler was placed at a height of 0.6–1.5 m above the ground, so that air would be aspirated from within the children breathing zone, according to the standard ISO7726:1998. The sampler head plate was thoroughly swabbed with 70% ethanol between samples.

Dust samples were collected from the child’s bed by using a hand vacuum (Clatronic 700 watts). The samples were collected in hermetic bags and transported to the laboratory the same day. After each sampling, the vacuum bag was swabbed and reused for the next sampling. In the laboratory, dust samples were immediately sieved using a Stainless Steel Fine Mesh Sieve (425-lm) to remove large particles; the obtained samples were stored in hermetic bags until fungi identification. For mold culturing, 1 g of sample dust was powdered in a Petri dish to pass through the same mentioned sieve.
Identification of Fungal Spores

Petri dishes used containing Sabouraud cloramphenicol as a culture medium was chosen for the enumeration of culturable fungi (Miguel et al., 1996). The covered plates of both air and dust samples were incubated at room temperature for an average of 7 days. The most common genera of fungi were identified by low- and high-power microscopy. Colony characteristics, color, evidence of sporulation and general morphology of sporulating structures were examined at low power directly on the culture plate. Some genera can be recognized by using these characteristics alone. The results are expressed as CFU m\(^{-3}\) of air for air samples and CFU g\(^{-1}\) of dust for dust samples.

Measurement of Meteorological Parameters

Temperature (°C) and relative humidity (%) were recorded in each place during sampling time using pocket weather (G.H. Zeal, London, UK).

Statistical Analyses

The t-test of the two independent samples was used to study the differences between the two groups of children with regard to biological parameters (total and specific IgE) and fungi rates. The variation of eosinophilia between the two groups was evaluated by the chi-square test, which was also performed to evaluate the association between the severity of asthma and biological parameters (total and specific IgE, eosinophilia). In addition, this same test was used to evaluate the association between specific IgE and fungi. Other correlations were calculated between the total IgE and specific IgE as well as the fungi and meteorological parameters using the Pearson correlation. Finally, the association between asthma severity and fungi was performed by the Fisher exact test. The values of p < 0.05 were statistically significant.

RESULTS

Description of the Population

The characteristics of the studied population are presented in Table 1. Actually our study showed a male predominance; there were 43 male (63.23%) and 25 female (36.77%). Positive family history of atopic disease was recorded in 72.05% of asthmatic subjects, with 43% with a maternal atopy. The group of non-asthmatic children had also 14% of a family-disease history. The main atopic manifestation was allergic rhinitis, which is recorded in 73.52% of asthmatic children. According to the description of asthmatic population, we found that the majority of them had moderate asthma (33.82%) and mild asthma (29.41%), and a few of them (23.53%) had severe asthma.

Biological Parameters and Asthma Severity

All studied subjects (asthmatic children) had an elevated serum concentration of total IgE compared with control subjects (p < 0.001) (Table 2). The distribution of the IgE totals according to the different degrees of asthma demonstrated that the subjects who had an elevated asthma degree had a high rate of IgE totals (Fig. 1). Moreover, the statistical analysis showed that the rate of IgE totals was significantly associated with the severity of asthma ($\chi^2$, p < 0.001). An elevated eosinophilia was also observed, and the difference between the two groups was highly significant ($\chi^2$, p < 0.01). In addition, blood eosinophilia was found to be associated with asthma severity ($\chi^2$, p = 0.01).

The group of asthmatic children was divided into three age intervals: 2 to 5, 6 to 10 and 11 to 15 (Fig. 2). The percentage of children with a total IgE level above the normal value increased with age, going from 68.19% above the reference value at [2–5] years, reaching 86.36% at [6–10] years and peaking at 88.88% at [11–15] years. Similarly, the percentage of positive cases for eosinophilia was found to increase according to the age brackets as well.

Sensitization to Aeroallergens and Asthma Severity

All children taking part in our study were sensitized to at least one specific pneumallergen. The highest concentration found was that of mites (Table 2), indicating that 76.50% of asthmatic children were sensitized to mites. The statistic analysis demonstrated that the specific IgE to

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy children (n = 50)</th>
<th>Asthmatic children (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F (%)</td>
<td>30/20 (60.00)</td>
<td>43/25 (63.23)</td>
</tr>
<tr>
<td>Average of age (years)</td>
<td>8.57</td>
<td>7.09</td>
</tr>
<tr>
<td>Familial atopy n (%)</td>
<td>7 (14.00)</td>
<td>49 (72.05)</td>
</tr>
<tr>
<td>Allergic rhinitis n (%)</td>
<td>7 (14.00)</td>
<td>50 (73.52)</td>
</tr>
<tr>
<td>Food allergy n (%)</td>
<td>0 (0.00)</td>
<td>6 (8.82)</td>
</tr>
<tr>
<td>Allergic conjunctivitis n (%)</td>
<td>3 (6.00)</td>
<td>24 (35.3)</td>
</tr>
<tr>
<td>Eczema n (%)</td>
<td>0 (0.00)</td>
<td>16 (23.52)</td>
</tr>
<tr>
<td>Intermittent asthma n (%)</td>
<td>-</td>
<td>9 (13.24)</td>
</tr>
<tr>
<td>Mild asthma n (%)</td>
<td>-</td>
<td>20 (29.41)</td>
</tr>
<tr>
<td>Moderate asthma n (%)</td>
<td>-</td>
<td>23 (33.82)</td>
</tr>
<tr>
<td>Severe asthma n (%)</td>
<td>-</td>
<td>16 (23.53)</td>
</tr>
</tbody>
</table>

n (%) = size (percentage).
Table 2. Mean of biological parameters in asthmatic and healthy children.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy children (n = 50)</th>
<th>Asthmatic children (n = 68)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgE (UI mL⁻¹)</td>
<td>122.48 ± 14.36</td>
<td>1857.66 ± 564</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Eosinophilia (elements µL⁻¹)</td>
<td>450 ± 34.4</td>
<td>520 ± 0.08</td>
<td>&lt; 0.01b</td>
</tr>
<tr>
<td>Specific IgE to pneumallergen (kU L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Dermatophagoides pteronyssinus, Der p1 (d1)</td>
<td>1.93 ± 0.445</td>
<td>23.39 ± 2.4</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>*Dermatophagoides farinae Der f2 (d2)</td>
<td>1.75 ± 0.376</td>
<td>20.28 ± 1.87</td>
<td>0.002a</td>
</tr>
<tr>
<td>*Acarus siro</td>
<td>0.27 ± 0.035</td>
<td>7.24 ± 0.932</td>
<td>0.09</td>
</tr>
<tr>
<td>*Animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat e1</td>
<td>0.02 ± 0.033</td>
<td>1.10 ± 0.35</td>
<td>0.662</td>
</tr>
<tr>
<td>Dog e2</td>
<td>0.03 ± 0.016</td>
<td>0.68 ± 0.047</td>
<td>0.02a</td>
</tr>
<tr>
<td>*Fungi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Cladosporium herbarum m2</td>
<td>0.21 ± 0.02</td>
<td>3.36 ± 0.7</td>
<td>0.369</td>
</tr>
<tr>
<td>*Alternaria alternata m6</td>
<td>0.02 ± 0.017</td>
<td>0.45 ± 0.2</td>
<td>0.121</td>
</tr>
</tbody>
</table>

*a The statistical analysis used was the t student test for two independent samples.

*b The statistical analysis used was the Chi square test: the samples which had values upper than the threshold value of eosinophilia were considered as positive cases and the values which were lower than the threshold value were considered as negative cases.

**Fig. 1.** Variation of total IgE level according to the asthma severity.

*Dermatophagoides pteronyssinus, Der p1 (d1) and Dermatophagoides farinae, Der f2 (d2) was considerably higher for asthmatic population in comparison control subjects (p < 0.001 and p < 0.01 respectively). Yet, there was no difference for the specific IgE to *Acarus siro*. Thus, the statistical analysis have proven that the rate of each IgE specific to mites correlates with the that of the total IgE: (r = 0.50), (r = 0.47) and (r = 0.32), respectively. It was found that asthma severity was associated with mites’ sensitization (r², p < 0.01). Furthermore, sensitization to cat and dog was also found in our population with 20%.

The difference between specific IgE to cat for asthmatic children and healthy subjects was not significant. However, sensitization to dog was significant among the two groups (p < 0.05). Sensitization to pets was also associated with the severity of asthma, which is highly significant with cats (χ², p = 0.001). Yet, there was no association between asthma severity and sensitization to fungi (χ², p > 0.05), despite the fact that the latter was recorded in 32.3% of asthmatic population.

**Home Environment and Fungi Exposure**

The average temperature was 30°C and 54% for the humidity in asthmatic children’s homes, whereas for healthy children’s homes, the average indoor temperature was 28°C and 49% for humidity.

On the basis of the questionnaire, we found that 69% of asthmatic children lived in the urban region of Annaba city and 89% of that same group had a visible mold in their homes.

Indoor fungal concentrations were available for 28 of 68 asthmatic children (40%) and for 16 of 50 healthy children.
Fig. 2. Percentage of positive cases of total IgE and eosinophilia levels according to the ranges of age.

(32%). Across the houses of asthmatic population, the culturable fungi most commonly found in indoor air and dust samples were *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor*, *Paecilomyces*, *Aureobasidium*, *Scytalidium*, *Fusarium* and *Rhodotorula*. Whereas some genera were more abundant in indoor air compared to dust samples (Table 3). The homes of healthy children were found to contain a similar spectrum of fungal genera, although some genera were absent.

All fungi found both in indoor air and dust in the houses of asthmatic children were more than those found in the houses of children without asthma. This increase was statistically significant ($p < 0.05$) for *Cladosporium* in air samples and *Penicillium* in dust samples.

The correlation between meteorological parameters and fungi rate have been investigated. Pearson test has revealed the existence of a significant correlation between the number of CFU m$^\text{-3}$ and the environmental indoor temperature ($p = 0.03$), especially the genus *Penicillium* where the correlation was highly significant ($p = 0.004$). Similarly, there are highly significant correlations between the humidity and *Aspergillus* genera ($p = 0.003$) and *Mucor* ($p = 0.008$).

Sensitization to fungi was strongly associated with the presence of fungi in indoor air ($\chi^2$, $p < 0.001$). A significant increase both of *Cladosporium* and of *Penicillium* in indoor air and dust samples according to asthma severity reached its highest rate in children with moderate and severe asthma (Fig. 3).

**DISCUSSION**

According to the description of asthmatic population (Table 1), it appears that 33.82% of them had a moderate asthma. Boumendjel et al. (2010) have also shown that most of asthmatic children had moderate asthma with 46%. These findings have shown that the majority of asthmatic population were at high risk to move up to the higher grade of the disease (severe asthma) and suffer from a lack of treatment or an excessive exposure to trigger factors.

Although the male predominance is often linked to the risk of asthma, as reported by various authors as Boumendjel et al. (2010) on pediatric population in Annaba, in our case, it is found in both asthmatic and healthy children. Thus, this male predominance could be simply the reflection of the structure of the selected population.

However, a family history of atopy is the most clearly defined risk factor for asthma in children. Indeed, 72.05% of asthmatic children were found to have a family history of atopy. A previous study in Annaba (Boumendjel et al., 2012) has shown a close percentage for familial atopy with 74%. Among these atopic children, 43% had a maternal atopy which also represents an important risk factor for the childhood onset of asthma and for recurrent wheezing that persists throughout childhood (Castro-Rodriguez et al., 2000).

Atopy is used to describe the genetic predisposition of humans to produce specific antibodies (IgE) against common allergens in food, indoor and outdoor environment (Fernando and Donata, 2013). That’s why the determination of the serum concentration of total and specific IgE antibodies plays a major role in the diagnosis of childhood asthma. Our results have shown that the serum concentration of the total IgE of asthmatic children is highly significant ($p < 0.001$) in comparison with healthy children (Table 2). The statistical analysis have also demonstrated that asthma severity is associated with the rate of the total IgE (Fig. 1). A recent study has reported a positive correlation between the total IgE and higher asthma severity in children under the age of 16 (Boumendjel et al., 2010). Asthma severity is also correlated with blood eosinophilia (Rothenberg and Mehogan, 2006). Moreover, in our results, an association between severity of asthma and eosinophilia has clearly been established. The percentage of positive cases of both total IgE and blood eosinophilia increases with the age (Fig. 2).

The severity of the disease, was proven clinically and through blood markers as associated with many allergic symptoms (Meharzi et al., 2015). So, atopic disease and an allergenic environment were strongly present in the pediatric population studied.
Table 3. The main genera found in indoor air and dust samples from homes of study subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Homes of healthy children n = 16 *</th>
<th>Homes of asthmatic children n = 28 *</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indoor air fungi (average CFU m⁻³)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cladosporium</td>
<td>20.40 ± 1.36</td>
<td>41.50 ± 4.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Penicillium</td>
<td>5.60 ± 2.19</td>
<td>19.14 ± 5.9</td>
<td>0.08</td>
</tr>
<tr>
<td>Alternaria</td>
<td>-</td>
<td>5.00 ± 2.58</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>-</td>
<td>32.50 ± 2.082</td>
<td>-</td>
</tr>
<tr>
<td>Aureobasidium</td>
<td>-</td>
<td>88.00 ± 3.32</td>
<td>-</td>
</tr>
<tr>
<td>Mucor</td>
<td>-</td>
<td>3.00 ± 1.19</td>
<td>-</td>
</tr>
<tr>
<td>Scytalidium</td>
<td>-</td>
<td>8.00 ± 1.51</td>
<td>-</td>
</tr>
<tr>
<td>Paecilomyces</td>
<td>-</td>
<td>4.00 ± 1.04</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium</td>
<td>2.00 ± 1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhodotorula (yeast)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Indoor dust fungi (average CFU g⁻¹ dust)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cladosporium</td>
<td>16.00 ± 2.19</td>
<td>14.21 ± 2.77</td>
<td>0.914</td>
</tr>
<tr>
<td>Penicillium</td>
<td>0.80 ± 0.178</td>
<td>13.80 ± 8.74</td>
<td>0.04</td>
</tr>
<tr>
<td>Alternaria</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>2.00 ± 0.45</td>
<td>1.22 ± 0.312</td>
<td>0.729</td>
</tr>
<tr>
<td>Aureobasidium</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucor</td>
<td>-</td>
<td>32.50 ± 8.53</td>
<td>-</td>
</tr>
<tr>
<td>Scytalidium</td>
<td>-</td>
<td>6.00 ± 1.13</td>
<td>-</td>
</tr>
<tr>
<td>Paecilomyces</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium</td>
<td>2.00 ± 0.273</td>
<td>0.50 ± 0.00</td>
<td>-</td>
</tr>
<tr>
<td>Rhodotorula (yeast)</td>
<td>-</td>
<td>31.00 ± 0.00</td>
<td>-</td>
</tr>
</tbody>
</table>

* Air and dust sampling was inadvertently omitted for the rest of the population. 

p values were obtained by using the independent two sample t test.

The literature reports that aeroallergens sensitization, including pets, is common, especially in people with asthma (Kim et al., 2013). In our study, it was recorded throughout our asthmatic population, with 29.4% of the asthmatic children sensitized to pets (dogs and cats). A previous study (Sharif et al., 2004) has also estimated that Pets causes allergy symptoms in 20 to 30 percent of asthmatic patients. Furthermore, literature has demonstrated that asthma severity was associated with sensitization to cat hair (Melén et al., 2001). In contrast, there was no significant difference between children with asthma and those without, as regards cat sensitization, while there was a significant increase sensitization to dog. This result seems to reflect the fact that in our society the dog does not belong to the indoor pets. Whereas does, environmental variables, such as temperature and moisture, can influence growth and reproduction in fungi and airborne spore concentrations, as it is well known that fungal concentrations are significantly correlated with meteorological parameters (Abdel Hameed et al., 2012). Thus, our study has shown that the average indoor temperature was approximately 30°C and 54% for humidity in the houses of asthmatic children, which represents favorable conditions for mold growth in indoor environments. Additionally, our results have clearly indicated that there is a strong correlation between the number of CFU m⁻³ and temperature indoor environments, in particular, genre members, as cited in the literature (Pakpour et al., 2015; Lal et al., 2017). We have also demonstrated that there are highly significant correlations between some fungi (including Aspergillus and Mucor) and humidity recorded in the visited houses because it is well known that fungi generally require moisture to grow and sporulate.

Our data (Table 3) thus show that the most common genera in indoor air are Cladosporium, Penicillium and Aspergillus, which is indicated by our previous report in Annaba (Meharzi et al., 2013). The same types were also found in the houses of healthy children at lower rates. According to a Taiwanese study (Hsu et al., 2012), Fusarium is mostly originates outdoors, but furrowing activity may have caused adsorbed microorganisms in soil and plants to be released into the outdoor air, which may explain the rather high levels of this fungal type in indoor environment.

Furthermore, the concentration of fungi in air and dust were more abundant in the samples from asthmatic children than those from the healthy ones. This result indicates that the indoor environment of the former contains an important aeroallergens source which could possibly be in favor of a significant worsening of their health. The difference between the two groups was not the result of seasonal variations, because, we think, in this study, they are small because sampling occurs in the same estival period which (in Algeria) lasts from late May to the middle of October. Nonetheless, seasonal differences in Mediterranean zone are not sufficiently documented in the literature.

Indeed, the exposure to these fungi has been associated with allergic and respiratory symptoms (Mendell et al., 2011). Our findings have shown that children who had the higher grade of asthma were exposed to high levels of fungi both in indoor air and dust (Fig. 3). This might be an
Fig. 3. Variation of fungi content both in indoor air (A) and indoor dust (B) according to asthma severity in comparison with healthy children.

origin for the development and exacerbation of asthma (Tischer and Heinrich, 2012). Thus, the determination of IgEs revealed that asthmatic children were also sensitized to fungi (Table 2). Indeed, 32.3% of them were sensitized both to Cladosporium and Alternaria. In addition, our study has demonstrated that the indoor fungi were associated with sensitization to fungi of both Cladosporium and Alternaria. sensitization fungi, particularly Alternaria alternata, have been linked to the presence, persistence and severity of asthma. This result is in accordance with those found by many previous studies (Khosravi et al., 2009; Baxi et al., 2013).

In the present study, the strongest sensitization was found with mites. Indeed, temperature and humidity also favor the development of mites indoor homes of asthmatic children. More than 50% of asthmatic children were sensitized to mites.

In addition, the statistical analysis have shown that the level of specific IgE to mites in asthmatic children was highly significant for Dermatophagoides pteronyssinus, Der p1 (d1) ($p < 0.001$) and Dermatophagoides farinae, Der f2 (d2) ($p < 0.01$) in comparison with healthy children, but not significant for Acarus siro.

Several studies have found positive associations between asthma and sensitization to dust mite (Gent et al., 2012), which was similarly found in the present research work with a highly significant value ($p < 0.01$). Mite allergens are also known to play an important role in inducing IgE-mediated sensitization and the development of bronchial hyperresponsiveness (BHR) and asthma (Glasgow et al., 2011). Moreover, it would be useful to take this research further by determining the rate of indoor mites and their main species in the houses of asthmatic children. Hence, we suggest that future studies need to be conducted to see the contributing role of other air pollutants at Annaba city. Indeed, the indoor environment also reflects the outdoor air quality and pollution. In fact, meteorological conditions are already favorable to increasing allergic diseases. Indeed, in Annaba, both the very high level of humidity (anonymous, 2004) and the average temperature contribute to the development of many indoor aeroallergens such as the cockroaches, mites and moulds. Moreover, the huge
consumption of recreational drugs through tobacco smoke provides important burdens of nicotine as harmful polar compounds in the air. In addition, Annaba is a dusty town, unventilated and highly industrialized (Khedairia and Khadir, 2012). These characteristics provide the gaseous and particulate pollution (such as carbon monoxide, ozone and sulfur dioxide) which is involved in intensifying allergic reactions and may explain the increasing prevalence of allergic respiratory diseases in this urban area. Indeed, such ambient fine and ultrafine particles are harmful, especially in infants and young children (Moya et al., 2004), and act as adjuvants in the course of allergic inflammation due to the sensitization by allergens. They penetrate deep into the lungs causing worsening of bronchial inflammatory response (mediated by specific IgE to aeroallergens), increased the incidence of acute respiratory illness and acute asthma exacerbations (Terzano et al., 2010).

CONCLUSION

In this study, carried out for the first time at Annaba, our results have demonstrated that most of the asthmatic children in our population were at the high grade of the disease. Additionally, the meteorological parameters in their houses favor the development of mites and fungi, which were indeed strongly present in all homes. Their presence, especially Cladosporium and Alternaria, were associated with sensitization to fungi and severity of asthma. The strongest sensitization was found with dust mites (Dermatophagoides pteronyssinus, Der p1 (d1) and Dermatophagoides farinae. Der f2 (d2)) which have a positive associations with asthma severity.

This high exposure to indoor pollutants, which is associated with the development of sensitization and severity of asthma, suggest that the reductions in the levels of indoor allergens would reduce the severity of disease and even morbidity in atopic asthmatic children at Annaba. Hence, in our opinion, the need to develop the profession of indoor environment medical consultant, which is almost nonexistent in Algeria has become urgent.

ACKNOWLEDGEMENTS

The authors would like to thank the DGSRTD (Directorate General for Scientific Research and Technological Development) of the Algerian Ministry of Higher Education and Scientific Research for the financial support of this research (PNR supervised by BOUMENDJEL Amel : Evaluation of biocontamination in indoor environments of patients with allergic respiratory diseases IgE-mediated. Health Impacts). They also wish to extend their thanks to Mrs. Leila MAHFOUDHI, an English teacher at the Sfax Faculty of Science, for having proofread and polished the language of this manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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*Received for review, June 1, 2016 Revised, July 14, 2017 Accepted, July 22, 2017*