Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece

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Introduction

Epidemiological surveys support that asthma affects 5-30% of children and 2-30% of adults [1, 2]. This wide range of inter-survey fluctuation is mainly due to the different geographic distribution and to the use of different criteria among studies. Researchers characterize it as “a chronic allergic disease of airways” [3]. Rhinitis affects 40% of the population.

The term respiratory allergy refers to clinical manifestations of both upper and lower respiratory tract due to allergy and is extensively used by scientists worldwide.

It is well established that inhalation of fungi spores induces respiratory allergy symptoms in sensitized individuals; among all patients suffering from respiratory allergy symptoms, 20-30% of them are sensitized to fungi spores [4]. These symptoms are clinically manifested as
rhinitis, bronchial asthma [4, 5, 6-10] and alveolitis [6, 9]. Many authors correlate the onset of respiratory allergy symptoms with the presence of fungi spores in the atmosphere [5, 6, 11-13]. A lot of different species of fungi spores disperse in the atmosphere, which are characteristic of each region. Fungi spores involved in allergic diseases belong to 80 genera, the most significant of which belong primarily to Ascomycetes and Deuteromycetes and, secondly, to Basidiomycetes [4-6, 9]. They are either specialized single cells or, more often, composed of branching threads, termed hyphae; they are responsible for the reproduction and growth of fungi [5-7, 9]. Fungi spore antigenicity appears to significantly differ from that of mother cultures [4-6, 9]. The recording and forecasting of fungi spore dispersion in the atmosphere attract the interest of most scientists and specialists, since they form the basis of diagnosis and treatment of allergic diseases caused by fungi spores [6, 7, 9, 11, 14, 15].

Aim of the study

Until today, no long-term records of allergenic fungi spore circulation in the atmosphere has been conducted in Greece, so as to draw useful conclusions for the protection of relevant allergic diseases. There is, however, a study of ours published in 1996 [16], referring to 4-year records in the area of Thessaloniki. We therefore decided to continue this research and create a database including long-term records of allergenic fungi spores performed on a daily basis in the city of Thessaloniki (Fungi Spore Calendar). It was considered necessary to simultaneously detect allergic patients sensitivity to the fungi spores most commonly involved in respiratory allergy symptoms in the region of Thessaloniki [5-8, 10].

Study area

Thessaloniki is an old city that celebrated its 2300-year anniversary of foundation in 1984. The city is built in the heart of Thermaikos Gulf in the Aegean Sea and its geographical location is 40° 37’N and 22° 57’E. Nowadays, Thessaloniki is an industrialized modern city of 1.1 million inhabitants; it is the second biggest city in Greece, the cultural and economic center of Macedonia and northern Greece and an important seaport.

In the north-east of Thessaloniki there is a large forest of pines, cypresses, cedars and oaks, called “Kedrinos Lofos”. In the western side, where the industrial zone is well developed, the city is in contact with extensive fields of cultivated cereals. The eastern part of the city is close to grass areas and olive trees, neighbouring the peninsula of Chalkidiki. To the south the city borders the Mediterranean Sea.

Material and methods

The study design included:

A. Daily records and identification of more than 40 fungi spore species belonging to different families. Selection of the 15 most significant for presentation, as far as their allergenicity is concerned.

B. Creation and presentation of the Fungi Spore Calendar for the 15 most allergenic fungi spore species in the area of Thessaloniki.

C. Detection of skin sensitivity (allergy) to fungi spores in asthmatic population in our region, simultaneously with the records of fungi spores.

A. Records, identification and presentation of fungi spores in the air of Thessaloniki.

This study began in September 1986, when the Burkard volumetric spore trap was installed in the center of Thessaloniki. Daily sampling of airborne fungi spores started on February 1st, 1987 and continues until today. In this study, 15-year records are presented, a period long enough for the creation of Fungi spore Calendars in an area [17].

The records of fungi spores were conducted using a 7-day recording Burkard volumetric spore trap, standard equipment for aerobiological sampling worldwide [5-7, 9, 17-18]. A Burkard volumetric trap was placed on the roof of a six-floor building in the center of Thessaloniki and at a height of 25 m above the ground, measuring concentrations of pollen grains in the ambient air throughout the study [7, 18]. Fungi spore counts are expressed as fungi spores per cubic meter of air [6-7, 18].

The aeroallergens selected for presentation comprise the most commonly implicated fungi spores to respiratory allergy symptoms both in European countries and worldwide [5-6, 9, 12, 19]. These are: Agrocybe spp., Alternaria spp., Ascospores, Botrytis spp., Cladosporium spp., Drechslera/Helminthosporium spp., Epicoccum spp., Fusarium spp., Leptosphaeria spp., Nigrospora spp., Phoma spp., Pleospora spp., Stempylhum spp., Torula spp. and Ustilago spp.

B. Creation and presentation of the Fungi Spore Calendar.

Data of fungi spore records were inserted in an automated computer worksheet and processed with Microsoft Excel for Windows [17, 20]. The Fungi Spore Calendar was created according to worldwide-established methodology [17]. Fungi spores were expressed in daily annual values and then, in our case, as summed average values per 10 days for the 15-year period (1987-2001). They were presented on a particular scale, each level corresponding to a specific total amount of spores. In this way, all interactions between external factors and fungi spore concentrations were significantly decreased, therefore enabling us to compare different fungi spore species concentrations throughout the year.
C. Detection of skin allergic sensitization to fungi spore extracts in asthmatic patients.

Skin sensitivity to fungi spores was detected in a sample of 1311 asthmatics admitted to the OutPatients Clinic for Asthma of the Pulmonary Department of Aristotle University of Thessaloniki in the General Hospital “G. Papanikolaou” from 1/1/90 until the end of 2001. The patients included in the study had to present annual periodicity, seasonal occurrence of symptoms and/or worsening of respiratory allergy symptoms. The examination included:

a) Filling of a questionnaire of 200 questions specific for asthma. The aim was to examine the relation between the skin allergic sensitization (skin prick tests) and the onset of clinical symptoms.

b) Clinical examination.

c) Spirometry and, in some cases, bronchial challenge test with metacholine or histamine.

d) Chest x-rays film.

e) Specific IgE (detected only in some cases).

f) Skin tests. Skin tests were performed by a modified skin prick technique [21- 25]. The most common inhalant and food allergens were used, counting up to a total 55 extracts, among which 5 fungi spore extracts. The selection of fungi spore species was made according to expected sensitization, as documented by past studies made in our country, Europe and the U.S.A. [5-7, 9, 12, 19, 23, 26, 27]. The allergens were either isolated or in groups. All the allergens were produced by Allergofarma®, Germany. The fungi spore extracts used were: *Alternaria* spp., *Cladosporium* spp., *Aspergillus* spp., *Fusarium* spp. and *Rhizopus* spp. Skin positivity was recorded 15 minutes after the test and the occurring wheal and flare reaction was

Figure 1. Fungi Spore Calendar of 15 fungi spore species in summed average values per 10 days for the 15-year period of records (1987-2001) in the area of Thessaloniki. The scale with the particular levels of total amount of spores is presented.
impressed on a transparent cello tape. Positivity was expressed in wheal diameter > 3 mm and flare diameter > 10 mm compared to negative and positive controls [21-25].

Results

A. Aerobiological records of fungi spores
(Fungi spore records are presented in Table 1)

More than 40 fungi spore species were recorded and identified during the 15-year period sampling, however, the 15 of them, which are the most common implicated in respiratory allergy symptoms worldwide, are presented in this study. The airborne fungi spore concentrations in the area of Thessaloniki during the 15-year period 1987-2001 are presented in Table 1.

B. Fungi Spore Calendar

In Figure 1, the Fungi Spore Calendar including all recorded aeroallergens is displayed.

C. Detection of skin allergic sensitization to fungi spores.
(Patient sensitization to fungi spores is presented in Table 2)

The sample of asthmatics examined for allergic sensitization to fungi spores consisted of 1744 patients suffering from respiratory allergy and displaying clinical symptoms mostly during May-October. Their average age was about 35 years old (6-68 years old) and average duration of asthma was 23 months (3 months to 10 years). Skin positivity to one or more allergen extracts of the 55 tested occurred in 1311 (75.2%) patients, while 433 patients (24.8%) did not react to the skin tests at all. About 421 (32%) out of the 1311 patients reacted positively to at least one fungus spore extract, from whom 172 (40.9%) were women, whereas 249 (59.1%) were men. On the contrary, regarding the 433 patients having presented negativity to skin prick tests, 260 (60%) were women and 173 (40%) were men.

Among the above 1311 patients, 177 (13.5%) presented positive skin allergic reaction to Alternaria spp., 98 (7.4%) to Cladosporium spp., 65 (5%) to Aspergillus spp., 45 (3.4%) to Fusarium spp. and 36 allergic patients (2.7%) displayed skin sensitivity to Rhizopus spp.

In Table 2, age and sex distribution of sensitized allergic patients is also presented for all fungi spore extracts.

Discussion

Not all components of the atmosphere present the same allergenic significance to humans. Pollen grains have been extensively studied as aeroallergens. On the contrary, allergy to airborne fungi spores has hardly been studied, as the cause of respiratory allergy symptoms. It must be mentioned that bronchial asthma incidents related to fungi spore allergy very often increase in summer. Moreover, sensitivity to some particular species, such as Alternaria spp., is one of the most severe

Table 1. Annual sum of fungi spore concentrations, their annual average values and their percentages (%) for each one of the 15 fungi spore species recorded in the area of Thessaloniki during 1987-2001

<table>
<thead>
<tr>
<th>Fungi Species</th>
<th>Sum</th>
<th>Average</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladosporium spp</td>
<td>1882560</td>
<td>125504</td>
<td>72.22%</td>
</tr>
<tr>
<td>Alternaria spp</td>
<td>255895</td>
<td>17060</td>
<td>9.82%</td>
</tr>
<tr>
<td>Ustilago spp</td>
<td>210970</td>
<td>14065</td>
<td>8.09%</td>
</tr>
<tr>
<td>Ascospores</td>
<td>70375</td>
<td>4692</td>
<td>2.70%</td>
</tr>
<tr>
<td>Drechslera/Helminthosporium spp</td>
<td>36190</td>
<td>2413</td>
<td>1.39%</td>
</tr>
<tr>
<td>Leptosphaeria spp</td>
<td>32175</td>
<td>2145</td>
<td>1.23%</td>
</tr>
<tr>
<td>Agrocybe spp</td>
<td>29925</td>
<td>1995</td>
<td>1.15%</td>
</tr>
<tr>
<td>Botryis spp</td>
<td>16770</td>
<td>1118</td>
<td>0.64%</td>
</tr>
<tr>
<td>Stemphylium spp</td>
<td>16750</td>
<td>1117</td>
<td>0.64%</td>
</tr>
<tr>
<td>Pleospora spp</td>
<td>15235</td>
<td>1016</td>
<td>0.58%</td>
</tr>
<tr>
<td>Nigrospora spp</td>
<td>14705</td>
<td>980</td>
<td>0.56%</td>
</tr>
<tr>
<td>Epicoccum spp</td>
<td>9770</td>
<td>651</td>
<td>0.37%</td>
</tr>
<tr>
<td>Fusarium spp</td>
<td>7640</td>
<td>509</td>
<td>0.29%</td>
</tr>
<tr>
<td>Torula spp</td>
<td>6170</td>
<td>411</td>
<td>0.24%</td>
</tr>
<tr>
<td>Phorma spp</td>
<td>1630</td>
<td>109</td>
<td>0.06%</td>
</tr>
</tbody>
</table>

**Total**                         **2606760** | **173784** |
forms of asthma, under conditions of high fungi spore concentrations in the atmosphere [11, 28, 29, 30, 31].

Allergy caused by fungi spores is necessarily relevant to regional vegetation and to the way and duration of exposure to each aeroallergen [5, 7, 8, 14, 20, 29]. Some fungi spore species may provoke respiratory allergy symptoms, as they enter the houses through air-conditioning equipment [19, 30, 32, 33].

For the first time in the area of Thessaloniki, and more generally in Greece, 15-year allergenic fungi spore records have been conducted. Allergenic fungi spores recorded in the area of Thessaloniki are usually present throughout the year (Figure 1). However, high concentrations of fungi spores appear in the atmosphere of the city at the beginning of March and spore circulation ends by the end of October (Figure 1). Similar results, concerning the very same fungi spore species, are also documented by more researchers in other areas, hence, with climatological conditions and prevailing meteorological factors very much like ours [14]. However, spore concentrations within the same species greatly differ, a fact due to the variety of the vegetation existing in each region [14, 35].

The exact numbers of fungi spores that are going to disperse in the air and the duration of their seasonal circulation in a specific region depend on the wind direction and speed, the presence of rain and its duration and relative humidity during each season [6, 9, 14, 19, 20].

As far as skin prick tests are concerned, they present a high diagnostic value, especially if combined with the patient’s medical history [2, 8, 36]. Positivity in skin tests is not usually observed in individuals lacking clinical symptoms [2, 24, 37]. The method of modified skin prick test proves to be a reliable tool for detection of type I sensitivity of the human body worldwide. Also, the results can be stored in transparent cello tapes, which enables us to compare the results of the test at a given time with those at different periods or with other patients’ tests, even from abroad [21, 22, 23, 37, 38]. The positivity of skin prick tests strongly correlates with bronchial hyper-responsiveness and other clinical aspects of bronchial asthma. Moreover, it is reported that skin prick tests are more reliable than detection of total and special IgE tests with Phadiatop [37]. In a study of D’Amato et al [8], European citizens were examined (among whom Greek patients were also tested by our research team); the relationship between skin prick test sensitivity to fungi spores and specific IgE was particularly investigated. As documented, increase in skin prick test sensitivity to fungi spores is not necessarily followed by a relevant increase in IgE; hence, this study refers only to fungi Alternaria and Cladosporium. In everyday medicine and asthma diseases, the problem of respiratory allergy caused by fungi spore inhalation is often confronted. Not many references exist in Greek literature about fungi spore allergy [8]. In a study, 32% of patients were discovered to present at least one positive skin prick test to fungi spores, a fact revealing the necessity of detection of sensitization to fungi spores in patients suffering from respiratory allergy. Recent Greek references document increase in sensitization to airborne fungi spores of Greek citizens [39, 40]; children, particularly, are greatly sensitive (19.5%) to Alternaria spp. [41]. Our data of skin prick test positivity are considered quite useful, since they derive from a sample of 1744 patients with respiratory allergy. They correspond to individuals from all over the county of Thessaloniki and were obtained during the same period of fungi spore aerobiological records. The data concern sensitization to the fungi spores most commonly involved in respiratory allergy symptoms worldwide [6-8, 11, 14-15, 18, 32, 34, 36, 38].

Sensitization provoked by Alternaria spp. spores counts up to 13.5%. It is worth mentioning that younger individuals suffer mostly from allergic diseases due to fungi spores (Table 2). Furthermore, men are more usually offended by respiratory allergy than women (Table 2), especially in the case of Alternaria spp. [11, 15, 31, 38, 42]. The frequent occurrence of skin positivity to Alternaria extracts is observed in other countries as well. In the USA, the allergenic factor most commonly reported as involved in respiratory allergy symptoms is the spores of the fungus species Alternaria. [5, 10, 11, 15, 31, 36], while in California it is the third most significant factor of occurrence of clinical symptoms, just after the pollen grains of grass and

Table 2. Patient sensitization to 5 fungi spores, presented as percentages of the total 1311 patients with respiratory allergy examined. Sensitization distribution between both sexes is also presented

<table>
<thead>
<tr>
<th>Fungi Species</th>
<th>Number of patients</th>
<th>% patients</th>
<th>Men</th>
<th>%</th>
<th>Women</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria spp.</td>
<td>177</td>
<td>13.5%</td>
<td>112</td>
<td>63.3%</td>
<td>65</td>
<td>36.7%</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>98</td>
<td>7.4%</td>
<td>54</td>
<td>55.1%</td>
<td>44</td>
<td>44.9%</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>65</td>
<td>5.0%</td>
<td>41</td>
<td>63.1%</td>
<td>24</td>
<td>36.9%</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>45</td>
<td>3.4%</td>
<td>22</td>
<td>48.9%</td>
<td>23</td>
<td>51.1%</td>
</tr>
<tr>
<td>Rhizopus spp.</td>
<td>36</td>
<td>2.7%</td>
<td>20</td>
<td>55.6%</td>
<td>16</td>
<td>44.4%</td>
</tr>
</tbody>
</table>

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ragweed [43]. On the contrary, in the Netherlands, Alternaria's role as an aeroallergen proves to be of minimal importance [19], resulting from the low airborne concentrations of fungus spores.

As far as Cladosporium spp. is concerned, it is also considered as a major allergenic factor responsible for causing of respiratory allergy symptoms in our area, with a percentage of skin positivity in asthmatics of about 7.4%. The spores of Cladosporium spp. are the most abundant in the air of all countries worldwide [5, 6, 7, 9, 14, 19, 20, 34]; however, their allergenicity appears to be rather low [7, 23, 34, 36]. Therefore, although in Thessaloniki 72.2% of the total amount of fungus spores circulating in the air belong to Cladosporium spp. (Table 1), there was not a corresponding hypersensitivity in the asthmatics examined (Table 2).

Aspergillus spp. extract was included in the study of patient sensitization because it comprises a common indoor fungus spore species (house dust, flowerpots etc), but also because it is often involved in various bronchopulmonary diseases (bronchopulmonary aspergillosis, aspergilloma, alveolitis, etc.) [4, 6, 9]. Skin prick test sensitivity to Aspergillus spp. reached up to 5% in asthmatic individuals (Table 2). This percentage might have been expected to be higher, inasmuch as Aspergillus spores are very common in domestic environment. Unfortunately, their very small size does not permit us to record their concentrations in the ambient air with the methodology used [5, 6]. Similarly to Alternaria spp., skin positivity was more often observed in men than in women (Table 2).

The fungus species of Rhizopus spp. belongs to Zygomycetes and comprises the most common indoor allergenic fungus spore family, along with mucor species, altogether known as moulds [6, 7, 9]. Rhizopus spp. offends cultivations and stored agricultural products (e.g. potatoes, peanuts, cereals, peaches etc). Its frequent existence in domestic environment urged researchers to detect its sensitization in asthmatics [5, 6]. Likewise, Fusarium spp. is also growing and reproducing in agricultural products, such as potatoes, cotton, tomatoes, garbage, watermelons, bananas, etc. [5, 6, 12, 26]. Skin sensitization to the fungus spore extracts of Rhizopus spp. and Fusarium spp. were positive for 3.4% and 2.7% of asthmatic patients respectively, percentages not very high compared to those reported in other countries worldwide [6, 34].

Finally, in a study of ours in 1994 [44], we investigated the relationship between skin prick test sensitivity to fungus spores, specific IgE positivity and positive medical history concerning respiratory allergy symptoms occurring during the main circulation season of fungus spores. No positive correlation was found between skin prick test sensitivity and specific IgE sensitivity. Particularly, Alternaria presented skin prick test positivity in 20 out of 21 patients, whereas specific IgE was found positive only in 15 patients. In contrast, Aspergillus presented skin prick test positivity in 8 out 21 patients, whereas specific IgE was found positive in 17 patients. It is worth mentioning that specific IgE generally revealed a low level of reaction. It is therefore concluded that there is no certain relation between skin prick test sensitivity and specific IgE sensitivity, a fact possibly due to the lack of completely purified fungi materials used in both techniques.

Conclusions

Fifteen allergenic fungi spore species were recorded in the area of Thessaloniki for the last 15 years (1987-2001) and are presented for the first time in a Fungi Spore Calendar, a fact very important for our city and country, both from the medical and biological viewpoint.

Sensitization (allergic reactions) to 5 fungi spore species was detected in patients suffering from respiratory allergy symptoms in the region of Thessaloniki. Clinically significant are mostly the fungi spores of Alternaria and Cladosporium, as evidenced both by great skin prick test positivity and by their abundant concentrations in the air of the city.

However, no safe conclusions were drawn concerning the relationship between skin prick test sensitivity and specific IgE sensitivity.

Finally, this kind of data is being presented for the first time in our area and could constitute the infrastructure of many medical, biological and other scientific field applications.

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Allergic fungi spore records and sensitization in asthmatics in Greece


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