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ORIGINAL ARTICLE/ARTICLE ORIGINAL

# Environmental and molecular study of fungal flora in asthmatic patients

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## KEYWORDS

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ITS sequencing

**Summary** The aim of the present study was to investigate the epidemiological and fungal environmental profile in asthmatic patients. We conducted a prospective study involving 49 patients with allergic asthma. One hundred and forty-five clinical samples and 289 environmental samples were performed. Only 30 patients accepted to participate to the environmental study at their home. For specific IgE antibodies, ELISA assay was conducted for 21 patients. Molecular ITS sequencing was performed for 37 isolates. The frequency of attacks was significantly associated with the seasonality, which was closely related to climate ( $P = 0.024$ ), exposure to animals (cats,  $P = 0.025$ ), plants (olive,  $P = 0.018$ ), physical effort ( $P = 0.04$ ) and the number of permanent occupants in house ( $> 6$ ) ( $P = 0.026$ ). Fungal contaminants were detected from 78.6% of biological samples and 97.8% of environmental samples. Antibodies corresponding to the studied allergens were detected in 10 patients (10/21). PCR sequencing allowed as rectified morphological identification for 27.02% (10/37) strains of *Aspergillus*. The allergy in molds is an indisputable reality that is necessary to look for in front of any severe asthma. So, it is important to establish clearly a relationship between exposure to fungi and health disorders in order to set up specific and effective preventive measures.

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## Introduction

Asthma is a clinical syndrome characterized by episodic reversible airway obstruction, increased bronchial reactivity, and airway inflammation. Asthma results from complex interactions among inflammatory cells, their mediators, airway epithelium and smooth muscle, and the nervous system.

It is a major public health problem, affecting more than 20% of the pediatric population and 5–10% of adults [1,2]. It is also the most common chronic childhood disease in developed nations [2,3]. The increasing prevalence of respiratory allergic diseases noted in recent years in industrialized countries raised questions about environmental changes that could be responsible [1,3].

Causes or triggers of asthma can be divided into allergic and non-allergic etiologies. Aeroallergens can include seasonal pollen, mold spores, dust mites, animal allergens, and food (especially in children) [4,5]. Indoor asthmatics environment play a major role in modulating asthma severity. Therefore, many surveys opted to study risk factors for asthma including a questionnaire about housing characteristics [1,6].

There is consistent evidence that environmental factors acting during early childhood play a key role in the pathogenesis of atopic disease [7]. Because of the clinical, social and economic importance of asthma and other atopic disease, environmental factors must be sought in order to take the necessary preventives measures.

Mold is one of the many triggers of asthma exacerbations in some individuals with atopic asthma, although no asthma exacerbation threshold for mold exposure is known to exist. However, it seems likely that all molds are not equally relevant to the disease.

Studies in different countries showed that occupants of damp or moldy buildings are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma [8,9]. However, associations between symptoms and concentrations of fungi seem to be dependent on distinctive genera or species [10,11].

Identifying specific molds that are potentially associated with asthma could narrow down our search for the most important molds.

In present study, we conducted an epidemiological and environmental survey for fungal flora in asthmatic patients.

## Materials and methods

### Patients study

We released a prospective study (February 2014–October 2014) involving 49 patients with allergic asthma hospitalized in Pneumology department (University Hospital Hedi Chaker Sfax, Tunisia). The stages of asthma of patients were classified according to the Global Initiative for Asthma (GINA) 2008 [12].

Only 30 patients accepted to participate in the environmental study at their home. The demographic data included: sex, date of birth, gender, underlying diseases. The additional data collected regarding housing were: type of home, building material, floor construction, age of home, number

of bedrooms, number of people usually living in the home, and type of floor covering in each room of the house. Antibiotic and antifungal therapy history was also noted for each patient.

In total, 145 biological samples: nasal samples (75) by swab and sputum (70) in sterile jars were taken for all patients. For environmental study, we performed 289 samples: by swabbing ( $n = 135$ ) (bed, table, rug, carpet, floor, wall, covering, headset), and by using contact Petri ( $n = 154$ ) on doors, sheets and walls. For air sampling ( $n = 75$ ), we used a bioimpactor (180 liters of air/minute) (SAS super 180). A total of 509 samples were then collected from patients and their environment. Culture plates in Sabouraud dextrose agar were incubated at 25 °C for 4 to 15 days.

The number of colonies per plate of each fungal genus/species was counted. The results were expressed as number of colony forming units per cubic meter (CFU/m<sup>3</sup>) in air samples.

For the morphological identification of different fungi, we used the Biforma manual (*cahier de formation Bioforma: Molds of medical interest*).

For measurement of fungal specific IgE antibodies, we used ELISA assay combining enzyme-labelled conjugates and chromogenic substances (Kit EUROIMMUN «Antibodies of class IgE against inhalation allergens»: EUROLINE Inhalation 2).

Mold sensibilization study was released for 21 patients by dosage of specific IgE of the following allergens: *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*.

### Molecular study

#### Identification of *Aspergillus* isolates and DNA isolation

Fungi strains were identified on the basis of macroscopic and microscopic morphological characteristics.

The morphological identification of 37 *Aspergillus* isolates was confirmed by the rRNA ITS1–5.8S–ITS2 region sequence analysis, as previously described by De Hoog et al. [13]. DNA was extracted by using a QIAmp kit (QIAGEN), following the manufacturer's instructions, and eluted with 50 µL sterile water.

### Statistical analyses

Statistical analyses were carried out using IBM SPSS software version 20. Bivariate analysis was performed using Chi<sup>2</sup> tests in the case of pairs of categorical variables or two tailed *t*-tests in the case of a categorical and a continuous variable. We studied the frequency of attacks according to the different risk factors and triggering factors (physical effort, dust, smoking, pollen, strong odors, perfumes, detergents, felines, olive, cold, heat, and climate change, exposure mold), sex, age, symptoms, seasonality characteristic of the home, improvement in case of change of habitat and humidity.

## Results

### Patients study

There were 13 men and 36 women (sex-ratio = 0.36). The average age was 50 years (extremes: 28–91 years).

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