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ORIGINAL ARTICLE

# Airway Colonization with *Alternaria* and *Cladosporium* spp. in Fungi-Sensitized Asthma Patients in Sharkeya, Egypt

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***Key words:***

***Alternaria, Cladosporium, Bronchial asthma, Sputum***

## ABSTRACT

***Background:*** *Sensitization to outdoor airborne fungi, as Alternaria and Cladosporium, is common in asthmatic patients, but the clinical relevance of this and the relationship with airway colonization by those fungi remain unclear. The range of fungi that may colonize the airways in asthmatic patients in Sharkeya, Egypt is unknown.* ***Objectives:*** *To assess the prevalence of colonization with Alternaria and Cladosporium spp. in a sample of asthmatic patients who are sensitized to those fungi in Sharkeya, Egypt.* ***Methodology:*** *In a case-control study, 103 asthmatic patients who had positive skin prick test (SPT) for Alternaria and /or Cladosporium, were compared with a well-matched control group of 100 healthy volunteers for detection of Alternaria and Cladosporium in their sputum by conventional methods (microscopy with lactophenol cotton blue stain and culture on Sabouraud dextrose agar). Specific IgE (sIgE) for Alternaria alternata and Cladosporium cladosporoides was measured in both groups.* ***Results:*** *Common environmental molds; Aspergillus (38.2%), Alternaria (35.3%), Zygomycetes(13.2%), Aeurobasidium(4.4%), Gliocladium (4.4%), Cladosporium (3%) and Epicoccum (1.5%), were isolated from patients sputa in a significantly higher frequency than in controls ($P < 0.001$). Colonization with Alternaria was significantly higher in sensitized asthmatic patients ($P<0.001$) compared to the control group. However, no statistically significant difference was observed in mean sIgE titers between patient and control groups ($P>0.05$).* ***Conclusion:*** *This study suggests Alternaria as a major allergen that its presence in the sputum and subsequent development of sensitization could have a significant role in the induction of asthma in our locality.*

## INTRODUCTION

Bronchial asthma is a disease of major public health importance that affects more than 300 million people worldwide with a growing burden in terms of morbidity, quality of life and healthcare costs [1]. In many people with asthma, particularly in those with onset in childhood, there is a clear association between immunoglobulin E (IgE) sensitization and common aeroallergens (atopy) [2].

Exposure to outdoor airborne fungi such as *Alternaria* and *Cladosporium* is especially important in inducing IgE antibody formation and sensitization of atopic individuals. Moreover, the exposure to high levels of fungal spores, particularly in the case of *Alternaria*, has been implicated in worsening and exacerbation of asthmatic attacks and even death [3].

In a retrospective study, that included 1230 patients undergoing skin prick test (SPT) performed at the Allergy and Immunology Unit of the Zagazig University, Egypt, between January 2012 and February 2013, it was revealed that the local population is more sensitized to outdoor than indoor allergens, in particular, *Alternaria* and *Cladosporium* (unpublished data).

Previous epidemiologic studies demonstrated the prevalence of sensitization to inhaled allergens in asthmatic patients [4]. However, the range of fungi that may colonize the airways in those patients has not been studied extensively, so far.

This study aimed to assess the prevalence of airway colonization with *Alternaria* and *Cladosporium* spp. in asthmatic patients who are sensitized to both fungal types in Sharkeya, Egypt as a preliminary step to clarify the exact role of those fungi in the pathogenesis of asthma.

## METHODOLOGY

This study was carried out at the Mycology unit & Immunology Research Lab., Department of Microbiology and Immunology, Faculty of Medicine, Zagazig, Egypt between August 2013 and December 2014. One hundred and three clinically diagnosed

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asthmatic patients (ages15-65 years, 65 females and 38 males), who were sensitized to *Alternaria* and/or *Cladosporium*, and 100 apparently healthy individuals (ages 15–65 years, 61 females and 39 males) were studied. The study was approved by the Institutional Review Board of Zagazig University Hospitals. Written informed consent was obtained from each individual. A detailed clinical history and a complete physical examination were carried out for each patient. Asthmatic patients were chosen from those attending the allergy outpatient clinic. All enrolled patients were sensitized to *Alternaria* and/or *Cladosporium* i.e. had positive skin test either for *Alternaria* and/or *Cladosporium.* All control individuals had negative skin test for both fungal allergens. A checklist including age, sex, symptoms and signs of the disease, severity, history of related (e.g. urticaria or eczema) or unrelated diseases (other clinical or surgical diseases), and family history was completed.

**Skin prick tests (SPTs):**

Tests were performed at the volar site of each patient s forearm by the same experienced personnel through the application of one drop of each allergen extract of a panel containing housedust mite (*Dermatophagoides pteronyssinus*), *Aspergillus* species mix, cottonwood mix, ash mix, tobacco leaf, ryegrass, *Alternaria, Cladosporium* and *Phoenix dactylifera* pollen (AL, AllergyLaboratories, Inc., USA) to the skin, at least 3 cm apart, according to a previously validated protocol [5]. Histamine was used as positive control and diluents of each allergen as a negative control (AL, Allergy Laboratories).The sensitivity of the skin test was estimated by the size of the wheal. The largest diameter of the wheal was evaluated as the size of the wheal after 20 min. A wheal diameter 3 mm or greater, accompanied by erythema, was defined as a positive reaction.

**Sputum examination:**

Morning sputum was collected from all participants under strict hygienic conditions. All sputum samples were subjected to direct microscopic examination using lactophenol cotton blue stain and culture on Sabouraud dextrose agar (SDA) (Oxoid, Ltd., Hampshire, England) with chloramphenicol (0.05 g/L). Cultures were incubated at room temperature (25-30˚C) and inspected daily for growth for at least four weeks. Identification was made to the genus level based on macroscopic and microscopic morphology of the fungal growth.

**Determination of specific IgE:**

Five ml blood were collected from each participant under complete aseptic conditions, were left to stand at 37˚C to coagulate. Separated sera were collected, stored at -20˚C till their use in ELISA tests. Quantitative determination of specific IgE for *Alternaria alternata* (*A. alternata*) and *Cladosporium cladosporoides* (*C. cladosporoides*) in serum samples was performed using Specific IgE R-Biopharm (AG, Ridascreen, Germany) according to the manufacturer's instructions. Those having ≥ 0.35 IU/ml were considered positive.

**Statistical analysis:**

Data was analyzed using Statistical Package of Social Sciences (SPSS) version 19. Chi- square ($\chi^2$) test was used to compare qualitative data. *P* value of < 0.05 was considered significant.

## RESULTS

**SPTs:**

Out of 103asthmatic patients and 100 control subjects, 65(63.1%) and 61 (61%) respectively were female. The mean of age of the patient and control group was 33.7 ± 11.9 and 35.11 ± 11.4, respectively. Both groups were matched in gender and age (*P* 0.8 and 0.2, respectively). All chosen patients (n=103) were sensitized to *Alternaria* with positive SPT, while only nine patients (8.7%) were found to be sensitized to *Cladosporium* in addition to *Alternaria*.

**Sputum examination:**

Direct sputum examination revealed that 64% (n=66) of asthmatic patients had fungal elements in their sputum with a highly significant difference (*P*<0.001) compared to the control group (5%, n=5). Furthermore, the macroconidia of *Alternaria* were detected in the sputa of 8.7% (n=9) of asthmatic patients. All sputum samples from patients yielded fungal growth. One type of fungal growth was obtained from each patient sample where molds were isolated from 66% (n=68) of the sputum samples, while 34% (n=35) of samples yielded the growth of yeasts. Only 11% (n=11) of sputum samples from the control group yielded fungal growth where 7% (n=7) of the samples yielded mold growth and 4% (n=4) yielded yeast growth. Both hyaline and dematiacous molds were isolated from patients with a ratio of 55.8: 44.2, respectively **(Fig. 1A)**. Among the molds isolated from patients, *Aspergillus* and *Alternaria* had the highest colonization percentage forming 38.2% and 35.3%, respectively. However, *Cladosporium* and *Epicoccum* had low isolation frequency, 3% and 1.5%, respectively. Other isolated molds included *Zygomycetes* (13.2%), *Aeurobasidium* and *Gliocladium* (4.4%for each) **(Fig. 1B)**. Colonization with *Alternaria* **(Fig. 2)** was significantly higher in sensitized asthmatic patients (*P*<0.001) compared to the control group **(Table 1)**. Furthermore, there was a significant relation between sputum colonization and sensitization to *Alternaria* (*P* < 0.0001) where 24 (23.3%) patients out of the examined 103 sensitized patients had *Alternaria* growing in their sputum following culture compared to 2% (n=2) of normal subjects. Only 1.9% (n=2) of patients' samples yielded *Cladosporium* growth after culture **(Fig. 3)** while among the control group, no sputum samples yielded *Cladosporium* growth **(Table 1)**.

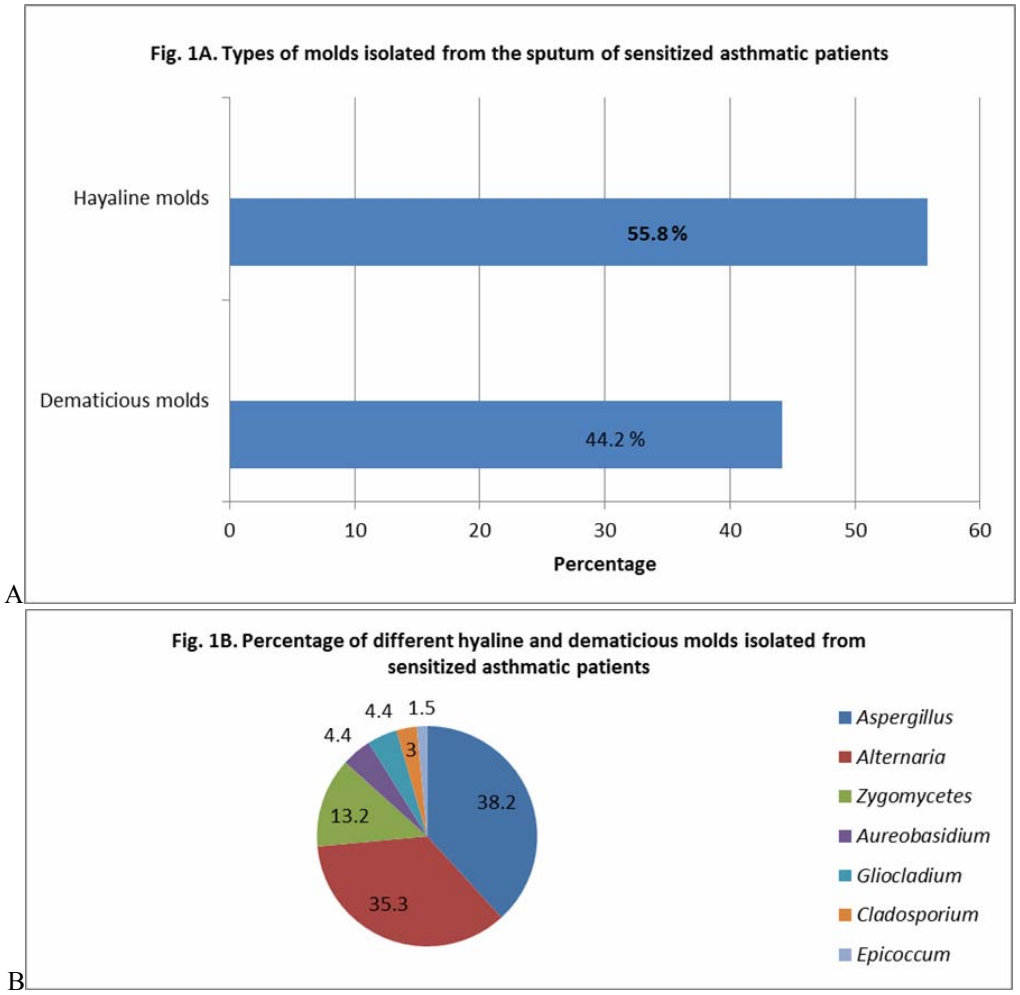


**Fig. 1:** Types (A) and percentages (B) of different molds isolated from sensitized asthmatic patients

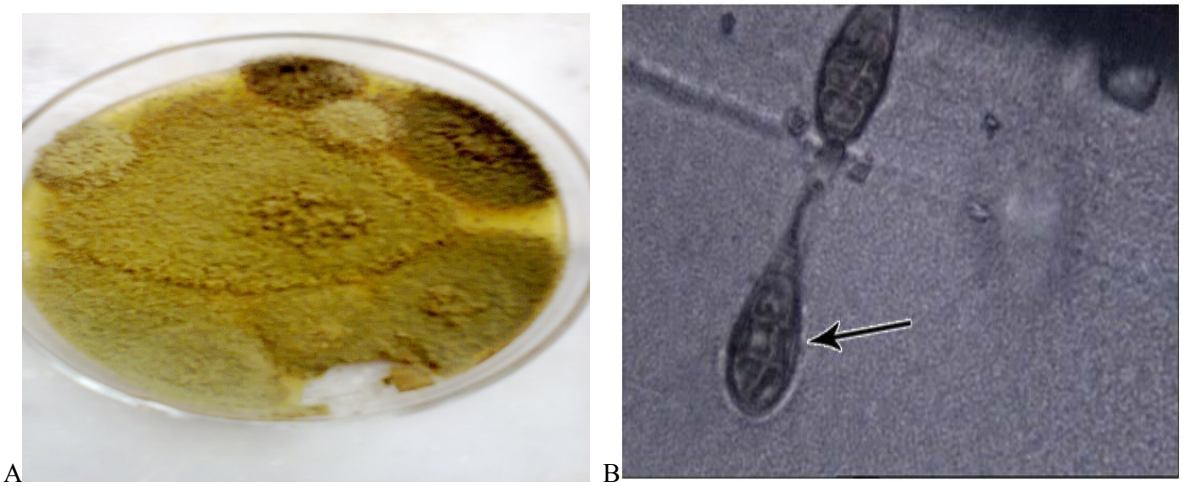


**Fig. 2:** **A.** Appearance of *Alternaria* colonies isolated from sputum on SDA plate. Colonies appear flat, downy to woolly black to olivaceous in colour. **B.** Microscopic appearance of *Alternaria* after culture on SDA. The arrow points to the characteristic conidia with club-shaped bases and tapered apices. Conidia appear in chain and both horizontal and transverse septa can be seen. (40X lens, light microscope) unstained.

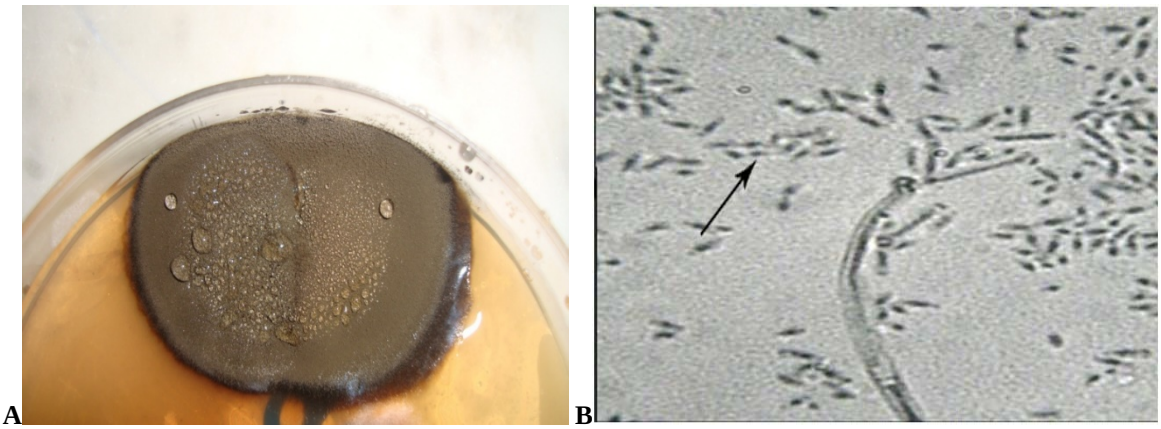

**Fig. 3: A.** Appearance of *Cladosporium* colonies isolated from sputum on SDA plate. Colonies are velvety and appear dark-gray greenish with slight folding. **B.** Microscopic appearance of *Cladosporium* after growing on SDA. It shows septate dark hyphae and forked conidiogenous cell. The arrow points to blastoconidia that appear in short chains with scars at each point of attachment. (40X lens, light microscope) unstained.

**Table 1: Results of sputum direct smear, sputum culture and specific IgE (sIgE) in sensitized asthmatic patients and control groups**

| | ***Sensitized asthmatic group (n=103) No. (%)*** | ***Non sensitized control group (n=100) No. (%)*** | $\chi^2$ | **P** |
|---|---|---|---|---|
| ***Alternaria*** | | | | |
| **Stained smear** | 9 (8.7) | 0 (0) | 9.14 | 0.002** |
| **Culture (colonization)** | 24 (23.3) | 2 (2) | 20.62 | <0.001** |
| **Positive sIgE against *A. alternate*** | 3 (2.9) | 1 (1) | 0.96 | 0.33 |
| ***Cladosporium*** | | | | |
| **Stained smear** | 0 (0) | 0 (0) | ------ | ------- |
| **Culture (colonization)** | 2 (1.9) | 0 (0) | 1.96 | 0.16 |
| **Positive sIgE against *C. cladosporides*** | 1 (0.97) | 0 (0) | 0.98 | 0.32 |

**Highly significant

**Specific IgE:**

Sensitization to *Alternaria* and *Cladosporium* did not have a significant relation with sIgE. The results are shown in **Table 1**. Additionally, no significant differences (*P* 0.1, 0.07 respectively) were observed between patients and controls in sIgE levels of *A. alternata* (mean of 0.25±0.8 in patients compared to 0.12±0.1 in controls) or that of *C. cladosporoides* (mean of 0.13±0.1 in patients compared to 0.1±0.1 in controls).

## DISCUSSION

Different studies had demonstrated that allergic diseases, including asthma, are more frequent in areas with high *Alternaria* and *Cladosporium* spore concentration. Furthermore, it was demonstrated that sensitization to *Alternaria* is considered an important risk factor for asthma development and can sometimes lead to severe, even potentially fatal, asthma[6]. This study was conducted to verify the extent of colonization by both types of fungi in 103 sensitized asthmatic patients compared to 100 apparently healthy well-matched volunteers who had negative skin test for both types of fungi, in our locality.

All enrolled patients, in our study, had positive skin test for *Alternaria*. Nine patients (8.7%) had positive skin test for both *Alternaria* and *Cladosporium*. Monosensitization to *Cladosporium* was not found. This comes in accordance with a previous Australian study where all the studied schoolchildren who were sensitized to *Cladosporium* allergens were also sensitized to *Alternaria* allergens[7]. This also comes in agreement with D'Amato et al. who found that all mold-positive skin prick test patients with respiratory allergy were sensitized to *Alternaria* with absence of monosensitization to *Cladosporium*[8]. In the same study[8], it was reported that 45 patients among 83 (54.2%) had sensitization to both *Alternaria* and *Cladosporium* which is much higher than our results.

This difference could be explained by the different geographical location as *Alternaria* seems to be one of the most common sensitizing agents in the Mediterranean compared to *Cladosporium* which is more common in northern Europe [8,9].

All sputum samples from asthmatic patients, in our study, yielded fungal growth compared to only 11% of sputum samples of healthy individuals with a significant difference ($P< 0.001$) between both groups. This is supported by a previous Indian study where a significantly higher rate of fungal culture was detected in the sputum of subjects with asthma compared with healthy controls [10]. It also comes in accordance with Agbetile et al. who isolated filamentous fungi from 54% of sputum samples of asthmatic patients compared to only 17% of healthy individuals' sputa [11].

Considering the molds isolated from asthmatic patients in our study, we found that *Aspergillus* and *Alternaria* had the highest colonization percentage. This almost comes in accordance with Pashley et al. [12] where most of the fungi cultured from asthmatic airways belonged to *Aspergillus* and *Penicillium* genera being more able to colonize the human airways as the small size of their spores permits them to bypass the filtering system of the upper airways and continue deposition in the distal small airways. In addition, many members have thermotolerant growth properties allowing them to grow at body temperature in the airways.

The role of airway colonization by fungi in bronchial asthma has been studied previously and has been related most notably to *Aspergillus fumigatus* [13,14]. Regarding black molds, a significant relation between *Alternaria* colonization and another related allergic disease  allergic rhinitis was reported previously [15]. Nevertheless, a number of studies have confirmed the association between sensitization to *Alternaria* and the severity of bronchial asthma. It was further shown that children sensitized to *Alternaria* were more likely to have persistent asthma in adulthood [16]. Our study indeed confirms the significant relation between sputum colonization with *Alternaria* and bronchial asthma ($P< 0.001$) as well as the significant relation between colonization and sensitization to *Alternaria* ($P< 0.001$). This, in turn, may highlight the importance of the diagnosis of fungal infection/colonization in asthmatic patients. Unexpectedly, we did not find such a significant relation with *Cladosporium*, though being one of the most common outdoor fungi in our locality as shown in a previous local study [17]. Although we could not fully explain this finding, it seems that the unicellular small sized conidia of *Cladosporium* may favor invasion rather than causing atopy.

The low ratio of patients that were positive for sIgE in our study (2.9% (n=3) for *A. alternata* sIgE and only 0.97% (n=1) for *C. cladosporoides* sIgE comes in contrast to what was reported previously where high ratio of asthmatic patients (38%) had specific IgE against *A. alternata* [18]. However, in another study, Reijula et al. reported lower levels of specific IgE against *A.alternata* in asthmatic patients [19]. The different geographic and climatic factors may be the cause for this difference though it seems that these factors play a less significant role in sensitivity to *Alternaria*, as suggested previously [20]. In addition, the possible differences in prick test solutions and allergen extracts used for *in vitro* tests could be another factor explaining this low ratio recorded in our study.

Finally, there were some limitations to our study. First, the absence of a gold standard for diagnosis which is the provocation test. However, this could only be practical in a controlled hospital environment as it may trigger a severe allergic reaction. Second, the choice of the patients that were all positive for *Alternaria* and/or *Cladosporium* skin test did not allow assessing the prevalence of sensitization to these fungi among asthmatic patients.

## CONCLUSION

In conclusion, this study demonstrated that *Alternaria* colonization was significantly higher in asthmatic patients sensitized to *Alternaria* compared to normal individuals, which suggests *Alternaria* as a major allergen that its presence in the sputum and subsequent development of sensitization have significant role in the induction of asthma.

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