

## ORIGINAL ARTICLE

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# Alternaria in Patients with Allergic Rhinitis

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## ABSTRACT

Inhalation of fungal spores is shown to participate in the development of allergic rhinitis symptoms. In this study, relation between presence of *Alternaria* in the human nasal cavity and allergic rhinitis is assessed.

In a case-control study, 58 allergic rhinitis patients were compared with a well-matched control group of fifty healthy volunteers for sensitization to *Alternaria* (by skin prick test) and detection of *Alternaria* in their nasal mucous by conventional methods (microscopy with Methylene Blue stain and culture in Sabourad dextrose agar). Severity of the disease was determined according to the ARIA classification. Pearson chi-square test was applied to compare the proportional difference between the study groups for detection of *Alternaria* in the nasal cavity, and sensitization to *Alternaria*.

Relation between detection of *Alternaria* and allergic rhinitis was significant [OR = 18.18 (4.02-82.50)] In addition, sensitization to *Alternaria* showed a significant relation with the disease [OR = 2.8 (2.1-3.8)]. There was a significant relation between the presence of *Alternaria* in the nasal cavity and sensitization to *Alternaria* [OR = 10.4 (3.8-28.3)]. Both sensitization to *Alternaria* and presence of *Alternaria* in the nasal cavity did not have a significant relation with the severity of allergic rhinitis. This study suggests *Alternaria* as a major allergen that its presence in the nasal cavity and subsequent development of sensitization have significant role in the induction of allergic rhinitis.

**Key words:** Allergy; *Alternaria*; Rhinitis; Sensitization; Severity

## INTRODUCTION

Allergic diseases impose a considerable morbidity

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on the people in society.<sup>1</sup> Allergic rhinitis alone, led to 3.5 million work days and 2 million schooldays losses annually in the United States.<sup>2</sup> Impairing social and professional lives of the patients. Allergic rhinitis obviously places a substantial adverse economic burden on the societies.<sup>3</sup> Thus, determination of factors which contribute to developing or aggravating allergic

symptoms have a considerable role in prevention. Fungi are among allergens shown to have a direct association with allergic diseases such as asthma and allergic rhinitis. *Aspergillus*, *Cladosporium*, and *Alternaria* are three common allergenic fungi,<sup>4</sup> which their extracts being frequently used for the evaluation of sensitization these days.<sup>5</sup>

*Alternaria* is a dark cell-walled mould, growing as a saprophyte or pathogen organism on decaying wood, compost, plant, food, and different types of soil. It seems that *Alternaria* induces allergic reactions through an IgE-mediated hypersensitivity.<sup>6,7</sup> Presence of *Alternaria* in the upper airways might be the cause of all these reactions, a hypothesis that is not thoroughly investigated so far. However, there is some supporting evidence that shows allergic rhinitis is more frequent in areas of high *Alternaria* spore concentration.<sup>8-10</sup> Andersson suggested a relation between higher concentration of airborne *Alternaria* spores and rhinitis symptoms.<sup>8</sup>

For evaluating this hypothesis, this study was designed. First, association between the presence of *Alternaria* in the nasal mucous and sensitization to it was assessed. Then, relation between the detection of *Alternaria* and allergic rhinitis (and its severity) was discussed. The results might complement the findings of studies on the relation between the *Alternaria* spores' airborne concentrations and the disease. In addition, it might lead to more clarification of the exact role of this fungus in the pathogenesis of allergic rhinitis.

## PATIENTS AND METHODS

For evaluating the hypothesis, a case-control study was designed by participation of 58 allergic rhinitis patients and a well-matched control group of 50 healthy volunteers. Sample size was determined according to the related formula for comparing the ratios.<sup>11</sup> Case and control groups were selected non-randomly from those referring to the otolaryngology and elective surgery clinics of Ghaem Hospital, Mashhad, Iran, following explaining thoroughly about the research objectives and process and acquiring a written consent. The research project was approved by the Ethics Committee of the Mashhad University of Medical Sciences (MUMS) as it was thoroughly

congruent with the provisions of the Declaration of Helsinki.

The patients in the case group enrolled in the study were from those who referred to the clinic from July to September 2006. Only those patients that fulfilled the inclusion criteria were selected. The criteria were symptoms and signs of rhinitis, documented allergic nature of the disease (positive skin prick test to at least three common aeroallergens), and no history of local or systemic antifungal therapy and sinus lavage or endoscopy. It is worth noticing that control group had neither present or past history of rhinitis symptoms nor an atopic state. Matching was performed case by case, with consideration of age, sex, medical and drug history of cases.

All the cases were examined by skin prick test for sensitization to *Alternaria*. Then, 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. Based on the previous month symptoms, severity of the disease was determined. ARIA classification was used for this purpose:<sup>12</sup> patients were classified as "moderate/severe" if at least one of the criteria "sleep, daily activities and work/school attendance" was impaired (sometimes, regular or all the time). Other conditions considered as "mild".

Afterwards, in the otolaryngology examination room, and under direct observation, sampling from the nasal cavity by applicators was performed from both sides- mainly from the middle meatus, due to the discharge of para-nasal sinuses in these areas. Each applicator was placed in a specific sterile tube with no solution and sent to the mycology laboratory. Direct microscopy, staining with Methylene Blue and culture were the tests for diagnosis of *Alternaria*. Culture tubes containing SCC (Sabourad dextrose agar with Chloramphenicol without Cyclohexamide) were used in this study for detection of *Alternaria*.

After incubation at 25°C for 48 hours, culture tubes were evaluated for colony formation every day. Colonies with indefinite characteristics were re-examined by direct microscopy for confirmation of the result.

## Alternaria and Allergic Rhinitis

**Table 1. Relation between presence of Alternaria and allergic rhinitis**

Topic	Result	Study groups		P-value	OR (CI 95%) <sup>1</sup>	Relation
		Rhinitis N (%)	Control N (%)			
Direct Examination	Positive	19 (32.8)	3 (6.0)	0.001	7.63 (2.10-27.71)	+
	Negative	39 (67.2)	47 (94.0)			
Staining	Positive	20 (34.5)	2 (4.0)	< 0.0001	12.63 (2.77-57.44)	+
	Negative	38 (65.5)	48 (96.0)			
Culture	Positive	25 (43.1)	2 (4.0)	< 0.0001	18.18 (4.02-82.50)	+
	Negative	33 (56.9)	48 (96.0)			

<sup>1</sup> OR: Odd Ratio, CI: Confidence Interval.

### Analysis

Data was analyzed with SPSS 13, using Pearson chi-square test and multi-variants analysis to compare the proportional difference between the study groups for detection of Alternaria in the nasal cavity, and sensitization to Alternaria. For evaluation of the strength of relations, we also calculated respective odds ratios.

### RESULTS

Out of the 58 allergic rhinitis patients and control group, 26 and 31 respectively were male. The mean of age in the case and control group was  $29.8 \pm 16.1$  yrs (5 - 66 yrs) and  $35.4 \pm 20.5$  (7-80), respectively. The difference was not significant between case and control groups regarding sex and age, with *P*-values of 0.562 and 0.676 respectively. In the study group, 22 patients (37.9%) showed mild allergic rhinitis, while 36 (62.1%) showed moderate to severe disease. History of eczema and urticaria was positive in 34.5% and 27.6%

of cases, respectively. Family history of atopic diseases (including eczema, urticaria, allergic rhinitis, and asthma) was positive in 72.4% of patients. Direct examination for Alternaria was positive in 32.8% of allergic rhinitis cases. Positive results for Alternaria by staining and culture were seen in 34.5, and 43.1 of cases, respectively.

There was a significant relation between the presence of Alternaria in the nasal cavity and sensitization to Alternaria [*P* < 0.0001, OR = 10.4 (3.8-28.3)]. While this relation was also significant within allergic rhinitis group [*P* = 0.018, OR = 3.9 (1.2-12.1)], it was not significant in the control group. Relation between allergic rhinitis and detection of Alternaria in the nasal cavity was significant (Table 1). In addition, sensitization to Alternaria showed a significant relation with the disease [*P* < 0.0001, OR = 2.8 (2.1-3.8)]. Severity of the disease was assessed within the case group. Both sensitization to Alternaria and presence of Alternaria in the nasal cavity did not have a significant relation with the severity of allergic rhinitis (Table 2).

**Table 2. Alternaria in the nasal cavity and severity of allergic rhinitis**

Topic	Result	Severity of Rhinitis		P-value	Relation
		Mild N (%)	Moderate/Severe N (%)		
Direct Examination	Positive	5 (22.7)	14 (38.8)	0.443	NS *
	Negative	17 (77.3)	22 (62.2)		
Staining	Positive	7 (31.8)	13 (36.1)	0.903	NS *
	Negative	15 (62.8)	23 (63.9)		
Culture	Positive	8 (36.4)	17 (47.2)	0.622	NS *
	Negative	14 (63.6)	19 (52.8)		
Sensitization	Positive	10 (45.5)	21 (58.3)	0.629	NS *
	Negative	12 (54.5)	15 (41.7)		

\* NS: Not Significant

## DISCUSSION

Fungal allergenicity and their role in the pathogenesis of rhinitis are increasingly investigated these days<sup>13-15</sup> and several allergenic fungi including *Alternaria* have been determined.<sup>16,17</sup> Several studies showed that allergic rhinitis is more frequent in the areas of high *Alternaria* spore concentration.<sup>8-10</sup> However, there are several aspects in the allergenicity of *Alternaria*, which needs to be clarified more. An important aspect to be discussed is that whether inhalation of *Alternaria* spores and presence of *Alternaria* in the nasal cavity play role in induction of allergic rhinitis or not.

This study supports the hypothesis "Presence of *Alternaria* in the upper airways might induce allergic rhinitis". Findings of this study can also be interpreted from another point of view: that is higher detection of *Alternaria* and sensitization to it might be due to a poorer discharge from edematous nasal cavities in the allergic condition. However, results of the following investigations which show allergenic nature of *Alternaria* and higher prevalence of *Alternaria* in the environments of allergic patients confirm the abovementioned hypothesis.

In another study by Lipiec, *Alternaria* was suggested as the most common cause of allergic rhinitis among all the fungi investigated in that study.<sup>18</sup> Approval of this hypothesis could also be inferred from the investigations, which showed the relation between concentration of airborne spores of *Alternaria* and the disease.<sup>8-10</sup> Stark, *et al.* discussed fungal levels in the home of children with allergic rhinitis and revealed that dust-borne *Alternaria* was among the predictors of allergic rhinitis.<sup>19</sup>

This study revealed that presence of *Alternaria* in the nasal cavity is related to allergic rhinitis. Some studies previously had shown the presence of *Alternaria* in the human nasal cavity in the rhinitis patients.<sup>20-22</sup> *Alternaria* was among the fungi detected in the nasal mucosa of chronic rhinosinusitis patients.<sup>20</sup> Gosepath confirmed the presence of *Alternaria* DNA was present in the nasal tissue of chronic rhinosinusitis patients.<sup>22</sup> However there is reverse evidence, in which *Alternaria* was not among the common cultured fungi in allergic fungal sinusitis.<sup>23</sup>

This study showed that presence of *Alternaria* in the nasal cavity is related with *Alternaria* sensitization. Previously, relation between exposure to fungi and

development of sensitization had been demonstrated.<sup>24</sup> Baybek revealed a correlation between fungi airborne concentrations and sensitization to the fungi.<sup>25</sup> Although our study is a complement to the reports of this kind, it adds a point that, in the same group, sensitization with *Alternaria* was significantly related to allergic rhinitis. Thus, it could be understood that as sequential events, inhalation of *Alternaria* spores, settling in the nasal cavity, induction of hypersensitivity reactions towards *Alternaria* surface antigens and *Alternaria* sensitization are the proceedings of allergic rhinitis.

The causative role of sensitization to *Alternaria* in allergic rhinitis was formerly described in several studies.<sup>18,26,27</sup> In addition, the seasonal aggravation of allergic rhinitis symptoms might have relation with seasonal peak of *Alternaria* spore concentration in the air and higher rate of hypersensitivity to it, as described by Lipiec.<sup>18</sup> Andersson demonstrated that rhinitis symptoms are higher in summer because the concentration of airborne spores of *Alternaria* is higher in this time of the year.<sup>8</sup>

In this study, there was no significant relation between the presence of *Alternaria* in the nasal cavity and severity of allergic rhinitis. Furthermore, relation between sensitization to *Alternaria* and severity of the disease was not significant. However, there is enough evidence to suggest the possibility of this relation. Higher frequency of the disease in the geographic areas with higher *Alternaria* spore concentrations is one example. Aggravation of symptoms in the seasons that concentration peaks, and relation between sensitization to *Alternaria* and severity of allergic diseases,<sup>28-30</sup> are other supporting evidence. Thus, it seems sensible that a relation with severity should exist, although this study could not show it. Further studies in larger scales might be useful in this case, to either approve or disapprove this finding.

Due to our practical limitations, we did not assess the co-relation of colonization of *Alternaria* with concentrations of *Alternaria* spore in the air. However, we performed sampling both in the case and control groups within three month, from July to September, to avoid any possible bias in this case. It should be kept in mind that these months are the time when airborne *Alternaria* spores peaks in Iran, according to a study in Turkey, which has an ecological and climate similarity to our region.

Another limitation was about the sample size. While it seems that a greater sample size is needed for evaluation of this hypothesis, but the relations were strong enough to support the hypothesis. Findings of this study necessitate further investigations with larger sample size and application complementary tests such as evaluation of specific IgE.

### CONCLUSION

Presence of *Alternaria* in the nasal cavity is related to sensitization to it and also associated with allergic rhinitis. Future studies approving this finding can be used as a base for thinking to newer treatment methods.<sup>31</sup>

Although no relation with severity of the disease is shown in this study, it seems logical to avoid from fungi such as *Alternaria*, and as it was previously approved, it serves as an important measure in the management of allergic rhinitis.

### Limitations

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