**Abstract.** – *Alternaria alternata* (A. alternata) is one of the most common airborne fungi in outdoor and indoor environment. *A. alternata* has also considered as an aeroallergen. So, it could be responsible for an allergen release and may be responsible for allergic reactions in sensitive patients. The aim of this study was the analyzing of specific IgE against *A. alternata* in atopic dermatitis (AD) and asthma patients.

A total of 50 AD patients (male 17 and female 33) and 50 asthma patients (male 20 and female 30) were entered in study. The range age vary from 4 months to 60 years. To analyzing of specific IgE, *A. alternata* was cultured in Sabouraud’s dextrose agar. The grown fungi were harvested and ruptured by liquid nitrogen and glass beads. Samples were centrifuged at 3000rpm in 15 minutes and then at 15,500 rpm (4ºC) in 2 hours and then supernatant were collected as crude extract. The crude extract was separated by Sodium Dodecyl Sulfate-Polyacryl Amide Gel Electrophoresis (SDS-PAGE). The separated proteins transferred to nitrocellulose filter and then socked with atopic dermatitis and asthma patient’s sera. The responsive bands to IgE were revealed by antihuman IgE antibodies conjugated with enzyme in chromogenic substrate.

16 (32%) and 19 (38%) of AD and asthma patients had specific IgE against *A. alternata*, respectively. Among the AD and asthma patients who were positive for specific IgE to *A. alternata*, 14 (87.5%) and 9 (47.4%) were women, respectively. Of the 16 AD patients for specific IgE positive, 9 (56.3%) were >12 years old. Of the 19 asthma patients for specific IgE positive, 10 (52.6%) were 20-39 years old.

This study suggests that *A. alternata* is a major aeroallergen. Our previous studies as well as different studies from other countries have shown that *A. alternata* is one of the most common indoor and outdoor airborne fungi, so it could permanently present some allergens to susceptible individuals. Therefore, control of *A. alternata* growth in indoor areas and avoidance with *A. alternata* propagules could play an important role in reducing allergic reaction in susceptible individuals.

**Key Words:**

**Introduction**
Fungal allergy is a worldwide problem because fungi grow almost everywhere, and exposure to allergenic molds can lead to IgE-mediated rhinitis and asthma and atopic dermatitis.1 Depending on geographic and climate conditions, the prevalence of allergy to molds might be as high as 30%.3

*Alternaria alternata* (A. alternata), a cosmopolitan saprophyte commonly found in soil and plants, is usually considered an outdoor allergen1-7. Although most intense exposure is likely to occur outdoors, *Alternaria* and other allergenic fungi are also found in indoor environments6-8. In our previous studies we have also shown that *Alternaria* is one of the most common airborne fungi both in indoor and outdoor environment8,10. So, it could be responsible for an allergen release and may be responsible for allergic reactions in sensitive patients. To look for serum specific IgE is one of the methods to define allergy to specific allergen. The aim of this study was the analyzing of specific IgE against *A. alternata* in atopic dermatitis (AD) and asthma patients.
**Material and Methods**

A total of 50 AD patients (male 17 and female 33) and 50 asthma patients (male 20 and female 30) were entered in study. The range age vary from 4 months to 60 years. The patients filled out the consent form to participate in research and it was approved by the ethical committee of Mazandaran University of Medical Sciences. All patients had total IgE higher than normal range. Specific IgE against *A. alternata* was analyzed by immunoblotting technique.

**Preparation of *A. Alternata* Extract**

*A. alternata* was cultured in Sabouraud’s dextrose agar. The grown *A. alternata* were harvested and ruptured by liquid nitrogen and glass beads. Samples were centrifuged at 3000 rpm in 15 minutes and then at 15,500 rpm (4°C) in 2 hours and then supernatant were collected as crude extract.

**Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

SDS-PAGE was performed in a vertical slab gel apparatus (Akhtaryan-Tehran, Iran) according to the method of Laemmli11, 12. 5% separation gel (the separation gel buffer was Tris – Hcl 3M pH 8.8) and 4% stacking gel (the stacking gel buffer was 0.5 M Tris – Hcl pH 6.8) were used in a discontinuous buffer system (Tris 0.025 M, glycine 0.192 M, SDS 0.1% (w/v) pH 8.3). The extracts were dissolved in PBS containing 5% (w/v) SDS, 10% 2-Mercaptoethanol (v/v), 20% glycerol (v/v), 0.02% bromophenol blue (w/v) and stacking gel buffer. The sample mixtures were boiled at 100°C for 5 min. One well was used for protein standard (MBI Fermantas SMO431). Each of samples was loaded in separate lanes. In the end of electrophoresis, one sample was stained with coomassie brilliant blue R-250 (sigma), and other used for immunoblotting.

**Immunoblotting**

After electrophoresis, the separated *A. alternata* components and the standard were transferred electrophoretically to nitrocellulose (NC) membrane (pore size 0.45 μm Amersham life, Hybond-c Extra, Science) in a mini – Trans blot cell (Akhtaryan, Tehran, Iran) according to the method of Towbin et al12. Before transfer the gel equilibrated for at least 30 min in pre cooled 4°C. Blotting buffer containing 25 mmol/l tris, 192 mmol/l glycine, 0.03% SDS (w/v), 25% methanol (v/v) (pH 8.3). The transfer was run for 3 h at 100v at 4°C. Protein binding sites still available on the NC membrane after completed transfer were blocked by incubation overnight at 4°C with PBS, (1%) bovine serum albumin and 0.05% Tween (PBS, BSA, TW). The NC membrane with the protein standard was stained with panceau S.

The NC membrane with blotted *A. alternata* components incubated for 2 hours at room temperature with sera samples (separately for each patient) diluted 1/1 in the (PBS-BSA-TW). After washing three times, the strips were incubated for 1h with Anti – human IgE conjugated with horseradish peroxidase (sigma) (diluted 1/1000 in PBS-BSA-TW) and subsequently after washing color was developed with 6 mg diaminobenzidine (sigma) in 9 ml of 0.01 M tris pH 7.4 and 10 µl of 30% hydrogen peroxidase. The reaction was stopped by washing the membrane with distilled water. The MW of respective IgE binding compounds was determined by comparison of their relative mobility with those of the protein standard.

**Results**

16 (32%) and 19 (38%) of AD and asthma patients had specific IgE against *A. alternata*, respectively. Among the AD and asthma patients

<table>
<thead>
<tr>
<th>Specific IgE</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of disease Gender</td>
<td>AD n (%)</td>
<td>Asthma n (%)</td>
</tr>
<tr>
<td>Male</td>
<td>2 (12.5)</td>
<td>10 (52.6)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (87.5)</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td>Total</td>
<td>16 (100.0)</td>
<td>19 (100.0)</td>
</tr>
</tbody>
</table>

Table I. The frequency of specific IgE against *A. alternata* in atopic dermatitis (AD) and asthma patients on the basis of gender.
Specific IgE against Alternaria alternata in atopic dermatitis and asthma patients

In our study, 35% of the patients with AD and asthma showed specific higher levels of specific IgE against *A. alternata* compared to other studies. Studies even from areas with the same climate showed a range of different sensitivity to *Alternaria*. The results of our study showed that the asthmatic patients are relatively more sensitive than AD patients, a finding observed by some authors. As *Alternaria* is one of the most common airborne fungi, different studies showed that exposure to the fungus *A. alternata* is a risk factor for asthma. Interestingly, studies have also shown that the allergic reactions to skin commensally fungi (*Malassezia* spp.) in AD patients are obviously higher in comparison to airborne fungi.

We observed that specific IgE against *A. alternata* occurs more frequently in female patients with AD (87.5%) than males, whereas in asthmatic patients it was not gender dependent.

Nolles et al did not find any significant difference for specific IgE against fungi including *A. alternata* between males and females.

The higher incidence of specific IgE against *A. alternata* in AD and asthma patients was observed in those who were positive for specific IgE to *A. alternata*, 14 (87.5%) and 9 (47.4%) were women, respectively. Of the 16 AD patients for specific IgE positive, 9 (56.3%) were >12 years old. Of the 19 asthma patients for specific IgE positive, 10 (52.6%) were 20-39 years old.

### Discussion

Sensitivity to *A. alternata* is a common cause of asthma. Some investigators have shown that exposure and sensitization to *Alternaria* is important risk factors for asthma, particularly among children. Importantly, *Alternaria* sensitivity can also lead to severe and potentially fatal asthma. Fungal exposure differs from pollen exposure in quantity (airborne spore count are often 1,000-fold greater than pollen counts) and duration (*Alternaria* exposure occurs for months, whereas ragweed exposure occurs for weeks). This prolonged intense exposure mimics that of other allergens such as cat dander and dust mite which likely contributes to both the chronicity and severity of asthma in *Alternaria* sensitive subjects. Therefore, in present study to show the importance of allergic reaction to *Alternaria* as one of the most important aeroallergen we analyzed specific IgE against *A. alternata* in Iranian patients with AD and asthma.

In our study, 35% of the patients with AD and asthma showed specific higher levels of specific IgE against *A. alternata* compared to other studies. Studies even from areas with the same climate showed a range of different sensitivity to *Alternaria*. The results of our study showed that the asthmatic patients are relatively more sensitive than AD patients, a finding observed by some authors. As *Alternaria* is one of the most common airborne fungi, different studies showed that exposure to the fungus *A. alternata* is a risk factor for asthma. Interestingly, studies have also shown that the allergic reactions to skin commensally fungus (*Malassezia* spp.) in AD patients are obviously higher in comparison to airborne fungi.

Although Reijula et al and D’Amato et al reported lower levels of specific IgE against *A. alternata* in AD patients we detected higher levels of specific IgE, a finding suggested by Scalabrin et al. Geographic and climatic factors seemed to play a less significant role on sensitivity to *Alternaria*; however, it has considered playing a role in distribution of fungi in nature.

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The higher incidence of specific IgE against *A. alternata* in AD and asthma patients was observed in those who were positive for specific IgE to *A. alternata*, 14 (87.5%) and 9 (47.4%) were women, respectively. Of the 16 AD patients for specific IgE positive, 9 (56.3%) were >12 years old. Of the 19 asthma patients for specific IgE positive, 10 (52.6%) were 20-39 years old.
observed in >12 and 20-39 years old age groups, respectively. An age-dependent distribution for various aeroallergens, including Alternaria, was also reported by two studies\textsuperscript{30,31}. Similar to the finding of Bogacka et al\textsuperscript{32} study, we found that sensitization to Alternaria is more prevalent in adulthood, an observation in contrary to reports by Nolles et al\textsuperscript{10} and Mari et al\textsuperscript{13}.

This study in concordance with other researches, suggest that A. alternata is a major aeroallergen. Our previous studies\textsuperscript{9,10} as well as different studies from other countries\textsuperscript{27-29} have shown that A. alternata is one of the most common indoor and outdoor airborne fungi, so it could permanently present some allergens to susceptible individuals. Therefore, control of A. alternata growth in indoor areas and avoidance with A. alternata propagules could play an important role in reducing allergic reaction in susceptible individuals.

References


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