European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2009; 13: 187-191

Specific IgE against *Alternaria alternata* in atopic dermatitis and asthma patients

M.T. HEDAYATI, A. ARABZADEHMOGHADAM*, Z. HAJHEYDARI**

Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari (Iran)
*School of Medicine, Mazandaran University of Medical Sciences, Sari (Iran)
**Department of Dermatology, School of Medicine, Mazandaran University of Medical Sciences, Sari (Iran)

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:

Specific IgE, *Alternaria alternata*, Atopic dermatitis, Asthma.

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%[2].

Alternaria alternata (*A. alternata*), a cosmopolitan saprophyte commonly found in soil and plants, is usually considered an outdoor allergen[1-5]. Although most intense exposure is likely to occur outdoors, *Alternaria* and other allergenic fungi are also found in indoor environments[1,6-8]. In our previous studies we have also shown that *Alternaria* is one of the most common airborne fungi both in indoor and outdoor environment[9,10]. 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.

Corresponding Author: Mohammad T Hedayati, MD; e-mail: hedayaty2001@yahoo.co.uk

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 (4ºC) 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 Laemmli[11], 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, glycin 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 ºC 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 al[12]. Before transfer the gel equilibrated for at least 30 min in pre cooled 4ºC. 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 4ºC. Protein binding sites still available on the NC membrane after completed transfer were blocked by incubation overnight at 4ºC 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 I. The frequency of specific IgE against *A. alternata* in atopic dermatitis (AD) and asthma patients on the basis of gender.

Specific IgE	Positive		Negative	
Type of disease Gender	*AD n (%)*	*Asthma n (%)*	*AD n (%)*	*Asthma n (%)*
Male	2 (12.5)	10 (52.6)	15 (44.5)	10 (32.3)
Female	14 (87.5)	9 (47.4)	19 (56.0)	21 (67.7)
Total	16 (100.0)	19 (100.0)	34 (100.0)	31 (100.0)

Table II. The frequency of specific IgE against *A. alternata* in AD patients on the basis of age.

Total n (%)	Negative n (%)	Positive n (%)	Specific IgE Age (year)
4 (8.0)	3 (8.8)	1 (6.2)	0-2
20 (40.0)	6 (37.5)	6 (37.5)	2-12
26 (52.0)	9 (56.3)	9 (56.3)	< 12
50 (100.0)	16 (100.0)	34 (100.0)	Total

who were positive for specific IgE to *A. alternata*, 14 (87.5%) and 9 (47.4%) were women, respectively (Table I). Of the 16 AD patients for specific IgE positive, 9 (56.3%) were >12 years old (Table II). Of the 19 asthma patients for specific IgE positive, 10 (52.6%) were 20-39 years old (Table III).

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[1,3,13]. In addition, up to 70% of mold-allergic patients have skin test reactivity to *Alternaria*[14]. Importantly *Alternaria* sensitivity can also lead to severe and potentially fatal asthma[1]. 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)[15]. 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[15]. 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[16-19]. Studies even from areas with the same climate showed a range of different sensitivity to *Alternaria*[16-19].

The results of our study showed that the asthmatic patients are relatively more sensitive than AD patients, a finding observed by some authors[18,20,21]. 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[22-24]. 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[21,25].

Although Reijula et al[17] and D'Amato et al[18] 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[26]. 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[27-29].

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[30] 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 ob-

Table III. The frequency of specific IgE against *A. alternata* in asthma patients on the basis of age.

Total n (%)	Negative n (%)	Positive n (%)	Specific IgE Age (year)
7 (14.0)	4 (12.9)	3 (15.8)	0-19
22 (44.0)	12 (38.7)	10 (52.6)	20-39
21 (42.0)	6 (31.6)	6 (31.6)	40-60
50 (100.0)	19 (100.0)	19 (100.0)	Total

served 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[30,31]. Similar to the finding of Bogacka et al[32] study, we found that sensitization to *Alternaria* is more prevalent in adulthood, an observation in contrary to reports by Nolles et al[30] and Mari et al[33].

This study in concordance with other researches, suggest that *A. alternata* is a major aeroallergen. Our previous studies[9,10] as well as different studies from other countries[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

1) O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med 1991; 324: 359-363.

2) Horner WE, Helbling A, Salvaggio JE, Lehrer SB. Fungal allergens. Clin Microbiol Rev 1995; 8: 161-179.

3) Bush RK, Prochnau JJ. Alternaria-induced asthma. J Allergy Clin Immunol 2004; 113: 227-234.

4) Corden J, Millington W. The long-term trends in seasonal variation of the aeroallergen Alternaria in Derby, UK. Aerobiologia 2001; 17: 127-136.

5) Perzanowski MS, Sporik R, Squillace SP, Gelber LE, Call R, Carter M. Association of sensitization to Alternaria allergens with asthma among school-age children. J Allergy Clin Immunol 1998; 101: 626-632.

6) Licorish K, Novey HS, Kozak P, Fairshter RD, Wilson AF. Role of Alternaria and Penicillium spores in the pathogenesis of asthma. J Allergy Clin Immunol 1985; 76: 819-825.

7) Mitakakis TZ, Tovey ER, Xuan W, Marks GB. Personal exposure to allergenic pollen and mould spores in inland New South Wales, Australia. Clin Exp Allergy 2000; 30: 1733-1739.

8) Bush RK, Portnoy JM. The role and abatement of fungal allergens in allergic diseases. J Allergy Clin Immunol 2001; 107: S430-440.

9) Hedayati MT, Mayahi S, Aghili R, Goharimoghadam K. Airborne Fungi in Indoor and Outdoor of Asthmatic Patients Home, Living in the City of Sari. Iran J Allergy Asthma Immunol 2005; 4: 189-191.

10) Aghili R, Hedayati MT, Shokohi T, Mahdavi S. Proceeding of the 4th National Iranian congress of Parasitology and parasitic disease, Mashhad, Iran, 2003: Oct 13-16.

11) Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-650.

12) Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of protein from polyacrylamide gel to nitrocellulose sheets. Procedure and some applications. Proc Nalt Acad Sci 1979; 76: 4350-4340.

13) Downs SH, Mitakakis TZ, Marks GB, Car NG, Belousova EG, Leuppi JD. Clinical importance of Alternaria exposure in children. Am J Respir Crit Care Med 2001; 164: 455-459.

14) Schonwald P. Allergenic molds in the Pacific Northwest. J Allergy 1938; 9: 175-179.

15) Sanchez H, Bush RK. A review of Alternaria alternata Sensitivity. Rev Iberoam Micol 2001; 18: 56-59.

16) Cantani A, Ciaschi V. Epidemiology of Alternaria alternata allergy: a prospective study in 6840 Italian asthmatic children. Eur Rev Med Pharmacol Sci 2004; 8: 289-294.

17) Reijula K, Leino M, Mussalo-Rauhamaa H, Nikulin M, Alenius H, Mikkola J, Elg P, Kari O, Makinen-Kiljunen S, Haahtela T. IgE-mediated allergy to fungal allergens in Finland with special reference to Alternaria alternata and Cladosporium herbarum. Ann Allergy Asthma Immunol 2003; 91: 280-287.

18) D'Amato G, Chatzrgeorgiou G, Corsico R, Gioulekas D, Jäger L, Jäger S, Kontou-Fili K, Kouridakis S, Liccardi G, Meriggi A, Palma-Carlos A, Palma-Carlos ML, Pagan Aleman A, Parmiani S, Puccinelli P, Russo M, Spieksma FT, Torricelli R, Wüthrich B. Evaluation of the prevalence of prick skin test positively to Alternaria and Cladosporium in patients with suspected respiratory allergy. Allergy 1997; 52: 711-716.

19) Soares FA, Segundo GR, Alves R, Ynoue LH, Resende RO, Sopelete MC, Silva DA, Sung SS, Taketomi EA. Indoor allergen sensitization profile in allergic patients of the allergy clinic in the University Hospital in Uberlândia, Brazil. Rev Assoc Med Bras 2007; 53: 25-28.

20) Eggleston PA, Rosenstreich D, Slavin R, Malveaux F. Relationship of indoor allergen exposure to skin test sensitivity in inner city asthma. J Allergy Clin Immunol 1998; 102: 563-570.

21) Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M. The Spectrum of Fungal Allergy. Int Arch Allergy Immunol 2008; 145: 58-86.

22) Salo PM, Arbes SJ Jr, Crockett PW, Thorne PS, Cohn RD, Zeldin DC. Exposure to multiple indoor allergens in US homes and its relationship to asthma. J Allergy Clin Immunol 2008; 121: 678-684.

23) Pulimood TB, Corden JM, Bryden C, Sharples L, Nasser SM. Epidemic asthma and the role of the fungal mold Alternaria alternata. J Allergy Clin Immunol 2007; 120: 610-17.

24) Salo PM, Arbes SJ Jr, Sever M, Jaramillo R, Cohn RD, London SJ, Zeldin DC. Exposure to Alternaria alternata in US homes is associated with asthma symptoms. J Allergy Clin Immunol 2006; 118: 892-898.

25) Faergemann J. Atopic Dermatitis and Fungi. Clin Microbiol Rev 2002; Oct: 545-563.

26) Scalabrin DM, Bavbek S, Perzanowski MS, Wilson BB, Platts-Mills TA, Wheatley LM. Use of specific IgE in assessing the relevance of fungal and dust mite allergens to atopic dermatitis: a comparison with asthmatic and nonasthmatic control subjects. J Allergy Clin Immunol 1999; 104: 1273-1279.

27) Sen B, Asan A. Fungal flora in indoor and outdoor air of different residential houses in Tekirdag City (Turkey): Seasonal distribution and relationship with climatic factors. Environ Monit Assess 2008 May 29.

28) Peters JL, Muilenberg ML, Rogers CA, Burge HA, Spengler JD. Alternaria measures in inner-city, low-income housing by immunoassay and culture-based analysis. Ann Allergy Asthma Immunol 2008; 100: 364-369.

29) de Ana SG, Torres-Rodríguez JM, Ramírez EA, García SM, Belmonte-Soler J. Seasonal distribution of Alternaria, Aspergillus, Cladosporium and Penicillium species isolated in homes of fungal allergic patients. J Investig Allergol Clin Immunol 2006; 16: 357-363.

30) Nolles G, Hoekstra MO, Schouten JP, Gerritsen J, Kauffman HF. Prevalence of immunoglobulin E for fungi in atopic children. Clin Exp Allergy 2001; 31: 1564-1570.

31) Ezeamuzie CI, Al-Ali S, Khan M, Hijazi Z, Dowaisan A, Thomson MS, Georgi J. IgE-mediated sensitization to mould allergens among patients with allergic respiratory diseases in a desert environment. Int Arch Allergy Immunol 2000; 121: 300-307.

32) Bogacka E, Nittner-Marszalska M, Fal AM, Kuzniar J, Nikiel E, Małolepszy J. Allergy to mould allergens as a risk factor for bronchial asthma in patients suffering from allergic rhinitis. Pol Merkur Lekarski 2003; 14: 388-392.

33) Mari A, Schneider P, Wally V, Breitenbach M, Simon-Nobbe B. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. Clin Exp Allergy 2003; 33: 1429-1438.

Acknowledgements

The participating patients are thanked for their kind co-operation, which was essential for the completion of the study. This study was supported by Mazandaran University of Medical Sciences, Sari (Iran).