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Assessment of allergen- specific IgE by west blotting method in resistant atopic dermatitis patient

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A B S T R A C T

Atopic dermatitis (AD) is a chronic and relapsing inflammatory disease characterized by typically distributed eczematous skin lesion with itches. Being confronted to different allergens which causes irritation of immune system and production of specific IgE, has an important role in forming of this disease. Recognition of the major allergens, which stimulate immune system and cause disease, avoiding to be confronted by these allergens has an important role in treatment of disease; the aim of study was to highlight the frequency of the allergens among the patient who are affected by resistant atopic dermatitis in the Eastern Azerbaijan. In this descriptive and analytical study, the serum's level of total IgE and frequency of specific IgE were measured by western blotting method against 20 common allergens about 35 atopic dermatitis patients who visited Sina Hospital in 2010-2011. The control group included persons who have been diagnosed healthy-Demographic situation, minor and major criterions of AD, previous treatment and the degree of patient's cure were studied. The mean age of patients was 29.2±14.79 years. In this study 51.4±% patients were male and 48.6% patients were female. During this assessment, we have seen 97.1% of the patients who have at least 3 main criterions. Common minor criterions were: pruritus when sweating 68.6%, xerosis 54.3, Dennie-Morgan lines 17.1% and Keratosis Pilaris 11.4% serum total was more than normal limits among 80% of patients. The mean of serum total IgE was 227.51±103 U/ml. in this study, 32 patients (91%) had specific IgE against at least one allergen. The most frequent allergens related to: cultivated rye (48.6%), Timothy grass (42.9%) house dust mite (22.9%), Alternaria (20%) cat (20%), Cladosporium (14.3%), Horse (14.3%) Birch (11.4%), potato (11.4%), dog (11.4%), Egg white (8.6%), Cow milk (8.6%) Mugwort, wheat, Rice Fish, soya, Apple and hazel nuts had little frequency. Carrot was not detected in none of the patients. The frequency of positive allergens among the patients who had been studied was in: plants and fungus allergens group 53.34% animal allergens group 26.8% food allergens group 19.56%.60% of patients after avoiding of the allergens which they had been sensitized to, and some cases immune therapy, were cured. In the control group there was no positive allergen serum total IgE elevation. Recognition of the frequent allergens such as: cultivated rye, Timothy grass, House dust mite Alternaria, Cat, Cladosporium, Horse, Birch, potato, Dog, Egg white cow milk in order to remind to the patients to avoid to be confronted to these allergens and immunotherapy or desensitization is useful in this area.

Introduction

Atopic Dermatitis (AD) is one of the chronic inflammatory diseases, manifesting itself in the form of recurrent popular and vesicular dermatitis's with pruritus (1). In this disease, exposure to various allergens and antigens, and subsequently, the activation of different immunological pathways, leading to an overproduction of specific IgE by B cells, could be observed. Specific IgE plays a significant role in inducing allergic reactions in individuals who demonstrate sensitivity towards allergens, while, it also aggravates allergic symptoms and causes further stimulation of the immune system (2).

In recent years, there has been an increase in the incidence of AD, the main cause of which has been attributed to an increase in the extent of exposure to various allergens or allergic substances (3). Furthermore, it has been proven that a positive correlation exists between AD and developing other respiratory allergic disease such as, asthma, hay fever, and so on. Various studies have suggested that, the occurrence of AD in under-3-month infants would increase the risk of developing respiratory diseases in them, with half of the children suffering from AD exhibiting symptoms of asthma or allergic rhinitis before reaching the age of 7 (4).

Considering the high incidence of AD, it increasing prevalence rate, and the impact it has on people's quality of life, many of the difficulties that susceptible individuals to this disease are confronted by could be resolved and their quality of live be enhanced by preventing such a disease and providing them with normal skins. Moreover, as it was noted earlier, there is a positive correlation between AD and developing other allergy-related diseases, the prevention of which could diminish the risk of developing such diseases, and in particular those of the respiratory type. Common treatments are not effective in some of the patients with AD, resulting in long-term chronic disease courses, the only treatment for which is the identification of responsible allergens.

Allergens play a vital role in the stimulation of the immune system and causing disease. Therefore, the first and foremost way of "treating" AD is by avoiding exposure to those allergens that stimulate the body's immune system (5). Identifying and avoiding exposure to major allergens that contribute to developing AD by stimulating the immune system, and in most cases, vaccination and immunotherapy against allergens would play a significant role in treating this disease.

There are many diverse factors in general, and allergens in particular, that contribute to developing AD and aggravating its clinical conditions, among which are: environmental, plant, animal, and food allergens, as well as emotional stress (1). Specific IgE is of diagnostic value to various allergens. According to various studies, the specific IgE level is more positively correlated with disease severity that that of total serum IgE (3).

Western blot (immunoblotting) is one of the latest techniques by means of which the exact level of specific IgE could be measured in a shorter time and at a lower price than with conventional methods. Since no study had been conducted on the incident of prevalent allergens in East Azerbaijan province, we sought to identify the allergens with the highest incidence in patients diagnosed with refractory atopic dermatitis admitted to the Sina hospital in the Azerbaijan region; which is imperative in

contributing to the prevention and treatment of allergic disease in this region.

In previous studies carried out throughout the world, only some of the allergens have been identified and measured in patients with AD, using such conventional methods as: Priktest, RIa, Rast, Pachtest, and Elisa, with no comprehensive study conducted on the whole spectrum of allergens.

Methods and Materials

In a descriptive-analytical study conducted on patients with AD in Department of Dermatology of Tabriz University of Medical science, we investigated the levels of total serum IgE and specific IgE in 20 common food, plant, animal, pollen, fungus, and mite allergens in 35 patients with AD for whom the common treatments were not effective.

After obtaining their informed consents, patients who had been diagnosed with AD for at least 6 months, and who, despite receiving conventional treatments, had displayed resistance to treatment with no sign of recuperation, were included in the study. The control group consisted of individuals who had not been diagnosed with AD.

After selecting the patients with AD, who conformed to the inclusion criteria of this study, standard questionnaires, consisting of such entries as demographic information, personal/family history, disease duration, and skin or clinical symptoms, was filled. Before treatment, serum samples were obtained from all patients in order to measure their total serum IgE and specific IgE levels. The control group was simultaneously put under examination. Monobind kits (MFD in USA) were used to measure total serum IgE levels.

Specific IgE levels in 20 common food, plant, pollen, fungus, and animal allergens were measured, which were subsequently divided into three categories.

Immunoblotting kits were employed in order to determine the incidence of specific IgEs.

Inclusion Criteria

Patients with AD for whom the conventional treatments were not effective, after obtaining their informed consents.

Exclusion Criteria

Unwillingness on the part of individuals to participate in the study;
Patients consuming generic or topical steroids, immunosuppressive, or cytotoxic drugs;
Pregnant or breastfeeding women and those with underlying disorders.

Ethical Considerations

Informed consents were obtained from the control and patient groups to collect blood samples and perform tests. The patients' medical information has been kept confidential. Additionally, this study has been approved by the Ethics Committee of Tabriz University of Medical Sciences.

Statistical Analysis

The collected data were analyzed by SPSS-17 statistical software. The collected data were expressed as percentage and mean ± SD. Continuous (quantitative) variables were compared by Independent samples and Paired t test. Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's exact test. P-value ≤ 0.05 was considered statistically significant.

Result and Discussion

In this study, 25 patients diagnosed with refractory atopic dermatitis admitted to the Sina hospital, as well as 35 individuals from the control group were investigated. The following results were obtained:

The mean age of patients was 14.79±29.02, in the range of 1.5 – 54 years. The mean age of individuals from the control group was 31.33±11.93, in the range of 1.5 – 55 years. There were 18 males (51.4%) and 17 females (48.6%) in each group.

In this study, 9 individuals (25.71%) were housekeepers, 9 (25.71%) were employees, 6 (17.1%) were university students, 4 (11.4%) were self-employed, and 7 (20%), who were either under 6 years of age or students, were unemployed.

Pruritus could be observed in all patients. Involvement in facial areas was present in 3 patients (71.42%) from the infant and children groups and 3 others (9.37%) from the adult group, equaling to a total of 6 patients (17.14%).

3 patients from the infant group (100%) and 1 other from the children and adult groups (3.12%) suffered from involvement in neck areas, equaling to a total of 4 patients (11.42%) out of the 35 ones investigated.

Involvement in extensor areas could be seen in 3 patients (100%) from the infant group, with no case seen in either of the children or adult groups. Overall, 3 patients (8.5%) suffered from involvement in extensor areas. Involvement in flexor areas could not be observed in infants, while it was present in 29 patients (90%) from the children and adult groups. Overall, the same number of patients, i.e. 29 (82.85%), suffered from involvement in flexor areas.

In this study, the most skin diffusion belonged to flexor areas (82.85%), face (17.14%), and neck (11.42%), and extensor areas (8.5%), respectively.

Chronic or recurrent chronic disease courses (at least 1 year) could be seen in 34 patients (97.14%). The mean chronic course in the "infant and children" groups, as well as the "adult" one were 3.28±3.02 and 8.01±9.92 years, respectively (P = 0.22).

19 patients (54.3%) in this study had a positive Family History (FH) for atopic dermatitis or allergic rhinitis.

The onset of AD in 7 of the patients (20%) was under the age of 2. 19 patients (54.3%) suffered from skin xerosis, 1 (2.9%) from ichthyosis, 4 (11.4%) from Keratosis Pilaris, 2 (5.71%) from nonspecific hand/foot dermatitis, another 2 (5.71%) from nipple dermatitis, 3 (8.6%) from perioral fissures, 1 (2.9%) from recurrent conjunctivitis, 6 (17.1%) from Dennie-Morgan lines, and 24 patients (68.6%) from itch when sweating.

The mean total serum IgE level was 227.51±103, in the range of 45-410. The mean total serum IgE levels in female and male patients were 221.64±111.53 and 333.75±97.13, respectively (P = 0.74). 28 of the examined patients (80%) displayed higher-than-normal total serum IgE levels. 32 patients displayed positive specific IgEs for at least 1 allergen: 11 patients (31.42%) for 1 allergen, 5 patients (14.28%) for 2 allergens, 6 patients (17.14%) for 3 allergens, 4 patients (11.42%) for 4 allergens, 1 patient (2.85%) for 5 allergens, 2 patients (5.71%) for 6 allergens, 1 patient (2.85%) for 7 allergens, 1 patient (2.85%) for 9 allergens, and another patient (2.85%) displayed positive specific IgEs for 10 allergens.

Table.1 Frequency of specific Allergens in patients with Atopic dermatitis

	Sex		Total
	Male(n=18)	Female(n=17)	(n=35)
Cat tail grass	7(38.9%)	7(47.1%)	15(42.9%)
Rye	8(44.4%)	9(52.9%)	17(48.6%)
Birch	3(16.7%)	1(5.9%)	4(11.4%)
Cladosporium	1(5.6%)	4(23.52%)	5(14.3%)
Alternaria	6(33.3%)	1(5.9%)	7(20%)
Domestic mites	2(11.1%)	6(35.2%)	8(22.9%)
Cat	-	7(41.17%)	7(20%)
Dog	1(5.6%)	3(17.6%)	4(11.4%)
Horse	3(16.3%)	2(11.8%)	5(14.3%)
egg white	1(5.6%)	2(11.8%)	3(8.6%)
cow milk	-	3(17.6%)	3(8.6%)
Fish	-	1(5.9%)	1(2.9%)
Wheat	1(5.6%)	1(5.9%)	2(5.7%)
Rice	2(11.1%)	-	2(5.7%)
Soya	1(5.6%)	-	1(2.9%)
Hazelnut	1(5.6%)	-	1(2.9%)
Sagebrush	3(16.67%)	-	2(5.7%)
Potato	1(5.6%)	3(17.6%)	4(11.4%)
Apple	1(5.6%)	-	1(2.9%)

On mean, the patients reacted positively to 3.06±2.31 allergens out of the total 20 ones examined with respect to specific IgE. No positive reaction to allergens with respect to specific IgE was observed in the control group.

The incidence of the fungus Cladosporium, an allergen, was demonstrated to be significantly higher in women compared to men (P = 0.013). The incidence of cat allergens was also significantly higher in women (P = 0.02). However, the incidence of the fungus Alternaria was significantly higher in male patients compared to female ones (P = 0.042). There was no statistically significant difference between men and women with respect to other allergens.

The incidence of positive allergens in the examined patients was 54.34% for the plant and fungus groups, 26.8% for the animal group, and 19.56% for the food group. No plant, animal, or food allergens were found in the control group. Before being included in the research, all patients had received oral and topical treatment courses. After the responsible allergens were identified, 21 patients (60%), including 12 women (70.6%) and 9 men (50%), experienced recovery by avoiding the respective allergens, receiving immunotherapy, or vaccination.

The results obtained from the chi-square correlation test pointed to no significant difference between male and female patients in terms of the extent of recovery (P = 0.21).

In examining 35 patients with refractory atopic dermatitis, the mean age was 29.02±14.79. 8.75% of patients were from the infant group (0-2 years of age), 11.4% from the children group (2-12 years of age), and 80% from the adult group (12 years of age and above). According to previous studies, AD could occur at any given age; yet, it generally appears during childhood or infancy, going through periods of aggravation and remission, and recovery is normally achieved with age (1,6).

In a study conducted by Sampson et al. (2003) in The United States, it was demonstrated that, 50% of patients with AD would display symptoms of the disease within the first year of life, and 80% of them would display such symptoms during the first 5 years of life (7).

Examining 59 patients diagnosed with AD in Thailand, Kulthanan et al. (2007) reveal that, the onset of symptoms was not uncommon in adulthood, with previous studies reporting a 13-47% prevalence rate for the onset of AD in adulthood, and the symptoms appearing within the third decade of life in the majority of cases (8).

Examining 56 patients diagnosed with AD, whose symptoms appeared within the third decade of life, in Thailand, Kulthanan et al. (2011) reported a 34.1±11.7 mean age in the patients (9).

In our study, 51.4% of patients were male and 48.6% of them were female. In an epidemiological study conducted on 2454 individuals, Mgeladze et al. (2009) reported an equal incidence of AD in both sexes (10). The diagnostic criteria for AD are clinical demonstrations. Several guidelines have been suggested for the diagnosis of AD; however, the primary and secondary criteria put forth by Hanifin & Rajka appear to be more adequate. We, too, adopted Hanifin & Rajka primary and secondary criteria for the diagnostic procedure. In a study carried out in India on 73 patients with AD, Shama et al. (2001) demonstrated that, Hanifin & Rajka criteria were of diagnostic nature (11). In another study performed in India on 110 patients with AD, De et al. (2006) demonstrated that, Hanifin & Rajka diagnostic criteria for AD had a statistical advantage over other methods (sensitivity 90%, specificity 93.75%, positive predictive value (PPV) 97%, and negative predictive value (NPV) 91.89%) (12).

Pruritus was present in 100% of patients, which corresponds to the previous studies; making it the diagnostic hallmark (1). Borirakchanyvat et al. (2001) observed itchy skins in all patients under examination (13). In a study conducted in Thailand on 56 adult patients with AD, Kulthanan et al. (2011) reported pruritus to be present in all of them(8).

Studying the lesion distribution in infants, 100% of patients had afflictions in facial, neck, and extensor areas. In children and adult groups, 90% of patients were afflicted in flexor areas, 9.25% in facial areas, and 3.12% in neck areas. In general, 82.25% of patients were afflicted in flexor areas, 17.14% in facial areas, 11.42% in neck areas, and 8.5% in extensor areas.

In a study conducted in The United States by Borirakchanyvat et al. (2001), it was revealed that, lesion distribution varied depending on the age of patients, with infants demonstrating greater involvement in flexor areas, causing traumas when crawling, or in neck or facial areas which they were capable of scratching. In children and adults, however, the involvement areas are antecubital and popliteal fossa (extensor areas) (13). In a study in Thailand on 59

adult patients diagnosed with AD by Kulthanan et al. (2007), the most involvement in adults belonged to flexor areas (8). Examining 56 adult patients with AD, Kulthanan et al. (2011), demonstrated that, typical specific morphology of AD existed in 73.5% of patients(8). Flexor areas had 72.1% involvement rate, neck areas 27%, extensor areas 23%, and facial areas 18.1% (9).

Concluding the specified studies, the most involvement was seen in facial and extensor areas in infants, experiencing trauma when crawling or scratching; while, the most involvement in children and adults belonged to flexor areas, which corresponds to the findings of this study.

In the present study, 54.28% of patients had a positive Family History (FH) for AD. In the study done by Borirakchanyvat et al. (2001), 75-80% of patients had a positive FH (13), while in the study done by Kulthanan et al. (2007), 84.7% of patients with AD had a positive FH (8). In the current study, the incidence of positive FH is lower than in specified ones, which could be accounted for by genetic and racial differences.

The onset of involvement was observed in 20% of patients at early ages, i.e. 2 cases from the adult group. Examining 59 patients diagnosed with AD in Thailand, Kulthanan et al. (2007) reveal that, the onset of symptoms was not uncommon in adulthood, with previous studies reporting a 13-47% prevalence rate for the onset of AD in adulthood, and the symptoms appearing within the third decade of life in the majority of cases (8). Studying 210 patients with AD, Wahab et al. (2011) observed a 31% prevalence rate for the onset of AD in adulthood (14).

The results from the abovementioned studies correspond to the findings of this research. However, according to various studies, AD generally occurs during childhood or infancy, going through periods of aggravation and remission, with recovery normally achieved with age (1, 6). The onset of AD in adulthood is probably responsible for cases with refractory atopic dermatitis.

The secondary criteria in this study, in order of incidence, were: itch when sweating (68.6%), skin xerosis (54.3%), Dennie-Morgan lines (17.1%), Keratosis Pilaris (11.4%), perioral fissures (8.6%), nipple dermatitis (5.71%), nonspecific hand/foot dermatitis (5.71%), recurrent conjunctivitis (2.9 %), and ichthyosis vulgaris (2.9 %). No incidence was found for Pityriasis Alba.

Examining 221 patients with AD, Bohme et al. (2000) observed skin xerosis (100%), environmental factors (87%) in facial erythema (54%), skin reactions provoked by ingested food (39%), itch when sweating (34%), and nonspecific hand/foot dermatitis (28%), to be present among the secondary criteria in patients (14).

Eigenmann et al. (2001) demonstrated the most prevalent of secondary criteria in children with AD to be Dennie-Morgan lines, and infra-auricular fissures, and in adults with AD to be, nonspecific hand/foot dermatitis, and perioral fissures (15).

Studying 108 patients with AD, Wisuthsarewong et la. (2004) demonstrated skin xerosis, ichthyosis vulgaris, food allergy, Dennie-Morgan infraorbital folds, orbital darkening, Pityriasis Alba, preauricular dermatitis, positive dermographism, diffuse scaling of scalp, and periorbital dermatitis to be significantly more frequent than other criteria. Moreover, they maintained that periorbital dermatitis,

truncal dermatitis, and skin xerosis could be used in diagnostic procedures (16).

In a study conducted by Wahad et al. (2011) on 210 patients with AD, the following conditions were observed in the patients: skin infections (80%), environmental factors (66%), wool allergy (50%), skin xerosis (43.8%), Dennie-Morgan lines (39.5 %), ichthyosis (34.3 %), itch when sweating (26.7 %), hyperlinear palms (24.8 %), allergy (19 %), Keratosis Pilaris (14.8 %), Pityriasis Alba (14.25 %), facial erythema (1.9 %), nonspecific hand/foot dermatitis (16.5 %), perioral fissures (10.5 %), diffuse scaling of scalp (5.2 %), and infra-auricular fissures (4.8 %); all of which are important in the disease diagnosis (17).

As can be noticed, there are many points of similarity among various studies with respect to the order of the secondary criteria incidence. The existing distinctions could be accounted for by various genetic or ethnic differences. Climatic differences appear to be playing a vital role in defining secondary criteria as well. In the study conducted by Bohme in Sweden, due to the existing cold and dry climate, 100% of patients displayed skin xerosis; while, in the study carried out by Wahab in Bangladesh, merely 43.8% of patients suffered from xerosis due to its hot and humid climate. The results from the present study correspond to those obtained from Kulthanan et al's(8).

In our study, 28 patients displayed higher-than-normal total serum IgE levels. The mean total serum IgE level was 227.51±103. The mean total serum IgE levels in female and name patients were 221.64±111.53 and 233.75±97.13, respectively (P > 0/05). Within the age 0-2 year group, 100% of cases displayed a higher-than-normal total serum IgE level, with the mean of 135.66±73.05. Within the age 2-12 year groups, 75% of cases displayed a higher-than-normal total serum IgE level, with the mean of 315±47.69. Within the age>12 year group, 78.57% of cases displayed a higher-than-normal total serum IgE level, with the mean of 268.5±67.51. According to previous studies, about 70-80% of patients would experience a higher-than-200 ml/IU increase in the total serum IgE level (1). Hugh et al. (2003) stated that, 85% of patients with AD have total serum IgE levels that are higher than those from the general public (3).

Ellis Hon et al. (2007) reported the total serum IgE levels in patients with AD to be high, with no significant difference in their levels observed among males and females (18), which corresponds to the findings of this study. Wahab et al. (2011) stated that, there was an increase in the total serum IgE levels in 60% of patients with AD (17).

The findings of the present study correspond to those obtained from the previous ones. Warner et al. (1997) maintained that, the increase of total serum IgE levels was more positively correlated with infections and environmental pollutants in patients with AD (19).

In our study, 32 patients (91%) displayed specific IgEs for at least 1 allergen. In a study conducted on patients with AD in Zanja, Afshar et al. (1999) stated that, these patients reacted positively to 3.35±3.15 allergens on mean out of the total 21 ones (20).

Studying 817 children diagnosed with AD, Warner et al. (1997) reported allergy to eggs (40.5%), cow milk (32.3%), cats (20%), and domestic mites (16.6%) on the part of patients (19).

Eigenmam et al. (1998) specified that, about one third of children with AD exhibited increased levels of total serum IgE in response to food proteins. The incidence of food allergies was considerably higher than those from the general public (21). In another study conducted on 492 students in Japan, Wakamori et al. (2009) realized that, there was a significant relationship between the increase of total serum IgE and specific IgE levels in 2 respiratory allergens (domestic mites, and pollens from the Japanese cypress) and the occurrence of AD. Furthermore, it was noted that, total serum IgE levels were adequate diagnostic indicators, and that they could be interpreted as symptoms of sensitivity to atopic allergens (6).

Conclusion

The mean age of patients was 29.2±14.79 years. In this study 51.4±% patients were male and 48.6% patients were female. During this assessment, we have seen 97.1% of the patients who have at least 3 main criterions. Common minor criterions were: pruritus when sweating 68.6%, xerosis 54.3, Dennie-Morgan lines 17.1% and Keratosis Pilaris 11.4% serum total was more than normal limits among 80% of patients. The mean of serum total IgE was 227.51±103 U/ml. In this study, 32 patients (91%) had specific IgE against at least one allergen. The most frequent allergens related to: cultivated rye (48.6%), Timothy grass (42.9%) house dust mite (22.9%), Alternaria (20%) cat (20%), Cladosporium (14.3%), Horse (14.3%) Birch (11.4%), potato (11.4%), dog (11.4%), Egg white (8.6%), Cow milk (8.6%) Mugwort, wheat, Rice Fish, soya, Apple and hazel nuts had little frequency. Carrot was not detected in none of the patients. The frequency of positive allergens among the patients who had been studied was in: plants and fungus allergens group 53.34% animal allergens group 26.8% food allergens group 19.56%.60% of patients after avoiding of the allergens which they had been sensitized to, and some cases immune therapy, were cured. In the control group there was no positive allergen serum total IgE elevation. Recognition of the frequent allergens such as: cultivated rye, Timothy grass, House dust mite Alternaria, Cat , Cladosporium, Horse, Birch, potato, Dog, Egg white cow milk in order to remind to the patients to avoid to be confronted to these allergens and immunotherapy or desensitization is useful in this area.

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