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ORIGINAL ARTICLE

The Role of Toll-Like Cell Receptor 2 (Arg753gln) Polymorphism in Allergen Sensitization and Disease Severity in Atopic Dermatitis Patients

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ABSTRACT

Background: *Variations in Toll-like receptor 2 (TLR2) encoding gene have been associated with atopic conditions.* ***Objective:*** *The present work aims to analyze single nucleotide polymorphism (SNP) of TLR2 gene Arg753Gln in atopic dermatitis (AD) and its association with allergen sensitization and disease severity.* ***Methodology:*** *110 AD patients and 75 healthy controls were enrolled and subjected to genotyping of TLR2 gene Arg753Gln by restriction enzyme analysis and allergy investigations.* ***Results:*** *TLR2 Arg753Gln SNP was significantly more frequent in the patients (48%) in comparison to the healthy group (32%) (0R= 1.7). Individuals with the G/A genotype were at a higher risk for AD development by two times. Inhalant allergens specific IgE were distinguished in 80 % of patients with TLR2 gene polymorphism.* ***Conclusion:*** *GA genotype of TLR2 gene is more dominant in severe cases of atopic dermatitis and associated with sensitization to certain inhalant allergens as house dust mites and pollens.*

INTRODUCTION

Atopic dermatitis (AD) is a frequently occurring inflammatory skin disorder with a higher prevalence in children (15%) and lower rate in 7–10% of adults [1, 2]. The recurrent rash, itching and high serum IgE are characteristic features, so it is also known as atopic eczema [3]. Atopic dermatitis is commonly accompanied with concomitant atopic comorbidities, as well as with non-atopic disorders as psychological and inflammatory diseases [4, 5].

Immunopathogenesis of AD is a complex process due to interactions between susceptibility genes, environmental factors, impaired skin integrity, and immune dysfunction [6, 7]. Inhalant allergens play vital role as house dust mites (HDM) and pollens [8]. Common food allergens triggering AD are milk, seafood, eggs, and shellfish [9].

The innate and adaptive immune responses mediate the pathogenesis in atopic eczema [10]. Innate immune responses are mediated by innate immune receptors, mostly Pattern Recognition Receptors (PRRs) that are important in defining pathogens [11, 12].

Toll-like receptors (TLRs) are one of PRRs which are involved in inflammatory responses to multiple antigens as TLR2 and TLR4 [13]. TLR2 facilitates cellular immune responses to different microbial sources including cell wall, heat-shock-proteins, and zymosan[14,15].

Activation of TLR2 strengthens Tight Junctions (TJs) which covers the gap between the cells of epithelial line, and restores the skin barrier[16]. Known gene polymorphisms within the TLR2 gene are sixteen, eight of them produce different amino acid. TLR2 Arg753Gln (rs5743708) polymorphism is the only SNP which was associated with multiple clinical disorders [14].

The present study aimed to investigate the relationship between TLR2 Arg753Gln (rs5743708) SNP, allergen sensitization, and severity in patients with AD.

METHODOLOGY

Patients selection by atopic dermatitis criteria

The study is a case control study carried out from June 2018 to December 2019. Patients were selected from Allergy and Immunology Unit, Zagazig University. A written informed consent was obtained from patients and controls after approval of the IRB of the Faculty of Medicine # 6313.

The study included 75 adult healthy controls and 110 adult subjects (18-60 years) with atopic dermatitis (AD) based on the diagnostic criteria determined by Liu et al.[17]. These patients must have eczema > 6 months plus one of these criteria: family history, and/or positive specific IgE [17].

Inclusion criteria of these patients were assessed according to REACH characterization that consisted of 11 questions. Question (Q1) and Q2 determine two

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major features, and Q3–11 detect minor criteria. Major criteria included itching, and rash. Minor criteria were atopic family history, kin thickening, dryness, oozing ear, oozing lip, darkening around the eyes, or wrist oozing [18].

Exclusion criteria of both patients and control groups were; attendance of with a chronic or acute infection, psychological disorders, tumor, liver, or hematopoietic disorders.

Blood samples were collected from all subjects and centrifuged at 1500g for 10 minutes to obtain serum for total and specific IgE assay.

Clinical scoring of atopic dermatitis

The severity of AD was assessed using the Scoring Atopic Dermatitis score (SCORAD) [19]. The eczema extent was scored from area of the body, and the intensity was consisted of six items: erythema, edema, excoriations, lichenification, oozing, and dryness in which every item was scored from 0 to 3[20].

Atopic dermatitis was reported as mild if the SCORAD was < 25, moderate if ranged from 25 to 50, and severe if SCORAD Index was more than 50 [21].

Allergy testing by skin prick method

Subjects were tested to the following aero-allergens *(D. pteronyssinus, D. farinae*, grass mix, Birch pollen, sunflower seeds, *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger*, *Alternaria* species, cat epithelium, dog epithelium, feather mix, and cockroach) and to the food by peach, strawberry, banana, pepper, tomato, mango, chicken, fish, shrimp, crab, wheat, oat flour, cacoa, milk, egg, peanut and soybean (Omega Laboratory) (Montreal, Canada). A drop of solution of each allergen, saline, and histamine (negative control, positive control; respectively) was pricked on the forearm. The test was positive if allergen produced a wheal size of 3 mm or more than that of the saline [22].

Total serum IgE Assay

Total IgE levels were determined by enzyme immunoassay (Allergozyme® Total Ig E. Omega Diagnostics GmbH, Herrengraben; Germany). In summary, 10 µl of the standard vials (5) and 10 µl of the patient's serum samples were used. The color intensity was measured with ELISA reader at 405 nm. Patients with total serum IgE levels > 100 kU/l were classified as AD patients [23].

Assay of specific serum IgE

Serum samples were tested for allergen sensitivity to inhalants and food allergens using the Allergy Screen Panel 2A EGY, and 2F EGY panel (MEDIWISS analytic GmbH, Germany). The assay was immune blot detection with a positive control of anti-goat IgG. The precipitates line intensity was determined by Rapid Reader (Improvio, Germany). The test was considered valid if anti-goat Ig G > 3.5 IU/ml. Atopy was determined by elevated specific IgE more than 0.35 IU/L.

Analysis of TLR2 Arg753Gln gene polymorphisms

One ml of each blood sample was withdrawn from patients and controls for genomic DNA extraction using Gene JET Whole Blood DNA Purification Mini Kit (Thermo-Scientific, Waltham, USA) according to manufacture instruction.

For all PCR reaction all primers were added to PCR Master Mix (Bioron, Germany) containing 1x PCR buffer (50 units Taq DNA polymerase, 0.2 mM of each dNTP) in a total volume of 20 µl. Amplification was performed using the following primers (operon, Invitrogen): forward 5′--GCCTACTGGGTGGAGAACCT-3′ and reverse 5′-GGCCACTCCAGGTAGGTCTT-3′. Also, a negative control was included by addition of water to the reaction mixture [24].

PCR Amplification was performed in Biometra thermocycler (T-Gradient Biometra, Germany). The cycling condition were, first: 95 °C for 15 min followed by forty-five cycles of 94 °C for thirty seconds, 58 °C for thirty seconds, and 72 °C for thirty seconds. PCR product of 340 bps length indicating positive amplification of TLR 2 gene [24].

TLR2 genotypes were investigated by *Aci*I restriction enzyme digestion. Seven microliters of the amplicon was incubated with 0.5U *Aci* I enzyme (New England Biolabs, Beverly, MA, USA) in a total volume of 10 µL at 36 °C for 2 h. The electrophoresis was done onto a 3% agarose gel with product of digestion (5 µl) (Invitrogen, Spain) [25, 26]. When the wild-type of the gene was digested by *Aci* I, three bands of 38, 75 and 227 bps will be visualized. However, two bands of 38 and 302 bps were presented when the mutation of one polymorphism of *TLR2* had occurred [24].

Statistical Analysis

The data were analyzed using Statistical Package of Social Services version 25 (SPSS). Continuous Measurable variables were summarized as the mean ± SD & median (range). Categorical variables were presented as number & relative frequencies (percentage). Appropriate statistical tests were used to determine the significance of any comparison.

RESULTS

The result demonstrated that the mean age of Atopic dermatitis (AD) group was 38.6± 9.0 years old, while mean age of healthy control was 43.7 years. Regarding gender distribution among both groups, there was no significant difference (P=0.87). Also, the results revealed that the frequency of family history of atopy was higher in AD patients than that of the control (30% vs 12%, P=0.004). Also, total IgE level in AD patients was elevated (127-265) IU/mL (table 1).

Table 1: Clinical and Laboratory data among the studied patients with Atopic dermatitis and healthy controls

Variable	AD group (N=110)		Healthy control group (N=75)		P-value	OR (CI)
Allergic conjunctivitis	23	20.9	---		---	---
Atopic asthma	23	20.9	---		---	---
Allergic rhinitis	22	20.0	---		---	---
Family history of atopy	33	30.0	9	12.0	0.004	3.14(1.4-7.04)
Total serum IgE, IU/mL	182(127-265)		20(17-22)		0.000	---
Serum aero-specific IgE, IU/mL	29.4±8.58		0.94±0.10		0.000	---
Serum food specific IgE, IU/mL	14.7±1.77		0.99±0.02		0.000	---

Regarding allergen sensitization, specific IgE against inhalant allergens was detected in 50 % of AD group and in 10% of control group (P<0.05). It was found that 50% of AD patients were sensitized to *Dermatophagoides pteronyssinus* (figure 1). Also, this specific IgE level in patients was 29±8.58 IU/L (table 1). Food specific IgE was demonstrated in 25 % of patients with AD and in 8% of the healthy group (P<0.05). Food specific IgE assay revealed that 23% of AD patients were sensitized to tomato, and 21% to egg white (figure 2).

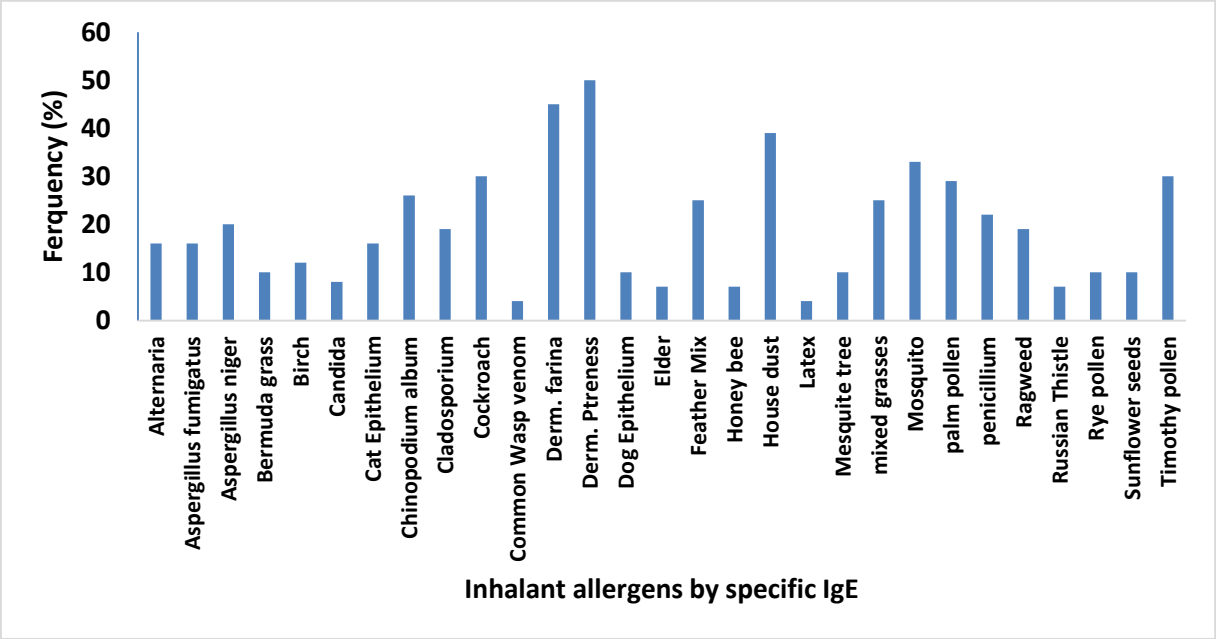

Fig. 1: The frequency of atopic dermatitis patients with inhalant allergy by aero-specific IgE: 50% of AD patients were sensitized to *Dermatophagoides pteronyssinus*, 45% to *Dermatophagoides Farinae*

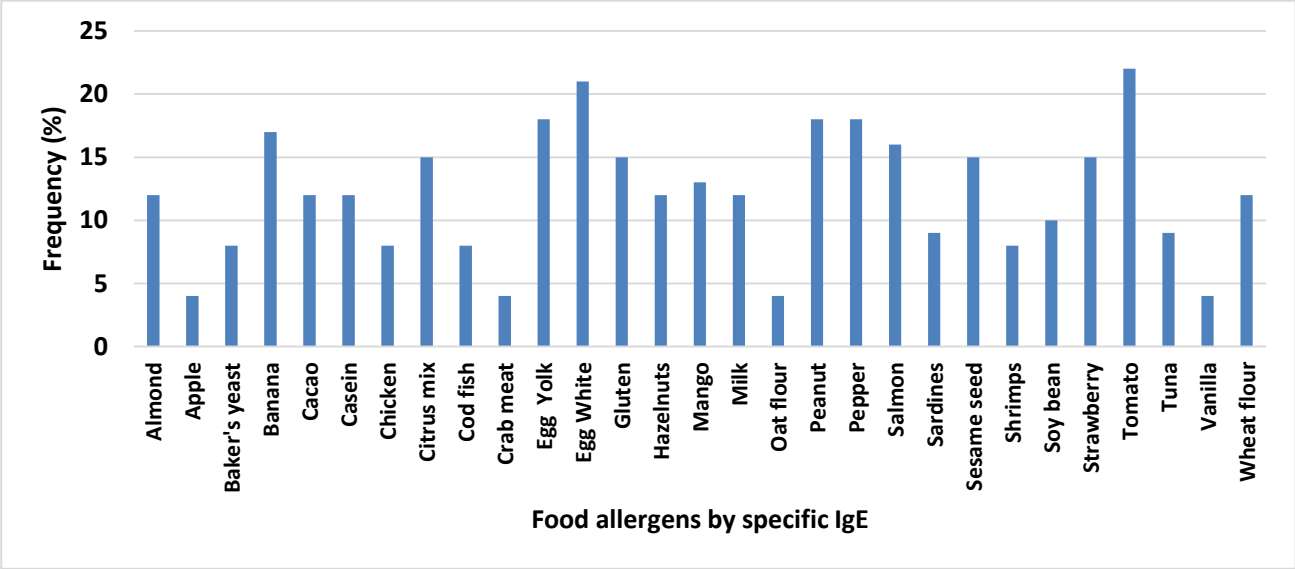

Fig. 2: The frequency of atopic dermatitis patients with food allergy by food specific IgE :23% were sensitized to tomato, 21% to egg white, followed by pepper

Severity of atopic dermatitis that was assessed by SCORAD index, revealed that most cases were moderate with SCORAD index of 39.7 by frequency of 60% (table 2). The results demonstrated that there were statistically significant differences among different phenotypes of AD. Analysis of correlation between SCORAD index and total IgE revealed strong positive correlation between them (r=0.865, P<0.001). Also, inhalant and food specific IgE level were significantly correlated with SCORAD index of the patients (r=0.9, P<0.001).

Table 2: Clinical and laboratory parameters in atopic dermatitis patients stratified according to the SCORAD index classification

Variable	Mild (N=32)		Moderate (N=66)		Severe (N=12)		P-value
Gender							
▪ Male	16	50.0	23	34.8	3	25.0	0.214
▪ Female	16	50.0	43	65.2	9	75.0	
Age, years	41.77 ±6.06		37.2 ±9.5		37.7± 10.8		0.062
Allergic conjunctivitis (No.)	4	12.5	16	24.2	3	25.0	0.380
Atopic asthma (No.)	4	12.5	16	24.2	3	25.0	0.380
Allergic rhinitis (No.)	8	25.0	8	12.1	6	50.0	0.007
Family history of atopy (No.)	4	12.5	20	30.0	9	75.0	0.000
SCORAD index	15.58 ± 3.6		39.7 ± 5.4		62.6± 7.8		0.000
Total serum IgE, IU/mL	115.3±15.4		215.7± 72.8		424.2± 46.5		0.000
Serum aero-specific IgE, IU/mL	15.5±1.7		32.1 ± 3.6		40.0 ± 2.5		0.000
Serum food specific IgE, IU/mL	13 ± 0.42		14.3± 0.63		17.9 ± 0.38		0.000
Genotype GG (No.)	20	62.5	31	47.0	6	50.0	0.03
Genotype AA (No.)	0	0.0	12	18.2	0	0.0	0.011
Genotype GA (No.)	12	37.5	23	34.8	6	50.0	0.043

Toll like receptor 2 Arg753Gln SNP was observed in 48% of the patients and in 32% of the healthy group (0R=1.7, 95% CI=0.8- 3.3, P =0.05) (table 3, figure 3). Also, the occurrence of the homozygous AA genotype in AD patients, presented more frequently (10.9%) than in control (8%). For TLR2 Arg753Gln gene polymorphism, patients with A allele were one and half time at a higher risk to develop atopic dermatitis (OR = 1.67, p = 0.03) (table 3).

Table 3: TLR2 Arg753Gln genotypes and alleles distribution among the patients with Atopic dermatitis and healthy control

Variable	AD group (N=110)		Healthy control group (N=75)		P-value	OR (CI)
	N	%	N	%		
Genotype GG (No.)	57	51.8	51	68.0	0.034	0.5(0.27-0.93)
Genotype AA (No.)	12	10.9	6	8.0	0.068	1.4(0.5-3.9)
Genotype GA(No.)	41	37.3	18	24.0	0.05	1.88(0.97-3.6)
	(N=220)		(n=150)			
Allele G (No.)	155	70.4	120	80.0	0.039	0.59(0.36-0.97)
Allele A(No.)	65	29.6	30	20.0		1.67(1.02-2.7)

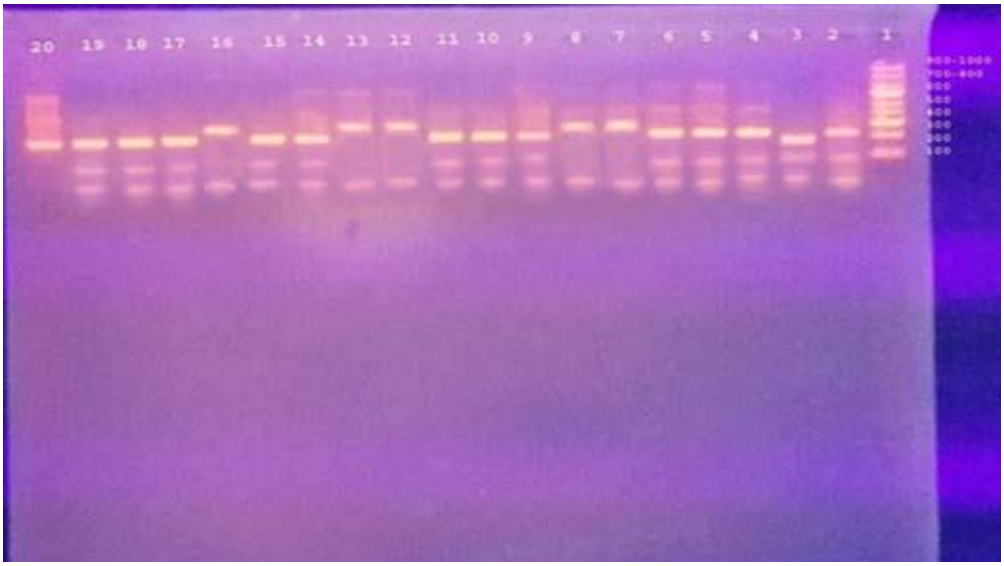

Fig. 3: Toll Like Receptor -2 polymorphism (Arg753Gln) *in patients with atopic dermatitis:* Digested fragments produced by Aci I restriction enzyme. Electrophoresis was carried out on 3% agarose gel. Lane No.1 and 20 represented 100 bps DNA ladder. The wild-type (GG) is presented by three bands of 38, 75 and 227 bps length as seen in lanes 2, 3, 4, 5, 6, 9, 10, 11, 14, 15, 17, 18 and 19. GA genotype is presented by two bands of 38, 302 bps length seen in lane 7, 8, 12, 13, and 16.

Inhalant allergens specific IgE was demonstrated in 80 % of patients with AD with TLR2 Arg753Gln gene SNP. Food specific IgE was demonstrated in 32 % of patients with this polymorphism. Regarding aero-specific IgE, it was found that 82 % of patients with TLR2 Arg753Gln gene polymorphism were sensitized to *Dermatophagoides pteronyssinus*, and 53% of those patients were sensitized to *Dermatophagoides Farinae*.This followed by timothy pollen (51%). Forty-four percent of patients with TLR2 Arg753Gln gene polymorphism were sensitized to house dust and mosquito (P<0.005). Food specific IgE assay revealed that 24% of AD patients with TLR2 Arg753Gln gene polymorphism were sensitized to tomato, 22% to egg white, followed by cacao and milk. The result of food specific IgE in patients with TLR2 Arg753Gln gene polymorphism was insignificant (P=0.63).

Analysis of clinical characteristic of different genotypes revealed a statistically significant association between TLR2 Arg753Gln polymorphism in AD patients with other atopic comorbidities. According to SCORAD index, most mild cases of atopic dermatitis was of GG genotype (62%) (table 4).

Table 4: Clinical and laboratory parameters in atopic dermatitis patients with respect to Toll-like receptor-2 gene Arg753Gln polymorphism

Quantitative parameter	TLR2 Genotype			P-value
	GG	GA	AA	
Atopic asthma (No.)	11(19.3%)	4(9.8%)	8(66.7%)	0.000*
Allergic rhinitis (No.)	11(19.3%)	7(17.1%)	4(33.3%)	0.456
Allergic conjunctivitis (No.)	8(14.0%)	15(36.6%)	0(0%)	0.004
Family history of atopy (No.)	10(17.5%)	15(36.6%)	8(66.7%)	0.002
SCORAD index	31.6(17.2-43.7)	41(21.5-42.9)	44.3(44.2-46.3)	0.000
Total serum IgE, IU/mL	153(125-249)	182(133-195.5)	256(254-264)	0.002
Serum aero-specific IgE, IU/mL	25.9(16.9-35.8)	30.6(28.2-34.7)	34.2(34-36.4)	0.011
Serum food specific IgE, IU/mL	14.5±1.7	14.7 ±2.3	15± 0.32	0.834

DISCUSSION

Atopic dermatitis as a chronic allergic skin disease is precipitated by many consecutive disorders including genetic, immunological and environmental interactions[2]. The majority of these genes are immune cells genes that are associated with neutrophil degranulation as TLR2[27,28].

This study demonstrated that patients with atopic dermatitis (AD) were younger than healthy controls and there was no significant difference in sex distribution. Similarly, Sanyal et al[29] demonstrated the same result. This may due to exposure to different sensitizing allergens and environmental factors in different age group. Other colleagues[27] revealed that females constituted 44.3% of the cases.

Atopic dermatitis severity is determined by many methods to select the suitable treatment, and to monitor the progression[20]. According to SCORAD assessment of patients, and in agreement with the other researches, it was shown that the mean SCORAD index in these patients was 35.22 [27]. Another study recorded a higher SCORAD mean of 69.66 [30]. Regarding the severity and in agreement with the study of Salpietro et al[31] and Can et al[27], moderate cases were the most frequently occurring phenotype. The high prevalence of moderate cases indicating that the disease pathogenesis was controlled by immune system and good response to the therapy.

Family history in the case group of the current research was registered in 33% of the cases and 12% of the control. This is the same result obtained by a previous study [32]. The current results supported the demonstration of other researchers who revealed an association between AD presence and aeroallergen sensitization as extrinsic factors [33]. In consistence with the result concluded by Vaneckova and Bukač[34], our result revealed a higher level of total Ig E (182 IU/ml) and a significant positive correlation with disease severity. This high level indicated that allergens sensitization is a characteristic diagnostics marker of atopic dermatitis [31].

In this study and in agreement with Vaneckova and Bukač[34], the result revealed that inhalant specific IgE level in cases of atopic dermatitis was higher than that in healthy individuals ($p<0.001$). Also, the present work, revealed that house dust mites sensitization was demonstrated in 50% of our patients, followed by timothy pollen as demonstrated previously [8, 11].

Regarding other types of allergens, the current study and in agreement with the research of Zhang et al[11], revealed the presence of food allergen sensitization in 25% of atopic dermatitis patients ($p<0.001$). The food allergens specific IgE level in serum revealed that most patients were allergic to tomato followed by egg. However, a previous study demonstrated that patients with AD did not show increased sensitivity to food in comparison to healthy subjects [31]. This inconsistency might result from the fact that the research studied hospitalized patients with atopic dermatitis, and different population demographic data [35].

A part of pathogenesis process in atopic dermatitis is TLR activation [13]. So, genes changes of TLR may act as a risk factor for development of variable immunological disorders as demonstrated by previous studies [36, 37].

In consistence with the previous research of Zhang et al[11], our result demonstrated that TLR2 G/A polymorphism was more frequently expressed in 48% of patient with atopic dermatitis which was more frequently than healthy group (32%). Also, another study demonstrated that the incidence of SNP was significantly increased in patients than controls [31]. Another previous study reported a lower percentage of 10% [37]. The current study, and agreement with a previous research, demonstrated that G allele occurrence was lower in atopic eczema cases than in control[13].

This study, and in association with the study of Salpietro et al[31] revealed that the GA genotype frequency was significantly increased in atopic dermatitis patients (37.3%). Previous studies reported

the significant role of TLRs polymorphisms in AD in Russian patients [11, 13]. However, one of the studies revealed an absence of association of TLR SNP and atopic eczema [27]. This could be due to the fact of differences in hereditary factors and environmental exposure to different precipitating agents [2].

Regarding the distribution of certain genotypes in different phenotypes of atopic dermatitis and in agreement with the studies of Salpietro et al[31], and Tyurin et al[13], it was demonstrated that A allele was concomitant with severe AD phenotype (P =0.05). However, other researchers revealed that there was no association between TLR polymorphism and severity [38]. This may due to the variation in genetic backgrounds and environmental interaction possibilities in different studies.

The current study is in association with Mrabet-Dahbi.et al[39], who demonstrated that TLR2 Arg753Gln SNP had a specific allergen sensitization pattern. Also, it was demonstrated that the most significant precipitating allergens were *D. farinae, and D. pteronyssinus* due antigenic similarity between *D. pteronyssinus*, and MD2 which is the TLR signaling chaperone[40].

CONCLUSION

In conclusion, the study confirms the claim that patients with atopic eczema had a characteristic genotype (Arg753Gln) of TLR-2. Arg753Gln SNP which is more dominant in severe cases, is associated with sensitization to certain inhalant allergens as house dust mites and pollens.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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