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Toll-Like Receptor-4 Gene (Asp299Gly) polymorphism in allergic conjunctivitis

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Abstract

Allergic conjunctivitis (AC) is an allergic reaction that causes inflammation of the conjunctiva. Toll-like receptors (TLRs) are essential innate immune receptors that contribute to developing various allergic diseases. This case-control study aims to determine the correlation between TLR-4 gene (Asp299Gly) polymorphism and AC incidence and severity. The study included 70 AC patients and 70 non-allergic controls. All included subjects were subjected to a skin prick test, total immunoglobulin E (IgE) measurement, and TLR-4 gene (Asp299Gly) polymorphism detection by PCR restriction fragment length polymorphism (PCR-RFLP) technique**.** AC patients had significantly higher total IgE levels than controls ($P \leq 0.001$). The frequency of the wild-type AA and heterozygous AG genotype were significantly lower in AC patients compared to controls (60 % vs. 80 % and 8.6% vs. 12.9 %, respectively). In contrast, the homozygous mutant GG genotype was significantly more prevalent among AC patients than controls (31.4 % vs. 7.1 %). Furthermore, the wild AA genotype was strongly associated with mild disease (68.2%); nonetheless, the homozygous mutant GG genotype was linked to severe disease (53.8%). The heterozygous AG genotype was only found in moderate AC patients (17.1%). AC patients with the mutant G allele may be more likely to have a severe course of AC.

Keywords: Allergic; Asp299Gly; Conjunctivitis; Polymorphism; Toll like receptor

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Introduction

Ocular allergy (OA) is a hypersensitivity disorder identified with a clinical history of signs and symptoms and in vivo and in vitro allergen testing.[1] Allergic conjunctivitis (AC) is the most common OA, which is usually mediated by the immunoglobulin (Ig)-E. It significantly impacts patient quality of life due to its frequent association with other allergic disorders, particularly rhinitis (rhino-conjunctivitis).[2] AC is divided into two types: seasonal (SAC) and perennial (PAC); with the former being the more frequent, they differ in frequency of

symptoms depending on the sensitizing allergen, affecting 15–20% of the general population.[3,4] Seasonal allergens such as tree or grass pollens induce seasonal symptoms, while indoor allergens like house dust mites, mold spores, cockroaches, and rats cause perennial symptoms.[5] OA intervention requires collaboration between ophthalmologists, dermatologists, pediatricians, and immunologists.[6] According to Bernstein et al., 2008, skin prick tests are used to analyze a panel of common aeroallergens often encountered (house dust mites, mixed grass, mixed pollens, molds, cockroaches, cotton, wool, pigeon feathers, cat hair).[7]

Toll-like receptors (TLRs) in humans are pattern recognition receptors that contribute to the early pathogen detection and subsequent initiation of the innate immune response. They also can influence the cellular immune response that changes the disease vulnerability.[8] TLRs-induced activation of the immune system results in the production of proinflammatory cytokines like tumor necrosis factor-alpha, interleukin (IL)-1, and IL-6, as well as regulatory cytokines (IL-12 and IL-18), leading to T-helper (Th)1 differentiation and reduction in Th2 response.[9] TLR-4 is expressed in both the epithelium and the stroma of the conjunctiva, and its activation results in cytokine production.[10] Bonini et al., 2005, compared the expression of TLR2, 4, 9 in conjunctival specimens of AC patients and patients with cataract. The study revealed that the expression of TLR-4 was significantly increased in patients with AC and was localized in eosinophils, CD4+ve T-cells, and mast cells.[11]

TLRs were investigated to find links between their variations and the incidence of allergic diseases.[12] TLR genes demonstrate many differences in human subjects, with ten different TLRs identified in humans may be involved in the development of allergic diseases.[13] However, the impact of these genetic differences on immunological responses or the risk of immune-mediated illnesses remains under investigation.[14]

Single nucleotide polymorphisms (SNPs), the most common alterations in the human genome, have been widely used to detect their association with disease susceptibility in various allergic disorders.[15] Previous studies have not found conclusive evidence for an association between genetic variations of TLR-4 and AR.[16-18] However, in 2000, Arbour et al., reported that the TLR-4 (Asp299Gly) SNP is linked to airway hypo-responsiveness in humans.[19] In addition, numerous studies have found a link between the TLR-4 (Asp299Gly) SNP and asthma, particularly atopic asthma.[20-22] To the best of our knowledge, studies to assess the genetic variations of TLR-4 and AC are lacking. Consequently, the current study aims to assess the role of TLR-4 (Asp299Gly) SNP in AC susceptibility and severity.

Subjects and Methods

Study design and subjects

In this case-control study, patients were recruited from Zagazig and Ain Shams Universities hospitals in the period between January and March 2021.

Since no previous studies have been conducted to assess the genetic variations of TLR-4 and AC, we depended on data of Böttcher et al., 2004, who studied the TLR4 (Asp299Gly) SNPs in the presence of asthma and allergic rhino-conjunctivitis (ARC) in children.[37] We assumed that the percentage of AA genotypes in the case group versus the control group was 81% versus 97%, with a 95% confidence and a power level of 80%. In addition, the total sample size calculated by Open Epi was 140, 70 in each group.

The control group included 70 non-allergic subjects, and the AC group included 70 patients with SAC or PAC without any corneal involvement as detected by slit-lamp microscopic examination. AC patients had a positive skin prick test for common allergens with a typical response to topical therapy (antihistamines or mast cell stabilizers). Patients with eczema, a history of infectious conjunctivitis (e.g., viral) in the past few months, non-allergic causes of conjunctivitis (e.g., superior limbic kerato-conjunctivitis, drug toxicity conjunctivitis, and cicatricial conjunctivitis) were excluded from the study. Contact lens wearers were also excluded.

All participants were subjected to the following procedures: 1) History taking, which included age, gender, residence, occupation, and family history of atopy. 2) Complete ophthalmological examination to determine AC severity, according to the 2020 Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines [23]. 3) Skin prick testing (SPT): A week prior to the skin testing, subjects were urged to stop taking antihistamines. The positive control was histamine dihydrochloride (10 mg/ml), while the negative control was saline. The diameter of the largest developed wheal was measured and termed positive if it was ≥ 3 mm.[24] One drop (0.05 mL) of each allergen extract was pricked into the skin. Aqueous allergen extracts (1:100 wt./vol) for skin testing were manufactured locally in the Allergy and Immunology Unit, Faculty of Medicine, Ain Shams University, according to guidelines developed by the American Academy of Allergy, Asthma & Immunology (AAAAI).[25]

Blood sample collection

From each participant, 4 mL of blood was collected via venipuncture under strict aseptic conditions using two vacutainer tubes: an EDTA-containing tube that was stored at -80 °C until further processing for PCR restriction fragment length polymorphism (PCR-RFLP) of the TLR-4 gene, and a plain tube for serum separation by centrifugation at 1000 ×g for 15 minutes. Sera were stored at-20°C until used for analysis of total IgE level.

Total IgE serum level analysis by ELISA

A commercial human sandwich ELISA Kit (EUROIMMUN, Germany; Cat No. EV 3840-9601 E) was used to measure serum total IgE levels, according to the manufacturer's instructions. A microtiter plate ELISA reader (Biotek, USA) was used to measure the absorbance of standards and samples at 450 nm. The upper reference range for normal subjects of 10-15 years old was 199 IU/ml, while the upper reference range for participants of > 16 years old was 100 IU/ml.

Determination of TLR-4Asp299Gly SNP (rs4986790) by PCR-RFLP

-PCR for the amplification of TLR-4 gene: The PCR was performed using a Phusion™ Blood Direct Master Mix kit (pub no. MAN0012900 Thermo Scientific™, USA) which was designed to perform PCR directly from whole blood with no prior DNA extraction or sample preparation according to the manufacturer's instructions using primers for human TLR-4 gene:[26] (forward primer 5'dAGCATACTTAGACTACTACCTCCAT 3'; Reverse primer: 5'-d GAGAGATTTGAGTTTCAAT GTGGG-3'. The following are the PCR cycling conditions in the thermal cycler (Biometra, Germany): Cell lysis at 98°C for 5 minutes, then 40 amplification cycles of denaturation, annealing, and extension (at 98°C for 5 minutes, 58°C for 30 seconds, and 72 °C for 30 seconds, respectively); finally, a final extension cycle at 72°C for 1 minute.[27] After amplification, the PCR reaction product was centrifuged at 1000 ×g for 3 minutes to collect the supernatant for analysis. Then, 10 µL of PCR product and 5 µL of O'GeneRuler Express DNA Ladder (100-1000 bp) were directly loaded directly in a 1.5% agarose gel (BioShop Canada Inc., Burlington, Ontario, Canada). The amplified DNA product was separated by electrophoresis and visualized under ultraviolet light by ethidium-bromide staining.

-Restriction fragment length polymorphism -(RFLP): The Fast Digest *Nco*1 (Thermo Scientific™, USA) restriction enzyme kit (Nco1; Thermo Scientific™, USA) was used to treat the resulting PCR product of the TLR-4 gene (188bp) according to the manufacturer's instructions. *Nco*1 recognizes 5'-CCATGG sites and cleaves between the first and second nucleotides. The digested products were then loaded into a 2% agarose gel and analyzed by electrophoresis. The product was visualized on an Ultra-Violet Transilluminator (Syngene, Frederick, Maryland, USA) and interpreted as follows: The wild AA genotype of the TLR-4 gene was indicated by an uncut band at 188 bp, while the mutant GG genotype was indicated by bands at 168 and 20 bp. The heterozygous AG genotype was indicated by bands at 188, 168 bp and 20 bp.

Statistical analysis

Data were analyzed using the Statistical Package of Social Science (SPSS) for Windows (Standard version 20). The Shapiro-Wilk test was used to determine the normality of data. We described

data using numbers and percentages (qualitative) or means and standard deviations (quantitative parametric). Comparisons were made using the Mann-Whitney U and Kruskal-Wallis tests (in quantitative non-parametric groups of data) and the Chi-square test for groups of qualitative types of data. Significance was set at $P < 0.05$.

Results

This study included 70 non-allergic subjects in the control group and 70 patients in the AC group. The mean age was 11.92 ± 4.04 years and 12.71 ± 4.78 years for the AC and control groups, respectively. While the AC group consisted of 64.3% males and 35.7% females, the control group included 61.4% males and 38.6% females. We found no significant differences between the two groups regarding age and gender.

The AC group showed a significant association ($P = 0.001$) with a family history of allergy, 68.6% of AC patients had a positive family history of allergy compared to 32.9% of the control group. There were higher levels of total serum IgE in the AC group than the control group (249.21 ± 60.25 IU/ml vs. 168.53 ± 37.64 IU /ml; $P \leq 0.001$). With respect to residence and socioeconomic status, no significant differences between the AC patients and controls were observed.

Homozygous wild-type AA was the most prevalent genotype in both groups, with a statistically significant lower expression in the AC group compared to the control group (60% vs. 80%; $P = 0.001$). Also, the heterozygous AG genotype expression was significantly lower in the AC group than the control group (8.6% vs. 12.9%; $P = 0.001$), but the mutant homozygous GG genotype was significantly higher (31.4% vs. 7.1%; $P = 0.001$**),** Table 1.

Table 1. Comparison between allergic conjunctivitis patients and the control subjects.

	AC Patients (N=70) N (%)	Controls (N=70) N (%)	*P value
Age (Years)	11.92 ± 4.04	12.71 ± 4.78	NS
Gender			
Male	45 (64.3%)	43 (61.4%)	NS
Female	25 (35.7%)	27 (38.6%)	
Residence			
Urban	39 (55.7%)	48 (68.6%)	NS
Rural	31 (44.3%)	22 (31.4%)	
Socioeconomical			
Student	32 (45.7%)	28 (40%)	
Unemployed	3 (4.3%)	7 (10%)	
Housewife	11 (15.7%)	9 (12.9%)	NS
Office Worker	10 (14.3%)	13 (18.6%)	
Agricultural Workers	11 (15.7%)	5 (7.1%)	
Educationist	3 (4.3%)	8 (11.4%)	
Family History Of Allergy	48 (68.6%)	23 (32.9%)	0.001
Total IgE (IU/ml)	249.21 ± 60.25	168.53 ± 37.64	≤ 0.001
TLR-4 Genotype			
AA	42 (60%)	56 (80%)	
AG	6 (8.6%)	9 (12.9%)	0.001
GG	22 (31.4%)	(7.1%)	

*P value > 0.05 is not significant (NS).

Of the AC patients, 55.7% were PAC type and 51.4% had a history of rhinitis. The most frequently reported symptoms were itching (100%), tearing (85.7%), and redness (82.9%). Regarding severity, 50% showed persistent moderate severity, 31.4% had persistent mild disease, and only 18.6% were severely persistent, Table 2.

Table 2. Characteristics of the allergic conjunctivitis (AC) patients.

	AC PATIENTS	
	N	%
Type of allergy		
SAC	31	44.3%
PAC	39	55.7%
Associated allergy (%)		
Rhinitis	36	51.4%
Asthma	21	30%
Urticaria	16	22.9%
None	11	15.7%
Symptoms		
Itching	70	100%
Redness	58	82.9%
Tearing	60	85.7%
Foreign body sensation	44	62.9%
Eyelid edema	29	41.4%
Ocular dryness	37	52.9%
Blurred vision	28	40%
Photophobia	13	18.6%
Severity score		
Mild persistent	22	31.4%
Moderate persistent	35	50%
Severe persistent	13	18.6%

PAC: Perennial Allergic Conjunctivitis; SAC: Seasonal Allergic Conjunctivitis

AC patients with different disease severity showed significant differences in the skin prick test ($P \leq 0.001$). The most frequently positive skin tests (58.6%) were attributed to mixed pollen in all AC patients. Positive skin tests were due to mixed grass (50%) and mites (45.5%) in mild AC cases. In the moderate AC group, mixed pollens (68.6%) and mites (60.0%) were the most common associated allergens. Severe AC patients showed reactions to mixed pollen (76.9%), molds (23.1%), pigeon feathers (23.1%), and cotton (23.1%) as determined by skin tests, Table 3.

Table 3. Comparison of the skin prick test reactions according to allergic conjunctivitis severity.

	Total (N=70)		Mild(N=22)		Moderate(N=35)		Severe(N=13)		*P value
	N	%	N	%	N	%	N	%	
Skin prick test									
Mixed pollens	41	58.6%	7	31.8%	24	68.6%	10	76.9%	
Mixed grass	27	38.6%	11	50%	16	45.7%	0	--	
Molds	19	27.1%	4	18.2%	12	34.3%	3	23.1%	
Wool	12	17.1%	8	36.4%	4	11.4%	0	--	
House dust mites	31	44.3%	10	45.5%	21	60%	0	--	≤ 0.001
Cat hair	4	5.7%	4	18.2%	0	--	0	--	
Pigeon feather	11	15.7%	4	18.2%	4	11.4%	3	23.1%	
Cotton	7	10%	0	--	4	11.4%	3	23.1%	
Cockroach	11	15.7%	4	18.2%	7	20%	0	--	

*P value < 0.05 is statistically significant.

TLR-4 genotype expression was significantly different (*P*=0.049) between severity groups of AC patients. While the wild AA genotype was the most prevalent in mild AC patients (68.2%), the homozygous mutant GG genotype was more prevalent in severe patients (53.8%). The AG genotype was only found in moderate AC patients (17.1%), Table 4 & Figure 1**.**

Table 4. TLR-4 genotypes according to the severity of allergic conjunctivitis.

		Severity score			*P value
		Mild	Moderate	Severe	
TLR-4 genotype	AA	15 (68.2%)	21 (60%)	6 (46.2%)	0.049
	AG	0	6 (17.1%)	0	
	GG	7 (31.8%)	8 (22.9%)	7 (53.8%)	

*P value < 0.05 is statistically significant.

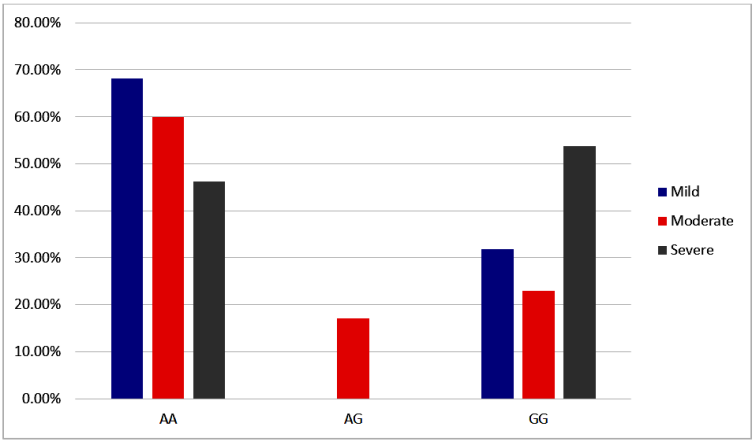

Figure 1. The frequency of the various TLR-4 genotypes according to the severity of allergic conjunctivitis (*P*=0.049). The wild AA genotype was the most prevalent among the mild AC patients (68.2%), whereas the homozygous mutant GG genotype was more prevalent in severe patients (53.8%). The heterozygous AG genotype was only found in moderate AC patients (17.1%).

In terms of AC types, there was a significant gender difference between the PAC and SAC groups of patients, with males being more significantly affected by PAC (53.8%; *P*= 0.041), while 77.4% of females by SAC. Residents of urban areas were more significantly affected by PAC (74.4%; *P* ≤ 0.001), while those in rural regions were more affected by SAC (67.7%). Subjects presented with PAC showed a significantly higher association with a family history of allergy (84.6%; *P*= 0.001), lower levels of total serum IgE (222.05 ± 42.01IU/ml IU/ml; *P* ≤ 0.001), and higher moderate persistent severity (71.8%; *P* ≤ 0.001). Such association was not observed for SAC patients. However, severe persistent cases were only found in 41.9% of the SAC group of patients. With regard to the TLR-4 genotype, the wild AA genotype was the most prevalent in both PAC and SAC groups, 46.2% and 77.4%, respectively, Table 5**.**

Table 5. Comparison of the characteristics of allergic conjunctivitis patients according to the type of allergy (PAC and SAC).

	PAC patients (N=39) N (%)	SAC patients (N=31) N (%)	*P value
Age (years) mean ±SD	11.69 ± 3.67	12.21 ± 4.51	NS
Gender			
Male	21 (53.8%)	24 (22.6%)	0.041
Female	18 (46.2%)	7 (77.4%)	
Residence			
Urban	29 (74.4%)	10 (32.3%)	≤ 0.001
Rural	10 (25.6%)	21 (67.7%)	
Socioeconomical			
Student	16 (41%)	16 (51.6%)	
Unemployed	3 (7.7%)	0	
Housewife	11 (28.2%)	0	0.002
Office Worker	6 (15.4%)	4 (12.9%)	
Agricultural Workers	3 (7.7%)	8 (25.8%)	
Educationist	0	3 (9.7%)	
Positive Family History Allergy	33 (84.6%)	15 (48.4%)	0.001
Total IgE (UI/ml) Mean ±SD	222.05 ± 42.01	283.39 ± 62.83	≤ 0.001
Severity Score			
Mild Persistent	11 (28.2%)	11 (35.5%)	
Moderate Persistent	28 (71.8%)	7 (22.6%)	≤ 0.001
Severe Persistent	0	13 (41.9%)	
TLR-4 Genotype			
AA	18 (46.2%)	24 (77.4%)	
AG	6 (15.4%)	0	0.011
GG	15 (38.5%)	7 (22.6%)	

*P value > 0.05 is not significant (NS).

When comparing the characteristics of AC patients based on their TLR-4 genotype, no significant differences were observed in terms of age, gender, residence location, or family history of allergies. Only total serum IgE levels were significantly higher in homozygous mutant GG patients (274.55 69.13 IU/ml; *P* = 0.004) than in homozygous wild-type AA (245.12 ± 51.89 IU/ml) and heterozygous AG patients (215.7 83.3 IU/ml), Table 6**.**

Table 6. Comparison of the characteristics of allergic conjunctivitis patients according to the TLR-4 genotype.

	AA patients (N=98) N (%)	AG patients (N=15) N (%)	GG patients (N=27) N (%)	*P-value
Age (years) mean ±SD	11.15 ± 3.31	13.25 ± 2.46	13.02 ± 5.27	NS
Gender				
Male	63 (64.3%)	9 (60%)	16 (59.3%)	NS
Female	35 (35.7%)	6 (40%)	11 (40.7%)	
Residence				
Urban	61 (62.2%)	10 (66.7%)	17 (63%)	NS
Rural	37 (37.8%)	5 (33.3%)	10 (37%)	
Socioeconomical				
Student	41 (41.8%)	6 (40%)	13 (48.1%)	
Unemployed	7 (7.1%)	2 (13.3%)	1 (3.7%)	
Housewife	14 (14.3%)	2 (13.3%)	4 (14.8%)	NS
Office worker	16 (16.3%)	2 (13.3%)	5 (18.5%)	
Agricultural workers	13 (13.3%)	1 (6.7%)	2 (7.4%)	
Educationist	7 (7.1%)	2 (13.3%)	2 (7.4%)	
Family history of allergy	50 (51%)	7 (46.7%)	13 (48.1%)	NS
Total IgE (IU/ml) mean ±SD	245.12 ± 51.89	215.8 ± 43.35	274.55 ± 69.13	0.004

*P value > 0.05 is not significant (NS).

Discussion

AC is a prevalent hypersensitivity disorder affecting up to 40% of the population worldwide. Itching, redness, and swelling are common symptoms which have a substantial impact on patients' quality of life. According to the duration of allergen exposure, OA is classified into seasonal and perennial forms. The seasonal form is based on the seasonal presence of a specific aeroallergen, while the perennial form is based on continual exposure to an allergen such as house dust or pet dander.[28] The current study was conducted on 70 AC patients and 70 non-allergic control subjects with the aim of investigating the association between the TLR-4 (Asp299Gly) gene polymorphism and patients' susceptibility to AC.

In the present study, the AC group comprised 64.3% males and 35.7% females and showed a significant association with family history of allergy (68.6%). Similarly, Haggag et al., 2017, reported male predominance (53.1%), and a significant association with positive family history (62.5%) in their included AC patients. They also found that 25% of cases were presented with AC alone, while 31.3% had associated AR (rhino-conjunctivitis), and 21.9% presented with an allergy triad, which means that rhino-conjunctivitis are associated with asthma.[29]

Furthermore, Oliveira et al., 2007, observed that 50% of their study subjects demonstrated a positive family history of atopy and 75% of cases had other atopic associated disorders, with AR (70%) and asthma (35%) being the most predominant ones.[30] In addition, in a study by Arej et al., 2018, the majority of AC cases lived in cities (72.7%) and 29.5% of them were students. Associated allergies (rhinitis, asthma or urticaria) were found in more than 70% of AC patients included in their study.[4]

In our study, mixed grass (50%) and mites (45.5%) were the most common allergens linked to positive skin tests in mild AC patients. On the contrary, in the moderate AC group, mixed pollens (68.6%) and mites (60%) were the most common related allergens. Skin testing in severe AC patients only reacted to mixed pollens (76.9%), molds (23.1%), pigeon feathers (23.1%), and cotton (23.1%). This is in line with the findings of Rasheed et al., 2015, who reported that the most prevalent offending allergens were Bermuda grass pollens, followed by animal dander, mites, and molds.[30] Also, according to Haggag et al., 2017, the most common allergens that resulted in a positive skin prick test were house dust mites, mold, and cockroaches (96.9%), followed by grass pollen (87.5%).[29]

Pruritus is the most prevalent symptom of AC, and its severity can range from moderate to highly debilitating. Besides, it can be painful in rare cases. Tearing, redness, a gritty sensation, mucous discharge, and swelling of the eyelids are some of the other symptoms.[31] The symptoms are typically bilateral and linked to rhinitis. In severe cases, blurred vision and photophobia were recorded.[32] Likewise, the most frequently reported symptoms of AC patients in our study were itching (100%), tearing (85.7%), and redness (82.9%).

In our study, 55.7% of AC patients were of the PAC type, 51.4% had a history of rhinitis, and 50% with moderate persistent severity. Our AC patients had higher levels of total serum IgE (249.21 ± 60.25 IU/ml) compared to the control group (168.53 ± 37.64 IU/ml; $P \leq 0.001$). Moreover, there was a significant difference between SAC and PAC patients as it was significantly lower in the PAC group (222.05 ± 42.01 IU/ml) than SAC group (283.39 ± 62.83 IU/ml; $P \leq 0.001$). This is partially similar to a study by Gurses et al., 2009, in which 32% of the included AC patients were diagnosed as PAC, and 68% diagnosed as SAC. The mean levels of total serum IgE of their controls and AC patients were 36.36 IU/ml and 227.40 IU/ml, respectively.[33] Furthermore, Arej et al., 2018, found a four-time predominance of SAC (80%) over PAC (20%; P=0.002) in their study patients, and significantly higher levels of total serum IgE.[4] Mimura et al., 2011, demonstrated that the high total serum IgE levels were correlated with the total tear IgE score and specific serum IgE levels in OA patients.[34]

Increased cytokine synthesis after TLR stimulation can lead to inflammation and allergies. Involvement of innate immunity, specifically TLRs, induces diversity in the mechanism of genesis and clinical manifestations of allergic reactions, particularly in IgE-independent variation.[35] Consequently, SNPs in the TLR-4 gene may be linked to atopic disorders.[33] TLR-4 (Asp299Gly) has been identified in humans and was found to be associated with hypo-responsiveness to inhaled allergens.[19]

In 2013, Levchenko et al., investigated the frequencies of several TLR gene SNPs, including the TLR-4 896A/G SNP in children with atopic dermatitis (AD) against controls. They found that the TLR-4 gene's mutant 896G allele was more frequently associated with AD in children who were susceptible to acute respiratory viral infections (ARVIs) compared to the control group (P = 0.038). In the control group, the frequencies of TLR-4 genotypes were 96.3%, 3.7% and 0.0% for the wild-type AA, the heterozygous AG and the mutant GG genotypes, respectively. While in AD children who were predisposed to frequent ARVIs, their frequencies were 85.19%, 11.11% and 3.7%, respectively.[36]

In contrast, Böttcher et al., 2004, who studied the TLR4 (Asp299Gly) SNPs in the presence of asthma and ARC in children, revealed that the frequencies of the wild AA and the heterozygous AG genotypes in children with asthma were 23% and 55%, respectively (P=0.03), while their frequencies in children

with rhino-conjunctivitis were 22% and 36%, respectively ($P > 0.05$). They also found that TLR-4 (Asp299Gly) SNP was only among children with atopic asthma and not with ARC.[37] On the contrary, a previous study by Raby et al., 2002 which included a heterogeneous North American and Canadian cohort, showed no association between genetic variation in the TLR-4 gene and asthma susceptibility.[38]

The TLR-4 SNP was not associated with positive SPT or atopic illnesses other than asthma. However, Böttcher et al., 2004, reported that the TLR-4 SNP was only present in asthmatic children who were SPT-positive, indicating that the TLR-4 SNP may be linked to atopic children's vulnerability to asthma but not associated with the development of atopic reactivity and IgE production and reinforcing mechanistic ideas based on immune cell polarization. Consequently, the alternative TLR-4 activated pathways could be involved in the development of atopic asthma.[37] TLR-4 expression was low in the human airway epithelium of people with the Asp299Gly mutation, suggesting that the SNP had local consequences in the airways,[19] and the TLR-4 Asp299Gly variant was linked to a higher incidence of infections that lead to the development of atopic diseases.[39] Previous research has found a correlation between environmental variables and AR when TLR-4 SNPs are considered. However, no evidence was found to demonstrate that SNPs in TLR genes contribute to the development and prognosis of AR and ARC.[40]

Based on the current study findings, we may conclude that TLR-4 (Asp299Gly) SNP may be associated with AC in which homozygous mutant GG genotype presence has higher odds of severe course. Specific sensitization, in combination with genetic SNPs in innate immunity pathways, could act as triggers for AC.

Author Contributions

SAB and SIT; performed the laboratory work. DES, AIAA and SHF; made the statistical analysis. MAH; examined the patients. SAB, DES, SHF and MAH; collected samples. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board (IRB) (approval no. 6877-13-12-2020) at Zagazig University.

Informed consent

At the time of recruitment, all subjects (or guardians of children, less than 18 years of age) signed an informed written consent.

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