CD14 promoter polymorphisms associated with different allergic phenotypes and modulated by house dust mite allergy

Original Article

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ABSTRACT

Background: The house dust mite (HDM) constitutes a major cause of allergic disease all over the world; meanwhile interaction between genetic control, environmental factors in the context of allergen exposure may affect allergic phenotype. Cluster of differentiation 14 (CD14) polymorphisms play a major role in genetic control of allergic phenotypes.
Objective: We aimed to assess the role of CD14 genetic polymorphisms at two loci A(-1,145)G, and G(-1,359)T in expression of atopic asthma and allergic rhinitis in the context of HDM exposure in Jazan, KSA.
Subjects and Methods: Through a case control study, 160 subjects served as 60 atopic asthmatic patients, 40 allergic rhinitis patients and 60 healthy non-allergic controls. Clinical and immunological parameters for the studied subjects were assessed. Then, genotyping of two single nucleotide polymorphisms (SNPs) at A(-1,145)G, and G(-1,359)T, in the promoter region of the CD14 gene was conducted using restriction fragment length polymorphisms (RFLP-PCR).
Results: The present study showed that in HDM sensitive subjects there was a significant association between GG genotype variant at A(-1,145)G with atopic asthma patients and another significant association between TT genotype variant at G(-1,359)T with allergic rhinitis patients.
Conclusion: The impact of allergy induced by HDMs may be enhanced in individuals with specific CD14 gene variants resulting in exaggerated allergic phenotype.

Keywords: allergic rhinitis, atopic asthma, CD14 gene polymorphism, house dust mites.

Received: 5 February 2019, **Accepted:** 23 February 2019 .

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Print ISSN: 1687-7942, **Online ISSN:** 2090-2646**, Vol. 12, No. 1, April, 2019.**

INTRODUCTION

House dust mites (HDMs), are tiny microscopic Acarida. The most predominant HDM isolated from dust samples are *Dermatophagoides pteronyssinus* (*D. p*) and *Dermatophagoides farina* (*D. f*). Dust mites thrive at a temperature of 20-25°C and humidity levels of 70-80%. Most mites die in low humidity levels or extreme temperatures, leaving the dead bodies and waste behind, predisposing to allergic reactions[1]. More than twenty different allergens that elicited specific IgE responses in sensitized patients were identified in both species[2]. These allergens are categorized into four groups: proteases, proteins that possess affinities for lipids, non-proteolytic enzymes, and non-enzymatic components[3]. They live in household dust and are recognized as one of the most common air born allergens sources all over the world. A record of more than 15-20% of the population from industrialized countries are affected by HDM sensitization[4]. Allergic sensitization is defined by IgE production against environmental antigens such as HDMs, grass pollen, and animal proteins which can lead to diseases including asthma, rhinitis, and atopic dermatitis[5].

Asthma is a complex disease in which genetic and environmental factors both lead to the diseased state[4]. Pathogen associated molecular patterns (PAMPs) are complexes present in pathogens but absent in the host[6]. During an infection, PAMPs are recognized by macrophages via toll-like receptors (TLRs). Stimulation of TLRs causes dendritic cell activation that establishes contact between peripheral and lymphatic tissues; which in turn activates the T cells, this being the most important part of the acquired immune system[7]. Experimental evidences suggest that HDM allergen specific Th2 cells play a key role in the allergic inflammatory response which leads to allergen specific IgE production, the drafting of eosinophils in tissues, the permissiveness of endothelium for the recruitment of inflammatory cells to inflamed lungs, the reproduction of mucous, and the modulation of the airway smooth muscle contraction[8].

It is becoming clear that as compared to the single gene expression, the complex gene interaction with the environment enhances the allergic reaction and disease with variable degrees[2]. Polymorphisms

 DOI: 10.21608/puj.2019.7917.1031

within innate immunity genes are associated with different allergic phenotypes, with variable results[9]. A multifunctional receptor CD14 is present on macrophages and monocytes where it binds with endotoxin and other bacterial wall components then facilitates the lipopolysaccharide presentation to TLR4 and thus promote immune activation[10]. The gene encoding CD14 seems to be among one of many genes that add to the allergic phenotype expression because it is localized on chromosome 5q31.1; a region which is linked to both asthma and total serum IgE concentration[11]. CD14 exists as a single-copy gene, having its protein in two distinct forms: a 55-kDa membrane molecule (mCD14) expressed primarily on the surface of monocytes/macrophages, dendritic cells and neutrophils, and a soluble form (sCD14) in serum[12]. Furthermore, Vercelli *et al.,*[13] characterized five SNPs (at positions -1,619, -1,359, -1,145, - 809, and -159) in the promoter of the gene encoding CD14. The effect of the CD14 -159 genotype (CC, TT or CT) on the asthma phenotype in terms of total IgE levels, can also be different. Numerous studies have been conducted to investigate the relationship between CD14 variants and total IgE levels[14-16]. It is noted that carriers of the -1,359T/-1,145A/-159C haplotype had the highest levels of IgE, and the lowest levels of sCD14 and, conversely, carriers of the -1,359G/-1,145G/-159T haplotype had the highest levels of sCD14 and the lowest IgE values[13].

Exposure to endotoxins may trigger allergic disease, but the genetic factors may affect the degree of response[17,18]. Similarly the atopic condition of the host may modulate the effect of endotoxins. It has been found that during allergy development, the genetic variants C-159T, C-260T and C-1,721T within CD14 interact with environmental factors[19]. A CD14/-159 CC genotype was associated with increased specific bronchial hyper-reactivity to *Der p* 1 allergen and higher concentrations of HDM specific IgE[15].

The aim of our study is to assess the role of CD14 A(-1,145)G, and G(-1,359)T polymorphisms in the expression of atopic asthma and allergic rhinitis in the context of HDM exposure in Jazan, KSA.

SUBJECTS AND METHODS

Through a case control study, a total of 160 individuals were enrolled, serving as 100 patients: 60 with atopic asthma, and 40 with allergic rhinitis, and 60 non-allergic healthy controls. The study subjects were Saudi citizens recruited from three Jazan general hospitals (Prince Bin- Nasser, Abu-Erish, and Sabia) during the period between September, 2017 to September, 2018.

Subjects: Selection of patients was based on the complaint of recurrent dyspnea, cough, wheeze, chest tightness, history of short attacks of breathlessness and allergic symptoms or a history of allergy. Patients who were suffering from upper or lower respiratory tract infections at the time of the study or who were on steroid therapy, on specific allergen immunotherapy or those who had a personal or family history of tuberculosis, were excluded. Asthma was diagnosed by the physician according to the global initiative for asthma (GINA) guidelines[20]. Allergic rhinitis patients had symptoms of sneezing, rhinorrhea, nasal obstruction, runny nose and/or itchy nose.

Clinical and laboratory evaluation: All subjects completed a uniformly structured detailed questionnaire to obtain their sociodemographic and clinical data. In addition, an objective evaluation of bronchial responsiveness and involvement was performed by a spirometric test to assess forced expiratory volume in one second (FEV1)[21].

Environmental exposure: Dust samples were collected from all subjects' houses. Dust of one meter square of the carpet, armchair, sofa, or other upholstered piece of furniture in the bedroom for 2-3 min was obtained using portable vacuum cleaner 300 W. Presence of HDM was determined by sieving of 1 gm house dust sample through 300 mesh brass sieve of 6 mm diameter to remove large particles and fibrous materials. Then mites were isolated from the dust samples in a 5 cm glass Petri dish under a dissecting microscope 20x with the aid of a fine camel brush (000)[22]. Positive cases selected were those with positive house dust specimen, while control subjects who had a positive specimen were excluded and replaced with another who had a negative specimen.

Measurement of *Der p* 1 allergen concentrations[16]: Evaluation in house dust samples were done using ELISA kits for antigen quantitation (INDOOR Biotechnologies Inc., Charlottesville, VA, USA) according to manufacturer's instructions.

HDMs allergy evaluation: All atopic patients were subjected to skin prick testing for responsiveness to the following aeroallergens (HDM, mould mix, grass pollen mix, thresher dust, paper mulberry and mixed food), (Allergopharma, Reinbek bei Hamburg, Germany). Positivity was identified as presence of a reaction to one or more aeroallergens with a wheel diameter ≥ 3 cm. Measurement of total IgE[16] was done for all studied subjects by ELISA test kit (Human Gesllschaft for biochemical and diagnostic, Max Plank, and Germany) following the manufacturer's instructions. Samples absorbance were read using microtiter plate reader at absorbance of 450/620 nm. Samples were tested in duplicate to ensure reliability, under strict sterile conditions. Measurement of IgE specific to *Der p* 1[16] was measured by ELISA test kit (Astra Biotech GmbH Rudower Chaussee 29 12489 Berlin, Germany) according to manufacturer's instructions.

Genotyping of CD14 promoter region polymorphism by PCR-RFLPs[23]: Genomic DNA was isolated from peripheral blood cells, and DNA was extracted from the whole blood using a DNA extraction kit, (QIA amp DNA Mini kit (Cat. No. 51304; Qiagen Inc, Valencia, CA), following the manufacturer's instructions. Genomic DNA was quality checked by agarose gel electrophoresis analysis, quantified spectrophotometrically, and stored at -80°C. Genotyping of CD14 promotor region was performed by PCR-RFLPs as follows: the polymorphism A(-1,145)G was typed using a sense (5'-CTCAGGAATCTGAGGCAAGA-3') and reverse (5'-AGTACAATCTCTGTGCCCTA-3') primer pair (Table 1).

The PCR amplification was performed in 25 μ reaction volume containing 12.5 μ 2x PCR master mix; 2x PCR buffer, 3 mM $MgCl_2$, 0.5 unit Taq DNA polymerase/μl, 400 µM of each dNTP, 1 μ of each primer (10 pmol), 2.5 μl of genomic DNA, and 8 μl sterilized nuclease free water. The reaction was performed in a Gene Ampl.9700 (A Hybaid thermal cycler, Promeg Corporation 2008 Woods Hollow Road Madison, W11 53711–5399, USA). The program was as follows: initial denaturation at 94°C for 5 min, 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 56° C and 1 min extension at 72° C, and a 5 min final extension at 72° C. The amplified product was detected in 2% agarose gel as a single band at 371 bp in length. Following PCR, the product was digested with HPYCH4V (Cat. NO. R0945) for 1 h at 37 °C. The product yielded bands of 300 bp in GG homozygotes, 230 bp in AA homozygotes and the two bands in heterozygotes.

The polymorphism G(-1,359)T was typed using the same primer pair as for A(-1,145)G. After PCR, the product was digested with 2 µ FOK1 (New England Bio Lab) for 1 h at 37°C. The PCR product was 371 bp in length. Digestion of the PCR product yielded bands of 178 and 146 bp in GG homozygotes, 146, 112, and 66 bp in TT homozygotes, and all bands in heterozygotes.

Statistical analysis: Statistical analysis was done using SPSS V16 (SPSS Inc, Chicago, USA). Genotype frequencies were determined, and comparisons of the frequencies were performed by Chi square test (χ^2) using 2·2 frequencies. Comparison between cases and controls were calculated using χ^2 test. Allele odds ratio (OR) and confidence intervals (CI) were computed. *P* value was considered statistically significant when less than 0.05.

Ethical considerations: Informed written consent was provided for each participant to sign, after being informed with the aim of the study. The study protocol was approved by ethical committee of the medical research center Jazan University.

RESULTS

Clinical and immunological characteristics of study population: A total of 160 Saudi subjects were enrolled in this study, they were classified as 60 atopic asthmatic patients, 40 patients with allergic rhinitis and 60 age and sex matched healthy controls. Recovery of HDMs from dust specimen from atopic patients was proved (Figure 1).

It was found that about 92% of atopic asthmatics and 80% of allergic rhinitis patients showed positive skin prick test to at least one allergen, meanwhile 12% of control showed positive skin prick test to allergens other than HDM. Spirometric assessment for the study population revealed lower predicted percentage of forced expiratory volume 1 (FEV1) in atopic asthmatics and allergic rhinitis patients compared to control group. Statistical difference for total IgE level was significant when comparing atopic asthma to control (P<0.001) and when comparing allergic rhinitis to control (P=0.03) (Table 2).

Genotype frequency of the CD14 promoter region: Frequencies of transition at nucleotide A to G at position -1,145 A(-1,145)G and a G to T at position -1,359 G(-1,359)T from the transcription start site in the promoter region of the CD14 gene were assessed in each of the study subjects using RFLP-PCR (Figures 2-5). Table (3) shows difference between atopic patients and

Table 1. Primers and restriction enzymes used for determination of polymorphisms of the gene encoding CD14.

Polymorphism	Primers	Enzyme	PCR product size
A (-1,145) G	'5-CTCAGGAATCTGAGGCAAGA-'3 5'-AGTACAATCTCTGTGCCCTA-'3	**HPYCH4V**	**371 bp**
G (-1,359) T	5'-CTCAGGAATCTGAGGCAAGA-'3 5'-AGTACAATCTCTGTGCCCTA-'3	**FOK1**	**371 bp**

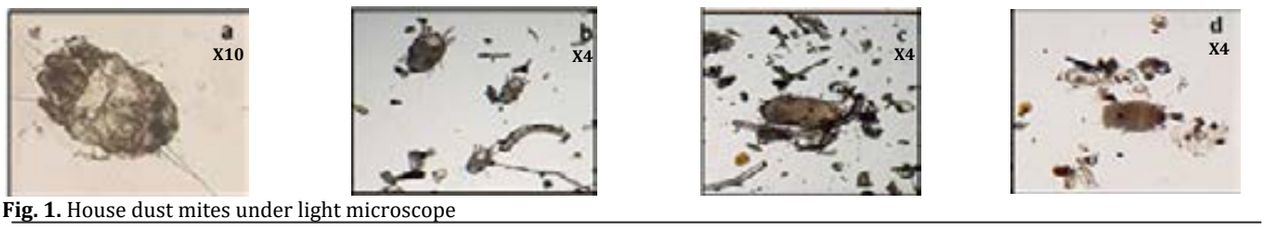

Fig. 1. House dust mites under light microscope

Table 2. Comparative characteristics of atopic asthma, allergic rhinitis and control subjects.

Characteristic	Patients		Control (60)	Statistical analysis *P* value
	Atopic asthma (60)	Allergic rhinitis (40)		
Age (years) (Mean ± SD)	18.6±7.9	20.4 ± 5.3	19.4 ± 1.7	Asthma:0.44, Rhinitis:0.17
Gender (M/F) No. (%)	27/33 (45/55%)	18/22 (45/55%)	38/32 (47/53%)	Asthma:0.27, Rhinitis:0.32
Positive SPT No. (%)	55 (92%)	32 (80%)	7 (12%)	Asthma: < 0.001*, Rhinitis: < 0.001*
Predicted FEV1 (%) (Mean ± SD)	69.3 ± 2.3	76.8 ± 2.4	89.7 ± 3.1	Asthma: < 0.001*, Rhinitis: < 0.001*
Serum total IgE (IU) (Mean ± SD)	1060.5 ± 410.9	812.4 ± 67.1	91.2 ± 31.6	Asthma: < 0.001*, Rhinitis: = 0.03*
Serum specific *Der p* I IgE (IU) (Mean ± SD)	85.06 ± 17.9	54.18 ± 12.4	0	Asthma: < 0.0001*, Rhinitis: < 0.0001*

M: Male, **F:** Female, **SPT:** Skin Prick Test, **FEV1:** Forced expiratory volume in one second, *: Significant.

control group, there was no difference between the two groups except for GG genotype (*P*=0.03). Although AA variant of CD14/-1,145 gave a statistically significant value (*P*=0.04), but it was not considered because its significance was toward number of controls, not number of patients (33% vs 19%).

When the study population was stratified according to phenotypic allergy, comparison of atopic asthma and allergic rhinitis with control group revealed association between A(-1,145)G and atopic asthma phenotype specially GG genotype (*P*=0.007), with OR (95% CI) = 3.05 (1.35-6.89), and significant dominance for G allele (*P*=0.02). Although in atopic asthma patients AA variant of CD14/-1,145 and A allele % gave statistically significant values (*P*=0.04, and 0.02, respectively), in comparison with controls, but it was not taken into consideration because its significance was toward controls and not toward patients with atopic asthma (33% vs 17%, and 57% vs 37%, respectively). On the other hand, G(-1,359)T was found to be associated with allergic rhinitis phenotype with significant difference for TT homozygous allele (*P*=0.01) (Table 4).

The association of CD14 promoter region genotypes with HDM antigen concentration: When the study population was divided according to HDMs allergen concentration (*Der p* 1), there was a significant difference of genotype distributions at A(-1,145)G for homozygote GG genotype only (*P*=0.02) (55% vs 31.5%, OR=2.63, 95% CI 1.12 to 6.13) (Table 5).

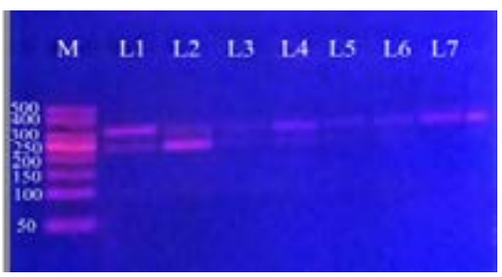

Fig. 2. Representative results of PCR-RFLP analysis and genotyping of atopic patients for A(-1,145)G after digestion by HPYCH4V. GG at 300 bp, AA at 230 bp, and AG at 300 bp and 230 bp. **M:** Marker, Lanes 1-4: AG, Lanes 5-7: GG.

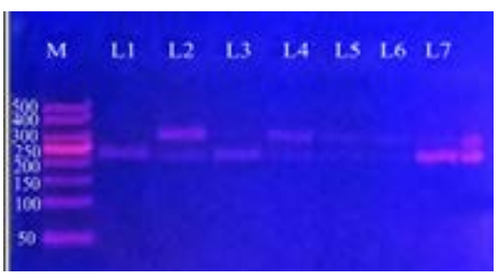

Fig. 3. Representative results of PCR-RFLP analysis and genotyping of control for A(-1,145)G, after digestion by HPYCH4V . GG at 300 bp, AA at 230 bp, and AG at 300 bp and 230 bp. **M:** Marker, Lanes 1, 3, 7: AA, Lanes 2, 4. 5, 6: AG.

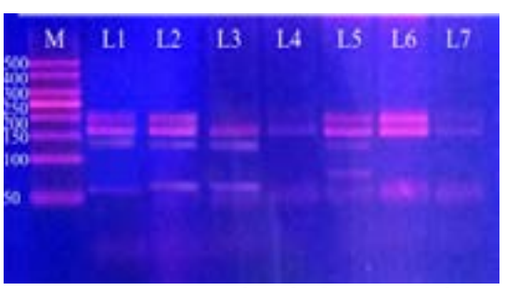

Fig. 4. Representative results of PCR-RFLP analysis and genotyping of atopic patients for G(-1,359)T, after digestion by FoK1. GG at 178 and 146 bp, TT at 146, 112, and 66 bp, GT at 178, 146, 112 and 66 bp. **M:** Marker, Lanes 1, 2, 5: GT, Lanes 3, 4, 7: TT, Lane 6: GG.

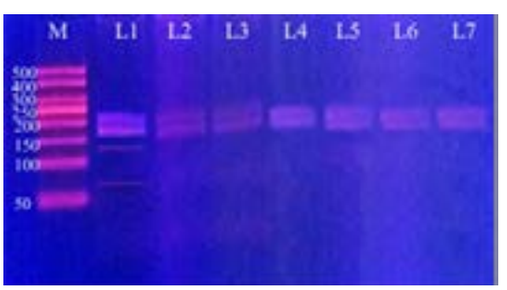

Fig. 5. Representative results of PCR-RFLP analysis and genotyping of control for G(-1,359)T, after digestion by FoK1. GG at 178 and 146 bp, TT at 146, 112, and 66 bp, GT at 178, 146, 112 and 66 bp. **M:** Marker, Lane 1: GT, Lanes 2-7: GG.

Table 3. Genotype - allele distribution in atopic subjects and control group using RFLP-PCR.

Genotypes/ Alleles (SNP)	Atopic patients (No. = 100) No. (%)	Control group (No. = 60) No. (%)	Statistical analysis	
			P value	OR (95% CI)
CD14/-1,145				
AA	19 (19%)	20 (33%)	0.04**	0.46 (0.22-0.97)
AG	45 (45%)	28 (47%)	0.83	0.93 (0.49-1.77)
GG	36 (36%)	12 (20%)	0.03*	2.25 (1.05-4.77)
A%	41 (41%)	34 (57%)	0.05	0.53 (0.27-1.01)
G%	59 (59%)	26 (43%)	0.05	1.88 (0.98-3.59)
CD14/-1,359				
GG	27 (27%)	18 (30%)	0.15	1.70 (0.81-3.57)
GT	39 (39%)	26 (43%)	0.58	0.83 (0.43-1.60)
TT	34 (34%)	16 (27%)	0.33	1.41 (0.69-2.87)
G%	46 (46%)	31 (52%)	0.48	0.79 (0.41-1.51)
T%	54 (54%)	29 (48%)	0.48	1.25 (0.66-2.38)

Chi square (χ^2) test was used. OR: Odds ratio; CI: Confidence intervals, * Significant, **Non significant because its significance is toward controls, not patients.

Table 4. Genotype-allele distribution in patients with atopic asthma/allergic rhinitis and control group.

Genotypes/ Alleles (SNP)	Control group (No. = 60)	Atopic asthma (No. = 60)			Allergic rhinitis (No. = 40)		
		No. (%)	Statistical analysis		No. (%)	Statistical analysis	
			P value	OR (95% CI)		*P* value	OR (95% CI)
CD14/-1,145							
AA	20 (33%)	10 (17%)	0.04**	3.5 (0.68-30.56)	09 (23%)	0.24	0.58 (0.23-1.45)
AG	28 (47%)	24 (40%)	0.43	0.85 (10.2-23.71)	21 (52%)	0.56	1.26 (0.56-2.81)
GG	12 (20%)	26 (43%)	0.007*	3.05 (1.35-6.89)	10 (25%)	0.55	1.33 (0.51-3.46)
A%	34 (57%)	22 (37%)	0.02**	0.44 (0.21-0.92)	19 (47.5%)	0.54	1.32 (0.53-3.25)
G%	26 (43%)	38 (63%)	0.02*	2.25 (1.08-4.69)	21 (52.5%)	0.36	1.44 (0.64-3.22)
CD14/-1,359							
GG	18 (30%)	19 (37%)	0.84	1.08 (0.49-2.34)	08 (20%)	0.26	0.58 (0.22-1.51)
GT	26 (43%)	27 (45%)	0.85	1.06 (0.52-2.91)	12 (30%)	0.18	0.56 (0.24-1.30)
TT	16 (27%)	14 (23%)	0.67	0.83 (0.36-1.91)	20 (50%)	0.01*	2.7 (1.18-6.39)
G%	31 (52%)	32 (53%)	0.85	1.06 (0.52-2.18)	14 (35%)	0.11	0.5 (0.22-1.14)
T%	29 (48%)	28 (47%)	0.85	0.93 (0.45-1.91)	26 (65%)	0.11	1.9 (0.87-4.52)

Chi square (χ^2) test was used. OR: Odds ratio; CI: Confidence intervals, * Significant, **Non significant because its significance is toward controls, not patients.

Table 5. Genotype allele distribution in HDM-sensitized patients with allergen concentration more and less than 2 µg/g dust.

Genotypes/ Alleles (SNP)	Antigen concentration (µg/g dust)		Statistical analysis	
	≤ 2 (No. = 62) No. (%)	> 2 (No. = 38) No. (%)	*P* value	OR (95% CI)
CD14/-1,145				
AA	14 (23%)	14 (37%)	0.12	0.50 (0.20-1.21)
AG	14 (23%)	12 (31.5%)	0.32	0.63 (0.25-1.56)
GG	34 (55%)	12 (31.5%)	0.02*	2.63 (1.12-6.13)
A%	21 (34%)	20 (53%)	0.06	0.46 (0.20-1.05)
G%	41 (66%)	18 (47%)	0.06	2.16 (0.94- 4.95)
CD14/-1,359				
GG	16 (26%)	8 (21%)	0.58	1.30 (0.49-3.42)
GT	18 (29%)	12 (32%)	0.78	0.88 (0.36-2.12)
TT	28 (45%)	18 (47%)	0.82	0.91 (0.40-2.05)
G%	25 (40%)	14 (37%)	0.72	1.15 (0.50-2.66)
T%	37 (60%)	24 (63%)	0.72	0.86 (0.37-1.98)

Chi square (χ^2) test was used. OR: Odds ratio; CI: Confidence intervals, *Significant.

DISCUSSION

Variable results for association between functional polymorphisms in CD14 and different allergic diseases were found. It depends, to a large extent, on both environmental and genetic factors. Innate immunity genes that regulate the relationship between the immune system and pathogens such as mite allergens, are supposed to have a critical function in the development of allergic diseases[24]. Results of the present study showed a significant correlation between total IgE levels and allergic patients when compared to control group. On the other hand, no significant difference was detected in total serum IgE on comparing atopic asthma to allergic rhinitis patients as all allergic subjects showed high level of IgE. Meanwhile, other studies that correlate serum total IgE to specific CD14 genotype revealed conflicting results. Kusunoki *et al.*,[25] postulated that CD14 can have a dual effect on atopy by inhibiting IgE production before allergic inflammation, then stimulating IgE after its onset.

A study conducted in Taiwan proved that carriers of the CD14 -159C/-1,145A/and -1,359T haplotype had the highest IgE and lowest serum CD14 levels as compared to other haplotypes[23]. The association between markers of atopy such as skin prick tests positivity or total serum IgE concentration and the C-159T polymorphism was demonstrated in non-Hispanic but not in Hispanic American population, suggesting environmental rather than genetic factor[17]. Activation of CD14 appears to up regulate IL-4 and IL-12- dependent IgE production[9,23,26,27]. In this way, the effect of endotoxins and consequently IgE may vary according to the atopic condition of the host[28].

Many hypotheses have been raised to investigate pathogenic mechanisms underlying allergy in HDM sensitization. *Der*-1 was found to target multiple proteins involved in the control of IgE synthesis and production[29], degradation of endogenous protease inhibitors[30], and surfactant proteins[31]. Moreover, it was suggested to increase the contact between allergens and dendritic cells (antigen presenting cells) beneath the bronchial epithelial barrier cleavage of the epithelial tight junctions by their proteolytic activities followed by release of chemokines and other mediators (e.g., IL-13, IL-33 and IL-25)[32]. A recent study proposed that *Der*-1 activates human receptor MRGPRX1 enhancing IL-6 production which contributes in pathogenesis of allergic rhinitis and allergic asthma[33].

Frequencies of transition at nucleotide A to G at position -1,145 A(-1,145)G and a G to T at position -1,359 G(-1,359)T from the transcription start site in the promoter region of the CD14 gene were assessed in each study subjects by using RFLP-PCR. In the present study, there was a significant difference (P=0.03) between atopic patients and the control group for GG genotype. Innate immunity genes that regulate the relationship between immune system and pathogens like mite allergens, are supposed to have a critical function in the development of allergic disease[24]. Most studies investigated C(-159)T; however, A(-1,145)G and G(-1,359)T rather than C(-159)T could be involved in various atopic conditions, since tight linkage disequilibrium has been observed in both polymorphisms[34].

When the study population was stratified according to phenotypic allergy (Table 4), there was a statistically significant association between GG homozygotes polymorphisms at CD14 A(-1,145)G in asthmatic patients as compared to control group (P=0.007) with significant dominance for G allele (P= 0.02) as shown in figures (2) and (3). In accordance with our study, in Pakistani population Micheal *et al.,*[35] proved that the A(-1,145)G polymorphism is associated with atopic asthma at GG genotype, while C(-159)T was associated with allergic rhinitis. Meanwhile in a Brazilian study conducted by de Faria *et al.,*[36] significant association between TT at C(-159)T genotype and severe asthma was found. In addition, they suggested a role of this genotype in modulation of asthma. A study conducted in Saudi Arabia[37] that assessed the role of C(-159)T, A(-1,145)G and G(-1,359)T in association with asthma, concluded that the heterogeneous genotypes CT, AG and GT were the highest prevalent genotypes among asthmatic patients.

Furthermore, in the current study it was shown that there was a statistically significant association between CD14 gene G(-1,359)T polymorphism in TT genotypes in case of allergic rhinitis phenotype as compared to control group (P=0.01). These findings are in agreement with different studies among other populations, e.g. in Chinese patients with allergic rhinitis, TT homozygotes are more common than other genotypes[38]. In a study for association of CD14, C(-159)T, G(-1,359) T with expression of asthma, croup, and allergy in Canadian children, it was established that those with TT combination were significantly more likely to have asthma[39]. Also, Tan *et al.,*[23] demonstrated that highest IgE levels were found in CD14 -159C/ -1,145A/ -1,359T haplotype as compared to other haplotypes; also they hypothesized that CD14 gene variants may play an important role in influencing allergen sensitization of children in Taiwan.

In this study, variability in the degree of allergy is explained by three successive steps: first, CD14 may recognize aeroallergens, such as dust mites. Second, it influences switching of the T helper cell response by enhancing production of interleukin (IL-12). Third, it leads to maturation of naïve T cells into type 1 helper T cells, and finally, it causes down-regulation of TH2 cells, and decreased immunoglobulin IgE production[40]. Polymorphisms in the CD14 promoter region have been associated with variability of atopic diseases and IgE levels.

When the study population was divided according to HDM allergen concentration (*Der p* 1) (Table 5), there was a significant difference of genotype distributions at A(-1,145)G for homozygote GG genotype only (*P*=0.02). Significant difference of GG genotype of G(-1,359)T polymorphism association to mite-sensitive subjects than other polymorphisms i.e of C(-159)T/A(-1,145) G among Taiwanese asthmatic children was reported by Tan *et al.,*[23]. Kowal *et al.,*[15] hypothesized that a possible genetic interaction between CD14 and serpin 1 may influence susceptibility to HDM-allergic asthma through an effect by those genes on different aspects of bronchial reactivity and IgE response. Moreover, in Poland, a study reported a significant positive correlation between HDM allergen concentrations in household and sensitization to HDM for CD14/-159 CC and CD14/-1,359 GG genotype[16]. Also, increased HDM exposure with reduced incidence of atopy was found for the whole population and subjects with CD14/-159 CC, CD14/-1,359 GT. On contrary, an opposite hypothesis was raised in another study which concluded that increasing endotoxin exposure was associated with reduced risk of allergic sensitization and eczema, but increased risk of non-atopic wheeze in children with CC genotype at -159 of the gene encoding CD14[19].

It is concluded that CD14 gene polymorphisms may affect susceptibility level of allergen sensitization and associated with the development of variable atopic diseases. However, reports are conflicting and further population studies are recommended.

Author contribution: WM Zaki proposed the research idea and the study design, contributed in sample collection, laboratory procedures, and prepared the final version for submission. HA Salem and ME Ajeeby shared in the study design, sample collection, and revised the manuscript. HA El-Azizy was responsible for acquisition, analysis and interpretation of resulting data.

Acknowledgment: The authors express their gratitude to Prof. Dr. Sherif M. Abaza, Medical Parasitology Department, Faculty of Medicine, Suez Canal University, for his beneficial revision of the manuscript. The authors would like to express their gratitude to the Medical Laboratory technicians at Prince Mohamed Bin-Nasser, Abu-Erish, and Sabia hospitals for their co-operation in sample collection.

Conflict of interest: There is no conflict of interest.

Financial support and sponsorship: This study received support and funding from the Deanship of Scientific Research, Jazan University, Jazan, KSA Funding number FS5-081.

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