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ORIGINAL ARTICLE

Association between viral Conjunctivitis and Genetic Polymorphisms related to IL-4 and IL-4R in Egyptian Population

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ABSTRACT

Background: Viral conjunctivitis is an inflammation of the conjunctiva caused by viruses. Interleukin-4 (IL-4) and IL-4R have been reported to associate with the pathogenesis of conjunctivitis; however, the role of IL-4 and IL-4R genetic polymorphisms in conjunctivitis remains unknown. Aim: This study aimed to evaluate the relation between IL-4 gene polymorphisms (rs2243250) and IL-4 gene receptor (rs1805010) with infectious conjunctivitis.

Methods: The study was conducted in 92 age-matched volunteers; 50 of viral conjunctivitis patients and 42 of healthy controls. The levels of both IL-4 and total IgE were measured using suitable methods. Genotyping of IL-4 (rs2243250) SNP was performed by Alleles Refractory Mutation Systems PCR (ARMS PCR) method, while the Genotyping of IL-4 receptor (rs1805010) SNP was performed by RFLP PCR method.

Results: Our results showed that there is a significant difference in genotype and alleles distributions in IL-4 (rs2243250) SNP between viral conjunctivitis and control. However, no significant difference in genotypes and alleles distribution IL-4 gene receptor (rs1805010) SNP between viral conjunctivitis and control. More ever, there is a significant increase in serum IL-4 and total IgE in viral conjunctivitis than control.

Conclusions: the analysis of our results indicates that IL-4 (rs2243250) SNP gene polymorphisms may be an indicator of increasing the incidence of viral conjunctivitis in the Egyptian population.

Keywords: Conjunctivitis; Gene polymorphism; IL-4; IL4R; Viral infection.

INTRODUCTION

Conjunctivitis is an inflammation of most of the white eye layers in the outer and inner eyelid surfaces. Pain, burning, scratching, or itching may also occur. Increased tears can occur in the eye or "stuck" in the morning [1]. Some common forms of conjunctivitis are infectious and non-infectious causes. Bacteria, viruses, and fungi are amongst the infectious causes. Allergies, foreign bodies, and chemicals are non-infectious causes [2].Signs and symptoms are often based on the diagnosis. The cause of conjunctivitis determines the treatment [3]. Adenovirus is the main cause of viral conjunctivitis, and it is spread mainly in adults. In one or two weeks, people usually get better. Irritation, photophobia, and watery discharge are the most common symptoms. Diagnosis is clinical; Viral cultures are sometimes indicated, or immune tests are reported. There is no specific treatment in most viral cases [4]. The virus infection triggers an immune response characterized by different production of cytokines with a strong influence on the mechanisms of immune effectors. The preferential activation of one form or the other of cytokines is thus associated with resistance to infectious diseases [5].Cytokines are pleiotropic molecules that control various aspects of immune responses and inflammatory effects. Cells of t-helper (Th) are the main source of regulatory cytokines and can be divided into Th1 cells that produce inflammatory cytokines such as interferon (IFN)-β, tumor necrosis factor-2 and TNF-α. whereas Th2 cells produce anti-inflammatory cytokines like IL-4, IL-5, IL-10, and IL-13[6].

IL-4 is a pleiotropic cytokine that promotes the development of T-cell and B-cell antibodies and plays a key role in the immune system [7]. It is produced mainly by activated T cells and mast cells, basophils, and eosinophils. In function, IL-4 is better known to define the so-called Th2 T-lymphocyte phenotype and to regulate cell proliferation, apoptosis, and expression of numerous genes in different cell types, including lymphocytes [8], macrophages, and fibroblasts, as

well as epithelial and endothelial cells [9]. It is an important regulator for adaptive immunity to humor. IL-4 decreases the production of Th1 cytokines such as IFN-gamma and IL-2. It is also responsible for the class switching of B-cells to IgE production [10].IL-4 mediates its role by complexing it with the receptor, which is present in endothelial, epithelial, muscle, fibroblastic, hepatocytic, and brain tissues. IL-4R is composed of IL-4Rα chain that complexes with IL-4 with high attraction followed by signaling based upon IL-4-mediated hetero-dimerization of the IL-4Rα chain with another chain called gamma common chain (γc), first identified as a part of the interleukin-2 receptor. Although the gamma chain can increase the observed affinity of the IL-4R complex for IL-4, it is also wanted for the activation of the signaling pathway after binding of IL-4 [11].IL-4 is present on chromosome 5 in the 5q31-q33 region, while IL-4R α is located on chromosome 16 in the region 16p12.1. The genetic variants in both IL-4 and IL-4R may be linked with susceptibility or resistance to infectious diseases. This is because some specific gene polymorphisms can modulate the genes expression and protein level. several studies showed that these polymorphisms are involved in the pathology of different diseases [12]. IL-4 gene polymorphisms have been described in association with some non-infectious diseases, such as rheumatoid arthritis [13], asthma and atopy and with infectious diseases [14] such as dental infection. IL-4 (-589 C/T) promoter polymorphism, a C-to-T base substitution, has been suggested to be associated with viral conjunctivitis [8]. Many previous studies investigated the association of IL-4 gene polymorphisms with conjunctivitis, but their results are conflicting, so the connection of IL-4 gene polymorphisms with conjunctivitis in Egyptians needs further studies. The IL-4R gene is also highly polymorphic. IL-4R SNPs have been identified for their implication in different diseases such as 175V (rs1805010) SNP which include A to G substitution at position 223. This, in turn, may affect IL-4 signaling and associated with conjunctivitis [15].In this study, we selected IL-4 as a candidate gene for viral conjunctivitis due to its biological properties in the regulation of immune responses. Aim: This study aimed to evaluate the relation between IL-4 gene polymorphisms (rs2243250) and IL-4 gene receptor (rs1805010) with infectious conjunctivitis.

METHODS

Study Design/Inclusion Criteria:

This case-control study was conducted at Zagazig University Medical Research Laboratory. The patients were collected from zagazig university patients out clinics in the period between March 2013 until August 2014. The study included 92 persons; 50 viral patients (aged between 18 and 61 years old, females N=16, males N= 34, and 42 patients of age-matched normal healthy controls (aged between20 and 57 years old, females N=10, males N= 32).

Exclusion Criteria:We excluded all bacterial conjunctivitis and allergic conjunctivitis.

Ethical Approvals:The study was approved by Zagazig University Ethical Committee. Written informed consent was obtained from all participants. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

METHODS

Blood Sampling and Biochemical Measurements:

5 ml of the blood sample was taken from every participant under complete aseptic condition and was divided into 2 portions; 2 ml of whole blood was collected in sterile EDTA containing tubes for DNA extraction, and the rest was left for 30-60 minutes for spontaneous clotting at room temperature then centrifuged at 3000 rpm for 10 minutes. Serum samples were separated into another set of tubes and kept frozen at -20◦C for biochemical analysis. For all subjects, IL-4 and IgE total were measured by enzymatic colorimetric methods using a Spectro photometry.

DNA Extraction:DNA was extracted from blood samples and collected in tubes containing EDTA with (TIAN amp Genomic DNA Kit Wizard® Genomic DNA Purification Kit). The extracted DNA was conserved at -20 °C till the genotyping steps.***Genotyping of IL-4 -589 C/T gene polymorphism using ARMS PCR method:***

Each sample was done in 2 PCR reactions. The first reaction for the wild allele, and the second reaction for the mutant allele. Each PCR reaction was done in 20 µL volume containing 5µL of template DNA, 5µL of working primer mixture (specific primer and common primer) and 10µL of master mix solution containing i-TaqTM (5U/µl), dNTPs 2.5Mm each, PCR reaction buffer, Gel loading buffer).***Primers used in ARMS PCR reaction for IL-4 -589 C/T:***Common:5`-AGT ACA GGT GGC ATC TTG GAA-3`. C allele:5`-TAA ACT TGG GAG AAC ATT GTC-3`. T allele:5`-TAA ACT TGG GAG AAC ATT GTT-3`. PCR cycling conditions were 5 min at 95º c for activation followed by 30 cycles of 30 s at 95 º c for denaturation, 27 s at 61 ºc for annealing, and 25 s at 72 ºc for elongation, with a final step at 72°C for 5 min to allow for complete extension of all PCR fragments. PCR products were visualized in 2% agarose gel stained with ethidium bromide [Figure 1]. Each sample was represented by two wells: one

for the wild allele and the other for the mutant allele. if the band with size 130 bp appears in the first well, it is homozygous wild allele CC. if the band appears the second well, it is homozygous mutant allele TT. if the band appeared at both first and second well, it is heterozygote CT

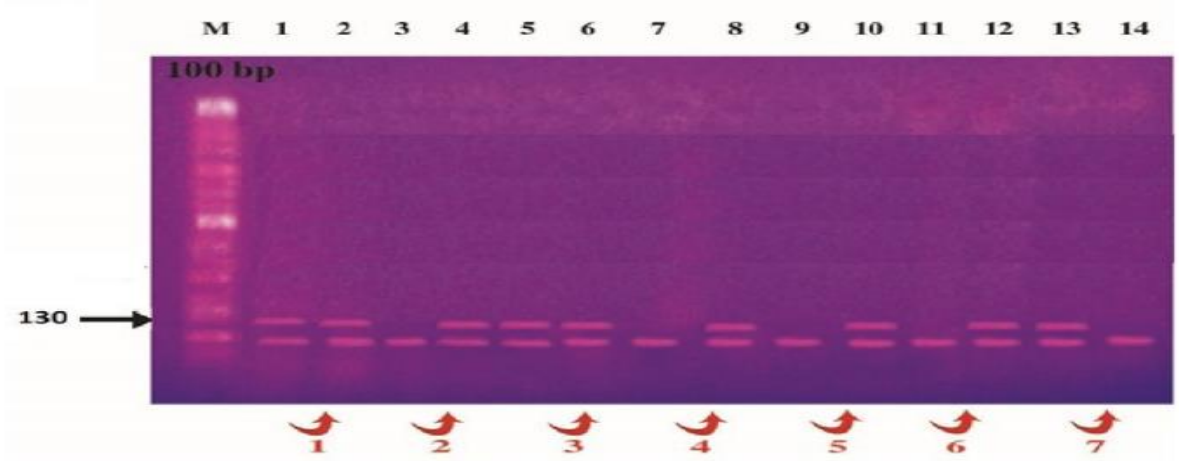

Figure (1): Gel electrophoresis for IL-4 (rs2243250) in the viral conjunctivitis patients.

Genotyping of IL-4R A/G gene polymorphism (c.223A>G p.Ile75Val) using RFLP PCR method: Each sample was done in one PCR followed by digestion enzyme and gel electrophoresis for visualization of genotypes. PCR reaction was done in 20 µl volume containing 5µL of template DNA, 5µL of working primer mixture (forward primer and reverse primer) and 10µL of master mix solution containing i-TaqTM (5U/µl), dNTPs 2.5Mm each, PCR reaction buffer, Gel loading buffer).

PCR primer sequences used in RFLP-PCR for IL-4R c.223A>G were:

Forward: 5`- GGC AGG TGT GAG GAG CAT CC-3`. Reserve: 5`- GCC TCC GTT GTT CTC AGG TA -3`

PCR reaction was run on thermal cycler device with the following conditions, three steps of PCR was standardized and carried out with an initial activation at 93° C for 5 minutes followed by 36 cycles of 3 steps; the first step for 30 s at 93 ° c for denaturation, the second step for 27 s at 60 ºc for annealing, and the third step for 25 s at 72 ºc for elongation, with a final step at 72°C for 5 min to allow for complete extension of all PCR fragments. PCR products were visualized in 2% agarose gel electrophoresis [Figure 2].

The amplified PCR products were digested using the RsaI restriction enzyme (R0167S NEW ENGLAND Bio labs) at 37° C overnight and visualized on 2 % agarose gel stained with ethidium bromide. This enzyme makes cutting at G allele and not A allele. The homozygous wild genotype (AA) gives one band at 273 bp. The heterozygote genotype gives three bands 273-254-19 bp. The homozygote mutant allele gives two bands at 254-19 bp.

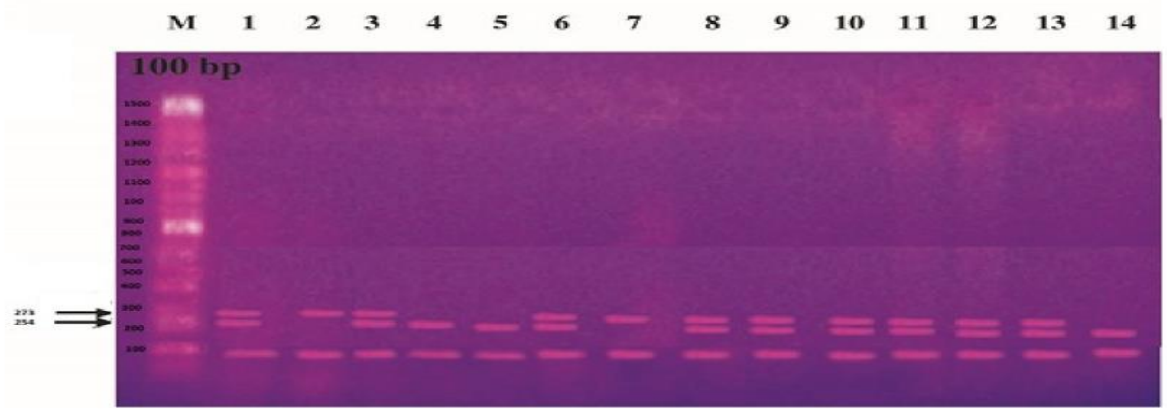

Figure (2): Gel electrophoresis for IL-4 receptor (rs1805010) in the viral conjunctivitis patients.

STATISTICAL ANALYSIS:

The statistical package for social sciences version 22.0 (IBM Corp., Armonk, NY, USA) was used for the analysis. Data were expressed as mean± standard deviation (SD) for continuous variables. While results for categorical variables were reported as number and percent of patients.For numerical data, a t-test was used. While for categorical data, the Chi-square test was used to compare patients and control subjects.

Armitage's trend test and Allele freq. difference test were used to test the significance in the

difference in genotype and allele frequencies distribution respectively between populations given each position. The strength of association was estimated by the crude odds ratios (OR), with 95% confidence interval (95% CI). The statistical significance was accepted for p<0.05.

RESULTS

There was no significant difference in age and gender between viral conjunctivitis and the control group (Table 1).For IL-4 -589 C/T polymorphism: Our results showed a significant difference in genotype and allele distribution between viral conjunctivitis patients and control (P<0.001) (Table 2).For IL-4R 223A/G polymorphism: Our results showed that no significant difference in genotype and allele distribution between viral conjunctivitis patients and control (P > 0.05) (Table 3).IL-4 and total IgE serum levels: There is a highly significant difference between viral conjunctivitis and control in both IL-4 and total IgE (P<0.001). However, there was no significant correlation between IL-4 with IgE total (P > 0.05) in the viral conjunctivitis group (Table 4).

Table 1: Demographic characteristics of patients and control enrolled in the study.

		Control n=42	Viral conjunctivitis n=50	P-Value
Age		(20-57)	(18-59)	**0.7**
Gender	**Female**	**10**	**0.245**	**0.245**

Table 2: Distribution of IL-4 (rs2243250) SNP c.-589C>T (Genotype and Allele) frequencies among viral and control groups.

Conjunctivitis types	Genotypes			Alleles	
	CC	CT	TT	C	T
Control (n=42)	5(12%)	2 (5%)	35(83%)	12/84	**72/84**
Viral (n=50)	14(15.1%)	19(20.4%)	17(18.3%)	47/100	**53/100**
X^2	23.7			**22.4**	
***P*- value**	**<0.001**			**<0.001**	

X2 =Chi square test. P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant.

Table 3: Distribution of IL-4 receptor (rs1805010) c.223A>G SNP (Genotypes and Alleles) frequencies among viral and control groups.

Conjunctivitis types	Genotypes			Alleles	
	AA	AG	GG	A	G
Control (n=42)	2	39	1	43	**41**
Viral (n=50)	1	46	3	48	**52**
X^2	1.22			**0.186**	
***P*- value**	**0.542**			**0.666**	

X2 =Chi square test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant.

Table 4: Comparison of clinical parameters among viral conjunctivitis and control group and correlation between them in viral conjunctivitis group.

	Control n=42	Viral conjunctivitis n=50	t-test	P-Value
IL-4	47.9±4.7	232.6±16.5	9.9	**<0.001**
Total IgE	55.3±2.14	108.5±2.9	14.1	**<0.001**
Correlations[a]				
			IL-4	**IgE total**
IL-4	**r** Pearson Correlation		1	**-0.114**
	P **Sig. (2-tailed)**		**-**	**0.431**

P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant.

DISCUSSION

Viral conjunctivitis is the most common overall cause of infectious conjunctivitis and usually does not require treatment; the signs and symptoms at presentation are variable. Our study aimed to evaluate the effect of SNPs of both IL-4 (c.-

589C>T) and IL-4R (c.223A>G p.Ile75Val) on the incidence of viral conjunctivitis in the Egyptian population.Several single nucleotide polymorphisms related to these two proteins were reported to be associated with allergic conjunctivitis in different populations with different ethnicity [16–19]. However, the impact of these polymorphisms was never estimated with the incidence of viral conjunctivitis.

IL-4 (rs2243250) SNP c.-589C>T is in the promoter region of IL-4 near 5' end [20]. The variation in this site from major wild allele C to the minor allele T may have an impact on increasing infection with the virus causing viral conjunctivitis. Our results showed a significant difference in the genotype distribution between patients and control. This is maybe interpreted by the alteration in the gene expression due to the variation in the c.-589C>T ($p < 0.05$) [21].

Regarding IL-4 receptor (rs1805010) c.223A>G, it is a missense variant in which the variation of ATC ⇒ GTC will lead to the variation of Isoleucine to Valine amino acid at the position 75 (p.Ile75Val) [22]. This variation is pathogenic and was linked with several diseases in several populations [23–27]. However, our results showed no significant difference in genotype and allele distribution ($p > 0.05$) which may be due to the similarity between Isoleucine and Valine in structure, properties, and function [28]. Our hypothesis is based on that the variation of these two polymorphisms could affect the gene expression level of IL-4 due to the variation and on the function of IL-4R due to the missense polymorphism, and consequently the incidence of viral infection to conjunctivitis will increase. Our results showed a significant difference in genotypes and alleles distribution of (rs2243250) -589C>T and (rs1805010) c.223A>G between both patients and control group.Also, our results showed a significant increase in serum IL-4 level in viral conjunctivitis patients than control. The cause of this increase may be explained by the variation in the two single nucleotide polymorphisms we have genotyped. IL- 4 is an anti-inflammatory cytokine and not responsible for the immunity state in viral infection. However, its increase in the pathogenesis of viral conjunctivitis may shed light on the impact of SNPs on the gene expression level and protein function of both IL-4 and IL-4 R proteins. The abnormal variation in the level of IL-4 can be one of the reasons that decrease the immunity of the body which can make it easier for viral conjunctivitis. Limitation of the study: There are several limitations in our study including, the small sample size, the low information about if any of the patients has any type of hypersensitivity, low information about the environment around the patients which may be a risk factor for more viral infection.

CONCLUSIONS

Single nucleotide polymorphisms have a great role in disease prognosis in early stage. In addition to its role in suspecting the action of the body against microbial attack due to their effect on cytokine production. The two SNPs used in our study (rs2243250) -589C>T and (rs1805010) c.223A>G showed significant difference between patients and control. They could interpret the increase of IL-4 in the blood which was associated with viral conjunctivitis incidence as a risk factor in the Egyptian population.

Conflicts of Interest/ Financial Disclosures: Nothing to declare.

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