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CLINICAL STUDY

Evaluation of TNF-α and IL-1β polymorphisms in Taiwan Chinese patients with pterygium

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Abstract

Purpose **Pterygium, a complex disease, is associated with ultraviolet radiation, immunoinflammatory process, genetic factors, and virus infection. Ultraviolet radiation induces secretion of proinflammatory cytokines by the ocular surface epithelium, inflammatory cells in the tear fluid, or both. Among these cytokines, tumour necrosis factor (TNF)α and interleukin (IL)-1β activate pterygium body fibroblasts, resulting in a phenotype capable of expressing various proteinases associated with extracellular matrix remodelling, angiogenesis, and fibroblast proliferation, which are important for pterygium formation and recurrence. The genetic factor was proposed to play a role in pterygium formation, but there were few studies to clarify this proposition. For investigating genetic factors, the association between pterygium and TNF-α and IL-1β polymorphisms is evaluated in this study.**
Methods **A total of 128 pterygium patients and 103 volunteers without pterygium were enrolled in this study. Polymerase chain reaction-based analysis was used to resolve the TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 receptor antagonist (IL-1 Ra) polymorphisms.**
Results **There were no significant differences in the frequency of genotypes and alleles of TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms between both groups.**
Conclusions **The correlation between pterygium and TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms does not exist and those polymorphisms are not useful genetic markers for pterygium susceptibility. Further studies on other polymorphisms or haplotypes of TNF-α and IL-1β are necessary.**
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Keywords: pterygium; tumor necrosis factor; interleukin-1; polymorphism

Introduction

Although the pathogenesis of pterygia is still poorly understood, epidemiologic evidence suggests that environmental stress may have a role. [1] Of the potential agents, ultraviolet (UV) irradiation has received the greatest attention.[1–3] UV irradiation can trigger secretion of such proinflammatory cytokines as interleukin (IL)-1, IL-6, IL-8, and tumour necrosis factor (TNF)α from the corneal, conjunctival, and pterygium epithelium.[4–7] Certain proinflammatory cytokines, specifically TNF-α and IL-1β, can stimulate proliferation of cultured Tenon's capsule fibroblast, and increase expression of matrix metalloproteinases in cultured pterygium body fibroblast.[6,8] Hence, Solomon *et al*[6] proposed that TNF-α and IL-1β play a key role in the development of pterygia.

As TNF-α and IL-1β are so important for pterygium formation, the TNF-α and IL-1β polymorphisms have been reported to be correlated with TNF-α and IL-1β expression,[9–11] and there is evidence that genetic factors may play a role in the development of pterygium,[12–18] it is logical to suspect a correlation between pterygium formation and TNF-α and IL-1β polymorphisms.

There are no reports about the association between pterygium and TNF-α and IL-1β polymorphisms. In this study, TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and

IL-1 receptor antagonist (IL-1 Ra), which had been reported to be associated with elevated TNF and IL-1β levels,[9–11,19,20] were evaluated in order to understand whether these polymorphisms are associated with increased susceptibility for pterygium.

Patients and methods

A total of 128 pterygium patients (71 male and 57 female patients) at the Department of Ophthalmology, National Cheng-Kung University Hospital from January 2003 to June 2003 were enrolled in the study with ages ranging from 35 to 90 years (mean, 64.6 years). A total of 103 volunteers aged 55 years or more without pterygium were enrolled as the control group. There were 64 male and 39 female volunteers in the control group (age range from 50 to 83 years with an average of 64.2 years).

The genomic DNA was prepared from peripheral blood by use of a DNA Extractor WB kit (Wako, Japan). Polymerase chain reactions (PCRs) were carried out in a total volume of 25 μl, containing genomic DNA, 2–6 pmol of each primer, IX *Taq* polymerase buffer (1.5 mM $MgCl_2$), and 0.25 U of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA, USA).

For TNF-α-308 promoter, the loci of the TNF-α gene were studied as previously described by Galbraith and Pendey.[11] The sequences of the primers were as follows: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-ACACTCCCCATCCTCCCGGCT-3′. The polymorphism was analysed by PCR amplification followed by *Nco*I restriction analysis.[11]

For IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra, six PCR primers were used to amplify the correlated gene. The sequences of the six primers were as follows (from 5′ to 3′ end): IL-1β promoter: upstream, TGGCATTGATCTGGTTCATC; downstream, GTTTAGGAATTCTCCCACTT; IL-1β exon 5: upstream, GTTGTCATCAGACTTTGACC; downstream, TTCAGTTCATATGGACCAGA; and IL-1Ra: upstream, CTCAGCAACACTCCTAT; downstream, TCCTGGTCTGCAGGTAA. The polymorphism was analysed by PCR amplification followed by restriction analysis.[9,19,20]

The PCR products from the same individual were mixed together and 10 μl of this solution was loaded into 3% agarose gel containing ethidium bromide for electrophoresis.

Statistical analysis for the distributions of TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms in the control group and pterygium group was carried out using the χ^2 test or Fisher's exact test. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($P<0.05$).

Results

There were no significant differences between both groups in age and sex. The frequency of the genotype and alleles of IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms in the pterygium group and control group is shown in Tables 1 and 2. There were no significant differences between both groups.

The frequency of the genotype and alleles of TNF-α-308 promoter is shown in Table 3. There were also no significant differences between both groups.

Discussion

Pterygium, a complex disease, is associated with UV radiation, immunoinflammatory process, virus infection, and genetic factors,[1] and there are several theories for its formation. Detorakis *et al*[1] proposed a 'two-hit' model for

Table 1 Genotypes of IL-1β polymorphisms in the pterygium and control groups

Genotype	*Pterygium n = 128 (%)*	*Control n = 103 (%)*	*P-value*
IL-1β-511 promotor			0.30
T/T	36 (28.1%)	25 (24.3%)	
T/C	66 (51.6%)	48 (46.6%)	
C/C	26 (20.3%)	30 (29.1%)	
IL-1β exon 5[a]			0.27
E1/E1	126 (98.4%)	99 (96.1%)	
E2/E1	2 (1.6%)	4 (3.9%)	
E2/E2	0 (0%)	0 (0%)	
IL-1 Ra[b]			0.15
I/I	112 (87.5%)	89 (86.4%)	
I/II	16 (12.5%)	10 (9.7%)	
II/II	0 (0%)	1 (0.98%)	
I/IV	0 (0%)	3 (2.92%)	

[a]E1: 135 base pairs (bp) + 114 bp; E2: 249 bp.
[b]I: 410 bp; II: 240 bp; III: 500 bp; IV: 325 bp; V: 595 bp.

Table 2 Allelic frequencies for IL-1β polymorphisms in the pterygium and control groups

Allele	*Pterygium n = 128 (%)*	*Control n = 103 (%)*	*P-value*
IL-1β-511 promotor			0.18
Allele T	138 (53.9%)	98 (47.6%)	
Allele C	118 (46.1%)	108 (52.4%)	
IL-1β exon 5			0.27
Allele E1	254 (99.2%)	202 (98.1%)	
Allele E2	2 (0.8%)	4 (2.0%)	
IL-1 Ra			0.15
Allele I	240 (93.75%)	191 (92.72%)	
Allele II	16 (6.25%)	12 (5.81%)	
Allele IV	0	3 (1.47%)	

Table 3 Genotypes and allelic frequencies for TNF-α-308 promotor in the pterygium and control groups

	Pterygium n = 128 (%)	*Control n = 103 (%)*	*P-value*
Genotype			0.54
G/G	114 (89.1%)	89 (86.4%)	
G/A	14 (10.9%)	14 (13.6%)	
A/A	0 (0%)	0 (0%)	
Allelic frequency			0.55
Allele A	14 (5.5%)	14 (6.8%)	
Allele G	242 (94.5%)	192 (93.2%)	

pterygium formation. The first hit could be either inherited or incurred by UV radiation, and the second hit could be caused either by solar light or by viral infection.

There is evidence that genetic factors play a role in the development of pterygium.[12–18] Several case reports have described clusters of family members with pterygium, and a hospital-based case–control study showed family history to be significant, suggesting a possible autosomal dominant pattern.[12–17] Besides, some races have a greater predisposition to pterygia; for example, Indians are affected more than Caucasians, Thais more than Chinese, dark-skinned Africans more than pale-skinned Arabs.[18] Although genetic factors were proposed to play a role in pterygium formation, there were few studies to clarify this proposition and no specific gene was identified.

In this study, we try to investigate the genetic factor of pterygium by single nucleotide polymorphism (SNP) marker. SNPs are the most abundant types of DNA sequence variation in the human genome, and the SNP marker has provided a new method for identification of complex gene-associated diseases.[21,22]

Solomon *et al*[6] proposed that certain environmental stimuli known to be associated with pterygium induce secretion of proinflammatory cytokines by the ocular surface epithelium, inflammatory cells in the tear fluid, or both. Among these cytokines, TNF-α and IL-1β activate pterygium body fibroblasts, resulting in a phenotype capable of expressing various proteinases associated with extracellular matrix remodelling, angiogenesis, and fibroblast proliferation. These traits are important for pterygium formation and recurrence.

Based on the theory of Solomon *et al*,[6] we evaluated the correlation between pterygium and TNF-α and IL-1β polymorphisms. As there were no earlier reports same as ours and there were several polymorphisms in TNF-α and IL-1β, we tried to evaluate the polymorphisms reported to be related to the production of TNF-α and IL-1β.

In TNF-α polymorphisms, biallelic G to A polymorphism, 308 nucleotides upstream from the transcription initiation site in the TNF promoter, is associated with elevated TNF levels, disease susceptibilities, and poor prognosis in several diseases.[10,11] The A 308 allele of the TNF-α promoter affects the binding of transcription factors and increases transcription promoter activity, which may further alter TNF-α production, immune response, and susceptibility to certain autoimmune, infectious, and malignant diseases.[23] Besides, the A 308 allele may inhibit repressors of transcription.[24] The presence of a G to A polymorphism at position 308 of the TNF-α promoter gene could increase transcription six- to seven-fold.[25]

The IL-1β gene is located on chromosome 2, in close linkage with another gene of the IL-1 gene family that encodes for IL-1Ra. Different polymorphisms have been described in the IL-1β gene and at least two of them could influence the protein production: one located in the promoter region at position −511,[19] and the other in exon 5.[20] Moreover, the action of IL-1β is regulated by its naturally occurring inhibitor IL-1Ra.[19,20] IL-1Ra has five polymorphic site variable number tandem repeats in intron 2 and 4 SNPs, including one in exon 2. These IL-1Ra gene polymorphisms have been associated with altered production rates of IL-1Ra protein.

In our series, there are no significant differences between pterygium and control groups in TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms. We suggest that the TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms maybe cannot become useful genetic markers for pterygium susceptibility. However, recent studies in genetic diagnostics showed that the genetic susceptibility sometimes cannot be found by one SNP of a gene, but can be revealed when multiple SNPs of the same haplotype are investigated together.[26–28] Hence, further studies by the haplotypes of the TNF-α and IL-1β genes are suggested.

In conclusion, the correlation between pterygium and TNF-α-308 promoter, IL-1β-511 promoter, IL-1β exon 5, and IL-1 Ra polymorphisms does not exist. This could be the basis of future surveys. Further studies on other polymorphisms or haplotypes of TNF-α and IL-1β are necessary for detection of a genetic predisposition to pterygium formation and its recurrence.

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