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Role of MDM2 SNP309 gene mutation in pterygium: a pilot study

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Abstract

Introduction: Pterygium is a common, vision impairing ocular surface disease whose etiopathogenesis is not yet fully established. Several factors, including ultraviolet radiation, heat, dry climate, microtrauma due to dust particles, and viral infections have been attributed to play a role in its pathogenesis. Recent studies have suggested that ptergyium might have a neoplastic origin. MDM2 SNP309 mutation has been associated with various tumors, however, its role in the pathogenesis of pterygium is not known.

Objectives: To study the association between MDM2 SNP309 mutation and pterygium.

Materials and Methods: In the present study, 37 pterygium patients and 49 controls were screened for MDM2 SNP309 mutation. Peripheral venous blood samples were collected from the study participants and the genomic DNA was isolated. This genomic DNA was used for polymerase chain reaction (PCR) amplification of MDM2 SNP309 mutation using the following set of primers: forward 5'-CGGGAGTTCAGGGTAAAGG-3' and reverse 5'-TCGGAACGTGTCTGAACTTG-3'.

Results: In the pterygium group, the frequency of genotypes GG, GT and TT were 32.6%, 40.8% and 26.5%; whereas in the controls it was 27.0%, 35.1% and 37.8% respectively. There was no statistically significant difference between the two groups in the genotype frequency of MDM2 SNP309 polymorphism ($p>0.05$).

Conclusions: The results showed no significant difference between the ptergyium patients and controls in the genotype frequency of MDM2 SNP309 polymorphism.

Keywords: MDM2 SNP309 polymorphism, Polymerase chain reaction, Pterygium.

Introduction

Pterygium is a fibrovascular fold of conjunctiva that encroaches onto the cornea in the interpalpebral fissure, and can be associated with significant visual morbidity. It is composed of epithelium and a highly vascular loose connective tissue. The mechanisms for the formation of pterygium are not completely understood; however, the importance of heredity as well as environmental factors, such as exposure to solar radiation (ultraviolet light), microtrauma by dust particles, viral infections, chronic inflammation, aberrant wound healing, immunologic mechanisms and epigenetic factors have been recognized.[1-3] The external or environmental factors may act as a trigger to induce aberrant fibrovascular proliferation resulting in development of pterygium in genetically predisposed individuals.[2] Since pterygium exhibits some characteristics of neoplasia like abnormal cell proliferation, local invasion of adjacent normal tissues and recurrence on excision; recent studies have advocated that pterygium might indeed be a neoplasm like growth disorder occurring in susceptible individuals.[3-6]

The tumor suppressor protein, p53, plays a crucial role in multi-cellular functions, including gene transcription, DNA synthesis and repair, growth arrest, cell senescence, and apoptosis. Mutations of p53 gene that disrupt the balance between cell apoptosis and repair are found in at least half of all human cancers, which highlight its critical role in the tumor suppression.[7,8] The human homolog of the mouse double minute 2 (MDM2) gene functions as an important negative regulator of p53 through an auto regulatory feedback loop. The elevated nuclear p53 levels activate MDM2 gene transcription and increase the protein expression of MDM2. The transcriptional activity of p53 is inhibited by MDM2 through its direct binding to p53[9] and MDM2 also serves as an E3 ubiquitin ligase, promoting the degradation of p53.[10-12] Thus, MDM2 over expression may disturb this feedback loop and cause the deficiency of p53, which results in an inefficient growth arrest and apoptosis. Amplification of MDM2 is observed in many human tumor tissues, including colorectal cancer (CRC),[13] and up-regulated expression of MDM2 and attenuation of p53 pathway has been observed in these cases.

MDM2 SNP309, which is located in the promoter of MDM2 gene, has been identified as a functional single nucleotide polymorphism (SNP). This SNP is a novel T to G substitution located at the 309th nucleotide in the first intron, showing a greater binding affinity for the transcription factor Sp1.[14] Therefore, it has been hypothesized that the genetic variant might have an impact on the expression of MDM2 and affects the individual's susceptibility to developing tumors. Previous studies have evaluated this association in different tumors, but their results are conflicting.[15-17] While some studies have reported a direct correlationbetween MDM2 SNP309 polymorphism and CRC risk,[18-20] the other studies have shown the oppositeand found no such correlation.[21,22] However,

the role of MDM2 SNP309 mutation has not been studied in pterygium patients. Based on this background, we aimed to investigate whether MDM2 SNP309 polymorphisms are associated with the formation of pterygium.

Materials and Methods

A total of 37 pterygium patients and 49 controls were included in the study. Peripheral venous blood samples were collected from the patients diagnosed with ophthalmic pterygium at Amritsar Eye Hospital. The diagnosis of pterygium was established on the slit lamp examination of the patients who had a triangular, fibrovascular fold of conjunctiva encroaching at least 1mm into the peripheral cornea. The control group constituted of patients who came to the clinic for the management of refractive errors unrelated to pterygium. The patients with any associated ocular or systemic hereditary diseases were excluded from the study. The study followed the tenets of Helsinki Declaration and all participants signed an informed consent. The study was performed with the approval of the university ethics committee.

Genotyping of MDM2 SNP309 polymorphism: Genomic DNA from blood was isolated using 7.5M ammonium acetate for removal of cell debris and 95% ethanol for DNA precipitation. This genomic DNA was used for polymerase chain reaction (PCR) amplification of MDM2 SNP309 using the following set of primers: forward 5'-CGGGAGTTCAGGGTAAAGG-3' and reverse: 5'-TCGGAACGTGTCTGAACTTG-3'. The PCR mixture contained 50ng of genomic DNA, 1U Taq DNA polymerase, 10pmol of each primer and 2.4µl of 10mM dNTP mix (Invitrogen, Thermofisher Scientific) of each dNTP. The PCR reaction was comprised of 3 minutes of denaturation at 94˚C followed by 35 cycles at 30 seconds at 94˚C (denaturation), 30 seconds at 52˚C (annealing), 30 seconds at 72˚C (elongation) with a final elongation of 10 minutes at 72˚C. The resulting 194 bp product was digested with restriction enzyme *MspAI*. Digested PCR products were separated in 4% agarose gel electrophoresis containing ethidium bromide and products were visualized under UV transilluminator. The MDM2 promoter SNP309 has two alleles, G has a unique MspAI site that is absent in other allele T. It results in different bands when subjected to digestion with *MspAI* which is as follows: Homozygous GG allele gives two bands 49bp, 145 bp; homozygous TT gives a single band of 194bp due to absence of MspAIsite; and heterozygous G/T gives three bands 49bp, 145bp and 194 bp.

Statistical Analysis: Chi square test was used to determine the genotype frequency of MDM2 309 polymorphisms in the study and control groups. The association between MDM2 309 polymorphisms and pterygium was modeled in terms of binary logistic regression analysis. To determine pterygium risk for the genotype, odds ratio (OR) and 95% confidence interval (95%) were calculated. The Medical software program was used for the statistical analysis and a value of $p \leq 0.05$ was considered to be statistically significant.

Results

A total of 76 patients were included in this study. The mean age of pterygium group (37 patients) was 53 ± 15 years and 21 (65.7%) were males, where as in the control group (49 patients) the mean age was 58 ± 11 years and 18 (36.7%) were males. There was no statistically significant difference for age between the two groups ($p>0.05$, t test). The frequency of genotypes for MDM2 SNP309 polymorphism is shown in Table 1. Although TT had a higher, and GG and GT a lower odds ratio in pterygium group as compared to controls, the difference was not found to be statistically significant ($p>0.05$). Fig. 1 shows a representative picture of gel analysis in a pterygium patient.

Table 1: Analysis of MDM2 SNPs genotype frequency in controls and pterygium patients

Genotype	Controls (n-49) Number (%)	Pterygium patients (n-37) Number (%)	Odds ratio	95% CI	Z stat	P value
GG	16 (32.6)	10 (27.0)	0.8	0.3 - 2.03	0.4	0.6
GT	20 (40.8)	13 (35.1)	0.8	0.3 - 1.9	0.3	0.7
TT	13 (26.5)	14 (37.8)	1.4	0.5 - 3.9	0.8	0.4

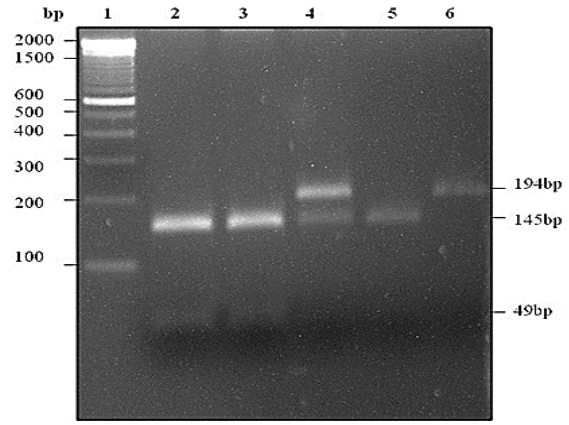

Fig. 1: DNA gel analysis of pterygium genotypes (Lane 1: DNA marker, 100 bp ladder; Lane 2,3,5: GG Phenotype; Lane 4: GT phenotype; Lane 6: TT Phenotype)

Discussion

The current study sought to analyze the role of MDM2 in the pathogenesis of pterygium. Although there was a trend towards a higher percentage of TT genotype, the difference was not statistically significant. Recently, a number of studies have shown the role of various genes in pterygium formation. Arish et al.[23] have studied the role of P14 and MDM2 gene promoter methylation and its association with pterygium. They analyzed 81 primary pterygium and 75 normal conjunctiva tissues for the assessment of methylation of P14ARF and MDM2 promoters by methylation-specific polymerase chain reaction. The study showed a significant relationship between MDM2 gene promoter methylation and the risk of disease in pterygium patients. Also, the expression of MDM2 gene was increased in pterygium compared to the control conjunctiva tissues.

Najafi et al.[24] reported that LATS1 and LATS2 promoter methylation have a role in pterygium formation. Using methylation-specific PCR, LATS1 and LATS2 mRNA expression was measured in 14 cases of pterygium and 14 normal specimens. They found statistically significant difference in gene expression for both LATS1 and LATS2 between two groups. Hypermethylation of the promoter regions of LATS1 and LATS2 promotes down-regulation of their mRNA levels in certain cancers and with a decreased expression of LATS1 or LATS2 mRNA promotes tumor formation. This study signifies the role of LATS1 and LATS2 in neoplastic origin of pterygium.

Ebrahimi et al.[25] reported that Cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) promoter hypermethylation might have a role in pterygium formation. Even though promoter methylation changes of CTLA4 gene were not statistically different between the two groups, the gene expression level of CTLA4 was markedly different in pterygium patients as compared to the healthy controls. CTLA4, encoded by the human CTLA4 gene is expressed on the surface of activated T cells and have been shown to a marker for certain cancers in humans. These studies have shown that the epigenetics changes that occur due to hypermethylation in promoter region of candidate genes might cause tumour like formation in pterygium patients.

The limitations of the current study include a low number of patients, and a selection bias in terms of it being a clinic based study which may not be fully representative of the general population. The current study found no correlation between MDM2 SNP309 gene mutation and pterygium. Further studies with a higher number of patients and controls may provide a better understanding of the role of MDM2 SNP309 mutation and its association with pterygium.

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