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CYP1A1 Gene Polymorphism in Inflammatory and non Inflammatory Pterygium

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Background and Objective: Pathogenesis of pterygium is not fully understood. CYP1A1 gene found to have role in carcinogen metabolisms and also detected in pterygium tissue. The purpose of this study was to assess the CYP1A1 gene polymorphisms in patients with inflammatory and non-inflammatory pterygium. **Methods:** The PCR was conducted on blood samples of 80 patients with pterygium which consisted of 40 inflammatory and 40 non-inflammatory pterygium. The rs 4646903 SNP genotyping T>C (m1) in the CYP1A1 gene was conducted using Restriction Fragment Length Polymorphisms-PCR (RFLP-PCR). The PCR products were cut with restriction enzyme *MspI*. Results of restriction were then electrophorised and then observed with GelDoc. **Results:** In inflammatory pterygium, 13 patients obtained TT alleles (wild type), 19 TC allele (heterozygous mutants) and 8 CC alleles (homozygous mutant). In non-inflammatory pterygium, 14 patients obtained TT allele (wild type), 18 TC allele (heterozygous mutant) and 8 CC alleles (homozygous mutant). **Conclusion:** The CYP1A1 gene polymorphisms may occur in pterygium patients either inflammatory or non-inflammatory. Polymorphisms occur in the form of heterozygous and homozygous mutant.

Key words: CYP1A1 polymorphism, inflammatory, non-inflammatory pterygium

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1 INTRODUCTION

Pterygium is epithelial hyperplasia characterized by fibrovascular tissue growth on the ocular surface, which grew out of conjunctival bulbar and proliferates all over the cornea¹. The disease is closely associated with exposure to ultraviolet (UV)-B radiations, primarily affect the stem cells in the limbus. Clinically, pterygium is associated with inflammation and neovascularization. Pterygium either primary or recurrent can cause changes in normal conditions such as astigmatism and changes in ocular surface, which eventually can lead to malfunctioning of vision due to worsen visual acuity²⁻⁴.

The pathogenesis of pterygium is still not fully understood. According to the researchers there is a relationship between pterygium with the involvement of environmental factors, such as viral infections or oxidative stress, anti-apoptotic mechanisms, mechanisms of immunology, cytokines, growth factors, extracellular matrix modulators, genetic factors and possibly other factors³⁻⁶.

Clinical symptoms of pterygium may be mild and often with no complaints at all (asymptomatic). However, some patients may also have complaints such as red eye, inflammation or irritation, burning sensation and foreign body sensation. Clinically, Donald Tan classified pterygium as atrophic, intermediate and fleshy⁷. In practice, complaints and symptoms of pterygium are composed of inflammatory and non-inflammatory.

Phototoxic ultraviolet radiation reacts directly or indirectly causing free radicals. According to previous studies free radicals are atoms or molecules that do not have a outer electrons partner, highly reactive and can react with molecules around them as discussed by Balci *et al.*⁸. Oxidative damage to cell membranes primarily experienced by unsaturated fatty acids and the DNA will cause damage in the form of DNA chain rupture, damage to deoxyribose and modified purine and pyrimidine. This damage will subsequently form DNA adducts⁸⁻¹⁰.

Pterygium has long been regarded as a chronic degenerative condition, but after the discovery of abnormal expression of p53 protein in the epithelium of pterygium, the pterygium is now regarded as an uncontrolled cell proliferation associated with ultraviolet, radiation like tumors. The P53 mutant has a direct or indirect effect to the increase in gene expression of growth by stimulating growth factors or growth receptors^{4,8}.

Environmental pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) are the result of incomplete combustion of organic materials derived from environmental carcinogens and are metabolized by different xenobiotic-metabolizing enzymes, one of which is cytochrome P450 (P450 or CYP)¹¹. This enzyme is mainly instrumental in the change of PAHs into more polar metabolites and soluble in water, so the metabolites formed can be excreted from the body. However,

during the metabolic process, various unstable and reactive metabolites of PAHs can be formed and these metabolites attack to DNA, causing toxicity and cell transformation. The polymorphism of CYP1A1 was previously found in pterygium tissue. Thus, it is stated that carcinogenic process was also involved in the pathogenesis of pterygium^{10,12}.

The CYP1A1 is a sub-family 1 of CYP gene superfamily, one of the important genes that encodes enzymes metabolizing carcinogens and is involved in the metabolism of carcinogenic PAHs. This gene has an important role in catalyzing the oxidative reaction of benzo(a)pyrene, which is one of the most important class of PAHs, into carcinogenic reactive metabolites. The CYP1 enzymes are responsible for the activation and detoxification of various metabolites of PAHs¹². The aim of the present study was to determine CYP1A1 gene polymorphisms found in pterygium tissue and its correlation with inflammatory and non inflammatory pterygium.

MATERIALS AND METHODS

Sample collection: Blood samples of 1 mL were obtained from 80 patients with pterygium (40 inflammatory and 40 non-inflammatory) from Central Eye Care (Balai Kesehatan Indra Masyarakat, BKIM) and private hospitals in Padang. Genomic DNA was isolated from blood samples of 300 mL using Genomic DNA mini kit (Blood/cultured cell) GB100, Geneaid. The DNA isolation kit was carried out according to procedures that outline sample preparation stage, the stage of cell lysis, DNA binding phase, washing steps and DNA elution stage.

Genotyping of rs4646903 (T>C): Genotyping of SNP rs4646903 T>C (m1) in the CYP1A1 gene was done using Restriction Fragment Length Polymorphism PCR (RFLP-PCR). The PCR reaction consisted of a total volume of 25 mL consisting of 0.2 µM deoxynucleoside triphosphates (dNTPs), 1 µg of genomic DNA, 1.25 U HotStarTaq bufermya DNA polymerase and its solution and 1.5 µM forward primer P79 (5'-AAGAGGTGTAGCCGCTGCACT-3') and 1.5 µM reverse primer P80 (5'-TAGGAGTCTTGTCTCATGCCT-3').

The PCR was performed with the following conditions: initial denaturation at a temperature of 95°C for 5 min, followed by 35 cycles of a series of processes consisting of denaturation at 94°C for 1 min, annealing at 55°C for 30 sec and elongation at 72°C for 30 sec. The PCR process ended with a final elongation step at 72°C for 10 min and PCR product size was 338 bp (Fig. 1).

The PCR products were cut with 1 U of restriction enzyme *MspI* (the restriction = C[^]CGG) at 37°C for 16 h. Then the restriction results were analyzed by electrophoresis on 2% agarose gel dyed with GelRed DNA and then observed with GelDoc.

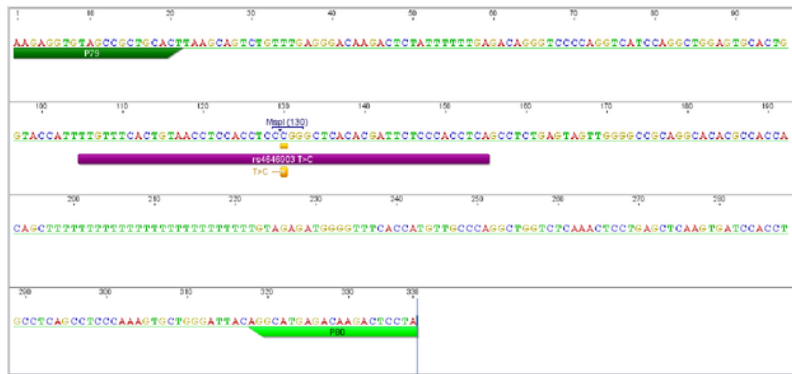


Fig. 1: Position of primer and the restriction enzyme on the gene CYP1A1 *MspI* allele m1

RESULTS

This study has been carried out an assessment of the CYP1A1 gene polymorphisms in patients with pterygium. Polymorphism is found in both groups of inflammatory and non-inflammatory, either heterozygous or homozygous mutant, in nearly equal numbers. The CYP1A1 gene is a sub-family 1 of CYP gene superfamily, that is involved in the metabolism of carcinogenic PAHs.

Blood samples from 80 patients with pterygium were examined, consisting of 40 inflammatory and 40 non-inflammatory. Clinical pterygium when viewed based on ages can be seen in Table 1.

Most people with inflammatory pterygium had more outdoor activities and otherwise people with non-inflammatory pterygium were more active indoors (indoor), as shown in Table 2. People with pterygium have a variety of symptoms, including a red eye due to irritation and inflammation, stinging, watering and foreign body sensation, but can also be found with no complaints at all. The correlation between duration of pterygium and patients complaints is shown in Table 3.

After PCR the product was cut with restriction enzyme *MspI*, the results were analyzed by electrophoresis restriction. Individuals who have wild-type genotype rs4646903 (TT alleles) produced a 338 bp DNA size. Individuals who have a heterozygous genotype rs4646903 (TC alleles) produced a 338 bp DNA size; 209 and 129 bp. Individuals who have a homozygous mutant genotype rs4646903 (CC allele) will produced a 209 bp DNA size and 129 bp, as shown in Fig. 2. A total of 27 individuals (13 inflammatory and 14 non-inflammatory) have a wild type genotype rs4646903 (TT alleles). Individuals who have a mutant heterozygous genotype rs4646903 (TC alleles) of 37 (19 inflammatory and 18 non-inflammatory) and individuals who have mutant homozygous genotype rs4646903 (CC alleles) were 16 (8 inflammatory and

Table 1: Frequency distribution of clinical pterygium by age groups

Groups of age (years)	Clinical manifestation		Total
	Inflammatory	Non-inflammatory	
30-39	4	2	6
40-49	9	9	18
50-59	13	12	25
60-69	11	7	18
70-79	3	6	9
80-89	0	4	4

Table 2: Frequency distribution of clinical pterygium by occupation

Occupation	Clinical manifestation		Total
	Inflammatory	Non-inflammatory	
Outdoor	24	13	37
Indoor	16	27	43

Table 3: Distribution of duration and complaints of pterygium

Duration of pterygium (year)	Symptoms		Total
	Yes	No	
<1	4	8	12
>1-2	11	16	27
>2-3	9	5	14
>3-4	0	0	0
>4-5	0	0	0
>5	18	9	27

16 non-inflammatory). Polymorphisms that occur in both groups of inflammatory and non-inflammatory were almost the same in mutant heterozygote, whereas mutant homozygote were equal in numbers (Table 4).

DISCUSSION

Pterygium is a disease closely associated with exposure to ultraviolet (UV)-B radiation, primarily affect the stem cells in the corneoscleral limbus. Di Girolamo *et al.*¹³ found that UV-B radiation was to be relevant with pterygium development. A stress-induced intracellular pathway and

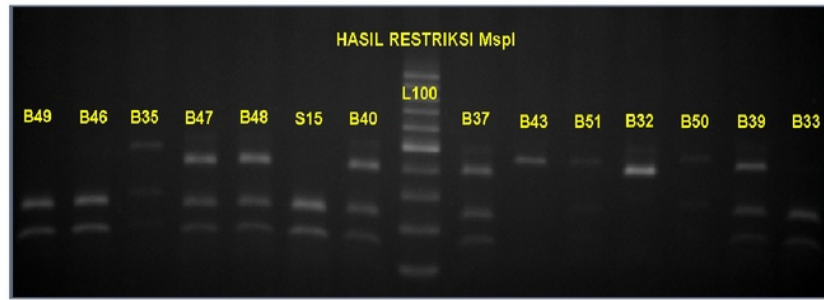


Fig. 2: Result of 2% agarose gel electrophoresis
 Wild type genotype produced 338 bp DNA size, while heterozygous genotype produced 338, 209 and 129 bp DNA size

Table 4: Distribution of CYP1A1 gene polymorphism frequency according to clinical pterygium

Clinical manifestation	Polymorphism of gene CYP1A1			Total
	Wild type (TT)	Mutant heterozygote (TC)	Mutant homozygote (CC)	
Inflammatory	13	19	8	40
Non-inflammatory	14	18	8	40
Total	27	37	16	80

potential cell-surface transmitters activated by UV radiation were identified in pterygium. Moreover, inflammatory cytokines and angiogenic factors were also identified in pterygium samples.

Clinically pterygium is associated with inflammation and neovascularization. Aspiotis *et al.*¹⁴ evaluated microvessel density (MVD) and expression of Vascular Endothelial Growth Factor (VEGF) and thrombospondin-1 (TSP-1) in pterygium samples. The study found especially high expression levels for VEGF compared to normal conjunctiva and it was concluded that the angiogenesis-related factors were proved to be highly expressed in pterygium tissue. Meanwhile, TSP expression level was low, allowing inducers of angiogenesis to act uninhibited which explained pathogenic basis of pterygium formation¹⁴.

The inflammation usually cause complaints including red eyes, pain, frequent watering and no foreign body sensation. Non-inflammatory type of pterygium can present with no complaints and all can be seen the white membrane that covers the cornea. Age involved can be started from second decades until old age. The prevalence generally increased in older age. Prevalence also tended to increase in outdoor workers, as they gain more exposure to ultraviolet B radiations^{15,16}.

The association of CYP1A1 protein and genetic polymorphism have been reported in several types of cancer, but less report was discussed about this in pterygium, especially in comparison between inflammatory and non-inflammatory pterygium. Therefore, in this study, the association of CYP1A1 gene polymorphism was analyzed in inflammatory and non-inflammatory pterygium. Young *et al.*¹⁷ found the correlation between CYP1A1 polymorphism with pterygium and stated that it might become a marker for the prediction of pterygium susceptibility.

Young also analyzed the CYP1A1 gene polymorphisms and GSTM1 using RFLP-PCR and PCR on 205 pterygium specimens and 206 control to understand how the CYP1A1 and GSTM1 polymorphisms increase the risk of pterygium. In the previous study, they found that CYP1A1 with genotype m1/m2 and m2/m2 was at risk of pterygium 1,553-fold greater than with genotype m1/m1. Young *et al.*¹⁷ formulated the hypothesis that after exposure to environmental PAH, genetic polymorphisms of CYP1A1 contribute to the risk of the pterygium formation thus CYP1A1 *MspI* polymorphisms can be used as a risk factor for pterygium¹⁸.

Peng *et al.*¹⁹ found that CYP1A1 expression in pterygium correlates with allelic variation and can be used as an independent risk marker. The study collected 150 pterygium samples and 50 normal conjunctiva samples, which served as control. Forty eight (33.3%) pterygium specimens tested positive for CYP1A1 protein expression. The CYP1A1 protein expression was significantly greater in the pterygium group than in the control group ($p < 0.0001$). In addition, CYP1A1 protein expression was associated with allelic variation. The CYP1A1 protein expression was significantly greater in the m2/m2 group than in the m1/m1 and m1/m2 groups ($p = 0.006$)¹⁹.

Tung *et al.*²⁰ found that the BPDE-like DNA adduct formation in pterygium samples was associated with CYP1A1 polymorphisms. Benzo[a]pyrene 7,8-diol 9,10-epoxide (BPDE), an ultimate metabolite of benzo[a]pyrene found to have relationship with CYP1A1. In this study, the pterygium was categorized into inflammatory and non-inflammatory. The results showed that CYP1A1 gene polymorphism was found in pterygium sample and it happened to be equal in both inflammatory and non-inflammatory pterygium.

CONCLUSION

The CYP1A1 gene polymorphisms may occur in the both types of pterygium, that is the inflammatory and non-inflammatory. Polymorphisms occur in form of mutant heterozygote and homozygote. In both groups, polymorphism of mutant heterozygote was found nearly equal in numbers while polymorphism of mutant homozygote was found just as much in both groups.

SIGNIFICANT STATEMENT

The pathogenesis of pterygium has long been hypnotized by many studies. The CYP1A1 gene is one of the genes known to have role in pathogenesis of pterygium. In this study, the CYP1A1 gene polymorphisms was determined in inflammatory and non inflammatory pterygium. To our knowledge, this study is the first study to prove gene mutation in inflammatory and non inflammatory pterygium.

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