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November 13-14th 2018

Mercure Hotel Padang, West Sumatra

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THE ASSOCIATION OF CYP1A1 GENE POLYMORPHISMS WITH CYP1A1 ENZYMES, p53 PROTEIN, AND VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN PTERYGIUM

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introduction

Pterygium is epithelial hyperplasia characterized by fibrovascular tissue growth on the ocular surface, which grows out of conjunctiva and proliferates all over the cornea.

Associated with :

- exposure to ultraviolet **(UV)-B**, primarily affect the limbal stem cells
- **Environmental** involvement

introduction

- Pterygium clinical symptoms may be mild and often with no complaints at all (asymptomatic).
- Complaints → red eye, inflammation, burning sensation, and foreign body sensation

- Clinically, Donald Tan classified pterygium as : atrophic, intermediate, and fleshy.
- In practice → inflammatory and non-inflammatory

- Formerly a chronic degenerative condition, but after the discovery of abnormal expression of p53 protein in the epithelium of pterygium → tumor-like uncontrolled cell proliferation

INTRODUCTION

VEGF

A heparin-binding vascular glycoprotein

Vascular permeability, cell proliferation, inflammation, tissue remodeling and angiogenesis

Important angiogenesis factors in the mechanism of pterygium formation

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) → result of incomplete combustion of organic materials from environmental carcinogens

Metabolized by different xenobiotic-metabolizing enzymes, one of which cythocrome P450 (P450 or CYP).

Attack DNA → toxicity and cell transformation

INTRODUCTION

CYP1A1 is a sub-family 1 CYP gene superfamily → encoding enzymes metabolizing carcinogens.

Hendriati et al (2017) classified pterigium based on its clinical symptoms into inflammatory and non-inflammatory pterygium and found that CYP1A1 polymorphisms may occur in both pterygium groups although the difference is not significant

The polymorphism of CYP1A1 was found in pteryigium tissue → carcinogenic involved in the pathogenesis of pterygium

Objective

This study aims to determine the association between CYP1A1*m1* (rs4646903) polymorphisms with the level of CYP1A1 enzymes, p53 protein, and vascular endothelial growth factor (VEGF) in patients with inflammatory and non-inflammatory pterygium.

Methods

- Cross sectional study comparative study design carried out in the Department of Ophthalmology Dr. M. Djamil General Hospital Padang, Community Health Center (BKIM) Padang, and private hospitals to collect samples.
- The study population were all pterigium patients

METHODS

Inclusion criteria

- Diagnosed with primary pterigium unilaterally and bilaterally, criteria of surgical indication, not receiving anti-VEGF therapy or steroids in the last 2 weeks, and agreed to participate in this study.

Exclusion criteria

- Anterior segment infection (conjunctivitis and keratitis) and other disorders in the conjunctiva, such as benign and malignant lesions of the conjunctiva.

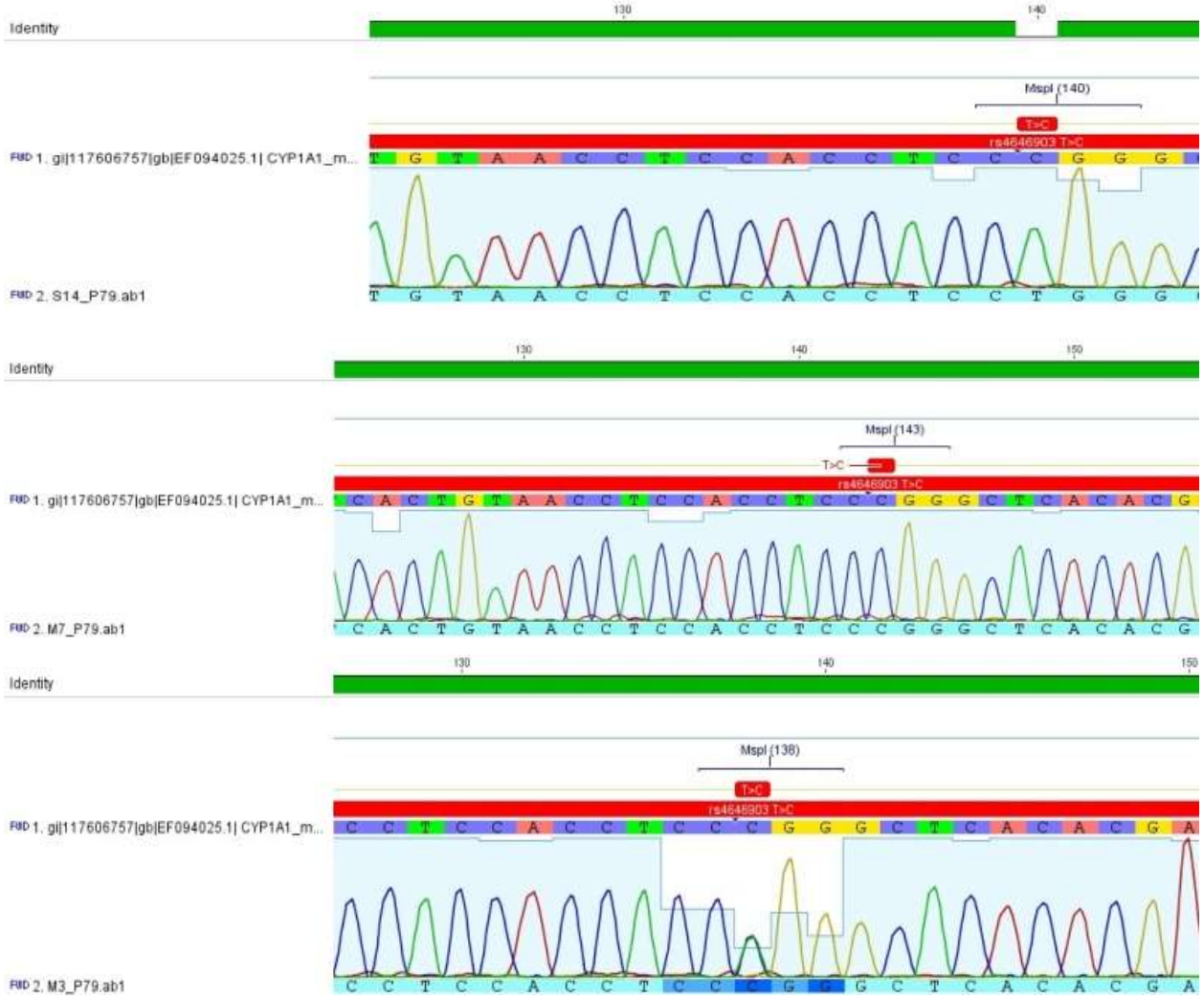
Methods

Isolation of Genomic DNA

Genotyping rs4646903 (T> C)

VEGF examination

RESULT



Sequencing results from 3 DNA samples; (a) sample S14 shows the sequencing results confirmed T> T wild type, (b) sample M7 shows the sequencing results confirmed C> C homozygote, and (c) M3 shows the sequences confirmed by T> C heterozygote

RESULT

CYP1A1*m1* Polymorphism in Pterygium

Polymorphism	Pterygium		Total f(%)	P
	Inflammatory f (%)	Non inflammatory f (%)		
Wild type (TT)	12 (54,5)	10 (45,5)	22 (100)	0,861 *
Mutant Homozygote	7 (50,0)	7 (50,0)	14 (100)	
Mutant Heterozygote	16 (47.1)	18 (52,9)	34 (100)	
Total	35 (50)	35 (50)	70 (100)	

CYP1A1 polymorphisms divided into :

- ✓ wild type
- ✓ mutant homozygote
- ✓ mutant heterozygote.

In both groups, mutant heterozygote polymorphisms were found to be higher than the wild type and homozygote types (34: 22: 14)

RESULT

CYP1A1 Level in Inflammatory and Non-Inflammatory Pterygium

Variables	Pterygium		p
	Inflammatory Mean (SD)	Non-inflammatory Mean (SD)	
CYP1A1 Enzymes Level	0,15 (0,06)	0,13 (0,07)	0,094*

The mean CYP1A1 enzyme level in the inflammatory pterygium group was higher than non-inflammatory pterygium, ie 0.15 (0.06) versus 0.13 (0.07).)

Protein p53 Level in Inflammatory and Non-Inflammatory Pterygium

Variables	Pterygium		p
	Inflammatory Mean (SD)	Non-inlammatory Mean (SD)	
P53 protein in pterygium tissue	13,21 (8,20)	13,66 (11,37)	1,00*

The average p53 protein content in pterigium group was not inflamed higher than inflammatory pterygium, ie 13.66 (11.37) versus 13.21 (8.20).

VEGF Level in Inflammatory and Non-Inflammatory Pterygium

Variables	Pterygium		p
	Inflamasi Rerata (SD)	Tidak Inflmasi Rerata (SD)	
VEGF level	11,99 (7,24)	9,71 (5,14)	0,136*

The mean VEGF levels in the inflammatory pterygium group were higher than the non-inflammatory pterygium, ie 11.99 (7.24) versus 9.71 (5.14).

Correlation of CYP1A1m1 Polymorphism with CYP1A1, Protein P53 and VEGF level on inflammatory and non-inflammatory Pterygium

CYP1A1 enzyme level in wild type and mutant heterozygote group is higher in inflammatory, non inflammatory was higher in mutant homozygote polymorphism.

p53 protein level in inflammatory pterygium group is higher in **mutant heterozygote**, while in non-inflammatory group the highest was **the wild type**

VEGF levels is highest in mutant homozygote in inflammatory group, whle in non-inflammatory group the VEGF level is highest in mutant homozygote group.

Discussion

CYP1A1m1 polymorphism (rs4646903) in inflammatory and non inflammatory Pterygium

- CYP1A1 m1 gene polymorphism was found in both pterygium groups.
- Mutant heterozygote polymorphism is a more common in both groups
- **Tung et al (2010**) → CYP1A1 protein expression in the mutant homozygote m2/m2 group is higher → did not match this study (mutant heterozygote group is higher)

Discussion

Different levels of CYP1A1 enzyme in inflammatory and non inflammatory Pterygium

- CYP1A1 enzyme level in the inflammatory pterygium group was higher than non-inflammatory group → no statistically significant different
- **Tung et al (2010)** → found a correlation between CYP1A1 polymorphism with BPDE-like DNA adduct formation in pterygium samples
- Cytochrome-P450 1A1 (CYP1A1) is involved in the metabolism of many xenobiotic compounds and endogenous lipophilic substances, which can activate procarcinogen into DNA reactive metabolites

Discussion

Differences in p53 protein levels in inflammatory and non inflammatory Pterygium

P53 mutants proven to activate Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF) and Vascular Endothelial Growth Factor (VEGF)

The results of this study showed no significant differences between p53 protein levels in inflammatory and non inflammatory pterygium

Differences in VEGF levels in inflammatory and non inflammatory Pterygium

VEGF : cell proliferation, inflammation, connective tissue remodeling and angiogenesis	No significant difference between VEGF levels in inflammatory and non inflammatory pterygium	**Peng et al (2014)** showed significant higher levels of VEGF in pterygium epithelial cells than normal conjunctival epithelium

The association of CYP1A1m1 polymorphism with CYP1A1, p53 and VEGF levels in inflammatory and non inflammatory pterygium

There was no significant correlation between CYP1A1m1 polymorphism with mean of CYP1A1, p53, and VEGF enzyme levels in pterigium in both inflammatory and non-inflammatory groups

Peng (2014) and Young (2010) → CYP1A1 protein expression of the m1/m1 genotype was not more significant than the m2/m2 genotype

Peng et al (2014) → variation of CYP1A1 allele could cause high BPDE-like DNA adduct formation rate, contributing to pterygium risk.

Conclusion

- Polymorphisms in the CYP1A1 gene either in the form of mutant homozygote (CC), mutant heterozygote (TC), and wild type (TT).
- Mutant heterozygote is the most common form of polymorphism between the two groups of inflammatory and non-inflammatory groups.
- The mean levels of CYP1A1 enzyme and VEGF levels in inflammatory pterygium tissue were higher than non-inflammatory pterygium while the higher p53 protein level in pterigium was found in non inflammatory pterygium compared to inflammatory pterygium, but statistically the difference was not significant.
- CYP1A1 gene polymorphisms have not been shown to be associated with levels of CYP1A1 enzymes, p53 protein and VEGF level in both pterygium groups.

THANK YOU