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Decreased Expression of Cytosolic Phospholipase A2 in Normal Conjunctiva May be Contributing to the Formation of Pterygium

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Reşat Duman and MT designed the study. Authors Reşat Duman and BDY performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Reşat Duman and Rahmi Duman managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Although there has been ongoing evidence about the role of inflammation in the pathophysiology of pterygium, exact inflammatory pathogenesis is not known. Cytosolic phospholipase A2 (cPLA2) is an inflammatory enzyme taking a role in various physiological and pathological responses. The present study aimed to investigate the expression of cPLA2 in human pterygium tissues.
Materials and Methods: Expressions of cPLA2 were analyzed by immunohistochemistry in biopsy specimens of 20 pterygium and 10 normal conjunctival tissues. Expression patterns and staining intensities were compared between pterygium and normal tissues.
Results: Intense expression of $cPLA_2$ was present in all normal conjunctival tissues (100%)

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whereas no expression was detected in 5 pterygium cases (25%), and in the remaining pterygium tissues (75%) mild/ moderate expression of $cPLA_2$ was shown. Furthermore, expression was shown throughout the epithelium being more intense in the superficial layers in normal conjunctivas, whereas it was mainly located in basal layers in pterygium tissues.
Conclusion: Downregulated expression of cPLA2 in pterygium tissues compared to normal conjunctiva shown in the present study supports the role of inflammatory enzymes in the formation of pterygium. cPLA2 enzyme seems to have a protective role against pterygium formation.

Keywords: Pterygium; phospholipase A2; inflammation; conjunctiva; fibrovascular tissue.

1. INTRODUCTION

Pterygium is a common ocular surface disease with an unknown etiopathogenesis, characterized by fibrovascular tissue formation extending from the bulbar conjunctiva to the cornea [1]. Although several previous reports suggested an etiopathogenetic role of ultraviolet exposure, temperature changes, microtrauma, infections, various immunologic and antiapoptotic mechanisms, the pathogenesis of pterygium still remains unclear [1,2].

Currently, the most accepted theory for pterygium formation includes ultraviolet-induced limbal damage, chronic inflammation, and increased angiogenesis. Although there is evidence about the role of inflammation in pterygium formation, exact inflammatory pathways leading to this lesion have not yet been clarified. Therefore, there is a need for new studies on pterygium formation.

Phospholipase A2 (PLA2) molecules are a family of enzymes catalyzing separation of free fatty acids and lysophospholipids from biological membranes and playing role in various inflammatory and allergic pathways especially through arachidonic acid metabolism [3]. PLA2 family contains several enzymes that can be categorized into different classes as secretory PLA2 (sPLA2), cytosolic PLA2 (cPLA2), calcium-independent PLA2 (iPLA2), platelet activating factor-acyl hydrolase, lysosomal PLA2, and adipose-specific PLA2 forms [3,4]. In addition to inflammatory actions, some PLA2s play role in cellular proliferation by activating cell signaling [3,4]. Furthermore, recent literature data showed a role of PLA2s in inflammation after ultraviolet exposure and also pathogenesis of several diseases including atherosclerosis, neuro-degeneration, arthritis, tumorogenesis, and angiogenesis in various cancers [5-7].

Recently, PLA2 expression in normal conjunctiva was shown, and some classes of PLA2 have been associated with ocular pathologies including glaucoma [8]. To our knowledge, there is no previous study on the expression of PLA2 in pterygium. Thus, the present study aimed to investigate immunohistochemically cPLA2 enzyme expression in pterygium tissues compared to normal conjunctiva.

2. MATERIALS AND METHODS

This comparative immunohistochemical study, performed by departments of Ophthalmology and Histology & Embryology, included biopsy specimens of 20 pterygium tissues and 10 normal conjunctival tissues. The study protocol was reviewed and approved by the institutional ethics committee and was conducted according to the Declaration of Helsinki. Before excision of the tissues written informed consent was obtained from all patients. All samples were obtained from patients without any systemic disease or another ocular disease including glaucoma. The study included only quiescent nasal pterygium cases, and inflammatory nasal and temporal cases were excluded from the study.

Normal conjunctival tissues were obtained from the healthy conjunctivas during the removal of donor conjunctival graft for pterygium surgery, and samples taken from the same areas were included in the study to avoid regional differences.

For immunohistochemical analysis, 5 µm thickness tissue sections were stained using antibodies against cPLA2 (Ab58375; Abcam, Cambridge, UK). cPLA2 expression levels were determined by the same blinded histologist using light microscopy, and intensity of epithelial and stromal stainings was categorized as follows: low expression, moderate expression, and intense expression.

Statistical analyses were performed using SPSS v.18.0 for Windows (SPSS Inc., Chicago, IL,

USA). Comparisons between groups were analyzed using Chi-square test, and p values <0.05 were determined to be significant.

3. RESULTS

The study included 20 pterygium and 10 normal conjunctival tissues of patients without any systemic or ocular disease other than pterygium. In all normal conjunctival tissues (100%) intense expression of cPLA2 was present whereas in pterygium group no expression was detected in 5 (25%) cases and in the remaining pterygium tissues (75%) mild/ moderate expression of cPLA2 was shown. In none of the pterygium cases (0%) intense expression was found (p=0.001). Furthermore, in both groups positively stained cells were mainly localized in the epithelium and stromal stainings were weak. In normal tissues, intense staining was observed throughout the epithelium with a little bit more intense staining in upper layers. In contrast, in pterygium groups positively stained cells were mainly located in the lower layers of the epithelium. Comparison of expression of cPLA2 between groups was given in Fig. 1.

4. DISCUSSION

In the present study, we evaluated immunohistochemically the expression of cPLA2 in the pterygium tissues. To our knowledge, this is the first study searching expression of this group of enzymes in the pterygium tissues compared to normal conjunctiva. And the major finding of the present study was that expression of cPLA2 was significantly lower in pterygium tissues compared to normal conjunctiva.

Literature data on expression of PLA2 in the human conjunctiva is limited. Helin M et al. firstly demonstrated that various classes of PLA2 including sPLA2, iPLA2, and cPLA2 were expressed in the conjunctiva [8]. In their study the expression intensity was low and positively stained cells were mainly localized in the surface of the epithelium. Similarly, we demonstrated positive expression of cPLA2 in the normal conjunctiva. However in contrast to their findings staining in our study was intense, and we observed positive staining throughout the epithelium despite a more intense staining in the superficial layers. These differences between two studies may be associated with several factors including immunohistochemical techniques, types of antibodies, and interobserver variabilities. Nevertheless, the findings of the two studies provide literature evidence about the positive expression of cPLA2 in the normal conjunctiva, especially being more intense in the superficial epithelium.

The present study findings also showed that expression of cPLA2 was significantly lower in pterygium tissues. Therefore, cPLA2 may be considered to have a protective role against pterygium formation. Previously several studies have reported an association between pterygium formation and ocular surface damage due to several factors such as solar injury, dry eye changes, and microtraumas [9-12]. In the present study, more intense superficial expression of cPLA2 in normal tissues and lack of immunostaining especially in superficial layers in pterygium tissues may support the potential protective role of cPLA2 against the superficial conjunctival damage leading to pterygium formation. Similarly, Helin M et al. also concluded that several PLA2 types including cPLA2 might take a role in the protection of conjunctiva against superficial risks including mechanical wear, tear stress, and infections [8]. In conclusion, according to the present study findings, cPLA2 seems to have a protective role for conjunctiva.

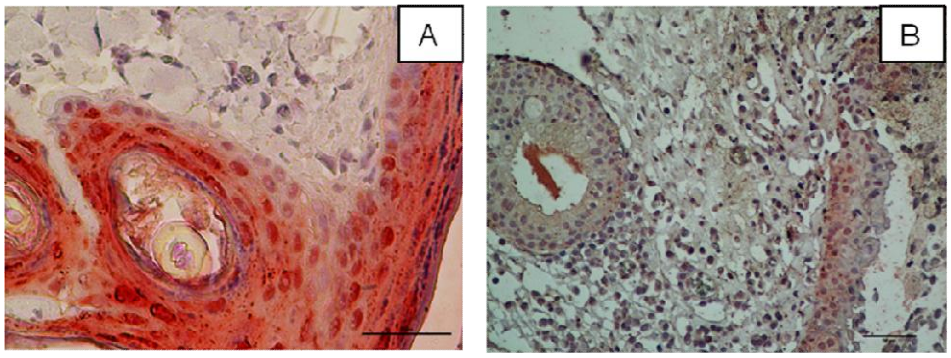

Fig. 1. Comparison of expression of cPLA2 in normal conjunctival tissue (A) vs. pterygium tissue (B)

One of the major functions of the cPLA2 enzymes is providing free arachidonic acid for the production of eicosanoids such as prostaglandin E2 (PGE2) that regulate inflammatory and immune responses to various stimuli [12]. Previously it has been shown that PGE2 and its receptors found in conjunctival epithelium contribute to regulation of ocular surface inflammation. In addition, there are some recent data about the role of PGE2 and its receptors in formation and recurrence of pterygium [13].

Fibroblast activation and abnormal extracellular matrix (ECM) accumulation appear to be major features of pterygium, as shown by previous studies indicating upregulation of ECM genes such as fibronectin, collagen, and versican in pterygium [14]. There is enough literature evidence that PGE2 negatively regulates fibroblast proliferation, deposition of ECM proteins, and the pathogenesis of fibrosis [12]. Similarly, cPLA2 deficient fibroblasts were shown to synthesize increased amounts of collagen compared with wild-type fibroblasts [12]. All these study findings suggest a role for cPLA2 and PGE2 in regulating collagen production and ECM deposition. The present study findings showing an association between decreased cPLA2 and pterygium formation may be explained by the negative regulatory role of cPLA2 on fibroblast activation and ECM remodeling.

The major drawback of the present study is a limited number of cases which is because of including only patients without any other ocular disease or systemic disorder, which may affect the expression of the inflammatory enzymes. In addition, control and pterygium tissue samples were taken from the same eye is another limitation of the study and It would have been better to consider a different sample of normal eyes. However, the study findings make a considerable contribution to the literature by supporting the role of cPLA2 in both prevention of fibrosis and ECM deposition and also the protection of conjunctiva against pterygium formation.

5. CONCLUSION

Downregulated expression of cPLA2 in pterygium tissues compared to normal conjunctiva shown in the present study supports the role of inflammatory enzymes in the formation of pterygium. cPLA2 enzyme seems to have a protective role against pterygium formation. Exact molecular pathways causing low expression of cPLA2 in pterygium tissues and the exact role of cPLA2 in the formation of pterygium need to be clarified with future studies. In addition, future studies may be planned to point out potential usage of cPLA2 analogs as a targeted therapy in pterygium tissues.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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