

Decreased expression of Cytosolic Phospholipase A2 in normal conjunctiva may be contributing to the formation of pterygium**Running Head:** Expression of Phospholipase A₂ in pterygium

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.

Material 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 cPLA₂ was present in all normal conjunctival tissues (100%) whereas no expression was detected in 5 pterygium cases (25%), and in the remaining pterygium tissues (75%) mild/ moderate expression of cPLA₂ 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.

16 **1. INTRODUCTION**

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

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

27 Phospholipase A2 (PLA2) molecules are a family of enzymes catalyzing separation of free
28 fatty acids and lysophospholipids from biological membranes and playing role in various
29 inflammatory and allergic pathways especially through arachidonic acid metabolism (3).
30 PLA2 family contains several enzymes that can be categorized into different classes as
31 secretory PLA2 (sPLA2), cytosolic PLA2 (cPLA2), calcium-independent PLA2 (iPLA2),
32 platelet activating factor-acyl hydrolase, lysosomal PLA2, and adipose-specific PLA2 forms
33 (3, 4). In addition to inflammatory actions, some PLA2s play role in cellular proliferation by
34 activating cell signaling (3, 4). Furthermore, recent literature data showed a role of PLA2s in
35 inflammation after ultraviolet exposure and also pathogenesis of several diseases including
36 atherosclerosis, neurodegeneration, arthritis, tumorigenesis, and angiogenesis in various
37 cancers (5-7).

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

43 **2. MATERIAL AND METHODS**

44
45 This comparative immunohistochemical study, performed by departments of Ophthalmology
46 and Histology & Embryology, included biopsy specimens of 20 pterygium tissues and 10
47 normal conjunctival tissues. The study protocol was reviewed and approved by the
48 institutional ethics committee and was conducted according to the Declaration of Helsinki.
49 Before excision of the tissues written informed consent was obtained from all patients. All
50 samples were obtained from patients without any systemic disease or another ocular
51 disease including glaucoma. The study included only quiescent nasal pterygium cases, and
52 inflammatory nasal and temporal cases were excluded from the study.

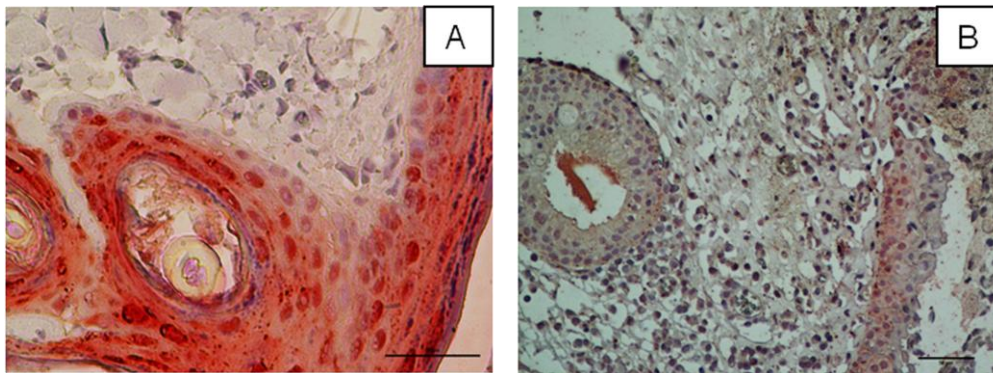
53 Normal conjunctival tissues were obtained from the healthy conjunctivas during the removal
54 of donor conjunctival graft for pterygium surgery, and samples taken from the same areas
55 were included in the study to avoid regional differences.

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

61 Statistical analyses were performed using SPSS v.18.0 for Windows (SPSS Inc., Chicago,
62 IL, USA). Comparisons between groups were analyzed using Chi-square test, and p values
63 <0.05 were determined to be significant.

64 3. RESULTS

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



76
77 **Figure 1:** Comparison of expression of cPLA2 in normal conjunctival tissue (A) vs. pterygium
78 tissue (B).

79 4. DISCUSSION

80 In the present study we aimed to compare the retinal layer effects of cruciate and circular In
81 the present study, we evaluated immunohistochemically the expression of cPLA2 in the
82 pterygium tissues. To our knowledge, this is the first study searching expression of this
83 group of enzymes in the pterygium tissues compared to normal conjunctiva. And the major
84 finding of the present study was that expression of cPLA2 was significantly lower in
85 pterygium tissues compared to normal conjunctiva.

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

96 expression of cPLA2 in the normal conjunctiva, especially being more intense in the
97 superficial epithelium.

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

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

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

126 The major drawback of the present study is a limited number of cases which is because of
127 including only patients without any other ocular disease or systemic disorder, which may
128 affect the expression of the inflammatory enzymes. However, the study findings make a
129 considerable contribution to the literature by supporting the role of cPLA2 in both prevention
130 of fibrosis and ECM deposition and also the protection of conjunctiva against pterygium
131 formation.

132 **5. CONCLUSION**

133
134 Downregulated expression of cPLA2 in pterygium tissues compared to normal conjunctiva
135 shown in the present study supports the role of inflammatory enzymes in the formation of
136 pterygium. cPLA2 enzyme seems to have a protective role against pterygium formation.
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138 **CONSENT**

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

141 **ETHICAL APPROVAL**

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

144 **COMPETING INTERESTS**

145 Authors have declared that no competing interests exist.

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