Original Article

Correlations Between Histopathologic Changes and Clinical Features in Pterygia

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Abstract

Purpose: To investigate the correlations between clinical findings and histopathologic changes in eyes with pterygium.

Methods: This prospective study included 70 eyes with primary pterygia undergoing surgical excision. Prior to surgery, clinical features of the pterygia including extension over the cornea, redness, fleshiness (based on obscuration of the underlying episcleral vessels), and obliteration of the plica semilunaris were determined. Postoperatively, pterygium specimens were examined by hematoxylin-eosin and trichrome staining to evaluate histopathologic characteristics including vascular density, leukocytic infiltration, stromal elastosis, stromal fibrosis and subepithelial fibrosis. Correlations between clinical findings and histopathologic changes were then investigated.

Results: There was a marginally significant correlation between the redness and the fleshiness of pterygium ($P = 0.06$). Both redness and fleshiness of the pterygium had significant positive correlation with dimensions of the lesion over the cornea. Moreover, larger pterygia were associated with obliteration of the plica semilunaris. Pterygium redness showed a significant correlation with vascular density ($P = 0.04$), and pterygium fleshiness had a significant correlation with stromal fibrosis ($P = 0.04$). Pterygium dimensions over the cornea demonstrated a positive correlation with vascular density and a negative correlation with stromal elastosis.

Conclusion: Redness and fleshiness of pterygium were only marginally correlated with each other, and each one showed a correlation with different histopathologic features. Larger pterygia were associated with more significant changes at the clinical and histopathologic levels.

Keywords: Elastosis; Fibrosis; Pterygium; Vascular Density

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INTRODUCTION

A pterygium is a lesion characterized by encroachment of fibrovascular tissue from the bulbar conjunctiva onto the cornea. Clinically, pterygia usually present as a

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triangular fleshy tissue which grow on the nasal side of the cornea within the interpalpebral fissure. Extension of pterygia over the cornea and its fleshiness vary widely among individual cases. Lesion fleshiness has previously been used to classify different pterygia,[1] and it has been shown that fleshy pterygia are associated with higher recurrence rates after excision, as compared to atrophic ones.[1,2]

Histopathologically, a pterygium is characterized by the presence of a combination of elastotic degeneration of collagen together with fibrovascular proliferation. Various histopathologic features have been reported in pterygia including epithelial changes, elastoid degeneration, fibrovascular proliferation, leukocytic infiltration, fibrosis, angiogenesis and extracellular matrix breakdown.[3-5] Histopathologic changes and clinical features in pterygia have been described in many studies; however, the correlations between these histopathologic and clinical characteristics have not been addressed in detail. These correlations may provide a better understanding of the pathogenesis and clinical manifestations of pterygium. Therefore, the present study was designed to evaluate the correlations between histopathologic changes and clinical features in pterygium.

METHODS

This prospective study included 70 eyes of 69 patients with primary nasal pterygia which underwent excision. The cases were consecutively selected from candidates for pterygium surgery. Exclusion criteria were previous medical treatment for pterygium including topical steroids or non-steroidal anti-inflammatory drugs, previous conjunctival surgery, conjunctival cicatricial disease, systemic autoimmune disease, and untreated dry eye disease. The study was approved by the Institutional Review Board of Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran and conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh, 2000). Written informed consent was obtained from all participants before surgery.

Prior to the procedure, all patients underwent a complete ocular examination including slit lamp biomicroscopy and photography. Pterygium dimensions over the cornea were measured by image analysis of calibrated digital photographs in primary position by Motic Images 2000 software (Motic Germany GmbH, Wetzlar, Germany). These dimensions comprised of length (distance from the edge of the pterygium apex to the limbus), width (distance between the two opposite pterygium edges intersecting the limbus), and the surface area of pterygium over the cornea. Measurements were performed by an examiner who was masked to other clinical and histopathological data. Other clinical morphologic features including: redness, fleshiness, and condition of the plica semilunaris were also evaluated for each pterygium.

Redness of the pterygium body was graded as grade I (no redness or faint pinkish hue), grade II (scattered areas with moderate redness) and grade III (significant diffuse redness) [Figure 1a-c]. Pterygium fleshiness was graded according to the description by Tan et al[1] through which pterygia were graded as grade T1 (atrophic pterygium) in which episcleral vessels were not obscured by the body of the lesion, grade T3 (fleshy pterygium) in which episcleral vessels were totally obscured, and grade T2 (those between grades T1 and T3) with partially obscured episcleral vessels Denion et al classification was modified to define the plica semilunaris as either present or obliterated.[6] All these clinical features were independently scored by two clinicians. In case of discrepancy, a third opinion was obtained.

All patients underwent operation using a similar technique for excision of the pterygia. The surgery included removal of the pterygium head from the cornea by blunt dissection followed by excision of the pterygium body with incisions at the borders of the body and 3 mm in front of caruncle. After application of mitomycin C, the procedure was concluded with conjunctival autograft or amniotic membrane transplantation.

Surgical specimens were immediately fixed in 10% buffered formalin (pH = 7.3), and then embedded in paraffin. Five micron-thick sections were prepared and stained with hematoxylin/eosin and trichrome staining methods. All specimens were evaluated by a pathologist who was masked to the clinical features of the pterygia. Histopathologic characteristics including vascular density, severity of leukocytic infiltration, stromal elastosis, subepithelial fibrosis, and stromal fibrosis were determined.

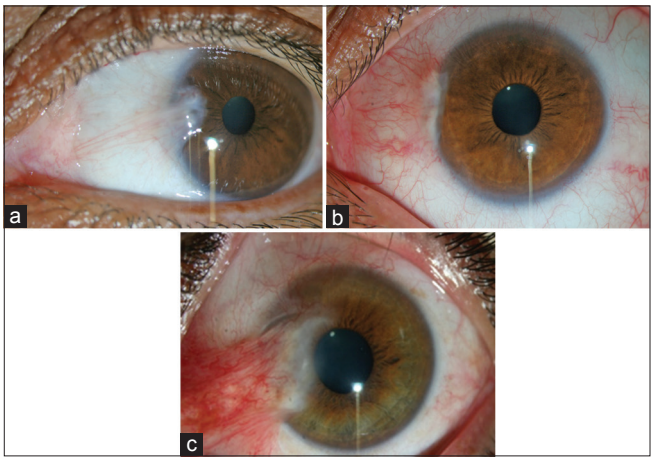

Figure 1. Slit-lamp photographs show pterygium redness severity, scored as (a) grade I: No redness or faint pinkish hue; (b) grade II: Scattered areas with moderate redness, and (c) grade III: Significant and diffuse redness.

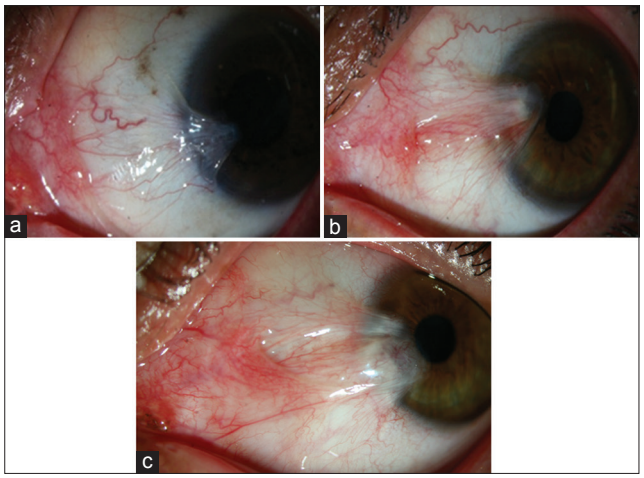

Figure 2. Slit-lamp photographs of pterygium fleshiness scored based on Tan classification as (a) grade T1, atrophic pterygium with visible episcleral vessels under the body of the pterygium; (b) grade T2, semi-fleshy pterygium with partial obscuration of episcleral vessels; and (c) grade T3, fleshy pterygium with total obscuration of episcleral vessels.

Vascular density was defined as the average vessel count in three high power fields (HPF, ×400) in the areas appearing as the most vascularized foci. For this purpose, the whole pterygium specimen was examined and 3 HPF which seemed to have the greatest vascular density were selected; only blood vessels lined by endothelium and containing RBCs were counted. Any space without an endothelial lining including all pseudo-vascular spaces and any real capillary without an observable endothelium were not counted. Lymphatic vessels were not considered. The presence of endothelium makes it possible to differentiate a vessel from pseudo-vascular channel, and RBCs differentiate a vascular channel from a lymphatic vessel.

The severity of leukocytic infiltration was graded with the following scale: Grade 0, unremarkable or few sparse lymphoid infiltrations; and grade I, significant patchy or diffuse leukocytic infiltration in at least one ×400 microscopic field [Figure 3a and b]. The percentage of fibrosis and elastosis were described as the proportion of dense fibrotic and elastotic changes, respectively, to the whole fibroconnective stroma.

Statistical analysis was performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). The Student's *t*-test, two-tailed Pearson correlation test, and Chi-square test were used to investigate the correlations between clinical findings and histopathologic characteristics. Furthermore, the analysis included evaluation of correlations among clinical features and among histopathologic features. *P* values of <0.05 were considered as statistically significant.

RESULTS

Seventy eyes of 69 patients including 46 male and 23 female subjects with mean age of 48.5 ± 14.6 (range, 20-83) years were included. Mean dimensions of the pterygia over the cornea consisted of the followings: length, 3.7 ± 1.9 (range, 1.7-6.6) mm, width, 6.7 ± 1.3 (range, 3.8-10.5) mm, and area, 17.7 ± 12.3 (range, 2.4-51.0) mm^2. The redness of the pterygium body was graded as grade I in 28 (40%) eyes, grade II in 30 (42.8%) eyes, and grade III in 12 (17.2%) eyes. Pterygium fleshiness was grade T1 in 18 (25.8%) eyes, grade T2 in 31 (44.2%) eyes, and T3 in 21 (30%) eyes. The plica semilunaris was present in 53 (75.7%) eyes and obliterated in 17 (24.3%) eyes.

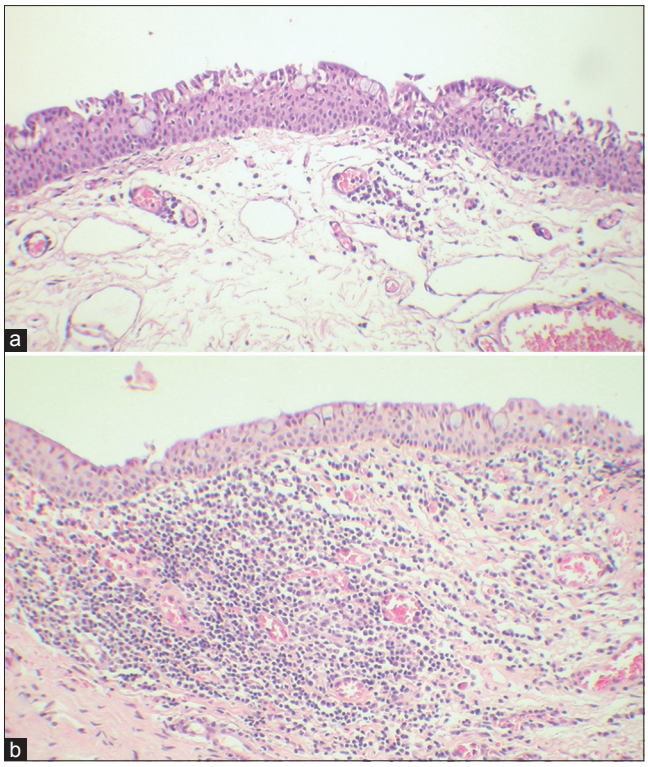

Figure 3. Leukocytic infiltration grading (H and E, ×400): (a) grade 0, unremarkable or limited few sparse lymphoid infiltrations; and (b) grade I, significant patchy or diffuse leukocytic infiltration.

Mean vascular density in the pterygia was 15.3 ± 4.6 (range, 6-28) vessel/HPF. Leukocytic infiltration was graded as grade 0 and grade I in 43 (61.4%) and 27 (38.6%) eyes, respectively. Mean percentage of subepithelial fibrosis and stromal fibrosis was 16.3 ± 19.4 (range, 0-66) % and 5.7 ± 7.7 (range, 0-30) %, respectively. Mean percentage of stromal elastosis was 6.1 ± 9.9 (range, 0-50) %.

Correlations among Clinical Features

There was a marginally significant correlation between pterygium redness and fleshiness ($P = 0.06$). Both redness and fleshiness of the pterygia had a significant positive correlation with dimensions of the lesion over the cornea [Table 1]. Moreover, all dimensional parameters were significantly greater in pterygia with obliterated plica

Table 1. Correlations between pterygium dimensions over the cornea and pterygium redness and fleshiness

Pterygium dimension	Redness			*P*	Fleshiness			*P*
	Grade I	Grade II	Grade III		Grade T1	Grade T2	Grade T3	
Length (mm)	1.3±2.8	4.1±1.7	4.7±2.2	0.001	3.1±1.5	3.9±2.1	4.2±1.7	0.060
Width (mm)	6.3±1.1	6.9±1.2	7.3±1.8	0.030	6.1±1.2	6.9±1.5	7.3±1.1	0.047
Area (mm^2)	11.6±6.9	19.8±11.4	24.5±14.1	0.001	12.1±6.9	18.7±15.0	20.1±12.3	0.032

mm, millimeter

semilunaris as compared to those without obliteration of the plica [Table 2].

Correlations between Clinical and Histopathologic Features

Pterygium redness showed a significant correlation with vascular density but not with other histopathologic features [Figure 4 and Table 3]. Pterygium fleshiness demonstrated a significant correlation with stromal fibrosis but not with other histopathologic features such as vascular density [Table 4]. A significant positive association was found between lesion dimensions over the cornea and vascular density ($P < 0.001$); dimensions, however, had a negative correlation with stromal elastosis [Table 5]. No other significant correlation was observed among other clinical and histopathologic features.

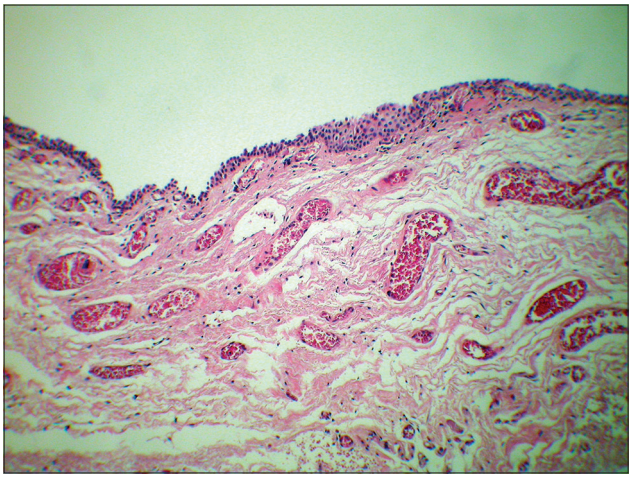

Figure 4. Marked vascular density (H and E, ×400) correlated with high grade pterygium redness as shown in Figure 1c.

DISCUSSION

Despite being a very common disease, there has been limited data on the correlation between clinical features and histopathologic changes in pterygia. Through evaluation of this correlation, this study found significant associations among clinical characteristics as well as between clinical and histopathologic changes in pterygia.

Pterygium dimensions over the cornea have been evaluated in many previous studies; however, other clinical features have been described infrequently. In order to grade pterygium fleshiness, visibility of the underlying episcleral vessels has been used in a few studies.[1] In addition, it has been shown that pterygia with higher grades of fleshiness have greater recurrence rates after surgery.[1,2] Nonetheless, the visibility of underlying vessels may be influenced by both the amount of fibrovascular tissue (as a sign of pterygium activity) and the amount of fibrosis (as a sign of pterygium quiescence). This is the reason why we separately categorized redness and fleshiness of pterygium. Interestingly, only a marginally significant correlation was observed between pterygium redness and fleshiness in the current study ($P = 0.06$). Thus in future studies, redness and fleshiness may be used separately to define pterygia with higher recurrence rates after excision.

The present study showed that pterygium redness and fleshiness had positive correlations with lesion dimensions over the cornea [Table 1]. Larger pterygium size may implicate current or old activity of the tissue, resulting in increased fibrovascular tissue or fibrous tissue, respectively, which in turn can be associated with increased redness and/or fleshiness. On the other hand, larger lesions over the cornea may be associated with greater thickness and volume of the pterygium body, leading to higher grades of redness and fleshiness. Our study also showed a positive correlation between pterygium dimensions and obliteration of the plica semilunaris [Table 2]. This obliteration may occur due to exertion of tractional forces at both pterygium ends; these forces are expected to be greater in larger pterygia.

We also noted increased vascular density in pterygia with higher grades of redness [Table 3]. The degree of pterygium redness is expected to be an indicator of its vascular content. Therefore, redness may be determined not only by vascular density but also by other parameters such as vessel diameter as well as total content of vascular tissue; the latter in turn depends on thickness of the pterygium body. This study did not show any other correlation between redness and histopathologic features [Table 3].

Pterygium fleshiness had significant correlation with stromal fibrosis but not with other histopathologic features [Table 4]. Thus, decreased visibility of underlying vessels, herein defined as fleshiness, may be due to increased fibrous content of the pterygium body. Zhang et al, by counting CD105-positive vascular endothelial cells, showed a correlation between

Table 2. Correlations between pterygium dimensions over the cornea and obliteration of the plica semilunaris

Pterygium dimension	Plica semilunaris		*P*
	Present	Absent	
Length (mm)	3.3±1.9	4.7±1.8	0.014
Width (mm)	6.2±1.3	7.4±1.3	0.004
Area (mm^2)	14.7±10.8	23.5±13.6	0.013

mm, millimeter

Table 3. Correlations between pterygium redness and various histopathologic features

	Pterygium redness			*P*
	Grade I	Grade II	Grade III	
Vascular density (vessel/HPF)	14.6±4.2	15.6±5.0	17.9±4.9	0.038
Subepithelial fibrosis (%)	17.6±21.5	17.7±18.7	14.4±20.1	0.913
Stromal fibrosis (%)	6.4±9.2	5.5±6.6	8.1±7.8	0.730
Stromal elastosis (%)	8.7±12.9	4.1±6.4	5.6±8.2	0.258
Leukocytic infiltration (Grade 0/Grade I)	15/13	16/14	6/6	0.665

HPF, high power field

Table 4. Correlations between pterygium fleshiness and various histopathologic features

	Pterygium fleshiness			*P*
	Grade T1	Grade T2	Grade T3	
Vascular density (vessel/HPF)	15.9±3.9	15.4±5.7	15.1±4.4	0.863
Subepithelial fibrosis (%)	15.8±19.9	16.5±17.1	19.0±23.9	0.885
Stromal fibrosis (%)	5.9±9.1	10.4±8.0	14.1±6.4	0.043
Stromal elastosis (%)	7.4±11.3	6.8±10.9	3.2±4.7	0.409
Leukocytic infiltration (Grade 0/Grade I)	10/5	11/10	16/18	0.247

HPF, high power field

Table 5. Correlations between pterygium dimensions over the cornea and vascular density and stromal elastosis

Pterygiuma dimension	Vascular density		Stromal elastosis	
	R	*P*	*R*	*P*
Length (mm)	0.51	<0.001	−0.28	0.027
Width (mm)	0.16	0.215	−0.19	0.132
Area (mm^2)	0.45	<0.001	−0.27	0.029

mm, millimeter

microvascular density in pterygia and its fleshiness.[7] Therefore, it seems that increased content of fibrous and/or vascular tissues in pterygia may result in increased lesion fleshiness.

Our study revealed grade 0 leukocytic infiltration in 43 (61.4%) eyes and grade I (patchy leukocytic infiltration) in 27 (38.6%) eyes. Moreover, there was no correlation between leukocytic infiltration and any clinical feature such as redness. Compared to normal conjunctival tissue, pterygium tissue has higher levels of inflammatory cells including lymphocytes, plasma cells and mast cells, as well as other inflammatory markers.[4,8-11] Thus, an inflammatory/immunologic pathogenesis has been suggested for pterygium.[12] Although Awdeh et al could not demonstrate a significant correlation between lymphocytic infiltration and clinical signs, of inflammation or use of topical anti-inflammatory agents,[13] Ribatti et al found a correlation between mast cells and vessel density in pterygia.[14] In the present study, leukocytic infiltration was not associated with vascular density.

Other clinical parameters such as duration of the pterygium may affect its histopathologic features,[15] but these were not evaluated in our study. There are other limitations to this study; the absence of immunohistochemistry could influence the sensitivity of the counting method. CD31 staining would specify some real but small capillary-sized blood vessels with no observable endothelium, thereby increasing the number of countable vessels which would enhance the sensitivity of study. The grading of redness and fleshiness were subjective, and also some histopathologic characteristics were graded by a semi-quantitative scoring. Moreover, this study included only surgical candidates who may be different from non-surgical cases. Further studies are required to determine correlations between surgical outcomes and ultrastructural characteristics of pterygia. Confirming such correlations will help adopt more aggressive treatment and achieve optimal surgical outcomes in high-risk pterygia.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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