tvst

Article

Evaluation of Primary Pterygia on Basis of the Loss of Vertical Length of Plica Semilunaris

Minjeong Kim[1], Yeoun Sook Chun[1], and Kyoung Woo Kim[1]

[1] Department of Ophthalmology, Chung-Ang University College of Medicine, Seoul, Republic of Korea

Correspondence: Kyoung Woo Kim, Department of Ophthalmology, Chung-Ang University College of Medicine, 102 Heukseok-ro, Dongjak-gu, Seoul 06973, Republic of Korea. e-mail: kkanssa@cau.ac.kr

Received: January 15, 2021
Accepted: May 31, 2021
Published: July 19, 2021

Keywords: LPS; MMP-9; plica semilunaris; primary pterygium

Citation: Kim M, Chun YS, Kim KW. Evaluation of primary pterygia on basis of the loss of vertical length of plica semilunaris. Transl Vis Sci Technol. 2021;10(8):18, https://doi.org/10.1167/tvst.10.8.18

Purpose: To propose a new grading system for primary pterygia based on the morphological loss of vertical length of plica semilunaris (LPS).

Methods: We included 50 eyes from 41 patients with primary pterygium. LPS was defined and quantified as the ratio of the length of loss of the normal vertical morphology at plica semilunaris to the vertical corneal diameter using anterior-segment photographs. Grades of tear metalloproteinase 9 (MMP-9) expression by point-of-care immunoassay, which is a well-known biomarker for inflammation, was correlated with the extent of LPS (%) of pterygia. Then, LPS was paralleled with the pre-established grading systems on the basis of tissue translucency (i.e., T grade) and vascularity (i.e., V grade) of the pterygium body.

Results: MMP-9 grades was 2.39 $\pm$ 1.12 in the group with LPS $\geq$50% and was 1.56 $\pm$ 1.12 in the group with LPS $<$50% ($P = 0.016$). In a linear regression, the extent of LPS was positively correlated with MMP-9 grades ($r = 0.315$, $P = 0.026$). MMP-9 expression did not differ between T grades or V grades. The extents of LPS were well correlated positively with both T grades ($r = 0.495$ and $P < 0.001$) and V grades ($r = 0.344$ and $P = 0.015$).

Conclusions: We devised a new grading system using LPS on the basis of morphological loss of the normal vertical plica semilunaris in primary pterygia. The extent of LPS correlated well with T grades and V grades and also reflected the expression of MMP-9 in tears.

Translational Relevance: The new clinical LPS grading system reflects severity and MMP-9 expression in tears in primary pterygia.

Introduction

Pterygium is a common disease in the ocular surface, characterized by triangle-shaped stromal proliferation, prominent vascularization, and frequent recurrence after excision.[1] With improved understanding of molecular mechanisms, the pathogenesis of pterygium has been diversified into cellular proliferation,[2] antiapoptosis,[3] inflammation,[4,5] immune activity,[6] oxidative stress,[7] extracellular matrix modulation,[8] viral involvement,[9,10] and inheritance.[11] Above all, one of the main factors leading to the development of pterygium is widely considered to be cell proliferation with pterygium viewed as a fibrotic disease.[1,2]

Pterygium stromal fibroblasts are largely responsible for fibrovascular tissue proliferation.[12] Previous studies revealed that the pathogenic mechanisms including upregulated stromal cell-derived factor 1 expression,[13] overexpression of angiogenin,[14] and tumor necrosis factor-alpha (α)–induced expression of hypoxia-inducible factor-1α[15] are involved in the stromal fibroblasts of the pterygium body. These suggest that the pterygium body and stromal fibroblasts in that layer may have a significant role in the proliferation of pterygia. Accordingly, Tan et al.[16] stressed estimating the amount of fibrovascular tissue by the visibility of the underlying episcleral vessel to predict the surgical outcome of primary and recurrent pterygia. In addition, a previous study devised new grading

system on the basis of the morphology of the lacrimal caruncle and performed a sealing-the-gap technique at the caruncle in patients with multirecurrent pterygia with the flattened caruncle.[17]

Given that fibrovascular tissue is generally abundant in the caruncle and semilunar fold, we hypothesized that stromal fibroblasts of the active and progressive pterygium may unfold the semilunar fold. Such a morphological change at the plica semilunaris may be more reliable in primary pterygia rather than in recurrent pterygia because the lacrimal caruncle and semilunar fold of primary pterygium have not been surgically disturbed before. In this study, we first estimated the loss of vertical length of plica semilunaris (LPS) in primary pterygia. Then, LPS values were paralleled with the grades according to pre-established grading systems based on tissue translucency,[16] and vascularity[14] of the pterygium body furthermore was correlated with the tear metalloproteinase 9 (MMP-9), which is a famous marker for inflammation level of ocular surface,[18,19] and its high level in pterygium fibroblasts is involved in the progression of pterygia.[20]

Materials and Methods

This study was an observational cross-sectional study, of which the whole process properly followed the tenets of the Declaration of Helsinki. The medical records were obtained through chart review. The study was approved by the Chung-Ang University Hospital Institutional Review Board.

Study Design

Our study design was outlined as follows:

- Estimation of LPS in the primary pterygia using the anterior segment photographs
- Analysis of tear MMP-9 expression according to the LPS values in primary pterygia
- Grading of the primary pterygia based on the new grading system using LPS and the pre-established grading system according to the translucency (i.e., T grading system) and the vascularity (i.e., V grading system) at the pterygium body stroma
- Correlation between the new grading system using LPS and the pre-established T or V grading system

Patients

Patients with primary pterygium at the nasal conjunctiva who visited our institution between March 2019 and September 2020 and underwent tear MMP-9 test were included in this study. Patients who had been diagnosed with systemic immunologic disease, dry eye disease, or ocular surface diseases that may elevate the level of tear MMP-9 including allergic conjunctivitis, sterile corneal ulceration, keratoconus, and conjunctivochalasis and who had applied topical steroid or cyclosporine within the previous three months were excluded. To exclude patients with dry eye disease in the present study, it was defined according to DEWS II criteria.[21] First, dry eye disease was screened with an ocular surface disease index questionnaire score $\geq$ 13, which then was confirmed when patients fulfilled one of the following: noninvasive tear break-up time (BUT) $<$10 seconds, tear osmolarity $\geq$ 308 mOsm/L in either eye or interocular difference $>$ 8 mOsm/L, ocular surface staining $>$ five corneal spots, $>$ nine conjunctival spots, or lid margin ($\geq$2 mm length and $\geq$25% width). Despite the fact that patients might have meibomian gland dysfunction (MGD), they were enrolled in this study if they had not met the diagnostic criteria for dry eye disease by DEWS II criteria. Patients with a history of the contact lens wearing within the previous three months and ocular surgery within the previous six months were also excluded.

Estimation of LPS in Primary Pterygia

The morphological change of the normal vertical plica semilunaris was identified with the aid of standard photographs of negative loss, partial loss, and complete loss of the normal vertical morphology of plica semilunaris (Figs. 1A–1C). LPS was estimated using digital photographs (magnification $\times$10) taken by a slit lamp imaging system (Topcon, Tokyo, Japan) during the lateral gaze with and without a yellow barrier filter. The detailed steps are shown in Figures 1D and 1E. First, the corneoconjunctival limbus was outlined with a circle in the photographs without filter. Then, the corneal circle is moved to the same-powered photographs with a yellow barrier filter. The center point (index *a*) of the corneal circle and the middle point (index *b*) at the lacrimal caruncle between the upper and lower eyelid margin were marked in the photographs with a yellow barrier. In cases with complete flattening of the caruncle, the middle point of the line that connects the upper and lower punctum was considered as *index b*. The line (index *c*) between those two points (i.e., indexes *a* and *b*) was created, and the upper (index *d*) and lower (index *e*) tangent lines to the circle that are parallel with the center line (index *c*) were created. The distance that is parallel with the normal vertical plica semilunaris between the two tangent lines (indexes *d* and *e*) was marked

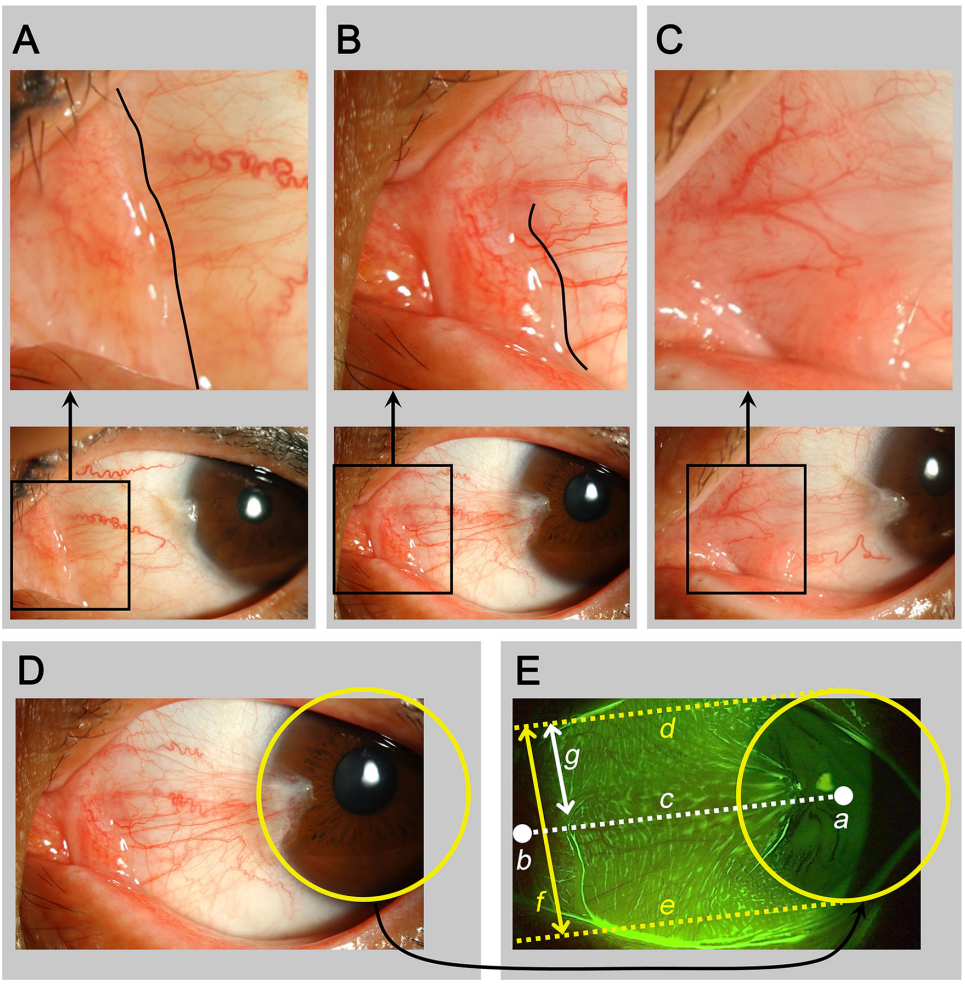

Figure 1. Grading of primary pterygium based on the LPS. The standard photographs of the negative loss (**A**), partial loss (**B**) and complete loss (**C**) of the normal vertical morphology of plica semilunaris. In high power photographs from rectangles in each **A** to **C**, the preserved length of the vertical plica semilunaris is noted along to the *black line* in **B** and **C**. The representative photographs without (**D**) and with yellow barrier filter (**E**) from an image in **B** to estimate a LPS value. The indexes *a* to *g* are landmarks that are required during the estimation of LPS (**E**). The ratio of the length of the index *g* to the length of the index *f* was defined as LPS.

with a line (index *f*) and was regarded as a "total line," which is the same as the vertical corneal diameter. The loss of the normal morphology of the plica semilunaris was identified as the disappearance of the vertical line in a pool of fluorescein dye at the plica semilunaris, was marked with a line (index *g*), and was regarded as a "lost line." The dragged plica semilunaris rather than a normal vertical line was thought abnormal and thus was incorporated into a "lost line." The length of a "total line" and a "lost line" (indexes *f* and *g*, respectively) was measured using Image*J* software (National Institutes of Health; http://rsbweb.nih.gov/ij/); then a ratio (i.e., the ratio of the length of the index *g* to the length of the index *f*) was defined as LPS.

Grading of Pterygia on the Basis of the Translucency and the Vascularity at the Pterygium Body Stroma

All eyes with pterygia were graded according to the two pre-established three-scale grading systems on the basis of the translucency[16] and vascularity[14] of the pterygium body stroma. Briefly, according to the T grading system, T1 was defined as the pterygium of which underlying episcleral vessels are clearly visible, T2 was defined as one of which underlying episcleral vessels are partially covered by the pterygium body, and T3 was defined as one of which underlying the episcleral vessels are completely covered by pterygium body. Pterygia with V1, V2, and V3 are characterized

by unidirectional minimal vascularization, moderate vascularization with unidirectional and enlarged vessels, and marked vascularization with unidirectional and engorged vessels, respectively. Pterygia with T1 or V1 grade were considered as mild cases at each grading system.

Immunoassay of Tear MMP-9

The test for tear MMP-9 was performed with the point-of-care MMP-9 immunoassay (InflammaDry; Quidel, San Diego, CA, USA) according to the manufacturer's instructions for use.[22,23] Brief, the operator gently dabbed the sample collector in multiple areas along the palpebral conjunctiva until the sampling fleece was saturated with tears. Next, the sample collector was assembled to the test cassette, and then the test pad was dipped in a buffer solution for 20 seconds for activation. After 10 minutes, the red test line in a readout window was read. Density evaluation of the red test line was performed on the basis of the five-scale grading system composed of grade 0 (i.e., negative), grade 1 (i.e., trace), grade 2 (i.e., weak positive), grade 3 (i.e., positive), and grade 4 (i.e., strong positive).[24]

Clinical Parameters for Ocular Surface Disease

To test signs for dry eye disease, the clinical parameters including meibomian gland (MG) plugging, quality of the secreted meibum, tear secretion using Schirmer I without anesthesia, tear BUT, tear osmolarity, and corneal sensitivity were evaluated. MG plugging was graded into grade 0 (i.e., expressible in all glands), grade 1 (i.e., expressible in three or four glands), grade 2 (i.e., expressible in one or two glands), and grade 3 (i.e., expressible in no glands) according to the meibum expressibility from five glands of the central upper lid as previously known.[25] The quality of the meibum secreted from the MG in the upper eyelid by manual compression was graded into grade 0 (i.e., clear), grade 1 (i.e., cloudy fluid), grade 2 (i.e., cloudy with debris) and grade 3 (i.e., toothpaste-like) as previously established.[26,27] Tear secretion was assessed by Schirmer I test, by applying Schirmer standard strip (Eagle Vision, Memphis, TN, USA) on lateral lower conjunctival sac for five minutes without analgesic eyedrops. To measure tear BUT, a fluorescein strip paper (Haag-Streit International, Koniz, Switzerland) was moistened with normal saline solution and then was gently touched in lower palpebral conjunctiva to stain tear film. Post-blinking time until the first occurrence of a black spot with using cobalt blue filter was considered as invasive tear BUT. Tear osmolarity was measured using I-PEN (I-MED Pharma Inc., Montreal, Quebec, Canada) from tear at the lower conjunctival fornix. Corneal sensitivity was measured using Cochet-Bonnet esthesiometer (Luneau Ophthalmology, Chartres, France) at corneal center.

Data Analysis

SPSS software version 20.0 (SPSS, Inc., Chicago, IL, USA) and the Prism software v.8.4.3 (GraphPad, La Jolla, CA, USA) were used for the statistical tests. To compare the means of the two groups, the data were analyzed using the parametric two-tailed Student *t*-test or nonparametric Mann–Whitney U test, depending on the normal data distribution. The categorical analysis between two grading systems was performed using the χ^2 test. The correlation between the two continuous variables was analyzed using Pearson's correlation test with a linear regression. And then, all variables that showed significant correlation with LPS values according to a univariate linear regression analysis were correlated with LPS values using multivariate linear regression analysis. The data are expressed as mean ± standard deviation (SD), and the differences were considered statistically significant at $P < 0.05$.

Results

Demographics and Clinical Parameters for the Ocular Surface Disease

Totals of 50 eyes of 41 patients with primary pterygia were included in this study. Among the total of 41 patients, male and female patients numbered 23 (56 %) and 18 (44 %), respectively. The mean age of the total patients was 64.0 ± 10.3 years (SD; range, 33 to 84 years). Twenty-three patients were male, and 18 patients were female. The grades for MG plugging and meibum quality were 1.41 ± 0.50 and 1.52 ± 0.62, respectively. Values of Schirmer I without anesthesia, tear BUT, tear osmolarity, and the corneal sensitivity were 10.38 ± 6.44 mm, 5.80 ± 2.02 sec., 315.0 ± 24.18 mOsm/L, and 5.80 ± 0.54 cm, respectively (Table 1). The total enrollee was divided into two groups according to the dichotomous grading of LPS with the 50% cutoff extent. The sex ration, age, and values of clinical parameters for the ocular surface disease including grades for MG plugging and meibum quality, Schirmer I without anesthesia, tear BUT, tear osmolarity, and corneal sensitivity were not significantly different between two groups (Table 1).

Table 1. Demographics and Clinical Parameters for the Ocular Surface Disease According to the Dichotomous Grading of the LPS in Primary Pterygia

Variables	Total	Group		*P* Value
		LPS < 50%	LPS ≥ 50%	
No. of patients/eyes	41/50	24/27	17/23	
Extent of LPS (%)	49.9 ± 29.5	28.2 ± 18.0	75.5 ± 17.1	<0.001*
Demographics				
Sex (male/female)	23/18	14/10	9/8	0.732
Age (y)	64.0 ± 10.3	63.3 ± 11.0	65.0 ± 9.5	0.615
Clinical parameters				
MG expressibility (Gr)	1.41 ± 0.50	1.44 ± 0.51	1.37 ± 0.50	0.763
Meibum quality (Gr)	1.52 ± 0.62	1.52 ± 0.64	1.53 ± 0.61	0.967
Schirmer I without anesthesia (mm)	10.38 ± 6.44	9.52 ± 5.49	11.36 ± 7.38	0.461
Tear BUT (sec)	5.80 ± 2.02	6.22 ± 2.33	5.46 ± 1.75	0.412
Tear osmolarity (mOsm/L)	315.0 ± 24.18	322.1 ± 30.4	309.1 ± 16.4	0.234
Corneal sensitivity (cm)	5.80 ± 0.54	5.69 ± 0.72	5.92 ± 0.19	0.622

Gr, grade.
*$P < 0.05$.

Tear MMP-9 According to Various Grading System in Primary Pterygia

The involvement of MMP-9 in the pathogenesis of pterygium has been identified through several studies. Previously, it was revealed that MMP-9 expression by pterygium fibroblasts contributed of MMP-9 both in the formation[28] and progression of pterygium.[20] Moreover, personal susceptibility to pterygium may be determined by the genotypes at MMP-9.[29] Recently, a study reported that ultraviolet exposure, which is a well-known culprit for the pathogenic factor of pterygium, led to the dramatic upregulation of the gene expression of MMP-9 in the corneal epithelial cells, conjunctival fibroblast, and primary pterygium fibroblast cells.[30] Accordingly, given that the semiquantitative five-scale grading system of MMP-9 using a point-of-care immunoassay kit is quite useful to clinically evaluate patients status,[24] we analyzed the tear MMP-9 expression according to the extent of LPS, T and V grading system. When cases were divided by dichotomous grading using three kinds of grading system (Extent of LPS: <50% vs. ≥50%; T grade: T1 vs. Non-T1; V grade: V1 vs. Non-V1), only LPS with cutoff value of 50% distinguished two groups with significant difference of tear MMP-9. Tear MMP-9 grades (2.39 ± 1.12) from LPS ≥ 50% group was higher than tear MMP-9 grades (1.56±1.12) from LPS<50% group ($P = 0.016$, Table 2). Tear MMP-9 grades were not different between T1 group (1.67 ± 1.09) and non-T1 group (2.19 ± 1.23, $P = 0.118$, Table 2) and were not different between V1 group (1.93 ± 1.19) and non-V1 group (1.95 ± 1.20, $P = 0.951$, Table 2). Furthermore, the extent of LPS and the grades of tear MMP-9 expression was positively correlated with significance ($r = 0.315$ and $P = 0.026$, Fig. 2).

Table 2. Tear MMP-9 Expressions in Patients With Primary Pterygia According to the Dichotomous Grading of the LPS, Pterygium Body Translucency, and Vascularity

Grading System	No. of Eyes	Tear MMP-9	
		Grade	*P* Value
Extent of LPS			
<50%	27	1.56 ± 1.12	0.016*
≥50%	23	2.39 ± 1.12	
Translucency (T grade)			
T1	24	1.67 ± 1.09	0.118
Non-T1 (T2 & T3)	26	2.19 ± 1.23	
Vascularity (V grade)			
V1	29	1.93 ± 1.19	0.951
Non-V1 (V2 & V3)	21	1.95 ± 1.20	

*$P < 0.05$.

Comparison Between a New LPS Grading System and the Translucency- or Vascularity-Based Grading System

Next, we investigated whether the new dichotomous LPS grading system may evaluate primary pterygia parallel with the translucency- or vascularity-based grading system. According to the χ^2 analysis, the

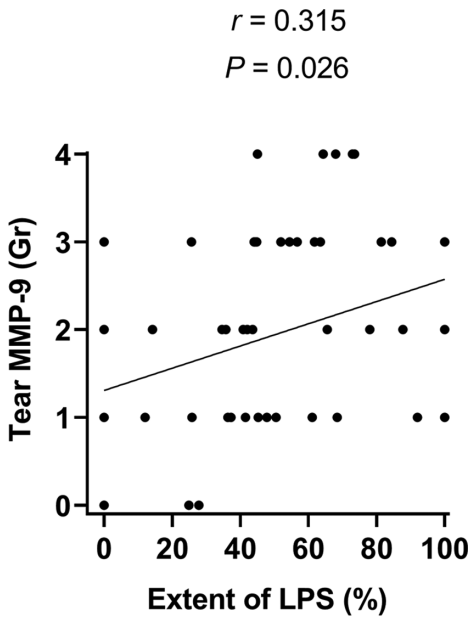

Figure 2. Scatterplots of tear MMP-9 grades according to the extent of the LPS.

composition of T grades was significantly different between in pterygia with LPS $\geq$ 50% and <50% (P = 0.005, Table 3). The proportion of T1 grade of pterygium was 67% and 26 % in the LPS < 50% group and in the LPS $\geq$ 50% group, respectively, while the proportion of T3 grade of pterygium was 0% and 17 % in the LPS < 50% group and in the LPS $\geq$ 50% group, respectively (Table 3). The composition of V grades was not distinguishable according to LPS grading system (P = 0.133, Table 3). The extent of LPS was well correlated positively with both T grades (r = 0.495 and P < 0.001, Fig. 3A) and V grades (r = 0.344 and P = 0.015, Fig. 3B).

Through the univariate linear regression analysis, T grades, V grades, and tear MMP-9 grades were found to be positively correlated with the extent of LPS (Table 4). To determine the difference of impart on the extent of LPS among those three variables, multivariate linear regression was performed. Among only T grades influenced the extent of LPS (β = 0.370 and P = 0.013, Table 4). Tear MMP-9 grades showed equivocal influence with marginal significance (β = 0.249 and P = 0.052, Table 4).

Discussion

In this study, we proposed a grading system using a newly devised LPS value to evaluate primary nasal pterygia reflecting the tear MMP-9 expression. Moreover, we correlated LPS with the pre-established and well-known grading system on the basis of the translucency of pterygium body. We identified that LPS values are quite well correlated with T and V grades and found that the more severe morphological loss of the vertical plica semilunaris is significantly associated with the higher expression of tear MMP-9.

Preservation of the morphology of caruncle and semilunar fold has been stressed, especially in patients with recurrent pterygium during the surgery.[17,31,32] Hirst[32] reported that the reconstruction of the semilunar fold contributed to lower recurrence rate of recurrent pterygium surgery. In a similar vein, Kim et al.[31] inserted a well-fitted, expanded polytetrafluoroethylene sheet with minimum dead space to separate the wound at the caruncle from the sclera, avoiding tissue-to-tissue contact that might lead to cicatricial adhesion formation.

Table 3. The χ^2 Analysis of the Dichotomous Grading of the LPS and the Grading System Based on the Pterygium Body Translucency and Vascularity in Primary Pterygia

	Group			
Grading System	LPS < 50%	LPS $\geq$ 50%	Total	*P* Value
Translucency (T grade)		No. of eyes		
T1	18 (67%)	6 (26%)	24 (48%)	
T2	9 (33%)	13 (57%)	22 (44%)	0.005*
T3	0 (0%)	4 (17%)	4 (8%)	
Total	27	23	50	
Vascularity (V grade)		No. of eyes		
V1	19 (70%)	10 (44%)	29 (58%)	
V2	7 (26%)	10 (44%)	17 (34%)	0.133
V3	1 (4%)	3 (12%)	4 (8%)	
Total	27	23	50	

*P < 0.05.

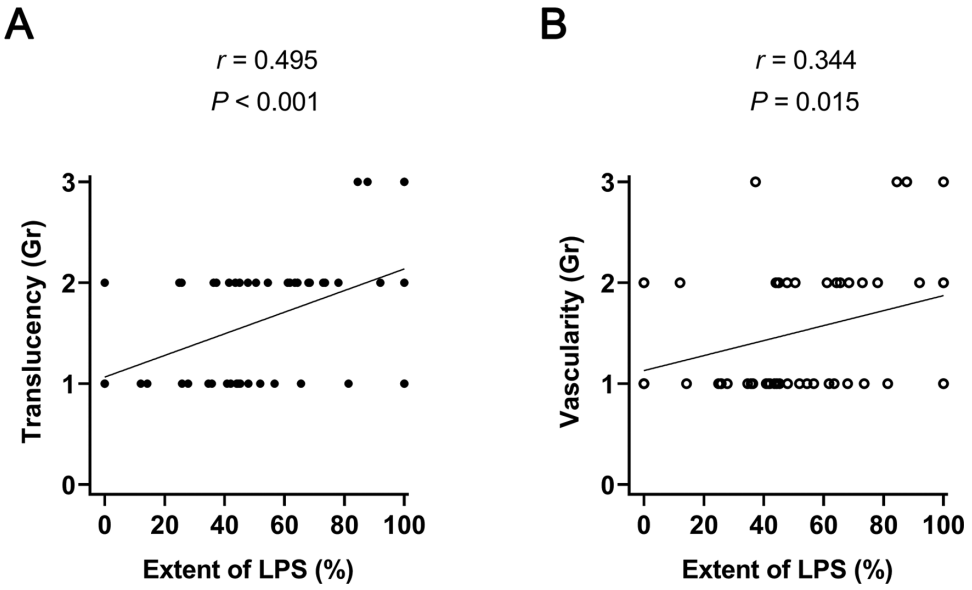

Figure 3. Scatterplots of grades based on the pterygium body translucency (**A**) and vascularity (**B**) according to the extent of the LPS.

Table 4. Univariate and Multivariate Linear Regression Analysis Between the Extent of LPS in Primary Pterygia and Variables Including Demographics, Pterygium Grading Systems, and Clinical Parameters for the Ocular Surface Diseases

	Extent of LPS (%)							
	Univariate Analysis				Multivariate Analysis			
Clinical Parameters	Unstandardized B	SE	Standardized β	*P* Value	Unstandardized B	SE	Standardized β	*P* Value
Demographics								
Age	0.369	0.430	0.123	0.395	—	—	—	—
Sex (female)	3.433	8.583	0.058	0.691	—	—	—	—
Pterygium grading systems								
T grade (Gr)	22.815	5.788	0.495	<0.001*	17.082	6.633	0.370	0.013*
V grade (Gr)	15.434	6.189	0.339	0.016*	7.252	6.441	0.159	0.266
Clinical parameters								
Tear MMP-9 (Gr)	7.831	3.407	0.315	0.026*	6.196	3.109	0.249	0.052
MG expressibility (Gr)	−14.406	8.854	−0.238	0.111	—	—	—	—
Meibum quality (Gr)	−0.008	7.282	<0.001	0.999	—	—	—	—
Schirmer I without anesthesia (mm)	0.446	0.630	0.102	0.482	—	—	—	—
Tear BUT (sec)	0.209	1.423	0.022	0.884	—	—	—	—
Tear osmolarity (mOsm/L)	0.007	0.027	0.039	0.789	—	—	—	—
Corneal sensitivity (cm)	2.521	11.052	0.048	0.822	—	—	—	—

Gr, grade; SE, standard error.
*$P < 0.05$.

However, in a real clinical setting, the preoperative evaluation for the vigorousness of pterygia is also very important because this may affect determining the time of surgery or selecting the surgical methods before surgery. The grading of pterygia on the basis of the pterygium body translucency has long been a standard to predict pterygium recurrence.[16] As another method to grade, Liu et al.[17] graded caruncle morphological characteristics into C1, C2 and C3 in multirecurrent. This C grading system correlated well with the preoperative severity of diplopia and postoperative recurrence of diplopia, furthermore, was useful to select sealing the gap technique for the restoration of the caruncle morphology and to lower the recurrence. Our study shares the concept that values the normal morphology at the area of caruncle and semilunar fold with a study of Liu et al.[17] However, unlike a previous study that targeted recurrent pterygia, we included patients with primary pterygia only. The development of primary pterygium may be started from epithelial

abnormalities where epithelial cells can obtain characteristics of mesenchymal cells via epithelial-mesenchymal transition (EMT) phenomenon as the origins of pterygium fibroblasts.[33] With viewing primary pterygium as an ongoing disease with EMT, we targeted primary pterygia only in this study.

The normal plica semilunaris is long and there is always a vertical furrow line between caruncle and plica semilunaris. So, we had observed partial or complete low of the vertical line carefully in patients, then we hypothesized that the loss of the normal vertical morphology of plica semilunaris might be caused by the bulging of the stromal tissue and stretching of the overlying the conjunctival epithelium (see Supplementary Fig. S1). Moreover, the bulged stromal tissue might also have led to the decrease of light transmission to the sclera. Such a decrease of pterygium body translucency reflects high correlation between LPS and T grading system. According to our previous study, an interactive action between stromal cell–derived factor 1 and chemokine receptor 4 contributed myofibroblast transformation in severe pterygia with T3 grade.[13] In some cases in the present study, the loss of the plica semilunaris accompanied the temporal dragging of the furrow line. It is plausible that alpha-smooth muscle actin secreted by myofibroblast in the pterygium body stroma contributed to the shortening, tightening and dragging between caruncle and plica semilunaris.

As is well known, the point-of-care immunoassay of tear MMP-9 has been suggested as an excellent indicator for inflammation in DED.[18,34,35] It is very easy to use, and it takes only a few minutes to get results with clinical usefulness. Despite the relevance of MMP enzyme in the pathogenesis of pterygia in a few of studies previously,[20,28,29] there has been no study that used tear MMP-9 immunoassay kit to reflect the inflammation in the ocular surface in patients with pterygia. Tsai et al.[28] revealed the expression of MMP-9 and MMP-10 in the cytoplasm of pterygium epithelium using the specimen. But Yang et al.[20] reported that mRNA expression of MMP-2 and MMP-9 in pterygium fibroblasts increased after the progression of pterygium. Although the origin of MMP-9 is unclear with only the immunoassay results of a tear MMP-9 kit, we speculate that both conjunctival epithelial cells under a process of EMT and the accordingly transformed pterygium fibroblasts may produce the MMP-9. In addition, because LPS grading system seems to represent the level of MMP-9 in tear better than the T or V grading system, clinicians can get hints from the extent of LPS regardless of whether anti-inflammatory treatment such as topical steroids would be strongly required before surgery when the immunoassay of tear MMP-9 is not allowed.

Because the prevalence of pterygium generally increases with age, MGD may be a common comorbidity with pterygium. We could exclude the effect of MGD on the different expression of MMP-9 according to the extent of LPS in pterygium in that MG expressibility and meibum quality showed no relationship with LPS grading. However, the comorbidity of MGD should be considered when we interpret the increased expression of tear MMP-9 in patients with pterygium because MGD itself reveals the altered level of MMP-9 in tear.[36]

Our study is limited in the fact that, because of its use of semiquantitative immunoassay for tear MMP-9 expression to correlated with the extent of LPS, the small number of cases to establish a new grading system. In addition, the strategies for preoperative anti-inflammatory treatments and for selecting surgical techniques according to LPS values need to be investigated in subsequent studies. Nevertheless, our results do carry clinical implications in that they provide an easily applicable new grading method for primary pterygia which may evaluate the progressive potency represented by MMP-9 expression, also which correlate well with the well-known T grading system.

Acknowledgments

Supported by the Basic Science Research Program through the National Research Foundation of Korea (2020R1C1C1007788).

Disclosure: **M. Kim,** (N); **Y.S. Chun,** (N); **K.W. Kim,** (N)

References

1. Kim KW, Park SH, Kim JC. Fibroblast biology in pterygia. *Exp Eye Res*. 2016;142:32–39.
2. Liang K, Jiang Z, Ding BQ, Cheng P, Huang DK, Tao LM. Expression of cell proliferation and apoptosis biomarkers in pterygia and normal conjunctiva. *Mol Vis*. 2011;17:1687–1693.
3. Tan DT, Tang WY, Liu YP, Goh HS, Smith DR. Apoptosis and apoptosis related gene expression in normal conjunctiva and pterygium. *Br J Ophthalmol*. 2000;84:212–216.
4. Chiang CC, Cheng YW, Lin CL, et al. Cyclooxygenase 2 expression in pterygium. *Mol Vis*. 2007;13:635–638.
5. Di Girolamo N, Kumar RK, Coroneo MT, Wakefield D. UVB-mediated induction of

interleukin-6 and -8 in pterygia and cultured human pterygium epithelial cells. *Invest Ophthalmol Vis Sci*. 2002;43:3430–3437.
6. Beden U, Irkec M, Orhan D, Orhan M. The roles of T-lymphocyte subpopulations (CD4 and CD8), intercellular adhesion molecule-1 (ICAM-1), HLA-DR receptor, and mast cells in etiopathogenesis of pterygium. *Ocul Immunol Inflamm*. 2003;11:115–122.
7. Kau HC, Tsai CC, Lee CF, et al. Increased oxidative DNA damage, 8-hydroxydeoxy- guanosine, in human pterygium. *Eye (Lond)*. 2006;20:826–831.
8. Naib-Majani W, Eltohami I, Wernert N, et al. Distribution of extracellular matrix proteins in pterygia: an immunohistochemical study. *Graefes Arch Clin Exp Ophthalmol*. 2004;242:332–338.
9. Gallagher MJ, Giannoudis A, Herrington CS, Hiscott P. Human papillomavirus in pterygium. *Br J Ophthalmol*. 2001;85:782–784.
10. Detorakis ET, Sourvinos G, Spandidos DA. Detection of herpes simplex virus and human papilloma virus in ophthalmic pterygium. *Cornea*. 2001;20:164–167.
11. Chui J, Di Girolamo N, Wakefield D, Coroneo MT. The pathogenesis of pterygium: current concepts and their therapeutic implications. *Ocul Surf*. 2008;6:24–43.
12. Chen JK, Tsai RJ, Lin SS. Fibroblasts isolated from human pterygia exhibit transformed cell characteristics. *In Vitro Cell Dev Biol Anim*. 1994;30A:243–248.
13. Kim KW, Park SH, Lee SH, Kim JC. Upregulated stromal cell-derived factor 1 (SDF-1) expression and its interaction with CXCR4 contribute to the pathogenesis of severe pterygia. *Invest Ophthalmol Vis Sci*. 2013;54:7198–7206.
14. Kim KW, Park SH, Wee SW, Kim JC. Overexpression of angiogenin in pterygium body fibroblasts and its association with proliferative potency. *Invest Ophthalmol Vis Sci*. 2013;54:6355–6362.
15. Kim KW, Lee SJ, Kim JC. TNF-alpha upregulates HIF-1alpha expression in pterygium fibroblasts and enhances their susceptibility to VEGF independent of hypoxia. *Exp Eye Res*. 2017;164:74–81.
16. Tan DT, Chee SP, Dear KB, Lim AS. Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. *Arch Ophthalmol*. 1997;115:1235–1240.
17. Liu J, Fu Y, Xu Y, Tseng SC. New grading system to improve the surgical outcome of multirecurrent pterygia. *Arch Ophthalmol*. 2012;130:39–49.
18. Messmer EM, von Lindenfels V, Garbe A, Kampik A. Matrix metalloproteinase 9 testing in dry eye disease using a commercially available point-of-care immunoassay. *Ophthalmology*. 2016;123:2300–2308.
19. De Paiva CS, Corrales RM, Villarreal AL, et al. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. *Exp Eye Res*. 2006;83:526–535.
20. Yang SF, Lin CY, Yang PY, Chao SC, Ye YZ, Hu DN. Increased expression of gelatinase (MMP-2 and MMP-9) in pterygia and pterygium fibroblasts with disease progression and activation of protein kinase C. *Invest Ophthalmol Vis Sci*. 2009;50:4588–4596.
21. Wolffsohn JS, Arita R, Chalmers R, et al. TFOS DEWS II Diagnostic Methodology report. *Ocul Surf*. 2017;15:539–574.
22. Sambursky R, Davitt WF, 3rd, Friedberg M, Tauber S. Prospective, multicenter, clinical evaluation of point-of-care matrix metalloproteinase-9 test for confirming dry eye disease. *Cornea*. 2014;33:812–818.
23. Sambursky R, Davitt WF, 3rd, Latkany R, et al. Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. *JAMA Ophthalmol*. 2013;131:24–28.
24. Park JY, Kim BG, Kim JS, Hwang JH. Matrix metalloproteinase 9 point-of-care immunoassay result predicts response to topical cyclosporine treatment in dry eye disease. *Transl Vis Sci Technol*. 2018;7:31.
25. Pflugfelder SC, Tseng SC, Sanabria O, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea*. 1998;17:38–56.
26. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci*. 2011;52:2006–2049.
27. Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. *Eye (Lond)*. 1991;5(Pt 4):395–411.
28. Tsai YY, Chiang CC, Yeh KT, Lee H, Cheng YW. Effect of TIMP-1 and MMP in pterygium invasion. *Invest Ophthalmol Vis Sci*. 2010;51:3462–3467.
29. Tsai CB, Hsia NY, Wang ZH, et al. The contribution of MMP-9 genotypes to pterygium in Taiwan. *Anticancer Res*. 2020;40:4523–4527.
30. Shibata N, Ishida H, Kiyokawa E, Singh DP, Sasaki H, Kubo E. Relative gene expression analysis of human pterygium tissues and UV

radiation-evoked gene expression patterns in corneal and conjunctival cells. *Exp Eye Res*. 2020;199:108194.
31. Kim KW, Kim JC, Moon JH, Koo H, Kim TH, Moon NJ. Management of complicated multirecurrent pterygia using multimicroporous expanded polytetrafluoroethylene. *Br J Ophthalmol*. 2013;97:694–700.
32. Hirst LW. Recurrent pterygium surgery using pterygium extended removal followed by extended conjunctival transplant: recurrence rate and cosmesis. *Ophthalmology*. 2009;116:1278–1286.
33. Kao WW, Liu H, Zhang J. Wakayama symposium: challenges of future research in ocular surface cell biology. *Ocul Surf*. 2013;11:19–24.
34. Acera A, Rocha G, Vecino E, Lema I, Duran JA. Inflammatory markers in the tears of patients with ocular surface disease. *Ophthalmic Res*. 2008;40:315–321.
35. Chotikavanich S, de Paiva CS, de Li Q, et al. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. *Invest Ophthalmol Vis Sci*. 2009;50:3203–3209.
36. Aragona P, Aguennouz M, Rania L, et al. Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. *Ophthalmology*. 2015;122:62–71.