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# Primary pterygium is not associated with corneal endothelial cell decrease in a rural Chinese population

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# Abstract

Purpose: To investigate corneal endothelial cell density (ECD) in eyes with primary pterygium. Methods: We first conducted a retrospective study to compare the ECD between 1670 eyes with primary pterygium and 4060 eyes without pterygium. Then we designed a prospective study to confirm the findings of our retrospective study in 95 patients with unilateral primary pterygium. Results: In the retrospective study, the mean ECD in eyes with primary pterygium (2453±306 cells/mm2) was significantly lower than those in eyes without pterygium (2529±313 cells/mm2, P<0.0001). However, the difference was not as high as previously reported (76 cells/mm2, 3.1%). In the prospective study, there was no significant difference in mean ECD in eyes with unilateral primary pterygium (2480±263 cells/mm2) and the contralateral eyes with no pterygium (2527±277 cells/mm2, P=0.20). There was also no significant difference in hexagonality (P=0.10) or coefficient of variation of size (P=0.15) of corneal endothelial cells between eyes with pterygium and the contralateral eyes with no pterygium. Conclusion: Primary pterygium may not be associated with a decrease in ECD in our study population of rural Chinese patients.


# Introduction

Pterygium is an ocular surface disorder involving a wing-like fibrovascular growth of the bulbar conjunctiva and underlying subconjunctival tissue onto the cornea. [1] The most important factor in the pathophysiology of pterygium appears to be over-exposure to ultraviolet (UV) light. [2-4] As such, it is most commonly seen in populations working outdoors or living in tropical climates. Studies have shown that apart from causing corneal surface changes, [5, 6] pterygium may also be associated with degradation of Bowman's layer and stromal scarring [7, 8] due to activation of cell proliferation factors, inflammatory mediators, growth factors, extracellular matrix modulators and angiogenic factors. [9] Theoretically, these mediators and UV light may also damage deeper corneal endothelial cells (ECC) and thus corneal endothelial cell density (ECD) may be decreased in eyes with pterygium. [10] Although results from previous clinical studies were consistent with this theory, [11-13] we found that ECD was not significantly decreased in eyes seen in our clinics with primary pterygium. We thus speculated that the development of pterygium might not be associated with ECD decrease.

# Materials And Methods

This article presents both retrospective and prospective studies on ECD in eyes with primary pterygium. Both studies were approved by the Institutional Review Board of Shanwei Project Vision Eye Hospital and are in agreement with the Declaration of Helsinki.

The retrospective study reviewed the medical records of eyes that were referred for cataract surgery between 2014 and 2016. A total of 1670 eyes with pterygium and 4060 eyes without pterygium were identified pre-operatively for analysis. Inclusion criteria were untreated primary pterygium and age greater than 51 years. To exclude possible co-existing corneal endothelial pathology and ECD measurement bias,

we only included eyes with a pre-operative ECD between 1800 and 3500 cells/mm$^2$. [14] Other exclusion criteria included eyes with pseudopterygium, recurrent pterygium, corneal dystrophy or corneal degeneration, history of corneal infection, glaucoma, uveitis, ocular trauma or intraocular surgery.

In the prospective study, a sample size of 95 patients was calculated at 80% power to detect a 5% difference in ECD at a 95% confidence level. Therefore, we prospectively recruited 95 consecutive patients with untreated unilateral primary pterygium between 2016 and 2017. Inclusion criteria and exclusion criteria were the same as the retrospective study, except for age limitation. Informed consent was obtained from each patient.

All eyes underwent routine pre-operative examination including best-corrected visual acuity (BCVA), intraocular pressure (IOP), slit-lamp examination. ECD was measured by the same technician using a specular microscope (SP-3000P; Topcon, Tokyo, Japan) and the center-to-center method. [11]

In the retrospective study, ECD was measured one time for each eye with a cell count of at least 60 cells. In the prospective study, we measured ECD three times for each eye, with a cell count of at least 80 cells. The coefficient of variation (COV), ECD, hexagonality and coefficient of variation of size (ECV) were then calculated from these three measurements. Data was presented as mean ± standard deviation (SD). Two-tailed Student's *t*-test was used for comparison of data with normal distribution and a Mann-Whitney test for other distributions. One-way ANOVA was used to compare three or more groups with normal distribution, with a Kruskal-Wallis test used otherwise. $P<0.05$ was considered to be statistically significant.

## Results

# Basic characteristics

In the retrospective study, there were 1670 eyes with primary pterygium (PT group) and 4060 eyes without primary pterygium (NPT group). There was a significant difference in age between patients in the PT group (71.4±8.1 years) and NPT group (70.5±8.3 years, $P=0.0002$). However, there was no significant difference in age among patients with bilateral pterygium (70.3±6.9 years), unilateral pterygium (70.1±8.1 years) or no pterygium (68.6±8.2 years, $P=0.10$). Gender distribution was not significantly different between patients in the PT and NPT groups, or among patients with bilateral pterygium, unilateral pterygium or no pterygium ($P>0.05$).

The mean age of patients in the prospective study was 63.7±8.8 years, with 40 males and 55 females. Forty-nine patients had pterygium in the right eye and 46 patients had pterygium in the left eye.

# ECD in the retrospective study

The mean preoperative ECD in the PT group (2453±306 cells/mm$^2$) was significantly lower than those in the NPT group (2529±313 cells/mm$^2$, $P$<0.0001). The difference in ECD between the PT group and the NPT group was 76 cells/mm$^2$ (3.1%).

There was a statistically significant difference in ECD among patients with bilateral pterygium (2444±329 cells/mm$^2$), unilateral pterygium (2486±309 cells/mm$^2$) and no pterygium (2535±303 cells/mm$^2$, $P$=0.001). In subgroup analysis, patients with bilateral pterygium had lower ECD than patients with no pterygium (91 cells/mm$^2$, 3.7%, $P$=0.01). However, there was no significant difference in ECD when comparing patients with unilateral PT (2487±313 cells/mm$^2$) to the contralateral NPT eye (2486±306 cells/mm$^2$, $P$=0.99). This finding raised the question of whether ECD was decreased in eyes with pterygium. We speculated that the small difference in ECD between the PT group and the NPT group in our population might have been due to the variation in ECD measurement.

# ECD in the prospective study

In the prospective study, the intraclass correlation coefficient (ICC) of single ECD measurement was 0.876, indicating good reliability. The 95% confidence interval (CI) was 3.0%-3.7% for the ECD COV and 76-91 cells/mm$^2$ for the ECD SD.

Eyes with pterygium had a mean ECD of 2480±263 cells/mm$^2$ while the contralateral eyes with no pterygium had a mean ECD of 2527±277 cells/mm$^2$ ($P$=0.20). We then calculated the ECD difference between the pterygium eye and the contralateral eye with no pterygium in each patient. The mean difference in ECD between these eyes (46 cells/mm$^2$, 3.1%) was within the 95% CI of the ECD SD and COV, which supported our hypothesis.

The ECC hexagonality was not significantly different between eyes with pterygium (52.8%±7.1%) and the contralateral eyes with no pterygium (51.4%±6.9%, $P$=0.10). The ECV was also similar between pterygium eyes (36.3%±4.2%) and the contralateral eyes (37.0%±4.6%, $P$=0.15).

# Discussion

Chronic irritation and/or inflammation in the peripheral cornea and limbus caused by dust, low humidity, microtrauma from smoke or sand, human papilloma virus infection and genetic factors have all been suggested as risk factors for the development of pterygium. [13] However, the strongest epidemiological evidence implicates the role of UV radiation, since pterygium is a condition commonly seen in areas within the 'pterygium zone' – a geographical latitude 40 degrees north and south of the equator. [15] It is also seen more commonly in people with outdoor occupations or hobbies. [1-4] In our retrospective study, which is based within the aforementioned 'pterygium zone', 29.1% (1670/5730) of eyes undergoing cataract surgery were found to have concomitant pterygium. This prevalence rate is slightly higher than what has been reported by previous studies, though there is great variability in the literature. [16]

Pterygium has been found to have a significant impact on the corneal surface, reducing the spherical power and surface regularity index while increasing astigmatism and the surface asymmetry index. [5, 6, 17] Studies have also shown that pterygium may be associated with changes in deeper corneal layers. [11-13] Mootha et al. first described changes in the corneal endothelium and Descemet membrane underlying or directly adjacent to pterygium. [13] In their study the mean ECD decrease was 367 cells/mm$^2$ in eyes with unilateral pterygium. [13] Hsu et al. also detected an ECD decrease of 230 cells/mm$^2$ (9.75%) in eyes with unilateral pterygium compared to the contralateral normal eyes. [11] They showed that 48.9% of patients with unilateral pterygium had a significant ECD decrease in the affected eye and the extent of this decrease correlated with the size of the pterygium. [11]

However, the results of our study contradicted these earlier findings. The difference in ECD between the PT group and the NPT group was 76 cells/mm$^2$ (3.1%) – significantly lower than previous studies. [11, 13, 18] Our retrospective study results demonstrated that the ECD of eyes with unilateral pterygium was almost the same as the ECD of the contralateral normal eyes. Our prospective study showed that ECC hexagonality and ECV in eyes with unilateral PT was also not significantly different from those in the contralateral NPT eyes. The mean difference in ECD between pterygium eyes and the contralateral eyes in the prospective study was only 46 cells/mm$^2$ (1.9%).

We also confirmed that the ECD decrease in the retrospective study was due to the variation of ECD measurement. The repeatability of ECD measurement by specular microscope is largely dependent on the cell count measured. [19] Doughty et al. found that a cell count of 25 cells resulted in a ±10% of ECD variation while an ECD variation of ±2% could be achieved if 75 cells were measured. [19] In our prospective study, a cell count of at least 80 cells was required for analysis. The 95% CI was 3.0%-3.7% for the ECD COV, and was 76-91 cells/mm$^2$ for the ECD SD. In our retrospective study, the minimum cell count for analysis was at least 60 cells and the variation of ECD measurement would be expected to be larger. Therefore, the difference of 76 cells/mm$^2$ (3.1%) in ECD between the PT group and the NPT group was very likely to be caused by variation of ECD measurement.

The mechanisms of ECD decrease in eyes with pterygium are not fully understood. Two possible mechanisms have been proposed. UV light over-exposure is considered to be the most significant risk factor for pterygium and UV radiation may trigger inflammation, oxidative damage and apoptosis of the ECC, leading to ECC loss. [10, 20] Outdoor workers have been shown to have lower ECD compared with non-outdoor workers [21] and this ECD decrease is correlated with the duration of UV radiation exposure. [18] The other purported mechanism of ECD decrease in eyes with pterygium involves a variety of pathogenic factors including inflammation, angiogenesis and extracellular matrix modulators. [9] These modulators have been shown to be expressed in pterygium tissue or the cornea underlying or adjacent to the pterygium. [7, 22] Moreover, the levels of these pathogenic factors may be correlated with the clinical features of the pterygium. [23, 24]

There might be several reasons why primary pterygium was not shown to be associated with ECD decrease in our study. Firstly, the UV exposure in our patients might not have reached the threshold to cause ECD decrease. Li X et al. divided patients with unilateral primary pterygium into two groups according to their daily UV exposure. [18] In patients with longer UV exposure, defined as spending >1 hour outdoors per day between 10am and 4pm in the summer, the ECD in eyes with pterygium was significantly lower than those in the contralateral eyes. However, in patients with less UV exposure, there was no significant difference in ECD between eyes with pterygium and those without. Moreover, patients with longer UV exposure had significantly lower overall ECD than patients with short UV exposure. [18] These results suggest that in eyes with pterygium, UV exposure needs to reach a certain threshold to induce ECD decrease. One limitation of our study is that the UV exposure of patients was not recorded. Therefore, it is possible our study population is comprised of patients with shorter UV exposure and thus did not meet the threshold for ECD decrease with pterygium. Secondly, the PT group in our study might have different clinical characteristics to those in previous studies. For example, Mootha et al. demonstrated that ECD decrease with pterygium was rarely seen (despite a high prevalence in their practice setting) and was associated with a mean duration of 43 years. [13] In addition, pterygium fleshiness, redness and extent may be correlated with the level of inflammatory and angiogenesis factors and extracellular matrix modulators involved in the pathophysiology of pterygium. [9] It has been shown that pterygia with at least intermediate fleshiness, redness and extent have higher expression of extracellular matrix modulators [23] and chronic inflammation in the corneal stroma. [24] The eyes in our population may not have these clinical features and thus not induce the inflammatory changes that may be associated with ECD reduction. Thirdly, UV radiation may cause an ECD decrease in both eyes of patients, irrespective of pterygium development. This is supported by the findings in both our retrospective study and prospective studies, wherein the ECD of eyes with unilateral pterygium was not significantly different from those in the normal contralateral eyes. Fourthly, corneal endothelial cells may adapt to UV radiation and chronic stromal inflammation under certain conditions and thus the ECD may not be reduced in all cases of pterygium.

In conclusion, the results of both the retrospective and prospective studies on our patient population suggest that primary pterygium is not associated with ECD decrease.

## Declarations

# Ethics approval and consent to participate

Both studies were in agreement with the Declaration of Helsinki and approved by the Institutional Review Board of Shanwei Project Vision Eye Hospital (SWPVEC20160701-1 and 2). Informed consent was obtained from each patient in the prospective study. Informed consent was not required in the retrospective study.

# Consent for publication

Not applicable.

# Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

# Competing interests

The authors declare that they have no competing interests.

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# Authors' contributions

YH, YL and LY designed the study. YH, WQ and ZL collected the dada. YH, LL and AA analyzed and interpreted the data. YH and AA wrote the article. YL and LY made critical revision to the article. All authors have read and approved the final manuscript.

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