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TO ASSESS THE CORNEAL TOPOGRAPHY AND ENDOTHELIAL CELL DENSITY IN PATIENTS WITH PTERYGIUM

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***ABSTRACT**--Pterygium is a very common affliction amongst the working population of our country. A large number of studies have been conducted to enhance our knowledge regarding the pathos behind development of pterygium and its common associations. With the advent of newer modalities, like specular microscopy, we are at an advantage to study the influence of pterygium on the endothelial cell layer. To investigate the effects of pterygium on corneal endothelial cell density (ECD) and astigmatism in patients of pterygium and evaluate a correlation, if any, between ECD and corneal astigmatism in patients of pterygium. An observational cross-sectional study with 90 patients will be conducted in a hospital setting utilising data of fellow eyes as control. The area of cornea affected by pterygium will be compared to the endothelial cell density. Astigmatism in eyes with pterygium will be compared to decreased ECD using the appropriate statistical tests. than in the controls. The Pearson correlation test will be used to show correlation between pterygium size and ECD, if any. In addition, increase in astigmatism will be correlated with the endothelial cell density. Based on the few studies at hand we expect to find that pterygium will be linked to a fall in corneal ECD which may be correlated to increase in corneal astigmatism in patients with pterygium.*

***KEYWORDS**-- Pterygium, endothelial cell density, astigmatism*

I. INTRODUCTION

Background/rationale:

Pterygium is a triangular ocular surface lesion which occurs as a consequence of invasion of the bulbar conjunctiva onto the cornea.[1] One of the most frequent symptoms patients present with in ophthalmic OPD are blurred vision[2] which can be explained by induced corneal astigmatism. A large number of studies have detailed the histopathology of pterygium. The most widely accepted description of the pathogenesis details a centripetal growth of limbal epithelial cells associated with hyperplasia supplemented by disbanding of Bowman's layer (BL) and activation of fibroblastic stroma. There is also inflammation, mesenchymal transition of epithelium, neovascularization and matrix remodelling.

The risk factors recognized to have a part in the pathogenesis of pterygium are proinflammatory cytokines like interleukin 1 and TNF-α and ultra-violet (UV) radiation. DNA damage caused by ultraviolet radiation may

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cause limbal stem cell deficiency probably due to relocation of the reserve stem cells. [1,3,4,5,6] UV irradiation may also induce mutations in TP53 tumor suppressor genes located in limbal basal cells. It adds to the level of proinflammatory cytokines by upregulating interleukin (IL) 1, IL-6, IL-8, and TNF-α production[4-5] and growth factors that weigh in on the vascular and fibrotic appearance of pterygia.

In addition, the expression of proteases released by the pterygium cells, such as matrix metalloproteinases (MMPs) that degrade basement membrane and BL have been found to be elevated in the leading edges of pterygia [6]. These proteases facilitate invasion by degrading basement membrane, and dissolving BL and adjacent stromal matrix (6,7). In addition, pterygium size and severity are also determined by hereditary factors which are multifactorial.[8]

According to Hsu et al [9] these steps in formation of pterygium could cause damage to deeper layers of the cornea such as Bowman's layer, Descemet's membrane and ultimately invade the endothelial cell layer. However, very limited research is available on the same. The primary aim of this study is to investigate the influence of pterygium on corneal ECD within our study population and add to the existing literature.

In a study conducted by Zhang, Y & Wen, J.-Q, the decrease of loss of corneal ECD had a linear correlation with the increase of corneal astigmatism [10]. Therefore, the secondary aim of our study will be to evaluate this correlation within the scope our study parameters by mapping the corneal topography of the patients. The amount of the pterygium induced astigmatism, however small will be measured using corneal topography.

The main rationale of the study is to assess endothelial cell involvement in cases of pterygium within a geographical area of central India's rural population.

II. OBJECTIVES

- To study the endothelial cell count, hexagonality, central corneal thickness and coefficient of variation using specular microscopy in patients with pterygium
- To study the induced corneal astigmatism using corneal topography in patients with pterygium
- To evaluate the age and gender distribution of the patients presenting with pterygium
- To establish a correlation if any between endothelial cell density and corneal astigmatism in patients with pterygium.

III. METHODS

Study design: This is an Observational Cross sectional Study.

Setting: The study shall be conducted over a period of two tears from September 2018 to September 2020. Patients with pterygium presenting to the Out Patient Department of Acharya Vinobha Bhave Rural Hospital will be selected sequentially for the study after taking the inclusion and exclusion criteria into consideration. All data will be collected by a single experienced ophthalmologist. The data will be collected in the form of a questionnaire to be filled by the investigator.

Participants: The participants will be sequentially selected on an OPD basis . The fellow eye of patients shall be considered as control. Following criteria will be utilized.

INCLUSION CRITERIA-

CASE:

Patients >18 years of age presenting with pterygium encroaching >1mm of cornea during study period.

CONTROL:

The other eye of the same patient with pterygium encroaching <1mm of cornea.

EXCLUSION CRITERIA-

1. Previous ocular surgery
2. h/o trauma to cornea
3. h/o Uveitis
4. h/o contact lens use in either eye
5. Central cell count of <1800 cells/mm^2
6. h/o keratitis
7. h/o glaucoma
8. Intraocular pressure of >21 mmHg
9. h/o Diabetes mellitus.
10. Those who did not give consent.
11. Ocular surface pathology in control eye
12. Patients with recurrent, pseudopterygium or diheaded pterygium
13. Patients with astigmatic error >2D in the fellow eye
14. Patients in whom ECD could not be recorded
15. Patients with pterygium encroaching >1mm in fellow eye.

Variables: The variables to be studied are age of patients, gender distribution of patients, pterygium to cornea ratio, endothelial cell density (ECD), coefficient of variation (CV), hexagonality(HEX), central corneal thickness(CCT), astigmatic error.

A difference between the endothelial cell density of study eye and fellow eye as well as difference in astigmatism between both eyes will be calculated as a percentage for statistical analysis. The difference in endothelial cell density as well as astigmatic error will be compared to the pterygium to cornea ratio independently. The coefficient of variation, hexagonality, central corneal thickness can be potential confounders therefore will be evaluated and compared to the same parameters in fellow eyes of patients.

Data sources/ measurement :

The study will adhere to the tenets of the Declaration of Helsinki, and it will be approved by an institutional ethics committee of DMIMSU.

- Informed consent will be acquired from all subjects after the nature of the study will be explained to them.
- An all-inclusive ophthalmic examination will be carried out for all the participants.
- Corneal diameter and pterygium length extending on to it, starting at the limbus, will be recorded on slit lamp using slit beam in horizontal direction. It will be expressed as percentage of the ratio for ease of quantification and comparison with other quantifiable parameters.
- An automated corneal topographer (*CA 800, Topcon*) will be used to calculate the induced corneal astigmatism from three reading in the central 3mm zone of cornea, the fellow eyes will be considered as controls.

The patients with fellow eyes in which astigmatic error will be more than 2 diopters will be removed from the study to avoid bias due to pre-existing astigmatism.

- Endothelial cell count will be measured using a noncontact specular microscope (*Topcon TSP-3000P*) at a resolution of 640 x 480 pixels.
- Three endothelial measurements will be required for each patient from central and paracentral areas using center-to-center method, the average of which will be taken as mean. All measurements will be made by one person at a single clinical site.
- Patients in whom pterygium is extensive thereby making it impossible to obtain a clear microphotograph of endothelial cell layer, will be excluded from the study. We will be using the dot method as it gives a more accurate estimation of area of cells as compared to the final cell area method. More cells will need to be counted by this method.[11]
- Statistical analysis will be performed for all measurable parameters.

IV. BIAS

There was a potential for observer bias in this study which was standardised by ensuring a single observer/investigator to record the measurements.

All variables will be recorded by a standard protocol which will be strictly followed for both study eyes and fellow eyes thus eliminating bias due to faulty or varied data measurement tools.

Since astigmatism can be a confounding factor in our study, an astigmatism >2D in fellow eye will be considered as an exclusion criteria thereby eliminating cases of astigmatism not attributable to pterygium.

As pterygium is a bilateral disease, fellow eyes with pterygium encroaching >1mm of cornea will be excluded to avoid skewing of data.

Study size:

- Using sample size formula with desired error of margin:

$$n=\frac{Z^2_{\alpha/2}*P*(1-P)}{d^2}$$

- Where,
- $Z_{a/2}$ is the level of significance at 5% i.e. 95% confidence interval=1.96
- P= Prevalence of unilateral pterygium in rural population of central India = 13% i.e. 0.13 [12]
- d = desired error of margin = 7% = 0.07
- Therefore n = $\frac{(1.96)^2 \times 0.13 \times 0.87}{(0.07)^2}$
- SAMPLE **SIZE:** 88.67 rounding off to 90 patients needed in the study
- There will not be any loss due to follow up accounting for attrition of cases

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Quantitative variables:

- The quantitative variables in this study are pterygium to cornea ratio, endothelial cell density (ECD), hexagonality (HEX), coefficient of variation (CV), central corneal thickness (CCT), astigmatic error.
- Pterygium to cornea ratio will be expressed as a percentage for standardisation and easy calculation against other quantitative variables.
- Decrease in endothelial cell density and increase in astigmatism will be expressed as a difference
- ECD (CASE)-ECD(CONTROL)/ECD(CONTROL) multiplied by 100 to express as a percentage decrease.
- ASTIGMATISM (CASE)-ASTIGMATISM(CONTROL)

A comparison between the area occupied by the pterygium to the depth of corneal invasion evaluated by the endothelial cell density will allow us to assess our study objective with a measurable outcome while comparison of the ECD difference with increase in astigmatic error will help in establishing a correlation if any.

Statistical methods:

- The normal distribution of each continuous variable will be assessed by performing the Shapiro-Wilk test. We will use Student's t-test for two independent samples to compare the ECD.
- Quantitative data will be described as the mean ― standard deviation (range). A two-sided p value of <0.025 will considered as indicating statistical significance. The Pearson correlation test and regression mode of analysis will be used to study the relation between dissimilar groups.
- The primary null hypothesis will be no between-group difference in the endothelial cell count. The alternative hypothesis will be that the endothelial cell count is lower in the pterygium group than in the contralateral eye group (without pterygium).
- Pearson test will be used to determine correlation between percentage of cornea invasion by pterygium and decrease in ECD as well as the induced astigmatism.
- All data analysis will be performed using the SPSS statistical program.

V. EXPECTED OUTCOMES/RESULTS

- Based on the previous study available on hand conducted by Hugo *et al* [13] we expect to find that pterygium exerts degradation of the deeper layers i.e endothelial cell layer of the cornea and that this will give rise to a fall in endothelial cell density when compared to the normative data.
- An increase in astigmatism was found to have a correlation with a fall in the endothelial cell density in eyes with pterygium, in a study conducted by Hsu My *et al[8]* We expect to find the same outcome here.

VI. DISCUSSION

Key results: A statistically significant decrease in endothelial cell density as compared to fellow eyes with pterygium <1mm size for all participants. A statistically significant increase in corneal astigmatism attributable to the pterygium. There is a direct negative link between the decrease in endothelial cell density and increase in

astigmatism. Consistent with other studies of similar nature we expect to find no noteworthy difference in the hexagonality, coefficient of variation and central corneal thickness.

Limitations:

1) Pterygium may also be classified into three stages based on vascularity which may have an impact on the input of pro inflammatory cytokines resulting in degradation of Bowman's layer and endothelial cell layer. As such there is no quantifiable standard for classifying pterygium into atrophic, intermediate or fleshy or measuring thickness of pterygium. Consequently, we cannot consider the vascularity of the pterygium as a factor in our study.

2) Since our sample size is small further larger scale studies are required to confirm the findings.

3) This study design doesn't provide the scope for follow-up, therefore it is not possible to determine the natural progression of involvement of deeper layers of cornea.

4) This study will establish association at most, not causality

Interpretation: The difference in ECD is directly suggestive of pterygium induced loss of endothelial cells and the increased astigmatism is a consequence of the flatter cornea caused by tractional forces exerted by the encroaching pterygium.

Generalisability: A large number of variables have been considered in this study with effort to minimise bias to a large extent. A larger scale study involving a larger population can be carried out to further validate the results of the present study.

VII. CONCLUSION

A very disturbing trend in recent time, is the increasing prevalence of non-communicable[14] eye diseases in the South-Asian population.

according to Mootha *et al [15]*, a pterygium potentially disrupts the deeper layers of cornea and replaces the stroma with fibrovascular tissue causing loss of endothelial cells. This study validates these findings by assessing similar parameters in a specific population of central India which is the first such study conducted in this geographical region. Most commonly, individuals involved in extensive outdoor work with exposure to UV radiation are affected by pterygium.[16,17] UV radiation is known to increase the level of proinflammatory cytokines such as interleukins 1-6 [18,19]. In addition smoking[20,21] has found to decrease the incidence of pterygium however this has not been considered in the current study.

The primary indication for removal of pterygium at present is decreased visual acuity or irregular astigmatism. The present study provides another quantifiable parameter that surgeons may use to advice pterygium surgery. A careful evaluation of the endothelial cell count and astigmatism will also help the surgeon in deciding whether to use mitomycin C (MMC), which may be responsible endothelial cell loss.

Different corneal measurements have a directly proportional impact on ECD values at the central of the cornea [22] The most commonly assessed parameter in this regard is central corneal thickness for its effect on ECD. According to the available data, thinner the cornea lower will be the ECD [23]. In our study, there was no statistically significant difference in central corneal thickness in eyes with pterygium and control eyes. In conclusion further large-scale studies are needed to comprehensively determine the whether the changes in ECD

are isolated or dependant on other parameters involving cornea and pterygium [24]. A number of articles on different aspects of this study from this region were reviewed [25-62].

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