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ORIGINAL ARTICLE

NUMBER OF TRABECULAR MESHWORK CELLS IN ADULT PRIMARY OPEN ANGLE GLAUCOMA PATIENTS FROM KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Background: Glaucoma is an irreversible optic neuropathy that results in a distinctive pattern of vision loss. The objective of this study was to determine the number of trabecular meshwork cells (TMCs) in adult POAG patients from Khyber Pakhtunkhwa, Pakistan.

Materials & Methods: This cross-sectional study was conducted in Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan from 01/07/2016 to 31/12/2016. Trabecular meshwork samples from 75 POAG patients who underwent trabeculectomy were included. After staining with haematoxylin-eosin, nuclei in TMCs were counted under 400X objective. Number of TMCs, sex, age groups and age in years were variables. Sex and age groups were analyzed as count and percentage. Two ratio variables were skewed, hence analyzed by median/quartile (Q2), Q1, Q3 and IQR with 95%CI. Hypothesis was tested with non-parametric one-sample Wilcoxon signed-rank test.

Results: Seventy-five patients included 58 (77.33%) men and 17 (22.67%) women, with 42 (56%) patients in 36-60 years, 25 (33.33%) in 61-70, 8(10.67%) in 71-80 and no (0.0%) patient in 81-90 years. The mean age was 57.53±10.86 (35-78, range 43) years, 95%CI 55.03-60.03. The median (Q2) number of TMCs/mm^2 was 260 (214-367, IQR 153), 95%CI 230-291. Null hypothesis was rejected ($p<.00001$), showing that the number of TMCs in glaucomatous adults of Khyber Pakhtunkhwa, Pakistan was statistically significantly lower than normative sample of non-glaucomatous enucleated eyes.

Conclusion: The number of trabecular meshwork cells in adult POAG patients from Khyber Pakhtunkhwa, Pakistan was significantly lower than normative sample of non-glaucomatous enucleated eyes.

KEY WORDS: Glaucoma; Primary Open Angle Glaucoma; Optic Neuropathy; Trabeculectomy; Trabecular Meshwork; Adults; Population; Pakistan.

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1. INTRODUCTION

1.1 Background: Glaucoma is an irreversible optic neuropathy that results in a distinctive pattern of vision loss.[1] It has different subtypes, among which 70% cases belong to Primary Open Angle Glaucoma (POAG). About 4.5 million people are blinded by glaucoma globally, making it the leading cause of irreversible blindness.[2] Several risk factors have been linked to the pathogenesis of POAG, among which increasing age is the most important factor.[3] With a better quality of life, the average age of population in

the developed countries has increased; leading to a significant increase in the prevalence of glaucoma.[4]

Another important modifiable risk factor linked to the pathogenesis of POAG is raised Intra-Ocular Pressure (IOP). Although glaucoma can be precipitated even when the IOP is normal; yet a strict monitoring of IOP is required to reduce the progression of glaucoma.[5] The steady-state IOP depends upon the relation between aqueous outflow, uveoscleral outflow and episcleral venous pressure. Anatomically, the episcleral vessels are well suited for the regulation of IOP, as the circulation is exclusively made up of frequently anastomosing veins, devoid of arteries and capillaries and also receiving autonomic innervation.[6]

Anatomically, Trabecular Meshwork (TM) can be divided into three distinct regions. The narrowest region close to the Shclemm's canal is the juxtacanalicular or cribriform layer that, together with the inner wall of Shclemm's canal, offers maximum resistance to the aqueous outflow.[7] The trabecular endothelial cells play a very important role in maintaining adequate aqueous outflow. These act as pressure sensors and have the ability to contract and relax in response to the flow through the meshwork, thus altering the dynamics of meshwork.[8] These also maintain a strict balance between the production and degradation of extracellular matrix; a major source of aqueous outflow resistance.[9]

Studies have concluded that in POAG, the number of trabecular cells is significantly reduced. This leads to a reversal of all the normal processes carried out by these cells. A characteristic 'plaque like' material and increased amount of debris are deposited in the meshwork; all contributing to the increased aqueous outflow resistance.[10] For a long time, it has been presumed that trabecular meshwork is the continuation of Descemet's membrane of cornea. A recent study has concluded that the collagen core of meshwork is the continuation of newly discovered pre-Descemet's layer, known as Dua's layer. Studies have shown that people who undergo surgical procedures where the pre-Descemet's layer is completely excised have a greater risk of developing glaucomatous optic neuropathy. Further insight into its anatomy and physiology may lead to the discovery of new concepts in the management of this sight threatening disease.[11,12]

1.2 Research Problem (RP) & Knowledge Gap (KG): We are unaware of the number of TMCs in adult POAG patients from Khyber Pakhtunkhwa, Pakistan. This unawareness is our RP. Literature search showed no such study for Khyber Pakhtunkhwa province or Pakistan. It is our KG. To fill this KG and to solve this RP is justification of our study.

1.3 Research Question: What is the number of trabecular meshwork cells (TMCs) in adult POAG patients from Khyber Pakhtunkhwa, Pakistan?

1.4 Research Objective: The objective of this study was to determine the numbers of TMCs in adult POAG patients from Khyber Pakhtunkhwa, Pakistan.

1.5 Research (null) hypothesis: The number of TMCs in adult POAG patients from Khyber Pakhtunkhwa, Pakistan is same as in normative sample of non-glaucomatous enucleated eyes.[14]

H_0=Number of TMCs= 2,800 cells/mm^2

1.6 Operational definition: Any patient with IOP >21 mmHg with corresponding optic disc cupping was labeled as a case of POAG.[13]

2. MATERIAL AND METHODS

2.1 Design, setting, duration and ethical consideration: This cross-sectional study was carried out in the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan from 01/07/2016 to 31/12/2016 under the supervision of Dr. Walayat Shah of the same institute. The TM samples were collected from the Department of Ophthalmology at Peshawar Institute of Medical Sciences, Peshawar. Ethical approval was granted by Ethical Committee of Khyber Medical University, Peshawar. The consent of patients was obtained before inclusion in the study.

2.2 Sampling: Seventy-five TM specimens were included for histological study through consecutive non-probability technique. All adults who underwent trabeculectomy for POAG were eligible.

2.3 Conduct of procedure: All patients underwent detailed ocular examination, including measurement of IOP and fundoscopy with cup-disc ratio measurement. IOP was measured on Perkins MK2 Tonometer (Clement Clarke Int., Harlow, Essex, UK). Cup-disc ratio was not available for 13 out of 75 eyes.

All trabeculectomies were performed by MD Khan by using standard surgical procedure. The specimens were immediately placed in 10% neutral buffered formalin solution and then processed for staining. Tissues were then stained with Haematoxylin-Eosin to be studied under light microscope. The nuclei in the trabecular meshwork were counted under 400X objective. (Figure 1 & 2)

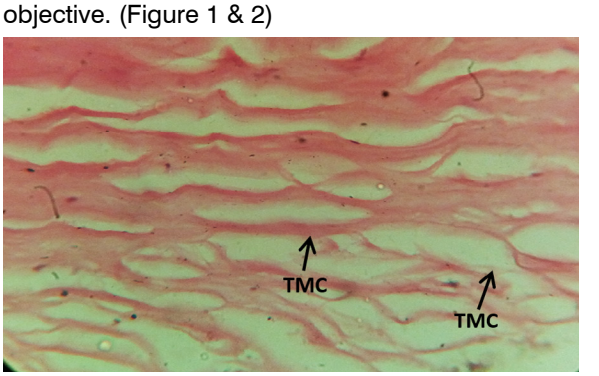

Figure 1: Photomicrograph of 52-year-old patient with primary open angle glaucoma (H&E; 400X), showing only few trabecular meshwork cells

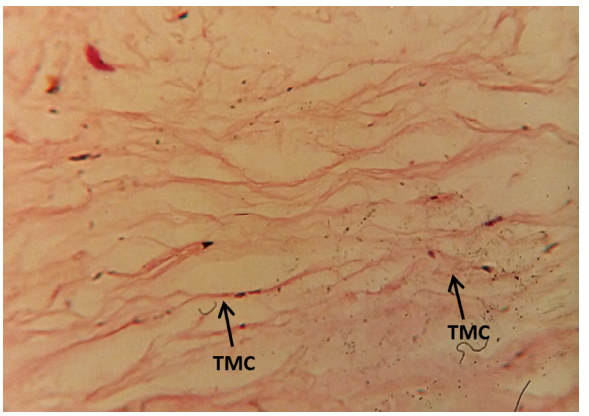

Figure 2: Photomicrograph of a 75-year-old patient (H&E; 400X) with primary open angle glaucoma, showing sparse trabecular meshwork cells

The count was carried out manually using a 10x10 grid reticule. The reticule was fitted in the eye piece and was calibrated against a micrometric slide of 1 mm length which was placed on the microscope stage. Such calibration was necessary to avoid magnification error. 23rd division on the stage micrometer coincided with 9th square on one side of the grid; thus the length of nine squares was 0.23 mm. If nine squares are considered on each side, then total area of the grid would be 0.23x0.23=0.0529. The count of cells was therefore done in 9x9=81 squares.

If 100 squares = "x" number of cells; then 81 squares = x ×81÷100 = y

The cell number per millimeter square was then calculated as:

If 0.0529 millimeter square = "y" number of cells; then 1 millimeter square = 1 × y ÷ 0.0529

2.4 Data collection and analysis plan: Data was collected for one research variable number of trabecular meshwork cells (TMCs)/mm^2 on ratio scale and three demographic variables; sex (men & women) on nominal scale, age groups (36-60 years, 61-70, 71-80 & 81-90 years) on ordinal scale and age in years on ratio scale. Sex and age groups were analyzed as count and percentage. The two ratio variables were subjected to Skewness, Kurtosis and Shapiro-Wilk test for normality. As their data was skewed, so these were analyzed by median/ quartile (Q2), Q1, Q3 and interquartile range (IQR=Q3-Q1) with 95%CI of median. Hypothesis was tested with a non-parametric one-sample Wilcoxon signed-rank test as done by Johnson and Tschumper.[14] All the data was analyzed using IBM SPSS Statistics for Windows, Version 22.0, released 2013 (IBM Corp., Armonk, NY).

3. RESULTS

3.1 Sample statistics & estimation of parameters: The sample of 75 patients included 58 (77.33%) men and 17 (22.67%) women. The sample included 42 (56%) patients in 36-60 years, 25 (33.33%) in 61-70, eight (10.67%) in 71-80 and no (0%) patient in 81-90 years.

The data for age in years and number of trabecular meshwork cells (TMCs) was subjected to tests of normality. Age in years was normal, while number of TMCs was shown to be skewed. (Table 3.1)

The mean age was 57.53±10.86 (35-78, range 43) years, 95%CI 55.03-60.03. The median (Q2) number of TMCs/mm^2 was 260 (214-367, IQR 153), 95%CI 230-291).

3.2 Hypothesis testing for number of TMCs in POAG: As the data distribution was skewed, therefore a non-parametric one-sample Wilcoxon signed-rank test was used. The test value of 2,800 cell/mm^2 was taken from a normative sample from Johnson and Tschumper. Johnson & Tschumper from Rochester, Minnesota, USA in 1987 successfully cultured 42 (78%) out of 54 human eye bank eyes, having mean age 73±12 (26-99) years, with four <60 years. Human TM cells remained in their normal position and viable on trabecular beams for at least four weeks. Four sections per eye were counted, averaged, and expressed as nuclei/mm^2 of overall TM area. In a subsample (count not given) of 61-70 years age, the average TMCs count was 2800±230 cells/mm^2 at two weeks in culture. Our sample of 75 included 42 (56%) eyes in 36-60 years, 25 (33.33%) in 61-70 and eight (10.67) in 71-80 years. Our median number of TMCs/mm^2 was 260. The test showed p-value (<.00001) less than alpha .05; the null hypothesis was proved to be false and hence rejected, showing that the number of TMCs/mm^2 in adult POAG

Table 3.1: Normality of data for age and number of TMCs in adult POAG patients from Khyber Pakhtunkhwa, Pakistan (n=75)

Variable	Skewness Statistic	Kurtosis Statistic	Kolmogorov-Smirnov test			Data distribution
			Statistic	d.f.	p-value	
Age in years	-.149	-.972	.099	75	.063607	Normal
Number of TMCs/mm^2	.577	-.717	.149	75	.000281	Skewed

Table 3.2: Number of TMCs in adult POAG patients from Khyber Pakhtunkhwa, Pakistan versus normative sample of non-glaucomatous enucleated eyes (n=75)

Median number of TMCs/mm^2	Test value from cultured eyes[15]	Difference of medians	Test statistic	Standard error	Standardized test statistic	p-value
260	2,800	-2,540	.000	189.276	-7.529	<.00001

patients from Khyber Pakhtunkhwa, Pakistan was statistically significantly lower than normative sample of non-glaucomatous enucleated eyes. (Table 3.2)

4. DISCUSSION

4.1 Number of trabecular meshwork cells in POAG patients: In our population of POAG patients, the median number of TMCs/mm^2 was 260 (214-367, IQR 153), 95%CI 230-291). It was significantly lower than normative sample of non-glaucomatous enucleated eyes (p<.00001).

Similar to our study, Sundaresan, et al.[15] from Madurai, Tamil Nadu, India in 2021 reported comparison of TM cellularity and trabecular meshwork stem cells (TMSCs) in donor eyes with POAG (n=6) having age 58-92 years and age-matched normal donors (n=8) with age 55-93 years. The donor eyes were received from the Rotary Aravind International Eye Bank, Madurai, and the eye banks from Aravind Eye Hospitals at Tirunelveli and Coimbatore, Tamil Nadu, India. Anatomical changes were evaluated by minimum three sections from each of the four anterior segment quadrants from each of the 14 eyes. TM cellularity was significantly lower in POAG (41.83±9.0 cells/section) than controls (69.33±12.77 cells/section) (p=.0007). Identification of TMSCs was made as cells with high ABCG2 and p75 positivity in the insert region of TM. TMSCs were also lower in POAG (0.14±0.17%) than controls (4.73±5.46%), but the difference was not statistically significant, probably due to small sample sizes (p=.0643).

Xiong, et al.[16] demonstrated that transplanting trabecular meshwork stem cells into the anterior chamber of Myoc Y437H mutant mice greatly increased the TM cellularity in the anterior chamber of these mice. The enhanced cellular count leads to the reversal of all the glaucoma symptoms in the form of IOP reduction, improved aqueous outflow facility and restoration of retinal ganglion cells as observed by electroretinogram.

Baleriola, et al.[17] from Madrid, Spain in 2008 detected the presence of apoptotic cells in trabeculectomy specimens of POAG eyes using TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) method of staining. They also found that the number of apoptotic cells in primary open angle glaucoma (POAG) samples (n1=7) was significantly higher than those of primary angle closure glaucoma (PACG) (n2=1). They concluded that one of the reasons of reduced cellularity in POAG could be increased apoptosis of TM cells that could possibly be attributed to the intense phagocytic activity of these cells.

Alvarado, et al.[18] observed that although the cellular count remained significantly lower in POAG patients, the rate of decrease in POAG and non-glaucomatous controls remained similar as the age progressed. They proposed that this could probably be due to some noxious stimulus to have occurred in an early period of life, which resulted in a marked decline and then the further decrease was simply an age related phenomenon. Till date, all the TM cellularity related studies has supported the notion of reduction in the cellular count and this phenomenon has not been negated by any author so far.

4.2 Establishing the platform for microscopic study at national level: So far various studies have been conducted at international level, but no such step has been taken on national level to interpret the histological findings in glaucoma patients. Since the pathogenesis of this disease is attributed both to the integrity of TM and optic nerve, our research can be of help to further work upon TM morphology and embark on experimental studies for establishing innovative treatment strategies.

4.3 Role of innovative stem cell therapy in treatment of POAG: One of the treatment options for high IOP in POAG is argon laser trabeculoplasty (ALT). The exact mechanism of how it reduces the IOP is not known, but it has been postulated that the TM cells located just beneath the Schwalbe's line in the anterior non filtering portion of TM have the ability to replicate and repopulate the burnt lesion sites of ALT. These cells are regarded as the stem cells of TM and are called "TM insert cells''. This study reveals the significance of TM histology and paves the way to the application of novel interventional procedures in the form of stem cell therapy.[19]

4.4 Marwat's logical trajectory of research process: We have followed this novel 8-steps logical flow including; identification of our research problem, verifying the knowledge gap, putting the research problem as research question, phrasing it as research objective and formulating it as a hypothesis; the tentative answer. Further relevant data was collected, analyzed and interpreted to get the true an-

swer for our research question, filling the knowledge gap and solving our research problem.[20-22]

5. CONCLUSION

The number of trabecular meshwork cells in adult POAG patients from Khyber Pakhtunkhwa, Pakistan was significantly lower than normative sample of non-glaucomatous enucleated eyes. We concluded that histological changes do occur in the TM tissue in POAG. There is a significant reduction of TMCs that could be a possible cause of consequential pathological events occurring in the tissue. Whether these changes are due to glaucomatous optic neuropathy or that the optic neuropathy occurs as a consequence of outflow resistance in the TM could not be established in this study and needs further exploration.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	FD, MK
Acquisition, Analysis or Interpretation of Data:	FD, MK, FD, MTK, MDK
Manuscript Writing & Approval:	FD, MK, FD, MTK, MDK

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.