Int.j.pathol.2023;21(1): 3 – 7

Original Article

Morphological Changes in the Trabecular Meshwork in Eyes with Primary Open Angle Glaucoma

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ABSTRACT

Introduction: Primary open angle glaucoma is a progressive optic neuropathy. It is a leading cause of blindness around the world. Exact etiology is not known although a number of risk factors identified. Glaucoma prevention and cure remains an enormous challenge and for that elaboration of pathogenesis may be helpful.

Objective: To determine the morphological changes in the trabecular meshwork in eyes with primary open angle glaucoma.

Materials and Methods: A total of 75 trabeculectomy specimens were obtained from Primary open angle glaucoma patients. The tissues were prepared for light microscopy and stained with haematoxylin and eosin. The lamellar thickness and inter-trabecular spaces were measured in micrometer. These numbers were compared with age matched controls. The data was analyzed using SPSS version 21. Student's t-test was used to compare the obtained values with controls.

Results: There was a statistically significant increase in the lamellar thickness (P value = 0.001). The normal trabecular thickness (4.7 μm) was compared with the mean value of trabecular thickness of the samples (10.35 μm). A significant reduction in the inter-trabecular spaces was observed (P value = 0.001) when compared to the values of age matched controls. (Normal inter-trabecular spaces = 25 μm, reduction in spaces = 11.78 μm).

Conclusion: This study confirmed and highlighted the histo-pathological changes occurring in the trabecular meshwork of Primary open angle glaucoma. The destructive changes obliterate the passages of delicate trabecular network that may lead to high aqueous resistance and a consequent rise in the IOP.

Key words: Glaucoma, primary open angle, trabecular meshwork, progressive optic neuropath.

Introduction

Primary open angle glaucoma (POAG) is a progressive optic neuropathy and a leading cause of permanent blindness around the world[1]. Around 64.3 million people have been affected by glaucoma in 2013, and the number is anticipated to reach over 120 million by 2040[2, 3]. The disease has a silent course and progresses without becoming clinically evident until sufficient nerve damage is done. It is therefore regarded as a "silent killer of sight"[4].

While a number of risk factors have been attributed to POAG, the exact etiology still remains unidentified.[5]

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The known risk factors include raised intra-ocular pressure (IOP), central corneal thickness, cerebrospinal fluid pressure and genetic predisposition.[6,7,8,9] Although glaucoma can occur with normal IOP, nearly all the treatment strategies aim at lowering or maintaining the IOP. Such interventions have been shown successful to halt to the progression of glaucoma.[10, 11]

Trabecular endothelial cells have a direct effect on the structural and function of the trabecular meshwork. Loss of these cells lead to marked disruption and thickening of trabecular lamellae. The amount of extra-cellular matrix (ECM) is increased and there is enhanced deposition of abnormal collagen and chondroitin sulphate in the ECM. [12] Lack of phagocytic activity by endothelial cells results in increased debris accumulation in the trabecular meshwork (TM).[13]

Genetics play a crucial role in the pathogenesis of glaucoma. An important gene associated with glaucoma is myocilin. It maintains the structural and

functional integrity of TM and ciliary body. Its mutation can therefore lead to high pressure POAG.[14]
Glaucoma prevention and cure remains an enormous challenge despite the vast and fast pace advancements in experimental medical sciences .[15]Advances in stem cell therapy bring in a new ray of hope in face of these challenges. A procedure called laser trabeculoplasty results in proliferation of trabecular stem cells that reoccupy the burn sites. These cells can rejuvenate the TM and maintain IOP homeostasis.[16]
Due to ethical issues and increased immunological rejection, the use of induced pluripotent stem cells (iPSCs) and embryonic stem cells remains controversial.[17] It has however, been noticed that iPSC-TM inoculation into the human donor eyes can result in significant increase of endogenous TM cells. [18]
This study aims to describe the events of changes occurring in the trabecular meshwork in POAG. Comparison of the microscopic changes to the standard normal architecture can help elaborate on the pathogenesis and consider experimental and targeted treatment options for the treatment of POAG.

Methodology

The study was conducted at Peshawar Institute of Medical Sciences in the department of ophthalmology, with the approval from ethical committee of Khyber Medical University Peshawar. POAG was defined as an IOP of more than 21mm Hg calculated with Perkins MK2 tonometer (Clement Clarke Int., Harlow, Essex, UK).[19] A total of seventy five trabeculectomy specimens were used for the study (male=58, female=17). Sample size has been calculated according to the given formula:

$$n = \frac{z^2 \times p\,(1-p)}{e^2}$$

$$n = \frac{(2.0537)^2 \times 0.024\,(1-0.024)}{(0.04)^2} = 62$$

(With the prevalence (p) of 2.4%, 2.0537 level of confidence (z) for 96% confidence interval and ***standard error (e) of 4%***).[20]
An informed consent was taken from the patients involved in the study. The specimens obtained were fixed in formalin solution, stained with haemotoxylin and eosin and analyzed under light microscope. The lamellar thickness was measured with an ocular micrometer, calibrated against a stage micrometer of 1 mm length. The coinciding divisions of the two micrometers were noted. The two coincided at 97th division of the reticule that corresponded to the 23rd line on stage micrometer. As each division on stage micrometer equals to 10 micrometer, 23rd division is therefore equal to 230 micrometer.
The number of divisions on the ocular micrometer covering the width of each lamella was noted. An average was taken for all the lamellae of each slide. The thickness was measured as:
If 97 division on ocular micrometer = 230 micrometer then,

"x" divisions = x × 230 ÷ 97.[21]

The measurements for inter-trabecular spaces were taken in a way similar to that of lamellar thickness. The final average was then calculated for all the spaces.
The data was analyzed using SPSS version 21. Mean $\pm$ SD was used to express the inter-trabecular spaces and lamellar thickness. Statistical significance was analyzed at a p value < 0.05 using student's t-test. Results were presented in the form of tables.

Results

The normal trabecular thickness (4.7 µm) [22] was compared with the mean value of trabecular thickness of the samples (10.35 µm). Standard Deviation was calculated as 5.47. Student's t- test applied for comparison was equal to -8.94. Trabecular thickness of the samples was significantly increased (p = 0.001).

Table 1: Trabecular thickness in POAG

Trabecular Thickness (micrometer)			t value	p value
Normal thickness	Sample mean	SD		
4.7	10.35	5.47	-8.94	0.001

The sample mean of trabecular spaces was calculated as 11.78 µm against the normal value of 25 µm.[23] The standard deviation of mean was 4.373. Student's t-test at p < 0.05 was calculated as 26.13. The decrease in the inter-trabecular spaces was statistically significant (P value was equal to 0.001).

Table 2: Width of inter-trabecular spaces in POAG

Inter-trabecular space (micrometer)			t value	p value
Normal space	Sample mean	SD		
25	11.78	4.373	26.13	0.001

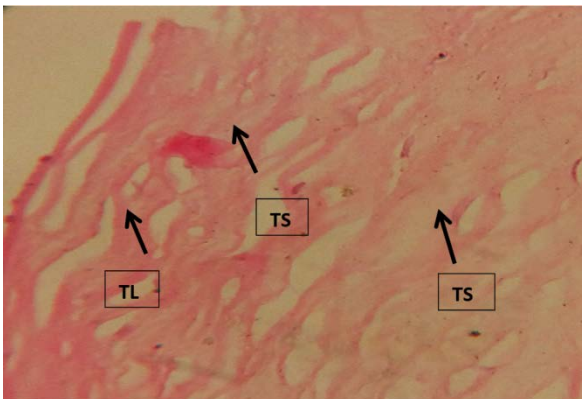

Figure 1: Photomicrograph of 50 year old patient (H&E; 400X). The collapsed inter-trabecular spaces (TS) and the thickened and merged trabeculae (TL) can be observed.

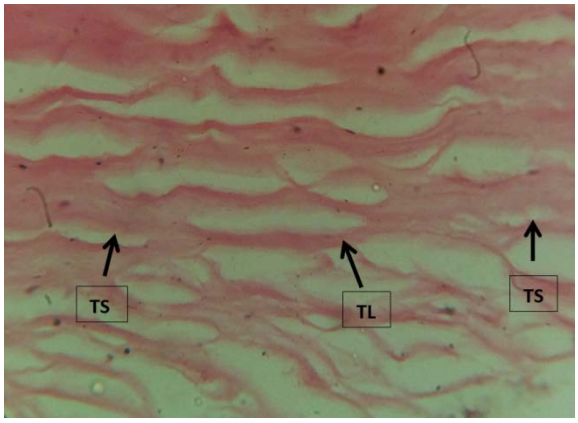

Figure 2: Photomicrograph of 53 year old patient (H&E; 400X).The trabeculae (TL) are mildly thickened and obliteration has initiated in the inter-trabecular spaces (TS).

Discussion

The trabecular meshwork forms the most essential route for the aqueous outflow and its distortion can markedly affect the IOP homeostasis.[24] In our study, we observed significant morphological changes in the glaucomatous TM. When compared with age matched controls, the trabecular lamellae were found to be markedly thickened and distorted. Watson and Grierson suggested that the trabeculae become "sticky" due to the loss of cellular cover, leading to their fusion and thickening.[25] Belmares et al observed fibrotic changes in TM due to the presence of much higher amount of collagen and characteristic banding sheath pattern in the glaucomatous eyes[26] Predominantly, the accumulation of type VI collagen is increased which is the major component of ECM. This contributes profoundly to the abnormal lamellar fusion, stiffening and distortion.[27,28]

The inter-trabecular spaces of the normal inner meshwork ideally measures more than 40 micrometer as reported by Gonzalez et al.[29] Our values fell significantly lower than this number. A number of factors have been attributed to the obliteration of these spaces. A transmission electron microscopy conducted by Taurone et al revealed various histological changes and presence of significant inflammatory response in POAG. They observed the presence of cellular fragments, ECM and collagen deposition, and collapsed trabecular lamellae. They also confirmed the presence of inflammatory cells like macrophages and lymphocytes .[30]

Since the pathophysiology is maximally attributed to the TM cell loss, innovative stem cell therapy has therefore been considered as the hallmark of current glaucoma treatment. Various studies support the notion that transplanting TM stem cells (TMSCs) into the tissue markedly reduce the IOP and effectively restore the ultrastructure and function of TM .[16, 31, 32, 33,34]. These stem cells however, require a number of ideal conditions to effectively home the trabecular meshwork [16] e.g. the expressions of specific type of integrins promote and enhance the anchoring of TMSCs to the TM, thus improving the efficacy of stem cell therapy.[35]

Some significant limitations apply to our study. We cannot interpret or apply the morphogenesis of trabeculectomy specimens to the entire meshwork due its very small size. Surgical and tissue processing artifacts are also unavoidable. Sophisticated tissue processing techniques can however, overcome this problem to quite an extent. Use of donor ocular globes of POAG can help us have a 360 degree evaluation of the TM architecture that can be compared to standard normal controls.

Conclusion

This study confirmed and highlighted the histopathological changes occurring in the trabecular meshwork of Primary open angle glaucoma. The destructive changes obliterate the passages of delicate trabecular network that may lead to high aqueous resistance and a consequent rise in the IOP.

RECOMMENDATIONS: Since the morphology of TM is directly related to the qualitative and quantitative regulation of trabecular endothelial cells, much of the research is therefore focused on innovative restoration of TM endothelial cells. Further insight into the

proliferative and protective function of TMSCs and the regenerative capacity of TM may generate novel and diverse therapeutic approaches in the treatment of POAG.

Conflict of Interest: Authors declare no conflict of interest.
Funding: No funding was received for this project

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HISTORY	
Date received:	19-11-2022
Date sent for review:	24-12-2022
Date received reviewers comments:	23-02-2023
Date received revised manuscript:	16-04-2023
Date accepted:	02-05-2023

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A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion