

A report on a series of nanophthalmos with histopathology and immunohistochemistry analyses using light microscopy

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We aimed to study the histopathological and immunohistochemistry features in clinically diagnosed cases of nanophthalmos using

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light microscopy. This was an observational comparative study. We enrolled four eyes of four consecutive patients with nanophthalmos and visually significant cataract, who underwent cataract surgery with prophylactic posterior sclerostomy. Histological analysis of the excised scleral tissue was done and compared with age-matched cadaver controls between January 2021 and October 2021. Hematoxylin and Eosin (H&E) stains were used for histological analysis, and was further supplemented with immunohistochemistry (IHC) and immunofluorescence (IF) analyses using a simple light microscope. The immunostained sections were analyzed using confocal microscope for the fibronectin expression level. The main outcome measure was demonstration of histological changes of sclera in nanophthalmic eyes undergoing cataract surgery. Light microscopic features of nanophthalmos revealed thick fibers with fraying and lightly stained cores, irregular serrated edges, and randomly interspersed fibroblasts compared to regular arrangement of collagen fibers seen in cadaver controls. Immunohistochemistry analysis with anti-fibronectin antibody showed strong positivity in clustered fibers in nanophthalmos, and less intense diffuse staining in cadaver tissue. Histoclinical correlation was observed in one nanophthalmic scleral tissue with axial length less than 17 mm

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showing severe disorganization with diffuse collagenization, loss of fibrillary architecture compared to another specimen with axial length more than 17 mm. Simple, cost-effective light microscopy using basic stains was effective in identifying the characteristic histopathological features in nanophthalmic eyes, and this was further highlighted by immunohistochemistry and immunofluorescence analyses.

Key words: Axial length, histopathology, light microscopy, nanophthalmos

Nanophthalmos is a rare developmental disorder characterized by an eye with short axial length, shallow anterior chamber, and an abnormally thickened sclera.^[1-3] A thickened sclera impedes vortex venous drainage and reduces the transscleral flow of proteins from the eye, causing accumulation of fluid in the choroid, subretinal space, or both, which may often lead to choroidal detachment, exudative retinal detachment, and uveal effusions.^[1-4]

An abnormally thickened sclera was found to be the main cause of primary uveal effusion syndrome, and hence, performing a subscleral sclerectomy as the window for the outflow of fluid was considered as the surgical treatment for this disease.^[4] Prophylactic sclerostomy (window surgery) has been proposed to prevent uveal effusions when performing cataract surgery in nanophthalmic eyes.^[5]

Trelstad *et al.* were the first to observe thickened sclera with a less orderly lamellar arrangement of the collagen bundles in nanophthalmic eyes compared to normal eyes.^[6,7] Similar histological features of intense and marked inter-fibrillary changes were found in a series of patients with high hyperopia and nanophthalmos.^[8]

Conventionally, transmission electron microscopy (TEM) is used to diagnose histopathological changes in scleral tissue. However it is expensive, needs expert interpretation, and is also not widely available. We used a low-cost, simple light microscopy to demonstrate histopathological characteristics in clinically diagnosed cases of nanophthalmic eyes undergoing cataract surgery. Additionally, immunohistochemistry and immunofluorescence with anti-fibronectin antibody were also done to highlight the underlying pathogenesis.

Methods

Our study was approved by the institutional review board of our hospital (IRB RES2017085CLI number). Informed consent was obtained from all subjects enrolled in the study. We enrolled four eyes of four consecutive patients with nanophthalmos and visually significant cataract who underwent cataract surgery with prophylactic posterior sclerostomy between January 2021 and October 2021. Inclusion criteria were as follows: (1) axial length less than 20.5 mm by IOL master (2) retinochoroidal scleral (RCS) thickness >1.7 mm on B-scan ultrasonography, and (3) significant cataract. We excluded eyes with corneal opacities, diseased sclera, preoperative uveal effusions, hypotony, intraocular tumor, rhegmatogenous retinal detachment, and intraocular inflammation.

All patients were carefully examined via routine ophthalmological examinations, including best corrected visual acuity (BCVA) by Snellen chart, retinoscopy with subjective refraction, measurement of the axial length, anterior chamber

depth, lens thickness and central corneal thickness using IOL master, intraocular pressure measurement (IOP) by Goldmann applanation tonometer, angle assessment using Zeiss four-mirror gonioscopy, slit-lamp biomicroscopic examination of anterior segment, fundus evaluation using 90D lens, and RCS thickness using B-scan ultrasonography. Preoperatively, all patients received antibiotic eye drops (ofloxacin 0.3% w/v) six times a day, before surgery. All patients were given 100 ml 20% intravenous mannitol, an hour before surgery to prevent intraoperative spike in IOP, and received sub-Tenon's anesthesia consisting of 3 ml of lignocaine hydrochloride (2%) mixed with hyaluronidase (1500 IU). All cataract surgeries (phacoemulsification) and all sclerostomies were performed by the same surgeon. A subscleral prophylactic posterior sclerostomy was performed in all four cases at the inferotemporal quadrant along with cataract surgery.

Histologic examinations of all excised scleral tissues were performed with routine Hematoxylin and Eosin (H&E) and special Alcian blue staining. Additionally, immunohistochemistry (IHC) for fibronectin staining and immunofluorescence (IF) were also done. Histopathological

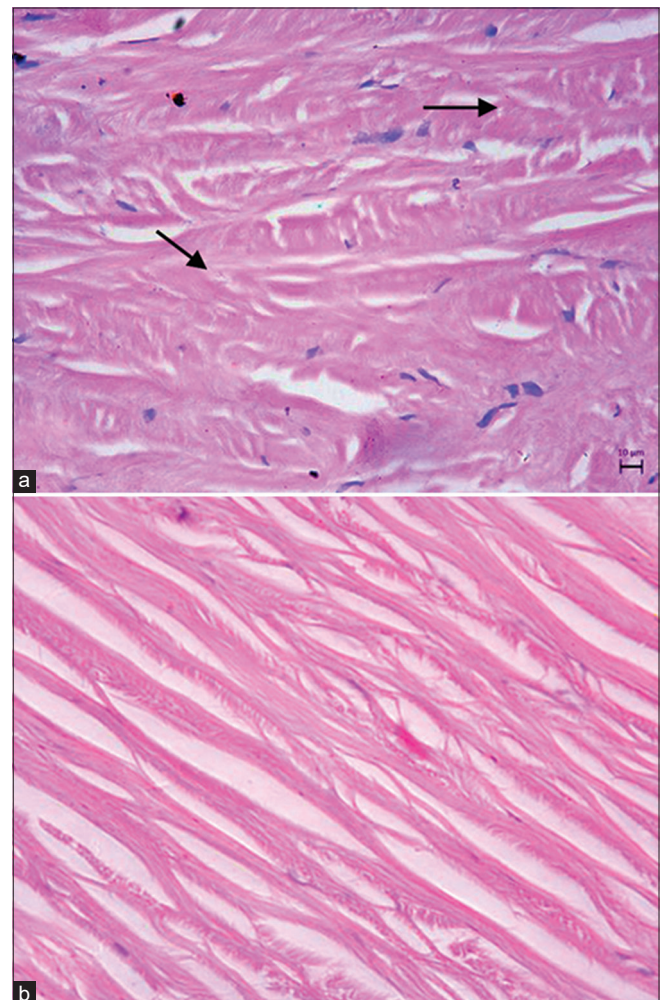


Figure 1: (Hematoxylin and Eosin magnification ×40): (a) Disordered collagen (black arrows) and proliferated fibroblasts, thickened fibers with fraying and lightly stained cores in nanophthalmic sclera, (b) Control shows regular arrangement of sweeping fibers

Table 1: Summary of preoperative ocular biometric details of the patients

Age (years)/ Sex	Eye	AXL (mm)	RCS (mm)	ACD (mm)	LT (mm)	Refractive error	Baseline BCVA	IOP (mmHg)	Grade of cataract	Complication	IOL power (D)	Post-op BCVA	Fundus
56/F	RE	16.19	2.82	2.48	4.27	+15	6/60	30	NS 2	NIL	54	6/9	Hypermetropic disc
46/F	RE	16.96	1.77	2.98	4.66	+10	5/60	14	PSCC	NIL	45	6/36	Pale disc
67/M	RE	19.45	1.96	1.58	5.65	+13	6/18	18	NS 2	NIL	36	6/18	CDR 0.6
60/M	LE	17.80	2.01	1.98	4.05	+12	6/60	16	NS 2	NIL	40	6/18	Hypermetropic disc

F: Female, M: Male, AXL: Axial length, RCS: Retinochoroidal scleral, ACD: Anterior chamber depth, LT: Lens thickness, BCVA: Best-corrected visual acuity, IOP: Intraocular pressure, IOL: Intraocular lens, mm: Millimeters, D: Diopters

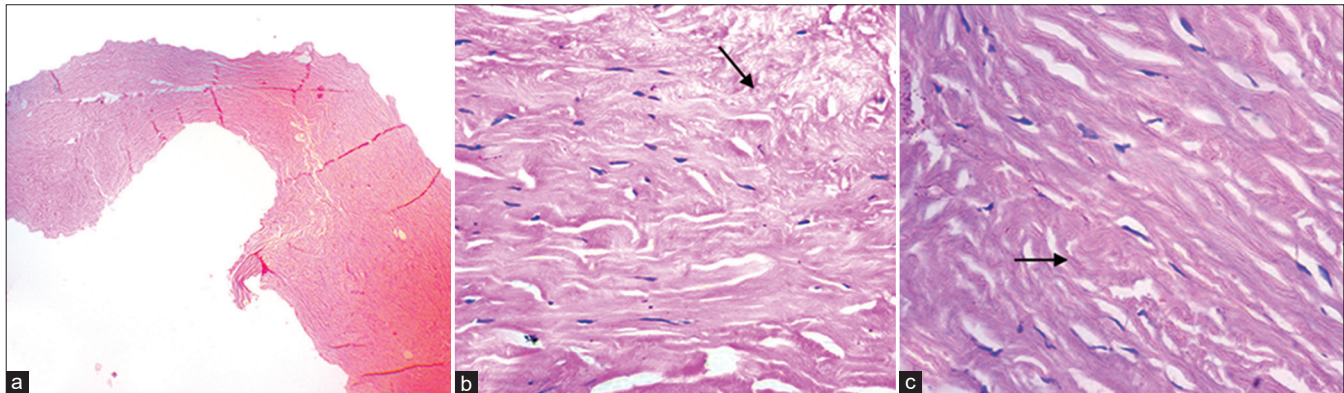


Figure 2: (a) [Axial length <17 mm ($\times 10$)] and (b) ($\times 40$) shows severe disorganization with diffuse collagenization (black arrow), loss of layering and fibrillary architecture with reduced sclerocytes, (c) [Axial length >17 mm ($\times 40$)] showed increased collagenization (black arrow) with preservation of layering and architecture

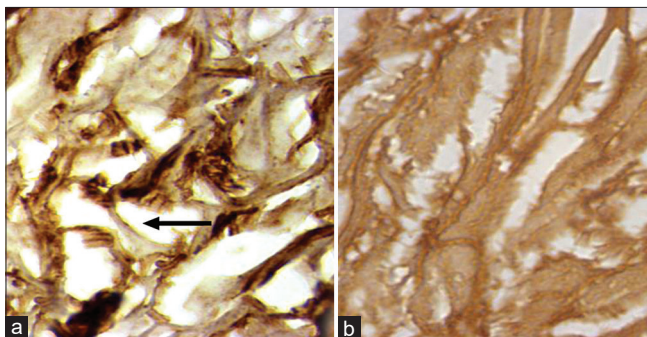


Figure 3: (IHC Magnification $\times 100$): IHC with anti-fibronectin antibody showing increased positivity (black arrow) in clustered fibers in nanophthalmic sclera (a). Less intense diffuse and uniform positive staining observed in cadaver sclera (b)

features of the cases were analyzed and compared with the age-matched cadaver controls.

All the specimens were examined by a single pathologist who was masked to the clinical parameters, and the same was reviewed by a second pathologist. As a control, two age-matched cadaver scleral tissues were dissected at the same anatomical location as that of the cases. We ruled out any abnormality in the cadaver eyes by measuring the length of the whole globe using a ruler and included only those eyes that measured more than 21 mm. Scleral tissues obtained by sclerostomy from both cases and cadaver eyes were fixed in 10% buffered formalin, and the tissues were processed and embedded in wax. Paraffin-embedded scleral tissues

were sectioned at 5 μ m on the Poly-L-Lysine coated slides with the help of Leica RM 2255 microtome. Scleral tissues from four nanophthalmic eyes and two cadaver controls were stained with routine H and E and special Alcian blue staining (pH 2.5).

IHC was done in two cases with anti-fibronectin antibody. Fibronectin (568): Sc-52331 mouse monoclonal antibody was used in this study. The IHC was carried out using commercially available kits (BioGenex, USA). The paraffin sections (5 μ m) of the scleral tissues were immunostained for analyzing the fibronectin expression, as described by Ashwinbalaji *et al.*^[9] Sections of normal human scleral tissue from donor eyes immunostained for fibronectin were used as positive controls while slides immunostained with no primary antibody were used as negative controls. Images were acquired using laser scanning microscope (Leica SP8 confocal microscope, Germany), as described by Arpitha *et al.*^[10]

Results

We report the results of four consecutive cases of nanophthalmos with visually significant cataract who underwent phacoemulsification with concomitant prophylactic posterior sclerostomy. Preoperative ocular biometry details were as shown in [Table 1]. Mean axial length was 17.60 ± 1.40 mm, and mean age was 57.25 ± 8.77 years. Patients 1 and 2 had very low axial length (<17 mm) and high RCS thickness, thereby requiring high IOL power. Mean RCS was 2.14 ± 0.16 mm and mean IOL power was 43.75 ± 7.76 . Histopathological interpretation of all the excised scleral tissue was compared with age-matched cadaver controls using basic light microscopy

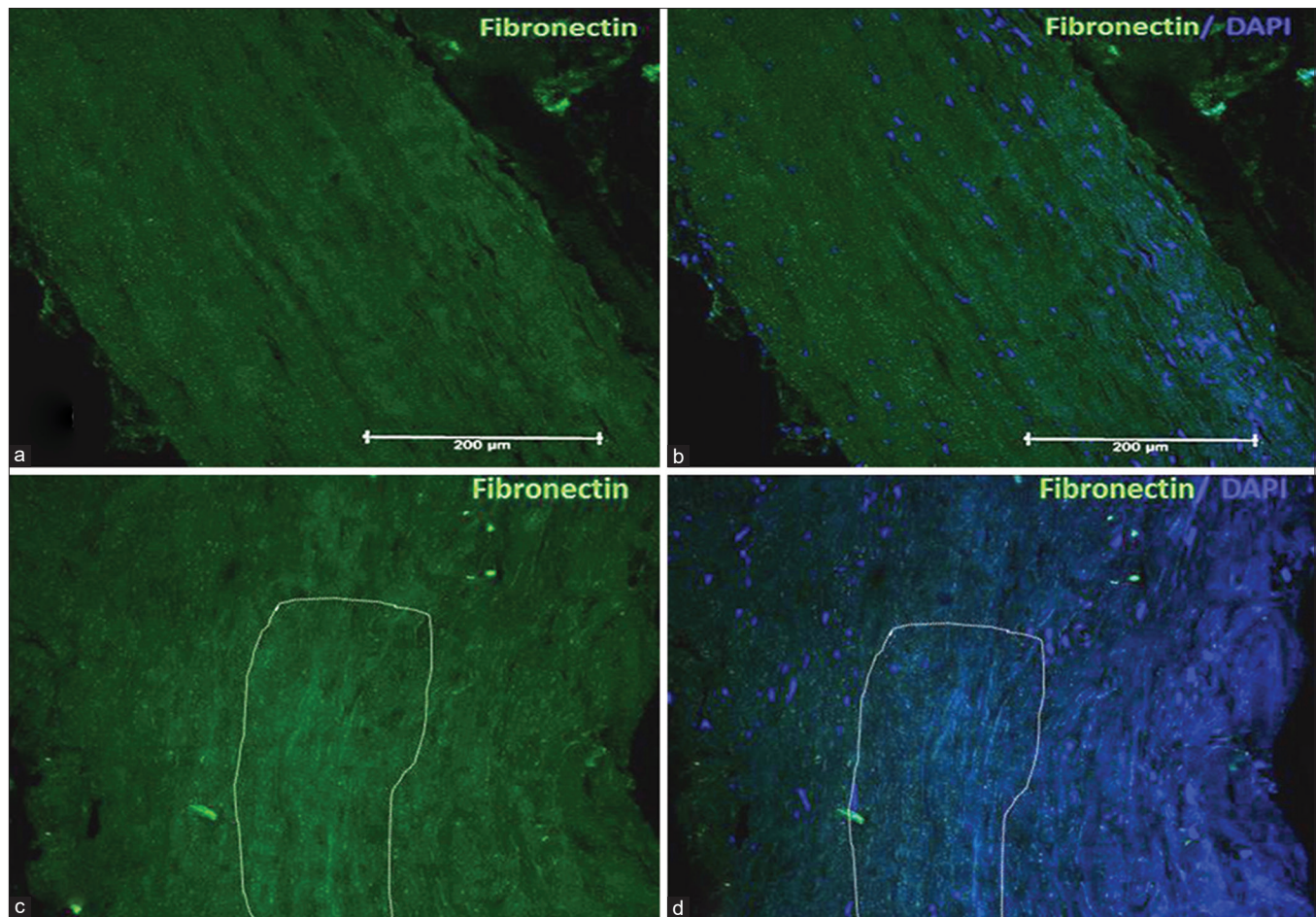


Figure 4: Representative confocal microscopic images of the fibronectin (green) immunostained scleral sections from a normal donor tissue (a and b) and a patient with nanophthalmos (c and d). The sections were counterstained with nuclear stain DAPI (blue). Highlighted region in c and d indicates the central scleral stroma with high fibronectin expression but with minimal nuclei

and confirmed by a second pathologist who was masked to the study. The H&E-stained slide of nanophthalmic sclera showed disorderly arranged collagen. Scleral fibers showed thickening with irregular packing and randomly interspersed proliferated fibroblasts in patient sclera [Fig. 1a] and regularly arranged fibers in cadaver controls [Fig. 1b]. However, no appreciable differentiation was observed between the case and control on histochemical staining for glycosaminoglycan with Alcian blue stain.

In order to study if a shorter axial length had an impact on the histopathological changes in nanophthalmic eyes, we examined the scleral tissue of a patient with axial length less than 16 mm and compared it with another patient with an axial length more than 16 mm, and documented the difference in histological characteristics between them. Severe disorganization with diffuse collagenization, loss of layering and fibrillary architecture with reduced sclerocytes was seen in sclera with axial length <17 mm [Figs. 2a and b], however the tissue with axial length >17 mm showed increased collagenization with preservation of layering and architecture [Fig. 2c].

Immunohistochemical analysis with anti-fibronectin antibody showed strong positivity, highlighting the irregularly clustered and closely packed fibers of varying size in nanophthalmic eyes [Fig. 3a] whereas less intense uniform

granular staining of sweeping fibers was found in cadaver controls [Fig. 3b].

Analysis of the immunostained sections of the normal human scleral tissue from donor eyes revealed the uniform and parallel distribution of the stromal fibroblasts (DAPI stain in Fig. 4b). In contrast, the arrangement of the stromal fibroblast nuclei was significantly disorganized in the scleral tissue of nanophthalmic subjects (DAPI in Fig. 4d). In addition, the immunofluorescence analysis revealed a higher expression of fibronectin in the scleral tissue of the patients with nanophthalmos, particularly in the central stromal region (highlighted in Fig. 4c); but the stromal nuclei were much reduced compared to the normal sclera [Fig. 4a and b].

Discussion

Nanophthalmos is a rare, potentially devastating ophthalmic entity that poses significant clinical challenges due to high risk of secondary angle closure glaucoma, spontaneous choroidal effusions, and perioperative complications with cataract and retinal surgeries.^[11–15] Thickened sclera and blockage of outflow from the vortex venous system is the reason for potential complications in these eyes. Performing a prophylactic sclerostomy along with cataract surgery had significantly reduced complications in short eyes.^[5] Various

reports have identified the contribution of extracellular matrix to scleral biomechanical properties, leading to changes in scleral shape, ocular size, and therefore the refractive state of the eye.^[6-8,16]

Owing to its rarity, there are only a few case reports published on the histopathological, and immunohistochemistry features of nanophthalmos and their clinical implication. Earlier studies on the excised scleral specimens of nanophthalmic eyes have shown distinct histological features using standard electron microscopy. Yamani *et al.*^[12] showed fraying and splitting of collagen fibers in all three scleral layers, with the degree of abnormality increasing inwards. The frayed fibrils were suggested to have contributed to the scleral inelasticity which caused sequestration of extracellular fluid, and consequently may lead to choroidal congestion, choroidal detachment and/or exudative retinal detachment.

We report similar histological features with severe disorganization and diffuse collagenization, loss of layering and fibrillary architecture with reduced sclerocytes in a nanophthalmic eye with very low axial length <17 mm compared to an eye with >17 mm using low-cost light microscopy. On comparing the histological features of nanophthalmic scleral tissue and cadaver controls with H and E staining, we observed characteristic alterations in the scleral fibers of nanophthalmic eyes with thickening, irregular packing and randomly interspersed proliferated fibroblasts compared to regularly arranged fibers in cadaver controls.^[6,7] Our findings on light microscopy were consistent with previous reports showing characteristic features of disordered arrangement of scleral fibers in nanophthalmic eyes using Electron microscopy (EM). However, on using special stains like Alcian blue, we could not observe any positivity for glycosaminoglycans in both cases and controls. In contrast to our findings, Trelstad *et al.*^[6] reported patches of Alcian blue-positive material throughout the sclera in nanophthalmic eyes. This variation may be related to differences in experimental conditions or may simply reflect intrinsic differences in the specimens.

Based on previous reports of Yue *et al.*,^[7] it was observed that nanophthalmic sclera contained more fibronectin (a glycoprotein) than normal sclera, and that nanophthalmic scleral cells secreted more fibronectin in tissue culture than did normal scleral cells. Likewise a study by Fukuchi *et al.*^[17] had revealed that scleral proteoglycans were qualitatively identical in nanophthalmic cases and controls but differed quantitatively. In our study, the immunohistochemistry analysis showed strong positivity for fibronectin in cases compared to uniform staining in cadaver controls, thus revealing the intense make up of scleral tissue with fibronectin in nanophthalmos. Additionally, immunofluorescence analysis showed disordered arrangement of stromal nuclei in fibroblasts of nanophthalmic eyes, which further highlighted the changes seen under light microscopy.

In our series, we incidentally observed one case with a shorter axial length (<17 mm) showing severe disorganization and reduced sclerocytes compared to another case of axial length >17 mm. However, we did not look into the histoclinical correlation with other clinical parameters like IOP, anterior chamber depth, and RCS.

Conventionally, EM is considered as a standard tool for histopathological diagnosis as it magnifies the image 250 times that of light microscopy, and also has a better resolution. However, it is technically challenging with specimen preparation taking several days, and is also more expensive with very high running costs. Hence these prohibitive features may make light microscopy an easier and more accessible option in developing countries.

Conclusion

Our case series demonstrated the characteristic histological and immunohistochemical features in nanophthalmic eyes using only a basic light microscope. We believe the low-cost, simple light microscope could possibly obviate the need for more sophisticated TEM in developing countries. We also identified a histoclinical correlation based on axial length in these eyes that could possibly relate to disease severity. However, in order to use this information into clinical practice, we need to undertake a larger sample size and also consider other clinical parameters to study histoclinical correlation for deciding the management protocols in short eyes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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