

Ocular surface evaluation in eyes with chronic glaucoma on long term topical antiglaucoma therapy

Manu Saini, Murugesan Vanathi, Tanuj Dada, Tushar Agarwal, Rebika Dhiman, Sudarshan Khokhar

Cornea & Ocular Surface Services, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi 110029, India

Correspondence to: Murugesan Vanathi. Cornea & Ocular Surface, Cataract & Refractive Services, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi 110029, India. vanathi_g@yahoo.com

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Abstract

• **AIM:** To evaluate ocular surface changes and its correlation with the central corneal subbasal nerve fibre layer in chronic glaucoma patients.

• **METHODS:** A prospective comparative study of ocular surface evaluation was performed in 50 eyes of 25 patients using two or more antiglaucoma medications for at least 6mo and 50 eyes of 25 normal subjects without any ocular problems as controls. The study parameters evaluated included visual acuity, intraocular pressure, ocular surface evaluation parameters [fluorescein break-up time (FTBUT), Schirmer's I test, ocular surface staining scores and ocular surface disease index score (OSDI)], central corneal sensation (Cochet Bonnett aesthesiometer), central subbasal nerve fiber layer density (SBNFLD) by confocal microscopy.

• **RESULTS:** The mean values in the glaucoma cases and control groups respectively were as follows: OSDI score ($35.89 \pm 16.07 / 6.02 \pm 3.84$; $P=0.001$), Schirmer's I test score (7.63 ± 2.64 mm/ 12.86 ± 1.93 mm; $P=0.001$), FTBUT (9.44 ± 2.76 s/ 11.8 ± 1.88 s; $P=0.001$), corneal ($5.7 \pm 2.33 / 1.1 \pm 0.58$; $P=0.001$) and conjunctival staining score ($5.06 \pm 1.94 / 0.84 \pm 0.46$; $P=0.001$), corneal sensitivity ($4.68 \pm 0.44 / 5.07 \pm 0.37$; $P=0.076$), mean subbasal nerve fiber number ($3.58 \pm 0.99 / 5.40 \pm 1.70$; $P=0.001$), SBNFL length (1101.44 ± 287.56 μ m/ 1963.70 ± 562.56 μ m; $P=0.001$) and density (6883.94 ± 1798.03 μ m/ 12273.15 ± 3516.04 μ m/ mm^2 ; $P=0.001$). Dry eye severity of level 2 and 3 was seen in 66% of glaucoma group. Corneal ($R^2=0.86$) and conjunctival staining ($R^2=0.71$) and OSDI score ($R^2=0.67$) showed statistically significant negative correlation with central corneal SBNFLD while FTBUT ($R^2=0.84$), corneal sensitivity ($R^2=0.52$) showed positive correlation to central corneal SBNFLD in the long term topical antiglaucoma medication group.

• **CONCLUSION:** Ocular surface changes and antiglaucoma therapy induced dry eye is found to be associated with decreased SBNFLD in eyes on long term topical antiglaucoma medications.

• **KEYWORDS:** confocal microscopy; glaucoma; ocular surface disease; subbasal nerve fiber layer; therapy

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INTRODUCTION

Ocular surface side effects occur due to chronic, long term use of antiglaucoma medications. Instillation of topical antiglaucoma drops for a period of three or more months has been found to cause significant subclinical inflammation, which has been detected as increased expression of HLA-DR on conjunctival epithelial cells^[1]. Pro-inflammatory cytokine secretion by conjunctival cells has been noted to occur as a result of instillation of antiglaucoma eye drops^[2-4].

Topical medication related ocular surface disease (OSD) results in worse symptoms, poorer compliance to treatment, poor surgical results, and decreases the quality of life in glaucoma patients^[5-7]. The major side effects include local allergic reactions, chronic conjunctival inflammation, tear film abnormalities, corneal epitheliopathy, punctate epitheliopathy, medically resistant herpetic keratitis, disruption of epithelial function, chronic inflammatory infiltration, expression of inflammatory markers, impaired wound healing, squamous metaplasia^[8-9].

The most commonly used antiglaucoma medications like timolol and latanoprost when used for a long term lead to chronic ocular surface disease. Noted ocular adverse effects of timolol-included corneal punctate erosions, burning sensation, hyperemia, tear film alterations and corneal anesthesia. Topical latanoprost causes increased pigmentation of the iris, hypertrichosis, hyperemia, allergic contact dermatitis and cystoid macular edema^[10].

Benzalkonium chloride (BAC, quaternary ammonium compound), the most commonly used preservative in topical antiglaucoma preparations has a slow turnover and the quaternary ammonium molecules may be retained in the ocular

tissues for as long as 168h after application^[11]. BAC promotes the activation of lipooxygenases, synthesis and secretion of eicosanoids, inflammatory mediators and many cytokines such as interleukin (IL)-1a, tumor necrosis factor, IL-8, IL-10, resulting in irritation, delayed hypersensitivity and allergic reactions^[12].

Delayed and prolonged effect of BAC is because of incorporation and persistence of BAC molecules in cell membranes^[13]. This affects the lipid layer of the tear film causing its instability^[14] thereby predisposing to inflammation of the ocular surface and conjunctival metaplasia. In addition, preservatives have direct destructive effects on the mucous gland, reducing the number of goblet cells and production of the protective mucus layer^[15]. Hence, the three mechanisms of BAC toxicity include a detergent effect causing loss of tear film stability, direct damage to the corneal/conjunctival epithelium and immune allergic reaction^[13].

Corneal innervation is vital for the maintenance of corneal epithelial integrity, proliferation function and in corneal wound healing after injury^[15-16]. The subbasal nerve plexus along with stromal keratocytes secrete a number of neuropeptides. These diffusible factors are believed to stimulate the epithelial growth, proliferation, differentiation, the production of collagen type VII, DNA synthesis, neurite survival and keratocyte proliferation^[17-19]. Alterations in corneal innervations impairs the wound healing ability of the epithelium and results in dry eye^[18-20].

Nerve degeneration that occurs in the scenario of chronic ocular surface inflammation, as in cases of dry eye, has been described to alter the subbasal nerve fiber layer (SBNFL) morphology^[21]. The role of *in vivo* confocal microscopy in ocular surface analysis of dry eye and glaucomatous patients has been elucidated^[20,22-27]. The rationale for the current study was to evaluate the correlation between the ocular surface changes and central corneal subbasal nerve fibre layer changes in cases of chronic glaucoma on long term medical control.

SUBJECTS AND METHODS

A prospective comparative open label study of ocular surface evaluation in 50 eyes of 25 patients using two or more antiglaucoma medications for at least 6mo and 50 eyes of 25 normal subjects without any ocular problems as controls was done. Patients on follow-up with the glaucoma clinic (during the period of November 2011 to November 2013) with chronic glaucoma on combination therapy with two or more topical antiglaucoma medications with preservatives (timolol 0.5%, brimonidine 0.1%, latanoprost 0.005%) for at least six months or more and consenting to participate in the study were included in the study. Patients with history of intraocular surgery, laser treatment in recent six months, contact lens use, autoimmune disease, recent ocular inflammation/injection,

eyes with trichomatous changes, dry eye related to other causes, previous or current use of other ocular medications such as artificial tear therapy were excluded from the study. Informed consent from all the enrolled patients was taken and institute Ethics Committee approval was sought and obtained. Demographic characteristics including age, gender, duration of therapy, and study parameters data were noted on a predesigned proforma. Comprehensive ocular examination, aided Snellen's visual acuity, intraocular pressure (Goldman applanation tonometry), ocular surface evaluation tests^[28] [tear break-up time (TBUT), Schirmer's I test, ocular surface staining score^[29], ocular surface disease index (OSDI), central corneal sensation and *in vivo* scanning slit confocal microscopy of the central cornea], dry eye severity (DEWS classification^[30]) and OSDI^[31] were done. Corneal sensation threshold measurement was done using Cochet Bonnet Anesthesiometer (CBA, Luneau, Paris, France)^[32] and measurement in both cases and control groups was taken in morning hours, between temperature 20 °C and 25 °C on the basis of out door patient services to avoid temperature variation and diurnal bias. We didn't asses any association in diabetic patients with loss of corneal sensitivity, as our cases and control groups did not have retinopathy and neuropathy clinical features. None of the enrolled subjects in our study had neurodegenerative diseases, ruled out after complete systemic evaluation

In vivo slit scanning confocal microscopy (ConfoScan 4, NIDEK Technologies, Padova, Italy) of the central cornea was done in automatic gain mode using a standard setting of 4 passes, with a scanning range of 200 µm to image the anterior layers of the cornea *i.e.* epithelium, SBNFL, stromal keratocytes at 40× magnifications^[33]. If satisfactory images were not obtained, procedure was repeated to get the desired images.

Each eye was scanned three times through its entire depth and the two best images were selected for analysis, of which the best one containing maximum number of SBNFL nerves imaged was selected for analysis. SBNFL image analysis was done in a masked manner using free downloadable custom NIH Image J software (<http://www.imagescience.org/meijering/software/neuronj/>). The tracing of subbasal nerves were performed using Neuron J, a semi-automatic Image J plugin to facilitate the tracing and quantification of elongated image structures. Then the total nerve number, total length/frame of subbasal nerves was measured automatically (Figures 1 and 2).

Nerve branches longer than 50 µm in length were counted as separated nerves. The total number of subbasal nerves was recorded. The mean subbasal nerve fiber layer density (SBNFLD) was calculated as total length of all main nerves and their branches divided by area of standard frame size

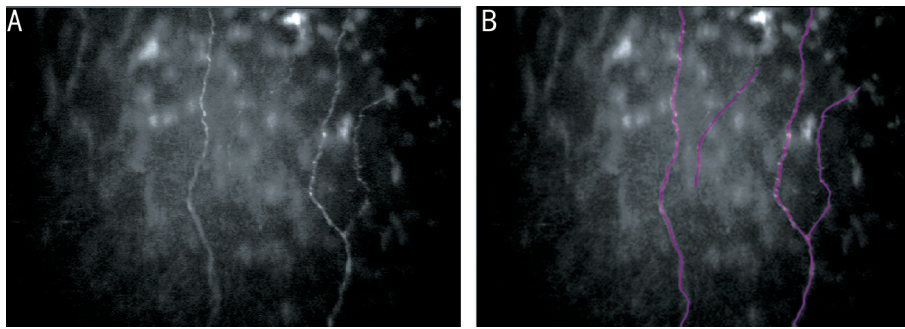


Figure 1 *In vivo* slit scanning confocal microscopy imaging of central cornea of eye on long term antiglaucoma therapy A: Subbasal nerve fiber layer; B: Tracing of the same using Neuron J (pink). Total nerve number, length/frame of subbasal nerves were measured automatically by Neuron J.

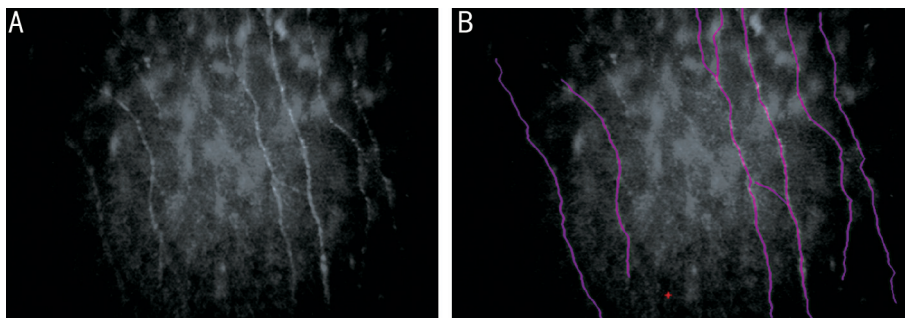


Figure 2 *In vivo* slit scanning confocal microscopy imaging of central cornea of control eye A: Subbasal nerve fiber layer; B: Tracing of the same using Neuron J (Pink). Neuron J, a semi-automatic Image J plugin facilitates the quantification of these elongated nerve structures.

Table 1 Demographic characteristics of eyes on antiglaucoma therapy and controls

Demographic data	Antiglaucoma therapy group	Controls	P
No. of eyes	50	50	-
Gender (M/F)	29/21	38/12	0.056
Mean age (mean±SD, range)	49.42±16.98 (22-75)a	40.68±13.73 (26-65)a	0.031
Treatment duration (mean±SD, range)	3.61±2.88 (0.6-12)a	-	-

containing images (460 $\mu\text{m} \times 345 \mu\text{m}$, area=0.16 mm^2)^[34]. Analysis of SNFL using custom software Neuron J was repeated on the same images at one week interval to measure the repeatability of the central corneal subbasal nerve layer parameters by the same observer (Saini M) to calculate the intraclass correlation coefficient (ICC).

The basal cells in the confocal images were identified in the scans manually and the density was calculated using the inbuilt software (all cells that intersected the edges of the frame of the image were not included in manual counting to avoid biasing the results with poorly illuminated or poorly defined cells)^[35].

Statistical Analysis Statistical analysis was done using the program SPSS version 15. Quantitative variables (expressed as mean, standard deviation, range) were compared between antiglaucoma medication group and control groups using two sample *t*-test with *P* value <0.05 considered statistically significant. Correlation between ocular surface evaluation parameters and SBNFLD was assessed using Pearson correlation coefficient. ICC was calculated to estimate repeatability of measurements between two occasions by the

same observer at one week interval (ICC for reproducibility was defined as: ≤ 0.4 , poor; 0.4 to 0.75, fair to good; ≥ 0.75 , excellent^[36]). Bland Altman plot summarized the agreement between the 2 data sets.

RESULTS

The demographic characteristics of enrolled eyes are shown in Table 1.

Of the 50 eyes on long term topical antiglaucoma therapy enrolled, 10 eyes were on combination of BAC preservative containing timolol 0.5% (one drop twice daily) and brimonidine 0.2% (one drop twice daily), 8 eyes on latanoprost 0.005% and brimonidine 0.2% (one drop twice daily) and 32 eyes on combination of timolol 0.5% (one drop twice daily) and latanoprost 0.005%. The 26 eyes had primary open angle glaucoma (POAG), 7 eyes had primary angle closure glaucoma (PACG), 6 eyes had juvenile open angle glaucoma (JOAG), 4 eyes had ocular hypertension, 4 eyes had normal tension glaucoma (NTG) and 3 eyes had mixed mechanism glaucoma. The control group comprised of 50 normal eyes of 25 subjects (age and sex matched) with normal eyes.

Table 2 Depicting results of ocular surface evaluation tests analysed in long term antiglaucoma medication group vs control group

Ocular surface evaluation tests	Antiglaucoma eyes	Control eyes	P
Mean OSDI (score)	35.89±16.07 (5.54-67)	6.02±3.84 (2.21-9.85)	0.001
Schirmer's I test (mm/5min)	7.63±2.64 (3-12)	12.86±1.93 (10-16)	0.001
FTBUT (s)	9.44±2.76 (4.3-15.72)	11.80±1.88 (9-15.26)	0.001
Corneal staining score	5.7±2.33 (2-9)	1.1±0.58 (0-2)	0.001
Conjunctival staining score	5.06±1.94 (2-9)	0.84±0.46 (0-2)	0.001

Table 3 Mean values of central corneal SBNFL parameters in eyes on long term antiglaucoma therapy and controls

SBNFLD	Antiglaucoma eyes	Control eyes	P
Nerve number	3.58±0.99 (2-6)	5.40±1.70 (3-10)	0.001
Nerve length	1101.44±287.64 (599.09-1990)	1963.70±562.56 (739.36-2697.74)	0.001
Nerve density	6883.94±1798.03 (3700-8757.5)	12 273.15±3516.04 (4621-19 351.96)	0.001
Corneal sensitivity	4.68±0.44 (4-5.5)	5.07±0.37 (4-5.5)	0.076

The mean values of the ocular surface evaluation tests (OSDI score, Schirmer's I test, fluorescein breakup time, conjunctival and corneal staining score) showed statistical significant difference between the antiglaucoma group and controls (Table 2). Dry eye disease (as per DEWS classification^[29]) of level 1 severity in 34% (n=17 eyes); levels 2 and 3 severity in 66% (n=33 eyes) was in the antiglaucoma therapy group. Level 1 severity 42% (n=21) was seen in control eyes.

Density of basal epithelial cells was found to be increased in antiglaucoma therapy group 4796.619±647.1526 cells/mm² compared to that of the control group 3926.819±571.8765 cells/mm² (P=0.0004). SBNFL number, length and density showed significant decrease in chronic glaucoma eyes compared to that of the controls (Table 3). Central corneal sensation threshold in antiglaucoma eyes was found to be decreased as compared to the controls (P=0.076). The OSDI score and ocular surface staining scores showed a statistically significant negative correlation with central corneal SBNFL in the antiglaucoma medication eyes (Figures 3-5). TBUT and central corneal SBNFLD showed a strong, statistically significant positive correlation (Figure 6), central corneal sensitivity also showed a good positive correlation with SBNFLD (Figure 7). Schirmer's I values did not show a significant correlation (Figure 8).

Intraobserver repeatability for *in vivo* confocal microscopy analysis of the SBNFL (nerve number, nerve length, nerve density) and ICC values given in Table 4 was calculated for both groups to measure the reproducibility of SBNFL measurements.

P value was found to be insignificant for subbasal nerve fiber length and density indicating good repeatability of measurements at two separate occasions.

The mean values were plotted against the differences between the measurements and the upper and lower limits

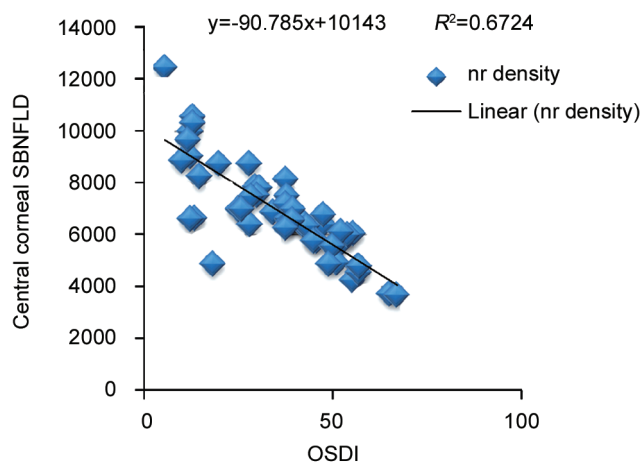


Figure 3 Correlation of OSDI scores with central corneal subbasal nerve (nr) fiber density in eyes on longterm antiglaucoma therapy.

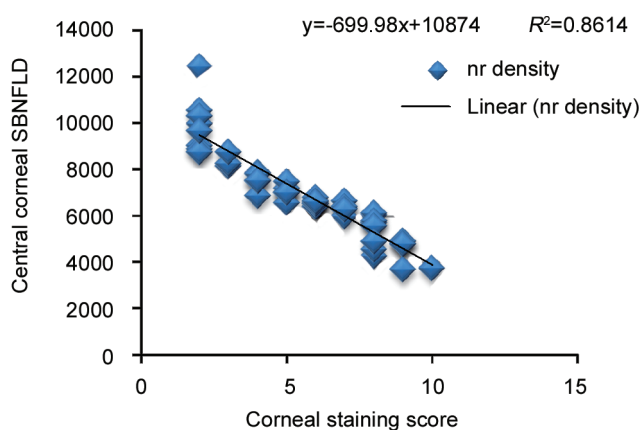


Figure 4 Correlation of corneal staining score with central corneal subbasal nerve (nr) fiber density in eyes on longterm antiglaucoma therapy.

of agreements (limits of agreement 1.96±SD) were obtained by Bland and Altman^[37] to appreciate the between occasion agreement as depicted in Figures 9 and 10.

Table 4 Intraclass correlation coefficient (ICC) for single image analysis in antiglaucoma medication group and control group

Variables	Control group				Antiglaucoma medication group			
	Observer 1	Observer 2	ICC	P	Observer 1	Observer 2	ICC	P
Nerve number	5.40±1.70	5.32±1.54	0.98	0.15	3.58±0.99	3.88±0.76	0.63	0.02
Nerve length	1963.70±562.56	1964.01±562.26	0.99	0.43	1101.44±287.64	1099.24±281.91	0.99	0.68
Nerve density	12 273.15±3516.04	12 275.29±3514.274	0.99	0.39	6883.94±1798.03	6870.24±1761.94	0.99	0.69

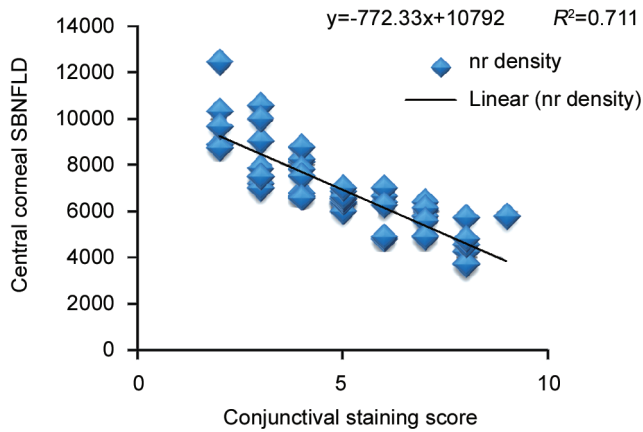


Figure 5 Correlation of conjunctival staining score with central corneal subbasal nerve (nr) fiber density in eyes on long-term antiglaucoma therapy.

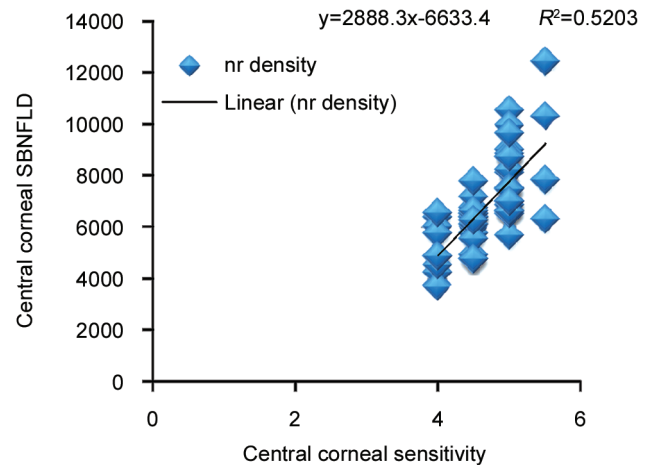


Figure 7 Correlation of central corneal sensation threshold with subbasal nerve (nr) fiber density in eyes on long-term antiglaucoma therapy.

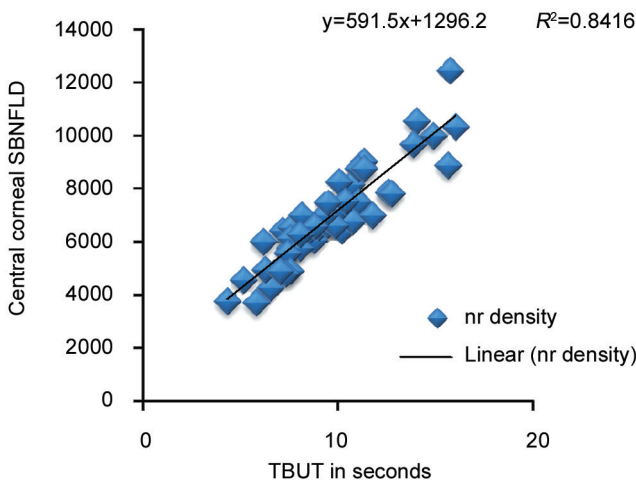


Figure 6 Correlation of TBUT with central corneal subbasal nerve (nr) fiber layer density in eyes on long-term antiglaucoma medication therapy.

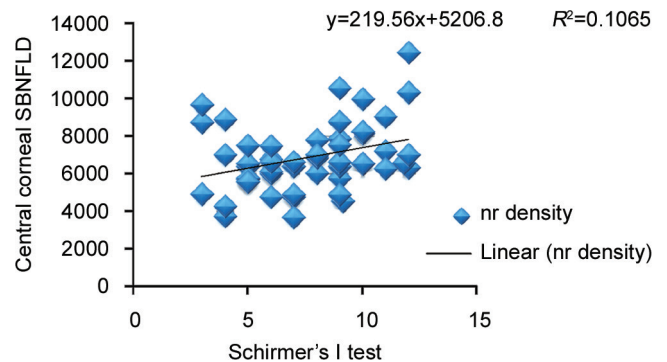


Figure 8 Correlation of Schirmer's I test with central corneal subbasal nerve (nr) fiber density in eyes on long-term antiglaucoma therapy.

DISCUSSION

Altered epithelial barrier function leads to exposure of corneal nerve ending to environment stimuli causing irritation, unstable tear film resulting in ocular surface epithelial changes, compromised visual function^[37-40]. The prevalence of ocular surface disease of 59% has been reported in glaucoma cases with higher prevalence in patients using BAC containing antiglaucoma medications^[41]. The frequency of eye symptoms and signs of ocular surface irritation are higher in patients treated with preserved than preservative-free eye drops^[42]. Our study also found a similar prevalence of dry eye in 66% (levels 2 and 3) in our cases. Patients enrolled in our study were using

combination therapy for mean duration of 0.61±2.88y (range 0.6-12y).

Corneal epithelial cells and stromal innervations influence corneal trophism and contribute to the maintenance of a healthy corneal surface. Alteration in corneal innervation will affect epithelial healing abilities and results in development of dry eye^[17]. A complex relationship seems to exist between ocular surface changes, dry eye disease and decreased SBNFL in eyes on chronic ocular hypotensive treatment. Our evaluation of ocular surface changes in eyes with chronic glaucoma on long term topical antiglaucoma medications showed statistical significant differences in ocular surface evaluation parameters. Central corneal *in vivo* confocal microscopic examination showed a statistical significant decrease in central corneal

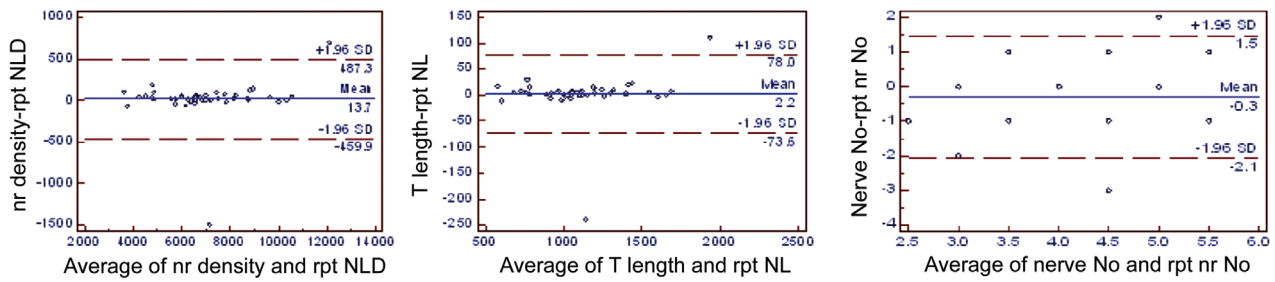


Figure 9 Bland-Altman plots for subbasal fiber layer density, length and number in eyes on antiglaucoma treatment indicating agreement between two separate measurements at different instances of the same eye, by same observer rpt: Repeat.

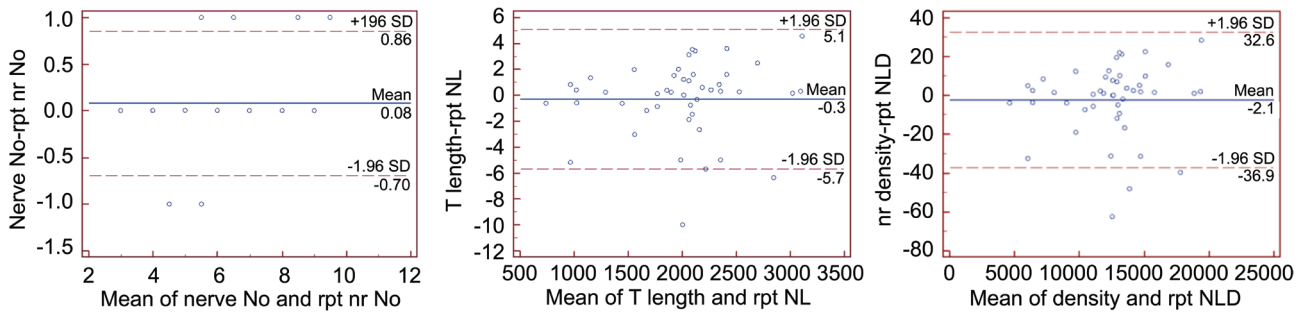


Figure 10 Bland-Altman plots for subbasal nerve fiber layer density, length, nerve number in eyes in the control group, indicating agreement between two separate measurements at different instances of the same eye, by same observer rpt: Repeat.

SBNFL nerve number, length and density and corresponding decrease in corneal sensitivity. Density of the basal epithelial cells was significantly increased in the eyes with chronic ocular hypotensive medications as compared to that in the control group. Central corneal sensation threshold was observed to be decreased in antiglaucoma medication group with corresponding decrease in SBNFLD (not statistically significant). This can probably be attributed to the inherent practical difficulty in the placement, positioning and force application of the nylon filament of the the Cochet Bonnet aesthesiometer.

The eyes on long term antiglaucoma therapy with reduced SBNFLD can probably still appreciate corneal sensations, but at higher stimulus intensity. The SBNFLD at which the threshold of corneal sensitivity become significantly decreased is not known.

Normal central cornea SBNFLD observed with NIDEK ConfoScan 4 in our study was $12\ 273.15 \pm 3516.04 \mu\text{m}/\text{mm}^2$. Patel *et al*^[43] reported a SBNFLD of $14\ 731 \pm 6056 \mu\text{m}/\text{mm}^2$. The variation in central corneal SBNFLD noted in different studies was because of difference in the methodologies adopted in computing the corneal nerve length.

Subbasal corneal nerve layer, keratocyte density and endothelial characteristics in ocular hypertensive patients with and without therapy has been studied earlier^[27], in which the medication group were found to have lower SBNFL nerve number and density. Similar results were also noted in other studies^[20,44]. Our study results also concur with their observations.

Our study also analyzed the correlation between the ocular surface changes in the antiglaucoma therapy eyes with their central corneal SBNFLD. We observed a strong negative correlation of FTBUT, OSDI score and ocular surface staining scores with decreased central corneal confocal SBNFLD, indicating that ocular surface changes due to chronic antiglaucoma therapy with preservative containing ocular hypotensives does result in a proportionate damage to the SBNFL of the cornea. As all patients of the antiglaucoma treatment group in our study were asymptomatic, further molecular level analysis can perhaps help to establish the cause effect relationship. A large sample size would have helped to establish the relation of decreased SBNFLD with the duration. In conclusion our study shows that long term antiglaucoma medication with preservatives results in significant alteration of ocular surface parameters producing ocular surface morbidity. SBNFLD is decreased in these eyes due to long term preservative containing antiglaucoma therapy with significant correlation to ocular surface staining scores, FTBUT and OSDI values. Concurrent topical lubricant and anti-inflammatory treatment may be considered in cases of chronic preservative containing antiglaucoma therapy showing ocular surface changes.

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