Original Article

Evaluation Of Peripapillary Nerve Fiber Layer Thickness And Macular Perfusion After Subscleral Trabeculectomy With And Without Mitomycin By OCT And OCT Angiography

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Abstract

Objectives: Evaluation of peripapillary nerve fiber layer thickness and macular perfusion after sub-scleral trabeculectomy with and without mitomycin by OCT and OCT angiography. **Subjects and Methods:** An interventional study conducted from May 2020 to end of December 2021 in the ophthalmology department at Beni-Suef University Hospital, on ninety-nine patients with glaucoma, participants were within three groups: (33 patients underwent sub-scleral trabeculectomy without use of antimetabolite, 33 patients underwent sub-scleral trabeculectomy with use of sub-scleral Mitomycin 0.02% and 33 patients underwent sub-scleral trabeculectomy with use of subconjunctival Mitomycin 0.02%) to assess peripapillary nerve fiber layer thickness and macular perfusion after sub-scleral trabeculectomy with and without mitomycin by OCT and OCT angiography. **Results:** The three techniques were effective in lowering IOP significantly, compared with preoperative levels, there was a statistically significant difference between the trabeculectomy alone and trabeculectomy + MMC groups, regarding the degree of decrease in IOP that was significantly lowest among trabeculectomy alone group (p-value =0.031, 0.042), there was non-statistically significant difference between group (B) and group (C) (sub-scleral or sub-conjunctival MMC), with regard to the mean decrease in IOP. Superior and Inferior RNFL were slightly decreased in all studied groups in post- as compared with pre-operative assessment; however, this reduction was of non-statistically significant difference. The superficial and deep macular vessel densities were significantly increased in some (not all sectors), in pre- and post-assessment in the three studied groups, however the improvement in VD (%) was more among groups (B) and (C) as compared with group (A), (p-values <0.05). **Conclusions:** Measurable changes occur in Vessel Density (%) but not in RNFL thickness after trabeculectomy as conducted by the three techniques (trabeculectomy, trabeculectomy + sub-scleral MMC and trabeculectomy+ sub-conjunctival MMC).

Keywords: Glaucoma, trabeculectomy, mitomycin MMC, OCT, OCT-A.

INTRODUCTION

Glaucoma is a group of eye conditions that damage the optic nerve, which is vital for good vision. This damage is often caused by an abnormally high pressure in the eye. If the damage worsens, glaucoma can cause total blindness within a few years **(McMonnies et al, 2017).**

The risk factors for getting glaucoma include age, race, sex, heridity, family history , systemic (Diabetes, Obesity, Hypertension, Hypotension, arterioscerosis and s moking) and socioeconomic factors as well as local factors (myopia, corneal thickness and scleral regidity) all will channel into the resultant level of IOP and disc damage **(2).**

There are many surgical techniques to reduce IOP. Trabeculectomy is the most common filtration procedure in the surgical treatment of glaucoma. This filtering technique provides good results in IOP reduction by creating an alternative drainage route for aqueous humor from the anterior chamber to the subconjunctival and/or Tenon capsule, namely the filtering bleb **(3).**

Despite improvements in surgical treatment of glaucoma, the complex pathophysiological mechanisms involved in conjunctival and scleral wound healing have been one of the most important factors limiting surgical success. So, anti-proliferative agents have been used in conjunction with trabeculectomy to inhibit subconjunctival scarring by decreasing fibroblastic activity and modulating wound healing at the bleb site **(4).**

Optical coherence tomography angiography (OCT-A) is a revolutionary complement to the OCT to visualize moving elements of blood vessels without injection of a dye. OCT-A is a non-invasive technique that can easily be repeated over time to follow disease progression and monitor treatment efficacy **(Kashani et al, 2017).**

AIM OF THE WORK

was to evaluation of peripapillary nerve fiber layer thickness by OCT ONH and macular perfusion after subscleral trabeculectomy with and without mitomycin by OCT angiography

PATIENTS AND METHODS

An interventional study conducted from May 2020 to end of December 2021 in the ophthalmology department at Beni-Suef University Hospital, on ninety-nine patients with glaucoma. Participants were divided into three groups to assess peripapillary nerve fiber layer thickness and macular perfusion after sub-scleral trabeculectomy with and without mitomycin by OCT and OCT angiography. Group A (thirty-three patients) underwent sub-scleral trabeculectomy without use of antimetabolite. Group B (thirty-three patients) underwent sub-scleral trabeculectomy with use of subscleral Mitomycin 0.02%. Group C (thirty-three patients) underwent sub-scleral trabeculectomy with use of subconjunctival Mitomycin 0.02%.

Inclusion criteria:

Patients with glaucomatous optic neuropathy, defined as the presence of at least two of the following characteristics (**6**): Vertical cup-to-disc ratio (VCDR)> 0.5 (based on OCT measurements) with corresponding reduced RNFL thickness and ganglion cell complex (GCC) defects on OCT analysis consistent with glaucoma, VCDR asymmetry C/D > 0.2 and IOP > 21 mmHg on examination, with or without antiglaucomatous eye drop.

Exclusion criteria:

Significant media opacity preventing high-quality imaging, age <18 years, refractive error > ± 6 D sphere and ± 2 D cylinder, previous intraocular surgery except for uncomplicated cataract extraction with IOL implantation in the bag, any ocular disease other than glaucoma or cataract, arterial hypertension, hypotension, diabetes or any other vascular diseases such as status post-heart failure, apoplexy or thrombosis, systemic drugs with an effect on vascular diameter either dilation or construction and any candidate with corneal melting, scleromalacia or any other contraindication with the use of Mitomycin C.

METHODS:

Initial assessment: All patients underwent full ophthalmological examination including uncorrected visual acuity (UCVA) using Snellen chart, best corrected visual acuity (BCVA) using Snellen chart, gonioscopy with Goldman three mirror lens, fundus examination with Volk +90D lens, slit lamp examination and Goldmann applanation tonometry and we did OCT angio measuring superficial capillary plexus and deep capillary plexus vessel density by (RTVUE XR, Optovue, USA) on the macula (6*6 mm) and OCT ONH measuring peripapillary nerve fiber layer thickness.

Surgical treatment of glaucoma as follows: Local or general anesthesia. A traction suture (placed partial thickness through the peripheral clear cornea) was applied for adequate exposure of the surgical site (superior quadrants of the eye). A limbal based conjunctival peritomy was created to expose the superior bare sclera. Gentle cautery was used to achieve hemostasis. A triangular partial thickness scleral flap was created in the superior sclera, hinged at the limbus. The scleral flap was dissected forward until the bluish gray zone at the limbus was exposed. Dissection of the subconjunctival space to provide a pathway for aqueous flow was then performed. A paracentesis was created in the peripheral clear cornea prior to creating an ostomy into the anterior chamber from underneath the scleral flap. Viscoelastic material was injected to stabilize the intraocular pressure as the ostomy is created to avoid anterior chamber shallowing. A peripheral iridectomy was created at this point. Interrupted sutures (10-0 nylon) were then used to close the scleral flap. The conjunctival peritomy was then closed using absorbable sutures.

In group A sub-scleral trabeculectomy was done as described without use of antimetabolite.
In group B sub-scleral trabeculectomy was done and Mitomycin 0.02% was applied subscleral. In group C sub-scleral trabeculectomy was done and Mitomycin 0.02% was applied subconjunctival.

Statistical methods:

The collected data were coded then entered and analyzed using the SPSS version 25 (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) for Microsoft Windows 10. **The following tests were used:**

Descriptive analysis of the results in the form of percentage distribution for qualitative data and (minimum, maximum, mean and standard deviation) calculation for quantitative data. **Cross tabulation and Chi Square test** ($\chi 2$)**:** For comparison between categorical variables and percentage values, exact test was used instead when the expected frequency is less than five. **Paired sample t-test:** to compare the means of two measurements taken from the same individual, (pre- and post-operative assessment) with a normal distribution. **One-way analysis of variance (ANOVA) test** was used to elucidate significance among group means, followed by Tukey's post-hoc test to compare mean values pairwise. Differences were considered significant at p-values ≤0.05. Total p-value for ANOVA was calculated and written, p-values of post hoc analysis were expressed as small letters [a,b,c]. **Pearson's correlation coefficient analysis** was done to evaluate linear relationship between studied baseline data in patient groups with the changes in IOP, OCT and OCTA parameters. Correlation graphs were drawn only for significant correlation which is considered significant at P ≤0.05. Correlation is considered positive (direct correlation) when r (correlation coefficient) had a + signal and negative (inverse correlation) in case of – signal and it is considered **(Schober et al., 2018):** 0.10–0.39 Weak correlation, 0.40–0.69 Moderate correlation, 0.70–0.89 Strong correlation, 0.90–1.00 Very strong correlation. P-values equal to or less than 0.05 were considered statistically significant. Simple graphs were used to illustrate some information.

RESULTS

The three studied groups were matched regarding their age, UCVA, BCVA and pre-operative IOP, (p-values >0.05). Participants' age was ranged from 49 to 73 years old without a statistically significant difference between the three studied groups (p-value= 0.119). Participants were 44 males and 55 females without a statistically significant difference between the three studied groups (p-value= 0.170). UCVA was ranged from 0.01 to 0.90 with an average acuity of (0.39 ±0.23) without a statistically significant difference between the three studied groups (p-value =292). BCVA was ranged from 0.10 to 0.90 with an average acuity of (0.72 ±0.18) without a statistically significant difference between the three studied groups (p-value =221). Pre-operative cup-to-disc ratio CDR was ranged from 0.50 to 0.90 with an average ratio of (0.642 ±0.09) without a statistically significant difference between the three studied groups (p-value =520). Pre-operative IOP was ranged from 22 to 42 with an average pressure of (30.69 ±4.54) without a statistically significant difference between the three studied groups (p-value =209), Table (1).

Table (1): Baseline assessment of the studied population

		Group (A) N= 33	Group (B) N= 33	Group (C) N= 33	Total N= 99	p-value
Age (years)	**Mean ±SD**	63.21 ±5.38	62.64 ±5.60	60.70 ±5.91	62.18 ±5.68	0.170
	Min Max	53.00 73.00	53.00 71.00	49.00 71.00	49.00 73.00	
Gender, N (%)	**Male**	18 (54.5%)	10 (30.3%)	16 (48.5%)	44 (44.4%)	0.119
	Female	15 (45.5%)	23 (69.7%)	17 (51.5%)	55 (55.6%)	
UCVA	**Mean ±SD**	0.34 ±0.23	0.40 ±0.22	0.43 ±0.24	0.39 ±0.23	0.292
	Min Max	0.01 0.90	0.10 0.90	0.01 0.90	0.01 0.90	
BCVA	**Mean ±SD**	0.70 ±0.22	0.76 ±0.12	0.69 ±0.18	0.72 ±0.18	0.221
	Min Max	0.10 0.90	0.50 0.90	0.20 0.90	0.10 0.90	
CDR	**Mean ±SD**	0.654 ±0.01	0.627 ±0.09	0.645 ±0.09	0.642 ±0.09	0.520
	Min Max	0.50 0.90	0.50 0.90	0.50 0.90	0.50 0.90	
IOP (mmHg)	**Mean ±SD**	30.3 ±4.6	31.8 ±4.1	30.0 ±4.8	30.3 ±4.6	0.209
	Min Max	25.00 42.00	22.00 39.00	22.00 39.00	22.00 42.00	

UCVA: uncorrected visual acuity, BCVA: best corrected visual acuity, CDR: cup-to-disc ratio, IOP: intra-ocular pressure,

There was non-statistically significant difference in pre-operative assessment of IOP between the three studied groups (p>0.05), also in post-operative assessment (p>0.05). The reduction in IOP was significantly lowest among group (A) as compared with group (B) and group (C) (p-value =0.031, 0.042), there was non-statistically significant difference between group (B) and group (C), regarding change in IOP (p-value >0.05), Table (3).

Table (2): Comparison between the three studied groups regarding the pre-operative, post-operative and change in IOP:

		Group (A) N= 33	Group (B) N= 33	Group (C) N= 33	p-value
IOP	**Pre-**	30.3 ±4.6	31.8 ±4.1	30.0 ±4.8	0.209
	Post-	16.8 ±5.3	15.4 ±3.0	15.6 ±4.8	0.413
	Change	-13.52 ±5.6[b,c]	-16.42 ± 5.1[a]	-15.37 ±5.5[a]	**0.085**

*p-value ≤0.05 is considered statistically significant

Each value represents a mean of 33 values ± SD.
Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.

- [a] Significantly different from Group (A) at p≤ 0.05.
- [b] Significantly different from Group (B) at p≤ 0.05.
- [c] Significantly different from Group (C) at p≤ 0.05.

Superior and Inferior RNFL were slightly decreased in all studied groups (A), (B) and (C) in post- as compared with pre-operative assessment, however this reduction was of non-statistically significant difference (p-values >0.05), Table (3).

Table (3) Comparison of RNFL pre- and post- operative within the three studied groups:

		Group (A)	p-value	Group (B) N= 33	p-value	Group (C) N= 33	p-value
RNFL Superior	**Pre-**	69.73 ±15.7	0.823	67.24 ±14.7	0.083	67.45 ±14.7	0.090
	Post-	69.76 ±15.6		66.98 ±15.2		67.23 ±14.7	
RNFL inferior	**Pre-**	66.91 ±14.9	0.070	64.27 ±13.4	0.129	68.12 ±15.0	0.060
	Post-	66.55 ±14.8		64.10 ±13.2		67.98 ±14.9	

*p-value ≤0.05 is considered statistically significant

Each value represents a mean of 33 values ± SD.

Statistical analysis was carried out using Paired-Sample-t-test.

Regarding the macular vessel density, whole macula VD (%) was significantly increase in group (B), (38.21 ±7.7 vs. 38.79 ±8.1, p=0.022) in pre- and post-operative assessment respectively, while in group (A) and group (C), there was non-statistically significant changes in pre- and post- operative assessment of whole macula. Inferior Hemi macula VD (%) was significantly increase in group (C), (36.43 ±6.8 vs. 37.21 ±6.3, p=0.041) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of inferior hemi macula. Foveal VD (%) was significantly increase in group (C), (7.76 ±4.0 vs. 8.30 ±4.5, p=0.050) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of fovea VD (%). Superior Hemi Para Fovea was significantly increase in group (C), (40.80 ±10.2 vs. 41.64 ±10.4, p=0.018) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of Superior Hemi Para Fovea VD (%). Nasal para fovea was significantly increase in group (B), (41.85 ±10.8 vs. 42.70 ±10.7, p=0.001) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of Nasal para fovea VD (%). Peri fovea was significantly increase in group (C), (42.47 ±8.3 vs. 43.09 ±8.4, p=0.024) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of Nasal para fovea VD (%). Temporal peri fovea was significantly increase in group (C), (39.52 ±7.2 vs. 40.76 ±7.8, p=0.001) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of Nasal para fovea VD (%). Inferior peri fovea was significantly increase in group (C), (39.87 ±6.9 vs. 41.03 ±6.2, p=0.006) in pre- and post-operative assessment respectively, while in group (A) and group (B), there was non-statistically significant changes in pre- and post- operative assessment of Nasal para fovea VD (%). The remaining sectors showed non-statistically significant changes in pre- and post- operative assessment of VD (%) within the three studied groups, (p-values>0.05), Table (4).

Table (4): Comparison of Superficial capillary plexus (SCP) vessel density (%) pre- and post- operative among the three studied groups:

Superficial		Group (A) N= 33	p-value	Group (B) N= 33	p-value	Group (C) N= 33	p-value
Whole Macula	**Pre**	36.92 ±7.1	0.056	38.21 ±7.7	**0.022***	36.74 ±7.0	0.867
	Post	36.61 ±6.8		38.79 ±8.1		36.79 ±7.1	
Superior Hemi Macula	**Pre**	37.38 ±7.4	0.919	38.13 ±7.7	0.218	37.11 ±7.2	0.132
	Post	37.39 ±7.5		38.55±7.7		37.45 ±6.3	
Inferior Hemi Macula	**Pre-**	36.53 ±6.9	0.612	38.05 ±7.5	0.235	36.43 ±6.8	**0.041***
	Post	36.45 ±7.2		37.15 ±7.3		37.21 ±6.3	
Fovea	**Pre**	8.53 ±4.7	0.414	7.62 ±4.5	0.790	7.76 ±4.0	**0.050***
	Post	8.48 ±4.6		7.67 ±4.5		8.30 ±4.5	
Para Fovea	**Pre**	40.14 ±11.5	0.371	41.12 ±11.8	0.765	40.32 ±11.3	0.641
	Post	40.03 ±11.3		41.20 ±11.6		40.48 ±11.1	
	Pre	40.69 ±10.5	0.142	41.37 ±10.9	0.827	40.80 ±10.2	**0.018***

Superior Hemi Para Fovea	**Post**	41.44 ±11.0		41.30 ±10.2		41.64 ±10.4	
Inferior Hemi Para Fovea	**Pre**	39.73 ±12.6	0.952	40.66 ±12.7	0.130	40.00 ±12.6	0.432
	Post	39.75 ±13.3		41.10 ±12.9		40.30 ±12.0	
Superior para fovea	**Pre-**	39.44 ±12.4	0.090	40.53 ±12.8	0.113	39.12 ±12.4	0.063
	Post	39.09 ±12.8		40.76 ±13.1		39.67 ±12.2	
Nasal para fovea	**Pre-**	40.07 ±10.0	0.592	41.85 ±10.8	**0.001***	40.38 ±9.6	0.754
	Post	39.98 ±10.1		42.70 ±10.7		40.27 ±10.4	
Inferior para fovea	**Pre-**	40.04 ±13.2	0.406	40.56 ±13.0	0.147	40.22 ±12.7	0.337
	Post	40.18 ±13.1		40.88 ±13.1		40.58 ±12.0	
Peri fovea	**Pre-**	40.31 ±11.9	0.512	41.63 ±12.0	0.353	42.47 ±8.3	**0.024***
	Post	40.42 ±12.1		41.92 ±12.5		43.09 ±8.4	
Superior hemi peri Fovea	**Pre-**	39.93 ±7.2	0.056	42.02 ±8.6	0.255	40.76 ±11.7	0.479
	Post	40.26 ±7.3		42.24 ±8.6		41.00 ±11.6	
Inferior Hemi Peri Fovea	**Pre-**	39.89 ±7.3	0.114	40.12 ±8.3	0.175	39.64 ±7.0	0.258
	Post	40.21 ±7.2		40.53 ±8.4		40.00 ±7.1	
Temporal peri fovea	**Pre-**	40.09 ±7.2	0.255	40.25 ±7.7	0.340	39.52 ±7.2	**0.001***
	Post	40.33 ±6.6		40.58 ±7.0		40.76 ±7.8	
Inferior peri fovea	**Pre-**	38.12 ±6.9	0.156	38.80 ±7.9	0.356	39.87 ±6.9	**0.006***
	Post	36.91 ±7.2		38.48 ±7.6		41.03 ±6.2	

According to Spearman's rank correlation analysis in each group separately and in the total population collectively, the postoperative change in IOP showed a statistically significant positive correlation with the change in superficial capillary plexus VD (%) in some sectors as follows: The change in Superficial Superior Para-foveal macular VD (%) showed a statistically significant linear positive correlation with change in IOP; ($r= 0.397$, $p= 0.022$). The change in Superficial Peri Foveal macular VD (%) showed a statistically significant linear positive correlation with change in IOP; ($r= 0.391$, $p= 0.024$). The change in Superficial Inferior Para Foveal macular VD (%) showed a statistically significant linear positive correlation with change in IOP; ($r= 0.465$, $p= 0.006$) in group (B) patients, and ($r= 0.252$, $p= 0.012$) in all patients collectively. In all other sectors, there was non-statistically significant linear correlation between the changes in superficial capillary plexus VD (%) with IOP change. Table (5)

Table (5): Correlation between postoperative change in Superficial capillary plexus (SCP) vessel density (%) with IOP change:

SCP	**Change in IOP**			
	Group (A)	**Group (B)**	**Group (C)**	**Total**
	N= 33	**N= 33**	**N= 33**	**N= 99**
Whole Macula	-0.259	-0.114	0.005	-0.049
	0.145	0.527	0.976	0.631
Superior Hemi	-0.205	0.077	0.149	0.095
	0.254	0.669	0.409	0.351
Inferior Hemi	-0.075	-0.120	0.142	-0.022
	0.680	0.505	0.432	0.825
Fovea	0.077	0.008	0.181	0.117
	0.670	0.965	0.313	0.250
Para Fovea	-0.143	-0.210	-0.022	-0.082
	0.428	0.240	0.905	0.419
Superior Hemi Para	0.081	-0.080	0.086	0.038
	0.653	0.660	0.634	0.710
Inferior Hemi Para	0.174	-0.033	0.160	0.130
	0.332	0.854	0.375	0.200

Temporal Para	0.094	0.210	0.070	0.138
	0.603	0.242	0.697	0.173
Superior Para	**0.397**	0.128	-0.005	0.161
	0.022*	0.477	0.978	0.110
Nasal Para	-0.110	-0.066	0.145	0.037
	0.544	0.716	0.421	0.718
Inferior Para	0.163	**0.465**	0.025	**0.252**
	0.365	**0.006***	0.888	**0.012***
Peri Fovea	**0.391**	-0.273	0.343	0.167
	0.024*	0.125	0.050	0.098
Superior Hemi Peri	0.241	0.082	0.224	0.155
	0.176	0.652	0.210	0.125
Inferior Hemi Peri	0.121	0.189	-0.015	0.094
	0.501	0.292	0.933	0.353
Temporal Peri	-0.023	0.336	0.025	0.113
	0.901	0.056	0.889	0.265
Superior Peri	-0.114	0.094	0.041	0.051
	0.528	0.602	0.820	0.619
Nasal Peri	-0.061	0.296	0.192	0.177
	0.736	0.095	0.285	0.080
Inferior Peri	-0.239	-0.130	0.194	0.068
	0.181	0.471	0.279	0.502

Case presentation

Case (I), Group (I): (Sub-scleral trabeculectomy without use of antimetabolite).

A. Pre-operative OCT:

Vessel Density & OCT Thickness

Density (%)	Section	Thickness
32.6	Whole Image	253
34.2	Superior-Hemi	260
30.9	Inferior-Hemi	246
19.3	Fovea	251
34.5	ParaFovea	300
34.1	-Superior-Hemi	306
34.9	-Inferior-Hemi	295
41.8	-Tempo	280
30.6	-Superior	304
32.6	-Nasal	322
33.0	-Inferior	295
31.7	PeriFovea	250
32.5	-Superior-Hemi	259
30.8	-Inferior-Hemi	240
27.5	-Tempo	236
28.6	-Superior	253
42.4	-Nasal	281
28.0	-Inferior	250

HD Angio Retina
Ring Diameters (mm): 1.00, 3.00, 6.00
Scan Quality 5/10
Right /
Export Angio
En Face
Edit Bnd
Vessel Density & OCT Thickness
Density (%) Section Thickness (µm)
32.6 Whole Image 253
34.2 Superior-Hemi 260
30.9 Inferior-Hemi 246
19.3 Fovea 251
34.5 ParaFovea 300
34.1 - Superior-Hemi 306
34.9 - Inferior-Hemi 295
41.8 - Tempo 280
30.6 - Superior 304
32.6 - Nasal 322
33.0 - Inferior 295
31.7 PeriFovea 250
32.5 - Superior-Hemi 259
30.8 - Inferior-Hemi 240
27.5 - Tempo 236
28.6 - Superior 253
42.4 - Nasal 281
28.0 - Inferior 230
26.3 29.8 40.2
33.7 31.2 43.8
27.2 28.5 33.0

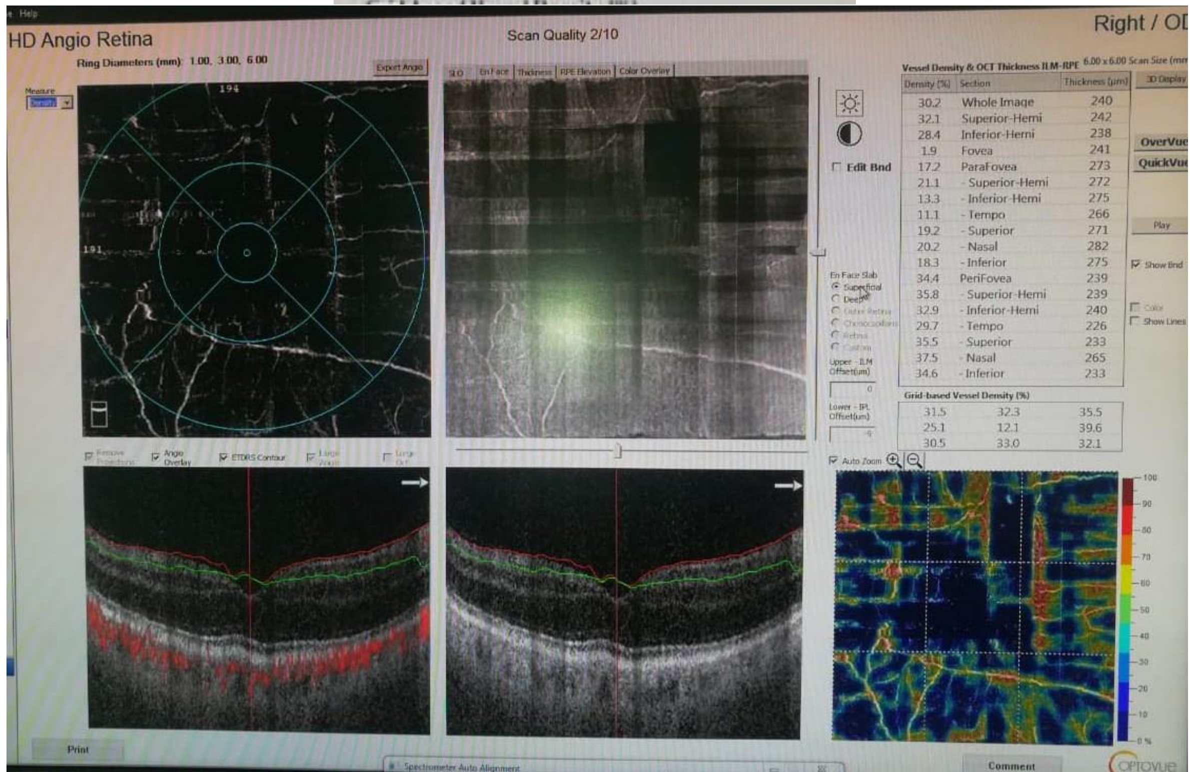
Post-operative OCT:

Density (%)	Section	Thickness (um)
31.1	Whole Image	247
34.2	Superior-Hemi	250
31.5	Inferior-Hemi	256
19.1	Fovea	272
34.7	ParaFovea	302
35.4	- Superior-Hemi	308
36.1	- Inferior-Hemi	285
41.3	- Tempo	270
30.2	- Superior	308
32.2	- Nasal	320
34.1	- Inferior	285
31.4	PeriFovea	246
34.1	- Superior-Hemi	265
32.3	- Inferior-Hemi	230
25.4	- Tempo	252
28.3	- Superior	250
41.5	- Nasal	277
27.1	- Inferior	285

Case (II), Group (II): (Sub-scleral trabeculectomy with use of sub-scleral Mitomycin 0.02%).

A. Pre-operative OCT:

Density (%)	Section	Thickness (µm)
30.2	Whole Image	240
32.1	Superior-Hemi	242
28.4	Inferior-Hemi	238
1.9	Fovea	241
17.2	ParaFovea	273
21.1	- Superior-Hemi	272
13.3	- Inferior-Hemi	275
11.1	- Tempo	266
19.2	- Superior	271
20.2	- Nasal	282
18.3	- Inferior	275
34.4	PeriFovea	239
35.8	- Superior-Hemi	239
32.9	- Inferior-Hemi	240
29.7	- Tempo	226
35.5	- Superior	233
37.5	- Nasal	265
34.6	- Inferior	233

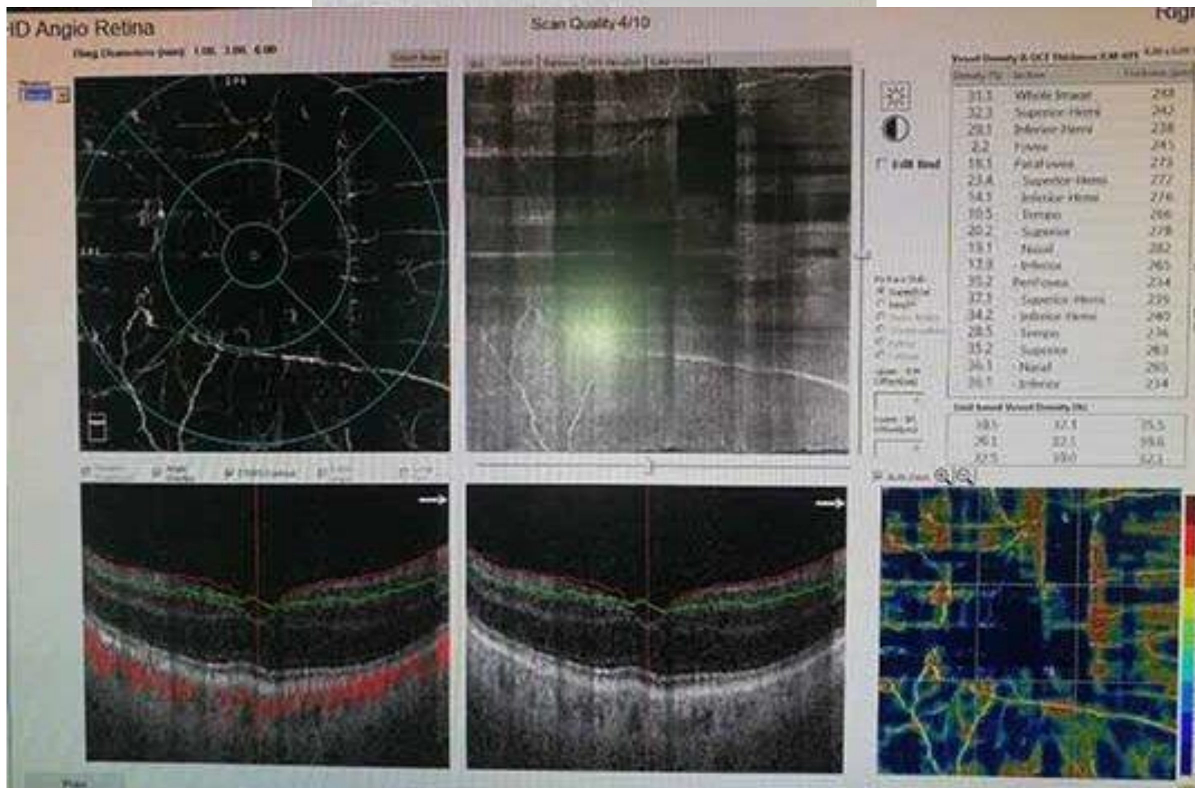


Density (%)	Section	Thickness (µm)
30.2	Whole Image	240
32.1	Superior-Hemi	242
28.4	Inferior-Hemi	238
1.9	Fovea	241
17.2	ParaFovea	273
21.1	- Superior-Hemi	272
13.3	- Inferior-Hemi	275
11.1	- Tempo	266
19.2	- Superior	271
20.2	- Nasal	282
18.3	- Inferior	275
34.4	PeriFovea	239
35.8	- Superior-Hemi	239
32.9	- Inferior-Hemi	240
29.7	- Tempo	226
35.5	- Superior	233
37.5	- Nasal	265
34.6	- Inferior	233

Grid-based Vessel Density (%)		
31.5	32.3	35.5
25.1	12.1	39.6
30.5	33.0	32.1

B. Post-operative OCT:

Density (%)	Section	Thickness (µm)
31.1	Whole Image	248
32.3	Superior-Hemi	242
29.1	Inferior-Hemi	238
2.2	Fovea	245
18.1	ParaFovea	273
23.4	- Superior-Hemi	272
14.1	- Inferior-Hemi	276
10.5	- Tempo	266
20.2	- Superior	278
19.1	- Nasal	282
17.3	- Inferior	265
35.2	PeriFovea	234
37.1	- Superior-Hemi	235
34.2	- Inferior-Hemi	240
28.5	- Tempo	236
35.2	- Superior	263
36.1	- Nasal	265
36.1	- Inferior	234



DISCUSSION

Glaucoma is a progressive optic neuropathy characterized by structural changes in the optic disc and retinal nerve fiber layers, along with specific patterns of functional abnormalities within the visual field that may eventually lead to severe visual impairment **(7).** Although glaucoma treatment options have improved, trabeculectomy remains the standard surgical procedure for glaucoma when maximal pressure-lowering medical treatment or LASER therapy fails to achieve the target intraocular pressure (IOP), **(8),** which is considered the major modifiable risk factor in the management of glaucoma **(9).**

Mitomycin C (MMC) has been widely used to augment the success rates of trabeculectomy. MMC reduces fibroblast proliferation in the subconjunctival space and in the Tenon capsule, thereby inhibiting scar formation. Several studies have reported that MMC decreases fibroblast activity in the conjunctival tissue and compared its efficacy to that of different substitutes **(10).**

In the current study we evaluate the peripapillary nerve fiber layer thickness (RNFL) and macular perfusion after subscleral trabeculectomy with and without mitomycin by OCT and OCT angiography. It was an interventional randomized controlled trial (RCT), conducted on 99 patients, randomly selected and randomly allocated to three groups: (33 patients underwent sub-scleral trabeculectomy without use of antimetabolite, 33 patients underwent sub-scleral trabeculectomy with use of sub-scleral Mitomycin 0.02% and 33 patients underwent sub-scleral trabeculectomy with use of subconjunctival Mitomycin 0.02%).

In the current study, IOP change was analyzed at the 6-month timepoint, the three studied groups showed a highly statistically significant reduction in the IOP in the post-operative as compared with the pre-operative assessment, (p-values <0.001). The three techniques (trabeculectomy, trabeculectomy + sub-scleral MMC and trabeculectomy+ sub-conjunctival MMC) were effective in lowering IOP significantly, compared with preoperative levels. However, there was a statistically significant difference between the trabeculectomy alone and trabeculectomy + MMC groups, the change in IOP was significantly lowest among group (A) –trabeculectomy alone– as compared with group (B) and group (C) (p-value =0.031, 0.042), there was non-statistically significant difference between group (B) and group (C) (sub-scleral or sub-conjunctival MMC), with regard to the mean decrease in IOP.

It had been reported that MMC-enhanced trabeculectomy had considerably increased the success rate of surgical treatments for glaucoma with a high risk of surgical failure **(11).** MMC caused inhibition of fibroblast proliferation, Histopathological studies in human eyes and experimental studies have demonstrated toxic effects of MMC to the ciliary body and its epithelium, which likely cause a reduction in aqueous secretion and a lowering of IOP **(12).** Our results were in line with previous published studies reported a higher rate of surgical success without glaucoma medications (i.e., complete success) was seen after trabeculectomy with MMC as copared with trabeculectomy alone, **Panarelli et al., (13)** reported on their study on 70 patients who underwent trabeculectomy with MMC, mean IOPs were lower at all postoperative time points after trabeculectomy with MMC compared with trabeculectomy alone, and these differences were statistically significant at 6 months and after one year. The trend toward greater IOP reduction after trabeculectomy with MMC was observed with the use of less adjunctive medical therapy, which was statistically significant throughout 3 years of follow-up **(13).** Similarly, in their study concluded that mitomycin C application with trabeculectomy led to a greater reduction in intraocular pressure and inhibition of fibroblasts **(14).**

In our study, comparison of Superior and Inferior RNFL sectors within the three studied groups showed a slightly decrease in all studied groups in post- as compared with pre-operative assessment, however this reduction was of non-statistically significant difference (p-values >0.05). This can be explained by the progressive nature of the disease and the multiple factors affecting it. Reported RNFL thickness change following IOP reduction in literature has shown conflicting results. Early studies using the OCT showed increased RNFL thickness following trabeculectomy **(15), (16).** However, later studies **Raghu et al., (17)** showed no change in RNFL thickness on OCT following IOP reduction. The variable results of these studies may be due to other factors such as stage of the disease and age.

Studies using the OCT for RNFL thickness measurement show similar results as ours. **Chang et al., (18)** showed no significant change in the RNFL thickness associated with the lowering of IOP by medical or surgical therapy. **Rebolleda et al., (19)** reported no change in peripapillary RNFL thickness 6 months following deep sclerectomy. They separately analyzed patients with <30% and >50% IOP reduction and reported no change in either group. **Lommatzsch et al. (20)** reported that no significant changes were detectable in the RNFL after trabeculectomy in open-angle glaucoma patients.

Some studies have reported RNFL thinning after IOP reduction. **Ely et al. (21)** reported that some pediatric glaucoma patients with IOP reductions and cupping reversal after glaucoma surgery showed continued RNFL thinning. **Aydin et al (15)** evaluated RNFL thickness change following trabeculectomy and reported a significant increase.

Some studies using OCTA in which the majority of study population had primary open-angle glaucoma (POAG) demonstrated decreased optic disc and peripapillary perfusion in glaucoma eyes **(22).** Structural reversibility after glaucoma treatment is well documented. Reversible optic disc cupping following acute IOP lowering has been known to occur for decades **(23).** Combination of both conventional OCT and OCTA allows simultaneous evaluation of RNFL structure, GCC thickness, optic disc, and macular perfusion **(24).**

In our study, we reported a statistically significant positive correlation between superficial peri foveal, deep superior para foveal, deep superior peri foveal, and deep nasal peri foveal VD (%) with reduction in IOP post-operatively, while the remaining sectors showed non-statistically significant correlation with reduction in IOP. Those results may suggest that the ocular perfusion impairment by high IOP can be improved by IOP reduction, and the reversal of microvasculature may contribute to the rate of glaucoma progression. Our results go in line with a study conducted on 25 patients to evaluate the microvascular changes at the peripapillary area in glaucomatous eyes after IOP lowering by trabeculectomy using OCT angiography that reported a significant influence of greater IOP reduction on the change of the VD of nasal and temporal region **(25).**

In contrast, **Zéboulon et al., (3)** who measured the influence of IOP lowering by filtering surgery on peripapillary and macular vessel density in glaucoma patients using OCT angiography demonstrated a very limited effect of surgically induced IOP reduction on peripapillary and macular vessel density**.** The discrepancy between our study and this study may be attributable to that in their study patients were observed for only up to 1 month after trabeculectomy, so the degree of IOP reduction was maintained for a relatively short period. Also, their patients had lower baseline IOP and lower IOP reduction (baseline IOP: 23.7 mmHg; mean IOP reduction: 11.5 mmHg). In contrast, the baseline IOP was 30.69 ±4.54 mmHg and the mean IOP reduction was 16.42 ± 5.1 mmHg at 6 months postoperatively in our study.

Also, **Liu et al. (26)** demonstrated higher repeatability and reproducibility of OCT angiography compared with other noninvasive techniques, such as Laser Doppler flowmetry (LDF) and laser speckle flowgraphy (LSFG). Several studies have suggested that vascular factors can play a pathogenic role in glaucoma, and decreased peripapillary retinal vasculature identified by OCTA is clinically useful to observe the glaucoma progression **(27).** Studies have evaluated the change of peripapillary and retinal microvasculature following a large amount of IOP reduction in glaucoma patients. Some studies reported limited or no significant VD change, while others have documented microvascular improvement after IOP reduction **(28).**

An interesting finding in the current study that some eyes showed a decrease of VD (%) despite successful IOP reduction. A significant IOP decrease after trabeculectomy is known to reduce the depth of the lamina cribrosa (LC), the LC depth reduction has shown an association with microvascular improvement after trabeculectomy **(20), (29).** However, in another study on the long-term shape and depth of LC after trabeculectomy, although most eyes showed long-term flattening and shallowing of the LC, some eyes showed a deepened LC from the baseline. Therefore, the authors concluded that a reduction of IOP plays an important role in the early phase of LC change; however, LC remodeling may play a crucial role in a stable IOP in the later phase. Hence, such remodeling of LC may lead to VD reduction and glaucomatous VF deterioration that occurred in some eyes in the current study **(30)**.

CONCLUSIONS

In conclusion, we have shown that measurable changes occur in Vessel Density (%) but not in RNFL thickness after trabeculectomy as conducted by the three techniques (trabeculectomy, trabeculectomy + sub-scleral MMC and trabeculectomy+ sub-conjunctival MMC). Further studies excluding the effects of glaucoma medications are needed to investigate the relationship between vascular changes and glaucomatous damages, investigate patients presenting with earlier stage of the disease and comparison of cases according to the severity of glaucoma might answer the question of whether structural glaucoma damage is reversible if intervened in time.

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