

Influence of Perfluorocarbon Liquids on Peripapillary Retinal Nerve Fiber–Layer Thickness Following Pars Plana Vitrectomy with Silicone Oil–Based Endotamponade

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Background: Inner retina–layer modifications after pars plana vitrectomy (PPV) can be objectively assessed through spectral domain optical coherence tomography (SD-OCT).

Methods: This study explored prospectively changes in retinal nerve-fiber layer (RNFL) thickness with SD-OCT in eyes undergoing PPV with silicone oil–based tamponade with and without use of perfluorocarbon liquids (PFCLs) during the early postoperative phase (up to 3 months) at the Research Institute of Ophthalmology, Egypt.

Results: Thirty patients were recruited who underwent PPV and silicone oil–based tamponade for either retinal detachment or diabetic retinopathy between April 2019 and September 2019. Mean RNFL thickness showed no significant change during follow-up at the first week (102.90 ± 30.68 mm), 1 month (107.30 ± 32.27), or three months (105.90 ± 36.68 ; $p=0.46, 0.68$). There were significant correlations noticed between RNFL thinning and axial length of eyes, intraocular pressure, and use of PFCLs during the follow-up period.

Conclusion: The RNFL tends to change postvitrectomy, but not significantly. Careful examination and consistent follow-up is required for postvitrectomy patients with larger axial length and intraoperative PFCL use.

Keywords: pars plana vitrectomy, PPV, ganglion-cell complex, GCC, retinal nerve-fiber layer, RNFL, perfluorocarbon liquids, PFCL spectral domain optical coherence tomography, SD-OCT

Introduction

Pars plana vitrectomy (PPV) was introduced in 1971 by Machemer et al and essentially encompasses vitreous body removal as one of the essential steps in the surgical management of various retinal disorders.¹ Despite technological advances, transconjunctival sutureless vitrectomy is not without complications, with reports of retinal tears,^{2,3} hypotony,^{4,5} and endophthalmitis^{6,7} in the early postoperative period and cataracts,^{8,9} retinal detachment,^{3,5} and recurrent macular edema⁸ as late-onset problems. The ganglion-cell complex has been defined as a region encompassing the retinal nerve-fiber layer (RNFL), ganglion-cell layer, and inner plexiform layer. The RNFL develops from retinal ganglion-cells axon converging on the disk to form bundles. Thickness of RNFL bundles is greater at the disc and becomes less so peripherally.¹⁰

Inner retina–layer changes after PPV have been a frequent topic of discussion in recent years.¹¹ Some studies have reported RNFL thickening after vitrectomy, either

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as a result of direct mechanical traction on the papillomacular bundle, resulting in tenting and elevation of nerve fibers,¹² or edema of the inner-retinal layers in the first postoperative week.¹³ Others have reported thinning, with possible mechanisms including NFL dehydration during fluid–air exchange,¹⁴ dye-related toxicity, mechanical injury, or increased intraocular pressure (IOP).¹⁵ There are several technologies that have been used for measuring the RNFL, these include scanning laser polarimetry, confocal scanning laser ophthalmoscopy, and ocular coherence tomography (OCT).¹⁶ The development of spectral domain (SD) OCT has made morphological study of different retinal layers with a high level of detail possible.¹³

Perfluorocarbon liquids (PFCLs) were first used in 1982 by Zimmerman and Faris to relocate a detached retina.¹⁷ Following reports on the efficacy and safety of intraoperative PFCL application in *in vivo* and *in vitro* studies, Chang et al utilized them in vitrectomies for retinal detachment with severe proliferative vitreoretinopathy.¹⁸ Their physical properties of low viscosity, high specific gravity (range 1.76–2.30), high surface tension, and transparency make them an ideal intraoperative tool.¹⁹ Short-term use of up to 48 hours has been declared safe in animal models, with no evidence of retinal toxicity at the microscopic level.^{20,21} This study investigated the effect of PFCL use on RNFL thickness using SD-OCT in eyes undergoing PPV with silicone-oil endotamponade with follow-up of 3 months.

Methods

This prospective noninterventional observational study of 30 eyes of 30 patients was conducted at the Research Institute of Ophthalmology, Egypt between April 2019 and September 2019. All patients underwent RNFL assessment after PPV with silicone oil–based endotamponade by SD-OCT. The study was approved as a prospective audit by the institutional review board at the Institute, and adhered to the Declaration of Helsinki. All patients provided informed consent for enrolment in the study.

All patients aged 18–70 years undergoing PPV with silicone oil–based endotamponade were enrolled during the study period. Patients with previous ocular trauma, ocular surgery, history of ocular vascular disease, uveitis, glaucoma, and high refractive errors were excluded from the study. All included patients underwent detailed ocular and systemic history, assessment of best-corrected visual acuity, IOP measurement, and peripapillary NFL-average measurement with SD-OCT using a Heidelberg OCT II

(Heidelberg Engineering, Heidelberg, Germany) at 1 week, 1 month, and 3 months.

Surgical Technique

Three-port 23-gauge PPVs were performed using the Alcon Constellation vision system. All patients underwent triamcinolone-assisted posterior vitreous detachment using a cutter with vacuum set to 450 mmHg. Core vitrectomy was then performed with parameters set to 4,000 cuts/second with linear vacuum set to 300 mmHg, followed by peripheral shaving with cut rate increased to 7,000 cuts/second with vacuum maintained to the same value. As per discretion of the surgeon, parameters were modified based on specific case circumstances. Infusion pressure was maintained at 30mmHg throughout surgery and increased to 45 mmHg during fluid–air exchange. PFL was used in cases with peripheral breaks to avoid the need for drainage retinotomies. Trypan blue–assisted internal limiting membrane (ILM) peeling was performed if considered necessary by the surgeon. Sclerostomies were assessed at the end of surgery and sutured only if considered necessary by the surgeon.

RNFL-Analysis Protocol

Peripapillary RNFL thickness–analysis parameters used were resolution mode high speed, circle diameter 3.5 mm, size X 768 pixels (10.9 mm), size Z 496 pixels (1.9 mm); scaling X 14.17 m/pixel, and scaling Z 3.87 m/pixel. RNFL-thickness parameters in the peripapillary area were automatically calculated by the SD-OCT device and categorized into regions: temporal quadrant thickness (90°), temporal superior quadrant thickness (45°), nasal superior quadrant thickness (45°), nasal quadrant thickness (90°), nasal inferior quadrant thickness (45°), temporal inferior quadrant thickness (45°), and average global thickness (360°).

Recorded data were analyzed using SPSS version 20.0. Quantitative data are expressed as means $\pm$ SD. Qualitative data are expressed as frequency and percentage. Independent-sample *t*-tests of significance were used when comparing between two means. Paired-sample *t*-tests were used when comparing between related samples. One-way ANOVA was used when comparing more than two means. Pearson's correlation coefficient (*r*) testing was used to assess the degree of association between two sets of variables. The confidence interval was set to 95% and the margin of error accepted was 5%. $p < 0.05$ was considered significant.

Results

Thirty patients (21 men and nine women) underwent uneventful PPV with silicone oil–based endotamponade for multiple etiologies (17 retinal detachments, 13 proliferative diabetic retinopathy) at the Research Institute of Ophthalmology, Egypt between April 2019 to September 2019. Mean patient age was 53.00 ± 12.21 (30–70) years. Mean axial length of vitrectomized eyes was 25.07 ± 1.72 (22–28) mm. Nineteen eyes underwent vitrectomy combined with cataract surgery. Ten cases underwent trypan stain–assisted ILM peeling, and PFCLs were used in ten cases. Endolaser, silicone endotamponade, and triamcinolone acetonide were used for all cases. Thirteen patients had no systemic disease, while eight were diabetic and nine suffered from both diabetes and hypertension (Table 1).

Changes in RNFL Thickness after Vitrectomy

Mean RNFL thickness showed no significant change during follow-up at the first week (102.90 ± 30.68 mm), 1 month (107.30 ± 32.27), or three months (105.90 ± 36.68 ; $p=0.46, 0.68$).

Table 2 shows mean differences in RNFL thickness compared to preoperative states, with paired *t*-test results in nasal, superior nasal, inferior nasal, temporal, superior temporal, and inferior temporal quadrants. Regional RNFL thickness in the superior nasal, superior temporal, inferior nasal, inferior temporal, temporal, and nasal quadrants did not differ significantly among 1-week, 1-month, and 3-month follow-up.

Table 1 Pathology, Intraoperative Variations, and Systemic Disease Distribution

	Total (n=30)
Pathology	
Retinal detachment	17 (56.7%)
Diabetic retinopathy	13 (43.3%)
Intraoperative variations	
Perfluorocarbon liquids (PFCLs)	10 (33.3%)
Stain	10 (33.3%)
ILM peel	10 (33.3%)
Phacoemulsification	19 (63.3%)
Systemic disease	
Healthy subjects	13 (43.3%)
Diabetics	8 (26.7%)
Diabetic + hypertensive	9 (30.0%)

Correlation Between Changes in RNFL Thickness and Visual Acuity and IOP

Changes in RNFL thickness were highly significantly correlated with visual acuity and IOP (Table 3 and Figure 1)

Changes in Retinal Nerve Fiber–Layer Thickness According to Eye and Patient Status

RNFL thickness in each quadrant was not significantly different by patient sex or age-group (paired *t*-test, $p>0.05$), pathology group (Table 4), or between healthy, diabetic and diabetic + hypertensive patient groups (ANOVA, $p>0.05$)

Changes in Retinal Nerve Fiber–Layer Thickness According to Operative Associations

RNFL thickness in each quadrant was not significantly different by use of stain-assisted ILM peeling or combining phacoemulsification with vitrectomy (paired *t*-test, $p>0.05$). RNFL thickness in average, nasal, temporal, superior temporal, inferior nasal, and inferior temporal quadrants was changed significantly by use of PFCs, as shown in Table 5.

Discussion

RNFL thickness measured by SD-OCT objectively and qualitatively is important, as RNFL damage often occurs before both visual field defects and optic nerve–head damage can be detected. Kerrigan-Baumrind reported that at least 25%–35% retinal ganglion–cell loss is associated with statistical abnormalities in automated visual field testing.²² The pathophysiology of visual field defects after vitrectomy is a matter of debate. A postoperative increase in IOP, retinal toxicity of intravitreal gas tamponade, phototoxicity from endoillumination, mechanical retinal damage during posterior vitreous–detachment induction, damage to the NFL in ERM or external limiting–membrane peeling, dehydration of the RNFL caused by fluid–air exchange, and chemical retinal damage caused by indocyanine green (ICG) staining are possible explanations given in the literature.^{11,23–25} The present study included 30 patients who underwent PPV combined by silicon endotamponade. Our results showed that RNFL parameters did not change significantly throughout the 3-month follow-up in any quadrant. This finding correlates well with studies reported by Lee and Kim et al.^{25,26}

Table 2 Peripapillary Nerve Fiber-layer Analysis at Various Quadrants and Comparison at 1 Week, 1 Month, and 3 Months Postoperatively

Period	NFL (Global)		Paired-Sample t-test		
	Range	Mean $\pm$ SD	Mean Difference	t	p-value
After 1 week	50–175	102.90 $\pm$ 30.68			
After 1 month	53–191	107.30 $\pm$ 32.27	4.4	–0.740	0.466
After 3 months	50–207	105.90 $\pm$ 36.68	3.0	–0.410	0.685
Period	NFL (nasal)		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	35–157	92.53 $\pm$ 31.43			
After 1 month	38–182	88.43 $\pm$ 28.80	–4.1	0.673	0.506
After 3 months	28–183	88.87 $\pm$ 36.78	–3.7	0.509	0.615
Period	NFL (temporal)		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	39–159	90.57 $\pm$ 30.06			
After 1 month	49–289	104.63 $\pm$ 46.20	14.1	–1.682	0.103
After 3 months	40–265	107.57 $\pm$ 62.23	17.0	–1.541	0.134
Period	NFL (superonasal)		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	43–208	112.07 $\pm$ 41.02			
After 1 month	22–315	111.47 $\pm$ 50.97	–0.6	0.069	0.946
After 3 months	20–251	110.70 $\pm$ 56.86	–1.4	0.132	0.896
Period	NFL (superotemporal)		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	14–200	120.13 $\pm$ 44.92			
After 1 month	24–242	124.90 $\pm$ 54.08	4.8	–0.407	0.687
After 3 months	24–288	131.70 $\pm$ 66.01	11.6	–0.804	0.428
Period	NFL (inferonasal)		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	28–280	117.30 $\pm$ 47.53			
After 1 month	59–241	120.10 $\pm$ 43.69	2.8	–0.391	0.699
After 3 months	53–187	112.93 $\pm$ 38.92	–4.4	0.589	0.560
Period	NFL (inferotemporal)		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	28–240	142.20 $\pm$ 51.41			
After 1 month	56–259	142.30 $\pm$ 52.32	0.1	–0.012	0.990
After 3 months	60–269	129.87 $\pm$ 51.66	–12.3	1.244	0.224

We observed that visual acuity improved significantly, which statistically correlated with changes in the RNFL. IOP was found to be significantly decreased during period of follow-up. Early postoperative IOP elevation is explained by

the inflammatory response that occurs postoperatively or due to the patient being a steroid responder. The subsequent decrease of IOP is assumed to be related to inflammatory recovery or steroid cessation. The spike in IOP was not

Table 3 Pre- and Post-Operative Comparison of Visual Acuity and Intraocular Pressure During Follow-up

Period	Visual Acuity		Paired-Sample t-test		
	Range	Mean $\pm$ SD	Mean Difference	t	p-value
After 1 week	0.001–0.004	0.002 $\pm$ 0.001			
After 1 month	0.004–0.03	0.010 $\pm$ 0.009	0.01	–4.623	<0.001
After 3 months	0.004–0.3	0.084 $\pm$ 0.099	0.08	–4.558	<0.001
Period	Intraocular pressure		Paired-sample t-test		
	Range	Mean $\pm$ SD	Mean difference	t	p-value
After 1 week	18–24	20.83 $\pm$ 1.82			
After 1 month	16–23	19.63 $\pm$ 2.09	–1.20	3.714	<0.001
After 3 months	13–23	18.77 $\pm$ 2.58	–2.06	4.520	<0.001

Note: Bold values points indicate statistically significance.

Table 4 Relationship of Axial Length and Etiology in Retinal Detachment (RD), and Diabetic Retinopathy (DR), and n RNFL Thickness at PostOperative Follow-up

Parameters	RD DR t-test					
	Mean	SD	Mean	SD	t	p-value
NFL 1 N	90.71	34.07	94.92	28.78	0.129	0.723
NFL 2 N	93.18	34.15	82.23	19.40	1.067	0.311
NFL 3 N	93.71	43.26	82.54	26.42	0.671	0.420
Change 1 week after 1 month	10.75	42.94	–7.19	32.73	1.567	0.221
Change 1 week after 3 months	13.56	58.34	–8.68	35.02	1.474	0.235
NFL 1 T	86.06	32.10	96.46	27.27	0.878	0.357
NFL 2 T	105.18	57.17	103.92	28.27	0.005	0.943
NFL 3 T	106.24	67.36	109.31	57.46	0.017	0.896
Change 1 week after 1 month	34.08	88.17	12.13	31.90	0.727	0.401
Change 1 week after 3 months	34.80	96.10	14.82	48.66	0.468	0.500
NFL 1 SN	105.82	41.57	120.23	40.45	0.906	0.349
NFL 2 SN	104.29	37.72	120.85	64.91	0.771	0.388
NFL 3 SN	111.88	60.05	109.15	54.78	0.016	0.899
Change 1 week after 1 month	11.12	75.71	2.28	39.59	0.146	0.705
Change 1 week after 3 months	18.34	79.94	–6.31	38.94	1.041	0.316
NFL 1 ST	114.59	52.56	127.38	33.03	0.589	0.449
NFL 2 ST	125.88	55.91	123.62	53.82	0.013	0.912
NFL 3 ST	134.53	60.82	128.00	74.65	0.070	0.794
Change 1 week after 1 month	75.62	224.77	14.64	101.50	0.823	0.372
Change 1 week after 3 months	105.91	322.13	16.43	98.90	0.929	0.343
NFL 1 IN	109.65	36.38	127.31	59.18	1.018	0.322
NFL 2 IN	122.47	39.89	117.00	49.72	0.112	0.740
NFL 3 IN	117.82	37.58	106.54	41.22	0.611	0.441
Change 1 week after 1 month	21.96	55.75	4.47	65.41	0.624	0.436
Change 1 week after 3 months	17.46	51.81	–4.29	55.48	1.221	0.279
NFL 1 IT	134.29	50.96	152.54	52.16	0.925	0.344
NFL 2 IT	134.47	50.84	152.54	54.48	0.875	0.358
NFL 3 IT	127.12	56.71	133.46	46.23	0.108	0.745
Change 1 week after 1 month	15.61	84.38	19.77	98.42	0.015	0.902
Change 1 week after 3 months	9.26	74.09	5.12	76.78	0.022	0.882

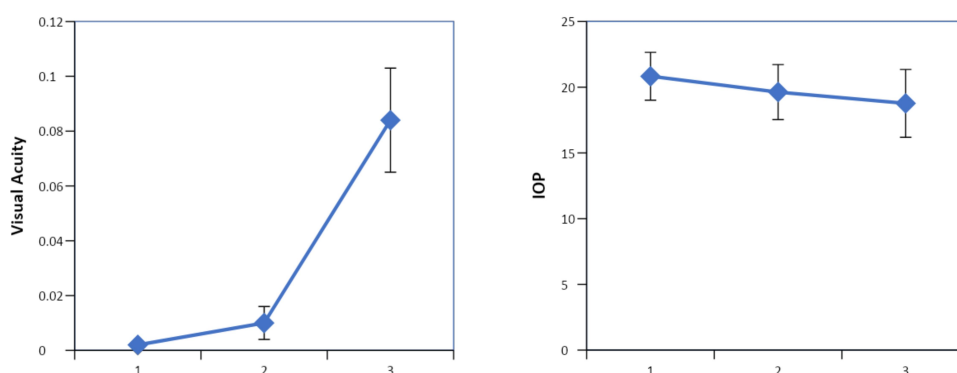


Figure 1 Visual acuity and intraocular pressure changes during follow-up.

alarming and was short-lived, and thus its effect on RNFL change is considered minimal if at all significant. PFCLs are useful and safe surgical tools in vitreoretinal surgery. Currently, retinal detachments associated with proliferative vitreoretinopathy, giant retinal tears, and penetrating trauma are the primary indications for their use. With growing evidence and advancements in surgical techniques, newer applications for PFCLs have been explored, including management of dislocated crystalline or intraocular lenses, retinopathy of prematurity, retinoschisis, retinal detachment associated with proliferative diabetic vitreoretinopathy, and management of suprachoroidal and submacular hemorrhage.²⁸ The use of PFCLs as a temporary tamponade has been controversial, due to corneal toxicity, retinal infiltration, and inflammatory reaction in experimental studies. Although most clinical studies have not been able to establish any proof of retinal toxicity, such as progressive visual acuity loss, electroretinography or retinal histological analysis has not been reported extensively.²⁹

SD-OCT is a relatively new technology, which explains why there is a significant paucity of literature on the effect of PFCLs on RNFL thickness. This study confirmed a positive correlation of RNFL thinning with use of PFCLs in ten patients. This was significant at the 1-week (global, nasal, superior temporal, and inferior temporal) and 3-month (inferior nasal and inferior temporal) follow-up. We expected that there might be a significant correlation of thickening of the RNFL due to rebound edema caused by compression by heavy liquids like PFCLs, but report thinning of the RNFL in this study could be attributed to the mechanical compression effect of the liquid on the RNFL. Although silicone oils have been used for years and are relatively safe, there have been reports of RNFL thinning after the use of silicone oil,^{27,30} while others reported

paradoxical thickening.³¹ In our study, we found that there were no statistically significant changes in RNFLs due to use of silicon endotamponade, in agreement with a study by Caramoy et al.³²

Wolf et al reported that substantial damage to Müller-cell end feet is caused by ILM peeling, even in the untouched retina directly adjacent to the peeled area. This suggests that the basal lamina can transmit mechanical forces to the inner retinal layers, causing cellular damage in these layers.³³ Kim et al and Yamashita et al assessed the RNFL after ICG-assisted vitrectomy and found that the RNFL was significantly reduced postoperatively.^{23,25} This can be explained by the mechanical damage due to retinal manipulation or by toxic effects of the dye. Although ICG is considered safe and widely used successfully, trypan blue has been demonstrated to have a larger safety margin.³⁴ In the present study, we observed no statistically significant differences in changes in RNFL thickness in correlation with trypan-assisted ILM peeling. Our findings matched those of Toba et al, who reported no significant correlation between the degree of change in RNFL thickness and the type of vital stain (triamcinolone acetonide, brilliant blue, trypan blue, and ICG) used during surgery.³⁵

It has been reported that, diabetic eyes that have been treated with panretinal photocoagulation have thinner RNFLs than nondiabetic eyes.³⁶ Eren et al reported initial thickening of RNFL in the first 3 months postprocedure due to ensuing axonal edema, followed by thinning at the sixth month, attributed to axonal loss secondary to the laser treatment.³⁷ All the cases in this study underwent panretinal photocoagulation, which was found to have no significant correlation with changes in RNFL thickness.³⁸ This study found that changes in the RNFL did not differ significantly

Table 5 Effect of Perfluorocarbon Liquids (PFCLs) on Retinal Nerve Fiber–Layer Analysis. Clinically Significant Values ($p>0.05$) Depicted in Bold Font

Parameters	No PFCLs		PFCLs		t-test	
	Mean	SD	Mean	SD	t	p-value
NFL 1 G	111.35	28.45	86.00	29.12	5.213	0.030
NFL 2 G	110.15	35.32	101.60	25.85	0.459	0.504
NFL 3 G	107.70	42.14	102.30	23.75	0.140	0.711
Change 1 week after 1 month	0.88	28.38	32.47	69.30	3.183	0.085
Change 1 week after 3 months	−0.57	37.61	33.59	61.56	3.571	0.069
NFL 1 N	96.15	32.25	85.30	30.00	0.789	0.382
NFL 2 N	85.45	30.96	94.40	24.29	0.636	0.432
NFL 3 N	88.15	42.56	90.30	23.06	0.022	0.883
Change 1 week after 1 month	−8.01	23.78	24.95	54.51	5.411	0.027
Change 1 week after 3 months	−4.78	43.74	21.31	59.57	1.860	0.183
NFL 1 T	99.95	26.54	71.80	28.95	7.069	0.013
NFL 2 T	112.60	52.30	88.70	26.08	1.836	0.186
NFL 3 T	113.00	62.85	96.70	62.78	0.449	0.508
Change 1 week after 1 month	18.55	76.84	36.61	53.52	0.441	0.512
Change 1 week after 3 months	18.66	77.64	41.11	82.48	0.535	0.471
NFL 1 SN	115.90	40.79	104.40	42.58	0.515	0.479
NFL 2 SN	114.05	58.13	106.30	34.55	0.150	0.702
NFL 3 SN	108.00	55.86	116.10	61.49	0.131	0.720
Change 1 week after 1 month	0.20	39.54	21.46	93.32	0.780	0.385
Change 1 week after 3 months	−3.46	48.29	29.90	90.19	1.768	0.194
NFL 1 ST	134.30	33.02	91.80	53.49	7.257	0.012
NFL 2 ST	126.40	56.36	121.90	51.98	0.045	0.834
NFL 3 ST	134.40	75.85	126.30	42.98	0.097	0.757
Change 1 week after 1 month	−3.02	46.66	153.63	289.53	5.756	0.023
Change 1 week after 3 months	5.54	71.76	190.33	409.76	3.962	0.056
NFL 1 IN	131.00	47.17	89.90	36.46	5.813	0.023
NFL 2 IN	122.25	43.48	115.80	46.12	0.141	0.710
NFL 3 IN	117.05	38.90	104.70	39.65	0.664	0.422
Change 1 week after 1 month	−5.38	17.64	53.89	90.68	8.205	0.008
Change 1 week after 3 months	−7.93	24.09	39.98	79.61	6.295	0.018
NFL 1 IT	158.25	38.79	110.10	60.19	7.073	0.013
NFL 2 IT	152.40	56.29	122.10	38.08	2.339	0.137
NFL 3 IT	143.35	54.89	102.90	32.04	4.594	0.041
Change 1 week after 1 month	−5.87	19.85	63.96	145.41	4.602	0.041
Change 1 week after 3 months	−9.01	27.34	40.43	119.36	3.203	0.084

with patient age, sex, pathology group, presence/absence of systemic diseases, or combined phacovitrectomy procedure, in agreement with the literature.^{25,27,39,40} Limitations of this study include the short follow-up and multiple pathologies possibly being confounding factors in interpretation of the results. However, the study results show realistic scenarios faced in clinical practice.

Conclusion

The RNFLi tends to change postvitrectomy, but not significantly. Careful examination and consistent follow-up is

required for postvitrectomy patients with larger axial length and intraoperative PFCL use. SD-OCT is a useful objective tool for evaluating RNFL thickness after vitrectomy, and large and long-term prospective studies are needed to evaluate changes in the RNFL with SD-OCT after vitrectomy.

Ethical Approval and Informed Consent

This study was approved as a prospective audit by the institutional review board at the Research Institute of

Ophthalmology, Giza, Egypt and adhered to the Declaration of Helsinki. All participants provided informed consent for participation in the study.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, took part in drafting the article or revising it critically for important intellectual content, agreed to submit to the current journal, gave final approval to the version to be published, and agree to be accountable for all aspects of the work.

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