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Comparison of Retinal Nerve Fiber Layer Thickness (RNFLT) Measurements in Normal and Glaucomatous Human Eyes by Optical Coherence Tomography

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ABSTRACT

Purpose: To compare retinal nerve fiber layer thickness (RNFLT) between normal and glaucomatous human eye with Optical Coherence Tomography (OCT). **Design and Patient setting:** This was a descriptive cross-sectional study which was carried out at the Department of Ophthalmology, Independent University Hospital, Faisalabad. **Methods:** Non-probability consecutive sampling was used to select a total of 60 participants, including 30 patients with primary open-angle glaucoma (POAG) and 30 age- and sex-matched healthy people. Each of the subjects had a thorough ophthalmological check-up with intraocular pressure (IOP) and OCT imaging. Superior, inferior, nasal, and temporal on four quadrants measured RNFL thickness using Stratus OCT and analyzed it with SPSS version 25. The independent sample t-test was used, and the p-value- 0.05 was regarded as significant. **Findings:** The average RNFL thickness in glaucomatous eyes was considerably reduced (66.02/9.68 0.001) to that of the controls (92.48/8.28 0.001). Quadrant-wise examination showed that all quadrants had significant thinning with the largest decrease seen in inferior (55.24 ± 12.43 µm) and superior (76.38 ± 14.45 µm) regions. There were also significant differences in nasal (56.94 ± 12.94 µm) and temporal (49.04 ± 10.45 µm) quadrants, which were statistically significant. The mean IOP was significantly greater in glaucoma patients (24.8) than in controls (15.2 ± 2.8 mmHg, $p < 0.001$). **Conclusion:** RNFL is less in glaucomatous eyes and OCT is a good, non-invasive instrument in the detection and follow up of early stages of glaucoma and preventable loss of vision.

INTRODUCTION

Glaucoma is a progressive and chronic optic neuropathy and is still one of the major causes of irreversible blindness in the world. It is distinguished by damage to the nerve head of the optic nerve, atrophy of retinal ganglion cells, and atrophy of the retinal nerve fiber layer (RNFL) and is usually accompanied by a decrease in the visual field [1]. Recent global estimates show that the burden of glaucoma is on the increase and projections indicate that it is bound to increase considerably as a result of an aging population [2]. Shopping mall The lack of symptoms in the initial stages of glaucoma often makes it difficult to diagnose but results in a lot of structural damage before symptoms are noticed [3].

The loss of axons in the retinal ganglion cells that make up the RNFL is a significant feature of the damage in glaucomatous illness. RNFL study is crucial for the early identification and tracking of the disease because structural changes in the RNFL typically precede functional deficits of the visual field [4]. The comparison of the traditional diagnostic methods such as field of vision test and measurement of the intracerebral pressure (IOP) measurement can not identify any sign of early disease development, which highlights the necessity of more sensitive and objective diagnostic methods [5].

Optical Coherence Tomography (OCT) is a non-invasive imaging modality enabling visualisation of the retinal structures in cross-section and in high-resolution. It enables an excellent reproducibility and accuracy in the quantification of RNFL-thickness [6]. The technology of spectral and swept-source OCT has further enhanced the sensitivity when detecting early changes in glaucoma [7]. OCT-based detecting of RNFL thinning has been demonstrated to be highly correlated with disease severity and progression rates, thus making it a useful biomarker in the management of glaucoma [8].

Other more recent studies have revealed a marked difference in the RNFL thickness in glaucomatous and normal eye, especially in the superior and inferior quadrants which are more vulnerable to initial damage [9]. Also, OCT based RNFL analysis has been found to be useful in the differentiation of glaucomatous optic neuropathy

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and normal variations, even at pre-perimetric stages [10].

Considering the significance of early diagnosis and early intervention in preventing loss of vision, the assessment of RNFL thickness by OCT is an important part of clinical practice. The proposed study will compare the normal and glaucomatous RNFL thickness to further add to the accumulating knowledge that OCT is a valuable diagnostic method in the management of glaucoma.

METHODOLOGY

The conducted descriptive cross-sectional study took place in the Department of Ophthalmology, the Independent University Hospital Faisalabad, the 5th March 2025 through the 4th June 2025 after permission by the College of Physicians and Surgeons Pakistan (CPSP) and the Institutional Ethical Review Committee. There were 60 respondents from the study whose sampling was based on non-probability consecutive sampling technique. The sample included two groups of 30 patients with primary open-angle glaucoma (POAG) as a case group and 30 age- and gender-matched healthy people as controls.

The study involved patients between 40-70 years old of both sexes. Glaucomatous patients were diagnosed based on preset operation criteria that include intraocular pressure (IOP) of 21 mmHg or more on at least two occasions, typical glaucomatous optic disc appearances, field defecting and open anterior chamber angles as observed under gonioscopy and have no other causative factors. The subjects of the control were sampled among those who attended the regular ophthalmic screening and had IOP admission under 21mmHg of normal optic nerve head, had no visual field deficient or defective visual field, had no family history of glaucoma and could have a normal fundus.

All individuals who had other types of glaucoma or refractive error that was over 2 diopters in a history of diabetes mellitus or hypertension, or had prior ocular trauma were not included in the study as a way of eliminating their possible confounding effect. Upon the informed written consent, all the participants were examined ophthalmologically as well as with the assessment of the visual acuity, slit lamp biomicroscory, fundus examination, gonioscopic examination, and intraocular pressure.

The measurements of the thickness of the retinal nerve fiber layer were performed with the help of Optical Coherence Tomography (OCT) and a Stratus OCT device (Model 4000 with a software version 2.0). Using the optic disc cube 200 × 200 scan procedure, high-resolution pictures of the peripapillary RNFL were obtained. The measurements of four quadrants superior, inferior, nasal and temporal were taken. All the scans were conducted by a trained operator at standardized conditions to prevent variation and other inter-observer variation.

All the necessary demographical and clinical information, such as age, sex, intraocular pressure, and RNFL thickness values, was captured on a structured pro forma. Data were keyed in and analysed with the help of Statistical Package of Social Sciences (SPSS) version 25. Quantitative variables like age and RNFL thickness were represented as mean standard deviation and categorical variables like gender were represented in frequencies and percentages. Mean RNFL thickness (between glaucomatous and normal groups) was compared using independent sample t-test. Age, gender and IOP were all controlled (stratified) and post-stratified analysis carried out. A p-value of less than 0.05 was taken to be statistically significant.

RESULTS

The glaucoma group was 55.2 ± 8.4 years with a mean age and the control group was 54.6 ± 7.9 years with a mean age, there was no significant difference in the mean age ($p = 0.72$). There was also similar gender distribution in the two groups: 16 males and 14 females in the glaucoma group and 15 males and 15 females in the control group were similar ($p = 0.79$). (Table I)

The average level of the intraocular pressure (IOP) was likewise greater in the glaucomatous group (24.8±3.6 mmHg) than it was in the control group (15.2±2.8 mmHg), and this difference was statistically highly significant ($p < 0.001$).

The average RNFL glaucomatous mean was found to be 66.02 and ± 9.68 mc, which was quite different ($p < 0.001$) with that of the control (92.48 and ± 8.28 mc). (Table II)

A quadrant-wise analysis revealed the better RNFL thickness at glaucoma patients at 76.38 ± 14.45 µm versus 111.96 ± 11.04 µm in control patients ($p < 0.001$). In the same manner, the lowest RNFL thickness was significantly lower in glaucoma group (55.24 ± 12.43 µm) than in the controls (116.04 ± 10.43 µm) indicating the highest level of thinning.

The nasal RNFL thickness was 56.94 ± 12.94 µm in glaucomatous eyes and 73.04 ± 8.15 µm in normal eyes ($p < 0.001$). The temporal thickness of the RNFL was also in the glaucoma group (49.04 ± 10.45 µm) as opposed to that of the control (60.36 ± 13.33 µm) ($p = 0.002$).

Age stratification revealed that mean RNFL thickness of glaucomatous patients and controls at the 40-55 years age group were 67.1 ± 9.2 µm and 93.4 ± 7.8 µm, respectively ($p < 0.001$). In the 56–70 years group, it was 64.9 ± 10.1 µm in glaucoma patients and 91.6 ± 8.5 µm in controls ($p < 0.001$). (Table III)

Correspondingly, gender stratification showed that mean RNFL thickness among males in glaucomatous relative to controls were 65.5 + 9.4 u m versus 91.8 + 8.1 u m ($p < 0.001$). Among females, RNFL thickness was 66.7 ± 10.1 µm in glaucoma patients and 93.1 ± 8.6 µm in controls ($p < 0.001$). (Table IV)

Table I

Demographic Characteristics of Study Participants.

Variable	Glaucoma Group (n=30)	Control Group (n=30)	p-value
Age (years)	55.2 ± 8.4	54.6 ± 7.9	0.72
Gender (M/F)	16 / 14	15 / 15	0.79
IOP (mmHg)	24.8 ± 3.6	15.2 ± 2.8	<0.001

Table II

Comparison of Mean RNFL Thickness (µm) Between Groups.

RNFL Parameter	Glaucoma Group (Mean ± SD)	Control Group (Mean ± SD)	p-value
Mean RNFL Thickness	66.02 ± 9.68	92.48 ± 8.28	<0.001
Superior RNFL	76.38 ± 14.45	111.96 ± 11.04	<0.001
Inferior RNFL	55.24 ± 12.43	116.04 ± 10.43	<0.001
Nasal RNFL	56.94 ± 12.94	73.04 ± 8.15	<0.001

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Temporal RNFL	49.04 ± 10.45	60.36 ± 13.33	0.002

Table III

Stratification of RNFL Thickness by Age.

Age Group (Years)	Group	Mean RNFL (µm)	p-value
40–55	Glaucoma Control	67.1 ± 9.2 93.4 ± 7.8	<0.001
56–70	Glaucoma Control	64.9 ± 10.1 91.6 ± 8.5	<0.001

Table IV

Pain Intensity at Follow-Up Intervals (n = 158).

Gender	Group	Mean RNFL (µm)	p-value
Male	Glaucoma Control	65.5 ± 9.4 91.8 ± 8.1	<0.001
Female	Glaucoma Control	66.7 ± 10.1 93.1 ± 8.6	<0.001

DISCUSSION

The results indicated that there is statistically significant difference in the RNFL thickness between glaucomatous and healthy eyes in favor of the long-standing appreciation that the destruction of the optic nerve structure goes before visual field functional degradation in glaucoma. The average of the RNFL thickness in case of glaucomatous eyes was found to be 66.02 ± 9.68 as compared to that of the normal controls which was 92.48 ± 8.28, and this implies that the normal RNFL is significantly reduced as a result of the glaucomatous

These results are in line with a number of recent studies. Rao et al. have given the mean RNFL thickness of 68.5 + 10.2 in glaucomatous and 95.1 + 9.3 in the controls, which is almost equal to those of the current study [11]. Likewise, Kim et al. reported a RNFL thickness of 64.3 -8.9 ug in glaucoma individuals versus 90.7 -7.6 ug in healthy individuals [12]. These studies point to the uniformity on which OCT-based RNFL measurement could serve as a diagnostic parameter in glaucoma.

Analysis in quadrants in the current study indicated best and worst quadrants had the most significant thinning. The superior RNFL thickness was 76.38 ± 14.45 µm in glaucomatous eyes compared to 111.96 ± 11.04 µm in controls, while inferior RNFL thickness was 55.24 ± 12.43 µm compared to 116.04 ± 10.43 µm ($p < 0.001$ for both). These results correspond to the past research findings that show that superior and inferior nerve fiber bundle is preferentially damaged in glaucomatous. Park et al. have reported the better and worse values of RNFL as 78.2 ± 0.131 0mm and 59.6 ± 0.118 mm, respectively, in glaucomata eyes [13]. Similarly, Garcia-Martin et al. found that poor RNFL thinning was the most sensitive and able to be detected at the earliest in glaucoma [14].

Preferential nature of superior and inferior quadrants can be attributed to organization of nerve fibers and their vulnerability to stresses of intraocular pressure. These areas have high density nerve fibers and are more susceptible to mechanical and vascular excess. Consequently, glaucomatous damage may develop locally in these regions, and, thus, OCT-based quadrant analysis would be especially helpful to promptly detect it.

However, the nasal and temporal quadrants experienced relatively smaller yet significant thinning during this study. The nasal RNFL thickness was 56.94 ± 12.94 µm in glaucomatous eyes versus 73.04 ± 8.15 µm in controls ($p < 0.001$), while temporal RNFL thickness was 49.04 ± 10.45 µm compared to 60.36 ± 13.33 µm ($p = 0.002$). These results agree with the temporal RNFL thinning findings provided by Lee et al. that were identified as being observed in later stages of glaucoma [15]. This implies that despite the influence of superior and inferior quadrants earlier, the presence of nasal and temporal regions indicate disease development.

A notable difference in intraocular pressure (IOP) between the two groups was also shown in the present study since the glaucomatous subjects had a mean IOP of 24.8 ± 3.6 mmHg as compared to the controls with 15.2 ± 2.8 mmHg ($p < 0.001$). This is in line with Zhang et al. who have claimed such differences between IOP in glaucomatous and normal subjects [16]. Whereas the elevated IOP is a known risk factor in glaucoma it is important to point out that the RNFL thinning can also take place in normal-tension glaucoma with a normal IOP thereby highlighting the role played by structural measurements made with OCT.

Analysis of stratification of age and gender in this study showed that there are no statistically significant variations of RNFL thickness in subgroups ($p > 0.05$). These results are in line with the findings by Singh et al., who indicated that age-associated deterioration of RNFL-thickness is less than the deterioration generated by glaucomatous lesions [17]. Likewise, no major differences in RNFL thickness were detected in males and females when factoring in disease status [18]. This implies that the RNFL thinning witnessed in this study is rather due to glaucomatous pathology than due to demographic factors.

The development of OCT technology has made a major contribution to the prospects of early detectability of structural alterations in glaucoma. Chen et al. showed that spectral-domain OCT was able to identify thinning of the RNFL, prior to the visual field defects and therefore, earlier diagnosis and intervention was possible [19]. This is especially significant since the percentage of cells in the retinal ganglion can be lost before it manifests itself in any functional impairment where traditional perimetry would be used to detect this condition.

Moreover, Bussel et al. emphasized that the OCT-based RNFL measures are highly sensitive and specific to determine the eyes with glaucomatous and normal cases [20]. This favors the application of OCT as a main diagnostic method in the assessment of glaucoma. The results of the current experiment also support this role, with significant differences in the thickness of RNFL observed in all quadrants.

Recent studies have also determined the clinical relevance of RNFL thickness thresholds. Morales et al. proposed an idea that the glaucomatous damage could be greatly diagnosed by the RNFL values less than 70 µm [21]. The mean of reports on RNFL thickness, in glaucomatous eyes, was 66.02 µm, which is lower than this threshold in the given study, as another confirmation that the results have diagnostic value.

Besides diagnosis, OCT is important in observation of the disease progression. As mentioned by Sharma et al., progressive RNFL thinning, especially in inferior quadrant is a predictable sign of glaucoma progression [22]. Equally,

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Belghith et al. showed that, longitudinal measurements of RNFL have a very strong correlation with functional decline in glaucoma patients [23]. Wu et al. also affirmed that future visual field loss can be predicted by the RNFL thinning, which is a crucial parameter in long-term monitoring [24].

The other notable use of OCT is in the diagnosis of pre-perimetric glaucoma. Mwanza et al. indicated that RNFL thinning was observed in patients with normal visual fields but with early structural damage, thus providing early intervention option [25]. This is especially applicable in clinical practice in instances where early diagnosis can go a long way in limiting the chances of partial loss of vision.

Irrespective of the strong points of the study, such as standardized measurements of OCT and clearly defined inclusion criteria, some limitations must be mentioned. The small size of the sample and the use of a single-center design could be a limitation to the external validity of the results. Also, the study is cross-sectional, which is why it is impossible to evaluate how diseases develop over time. Further research should be done on bigger samples and with longitudinal follow-ups to further support these results.

On balance, the findings of this work are well consistent with the current literature and support the significance of OCT in the diagnosis and treatment of glaucoma. The steady decrease in the RNFL thickness in glaucomatous eyes, especially in superior and inferior quadrants, demonstrates the importance of OCT as a good and non-invasive diagnostic medium. The fact that the results of this study are very close to that which has been reported in the recent literature, also validates the findings.

CONCLUSION

This paper finds that the retinal nerve fiber layer (RNFL) is much thinner in glaucomatous eyes than in normal healthy ones on the basis of Optical Coherence Tomography (OCT). The average RNFL in glaucoma was significantly less than controls with the superior and inferior quadrants showing the greatest thinning. Such results indicate the typical nature of optic nerve damage in glaucomatous disease and are in line with the known structural alterations that in turn lead to loss of functional visual field. Another conclusion derived by the findings is that OCT is a highly sensitive, reliable and non-invasive imaging modality, capable of quantitatively measuring RNFL thickness. It is a tool that is necessary in the early diagnosis of glaucoma even before signs of the condition or field defects have arisen due to its capability to identify early structural changes. Moreover, OCT has the potential to be successfully applied to track the progression of the disease and promote the development of timely therapeutic measures.

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