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Evaluation of Macular Thickness and Volume Using Optical Coherence Tomography in Preperimetric, Perimetric Early, Moderate and Advance Glaucoma

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ABSTRACT

Background: The objective of the study is to quantitatively compare the measured thickness and volume of macula by spectral domain OCT in glaucoma suspect & diagnosed case of glaucoma. Study: An observational study. Place and duration: Regional Institute of Ophthalmology, IGIMS, PATNA from January 2014 to January 2015. **Methods:** A total 110 eyes of 60 patientsof age between 40-80 years with Primary open angle glaucoma or labelled as glaucoma suspect were selected randomly who came as outdoor patient.Field analysis was done by automated Humphrey analyzer (carl zeiss), eyes were divided into four categories. 1. Preperimetric (30 eyes), 2. Perimetric early glaucoma (26eyes) 3. Moderate glaucoma (27 eyes)4. Advanced glaucoma (27 eyes). Subsequently all subjects underwent OCT testing of macular area for volume and thickness (inner and outer). The data of the preperimetric glaucoma were compared with perimetric early, moderate and advance glaucoma one by one using the unpaired t-test. The data were reported as mean ± standard deviation. A P value of less than 0.05 was considered statistically significant. **Results:** There were no clinically significant difference in the mean macular volume between preperimetric glaucoma and perimetric early glaucoma, however it was significant with moderate and advance glaucoma. Like macular volume the difference of the mean inner and outer macular thickness in all perimacular quadrants of preperimetric glaucoma and perimetric early glaucoma was not statically significant however it was statically significant in inner macular thickness (in all perimacular quadrants) and outer inferior and superior quadrant if compare preperimetric glaucoma and perimetric moderate and advanced glaucoma. **Conclusion:** the role of macular parameters such as macular volume and macular thickness in the diagnosis of early glaucoma is limited. Significant difference in macular volume and thickness in advanced glaucoma suggest that it may be useful method of documenting progression.

Keywords: *Glaucoma, Macular thickness, Macular volume, OCT & HFA.*

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness in the world. In 2013, glaucoma was estimated to affect 64.3 million people 40-80 years-of-age, with this number increasing to 76.0 million by 2020 and 111.8 million by 2040.[1]

Glaucoma is a chronic progressive optic neuropathy characterised by a loss of retinal ganglion cells (RCG) which result in visual impairment.[2-4] Clinically it is diagnosed by observing optic disc changes as characteristic cupping (Glaucomatous optic atrophy) and measurement of visual field by perimetry. Perimetry changes appear late when there is already loss of 20% - 40% ganglion cells.[4] Both perimetry and observation of optic disc changes are subjective examinations that are prone to variability.

Since the development of Optical Coherence Tomography (OCT) by Huang (1991), evaluation of early glaucomatous change has focused mostly on optic disc and peripapillary retinal nerve fibre layer (RNFL) changes. OCT provide an objective and quantitative measurement of RNFL thickness.

However, glaucomatous damage is primarily due to retinal ganglion cells, which are particularly abundant in the perimacular region, where ganglion cells are arranged in 4-6 layer making up to 30- 35 % of retinal macular thickness.[6]

Macular Ganglion cells complex (GCC):

It is defined as three inner most retinal layer.

1. RNFL – made up of ganglion cells axon
2. Ganglion cells layer (GCL) made up of ganglion cell bodies &
3. The inner plexyform layer (IPL) made up of ganglion cells dendrites.

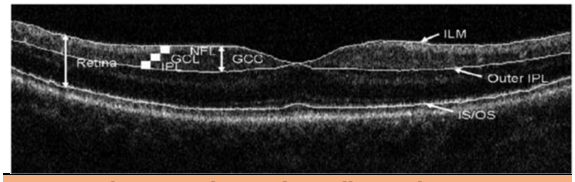

Figure 1: Shows macular ganglion cells complex.

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Topography of ganglion cells densities– polyak (1941),[46] divided human retina in to two regions on the basis of ganglion cells layer thickness: the central area, a foveocentric region 6 mm diameter where the ganglion cell layer was more than one cell deep, and a remaining peripheral retina, where ganglion cells formed single continuous or broken layer.

Central retina: the highest ganglion cells densities are found in a horizontally oriented, elliptical ring that at half height extend from 0.4 – 2.0 mm from the foveal center. Ganglion cells density is about 15% higher in nasal retina than at equivalent eccentricities in temporal retina from 0.4 – 2.0 mm eccentricity, but from 2 – 4 mm nasal and temporal retina have equal density. Densities along the vertical meridian are equal in superior and inferior retina from 0.4 – 2.0 mm, but by 4 mm eccentricity superior retina has 65% higher density than inferior retina.

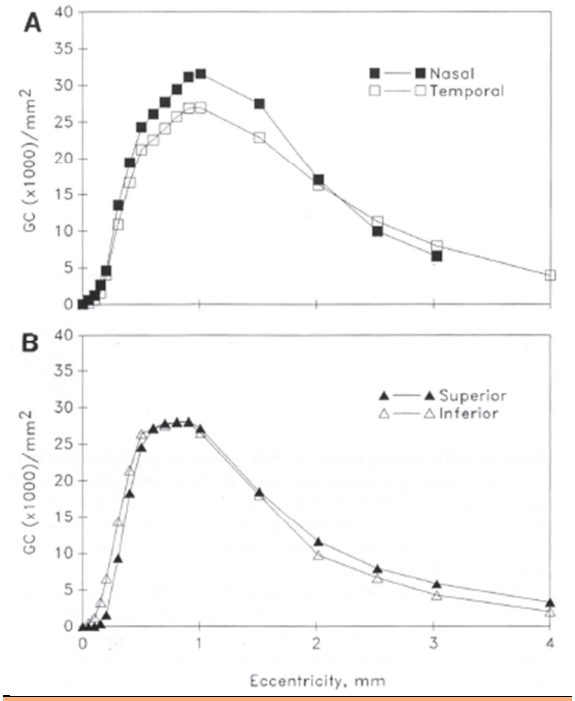

Figure 2: Shows Topography of ganglion cells densities in central (foveocentric) retina of 4 mm diameter.

Tan and colleagues suggested that glaucoma likely preferentially affects these layers,[5] rather than all macular layers, because they contain the axons, cell bodies, and dendrites of ganglion cells. This idea is supported by research findings that photoreceptors do not seem to be lost in glaucoma.[6]

Zeimer and colleagues first suggested that, since a significant portion of retinal ganglion cells (RGCs) reside in the macula, a loss of tissue in this region might be a sign of glaucomatous damage, and ganglion cell death could potentially result in a reduction of retinal macular thickness and volume.

Thus three layers of ganglion cell complex are slightly thinner in glaucoma patients due to death of ganglion cells.

The purpose of this study is to compare the measured thickness and volume of macula by SD-OCT (spectral domain-OCT) in open angle glaucoma patients in relation with the field loss on HFA.

MATERIALS AND METHODS

Study Design:

Types of study: Prospective, observational study.

Sample size and Place: A total 110 eyes of 60 patients were selected randomly who came as outdoor patient at Regional Institute of Ophthalmology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar.

Inclusion Criteria:

Age between 40-80 years with Primary open angle glaucoma or labelled as glaucoma suspect.

Exclusion Criteria:

Patient with a history of ophthalmic surgery (including refractive surgery), ophthalmic trauma or inflammation, and any form of macular pathology or any diagnosed neurological condition, diabetic and hypertensive retinopathy.

Stage 1 : Early defect **Mean deviation (MD) ≤ –6.00 dB and at least one of the following:**	
A	On pattern deviation plot, there exists a cluster of 3 or more points in an expected location of the visual field depressed below the 5% level, at least 1 of which is depressed below the 1% level
B	Corrected pattern standard deviation/pattern standard deviation significant at P < 0.05
C	Glaucoma hemifield test "outside normal limits"
Stage 2: Moderate Defect **MD of –6.01 to –12.00 dB and at least one of the following:**	
A	On pattern deviation plot, greater than or equal to 25% but fewer than 50% of points depressed below the 5% level, and greater than or equal to 15% but fewer than 25% of points depressed below 1% level
B	At least 1 point within central 5° with sensitivity of < 15 dB but no point within central 5° with sensitivity of < 0 Db
C	Only 1 hemifield containing a point with sensitivity < 15 dB within 5° of fixation
Stage 3: Advanced Defect **MD of –12.01 dB to –20.00 dB and at least one of the following:**	
A	On pattern deviation plot, greater than or equal to 50% but fewer than 75% of points depressed below the 5% level and greater than or equal to 25% but fewer than 50% of points depressed below 1% level
B	Any point within central 5° with sensitivity of < 0 Db
C	Both hemifields containing a point(s) with sensitivity < 15 dB within 5° of fixation

All subjects underwent general and systemic examination, through baseline ophthalmic evaluation, including medical history, Best Corrected Visual Acuity (BCVA), Intra Ocular Pressure (IOP) measurement by Goldmann Applanation Tonometry (corrected according to central corneal thickness), gonioscopy, undilated and dilated Slit Lamp Biomicroscopy using +90 Diopter lens.

Visual field testing

All subjects underwent automated Humphrey (Carl Zeiss meditecinc. Dublin, CA) visual field (24-2) white on white perimetry examination. A reliable VF test was defined as one with fewer than 30% fixation losses, false positive or false negative responses. Normal VF test results were defined as having no cluster of three or more adjacent points depressed more than 5 dB or two adjacent points depressed more than 10 dB in the pattern deviation plot visual field by humphrey field analysis (HFA). Abnormal VF test was defined as a cluster of abnormal points and classified as (Yanoff & Duker, table 10-5-3 page 1130)

The patient eyes which does not fulfill the diagnostic criteria of Humphrey included in preperimetric group, rest were divided on the basis of mean deviation (MD) in to early, moderate and advanced glaucoma

1. Preperimetric (30 eyes),
2. Perimetric early glaucoma (26eyes)
3. Moderate glaucoma (27 eyes)
4. Advanced glaucoma (27 eyes).

Subsequently all participants underwent OCT testing (3D OCT-2000, Topcon) of macular area for volume and thickness. For this purpose Fast Map software used which incorporates the latest layer detection algorithms, allowing automatically measurement of retinal thickness of Fovea, Inner macular thickness, and Outer macular thickness.

Spectral Domain (SD) OCT.[8-11&31] Were used for this purpose (3D OCT-2000, Topcon).Since sectoral loss occurs more often in the earlier stages of glaucoma, Macular layer thickness and volume were calculated globally, as well as sectorally, in the sectors indicated in figure. Global measurements are taken inside the outermost circle, the outer circle has a diameter of 6 mm, the middle circle has a diameter of 3 mm, and the innermost circle has a diameter of 1 mm (fovea).

Three concentric circles divide the macular thickness map in to three zones: [Figure 3]

1. Fovea (1 mm)
2. Inner macula (3 mm)
3. Outer macula (6 mm)

The inner and outer zones are further divided in to four quadrants by two diagonal lines. Thus a total of nine areas (fovea, inner-inferior, inner-superior, inner-nasal, inner-temporal, outer-inferior, outer-superior, outer-nasal, outer-temporal) are available for analysis.

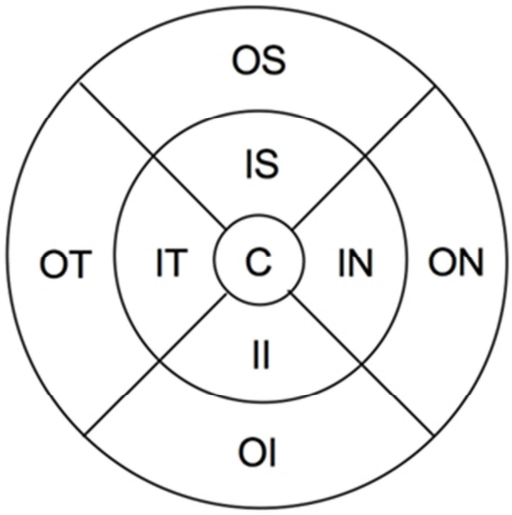

Figure 3: Shows 3 concentric (foveocentric) circle of 1 mm, 3 mm and 6 mm diameter respectively.

RESULTS

The study included 110 eyes of 60 patients with a mean ±SD age of 61±9 years (range 42- 76 years). Twenty nine patients were female and 31 male.

Table 1: Showing Age distribution of patients

Age Group (in yrs)	Age distribution				
	40-50	51-60	61-70	71-80	Total
Male	9	6	14	2	31
Female	3	6	14	6	30
Total	12	12	28	8	60

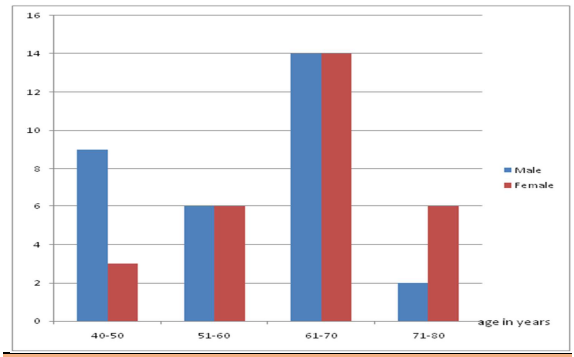

Figure 5: Bar chart showing age distribution of the patients.

There were 12 patients (9 male, 3female) in between age group of 40-50 years, 12 patients (6 male & 6 female) in between age group of 51-60 years, 28

patients (14 male & 14 female) in between age group of 61-70 years, 8 patients (2 male & 6 female) in between age group of 71-80 years.

Mean deviation (MD) of HFA was analysed and on the basis mean deviation of HFA result 110 Eyes was divided in to four categories.

Table 2: Showing Mean of Mean deviation (MD) of HFA 24-2 of category 1,2,3 and 4.

	Mean deviation			
CATAGORY	**CAT _1**	**CAT_2**	**CAT_3**	**CAT_4**
Mean±SD	-2.76±0.91	-4.45 ± 1.10	- 8.97± 2.00	- 15.49 ± 6.15

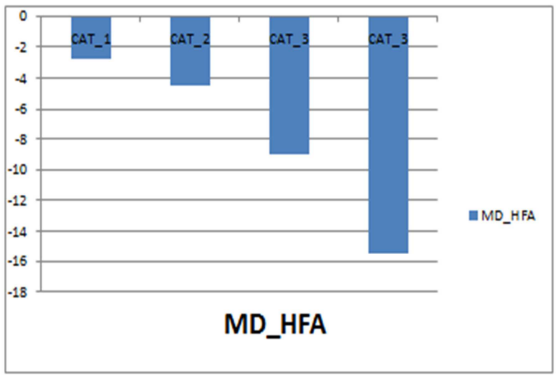

Figure 6: Bar chart showing mean of mean deviation (MD) of HFA (24-2) of category 1, 2, 3 & 4.

The mean of mean deviation (MD) and standard deviation (SD) of HFA (24:2) of category 1, 2, 3, & 4 were -2.76±0.91, -4.45 ± 1.10, - 8.97± 2.00, & - 15.49 ± 6.15. respectivelly.

Macular Volume

The mean macular volume (mm3) of category 1, 2, 3 & 4 were 7.24 ± 0.35, 7.02 ± 0.47, 6.71±0.42, & 6.12±0.47 mm3 respectively.

Table 3: Macular Volume Analysis (Mean ± SD) of Category 1, 2, 3, & 4.

	Macular volume			
Catagory	**CAT _1**	**CAT_2**	**CAT_3**	**CAT_4**
Mean±SD	7.24±0.35	7.02±0.47	6.71±0.42	6.12±0.47

The unpaired t-test was used to compare the mean macular volume of category 1(preperimetric glaucoma) with the mean macular volume of category 2, 3, & 4 (perimetric early, moderate and advance glaucoma)

Table 4: Showing unpaired t-test of category 1 with category 2, 3, &4.

Unpaired t-test	**CAT 1&2**	**CAT1&3**	**CAT1&4**
p-value	0.0538	0.0001	0.0001
Statistically significant?(<0.005)	No	Yes	Yes

The difference of mean macular volume of category 1 with category 2 was not statistically significant (unpaired t Test $p > 0.05$), however it was significant if compare mean macular volume of category 1 with category 3 and category 4 (unpaired t Test $p < 0.05$).

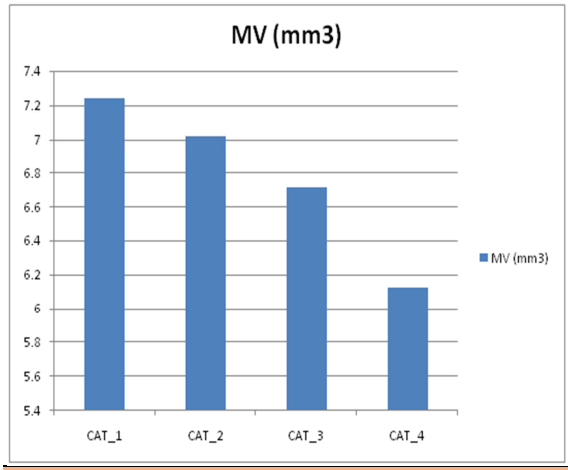

Figure 7: Bar chart showing mean macular volume of category 1, 2, 3, & 4.

The mean macular volume (mm3) of category 1, 2, 3 & 4 were 7.24 ± 0.35, 7.02 ± 0.47, 6.71±0.42, & 6.12±0.47 mm3 respectively.

Macular Thickness

The mean of inner and outer macular thickness parameters in all eight quadrants (inferior, superior, nasal, & temporal) of category 1, category 2, category 3 &category4 are expressed in table.

Table 5: Macular Thickness Analysis (Mean±SD) of Category 1, 2, 3, and 4.

Macular thickness (µm)	**CAT_1 Mean± SD**	**CAT_2 Mean± SD**	**CAT_3 Mean± SD**	**CAT_4 Mean± SD**
IMT_I	259±15.4	257.7 ±9.0	241.3±6.6	223.1±4.8
IMT_S	263.7±12 .2	256.4 ±15.6	238.9±6.7	222.5±3.9
IMT_N	266.4±5. 7	263.2 ±10.3	259.1±8.5	254.3±8.7
IMT_T	251.5±7. 5	248.9 ±5.0	239.1±6.6	222.0±3.9
OMT_I	221.2±14 .8	221.9±17. 3	211.9±19. 2	192.6±9.4
OMT_S	232.6±12 .2	227.5±13. 5	221.7 ±14.6	213.2±17.2
OMT_N	247.8±7. 1	247.0 ±10.9	247.7±15. 7	240.2±13.0 9
OMT_T	215.5±10 .0	215.4 ±13.0	210.4±21. 1	206.9±20.3

Figure 8: Bar chart showing inner macular thickness of category 1, 2, 3 & 4.

The mean inner macular thickness in inferior, superior, nasal and temporal of category 1 were 259 ±15.4, 263±12.2, 266.4±5.7, 251.5±7.5 respectively, category 2 were 257.7 ±9.0, 256.4 ±15.6, 263.2 ±10.3, 248.9 ±5.0 respectively, category 3 were 241.3±6.6, 238.9±6.7, 259.1±8.5, 239.1±6.6 respectively, category 4 were 223.1±4.8, 222.5±3.9, 254.3±8.7, 222.0±3.9 respectively.
[IMT_I = Inner macular thickness Inferior, IMT_S = Inner macular thickness Superior, IMT_N = Inner macular thickness Nasal, IMT_T = Inner macular thickness Temporal]

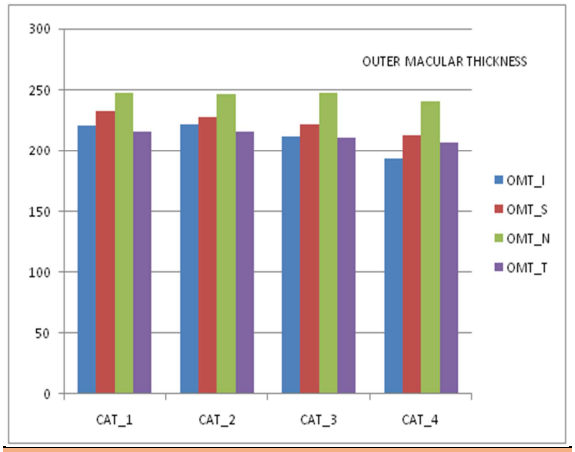

Figure 9: Bar chart showing outer macular thickness of category 1, 2, 3 & 4.

The mean outer macular thickness in inferior, superior, nasal and temporal of category 1 were 221.2±14.8, 232.6±12.2, 247.8±7.1, 215.5±10.0 respectively, category 2 were 221.9±17.3, 227.5±13.5, 247.0 ±10.9, 215.4 ±13.0 respectively, category 3 were 211.9±19.2, 221.7 ±14.6, 247.7±15.7, 210.4±21.1 respectively, category 4 were 192.6±9.4, 213.2±17.2, 240.2±13.9, 206.9±20.3 respectively.
OMT_I = Outer macular thickness Inferior, OMT_S = Outer macular thickness Superior, OMT_N = Outer macular thickness Nasal, OMT_T= Outer macular thickness Temporal]

Table 6: Shows Unpaired t-Test P Value and it significances

Unpaired t-Test	P –Value					
	CAT 1&2	Statistically significant	CAT 1&3	Statistically significant	CAT 1&4	Statistically significant
IMT_I	0.0227	No	0.0001	Yes	0.0001	Yes
IMT_S	0.0034	No	0.0001	Yes	0.0001	Yes
IMT_N	0.1578	No	0.0004	Yes	0.0001	Yes
IMT_T	0.1337	No	0.0001	Yes	0.0001	Yes
OMT_I	0.0004	No	0.0002	Yes	0.0001	Yes
OMT_S	O.0318	No	0.0025	Yes	0.0001	Yes
OMT_N	0.7577	No	0.9677	No	0.2626	Yes
OMT_T	0.9901	No	0.2468	No	0.0459	Yes

The unpaired t-test was used to compare the inner and outer macular thickness of category 1(preperimetric glaucoma) with category 2, category 3, &category 4 (perimetric early, moderate and advance glaucoma).
The difference of the mean inner and outer macular thickness in all perimacular quadrants of category 1with category 2wasnot statically significant (unpaired t test $p > 0.05$), however it was statically significant in inner macular thickness (in all perimacular quadrants) and outer inferior and superior quadrant if compare category 1with category 3, and category 4 (unpaired t test $p < 0.05$)
There were no statistical differences of the mean outer macular thickness in nasal and temporal quadrant of category 1 compare with category 2 and category 3 ($p > 0.05$), however it was statically significant if compare with category 1 and category 4. (Unpaired t test $p < 0.05$).

DISCUSSION

The macula includes the region surrounding the fovea with the highest density of retinal ganglion cells (RGCs). While this area represents less than 2% of the retinal area, it contains over 30% of the RGCs (Curcio and Allen, 1990). Glaucomatous optic neuropathy results in death of these retinal ganglion cell, which are more densely populated in the macular region.

Several studies have reported a decrease in macular retinal thickness and volume in established glaucoma.[36-40]

The ganglion cell analysis is a relatively recent concept that helps in measuring in an indirect manner the quantity of ganglion cells in the retina. The ganglion cell thickness is shown to correlate with the retinal nerve fiber layer (RNFL) loss, visual field loss, contrast sensitivity function, cup:disc ratio and relative afferent pupillary defect.[32-34] Ganglion cell layers analysis (thickness and volume) on OCT has been shown to have good reproducibility and may be used for long term follow up. In cases of parafoveal visual field loss, ganglion cell thickness measurement has shown better ability to diagnosed glaucoma than RNFL changes.[35]

David E Lederer et al,[27] assessed 272 eyes of 164 subjects as part of an institutional study at New England Eye Center in Boston, Massachusetts; 202 eyes were in the study group and 70 eyes in the control group. They found decreasing macular volume in eyes with more advanced disease.

Giovannini A et al,[28] evaluate macular volume in normal and glaucomatous eyes using Optical Coherence Tomography (OCT) and concluded OCT tomograms may be a useful method of documenting and monitoring patients with early glaucoma and advanced glaucoma.

Our data also shows the mean macular volume (mm3) of category 1, 2, 3 & 4 (preperimetric, perimetric early, moderate and advance glaucoma) as7.24 ± 0.35, 7.02 ± 0.47, 6.71 ± 0.42, & 6.12 ± 0.47 mm3 respectively. The difference of mean macular volume of preperimetric with perimetric early was not statistically significant (unpaired t Test $p > 0.05$), however it was significant if compare mean macular volume of preperimetric with perimetric moderate and advanced glaucoma (unpaired t Test $p < 0.05$). However there is reported study Parikh R. et al (BJO) 44where he reported out inferior thickness and volume in early glaucoma are significantly differ from the normal , further he states that, moderate sensitivity and specificity suggest that role of macular parameter in diagnosis of early glaucoma is limited.

In our study we found out there were no clinically significant difference in the mean macular volume between preperimetric glaucoma and perimetric early glaucoma (unpaired t Test $p > 0.05$), however it was significant with moderate and advanced glaucoma.

Significant difference in macular volume between preperimetric and perimetric advanced glaucoma correlates with a trend of decreasing macular volume in eyes with more advanced disease.

Like macular volume the difference of the mean inner and outer macular thickness in all perimacular quadrants of preperimetric glaucoma and perimetric early glaucoma was not statically significant (unpaired t Test $p > 0.05$). However it was statically significant in inner macular thickness (in all perimacular quadrants) and outer inferior and superior quadrant if compare preperimetric glaucoma and perimetric moderate and advanced glaucoma (unpaired t Test $p < 0.05$).

There were no statistical differences of the mean outer macular thickness in nasal and temporal quadrant of preperimetric glaucoma and perimetric early or moderate glaucoma; it was statically significant only with advanced glaucoma (unpaired t Test $p < 0.05$).

Arvanitaki V et al,[18] evaluated macular retinal thickness (RT) and retinal nerve fiber layer thickness changes in early glaucoma using optical coherence tomography and concluded retinal thickness was significantly lower in early manifest glaucoma patients and glaucoma suspects indicates that the transposition of OCT fast RNFL thickness (3.4) protocol from peripapillary area to the perimacular area can be used for early glaucoma diagnosis. Intra retinal changes in early glaucoma, likely precede nerve fiber changes.

However in our study the difference of the mean inner and outer macular thickness in all perimacular quadrants of preperimetric glaucoma and perimetric early glaucoma was not statically significant. It is significant only in moderate to advanced glaucoma. Therefore not supporting the above published study.

Present study shows significant reduction in superior and inferior quadrant of inner and outer macular thickness if compare with preperimetric glaucoma and perimetric moderate and advance glaucoma, which is partially matched with previous studies.[43,44]

Tan O et al,[21] evaluated segmental macula and reported reduced thickness in inner retinal layer in glaucomatous eyes was maximal for the inferior perimacular quadrant whereas other study by Vassiliki Arvanitaki et al,[18] shows significant reduction in superior quadrant.

Focal reduction in retinal thickness in the superior or inferior segments is more than temporal and nasal possibly reflects the retinotopic distribution of the affected ganglion cells.

There were few studies which were specially done to show correlation between macular thickness/ volume and glaucoma status.

Greenfield DS et al,[25] in his study of 59 eyes of 59 patients found that Macular thickness changes are well correlated with changes in visual function and RNFL structure in glaucoma and may be a surrogate indicator of retinal ganglion cell loss.

Viviane Guedes et al,[41] in their cross sectional OCT study of total 534 eyes of macular and RNFL thickness in normal and glaucomatous eyes concluded that macular thickness may be used as an additional parameter in clinical assessment of glaucoma.

FN Kanadani et al,[42] in their study of glaucoma patients studied macular parameters using OCT and correlated the same with visual field changes and multifocal visual evoked potential (mf VEP) of macular area. They found good correlation between assessment of structural changes of macula on OCT with functional changes of visual field and mfVEP.

Furthermore previous studies also reported that retinal nerve fiber layer thickness measurement obtained by stratus OCT may display significant variability especially early and moderate glaucoma.[45] The fact that retinal thickness reduction in the early manifest glaucoma group and glaucoma suspects was much more pronounced than RNFLT reduction in all perimacular quadrant imply that intra retinal changes may precede retinal nerve fiber changes in early glaucoma.

The results of this study imply that the role of macular parameters such as macular volume and macular thickness in the diagnosis of early glaucoma is limited. Significant difference in macular volume and thickness in advanced glaucoma suggest that it may be useful method of documenting progression

The limitations of our study are that sample was small. Age and sex control match was not done. Further study with large sample size and proper age and sex match may be required to validate our finding.

CONCLUSION

So our study concludes that the role of macular parameters such as macular volume and macular thickness in the diagnosis of early glaucoma is limited. Significant difference in macular volume and thickness in advanced glaucoma suggest that it may be useful method of documenting progression. It can be used in addition to RNFL thickness to aid in the diagnosis of moderate and advanced glaucoma especially in certain condition like disc abnormality and peripapillary atrophy.

The limitation of our study is small sample size and not taking into account a normative age/sex matched data. Further study in this regard is required to validate the findings.

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