International Journal of Medical Ophthalmology

E-ISSN: 2663-8274
P-ISSN: 2663-8266
www.ophthalmoljournal.com
IJMO 2021; 3(2): 39-46
Received: 23-05-2021
Accepted: 26-06-2021

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The correlation between corneal endothelial cell density, central corneal thickness and retinal nerve fibre layer thickness in glaucoma suspects and newly detected primary glaucoma patients.

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DOI: https://doi.org/10.33545/26638266.2021.v3.i2a.89

Abstract
Purpose: To correlate corneal endothelial cell density, central corneal thickness and retinal nerve fiber layer thickness in glaucoma suspects and newly detected primary glaucoma patients.
Patients and Methods: This duration-based, case-control study included 219 patients of which 80 were glaucoma suspects, 60 primary open- angle glaucoma and 79 were controls. The data was collected at the department of ophthalmology at Vydehi Institute of Medical Sciences and Research Center, Bangalore. Specular microscopy and Optical Coherence Tomography were performed on each patient and analysed.
Results: The mean endothelial cell density showed a significant difference in glaucoma suspects and primary open angle glaucoma in comparison with controls. The CCT showed only a minimal difference between the three groups. The comparative analysis of RNFL in controls, glaucoma suspects and primary open angle glaucoma showed that primary open angle glaucoma showed the highest thinning among the three groups. The mean value between the controls, glaucoma suspects and primary open angle glaucoma was significant with a p value <0.001.
Conclusion: Eyes with lower ECD were found to have thinner RNFL and Larger CDR in glaucoma suspects and newly detected cases of primary open angle glaucoma and the rate of RNFL and endothelial cell damage was directly proportional to increasing IOP. The CCT in the three groups showed only minimal difference. Thus, corneal parameters like ECD and CCT when used in combination with RNFL thickness are paramount in predicting the chances of future development of progressive glaucomatous optic neuropathy among glaucoma suspects and newly detected primary open angle glaucoma patients.

Keywords: Endothelial cell density, central corneal thickness, retinal nerve fiber layer thickness, specular microscopy, optical coherence tomography, primary open angle glaucoma, glaucoma suspects.

Introduction
Glaucoma is a progressive disease that causes irreparable damage before there is any vision loss, hence it is referred to as 'silent thief of the night'.
Thus, prevention and treatment has been a major focus of international directives including the World Health Organization's Vision 2020 campaign as blindness and vision impairment have a major impact on local and national economies. In developing countries like ours, blindness removes two people from the workforce: the blind patient, and a family member to care of the patient [1]. With an earlier accurate diagnosis and timely therapy, the goal for this century should be to prevent glaucoma-related blindness.
Glaucoma, a progressive optic neuropathy, causes damage to the optic disc, retinal nerve fibre layer (RNFL) and visual field. The most common type of glaucoma worldwide as per population-based studies is primary open angle glaucoma (POAG). Detecting the structural and functional changes is one of the most challenging aspects of glaucoma management [2]. Corneal involvement, especially Corneal Endothelial cell loss and Central corneal thickness (CCT), has grown to become a topic of great interest in the field of glaucoma. Corneal endothelial cells play an important role in maintaining corneal clarity by deturgescence, but the Human Corneal Endothelial Cells (HCEC) are non-regenerative and when they reach a critical limit to no longer be able to provide adequate deturgescence, the cornea becomes cloudy leading to decreased visual acuity [3].

Many factors affecting the HCEC in glaucoma patients are direct damage due to elevated intraocular pressure (IOP), pre- existing congenital changes, ocular surgery, and ocular trauma. Several studies reported a 31% reduction in ECD in primary open-angle glaucoma (POAG) patients compared with a normal control group [4]. It is believed that elevated intraocular pressure (IOP) causes decrease in ECD in eyes with glaucoma but it is not yet known if it also has an impact on glaucoma suspects, there has been little discussion on this in previous literature. A study done among glaucoma suspects with ocular hypertension, also showed lower corneal endothelial cell counts.5 The RNFL thinning seen on spectral domain OCT is a known risk factor in the progression of glaucoma but its association with the corneal changes needs further detailed evaluation. Hence, visualisation and study of HCEC is paramount in the complete assessment of a glaucoma suspect patient, as endothelial cell loss may be a significant risk factor [3].

The relationship between the changes in RNFL thickness and ECD among glaucomatous patients has been studied by Daisy Rani Das and Dipali Choudhary Deka in 2015 and revealed that lower ECD correlates well with thinner RNFL in primary glaucoma [2].

In patients with established diagnosis of glaucoma, evidence of progression influences the clinician's decision to modify glaucoma therapy, and in a glaucoma suspect, progression of the disease confirms the diagnosis and helps to decide the management.

Understanding the relationship may provide a new insight into the role of corneal ECD, CCT and RNFL thickness in early diagnosis and treatment of primary glaucoma and its suspects as well as aid in developing and administering a targeted treatment to the affected patients.

Objectives

To assess the association between ECD and mean IOP in newly detected cases of primary glaucoma and glaucoma suspects and correlate corneal ECD and central corneal thickness in relation to changes in RNFL thickness as well as to assess whether corneal ECD, CCT and RNFL thickness are predictable factors in glaucoma suspects.

Materials and methods

A duration-based case-control study was conducted which analysed 219 patients, i.e. 436 eyes, of which 80 were glaucoma suspects, 60 primary open angle glaucoma patients, and a sample of 79 participants as the control group. All patients had visited the out-patient department at Vydehi Institute of Medical Sciences and Research Center, Bangalore between 2016 and 2017 and were recruited after satisfying the inclusion and exclusion criteria. After receiving ethical approval from the Vydehi Institutional Ethical Committee, a pre-structured proforma was used to collect the baseline data and an informed written consent was obtained after explaining the need for the study.

Inclusion Criteria

All patients who were either newly detected cases of primary open angle glaucoma or glaucoma suspects.

Exclusion Criteria

(1.) Angle closure glaucoma Secondary glaucoma (2.) Contact lens wearers (3.) Myopes (4.) Diabetes mellitus (5.) History of corneal diseases (Fuch's dystrophy, central guttae) (6.) Uveitis (7.) Prior use of any antiglaucoma drugs (8.) Past history of ocular inflammation (9.) Ocular trauma (10.) Ocular surgery (PI, trabeculectomy, cataract surgery) (11.) History of laser treatment

Method of collection of data

Using the Preferred practice Patterns by AAO the patients were categorised into primary glaucoma and glaucoma suspects as follows: A glaucoma suspect was defined as an adult who had one of the following findings in at least 1 eye:

- An optic nerve or nerve fiber layer defect suggestive of glaucoma (enlarged cup–disc ratio, asymmetric cup–disc ratio, notching or narrowing of the neuro-retinal rim, a disc hemorrhage, or suspicious alteration in the nerve fiber layer)
- A visual field abnormality consistent with glaucoma (paracentral, arcuate or siedle's scotoma or a nasal step) an elevated IOP of more than 21 mm Hg.

Usually, if 2 or more of these findings are present, the diagnosis of primary glaucoma is supported, especially in the presence of other risk factors, such as age older than 50 years, family history of glaucoma, and black race. Diagnosis of a glaucoma suspect is also dependent on a normal open angle on gonioscopy.

Investigations

The following investigations and interventions were conducted on all participants:

1. Visual acuity was recorded with Snellen's chart.
2. Best corrected visual acuity was performed at a single sitting by the same vision examiner in the same room with standardized low-light conditions.
3. Slit lamp examination was done pre and post dilatation of the pupil.
4. Direct and indirect ophthalmoscopy, and 78D lens to view the posterior segment.
5. Specular microscopy for measuring endothelial cell density and central corneal thickness using the Model-Konan Cell check SL Premier Endothelial Analytics.
   Konan Cell Chek SL quantitatively analyzes the information and generates four numeric indices: cell density (CD), coefficient of variation (CV), percentage of hexagonal cells (HEX), number of cells used to calculate the results (NUM) and pachymetry (Pach). The first three indices are useful for measuring endothelial cell death. CD is a measurement of endothelial cell density in mm2 CV represents the coefficient, or degree, of variation in the sizes of the endothelial cells (polymegethism). A CV less than 40 is normal.
   HEX indicates the variability in hexagonal cell shape over time. A value above 50% is suggested to be normal.
   Pachymetry was done using the same non-contact specular microscope. This lowered the risk of infection and corneal epithelial damage or erosion. The average of three consecutive measurements was used for statistical analysis. Mean value of normal CCT for this machine was considered to be 554.78+/- 32.61 [6].
6. Optical coherence tomography of RNFL thickness was performed using Stratus OCT3; Carl Zeiss Meditech in a dim room after cycloplegia. The pupils were dilated to at least 5 mm diameter. The same operator performed the OCT examination. The scans only with signal strength > 7 were included in this study.
7. Glaucoma hemifield test (GHT) using Humphrey's 24-2

visual field analysis was done by the same perimetrist.

8. Goldmann Applanation Tonometry for measuring intraocular pressure was used. In the glaucoma patient groups, IOP was measured three times a day (9:00 a.m., 1:00 p.m., 4:00 p.m.). A single IOP reading of more than 21 mmHg resulted in a diagnosis of POAG or its suspects. Modified Ehler's correction factor was used to arrive at the corrected IOP value.
9. Gonioscopic examination with Zeiss Goldmann lens to assess anterior chamber angles and graded them using Shaffer gonioscopic classification of anterior chamber angle.

Statistical Methods

The statistical analysis was performed by STATA 11.2 (College Station TX USA). Shapiro-wilk test has been used to check normality. Analysis of variance were used to find the significance difference between the age, CD ratio, Intra ocular pressure, Cell density, coefficient of variation, hexagonality, central corneal thickness, RNFL thickness with the groups (Normal, Glaucoma suspect and POAG) respectively and these are expressed as mean and standard deviation. Chi square test were used to measure the association between the gender, family history and family history with groups respectively and these expressed as frequency and percentage. p value <0.05 considered as statistically significant.

Results

This study was conducted in Vydehi Institute of Medical Sciences and Research Center, Whitefield, Bangalore on the subjects who visited the outpatient department of Ophthalmology from January 2016 to June 2017. This was a duration based, case-controlled study of diagnosed patients of primary open glaucoma and glaucoma suspects which took into consideration a sample of sixty and seventy-nine patients respectively for the case group and a sample of eighty participants as the control group, all cases satisfied the inclusion and exclusion criteria.

The age wise distribution of patients as analysed showed a maximum number of patients (36.71%) in control group were above 60 years, (36.25%) in Glaucoma suspect group were between 41-50 years and (36.67%) in POAG group were 51-60 years of age. A gender-wise showed forty-five patients (57%) males and thirty-four (43%) females in control group, fifty-four (68%) males and twenty- six (32%) females in glaucoma suspect group and forty- two (70%) males and eighteen (30%) females in POAG group. Only the case study group showed family history in 6 patients (8%) among glaucoma suspects and 3 patients (5%) in POAG group. The rest had no significant family history of glaucoma. There was no significant difference in gender or family history between the three groups.

The mean CDR between the groups was 0.20-0.50 with a mean of 0.34 ± 0.08, in the control groups. 0.20 – 0.80 with a mean of 0.67 ± 0.09 in the glaucoma suspects, and among POAG it was between 0.40 – 0.90 with a mean of 0.68 ± 0.10. There was a significant difference between the CDRs among the three groups with POAG higher than the other two groups.

Table 1: Mean cIOP between the groups

Control		Glaucoma suspect (In mean ± SD)	POAG	p- value
Mean cIOP (mmHg)	14.99 ±2.06	17.09 ± 3.74	23.53 ± 2.99	<0.001
Range	11-21	10-24	14-36	

Table 2: Mean CDR between the groups

Control (mean± SD)		Glaucoma Suspect (mean ± SD)	POAG (mean ± SD)	p- Value
CD Ratio	0.34 ± 0.08	0.67 ± 0.09	0.68 ± 0.10	<0.001
Range	0.20 – 0.50	0.20 – 0.80	0.40 – 0.90	

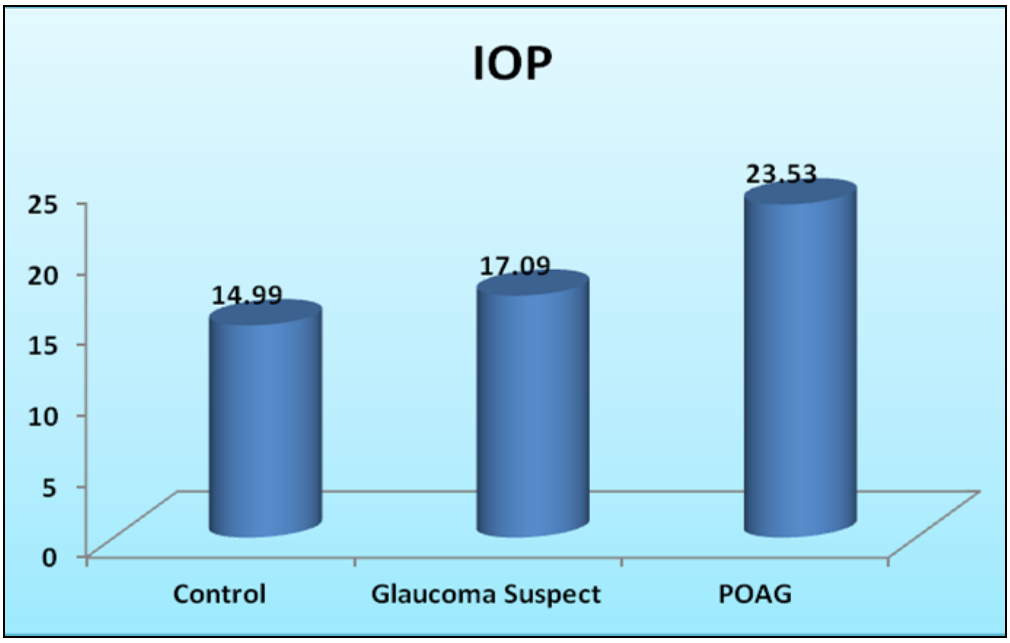

Fig 1: Mean cIOP between the groups

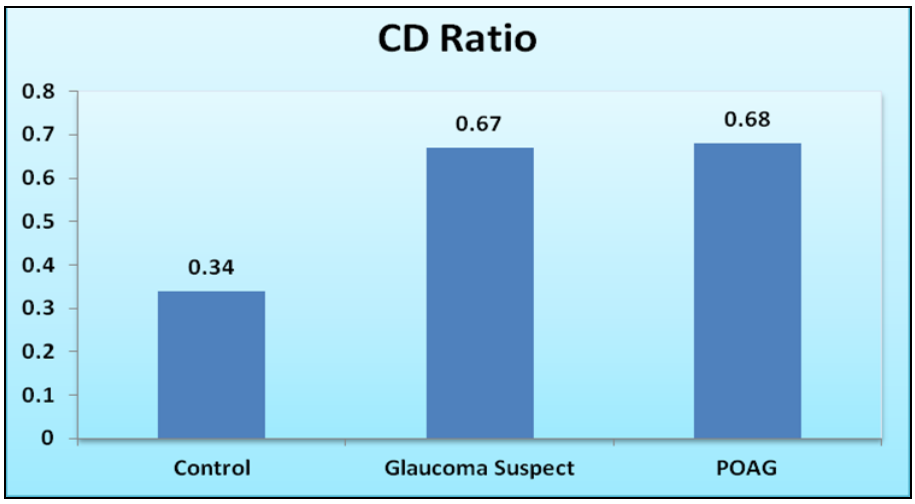

Fig 2: Mean CDR between the groups

Table 2 shows the mean corrected IOP between the groups. cIOP in the control group was 14.99 ± 2.06 with a minimum IOP of 11 mm Hg and maximum IOP of 21 mm Hg. The glaucoma suspects group had a mean of 17.09 ± 3.74 with a minimum IOP of 10 mm Hg and maximum IOP of 24 mm Hg. The POAG group had a mean of 23.53 ± 2.99 with a minimum IOP of 14 mm Hg and maximum IOP of 36 mm Hg. The difference of mean corrected IOP of the glaucoma suspects and POAG with the normal group was found to be statistically significant (p<0.001). Moreover, the mean cIOP between glaucoma suspects and POAG was also found to be statistically significant (p<0.001).

With respect to the distribution of the visual field defects and non-visual field defects among study groups. A moderately significant difference was noted among POAG patients in comparison to the control and glaucoma suspect patients. 98.33% of POAG patients had visual field defects compared to glaucoma suspects with 1.25%. None of the controls showed any VFD. Majority of the glaucoma suspects were in their pre-perimetric glaucomatous stage.

Table 3 describes the mean endothelial cell density between the groups. The mean ECD among the controls, glaucoma suspects and POAG was 2679.75 ± 306.64, 2587.35± 250.43 and 2367.23 ± 261.31 respectively. A significant difference between the ECD among the two study groups i.e. glaucoma suspects and POAG in comparison to their control group was seen.

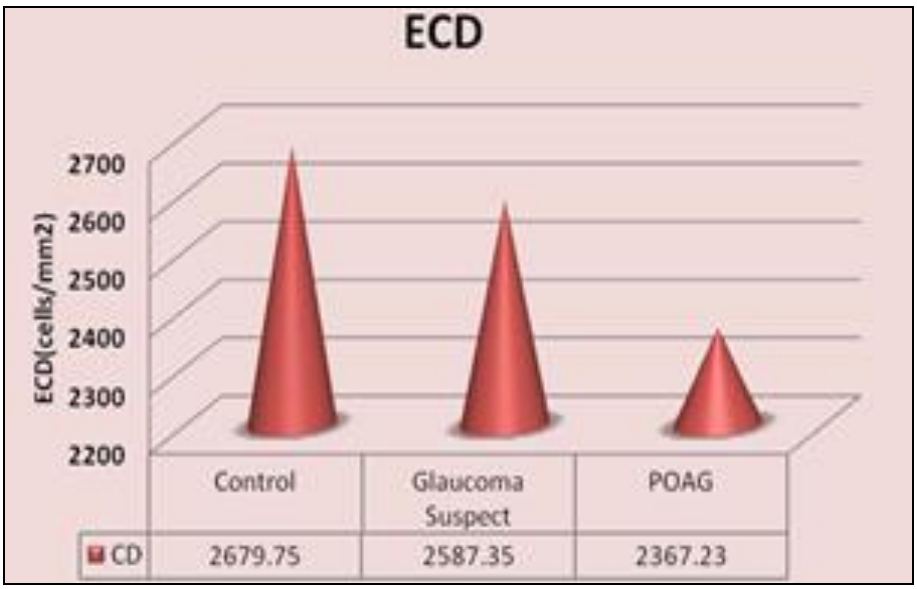

Fig 3: Mean ECD comparison between the groups

Table 3: Mean ECD comparison between the groups

	Control	Glaucoma Suspect (mean ± SD)	POAG	p-value
ECD	2679.75 ± 306.64	2587.35 ± 250.43	2367.23 ± 204.64	<0.001
Minimum density	2114	2080	1850	
Max Density	5622	3096	2959	

Table 4: Distribution of RNFL thickness

RNFL thickness (um)	Controls	Glaucoma suspects	POAG
20-35	0	1	2
35-50	0	0	9
50-65	0	14	17
65-80	9	39	52
80-95	72	83	32
95-110	77	22	7

Table 5: Correlation between ECD and CDR

	CDR				
ECD (cell/mm2)	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	>0.7
<2000	0	0	0	0	0
2000-2500	36	10	29	35	23
2500-3000	116	20	34	82	31
>3500	3	2	5	7	3

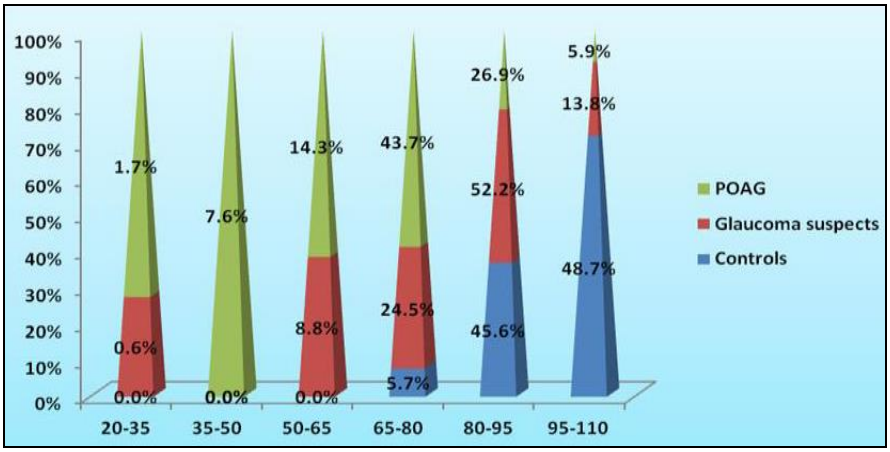

Fig 4: Distribution of RNFL thickness

Statistical analysis done to study the correlation between ECD and CDR among the study groups revealed that majority of the patients with a CDR less than 0.5 had an ECD between 2500-3000. Even with a CDR of 0.6-0.7, we encountered patients with similar ECD. We found that as the CDR increased beyond 0.6-0.7, nearly 26% of them showed an ECD between 2000-2500 cells/mm2 and 17% showed similar ECD with a CDR > 0.7 (Table 5).

The mean CV was 33.09 ± 6.98, 30.21 ± 8.12, 30.38 ± 9.07 among controls, glaucoma suspects and POAG patients respectively. The mean hexagonality (H) was 44.16 ± 7.74, 48.78 ± 14.69 and 47.61 ± 10.42 among controls, glaucoma suspects and POAG patients. The study showed a statistical significance for the hexagonality of the endothelial cells and for the coefficient of variation of these cells.

With regard to the distribution of RNFL thickness, our study revealed that the average RNFL thickness in majority of the patients in the control group were between 95-110µ m, glaucoma suspects between 80-95µ m and newly detected POAG between 65-80µ m (Table 4). The control group did not show RNFL thinning below 65µ m and the thinning noted in the study groups was as low as 20-35µ m.

The mean Central Corneal Thickness (CCT) among the controls, glaucoma suspects and POAG was 527.04 ± 38.89, 537.68 ± 31.04 and 531.42 ± 35.82 respectively, with statistical significance between the groups (Table 6). The mean difference between the three groups was minimal.

Table 6: Mean CCT between the groups

	Control	Glaucoma Suspect (mean ± SD)	POAG	p- value
CCT	527.04 ± 38.89	537.68 ± 31.04	531.42 ± 35.82	0.027
Range	418-627	408-603	451-621	

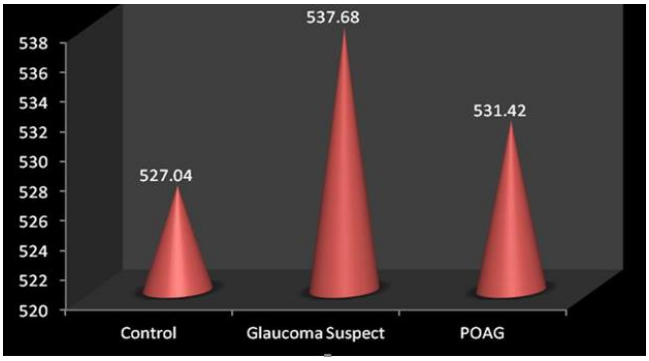

Fig 5: Mean CCT between the groups

The comparative analysis of mean values of RNFL parameters in controls, glaucoma suspects and POAG showed average RNFL thickness and its variation in each quadrant. Here we noticed the order of the mean RNFL thickness was 93.28 ± 7.38 among the controls, 83.74 ± 12.42 among the suspects, 73.73 ± 14.64 among the POAG patients (Table 7). The highest thinning among the three groups was in the POAG group. The mean difference between the controls, glaucoma suspects and POAG showed a strong significance with a p value of < 0.001.

The RNFL thickness for the four quadrants showed majority of our patients falling within the normal limits, unlike the glaucoma suspects who revealed a thinning in the inferior quadrant (17.61%) and POAG patients who showed significant thinning in the inferior (42.02%) and superior quadrants (42%), the thickness being significantly lesser than glaucoma suspects.

Studying the association between ECD and IOP, it was seen that patients with IOP of 10-15 mm Hg had an ECD of 2500- 3000 cells/mm2. With an increasing IOP beyond of 20-25 mmHg, a loss of ECD was seen (Table 8). Association between CCT and CDR has shown that a

majority of our patients had a CDR of 0.3-0.4. With an increase in the CDR, we saw a thinning of the CCT to the extent of it being lesser than 523um.

Table 7: Comparison of RNFL thickness in different study groups and quadrants

	Control	Glaucoma suspect	POAG	Total	P- value
Average RNFL thickness	93.28 ± 7.38	83.74 ± 12.42	73.73 ± 14.64		**<0.001**
Range	80 – 107	28 – 108	30-105		
Superior					
G	150 (95%)	118 (76%)	49 (42%)	317	<0.001
R	0	16 (10%)	49 (42%)	65	
Y	8 (5%)	21 (14%)	20 (17%)	49	
Nasal					
G	156 (98.7%)	129 (81.13%)	72 (60.5%)	357	<0.001
R	0	8 (5.03%)	24 (20.17%)	32	
Y	2 (1.27%)	22 (13.84%)	23 (19.33%)	47	
Inferior					
G	149 (94.30%)	112 (70.44%)	44 (36.97%)	305	<0.001
R	2 (1.27%)	28 (17.61%)	50 (42.02%)	80	
Y	7 (4.43%)	19 (11.95%)	25 (21.01%)	51	
Temporal					
G	154 (97.47%)	125 (78.62%)	68 (57.14%)	347	<0.001
R	0	14 (8.81%)	27 (22.69%)	41	
Y	4 (2.53%)	20 (12.58%)	24 (20.17%)	48	

Table 8: Association between ECD and mean IOP

ECD (cell/mm2)	IOP (in mmHg)				
	10-15	15-20	20-25	25-30	>30
<2000	0	0	0	0	0
2000-2500	33	34	52	14	0
2500-3000	122	92	58	10	2
>3500	9	4	7	0	0

Table 9: Case-control comparison of means of various parameters in the study

Control (mean ± SD)		Glaucoma Suspect (mean ± SD)	POAG (mean ± SD)	p-value
Age (in years)	56.15 ± 10.62	47.81 ± 11.23	50.97 ± 11.52	<0.001
ECD(Cells/mm2)	2679.75 ± 306.64	2587.35 ± 250.43	2367.23 ± 204.64	<0.001
Coefficient of variation (CV)	33.09 ± 6.98	30.21 ± 8.12	30.38 ± 9.07	0.002
Hexagonality (H) (%)	44.16 ± 7.74	48.78 ± 14.69	47.61 ± 10.42	0.001
IOP (mmHg)	14.99 ± 2.06	17.09 ± 3.74	23.53 ± 2.99	<0.001
CCT (µm)	527.04 ± 38.89	537.68 ± 31.04	531.42 ± 35.82	0.027
CDR	0.34 ± 0.08	0.67 ± 0.09	0.68 ± 0.10	<0.001
Average RNFL thickness (µm)	93.28 ± 7.38	83.74 ± 12.42	73.73 ± 14.64	<0.001

The mean age in the control group was 56.15 with a standard deviation of 10.62. In the case group, glaucoma suspects were in the age group of 47.81 ± 11.23 and newly detected POAG patients in 50.97 ± 11.52 years, which was statistically significant. The mean ECD in the control group was 2679.75 with a standard deviation of 306.64. In the case group, glaucoma suspects had 2587.35 ± 250.43 and newly detected POAG patients in 2367.23 ± 204.64, which was statistically insignificant.

The mean CV in the control group was 33.09 with a standard deviation of 6.98. In the case group, glaucoma suspects had 30.21 ± 8.12 and newly detected POAG patients in 30.38 ± 9.07, which was statistically significant. The mean H in the control group was 44.16 with a standard deviation of 7.74. In the case group, glaucoma suspects had 48.78±14.69 and newly detected POAG patients in 47.61 ± 10.42, which was statistically significant. The mean IOP in the control group was 14.99 with a standard deviation of 2.06. In the case group, glaucoma suspects had 17.09 ± 3.74 and newly detected POAG patients in 23.53 ± 2.99, which was statistically significant. The mean CCT in the control group was 527.04 with a standard deviation of 38.89. In the case group, glaucoma suspects had 537.68± 31.04 and newly detected POAG patients in 531.42 ± 35.82, which was statistically significant. The mean CDR in the control group was 0.34 with a standard deviation of 0.08. In the case group, glaucoma suspects had 0.67 ± 0.09 and newly detected POAG patients in 0.68 ± 0.10, which was statistically significant. The mean average RNFL thickness in the control group was 93.28 with a standard deviation of 7.38. In the case group, glaucoma suspects had 83.74 ± 12.42 and newly detected POAG patients in 73.73 ± 14.64, which statistically significant.

Discussion

This study was primarily aimed at correlating the corneal endothelial cell density, central corneal thickness and RNFL thickness of glaucoma suspects and newly detected primary glaucoma patients. The demographic data of the three groups showed that there was no statistical significance in age, gender or family history of glaucoma among the three groups.

There is a considerable amount of literature on low ECD among patients who fall into the category of incipient or terminal glaucoma like ocular hypertensive glaucoma/normal tension glaucoma/early primary glaucoma, established glaucoma or angle closure glaucoma, thus emphasising the vital role of endothelium in glaucoma. Gagon *et al.* reported a 13.0% reduction in ECD in POAG patients, and a 11.9% reduction in NTG (n=5) patients who

were on glaucoma medication, but in our study, we excluded patients on glaucoma medication.6 Sung Woo Cho *et al.* showed no significant reduction in corneal endothelial cell density in normal tension glaucoma patients, unlike the POAG patients who showed 13% reduction compared to normal subjects.7 ECD was significantly lower in patients with glaucoma (2514 ± 419 cells/mm2) compared with controls (2560 ± 306 cells/mm2). Despite this interest, to the best of our knowledge it is not yet known whether early changes in the endothelial cell count among glaucoma suspects can be used as a predictive factor in diagnosing glaucoma.

In this context, we evaluated glaucoma suspects and incipient primary glaucoma patients and we found that the ECD was significantly reduced ($p < 0.001$). The mean endothelial cell density among the controls, glaucoma suspects and newly detected cases of POAG was 2679.75 ± 306.64, 2587.35 ± 250.43 and 2367.23 ± 204.64 respectively. The mean ECD among the glaucoma suspects was towards the lower limit of normal while it was significantly low among the newly detected cases of primary glaucoma. The POAG patients and glaucoma suspects showed a mean difference of 92.4 cells (3.44%) and 312.52 cells (11.66%) compared with the normal group respectively. Another study evaluated the corneal endothelial cells in glaucoma suspects with ocular hypertension (2254 ± 277 cells/ mm2) revealed that there was significant reduction in ECD compared to normal (2496 ± 418 cells/ mm^2) [8].

Surprisingly, our analysis showed significant difference in coefficient of variation (CV) and hexagonality (H) among the groups. This is in complete disagreement with previous findings in the literature as changes in CV and hexagonality indicate acute corneal endothelial damage [9]. Justifying our finding is a study which states that an elevated or abnormal rate of polymegathism is usually the first sign of endothelial damage. These findings indicate physiological stress to the corneal endothelium and an overactive wound repair mechanism. We need not sound a note of caution when we see a CV above 40 or H less than 50% as they are more susceptible to additional trauma from insults such as glaucoma per se, and also intraocular surgery, diabetes, uveitis or contact lens wear.

Our study also highlighted that corneas in glaucoma suspects (537.68 ± 31.04) were thicker than those of known glaucoma patients and normal population (531.42 ± 35.82 and 527.04 ± 38.89 respectively). This lends support to the previous findings in the study on, role of CCT in diagnosis of glaucoma which also found to have significantly higher CCT in ocular hypertensives than in those of glaucoma and normal subjects [10]. Though this difference in our study was minimal and more on the normal limit, it was still significant. The Ocular Hypertension Treatment Study (OHTS) has now recognized lower central corneal thickness (CCT) as an independent risk factor for the development of POAG in eyes with ocular hypertension (OH) [7].

Our study provides considerable insight about the relationship between ECD, CCT and CDR which are derivatives of neural crest. We observed that with ECD and CCT are inversely proportional to CDR this phenomenon could be related with the underestimation of IOP measurement in eyes with thin corneas and probably with the high tolerance of lamina cribrosa in eyes with thick corneas. Based on that remark, we could consider that the coexistence of thin cornea and big C/D area ratio in a glaucoma suspect, may contribute to glaucoma progression. Pakravan and colleagues (2007) confirmed that CDR was inversely correlated with CCT in patients with POAG.11 Herndon *et al.* quantified this relationship by saying that, with an increase in CCT by 10µ m, the vertical CDR decreased by 0.008 and horizontal CDR decreased by 0.007 [11]. OCT evaluation of the same study population showed a significant decrease in average RNFL thickness in glaucoma suspects (80-95µ m) and more so among the newly detected cases of glaucoma (65-80µ m) than in the controls (95-110µ m). The RNFL thickness for the four quadrants (superior, nasal, inferior and temporal quadrants) showed significant difference. Majority of our normal patients were within the green zone unlike the glaucoma suspects who showed significant thinning in the inferior quadrant (17.61%) and POAG patients showed thinning in the inferior (42.02%) and superior quadrants (42%).The RNFL thickness of the four quadrants among the newly detected primary glaucoma patients (POAG) was significantly lesser than that of glaucoma suspects ($p<0.001$). Our results have a number of similarities with Safal Khanal *et al.* in 2014 who studied the "Retinal nerve fiber layer thickness in glaucomatous Nepalese eyes and its relation with visual field sensitivity", revealed that the mean RNFL thickness was significantly less in the POAG (64.30 ± 14.45 µm), NTG (85.43 ± 9.79 µm) and Glaucoma suspects (102.0 ± 9.37 µm) groups than in the healthy group (109.8 ± 8.32 µm). The RNFL was significantly thinner across all quadrants in all study group pairs ($p< 0.05$) except for normal vs. Glaucoma suspects (superior and inferior quadrant, significant thinning) [12].

This concurs with Maziar lalezary *et al.*'s findings where they compared baseline RNFL thickness between eyes with and without glaucomatous change which revealed that only the inferior quadrant was significantly different between the two groups [13]. As also reported by Alfonso Anton *et al.* (2007), the evidence we found points towards the mean RNFL thickness around the disc, and superior and inferior RNFL thickness, which were significantly thinner in glaucomatous eyes than in ocular hypertensive or normal eyes [14]. Chen *et al.* (2005) had shown that for differentiating early glaucoma from normal eyes, the average RNFL thickness was the most effective parameter (AROC 0.793) [15].

Further analysis showed that with increasing IOP there was a significant decrease in RNFL thickness among glaucoma suspects and newly detected primary glaucoma patients. Similarly, the ECD of the glaucoma suspects and newly detected cases of primary glaucoma were found to significantly decreasing with increasing IOP. Also, there seems to be a greater decrease in the ECD in the newly detected glaucoma patients in comparison to the glaucoma suspects. Therefore, lower endothelial cell density correlates with thinner RNFL thickness and is directly proportional to IOP. A similar study by Daisy Rani Das (2015) confirmed our findings in primary glaucoma patients which concluded that the rate of damage to RNFL and corneal ECD in primary glaucoma is directly proportion to IOP [2]. This correlation is worth mentioning as it substantiates the correlation between RNFL thickness and corneal ECD in glaucoma suspects and primary glaucoma patients while considering other confounding factors like CDR, CCT, IOP and VFD.

Thus ECD, CCT and RNFL thickness are significant predictors of glaucomatous damage in the early diagnosis of glaucoma suspects, in managing newly detected cases of

primary glaucoma and in assessing the progression of the disease.

We have worked on a relatively small sample size and the study period was only one year, hence we believe that a large population-based prospective study is needed and future studies should include all sub-groups of glaucoma for better understanding.

Conclusion and Summary

In our study we found that there was endothelial cell loss among the case groups with 3.44% in glaucoma suspects and 11.66% in newly detected cases of glaucoma. As the disease progressed, we saw a significant difference between the two study groups (glaucoma suspects and newly detected cases of glaucoma) with respect to mean cIOP, CDR, CCT and VFD. cIOP and CDR are indirectly proportional ECD whereas, CCT and VFD are directly proportional to ECD. Our study results revealed average, inferior average followed by superior average RNFL thinning helping to differentiate normal eyes from eyes with early glaucomatous changes and newly detected primary glaucoma.

On cumulating the above data, we concluded that eyes with lower ECD were found to have a thinner RNFL (inferior<superior quadrant) and larger CDR among glaucoma suspects and newly detected cases of POAG. The rates of damage of RNFL and endothelial cells were directly proportional to increasing IOP.

Thus, considerable insight has been gained with regard to the importance of evaluating corneal endothelium and has underlined the importance of incorporating it in the evolution of glaucoma screening, treatment and rehabilitation by using advanced technology. This will lend a hand in early diagnosis, more accurate follow-up to assess progression and most importantly, allow better therapeutic options.

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