Comparison of corneal endothelial cell parameters in four different groups by specular microscope

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Abstract

Background: Corneal endothelial cell is important for corneal function and viability. The advent of specular microscopy has greatly improved the study of human corneal endothelial morphology and allowed quantification of endothelial changes.

Objective: To compare the corneal endothelial cell parameters in four groups (i.e., young subjects, elderly persons, cataract patients, and primary open angle glaucoma patients) measured by specular microscopy.

Materials and Methods: This prospective, randomized, single-site clinical study included a total of 120 patients who were selected and divided into 4 groups of 30 patients each. Endothelial cell density (ECD), average cell size, and percentage of hexagonality (6A%) were measured by noncontact specular microscope.

Result: In our study, of 120 patients, 74 (61.66%) were male while 46 (38.33%) female subjects. In group A, ECD, average cell size, and percentage of hexagonality were 2,797.43 ± 171.96 cells/mm^2, 324.63 ± 40.61 μm^2, and 66.43 ± 2.56%, respectively. The values in group B were 2,364.10 ± 81.56 cells/mm^2, 362.27 ± 22.30 μm^2, and 59.40 ± 3.54%, respectively. In group C, these values were 2,534.37 ± 125.34 cells/mm^2, 414.93 ± 37.45 μm^2, and 60.13 ± 2.52%, respectively. While in group D, these values were 2,311.30 ± 100.87 cells/mm^2, 436.13 ± 41.47 μm^2, and 59.93 ± 2.55%, respectively.

Conclusion: From this study, it can be concluded that ECD is at its peak value in young age and significantly reduces with age and not influenced by cataract and glaucoma. Hexagonality of corneal endothelium is significantly high in young males when compared with young female subjects. Young subjects showed small average size of endothelial cell.

KEY WORDS: ECD, hexagonality, average cell size, specular microscope

Introduction

Corneal endothelial cell is derived from the neural crest from the corneal endothelial lining as a single layer of hexagonal cell, whose function is to maintain corneal clarity. Over 60% of the endothelial cells are six-sided in a normal endothelium. These cells are uniformly 5 μm in thickness, 20 μm in width, and polygonal (mostly hexagonal) in shape. The size and shape of the endothelial cell is significant as adjacent cells with homogenous proportions best maintain the fluid barrier function of the endothelium.[1] The endothelium is accountable for maintaining the relatively low level of stromal hydration required for corneal transparency. With specular microscopy, the corneal endothelium looks as somewhat-regular array of cells, the endothelial mosaic. All the endothelial cells appear to be roughly of the same size and shape in this mosaic configuration.[2] Corneal endothelial cells are capable of normal division during fetal development; however, the total corneal endothelial cell deserved is limited because cell division in adult cell either does not occur at all or occurs at a rate too slow to efficiently replace dead or injured cell.[3–6] Endothelial cell density (ECD) in the normal healthy corneal cell decreases with the age. The average density of corneal endothelial cell at birth is approximately ~4,500 cells/mm^2.[3] The overall rate of cell loss accelerates if the endothelial is injured as a result of aging, trauma, disease, or dystrophy.[6–8] Because of normal attrition, the central cornea loses 10–50 endothelial cells/year,[9] and ECD reduces by 0.5%–0.6%/year. To mend the gap, the endothelium depends on cellular migration and cellular fusion. In this wound repair process, the endothelial cell next to the defect, progress to fill

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in the space left by the sloughed cell. The cell either stretch or slide into a different position or combine together to reestablish total coverage of the posterior surface of cornea.[10] This movement is known as polymegathism. The rate of polymegathism is represented by the coefficient of variation (CV), with values between 0.22 and 0.31 considered normal.[11] Increased heterogenicity in cell shape (i.e., pleomorphism) also occur with age or trauma.[12–15] Decompensation (i.e., loss of monolayer integrity and function), occur when cell density falls below 300–400 cells/mm^2 or when the mean cell size reaches ~3,000–3,500 μm^2. The minimum number of cells, critical cell density, and average between 300 and 500 cells/mm^2 lead to development of corneal edema.[16,17] With aging, there is gradual decrease in ECD, polymegathism, pleomorphism, increased CV, and decrease of hexagonal cell, mosaic degeneration.

The advent of specular microscopy has greatly improved the study of human corneal endothelial morphology and allowed quantification of endothelial changes. Specular microscopy is used to view and record noninvasively the image of the corneal endothelial cell layer.[18–21] Analyzing specular micrograph can be done qualitatively by looking at the cellular morphology and giving an interpretation or quantitatively by counting cell density and performing morphometric analysis. Quantitative analysis includes ECD, central corneal thickness, percentage of regular hexagonal cell, and average size of endothelial cell. Using specular microscopy, endothelial disease may be characterized by one or more abnormalities of cell morphology.

Materials and Methods

This prospective, randomized, single-site study included a total of 120 patients who attended our outpatient department from July 2013 to June 2014. This study was approved by institutional review board. All patients were informed about the design of study, and informed consent was taken. Patients with chronic primary open angle glaucoma (POAG) on medical management, in which eye drops are effective in lowering intra ocular pressure (IOP) to normal values, and taking drops since more than 6 months, written consent was taken. Patients were divided into 4 groups of 30 each as follows:

Group A: Young healthy patients (25 ± 5 years) acting as a control group to the rest of the three groups.

Group B: Elderly patients (60 ± 5 years) acting as a control group to groups C and D.

Group C: Senile cataract patients; at least in one eye undergoing cataract surgery (ages 50–60 years).

Group D: Already diagnosed chronic POAG patients.

Patients with DM, hypertension, history of previous intraocular surgery or ocular trauma, corneal or conjunctival irritation, history of chemical trauma, uveitis, known corneal degeneration, dystrophies, opacity, high myopia, pregnant or lactating women, contact lens wearer, dry eye, and family history of corneal decompensation were excluded.

After enrolment, a thorough clinical examination with slit lamp was carried out. Visual acuity was recorded using Snellen's chart for distance and Jaeger's chart for near. IOP was recorded using Schiotz indentation tonometer. One eye was considered as one patient. Routine investigations—hemoglobin, bleeding time (BT), clotting time, urine albumin, and sugar and blood pressure measurements—were done.

Noncontact Tomey EM-3000 specular microscope with automated analysis was used to measure the corneal thickness and endothelium biometry parameters, but only endothelial biometry parameters were used.

Student's *t*-test was used for statistical analysis.

Result

In this study, 120 patients were divided in four groups of 30 each.

Group A: Young healthy patients (25 ± 5 years) acting as a control group to the rest of the three groups.

Group B: Elderly patients (60 ± 5 years) acting as a control group to groups C and D.

Group C: Senile cataract patients; at least in one eye undergoing cataract surgery (ages 50–60 years).

Group D: Already diagnosed chronic POAG patients.

Of 120 patients, 74 were male and 46 female subjects. Male outnumbered in all four groups. Sex distribution in all four groups is shown in Table 1.

The age group of patients included for the study ranged from 22 to 65 years. The mean age ± SD of the study was 53.24 ± 15.19 years. The mean age ± SD in various groups is displayed in Table 2.

ECD, average cell size, percentage of hexagonal cell (6A%) in young subjects (i.e., group A) were 2,797.43 ±

Table 1: Sex distribution

Group	Young		Elderly		Cataract		Glaucoma		Total	
	N	%	N	%	N	%	N	%	N	%
Sex										
Male	18	60	16	53.34	16	53.34	24	80	74	61.66
Female	12	40	14	46.66	14	46.66	6	20	46	38.33
Total	30	100	30	100	30	100	30	100	120	100

Table 2: Age distribution in defined groups

Group	Mean age ± SD*
A	26.06 ± 2.59
B	62.4 ± 1.96
C	61.8 ± 2.47
D	62.7 ± 2.00

*SD, standard deviation.

171.96 cells/mm², 324.63 ± 40.61 μm², and 66.43 ± 2.56%, respectively. These values in elder subjects (i.e., group B) were 2,364.10 ± 81.56 cells/mm², 362.27 ± 22.30 μm², and 59.40 ± 3.54%, respectively, shows that elderly subjects exhibit higher average cell size, lower ECD, and % of 6A than young subjects. In the group C (i.e., cataract patients), values were 2,534.37 ± 125.34 cells/mm², 414.93 ± 37.45 μm², and 60.13 ± 2.52%, respectively, which show that cataract patient exhibit higher average cell size, slight lower ECD, and low % of 6A when compared with young subjects. These values in glaucoma patients group (i.e., Group D) were 2,311.30 ± 100.87 cells/mm², 436.13 ± 41.47 μm², and 59.93 ± 2.55%, respectively, which show that glaucoma patient present lower ECD, higher average cell size, and lower % of 6A than young as displayed in Table 3.

It was observed that ECD distribution between gender in defined study groups that no statistically significant difference between male and female subjects is present ($p > 0.05$); hence, sex does not seem to influence ECD as shown in Table 4.

Table 5 shows average cell size distribution between gender in defined study groups. It was found that no statistically significant difference was seen in average cell size between sex in all four groups. Hence, sex does not influence average cell size; however, group A patients showed smaller average cell size when compared with the rest of the groups.

Table 6 shows 6A% distribution between gender in defined study groups. Group A showed higher 6A% when

Table 3: Parameter distribution in defined groups

Parameter	Group A	Group B	Group C	Group D
ECD (cells/mm²)	2,797.43 ± 171.96	2,364.10 ± 81.56	2,534.37 ± 125.34	2,311.30 ± 100.87
Avg. cell size (μm²)	324.63 ± 40.61	362.27 ± 22.30	414.93 ± 37.45	436.13 ± 41.47
% of hexagonality	66.43 ± 2.56	59.40 ± 3.54	60.13 ± 2.52	59.93 ± 2.55

Table 4: ECD (cell/mm²) distribution between sex in study group

Parameter	Group A	Group B	Group C	Group D
Male	2,821.83 ± 94.47	2,389.56 ± 63.90	2,569.25 ± 121.20	2,322.33 ± 87.79
Female	2,741.17 ± 175.63	2,335.0 ± 91.76	2,494.50 ± 121.49	2,308.54 ± 105.44
Overall mean	2,797.43 ± 171.96	2,364.10 ± 81.56	2,534.37 ± 125.34	2,322.30 ± 100.87
p	0.06	0.06	0.104	0.76

Table 5: Cell size (μm²) distribution between sex in study population

Parameter	Group A	Group B	Group C	Group D
Male	320.17 ± 15.34	357.25 ± 23.70	412.63 ± 36.10	427.33 ± 39.98
Female	329.27 ± 25.28	365.86 ± 20.47	417.57 ± 40.15	441.33 ± 27.62
Overall mean	324.63 ± 40.61	362.27 ± 22.30	414.93 ± 37.45	436.13 ± 41.47
p	0.059	0.13	0.72	0.11

Table 6: Percentage of hexagonality (6A%) between sex in study population

Parameter	Group A	Group B	Group C	Group D
Male	71.67 ± 2.61	61.31 ± 2.21	60.81 ± 2.41	60.21 ± 2.45
Female	60.67 ± 2.43	59.21 ± 3.53	59.36 ± 2.50	58.83 ± 2.86
Overall mean	66.43 ± 2.56	59.40 ± 3.54	60.13 ± 2.52	59.93 ± 2.55
p	0.042	0.073	0.115	0.218

compared with the rest of the groups. In group A, statistically significant difference was found between sex. Male subjects showed higher 6A% than female subjects ($p = 0.042$). However, the other three groups showed no statistically significant difference between sex, when hexagonality was compared.

Table 7 compares endothelial cell parameters between groups A and B. Group B showed low ECD when compared with group A, and difference was found to be statistically significant ($p < 0.001$). Therefore, it can be concluded that age has definite negative effect on ECD. Group A showed small average cell size in comparison to Group B and higher % of 6A than Group B. This difference was found to be borderline statistically significant ($p = 0.059$ and $p = 0.056$, respectively).

Table 8 compares between groups B and C. It showed that no statistically significant difference was found between groups B and C with respect to any parameters ($p > 0.05$). It signifies that there was no significant change in corneal parameter owing to disease process.

Table 9 compares between groups B and D. Group B serves as age-matched control group for group D. Group B patients showed higher average cell size than group B, which was statistically significant ($p < 0.001$), signifies that POAG was probably responsible for increased cell size. Change in ECD and 6A% owing to glaucoma was not found to be significant.

Table 10 compares between group A with groups B and C with respect to different parameters. It showed that no significant difference in 6A% between groups A and B, A and C, and B and C ($p > 0.05$), signifies that neither age nor cataract has any effect on 6A%. ECD differences were found significant between groups A and B and groups A and C ($p < 0.01$), but not significant between groups B and C ($p = 0.073$); it shows that age had definite decremental effect on ECD but not cataract. In group C patients, the significant difference in ECD than group A was because of aging process and not owing to cataract. In Group B, average cell size increased than Group A, but the difference was found not to be significant ($p = 0.059$). Difference between groups B and C in average cell size was not found significant ($p = 0.074$), but the average cell size difference between groups A and C was found significant ($p < 0.001$), probably it may be partially owing to aging.

Table 11 compares between group A with groups B and D with respect to different parameter. ECD difference was found significant between groups A and B and groups A and D ($p < 0.01$), but not significant between groups B and D ($p = 0.082$). This shows that age has negative effect on ECD but not glaucoma. In Group D subjects, the ECD was significantly lower than group A because of aging process not owing to glaucoma. In Group B, the average cell size was more than group A, but the difference was not found to be significant while in groups B and D and groups A and D was found significant. This shows that average cell size was significantly affected by glaucoma. Group B patients showed lower percentage of hexagonal cell than group A but not statistically significant. Group D showed similar percentage as group A shows that glaucoma has no additional decreasing effect on hexagonality.

Table 7: Comparison of different corneal parameters in groups A (young) and B (elder)

Parameter	Group A	Group B	*p*
ECD (cells/mm^2)	2,797.43 ± 171.96	2,364.10 ± 81.56	<0.001
Avg. cell size (μm^2)	324.63 ± 40.61	362 ± 22.30	0.059
% of 6A	66.43 ± 2.56	59.40 ± 3.54	0.056

Table 8: Comparison of different corneal parameters in groups B (elder) and C (cataract)

Parameter	Group B	Group C	*p*
ECD (cells/mm^2)	2,364.10 ± 81.56	2,534.37 ± 125.34	0.073
Avg. cell size (μm^2)	363.27 ± 22.30	414.93 ± 37.45	0.074
% of 6A	59.40 ± 3.54	60.13 ± 2.52	0.360

Table 9: Comparison of different corneal parameters in groups B (elder) and D (glaucoma)

Parameter	Group B	Group D	*p*
ECD (cells/mm^2)	2,364.10 ± 81.56	2,311.30 ± 100.87	0.082
Avg. cell size (μm^2)	362.27 ± 22.30	436.13 ± 41.47	<0.001
% of 6A	59.40 ± 3.54	59.93 ± 2.55	0.66

Table 10: Comparison of different corneal parameters in group A with groups B and C

Parameter	Group A	Group B	*p* (between groups A and B)	Group C	*p* (between groups A and C)	*p* (between groups B and C)
ECD (cells/mm²)	2,797.43 ± 171.96	2,364.10 ± 81.56	<0.001	2,534.37 ± 125.34	<0.001	0.073
Avg. cell size (μm²)	324.63 ± 40.61	362.27 ± 22.30	0.059	414.93 ± 37.45	<0.001	0.074
% of 6A	66.43 ± 2.56	59.40 ± 3.54	0.056	60.13 ± 2.52	0.061	0.36

Table 11: Comparison of different corneal parameters in group A with groups B and D

Parameter	Group A	Group B	*p* (between groups A and B)	Group D	*p* (between groups A and D)	*p* (between groups B and D)
ECD (cells/mm²)	2,797.43 ± 171.96	2,364.10 ± 81.56	<0.001	2,311.30 ± 100.87	<0.001	0.082
Avg. cell size (μm²)	324.63 ± 40.61	362.27 ± 22.30	0.059	436.13 ± 41.47	<0.001	<0.001
% of 6A	66.43 ± 2.56	59.40 ± 3.54	0.056	59.93± 2.55	0.059	0.66

Discussion

Cornea is the principal refractive surface of the eye, and vision can be significantly affected by relatively small changes in its structure and parameter. Measurement of corneal parameters is important in the diagnosis and management of various ocular diseases. Our study aimed at the comparison of corneal endothelial cell characteristics in healthy young subjects, elderly persons, and diseased group of cataract and glaucoma patients by specular microscopy. The patients were examined for corneal ECD, average cell size, and 6A% with a noncontact type TOMEY EM-3000 specular microscope with automated analysis. The age group of patients included for the study ranged from 22 years to 65 years. The mean age ± SD in our study was 53.24 ± 15.91 years. In groups A, B, C, and D, it was 26.06 ± 2.59 years, 62.4 ± 1.96 years, 61.8 ± 2.47 years, and 62.7 ± 2.00 years, respectively. Mean ECD in young subjects (i.e., group A) was found 2,797.43 ± 171.96 cells/mm², with average value in male subjects to be 2,821.83 ± 94.47 cells/mm² and in female subjects to be 2,741 ± 175 cells/mm². Females showed slightly lower ECD values than male subjects but, the difference was not so significant. Similar results were seen by Rao et al.,[22] Sarath et al.,[23] and Higa et al.[24] Mean ECD in elder patients as in group B was 2,364.10 ± 81.56 cells/mm²; comparison between male and female subjects showed no sex-related difference. In our study, it was observed that there is a significant decrease in ECD as age advances ($p < 0.001$). Same results were observed by Rao et al.,[22] Sarath et al.,[23] Laule et al.,[25] and Faragher et al.[26] No sex-related difference was observed. Mean ECD in cataract patients (i.e., group C) was 2,534.37 ± 125.34 cells/mm². Similarly, no significant difference in male and female subjects was observed as in previous group. In group C, ECD was at lower side owing to aging process. Almost similar results were observed by Galgauskas et al.,[27] while different results were obtained by Praveen et al.[28] In Group D, mean ECD was 2,311.30 ± 100.87 cells/mm². No difference between sex was observed. It was observed that glaucoma patients did not exhibit additional effect on ECD. Results differing to our study was observed by Gagnon et al.[29]

Sex showed no significant effect on average cell size in our study. In elder patients, the average cell size was increased in comparison to young subjects. It proves that, with aging, corneal endothelial compensates by increasing the size. While in glaucoma patients, average cell size was increased (polymegathism), and it was more than elder and young group patients suggesting that glaucoma significantly affects the average cell size ($p < 0.001$). It was observed that males had more percentage of hexagonal cells than female subjects, and the difference was found to be significant ($p = 0.042$). Similar results were found by Rao et al.,[22] while in young Thai eyes, the 6A% was lower than young Indians and has significant gender difference. The 6A% in group B was 59.40 ± 3.54 %, with average value in male of 61.31 ± 2.21%, while in female it was 59.21 ± 3.53%. There was no significant difference in gender in this group. Our study result shows that elderly persons show lower percentage of 6A when compared with young subjects, which is borderline significant ($p = 0.056$). The 6A% in cataract patients showed no difference with respect to sex, and no significant difference was found in 6A% between young and cataract subjects, elderly and cataract subjects, which signifies that cataract shows no effect on 6A%, while different results were obtained by Snellingen et al.,[30] which showed that in cataract patients group, females showed 7.8% higher percentage of hexagonal cells than male subjects, while in group D also no significant difference in 6A% was observed between male and female subjects. It was also observed that glaucoma itself showed no significant effect on 6A%.

Conclusion

Young subjects exhibit higher ECD value, smaller average cell size, and more percentage of hexagonal cell. The corneal endothelium in young males has more regular hexagonal cells than female subjects. Age is the most important factor of ECD, and its count decreases as one ages. From this study, it was concluded that cataract and glaucoma did not affect the ECD when compared with their age-matched population. The clinical use of cell analysis includes the assessment of donor corneal endothelium, effects of intraocular surgery and is essential in evaluating the safety of corneal surgical procedure.

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