Original Article

Comparative analysis of endothelial cell loss following phacoemulsification in pupils of different sizes

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Purpose: To compare Endothelial cell(EC) loss following Phacoemulsification (PKE) in pupils of different sizes. **Methods:** A prospective double masked observational study in which a total of 150 eyes of 150 patients between 50 & 70 years of age with senile cataract of nuclear sclerosis grade II were enrolled. Patients were allocated into three groups of 50 eyes each in Group A (pupil size <5 mm), Group B (pupil size 5–7 mm) and Group C (pupil size >7 mm). Pupillary size was measured by determining the height of slit on slit-lamp biomicroscope examination. PKE was done by the same expert surgeon using vertical chop technique and a foldable intraocular lens was implanted in the capsular bag. Corneal EC count and pachymetry were performed twice and average of 2 readings was taken for the purpose of this study. Measurements were taken preoperatively and postoperatively on day 1, day 7 and day 30. **Results:** The mean EC count loss on postoperative day 1 in Group A was 19.45%, Group B 14.89%, Group C 10.19% with statistical significant difference between Group A and Group B, as also Group A and Group C. The difference was not significant between Group B and Group C, though there was a fall in EC count in Group C as well. Increase in corneal thickness on postoperative day 1 in group A was 5.43%, Group B 3.55%, Group C 2.14% with statistical significant difference between Group A and Group B, as also Group A and Group C with no difference in Group B and Group C. **Conclusion:** PKE done in eyes with maximal pupillary dilatation of <5 mm causes a greater EC loss and results in thicker corneas postoperatively as compared to eyes with pupillary dilatation of >5 mm at the end of one month.

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The most common cause of preventable blindness and low vision is cataract.[1] Phacoemulsification (PKE) with the insertion of intraocular lens (IOL) has become the standard of care for treating cataract.[2] Ultrasonic energy dissipated during PKE surgery causes mechanical and thermal damage to the corneal endothelium, an essential tissue for maintaining transparency of the cornea. The endothelium is a monolayer of hexagonal cells of limited regenerative power. Loss of these cells is compensated only by the migration, enlargement and increased heterogeneity of these cells.[3,4] Specular microscopy is a noninvasive photographic technique that allows us to visualize and analyze the corneal endothelium. PKE is a closed chamber procedure performed in a confined space and thus causes mechanical and thermal damage to the endothelium during surgery because of its proximity to the corneal tissue. Pirazzoli *et al*. found that PKE was associated with 16.67% endothelial cell loss (EC) loss that correlated with the degree of trauma during surgery.[5] EC loss can be influenced by several pre- and intra-operative factors. Preoperative factors that influence EC loss include the age of the patient, hardness of the nucleus and shorter axial length (AL).[6] Intraoperative factors include the incision size and design, Descemet membrane detachment, toxic intraoperative medications, PKE technique, and ophthalmic viscoelastic device (OVD) used.[7,8] Smaller pupillary size restricts the operative space for the surgeon and is likely to result in increased proximity of the phaco tip to the endothelium. In one study small dilated pupil was associated with more EC loss.[9] However, in most of these studies, multiple factors were assessed simultaneously making it difficult to evaluate the exclusive impact of pupillary size on EC loss and health of the cornea after eliminating other confounding factors. Therefore, this study was planned to evaluate EC loss in different pupillary sizes where other pre-, intra-, and post-operative parameters were similar.

Methods

After obtaining clearance from ethical committee of the institution a prospective double-masked study was conducted at tertiary care hospital in eastern India from January 2016 to April 2017. A total of 150 patients between 50 and 70 years with senile cataract of nuclear sclerosis grade II were enrolled in the study.

Exclusion criteria

- Evidence of pseudoexfoliation.
- Corneal scar/dystrophy

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- History of angle closure
- Preoperative EC count <1500 cells/mm^2
- Anterior Chamber (AC) depth <2.25 mm
- History of laser photocoagulation/diabetic retinopathy
- Glaucoma
- History of intraocular surgery
- Total surgical time >15 min
- Intraoperative surgical complication
- Significant pupillary constriction requiring intracameral mydriatic or iris hooks.

Preoperative evaluation of the patients included measurement of uncorrected visual acuity, best corrected visual acuity, intraocular pressure measurement by Goldman applanation tonometry. Then the pupils were dilated by instilling one drop of commercially available combination of tropicamide 1% with phenylephrine 2.5% eye drop in both the eyes every 15 min for 45 min. After dilatation, a slit lamp biomicroscopic examination of each eye was done to measure the precise pupillary size on the basis of the height of the slit and lens nucleus was graded according to Emery and Little nuclear hardness classification.[10] Only those patients whose lens was assessed to be nuclear sclerosis grade 2 were included in this study. Two independent examiners measured the pupillary size and graded the nuclei and the patient was enrolled for the purpose of this study only if both evaluations were matching.

These 150 eyes of 150 patients were divided into three groups according to fully-diluted pupil size. Patients were allotted to Group A (pupil size <5 mm), Group B (pupil size 5–7 mm) and Group C (pupil size >7 mm). Corneal EC count and pachymetry readings were taken preoperatively and postoperatively on day 1, 7, and 30 for all patients by using SP 3000P noncontact specular microscope (Topcon America Corporation, Paramus, NJ). The EC density were determined by counting of cells after freezing the image involving the central 3 mm. Three such readings were taken and their mean was recorded. AL, AC depth, and corneal thickness were measured using IOL Master (Carl Zeiss 500, USA). The power of the IOL to be implanted was calculated by same IOL master in all the patients using Sanders, Retzlaff, Kraff-T formula. One day before surgery all patients were put on eye drop Moxifloxacin one drop four times a day in both eyes.

Surgical technique

On the day of surgery, every patient underwent dilatation of pupil by instillation of one drop of tropicamide 1% with phenylephrine 2.5% every 15 min for 45 min. Surgery was performed under peribulbar anesthesia using 2 ml of 0.5% bupivacaine and 4 ml of 2% lignocaine mixed with 150 IU of hyaluronidase. Surgery was done by single surgeon using Stellaris (Bausch and Lomb, NY, USA) PKE machine with CSS software. Main incision was a triplanar clear corneal tunnel incision starting at mid limbus superotemporally in the right eye with side port made superonasally. In the left eye main incision was made superonasally and side port superotemporally with both incisions placed 4 h apart. The main incision was made with a 2.75 mm angled slit knife (Alcon surgical, USA) and sideport entry was made by MVR blade. Hydroxypropyl methylcellulose 2% w/v was introduced in the AC. Capsulorrhexis was performed by a bent tipped 26G cystotome. Hydrodissection and hydrodelineation were performed to achieve free rotation of nucleus. PKE was done using direct chop technique all the cases. The following phaco parameters were used: 15° Stellaris phaco tip, maximum vacuum 400 mmHg, maximum US power 25%, duty cycle 60%, bottle height 120 cm above the patient's eye, and CSS software with waveform bursts. Effective PKE time was noted by independent observer in all the cases. Irrigation and aspiration of cortical matter were performed coaxially. Foldable single-piece hydrophilic acrylic lenses (Bausch and Lomb, NY, USA) were implanted by injecting through the viscojet cartridge using a disposable injector in all patients. On 1 postoperative day, patients were prescribed ofloxacin + prednisolone combination eye drops six times daily in reducing frequency for 4 weeks. Intraocular pressure was measured by Goldman applanation tonometer on postoperative day 1, 7, and 30. The examiner who performed postoperative measurements was masked about the group allottment. The tip of the Goldman applanation tonometer was wiped with gauze soaked in 70% isopropyl alcohol and dried before use in every patient.

Results

Categorical variables were expressed as number of patients and percentage of patients and compared across the groups using Pearson's Chi-square test for Independence of Attributes. Continuous variables were expressed as mean ± standard deviation and compared across the groups using unpaired *t*-test and one way ANOVA. The statistical software SPSS version 20 SPSS (IBM, USA) was used for the analysis. An alpha level of 5% was taken, i.e., if any $P < 0.05$ it was considered as statistically significant.

The groups were matched for age [Table 1], preoperative endothelial count and pachymetry. EC [Tables 2 and 3]: On postoperative day 1, cell loss was statistically significant in A versus B, A versus C with no difference in B versus C. On postoperative Day 7, cell loss was statistically significant in A versus B, A versus C with no difference in B versus C. On postoperative day 30, cell loss was statistically significant in A versus B, A versus C with no difference in B versus C. Corneal thickness [Tables 4 and 5]: On day 1 postoperatively, mean increase of thickness was statistically significant between

Table 1: Distribution of patients according to gender

	Group			Total	*P*			
	Group A	Group B	Group C		A versus B	A versus C	B versus C	Overall
Sex								
Female	28 (56)	26 (52)	28 (56)	82 (54.67)	0.688	1.000	0.688	0.898
Male	22 (44)	24 (48)	22 (44)	68 (45.33)				
Total	50 (100)	50 (100)	50 (100)	150 (100)				

Group A versus B, Group A versus C with no difference between Group B versus C. On postoperative day 7, mean increase of thickness was statistically significant between Group A versus B, Group A versus C with no significant difference between Group B versus C. However, on postoperative day 30, mean increase of thickness was statistically significant between Group A versus B, Group A versus C, and Group B versus C.

Discussion

ECs maintain cornea in a dehydrated state by their pumping activity assuring its transparency. This is an active process which is controlled by Na^+/K^+-ATPase and involves the generation of a bicarbonate ion gradient across the corneal endothelium.[11] Corneal endothelial protection is always a major concern in any form of cataract surgery and plays a vital role in postoperative vision of the patient following cataract surgeries. The mean density of endothelial cells considered normal for adults is approximately 2500 cells/mm^2, with corneal edema and decompensation occurring when it falls below 500 cells/mm^2.[3] Specular microscopy is a useful tool in preoperative assessment of cataract patients undergoing PKE.[12] There is ample evidence to indicate that once the mature endothelial monolayer has formed, human corneal ECs do not replicate *in vivo*.[13] In Indian population, the EC density has been estimated to be 2527+/-337 cell/mm^2.[14] During an uneventful cataract surgery, EC loss may be caused by ultrasound vibration, air bubbles, turbulent flow of irrigating solution, endothelial contact with the nucleus and its fragments and floating fragments of lens cortex. In one case report by Pong *et al*. it was found that occurrence of toxic endothelial cell destruction after use of intracameral epinephrine.[15] Hence, significant pupillary constriction necessitating intracameral mydriatic were excluded from the study.

Endothelial changes and alterations of central corneal thickness (CCT) are considered important parameters of

Table 2: Endothelial cell count both pre- and post-operatively in different groups

	Mean±SD			P			
	Group A	Group B	Group C	A versus B	A versus C	B versus C	Overall
EC Preoperatively	2508.66±315.99	2584.88±305.95	2473.42±327.15	0.223	0.585	0.082	0.201
ECC day 1	2020.82±252.66	2200.26±265.03	2221.26±293.23	0.001	<0.001	0.708	<0.001
ECC days 7	2062.78±260.54	2324.2±276.11	2272.96±301.01	<0.001	<0.001	0.377	<0.001
ECC days 30	2102.14±264.37	2365.38±265.97	2312.5±304.91	<0.001	<0.001	0.358	<0.001

ECC: Endothelial cell count, SD: standard deviation

Table 3: Endothelial cell loss in different groups

Day	Group A		Group B		Group C	
	Mean cell loss	Percentage cell loss	Mean cell loss	Percentage cell loss	Mean cell loss	Percentage cell loss
Preoperatively-day 1	488	19.45	385	14.89	252	10.19
Preoperatively-days 7	446	17.78	261	10.09	200	8.09
Preoperatively-days 30	407	16.22	220	8.51	160	6.47

Table 4: Corneal thickness both pre- and post-operatively in different groups

	Mean±SD			*P*			
	Group A	Group B	Group C	A versus B	A versus C	B versus C	Overall
CT preoperatively	515.98±19.99	506.9±35.15	513.54±19.77	0.116	0.541	0.247	0.198
CT day 1	543.98±20.97	525.24±36.3	525.42±20.09	0.002	<0.001	0.976	<0.001
CT days 7	532.26±20.63	518.72±36.1	520.92±20.22	0.023	0.007	0.708	0.027
CT days 30	523.44±20.31	512.56±35.65	515.78±19.9	0.064	0.06	0.578	0.109

CT: Corneal thickness, SD: Standard deviation

Table 5: Mean increase in pachymetry in different groups

Day	Group A		Group B		Group C	
	Mean corneal swelling	Percentage increase	Mean corneal swelling	Percentage increase	Mean corneal swelling	Percentage increase
1	28	5.43	18	3.55	11	2.14
7	16	3.10	12	2.37	7	1.36
30	7	1.4	6	1.18	2	0.04

surgical trauma and are indispensable in evaluating the safety of new surgical methods.[16] Al though complete endothelial recovery may take >1 month, there is no significant change in the endothelial cell count afterward. In a study, Hamid Gharaee compared corneal endothelial loss with the location of PKE incision at 1 week, 1, and 3 months postoperatively. There was no significant change in the endothelial cell count measured at 1 month and 3 months postoperatively in any of the three groups.[17] Age, nucleus grade, size of the nucleus, type of IOL, greater amount of total emitted US energy, and longer duration of surgery are associated with EC loss.[18-20] Intraoperative complications such as vitreous loss and Descemet's membrane detachments are known to be associated with increased EC loss. Corneal clarity was of the order that we could completely observe fine iris details in 147 of the total 150 eyes and did not differ in between two groups. Photograph of the corneal endothelium on the first postoperative day could be taken as significant stromal edema was not seen in any of cases. In 2 cases, there was posterior capsular rent and these 2 cases were excluded from the study.

Maximal pupillary dilatation was ascertained during initial evaluation and also when the patient was found to meet inclusion criteria. They were equal in nearly all cases, therefore, no reassessment was done on the day of surgery. K Ajay found that after achieving maximal dilatation with topical mydriatic, no significant change in pupillary size was noted after a peribulbar block.[21] Udai Devgan found that single most important predictor of good vision and a clear cornea postoperatively is the total amount of phaco energy delivered into the eye.[10] In our study, effective phaco time in Group A was 3.89 ± 2.44 s, Group B was 3.45 ± 2.51 s, and Group C was 3.31 ± 2.33 s with no statistically significant difference ($P = 0.462$). We did not observe any difference in the degree of phaco power used in pupils of different sizes in different groups. Nixon found that effective phaco time varied significantly in cataracts of different grades.[22]

Inoue *et al*. found that age is the major relevant factor in corneal EC morphology in patients before cataract surgery.[23] In our study, the patient age group was limited to a range of 50–70 years with the mean age in Group A 59.72 years, Group B was 60.58 years, and Group C was 59.74 years. No significant difference in age was present among the 3 groups.

In our study, mean EC loss on postoperative day 1 in Group A was 19.32%, Group B 14.25%, and Group C 10.12%. AC Sobottka Ventura compared corneal thickness and endothelial density before and after PKE.[24] They found that all patients incurred significant postoperative corneal swelling and EC losses after surgery, the mean loss being 346 cells/mm^2 (16%; $P = 0.001$) but this loss did not translate into decreased visual acuity. Hence, they concluded that as long as the numerical density of the corneal ECs does not fall below the physiological threshold, a moderate decrease in this parameter does not compromise the pumping activity of the corneal endothelium as a whole and hence does not have a bearing on the visual acuity. Stoll-Paulsen A and Park J found that significantly less PKE power was used during phaco chop surgery than during divide-and-conquer surgery.[25,26] Postoperatively, both groups had a significant but equal decrease in cell density. In this study, all the patients had undergone cataract surgery by stop and chop technique. In our study, direct chop technique was used in all the cases with 15° phaco tip in bevel up position. Raskin E found a significant difference in EC with phaco tip bevel up position as compared to bevel down position.[27] Nayak and Jain compared corneal EC loss in PKE using continuous AC infusion versus those using OVD and found that using AC maintainer for continuous AC infusion and omission of OVD during PKE did not cause significant difference in corneal swelling or EC loss in the immediate postoperative period up to 1 month.[28]

An attempt has been made in our study to reduce the effect of multiple variables by excluding cases with other ocular complications, including cases with cataracts of comparable nuclear sclerosis (grade II) and surgery was performed by the same surgeon using near identical in all cases.

On postoperative day 1, Group A had maximum EC loss of 19.32 (19.45)% followed by Group B with 14.25 (14.89)% and then Group C with 10.12 (10.19)%. The difference was statistically significant in A versus B and A versus C with no difference in B versus C. On postoperative day 7 and 30, EC counts improved in all groups probably due to cell migration but the trend of cell loss remained the same with statistically significant difference in A versus B, A versus C with no difference in B versus C. In another study by Pirazzoli PKE was associated with 16.67% EC loss which correlated with the degree of trauma during surgery.[5]

Increase in corneal thickness was maximum in Group A with a 5.49 (5.43)% increase, followed by Group B with 3.7 (3.55)% and minimum in Group C with 2.2 (2.14)%. Statistically significant difference of corneal thickness was observed between Group A versus Group B and Group A versus Group C on postoperative day 1 and day 7. However, on postoperative day 30, the difference of corneal thickness was not statistically significant. In a study by Sachin M Salvi, it was found that CCT remained increased by 6.44% on day 1 postoperatively compared with preoperative values and gradually reduced to preoperative levels by the 1-week postoperative period (0.57% difference).[29] We agree with them that pachymetric changes after PKE surgery are reversible. No statistically significant difference of corneal thickness was observed between Group B versus Group C on any postoperative day. In one study, post PKE increase in pachymetry varied from 4.86% to 5.95% in different groups.[28] We have not found any other study in the available literature that has compared EC loss and corneal thickness exclusively on the basis of pupillary size and has tried to evaluate its influence on these two parameters that are critical to the corneal transparency. Our hypothesis is that a smaller pupil (which becomes further smaller in the course of the surgery).

- A small pupil may result in a more anterior PKE close to the corneal plane as against in the bag emulsification resulting in greater EC
- Results in greater incidence of collision between loose nuclear fragments and pupillary margin or corneal endothelium
- Surgeon takes more time and manoeuvre to ensure complete removal of lens cortex.

Conclusion

PKE done in eyes with maximal pupillary dilatation of <5 mm causes a greater EC loss and results in thicker corneas postoperatively as compared to eyes with pupillary dilatation of >5 mm at the end of one month.

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Conflicts of interest

There are no conflicts of interest.

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