A PROSPECTIVE STUDY ON THE FACTORS THAT AFFECT CHANGES IN ENDOTHELIAL CELL DENSITY AND CENTRAL CORNEAL THICKNESS OVER A THREE-MONTH PERIOD AFTER PHACOEMULSIFICATION AT AN EYE HOSPITAL IN NAIROBI, KENYA

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DECLARATION

I declare that this dissertation is my original work and has not been presented for the award of a degree in any other university.

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LIST OF ABBREVIATIONS

ACD –	Anterior chamber depth
AGEs –	Advanced glycosylation end-products
AL –	Axial length
BSS –	Basic saline solution
CCT –	Central corneal thickness
DM –	Diabetes mellitus
ECD –	Endothelial cell density
IOL –	Intraocular lens
IOP-	Intraocular pressure
MMPs –	Metalloproteinases
PHACO –	Phacoemulsification
APT –	Absolute phacoemulsification time
EPT –	Effective phacoemulsification time

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ABSTRACT

Background: The ultrasonic energy during phacoemulsification cataract extraction results in pathophysiological mechanism that induce changes in endothelial cell density (ECD) and central corneal thickness (CCT).

Objective: To evaluate the change in ECD and CCT over three months following phacoemulsification and selected factors that affects these changes.

Methodology: Prospectively, we studied all consecutive eyes undergoing routine phacoemulsification over the 9-month study period. Preoperative ECD, CCT, anterior chamber depth, axial length, age and diabetic status were recorded in a data form. We recorded intra-operative infusion fluid type and volume, nucleus fraction technique, elapsed phaco time and ultrasound power. Serial postoperative ECD and CCT were recorded on day 1, month 1 and month 3 postoperatively.

Results: After excluding 14 eyes, we analyzed46eligible eyes of 36 patients. The mean age was 66.69 years and male: female ratio was 1.4:1.At the third month the average decrease in ECD was 7% and in CCT was 6%. Older age and diabetic status; use of Balanced Salt Solution (BSS), higher effective phacoemulsification time (a product of elapsed phaco time and Ultrasound average) were found to correlate with more decrease in ECD. Deep ACD or long AL were found to be associated with less decrease in ECD. Onmultivariate analysis, patient factors had statistically significant effects on ECD up to the first month only. ($p= <0.05$) However, the intra-operative factors had a statistically significant effect on ECD up to three months postoperatively.

Conclusion: Phacoemulsification is safe and was associated with minimal change in ECD and CCT. ECD cut off of 2000 cells/$mm^2$was not associated with increase in postoperative CCT.

CHAPTER 1: INTRODUCTION, BACKGROUND AND LITERATURE REVIEW

1.1 INTRODUCTION AND BACKGROUND

The cornea is the most powerful refractive surface of the eye and its clarity is paramount for vision. The endothelium, the innermost layer of the cornea is a single layer of hexagonal non-replicative cells that acts as a barrier to the aqueous humor. The endothelial metabolic pump keeps the corneal stroma in a state of dehydration, thus maintaining corneal transparency and normal corneal thickness.,[1][2]

The mean endothelial cell density (ECD) at birth is 5000 cells/mm^2 and declines physiologically with age. By the age of 5 years, it declines to about 3500 cells/mm^2 and between 14-20 years of age, the ECD is at 3000 cells/mm.[3]Thereafter, there is a slow decline of between 0.3-0.6% per year leading to about 2500 cells/mm in late adulthood.[2][3] Since endothelial cells are non-replicative, loss of these cells is compensated for by the migration, enlargement, and increasing heterogeneity of the residual cells. [3]

The ECD is determined using photographic Specular microscopy that analyses the maximum and minimum endothelial cell area, coefficient of variation, the percentage of hexagonal cells and central corneal thickness; both contact and non contact methods are available.

The average central corneal thickness (CCT) is about 540 micrometers(μm) in the adult eye, with slight variations among the races.[1][2][4]CCT is determined by optical or ultrasonicpachymetry. Newer methods of assessment of CCT such as scanning slit technology, Scheimpflug anterior segment imaging, optical coherence tomography, and high-resolution ultrasonography may produce precise maps of the entire corneal thickness, including corneal curvature.

Cataracts are the leading cause of reversible blindness in developing countries, causing 40-80% of all blindness.[5]with the expected increase in the prevalence of cataracts due to increasing life expectancy and no known prevention, surgery is the only management.

In 2015, 3.6 million cataract extractions were done in the United States, with global estimates being at 20 million.[6]Phacoemulsification with foldable intraocular lens insertion is the most common surgical procedures performed today. Since the first phacoemulsification in Kenya performed in 1993 in a private hospital, the technique is now widely performed even in high-volume eye centers countrywide.[7]

The Phacoemulsification ultrasonic energy is known to cause both mechanical and thermal damage to the endothelium during surgery.[7,8] Although a Low ECD below 1000 cells/mm^2 may still maintain cornea transparency, this precludes intraocular surgery because such corneas are at greater risk for corneal decompensation.[2] A low ECD below 500 cells/mm^2 will definitely cause corneal decompensation, an increase in CCT, cornea opacity and poor vision independent of other predisposing factors.[1]

Routine documentation of ECD is therefore important to determine the visual prognosis following phacoemulsification. This is the clinical basis of studies on ECD. However, few centers in Kenya perform routine ECD measurements.

1.2 LITERATURE REVIEW

The reported average ECD losses after phacoemulsification vary between 4% and 17%.[13–19] This loss is a function of several pre- and intra-operative factors. Studies have reported a subsequent postoperative increase in CCT which varies from 9-15%.[20,21] Ventura *et al* found that though there was a significant increase in CCT on the first postoperative day, but preoperative values were restored in 3 to 12 months despite endothelial cell loss as long as the endothelial cell count was above the physiological threshold.[20] However, Kohlhaas*et al* showed an increase in CCT of about 10 percent a year following phacoemulsification.[21]Following phacoemulsification, the causes of corneal oedema include type of viscoelastic used, type of fluid used intra-operatively and intraocular pressure (IOP) spike postoperatively.[9–12]

1.2.1 FACTORS AFFECTING ENDOTHELIAL CELL DENSITY (ECD) AND CENTRAL CORNEAL THICKNESS (CCT) FOLLOWING CATARACT SURGERY.

1.2.1.1 PATIENT FACTORS

1. **Age and cataract density**

Most of the patients undergoing phacoemulsification belong to the elderly age group, who have denser cataracts and an already reduced endothelial cell density.[2,22] Pre-existing low ECD values and further intra-operative endothelial cell loss become important factors to consider during case selection for surgery. Several studies have demonstrated that a denser and thicker nucleus leads to a greater loss of ECD.[14,20,23,24] O'Brien *et al* determined that denser cataracts which are mostly seen in older patients are associated with utilization of higher ultrasound energy and longer surgery time, thus more endothelial cell loss.[24]Walkow*et al*also concluded that a higher age correlated with increased lens thickness and a longer operation time. However, Walkow*et al*did not find any significant correlations between central endothelial cell loss and age, lens thickness, operation time, phacoemulsification energy, or intensity.[14]

2. Anterior Chamber Depth and Axial length

In a study by Mashige KP et al where he examined 600 African eyes, he found the normal anterior chamber depth (ACD) to be between 2.38-4.13mm.[25]In a prospective study by Hwang *et al* investigating the endothelial cell loss after phacoemulsification according to different anterior chamber depth (ACD), it was found that eyes with shallow ACD especially with hard cataracts are more vulnerable to endothelial cell loss.[23]In a similar study, Walkow*et al*2000 did not find a significant correlation between endothelial cell loss and anterior chamber depth.[14]Bhardwaj V et al and Dong et al described the normal axial length as between 22-25mm.[26,27] Both Walkow*et al* and Baradaran-Rafii A*et al* separately showed that shorter axial lengths were associated with greater ECD loss.[14,22] O'Brien *et al* concluded that neither a short axial length nor a shallow anterior chamber was associated with an increased risk of endothelial cell loss.[24]

3. Diabetes Mellitus

Diabetes mellitus (DM) causes several biochemical and ultra structural abnormalities in the cornea. Reduced endothelial cell function and dysfunctional repair mechanisms lead to corneal edema, delayed wound healing and reduced corneal sensitivity.[28] Hyperglycemia results in an increase in aldose reductase activity, the expression of metalloproteinase (MMP), and the formation of advanced glycation end products (AGEs). These processes cause dysmorphological changes in the corneal endothelium, damage to the basement membrane, abnormal cellular adhesion, and limitation of cell migration, resulting in poor healing.[28]

In a meta-analysis evaluating corneal changes after phacoemulsification in diabetic and non-diabetic cataract patients, Tang *et al* found that diabetic patients have a significantly lower ECD at preoperative and all postoperative time points than the non-diabetic group.[28]

Percent increase of CCT showed no significant difference between the DM group and the non-DM group at 1 day, 1 week and 3 months postoperative follow up visits. Statistically significant changes in CCT was found in diabetic patients at 1 month postoperative visit.[28]

In a comparative study evaluating corneal endothelium in patients with well controlled diabetes mellitus against non- diabetic controls, Sahu*et al* it found that age

and sex matched diabetic patients showed significantly higher loss in endothelial cell count than the controls despite good glycemic control.[13]Hugod et al similarly found more endothelial cell loss in diabetic patients than non-diabetic patients. The difference was statistically significant.[29]

4. **Pupillary diameter**

There is limited systematic documentation on the association between pupillary diameter and endothelial cell loss. However, Maggon*et al* found that phacoemulsification done in eyes with maximal pupillary dilatation of less than 5 mm had a greater endothelial cell loss and results in thicker corneas postoperatively as compared to eyes with pupillary dilatation of greater than 5 mm at the end of one month postoperatively.[8]

1.2.1.2 INTRAOPERATIVE FACTORS

1. **Phacoemulsification time and ultrasound energy**

The total energy delivered into the eye is a product of the phacoemulsification power and the duration of time that this power is on. This duration is more specifically referred to as theeffective phacoemulsification time (EPT). Several studies have shown that higher phacoemulsification energy and time are correlated to endothelial cell loss.[14,22–24] However, Walkow*et al* concluded that a direct correlation between relative phacoemulsification energy (as a function of phacoemulsification time/relative intensity of phacoemulsification) and endothelial cell loss could only be shown for lateral cell loss (not central). Furthermore, the same study found out that a higher age, correlated with increased lens thickness, was associated with a longer operation time.[14]

2. Phacoemulsification technique

In a study by Hayashi *et al* evaluating corneal endothelial cell loss after phacoemulsification using nuclear cracking procedures, it was found that there was less endothelial cell loss with cracking procedures than with non-cracking procedures. This is because less ultrasound energy is utilized in cracking procedures.(30)Surgeon experience may also strongly influence the endothelial cell loss in patients following phacoemulsification as was demonstrated by Orski*et al*.[31]

3. Infusion volume and type of fluid

In a comparative study to evaluate corneal endothelial cell count after phacoemulsification with continuous anterior chamber infusion, Milla*et al* found endothelial cell density values were lower in the group that had a continuous infusion as compared to the control.[32] However, Baradaran-Rafii A*et al* did not find a statistically significant relationship between the total volume of infused fluid and endothelial cell loss.[22]Lucena*et al* concluded that Ringers solution was similar to Basic Saline Solution (BSS) Plus for corneal preservation in traumatic cataract surgery. However, the study demonstrated that there was a trend towards lower postoperative endothelial cell density for surgeries with longer phacoemulsification time and higher irrigation volumes if Ringers solution were to be used.[33]

1.2.1.3 TECHNIQUES IN DETERMININGENDOTHELIAL CELL DENSITY

The endothelial cell density can be performed by four methods; (1) comparison method (2) Frame method (fixed or variable), (3) corner method, and (4) centre-to-center method (Annex 1). Regardless of the technique, accuracy is dependent on the quality of endothelial cell image to identify individual cells. The Corneal Study Group determined that the cell density analysis difference between technicians using excellent-quality images was 0-6%, whereas fair images had a difference of 6-11%.[34]

The comparison method visually compares the patient's endothelial cell pattern to a known set of hexagon patterns. (Appendix 3)

The frame method provides a numerical assessment of the cell density by counting cells within a frame, which may be a fixed rectangular frame, or a variable one with hexagonal borders. To adjust for cells extending beyond the frame, partial cells extending over 2 adjacent frames are counted as one. The counted cells in the frame are then converted to a value of cells/mm^2The frame method therefore depends on the subjective decision to define a cell, the location of the frame, the chosen size and type of frame and the image magnification.

The corner method identifies the corners of the hexagonally shaped endothelial cells in the captures image, which are then computed to give a count of cells that is then converted to cells/mm^2.

The center-to-center method determines the endothelial cell density by identifying the hexagonal-shaped endothelial cells within the captured image, mapping their centre, then counting each of the identified cells to give a value per mm^2.[35]

A study done by McCarey et al reviewing corneal endothelial specular microscopy, an error of about ±362 cells/mm^2He explained that a reading of 2500 cells/mm^2could have a value of between 2138–2862 cells/mm^2.[35]

The Tomey EM-3000 is a non-contact specular microscope that utilizes the frame analysis method to obtain the endothelial cells density. While reporting on his experience with the Tomey EM 3000, Kinoshita S et al reported a difference of up to 500 cells/mm^2 between manual and automatic modes on this machine.[36]

CHAPTER 2: JUSTIFICATION

2.1 Study Rationale

Currently, corneal changes in ECD and CCT following phacoemulsification, and the factors affecting these changes are not known in Kenya, despite increased use of phacoemulsification.

In our study, we determined the endothelial cell density (ECD) and central corneal thickness (CCT) changes following phacoemulsification. The results of our study can be used in patient selection and also guide surgeons in their practice as they would be informed of the factors that predispose to more corneal changes.

2.2 Objectives

2.2.1 Broad Objectives

To assess the factors that affect changes in endothelial cell density and central corneal thickness before and after phacoemulsification at eye hospital in Nairobi.

2.2.2 Specific Objectives

1. To determine the change on ECD and CCT associated with selected patient factors: age, diabetes status, axial length and anterior chamber depth.

2. To determine the change on ECD and CCT associated with intra-operative factors: fluid type and volume, effective phacoemulsification time (EPT) and cracking versus non-cracking techniques

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Design

Prospective cohort study

3.2 Study Setting

The study was conducted at Eagle Eye Laser Centre in Lavington, Nairobi, Kenya. (Refer to map in Appendix 3).

The catchment population is diverse; most are urban patients within the city but also receives referrals from outside Nairobi and outside Kenya.

In our pre-study survey to establish ideal study sites in Kenya, the centre was selected because it was the that satisfied our major criteria of availability of the specular microscope, Tomey EM-3000, and performed ECD routinely. With permission, we confirmed that all equipment was in excellent calibrated condition. The centre uses a cut off of ECD $\geq$2000 cells/mm^2 for all eyes to undergo phacoemulsification surgery.

During the study period at EELC all phaco surgeries were performed by two surgeons on the same Alcon Accurus phacoemulsifier; ECD, CCT and biometry recorded by one experienced technician and intra-operative data from the Alcon Accurus captured by one experienced theatre nurse.

3.3 Study Period

The study was carried out from10thAugust 2018 to 30th April 2019. Patients captured underwent surgery between 10th August 2018 to 31st January 2019; they were then followed up until 30th April 2019, to ensure three month follow up for each.

3.4 Study Population

All eyes which underwent phacoemulsification during the study period.

3.5 Sample Size

The following formula was used to calculate the required sample size for the study:

$$\frac{N=Z\alpha/2^2\{P(1-P)\}}{d^2}$$

Where:

Zα/2 is critical value for 95% confidence interval that is 1.96

P is estimated proportion of population value, in this case estimated failure rate = 15%

d is margin of error = 10%

$$\frac{N=1.96^2\{0.15(1-0.15)\}}{0.1^2}$$

N = 49 eyes

Correcting for finite population is done using the following formula:

$$\frac{N=N\ x\ X}{X+N-1}$$

Where:

N is population size (number of phacoemulsification surgeries done in the last 6 months = 196)

X is previous sample size calculated

$$\frac{N=196\ x\ 49}{49+196-1}$$

N = 39 eyes

The minimum sample size required for this study to have adequate power of 80% was calculated using this formula:

$$n'=\frac{n}{1+n/N}$$

Where:

n is sample size after population correction

N is previous sample size calculated

$$n'=\frac{39}{1+39/49}$$

n'=22 eyes

3.6 Inclusion Criteria

All eyes of patients undergoing phacoemulsification during the study period

3.7 Exclusion Criteria

1. Eyes with pre-existing corneal pathology
2. History of ocular infection and /or inflammation
3. Complicated phacoemulsification procedure intra-operative and postoperative complications
4. Pre-operative ECD of less than 2000 cells/mm^2
5. No phaco power used intra-operatively

3.8 Outcome measures

3.8.1 Primary outcome Measures

Endothelial cell density (ECD) and Central Corneal Thickness (CCT) at post-operative day one, month one and month three.

3.8.2 Secondary Outcome Measures

The association between endothelial cell loss and central corneal thickness with:

1.Patient factors: age, diabetic status, anterior chamber depth and axial length

2. Intra-operative factors: fluid type and volume used, effective phacoemulsification time and cracking versus non-cracking techniques.

3.9 Materials

A pre-designed questionnaire was used to collect the data (Appendix 2).

Tomey EM-3000© A non-contact specular microscope, utilizing frame method was used to obtain ECD and CCT

A-scan by Alcon©was used to obtain ACD and AL

Alcon Accurus© phacoemulsification machine

3.10 Data Collection Procedure

The principle investigator led a data collection team consisting of one technician, one scrub nurse and two surgeons.

Before the study was undertaken, after obtaining ethical approval, the principal investigator confirmed that the technician calibrated the Tomey EM-3000.This was done by resetting the machine back to its factory settings.

To test the accuracy of the machine, on 9th August 2018, the principal investigator took the ECD readings of five healthy volunteers. There was an ECDvariation of about ±300 cells/mm^2 found in this pretest.(Appendix 3)

One experienced technician used the Tomey EM 3000 to take measurements of the ECD and CCT both pre-operatively and postoperatively. This was from the central part of the cornea, using automatic mode. Two readings were made in the same sitting for each patient without switching off the machine. The average of these two readings was then calculated. This was to enhance accuracy. The machine was switched off between patients to avoid data mix up.

The anterior chamber depth and axial depth were taken from A scan results from the Alcon machine.

The center only performs surgery in patients with ECD of ≥2000 cells/mm^2. Two surgeons (JN and HG) performed all the phacoemulsification surgeries.

Intraoperative phacoemulsification auto-data was obtained by the scrub nurse taking a screenshot of the Alcon Accurus© phacoemulsification machine at the end of each surgery. She also noted the type and volume of fluid used per surgery.

The surgeons reported the type of phacoemulsification technique used, cracking versus non-cracking technique and if there were any intraoperative complications.

The principal investigator computed the effective phaco time for each surgery by multiplying the phaco ultrasound average with the elapsed time.

At the post-operative reviews at day 1, month 1 and month 3, the technician used the Tomey EM-3000 to take two readings each containing the ECD and CCT, the average of which was used for analysis. The eyes were also observed for any post-operative complications during each of these visits.

3.11 Data Analysis

The raw data was entered from the questionnaire into Microsoft Excel 2011 by the principal investigator and submitted to the statistician for analysis of the ECD and CCT. Any outlier data was analyzed separately to determine specific factors.

Descriptive analysis was used to determine the frequencies and proportions of the variables, which were presented in tables or graphs where appropriate. The normality of the data was assessed using histograms. If not normally distributed, transformation

of the data was attempted, where possible, to find the best possible line of fit. The mean with standard deviations was reported when the data was normally distributed and medians when it was not, or where it was appropriate. Student's t-test was used to study the statistical significance of differences between pre- and post-operative measurements at follow up visit. Statistical significance was set at a p-value of <0.05 with confidence interval of 95%.

Pearson coefficient was used to determine correlations between outcomes and measures and demographic characteristics and presenting features of the patients. The strength of these correlations was further tested using univariate regression analysis. A multivariate analysis, this was then used to build a model for factors affecting the changes in the ECD and CCT.

3.12 Ethical Consideration

Ethical permission was sought and granted from Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee.

Permission was also sought and granted from the administration of Eagle Eye Laser `Centre, Nairobi.

Patient details and identity were kept anonymous at all times through the use of coded questionnaires. The information on the questionnaire was only accessible to the principle investigator and the technician filling in the machines readings. Confidentiality was upheld and data collection standards were maintained and adhered to. Collected data was also encrypted to further facilitate confidentiality. The coded questionnaires were destroyed after data was analyzed. The investigator had no conflict of interest to disclose.

CHAPTER 4: RESULTS

After excluding 14 eyes, we analyzed 46 eligible consecutive eyes of 36 participants who underwent phacoemulsification surgery at the study site in Nairobi, between 10th August 2018 and 31st January 2019. They were followed up until 30th April 2019.

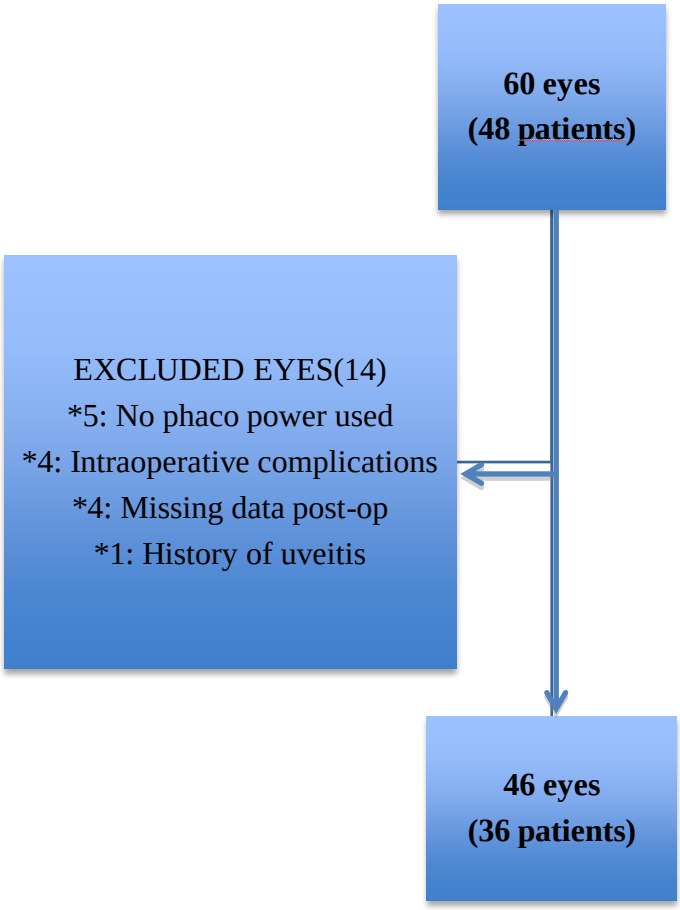
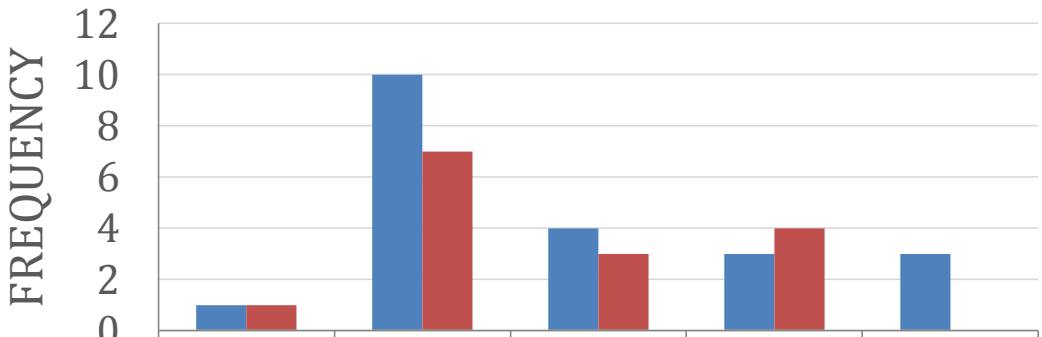


Figure 1: Flow Chart showing the selection of patients included in the study

4.1 Preoperative Statistics

4.1.1 Demographics

N=36 participants



	45- 54	55 - 64	65 - 74	75 -84	85-94
Male	1	10	4	3	3
Female	1	7	3	4	0

Figure 2: Number of patients that underwent phacoemulsification based on age and sex. There was a slight male preponderance. Most patients were aged between 55-64 years.

4.1.2 Participant characteristics

Table 1: **Summary of pre-operative statistics (N=46 eyes)**

Characteristics	**Mean ± SD/N(%)**
Number of participants	36
Age (years)	66.69±10.39
Diabetes Mellitus	Yes- 9 (25%)
	No- 27 (75%)
Gender	Female- 15 (42%)
	Male -21 (58%)
Average ECD (cells/mm^2)	2412±203
Average CCT (μm)	506 ±40
Average ACD (mm)	3.81 ± 0.82
Average AL (mm)	23.83±1.09

Male: Female Ratio: 1.4:1

There were more males than females.

There were more non-diabetics than diabetics.

4.1.3 Intraoperative Characteristics

Table 2: Summary of Intraoperative Variables (N=46 eyes)

Variables measured	**Mean±SD N(%)**
Fluid Type	BSS- 21 (46)
	RL-25 (54)
Volume of fluid (ml)	264 ± 89
Cracking versus Non-cracking	Cracking- 43 (93)
	Non-cracking- 3 (7)
EPT	17.11±15.43

Ringer's Lactate was used in more eyes than Balanced Salt Solution

Cracking technique was more commonly used than non-cracking technique

4.2TREND IN AVERAGE ECD AND CCT OVER THREE MONTHS

4.2.1 Trend in the Average ECD Over Three Months

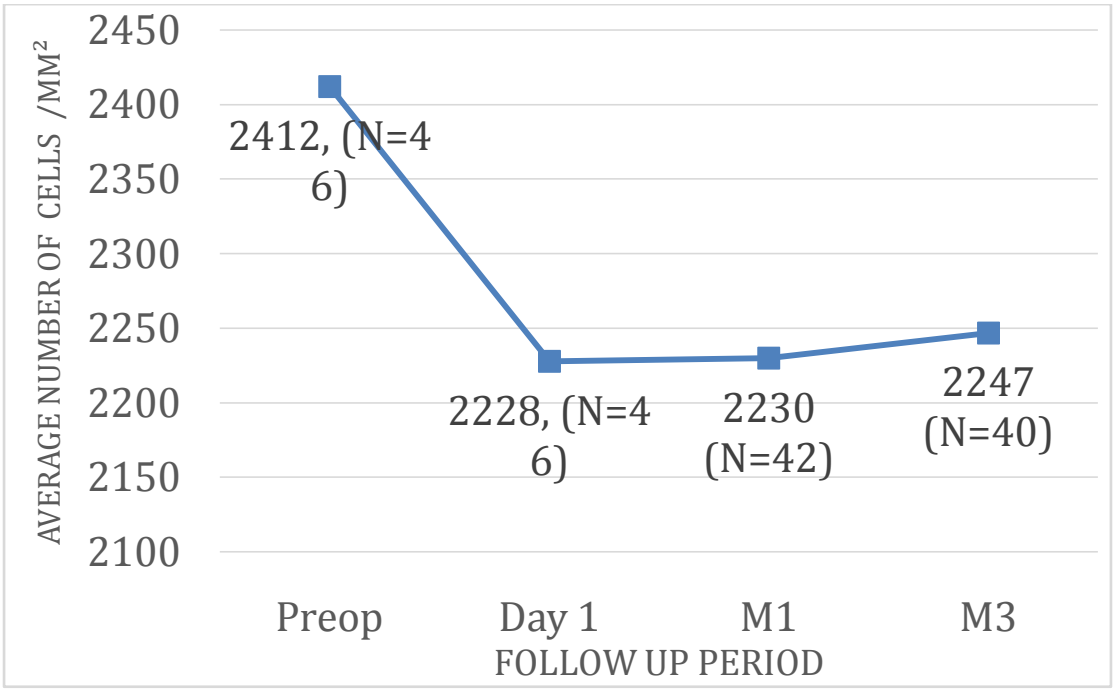


Figure 3: Trend in the Average ECD Over Three Months

There was a significant decrease in endothelial cell density on the first post-operative day, ($p < 0.05$) followed by a slight rise on the first and third month postoperatively.

4.2.2 Trend in the Average CCT Over Three Months

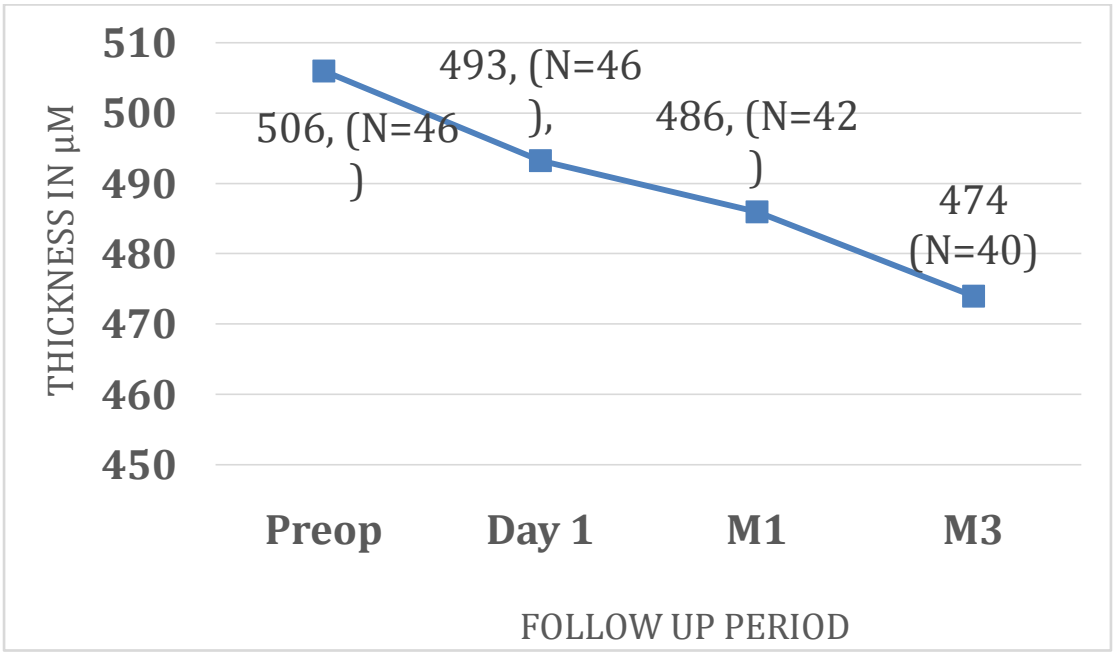


Figure 4: Trend in the Average CCT Over Three Months

There was a decline in central corneal thickness postoperatively, from first day postoperatively.

4.2.3 DIFFERENCE IN AVERAGE ECD AND CCT OVER THREE MONTHS

Table 3: Difference in Average ECD and CCT over Three Months

		PRE-OPERATIVE	POST-OPERATIVE	DIFFERENCE (%)	p-Value
DAY 1 N=46	ECD	2421	2228	-184(8)	0.00
	CCT	506	493	-13(3)	0.00
MONTH 1 N=42	ECD	2396	2230	-166(7)	0.00
	CCT	506	486	-20(4)	0.00
MONTH 3 N=40	ECD	2405	2247	-158(7)	0.00
	CCT	505	474	-31(6)	0.00

There was a statistically significant change in the ECD and CCT at each point post-operatively.

4.3 Effect of Selected Patient Factors on ECD and CCT Over Three Months

4.3.1 AGE

4.3.1.1 Effect of Age on ECD and CCT On Day One Postoperatively (N=46)

a) Change in Endothelial Cell Density (N=46)

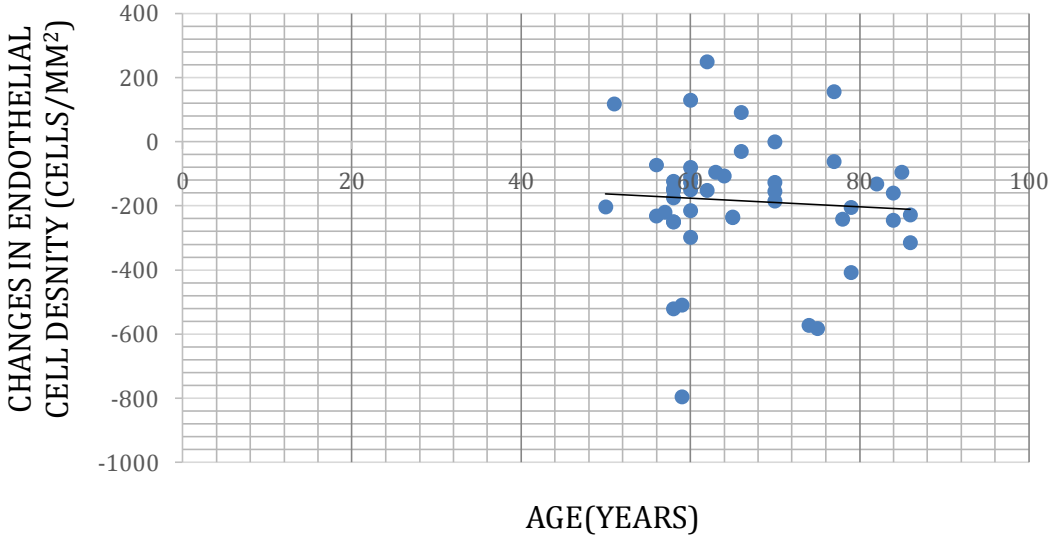


Figure 5: Age versus Change in ECD at One Day Postoperatively

There was no correlation between age and change in ECD (R2= 0.00)

b) Change in Central Corneal Thickness (N=46)

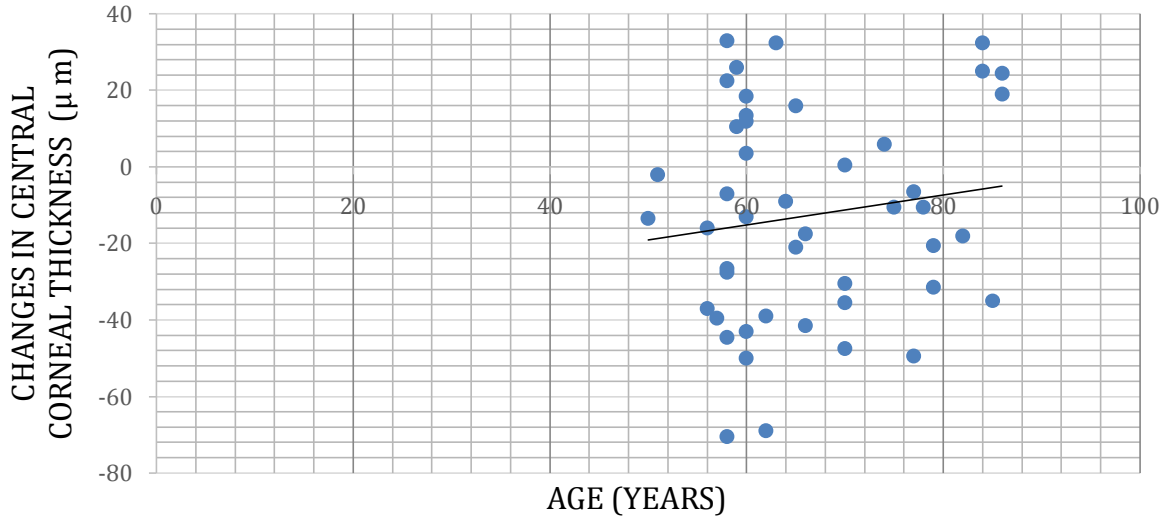


Figure 6: Age versus Change in CCT at One Day Post-operatively

There is a weak positive correlation between age and CCT change (R^2= 0.02).

4.3.1.2 Effect of Age on ECD and CCT on Month One Postoperatively (N=42)

a) Change in Endothelial Cell Density (N=42)

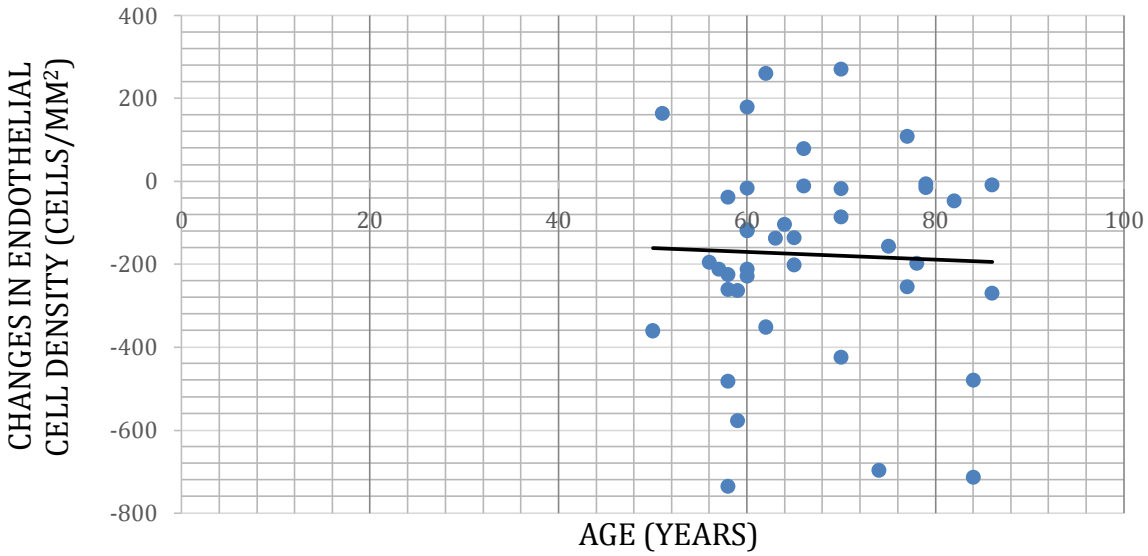


Figure 7: Age versus Change In ECD at One Month Postoperatively

There is no correlation between ECD change and age.(R^2=0.00)

b) Change in Central Corneal Thickness (N=42)

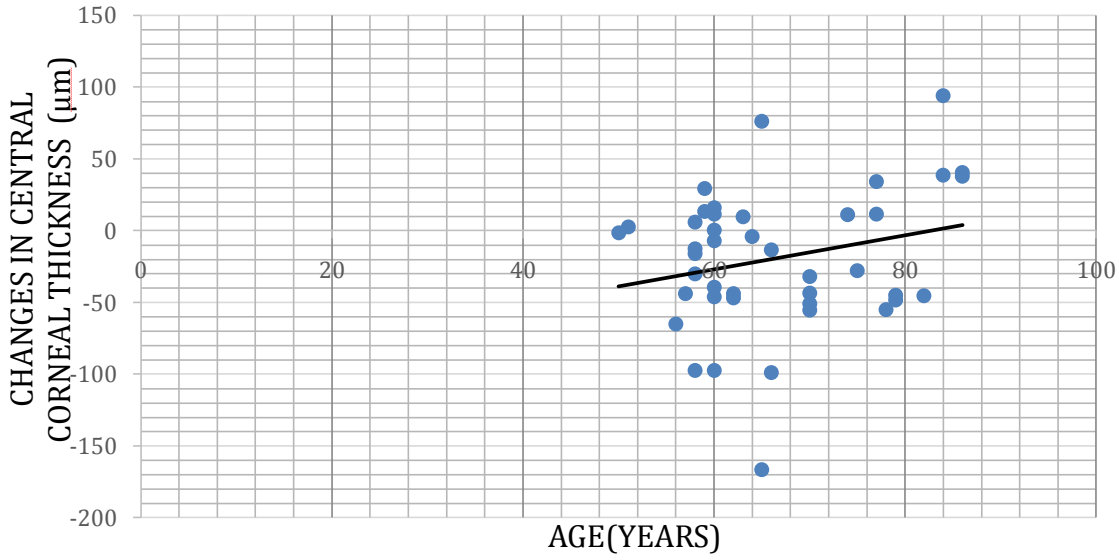


Figure 8: Age versus Change in CCT at One Month Postoperatively

There was a weak positive correlation between age and CCT change. (R^2= 0.05)

4.3.1.3Effect of Age on ECD and CCT on Month Three Postoperatively (N=40)

a) Change in Endothelial Cell Density (N=40)

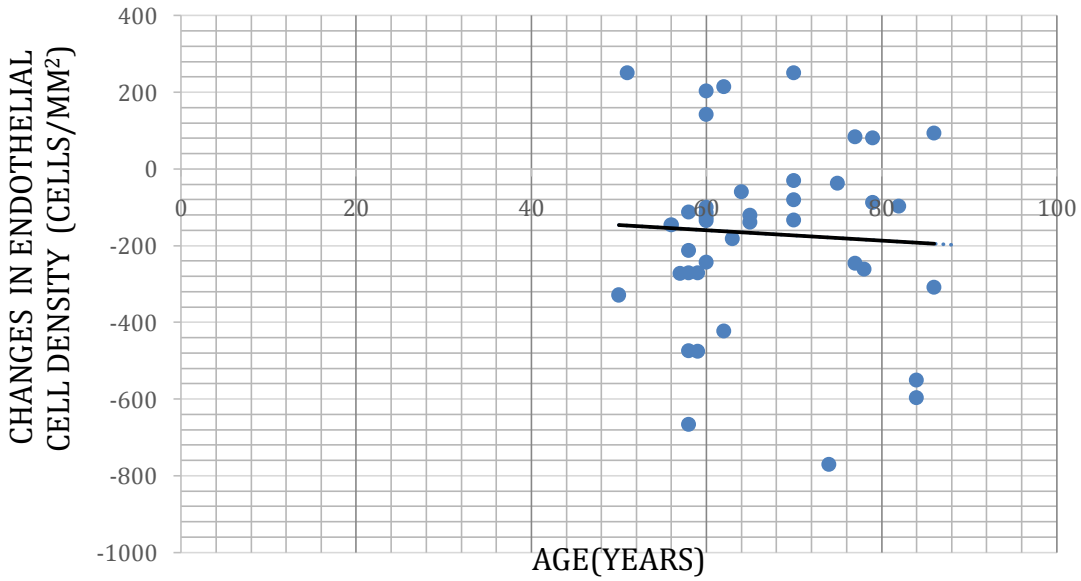


Figure 9: Age versus Change In ECD at Three Months Postoperatively

There is a weak negative correlation between Age and ECD changes (R^2= -0.04)

b) Changes in Central Corneal Thickness (N=40)

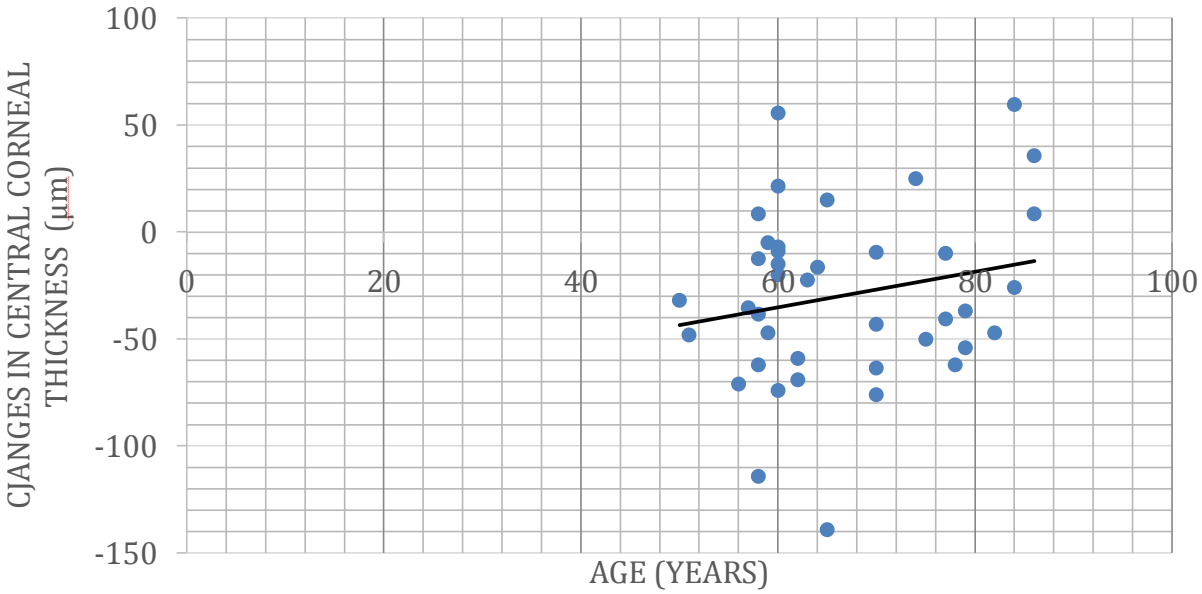


Figure 10: Age versus Change in CCT at Three Months Postoperatively

There was a weak positive correlation between age and change in CCT.(R^2= 0.06)

4.3.2DIABETES MELLITUS

Table 4: Change In ECD and CCT in Diabetics versus Non-Diabetics Over Three Months

DM STATUS	ECD CHANGE(cells/mm^2)	p-value	CCT CHANGE(μm)	p-value
Day 1 N=46		>0.05		<0.05
Yes (N=12)	-199		-18	
No (N=34)	-179		-11	
Month 1 N=42		<0.05		>0.05
Yes (N=11)	-189		-38	
No (N=31)	-158		-13	
Month 3 N=40		>0.05		>0.05
Yes (N=11)	-189		-38	
No (N=29)	-153		-27	

The decrease in ECD and CCT among diabetics was greater compared to non-diabetics at all time points up to three months.

There was a statistically significant decrease in the CCT on the first postoperative day and in the ECD on first postoperative month, among all diabetics.

4.3.3ANTERIOR CHAMBER DEPTH

Table 5: Change In ECD and CCT Compared To Anterior Chamber Depth Over Three Months

ACD (mm)	ECD CHANGE(cells/mm^2)	p-value	CCT CHANGE(μm)	p-value
Day 1 N=46		>0.05		>0.05
≤4.13(N=27)	-198		-8	
> 4.13(N=19)	-165		-17	
Month 1 N=42		>0.05		>0.05
≤4.13 (N=27)	-185		-15	
>4.13 (N=15)	-132		-25	
Month 3 N=40		>0.05		>0.05
≤4.13 (N=26)	-170		-23	
>4.13(N=14)	-137		-38	

Normal ACD is 2.38-4.13mm. No eyes were found to have shallow ACD.

The decrease in ECD was less in eyes with deep ACD at all time points up to three months.

There was no statistical significance between ACD and either the decrease in ECD or decrease in CCT.

4.3.4 AXIAL LENGTH

Table 6: Change In ECD and CCT Compared To Axial Length Over Three Months

AL (mm)	ECD CHANGE(cells/mm^2)	CCT CHANGE(μm)
Day 1 N=46		
≤25 (N=39)	-186	-9
>25(N=7)	-175	-17
Month 1 N=42		
≤25 (N=36)	-174	-11
>25 (N=6)	-167	-28
Month 3 N=40		
≤25 (N=34)	-189	-22
> 25(N=6)	-168	-39

No eyes were found to have short AL

The decrease in ECD and in CCT among eyes with normal AL(22-25 mm) was greater than eyes with long AL (>25 mm)at all time points up to three months.

4.4 Intraoperative factors affecting ECD and CCT over Three Months

4.4.1 Fluid type

Table 7: Change In ECD and CCT Compared To Fluid Type Over Three Months

FLUID TYPE	ECD CHANGE(cells/mm^2)	p-value	CCT CHANGE(μm)	p-value
Day 1 N=46		>0.05		>0.05
BSS (N=21)	-153		-3	
RL (N=25)	-211		-21	
Month 1 N=42		>0.05		>0.05
BSS (N=20)	-269		-4	
RL (N=22)	-72		-34	
Month 3 N=40		>0.05		>0.05
BSS (N=18)	-253		-15	
RL (N=22)	-80		-42	

The decrease in ECD was greater at first and third month postoperatively, in eyes where BSS was used intra-operatively

There decrease in CCT was greater at all time points in eyes where RL was used intra-operatively.

There was a statistically significant difference between the CCT decrease and type of fluid used intra-operatively (BSS and RL) only on the first day postoperatively.

4.4.2 Fluid Volume

Table 8: Change in ECD and CCT Compared To Fluid Volume Over Three Months

FLUID VOLUME (mls)	ECD CHANGE(cells/mm^2)	p-value	CCT CHANGE(μm)	p-value
Day 1 N=46 Median=265		>0.05		>0.05
≤265 (N=23)	-126		-12	
>265 (N=23)	-242		-13	
Month 1 N=42 Median=280		>0.05		>0.05
≤280 (N=20)	-172		-11	
>280 (N=22)	-160		-28	
Month 3 N=40 Median=280		>0.05		<0.05
≤280 (N=18)	-157		-17	
>280 (N=22)	-159		-41	

There were more ECD changes with more fluid used noted at first day and first month postoperatively.

There were more CCT changes with more fluid used over three months.

There was a statistically significant difference between the CCT changes and volume of fluid used at third month postoperatively

4.4.3 Effective Phacoemulsification Time

Table 9: Change in ECD and CCT Compared To EPT Over Three Months

EPT	ECD CHANGE(cells/mm^2)	p-value	CCT CHANGE(μm)	p-value
Day 1 N=46 Median=12.81		>0.05		>0.05
≤12.81 (N=23)	-102		-12	
>12.81 (N=23)	-265		-13	
Month 1 N=42 Median=10.8		<0.05		>0.05
≤10.8 (N=21)	-152		-14	
>10.8 (N=21)	-180		-26	
Month 3 N=40 Median=12.2		>0.05		<0.05
≤12.2 (N=20)	-135		-14	
> 12.2(N=20)	-181		-47	

There were more ECD losses with higher EPT over the three months.

There was also more reduction in CCT with higher EPT over the three months.

There was a statistically significant difference in the ECD and CCT at one month and three months postoperatively.

4.4.4 Cracking versus Non-Cracking Technique

Table 10: Change in ECD and CCT Compared to Surgery Type Over Three Months.

SURGERY TYPE	ECD CHANGE(cells/mm^2)	CCT CHANGE(μm)
Day 1 N=46		
Cracking (N=43)	-183	-13
Non-cracking (N=3)	-200	-3
Month 1 N=42		
Cracking (N=39)	-117	-17
Non-cracking (N=3)	-121	-58
Month 3 N=40		
Cracking (N=37)	-145	-30
Non-cracking(N=3)	-159	-38

43 of the 46 eyes studies had cracking technique performed.

There was more ECD loss in the non-cracking than in cracking techniques.

No clear relationship between CCT and surgery technique.

4.5 Multivariate analysis of patient and intra-operative factors affecting ECD and CCT over 3 months

Table 11: Multivariate Analysis of Factors Affecting ECD and CCT Change

		Patient Factors* p-value	Intra-operative factors* p value
DAY 1	ECD	>0.05	>0.05
	CCT	>0.05	>0.05
MONTH 1	ECD	<0.05	<0.05
	CCT	>0.05	>0.05
MONTH 3	ECD	>0.05	<0.05
	CCD	>0.05	>0.05

*Patient factors: Age, Diabetic status, ACD, AL

*Intra-operative factors: Fluid type, Fluid volume, EPT

Both patient and intra-operative factors had statistically significant effect on the ECD changes on the first month postoperatively.

CCT changes were statistically significantly affected by intra-operative factors.

CHAPTER 5: DISCUSSION

In our study we analyzed 46 eyes of 36 patients, 10 patients had bilateral surgery. There was good compliance of visits at all preset time points up to 3 months; only 6 eyes (13%) were lost to follow up. The average age was 66.79 years (range 46-87 years). This was similar to the study by Conrad-Henger I *et al and* Barandaran_Rafii*et al*.[18,22] The male to female ratio was 1.4:1 showing a slight male preponderance. Thisis similar to a study by Conrad-Henger et al. [18]

The decrease in overall ECD at three months was 7% in our study, which demonstrates that phacoemulsification leads to decrease in ECD. Our findings are within the reported ECD loss of 4-17%at three months in the UK, Iran, Japan, Germany, Italy and Ireland. [15–19,24] The slight increase in the ECD on the first and third month postoperatively was probably due to the known acceptable machine variability.

The decrease in overall CCT decrease at three months was 6% in our study, which suggests that there was no corneal oedemadue to loss of ECD immediately after phacoemulsification. Sobottka Ventura *et al*, Switzerland and Kohlhaas M *et al*, Germany reported increase in CCT of 2-10%in the first postoperative day.[20,21] However other factors apart from the CCT may be the main determinant of increase in CCT on the first day postoperatively. Behndig A *et al* and Glasser DB *et al*found that corneal oedemamainly depended on the type of viscoelastic. [9,10] Kim J *et al* described increase in the intraocular pressure was the main cause of corneal oedema on the first day postoperatively.[11] The findings in our study may be attributed to either the minimal loss of endothelial cells or the strict adherence to the minimum ECD for surgery at the center such that any endothelial cells loss was not significant enough to cause corneal oedema. In our study we did not assess the quality viscoelastic and the effect of intraocular pressure postoperatively. Furthermore, Edelhauser HF *et al*[12] found that newer fluids like BSS, used currently in phacoemulsification, has essential ions, buffers and dextrose which ensure minimal damage to endothelial tight junctions hence the cornea does not develop oedema. In our study almost half the patients used BSS as the infusion fluid intra-operatively.

Older patient lost more endothelial cells in our study compared younger patients. This is because older patients may have had denser cataracts that required more ultrasound

power and longer surgery time, hence more endothelial cell loss. This was similar to reports in Germany, Iran, Korea and Ireland. [14,22–24]There was a weak linear correlation between age and ECD in our study similar to reports by Walkow*et al*.[14]

Diabetic patients lost more endothelial cells than non-diabetic patients probably because of diabetic biochemical and ultra structural abnormalities in the cornea. Hyperglycemia causes dysmorphological changes in the corneal endothelium, damage to the basement membrane, abnormal cellular adhesion and limited cell migration.[28]Our findings were similar to those of Tang *et al* in a meta-analysis evaluating corneal changes after phacoemulsification in diabetics versus non-diabetics. In the same study, there was no significant difference in CCT between the two groups.[28]Hugod M *et al*, Denmark, also found that there was more endothelial cell loss among diabetics compared to non-diabetics.[29]

In our study, the ACD in 27 eyes was within normal range reported by Khathutshelo*et al* (2.38-4.13mm)[30]. We found 19 had a deep ACD more than 4.13mm. We observed that the eyes within the normal ACD lost more endothelial cells compared to eyes with comparatively deeper ACD, perhaps because phacoemulsification energy was restricted to a more confined space unlike eyes with deeper ACD. A deep ACD decreases the risk of corneal endothelial damage by increasing the distance between the phaco tip and the cornea, which also minimizes irrigation solution turbulence and the IOL cornea touch.[23]

We found greater ECD loss in the patients with normal AL (22-25mm) compared to eyes with longer AL. VeenaBhardwaj*et al*and Jing Dong *et al*found normal AL to be between 22-25mm.[26,27]we found 39 eyes had normal AL while 7 were found to have longer AL of more than 25mm (Table 6). There was a positive linear relation between ACD and axial length in our study (R^2=0.5). Longer axial lengths are therefore associated with deeper anterior chamber depths, which are associated with less ECD loss. Walkow*et al* and Baradaran-Rafii A*et al* showed similar results.[14,22]No statistical analysis was applicable due to the significantly larger number of eyes within normal AL range compared to long AL (39 versus 7 eyes).

We found greater ECD decrease in eyes that underwent phacoemulsification using BSS than those that used RL found to have more significant loss in endothelial cells, on the first and third month postoperatively. RL showed more decrease in CCT postoperatively at each of the time points investigated. Lucena*et al*[33]however found

that RL and BSS were similar for corneal preservation. In this study, there was however no statistical significance between type of fluid used and ECD and CCT changes. This was in keeping with Baradaran-Rafii A*et al* who had similar findings.[22]

In the reference studies we reviewed, no author provided a reference range of what is considered the normal volume of intraoperative fluid during phacoemulsification.[32,33] Therefore, we used our median to define low volume and high volume of intraoperative fluid, whereby there was no clear relationship between the volume of fluid used in relation to the ECD and CCT changes. We did not find any statistical significance between endothelial cell loss and volume if fluid used, similar to Hat described by Baradaran-Rafii*et al*.[22]

Effective phacoemulsification time is a product of the average ultrasound power and the elapsed time. Our reference studies do not define the normal EPT that should be used during phacoemulsification.[14,22-24] Therefore, we used our computed median EPT to as a define a high or low EPT, whereby higher EPT was associated with more ECD loss Our finding confirms that higher ultrasound power causes more damage to the endothelium; worsened by a longer intra-ocular exposure of the cornea to ultrasonic energy, the phaco tip itself, and irrigation fluid turbulence. [14] We found that the change in ECD and CCT was statistically significant at one month and three months respectively, similar to that reported by Walkow*et al*, Baradaran-Rafii A *et al*, Hwang H Bin *et al* and O'Brien PD *et al*. [14,22–24].

In our study 43 eyes underwent cracking technique and only 3 underwent non-cracking technique, whereby the loss in endothelial cells in the non-cracking was greater than at all time points up to three months. Our finding may imply less ultrasound phaco power was used with cracking compared to non-cracking techniques, but is statistically inconclusive because too few eyes underwent cracking technique. Our findings were similar to the report by of Hwang *et al*.[23]

On the multivariate analysis, the change in ECD was statistically significant in ECD both patient and intraoperative because the cornea is expected to have healed well by one month postoperatively depending on factors in both the patient and the surgical procedure. We found that the ECD changes were affected by the intraoperative factors up to three months postoperatively, implying a long-term effect on the cornea by phacoemulsification energy and fluid type .

Our study provides insight on the long-term consequences of phacoemulsification on the cornea endothelium, and the benefits of routine strict evidence-based assessment to guide safety and outcome of surgical patients.

CHAPTER 6: CONCLUSIONS, RECOMMENDATIONS, LIMITATIONS

6.1 Conclusions

1. Phacoemulsification is a safe procedure with minimal ECD loss and CCT changes when the minimum ECD of 2000 is observed
2. Patient factors associated with more ECD loss were age, diabetes; ocular factors longer ACD and AL were found to be associated with least ECD loss.
3. Intraoperatively, cracking technique and use of Ringer Lactate was associated with least ECD loss.

6.2 Recommendations

1. Guidelines for cataract surgeons in Kenya should adopt routine ECD measurements and perform phacoemulsification when ECD is above 2000 cells/mm^2 .

2. Guidelines for counseling patients in Kenya should address known high-risk patient factors and advice prognosis.

3. Future studies: A randomized control trial to evaluate the differences on the endothelial cell density between cracking and non-cracking surgical techniques, viscoelastic and infusion fluid type in Kenya

6.3 Limitations

ECD readings were made at the central cornea only without considering measurements from peripheral areas of the cornea to get a more comprehensive measurement of ECD on the whole human cornea.

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APPENDIX 1: CONSENT INFORMATION TO PARTICIPATE IN RESEARCH STUDY

I am **DR JUDY KARAGANIA,** the principle investigator in **A PROSPECTIVE STUDY ON THE FACTORS THAT AFFECT CHANGES IN ENDOTHELIAL CELL DENSITY AND CENTRAL CORNEAL THICKNESS OVER A THREE-MONTH PERIOD AFTER PHACOEMULSIFICATION AT AN EYE HOSPITAL IN NAIROBI, KENYA**

STUDY BACKGROUND

Phacoemulsification with foldable intraocular lens (IOL) insertion is the method of choice for cataract management at Eagle Eye and Laser Center, Nairobi, Kenya. The study will assess the changes in endothelial cell density and central corneal thickness after phacoemulsification over a three-month follow-up period

BROAD OBJECTIVE

To assess the changes in endothelial cell density and central corneal thickness before and after phacoemulsification at Eagle Eye and Laser Centre, Nairobi

DESCRIPTION OF THE STUDY PROCEDURE

If you agree to be in this study, your diabetes history and pre-operative ocular history and will be taken, your endothelial cell density (ECD), central corneal thickness (CCT), anterior chamber depth(ACD) and axial length (AL) will be measured. After the surgery your ECD and CCT will be measured again during your usual follow up visits at 1 day, 1 month and 3 months post-operatively.

VOLUNTARINESS OF PARTICIPATION

Your participation in this study is voluntary. It is up to you to decide whether or not to take part in this study. If you decide to take part in this study, you will be asked to sign below.

CONFIDENTIALITY

The data collected is confidential and will only be accessible to the investigator and her supervisors. Only your initials shall be used to collect information and records

will further be serialized to avoid your information being linked to any individual(s). All records will be destroyed upon conclusion of the study.

BENEFITS

The data collected will generate local data about the effects of phacoemulsification on the cornea. This will guide future techniques and patient selection.

RISKS

There are no risks of being part of this study. The data will be collected from an elective procedure, and the measurements taken after the surgery are part of a monitoring process for the progress of your eye(s) and are not invasive.

RIGHT OF WITHDRAWAL

You are free to withdraw at any time and without giving a reason. Withdrawing from this study will not affect the relationship you have with the institution. If you withdraw from the study before data collection is completed, your data will be returned to you or destroyed

UJUMBE WA RIDHAA YA KUSHIRIKI KWENYE UTAFITI

Mimi ni **DKT JUDY KARAGANIA**, mchunguzi mkuu katika **UTAFITI WA KUTARAJIWA WA VIFAZO VYA KUFANYA MABADILIKO KWA SELI ZA NDANI NA UNENE WA KATIKATI WA MBONI BAADA YA UTOAJI WA LENZI KWA NJIA YA KUYEYUSHA KWA MUDA WA MIEZI MITATU KATIKA HOSPITALI YA MACHO, NAIROBI, KENYA**

USULI WA UTAFITI

Utoaji wa lenzi kwa njia ya kuyeyusha na kuingizwa kwa lenzi bandia inayokunjika, ni njia chaguazi kwa ajili ya kurekebisha utandu wa macho katika hospitali ya Eagle Eye and Laser Center, Nairobi, Kenya. Utafiti huu utapima mabadiliko katika seli za ndani ya konea na unene wa kati wa konea baada ya operesheni kwa muda wa kipindi cha miezi mitatu ya kufuatilia

LENGO KUU

Kutathmini mabadiliko katika seli za ndani na unene wa kati wa konea kabla na baada ya kutolea kwa lenzi kwa njia ya kuyeyusha kwa Eagle Eye and Laser Center, Nairobi

MAELEZO YA UTARATIBU WA UTAFITI

Ikiwa unakubali kuwa katika utafiti huu, historia ya ugonjwa wa kisukari na historia ya macho kabla ya operesheni itachukuliwa. Hesabu ya seli za ndani za konea(ECD) , unene wa katikati wa konea(CCT), kina cha chumba cha ndani ya jicho(ACD) na urefu wa jicho (AL) pia itachukulia. Baada ya upasuaji ECD yako na CCT itapimwa tena wakati wa ziara yako ya kawaida ya kufuatilia siku moja, mwezi mmoja na miezi tatu .

USHIRIKI WA HIARI

Ushiriki wako katika utafiti huu ni kwa hiari,. Ikiwa unaamua kushiriki katika utafiti huu, niwewekuamuakuafiki au kukataa. Ukiafikikuwasehemuyautafitihuu, utaombwakutiasahihiyakohapomwisho.

USIRI

Takwimu zilizokusanywa ni za siri na zitapatikana tu kwa mchunguzi na wasimamizi wake. Vikwazo vyako pekee vitatumika kukusanya habari na rekodi zitastahili

kufungwa ili kuzuia maelezo yako yanayohusishwa na mtu yeyote . Rekodi zote zitaharibiwa baada ya kumalizika kwa utafiti.

FAIDA

Takwimu zilizokusanywa zitazalisha takwimu za mitaa kuhusu adhari za opareshen ya kutoa lenzi kwa njia ya kuyeyusha. Hii itaongoza mbinu za baadaye na uteuzi wa mgonjwa.

MADHARA

Hakuna hatari ya kuwa sehemu ya utafiti huu. Takwimu zitakusanywa kutoka kwa utaratibu wa uteuzi, na vipimo vimechukuliwa baada ya upasuaji ni sehemu ya mchakato wa ufuatiliaji wa maendeleo ya jicho lako na sio uvamizi.

HAKI YA KUJIONDOA

Ukohurukuafiki au kutokuafikimudawowotenabilasababuyoyote. Kutokuafikikwakohakutakuwanamadharayoyotekatiyako, mahusianoyakonataasisihii. Usipoafikikablayaukusanyajiwa data kukamilika, data zitarudishwakwako au kuharibiwa

CONSENT FORM

Participant's Consent

I have read and understood the consent information above. I have had my questions answered and the benefits and risks explained to me. I understand that my participation in this study is voluntary and I may choose to withdraw at any time and without reason. I freely agree to participate in this study.

Ridhaaya mshiriki

Nimesomananimeelewataarifayaridhaailiyotolewahapojuu.
Maswaliyanguyamejibiwananimeelezewafaidanamadharayakushirikikatikautafitihuu.
Ninaelewayakuwaushirikiwanguniwahiarinanawezakuchaguakujiondoawakatiwowote nabilasababu. Nakubalikushirikikatikautafitihuu.

SUBJECT'S SIGNATURE *(SAHIHI)*
***YAMUHUSIKA)*:_____**
DATE (*TAREHE*):_____

Investigator's Statement

I, the undersigned, have fully explained the relevant details of this research study to the participant above and believe that the participant has understood and willingly and freely given his/her consent.

INVESTIGATOR'S NAME _____SIGNATURE _____
DATE_____

CONTACT INFORMATION

A. PRINCIPAL INVESTIGATOR:

   Dr. Judy NjeriGathoni Karagania

   Telephone: (+254) 0738480454

   Email: njerikaragania@gmail.com

B. LEAD SUPERVISOR:

   Dr. Joseph Nyamori

   Telephone: (+254) 0721961411

   Email: joenyamori@yahoo.com

C. KNH-UoN ERC Secretariat

   Email: uonknh_erc@uonbi.ac.ke

   Facebook: https://www.facebook.com/uonknh.erc

Twitter:@UONKNH_ERC https://twitter.com/UONKNH_ERCs

APPENDIX 2: QUESTIONNAIRE

BIODATA

Initials: (*First name, then second name*) _____

Age: _____(*Years*)

Sex: Female ☐ Male ☐

Medical History

Do you have Diabetes Mellitus? No ☐ Yes ☐

-If yes, for how many years? _____

Ocular History

	RIGHT EYE	**LEFT EYE**
Do you have any history of the following conditions? (1) Glaucoma (2) Dry Eye Syndome (3) None of the above		
Do you have any history of corneal trauma that required surgery? (1) Yes (2) No		
Do you have any history of any other intraocular surgery? (1) anterior chamber wash out (2) lens washout (3) trabeculectomy (4) trabeculotomy (5) Other, specify (6) None		

PRE-OPERATIVE DATA

	RIGHT EYE	**LEFT EYE**
DATE		
VISUAL ACUITY		
IOP		
ECD	R1	R1
	R2	R2
CCT	R1	R1
	R2	R2
ACD		
AL		

INTRAOPERATIVE DATA:

Phacoemulsification date:_____/_____/2018

Absolute phaco time:_____minutes

Effective phaco time:_____minutes

Technique:

(1) Cracking

(2) Non-Cracking

Type of fluid used:

(1) Ringer's Lactate

(2)Normal Saline

(3) BSS

Volume of fluid used:_____mls

Intra-operative complications:

(1) Posterior capsule tear

(2) Vitreous loss

(3) IOL dislocated into the vitreous

(4) Other, specify

(5) None

POST-OPERATIVE DATA

FOLLOW UP VISIT	RIGHT EYE	LEFT EYE	VISUAL ACUITY	IOP	ECD	CCT
Day 1					R1	R1
					R2	R2
Month 1					R1	R2
					R1	R2
Month 3					R1	R2
					R1	R2

POST-OPERATIVE COMPLICATIONS

(1) Endophthalmitis

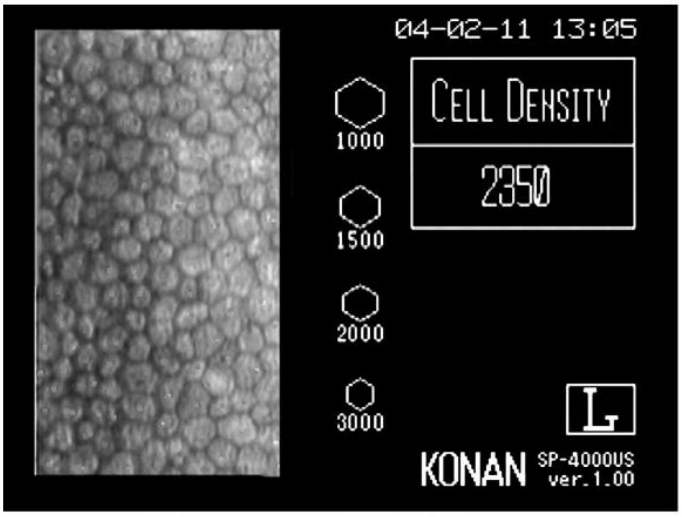
(2) Hyphema

(3) Other, specify

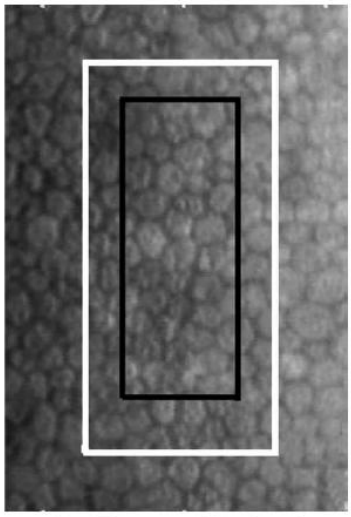
(4) None

APPENDIX 3: ANNEX

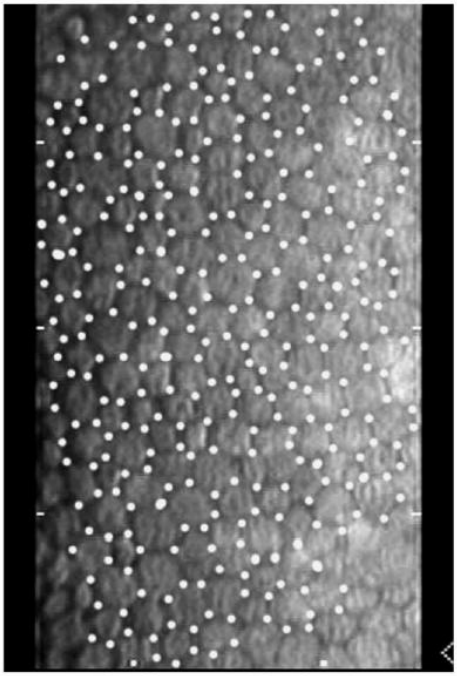
METHODS OF MEASUREMENT OF ECD



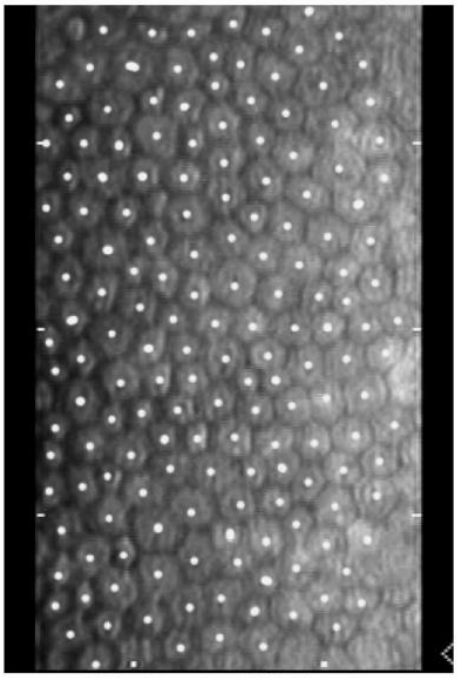
A: Compare to Fixed Pattern



B: Frame Method



C: Corner Method

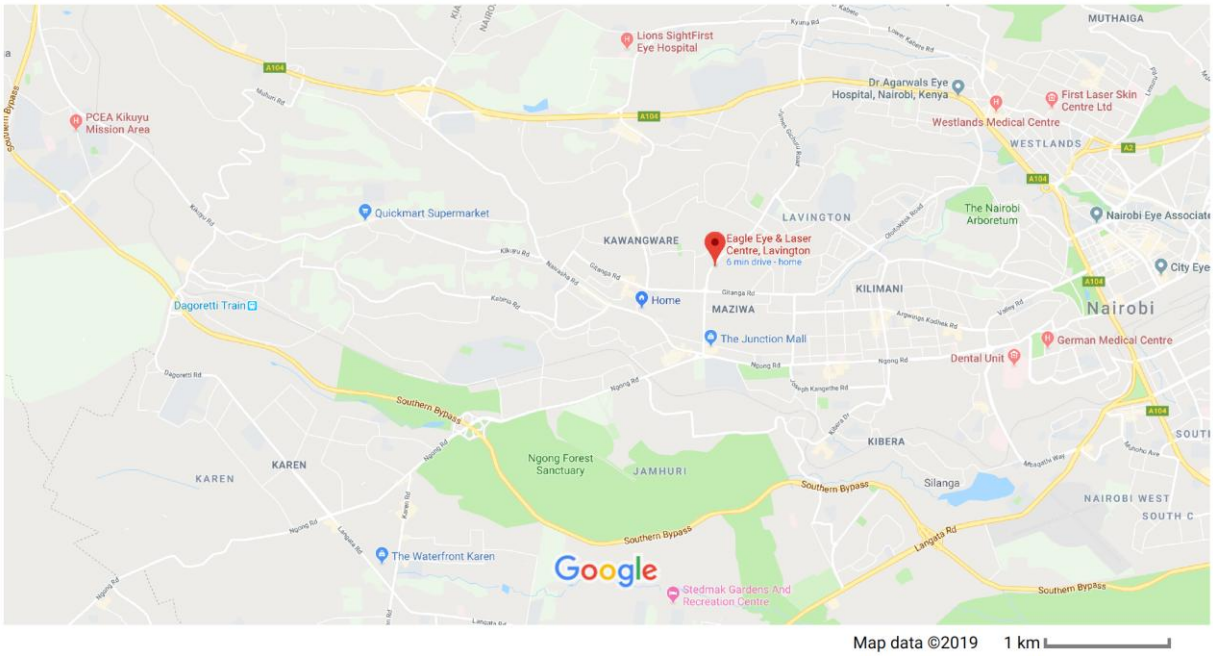


D: Center Method

PRETEST OF TOMEY EM-3000 ECD READINGS ON FIVE VOLUNTEERS

Volunteer	EYE	R1	R2	R3	R4	R5
1	R	2807	2854	2910	3005	2980
	L	2844	2844	2912	2943	3046
2	R	2692	2738	2763	2844	2911
	L	2862	2879	2886	2907	2910
3	R	2681	2741	2751	2803	2864
	L	2558	2613	2634	2636	2663
4	R	2430	2551	2710	2711	2759
	L	2793	2830	2884	2892	3172
5	R	2785	2786	2810	2814	2834
	L	2704	2744	2766	2782	2914

EAGLE EYE LASER CENTRE LOCATION MAP



APPENDIX 4: BUDGET

Item	Quantity	Unit Cost	Total Cost
Proposal/Ethical approval			
Proposal writing and printing (35 pages)	6 copies		1,000
Binding Proposal	6 copies		150
Ethics	1		2,000
Internet			1,000
		Subtotal	**4,150**
Data Collection			
Printing of Questionnaires	4 pages		50
Photocopy of Questionnaires	4 pages (400 copies)		800
Stationery –pens, rubbers etc.			500
Flash Disk 16GB Hp	1		2,000
Box files for filing questionnaires	10		400
		Subtotal	**3,750**
Transport		**Subtotal**	**2,000**
Contracted services			
Statistician	1	**Subtotal**	**40,000**
Printing costs and binding of final book			
Finished book printing (approx. 60 pages)	8 copies		2,400
	20 coloured pages		400
Binding	8 copies		800
		Subtotal	**3,600**
TOTAL			**53,500**

APPENDIX 5: ETHICAL APPROVAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/310

August 9, 2018

KENYATTA NATIONAL HOSPITAL APPROVED 09 AUG 2018 KNH/UoN-ERC P.O. Box 20723-00202 NRB

Dr. Judy Njeri Gathoni Karagania
Reg. No.H58/87845/2016
Department of Ophthalmology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Karagania

RESEARCH PROPOSAL – A PROSPECTIVE STUDY ON THE FACTORS THAT AFFECT CHANGES IN ENDOTHELIAL CELL DENSITY AND CENTRAL CORNEAL THICKNESS OVER A THREE-MONTH PERIOD AFTER PHACOEMULSIFICATION AT AN EYE HOSPITAL IN NAIROBI, KENYA (P207/04/2018)

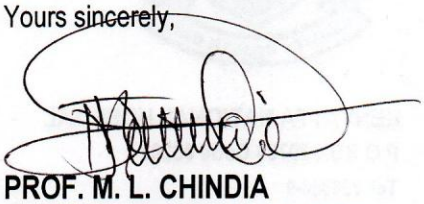
This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 9th August 2018 - 8th August 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,



PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH-UON ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UON
The Chair, Dept.of Ophthalmology, UoN
Supervisors: Dr.Joseph Nyamori, Dr. Millicent Bore