THE ROLE OF ASCORBIC ACID IN CORNEAL ENDOTHELIAL PROTECTION DURING SMALL INCISION CATARACT SURGERY

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CERTIFICATE

This is to certify that the dissertation entitled, **"The Role of Ascorbic Acid in Corneal Endothelial Protection during Small incision cataract surgery"** submitted by **Dr.SRUTHI.S,** in partial fulfillment for the award of the degree of Master of Surgery in Ophthalmology by The Tamilnadu Dr.M.G.R.Medical University, Chennai is a bonafide record of the work done by her in the Regional Institute of Ophthalmology, Government Ophthalmic Hospital, Egmore, Chennai, during the academic year 2009 – 2012.

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"THE ROLE OF ASCORBIC ACID IN CORNEAL ENDOTHELIAL PROTECTION DURING SMALL INCISION CATARACT SURGERY"** is a bonafide and genuine research work carried out by me under the guidance of Prof.Dr.K.Vasantha, M.S.,FRCS,(Edin), Department of Cornea Services, Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Egmore, Chennai – 600 008.

Date :

Place : Signature

Dr. Sruthi S.

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PART – I

INTRODUCTION

THE PLACE AND RELEVANCE OF MANUAL SMALL INCISION CATARACT SURGERY (SICS) :

Modern cataract surgery aims to achieve an unaided visual acuity with rapid post surgical recovery and minimal surgical complications. This has been made possible with a smaller incision size, appropriate use of ophthalmic viscosurgical devices (OVD) and irrigating solutions, better instrumentation and technology. The advantages associated with phacoemulsification have made it the ideal technique for cataract surgery and the preferred one where resources are available. However, this technique cannot be employed as the standard procedure in developing countries due to certain reasons. Manual small incision cataract surgery offers similar advantages with the merit of wider applicability, better safety, a shorter learning curve and lower cost.

ROLE OF STERILE INTRAOCULAR IRRIGATING SOLUTION:

After intraocular surgery, corneal endothelial cellular morphologic changes are noted that are indicative of a stressed endothelial monolayer. The stressors implicated are instrumentation, loss of aqueous humor, endothelial contact with nuclear and cortical fragments, postoperative formation of oxygen free radicals. An ideal irrigating solution combines an energy source, a buffer and an antioxidant to maximally protect and nourish the intraocular structures.

WHY THIS STUDY?

Studies of the harmful effects of cataract surgery on corneal endothelial cells suggest that much of this damage is mediated by free radicals. Subsequent articles have emphasized the potential protective effects of various free radical scavengers[1-6] and suggest that these substances may help prevent phacoemulsification-induced endothelial cell loss, a common and well-documented side effect of cataract surgery[7-13].

Holst et al. documented the formation of large amounts of free radicals and ultraviolet radiation in phacoemulsification, both in vivo and in vitro environments[14]. These formations were reduced by the addition of superoxide dismutase to the irrigating solution; however, they did not examine whether this reduction could effectively reduce endothelial cell loss.

Other studies have shown a nonspecific beneficial effect of irrigating solutions containing glutathione (a free radical scavenger) in reducing cell loss from prolonged endothelial exposure to irrigating solutions [2,5]. In a study in which a similar irrigation model in dogs was used, the investigators found no beneficial effect of glutathione [15]. A study of the protective effects of different viscoelastic materials against endothelial damage by intraocular hydrogen-peroxide solutions suggested that their protective effect may be due in part to free-radical scavenging properties [3].

Several researchers have compared the results of cataract surgery using a balanced saline ophthalmic irrigating solution to those obtained using solutions containing glutathione. Some have reported a protective effect of glutathione on the corneal endothelium, whereas others have found no effect [16-18]. These studies, while important in determining the clinical use of glutathione, do not exclude other causes of endothelial damage, such as intraocular lens implantation, release of inflammatory substances in the eye, or release of lens particles. Also, these studies examined only one concentration of one free radical scavenger (glutathione), since it is the only commercially marketed solution in this category.

We elected to examine the role of ascorbic acid, a well-known scavenger of free radicals. Since this chemical is found in naturally high concentrations in normal aqueous, it is less likely to cause chemical damage to intraocular structures, and also has not been previously tested in the present setting to our knowledge [22,23].

To elucidate the role of free radicals in endothelial cell loss secondary to manual SICS, we devised an experiment that would attempt to isolate the endothelial damage due to formation of free radicals during and following surgery as a result of manipulation and loss of natural aqueous humor.

HISTORY OF CATARACT SURGERY

5th century BC - Earliest written reference to cataract surgery is found in Sanskrit written by the Hindu surgeon Susruta. He practiced a type of cataract surgery known as couching or reclination, in which the cataractous lens was displaced away from the pupil to lie in the vitreous cavity in the back of the eye.

In the Western world, recent excavations in Babylonia (Iraq), Greece, and Egypt have uncovered bronze instruments that would have been appropriate for cataract surgery. The first written description of the cataract and its treatment in the West appears in 29 AD in De Medicinae, the work of the Latin encyclopedist Celsus.

History also records the use of bloodletting, antiphlogistics (agents to counteract inflammation and fever), and mercury to prevent or dissolve cataracts - all of which were unsuccessful.

1748 - Modern cataract surgery; in which the cataract is actually extracted from the eye, introduced by Jacques Daviel in Paris.

1753 - Samuel Sharp of London introduced the concept of intracapsular cataract surgery by using pressure with his thumb to remove the entire lens intact through an incision.

1867 - The use of sutures for cataract surgery, first described by Henry Willard Williams of Boston.

1884 - Anesthesia in the form of eyedrops (cocaine) was developed, obviating the hazards of general anesthesia and its postoperative complications

1902 - Small suction cups (erysiphakes) were introduced for this purpose as well as various capsular forceps to grasp the lens for removal.

1940s - Harold Ridley introduced the intraocular lens in England enabling efficient and comfortable visual rehabilitation became possible following cataract surgery

1957 - Barraquer of Spain used alpha-chymotrypsin to enzymatically dissolve the zonules for removal of the lens.

1961 - Cryo-surgery was introduced by Krawicz of Poland to remove the lens with a tiny probe that could attach by freezing a small area on the surface of the cataract.

Late 1960s - Charles Kelman of New York developed a technique for emulsifying the lens contents using ultrasonic vibrations and aspirating the emulsified cataract.

STEPS OF MANUAL SICS

1. Pupillary dilation and anaesthesia
2. Superior rectus suture
3. Fornix based conjunctival flap
4. The incision
   - Opening in the sclera
   - The tunnel
   - Opening in the cornea
5. Staining of anterior lens capsule (optional)
6. Opening the anterior capsule (can opener capsulotomy / continuous curvilinear capsulorrhexis)
7. Hydrodissection / hydrodelineation
8. Mobilization of nucleus into the anterior chamber
9. Removal of nucleus
10. Cortical wash
11. Insertion of artificial intraocular lens
12. Removal of ocular viscosurgical devices
13. Wound closure

ANATOMY OF THE CORNEA

The cornea is a transparent, avascular watch glass like structure forming the anterior one sixth of the eyeball. It forms the principle refractive surface, contains the intraocular pressure and provides a protective interface with the environment. These functions can be subserved by virtue of a specialized substructural organization.

Histologically, the cornea is composed of five layers;

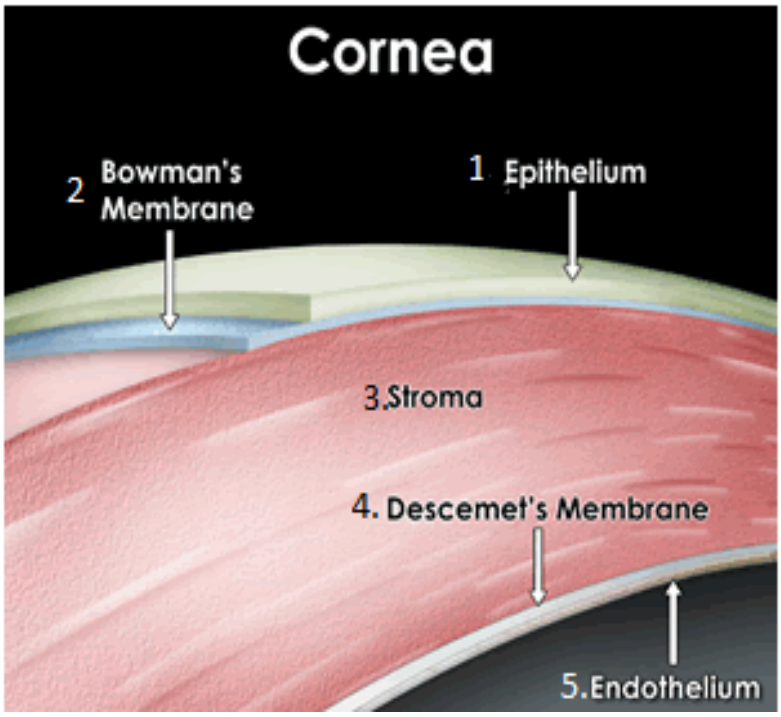
- Epithelium
- Bowman's membrane
- Stroma or substantia propria
- Descemet's membrane
- Endothelium

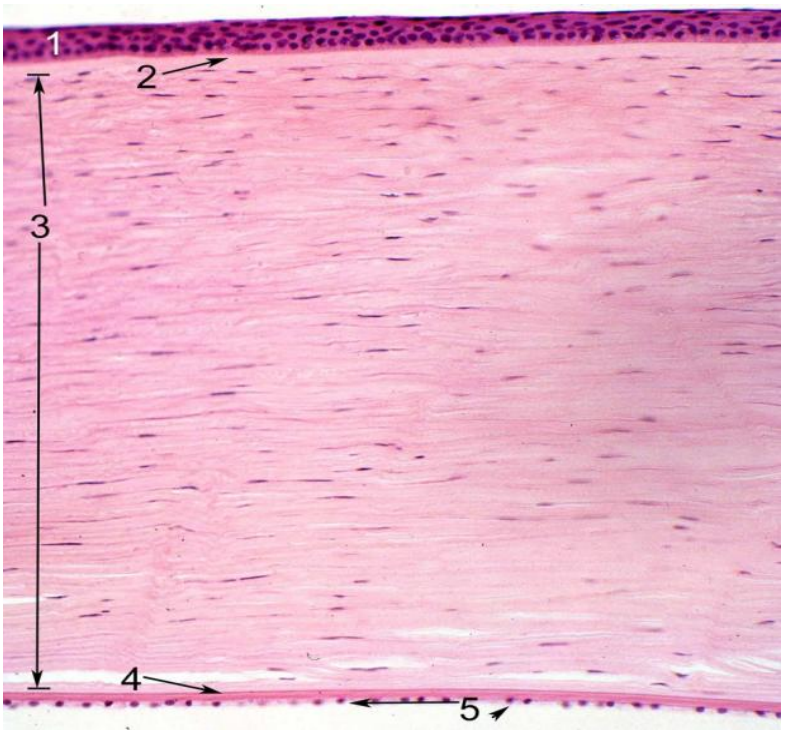
CORNEAL EPITHELIUM:

It is stratified and non keratinized, 50 to 90 micron thick and consists of 5 or 6 layers of nucleated cells

BOWMAN'S LAYER:

It is a narrow, acellular, homogenous zone, 8 to 14 micron thick, immediately adjacent to the basal lamina of the epithelium. It is relatively resistant to trauma due to the compact arrangement of collagen but once destroyed, it cannot regenerate.





1. Epithelium 2. Bowman's Membrane 3. Stroma
4. Descemet's membrane 5.Endothelium

STROMA or SUBSTANTIA PROPRIA:

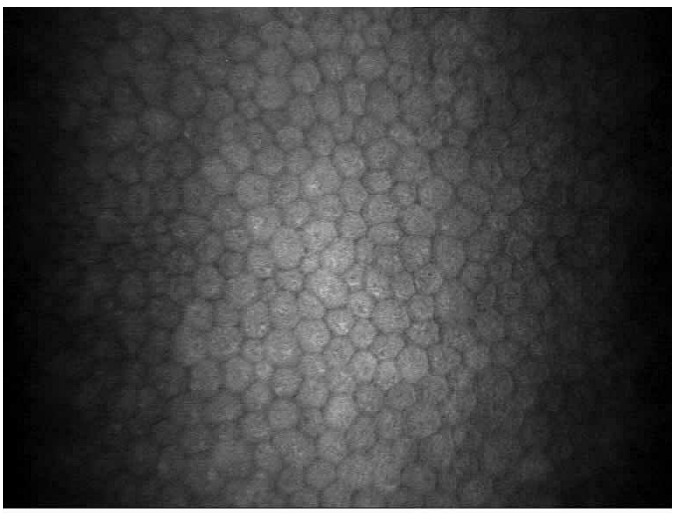
It comprises 90% of the total thickness of cornea, is 560 micron thick and comprises of regularly arranged lamellae of collagen bundles in a proteoglycan ground substance with cells called keratocytes.

DESCEMET'S MEMBRANE:

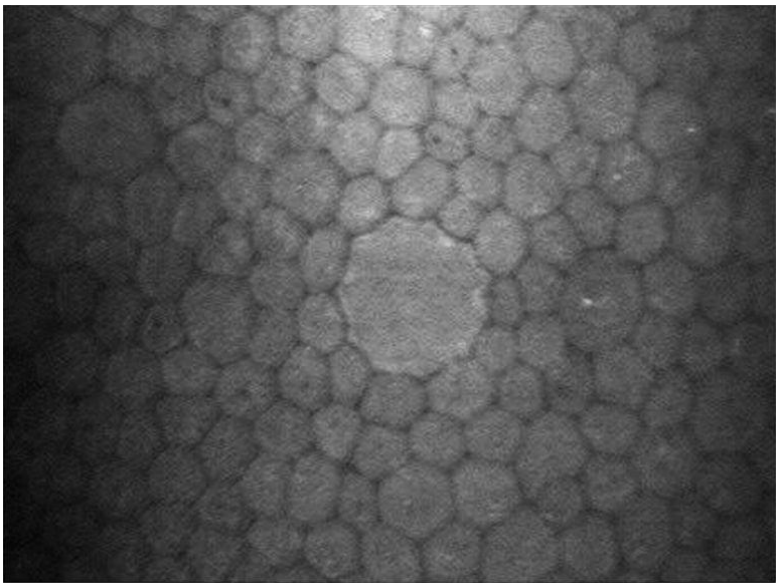
It is a thick basal lamina produced by the corneal endothelium and is 10 to 12 micron thick. Its peripheral termination is marked by the schwalbe's line. The major protein type is collagen type IV. It regenerates readily following injury.

ENDOTHELIUM:

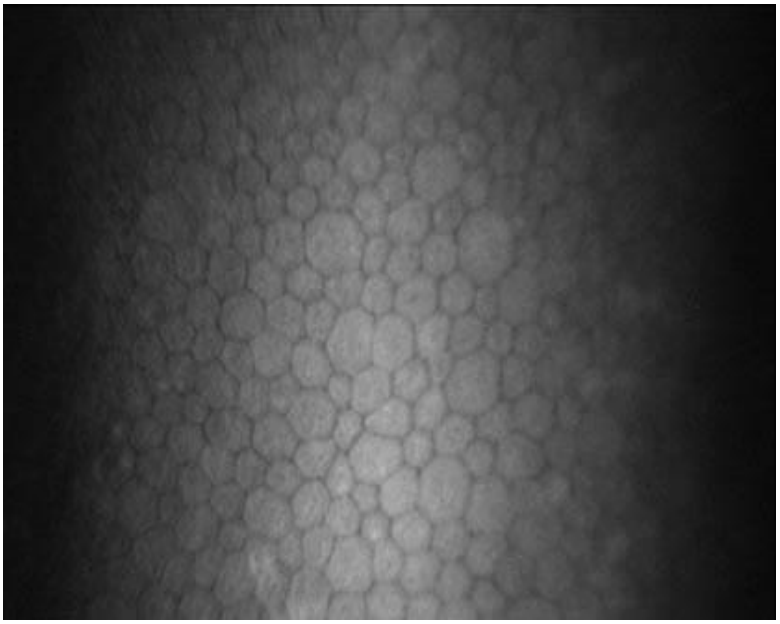
It consists of a single layer of hexagonal cells lying on the descemet's membrane, linked by hemidesmosomes and laterally to each other by tight junctional complexes. It has abundant mitochondria which aid in barrier function, protein synthesis and energy production.



Endothelium



Polymegathism



Pleomorphism

Corneal endothelial cell turnover :

The cell density of the endothelium is around 6000 cells/mm^2 at birth. The cell count falls by about 26% in the first year of life and a further 26% is lost over the next 11 years. The cell count comes down to 2400-3000 cells/mm^2 in young adults. The defect left by the dying cells is filled by enlargement (polymegathism) of the remaining cells. Hence, these cells vary in diameter from 18 to 20 micron early in life to 40 micron in aged. The response to direct wounding is to undergo 'cell slide'. If a sufficient number of endothelial cells are lost, it loses its function and the cornea imbibes water (decompensates) and becomes opaque. This corneal decompensation occurs only after more than 75% of the adult cells are lost; that is, when the endothelial cell count becomes less than 500 cells/mm^2.

The endothelial cells are best evaluated by specular microscopy. Deturgescence of the corneal stroma is controlled by the pumping action of the endothelial layer and can be monitored by measurement of central corneal thickness (pachymetry). Loss or damage of endothelial cells leads to an increase in corneal thickness, which may ultimately induce corneal decompensation and loss of vision.

SPECULAR MICROSCOPY

The corneal specular microscope is a reflected-light microscope that projects light onto the cornea and images the light reflected from an optical interface of the corneal tissue, most typically the interface between the corneal endothelium and the aqueous humor. Depending on the instrument used, the projected light can be in the form of a stationary slit, a moving slit, or a moving spot and the optical design can either be non-confocal or confocal.

The goal of endothelial specular micrsocopy is to enable the status of the endothelium to be obtained by visual observation and morphometric analysis of the endothelial image. In general, the more the endothelial image varies from the normal appearance, the more compromised the endothelium, and the less able is the endothelium to provide its necessary functions that maintain corneal clarity.

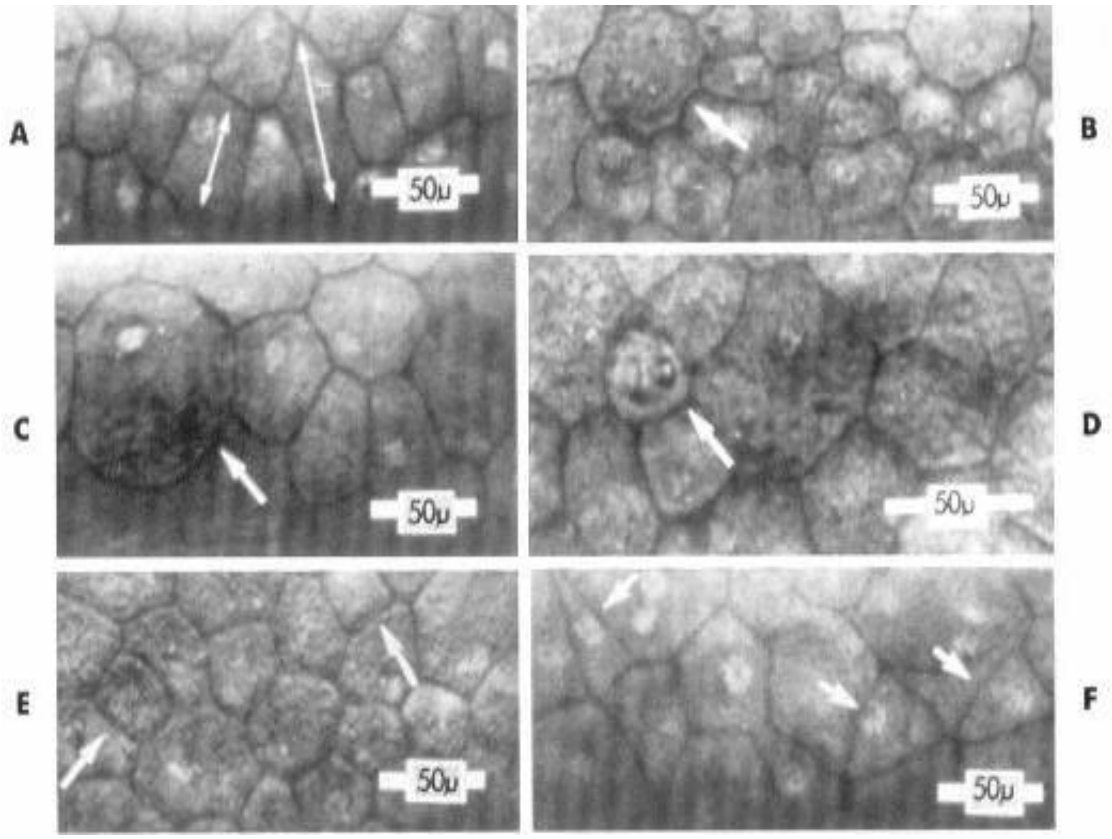
Optics of Specular Microscope:

Light striking a surface can be reflected, transmitted, or absorbed. Generally, some combination of the three effects occur, with the relative proportions depending on such conditions as the wavelength of the light, the relative transparency of the medium below its surface, and the relative refractive indices on each side of the surface. Of primary importance in clinical specular microscopy is the light that is reflected specularly (i.e. “mirror-like”) where the angle of reflection is equal to the angle of incidence.

As light strikes the posterior corneal surface, almost all of it is transmitted into the aqueous humor. Because there is a change in index of refraction at the endothelium-aqueous humor interface, about 0.022 per cent of the total incident light is reflected; this reflected light is captured by the clinical specular microscope and forms the endothelial image.

Examination of corneal endothelium:

Both qualitative and quantitative assessments of the corneal endothelium can be made. Qualitative cellular analysis identifies abnormal endothelial structures and grades the endothelium either according to the number or size of the abnormal structures present or on the basis of an overall visual assessment of endothelial appearance. The goal is to provide a subjective evaluation of the endothelium, not to assign a precise numerical value to the specular photomicrograph. This type of analysis provides a rapid clinical evaluation of the endothelium to assess the risks of intraocular surgery, to establish a diagnosis, or to decide upon treatment. Complete qualitative analysis requires that several parameters be evaluated including cell conformation, cell boundaries and their intersections, configuration of the dark boundary, and the presence of acellular structures. One must be careful to eliminate optical artifacts from consideration when performing either qualitative or quantitative analysis of the corneal endothelium.



Variations in the configuration of the corneal endothelium.

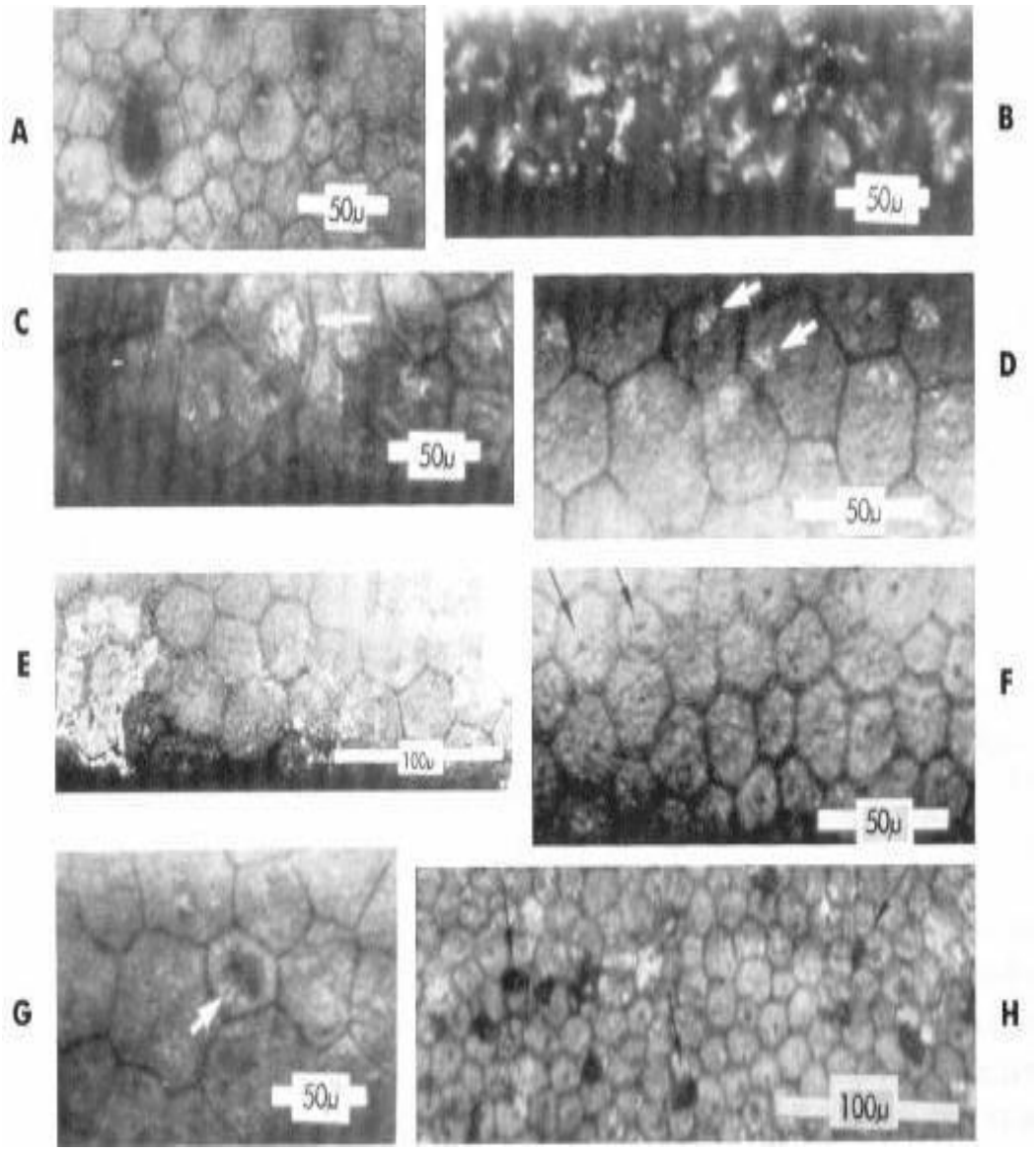
A- Elongated cells

B-Cell having scalloped edges

C and D- Round cells

E- Square cell

F- Triangular cell.



Miscellaneous endothelial structures.

A- Isolated smooth excrescences (cornea guttata)

B- Multiple coalesced excrescences.

C and D- Intracellular bright structures.

E- Pigmented endothelial deposits.

F and G- intracellular dark structures.

H- intercellular dark structures, believed to be invading inflammatory cells.

The clinical specular microscope can identify morphologic variations in endothelial cell configuration, cell surface properties, and intercellular boundaries, as well as the presence of numerous intracellular structures. Although the nature and significance of many of these abnormalities are not presently known, their recognition represents an initial step in the elucidation of their pathophysiological significance.

Various morphological parameters can also be quantified. These include cell size (cell area or cell density), polymegathism (variation of cell size such as coefficient of variation of mean cell area), pleomorphism (variation of cell shape such as percent of hexagonal cells or coefficient of variation of cell shape), cell perimeter, average cell side length, and cell shape. To date only cell size, pleomorphism, and polymegathism and several variables related to these parameters have proven useful in determining endothelial status.

Two equivalent parameters have been used to quantify endothelial cell size. They are mean cell area and cell density (or cell count). Cell area has most often been expressed in units of μm^2 per cell and cell density in units of cells per mm^2.

Fixed Frame Analysis of Cell Size

In fixed frame analysis one counts the number of cells within a frame or window of constant area. All cells lying completely within the frame are counted as whole cells. However, along the boundary of the frame there are

many cells that lie only partly in the frame, and for these cells it is usually impossible to determine the fraction of the cellular area that lies within the borders of the frame. Each cell that is only partially within the frame is counted as one half-cell regardless of the fractional area of that cell located within the frame. The total number of cells (the cell count) is then taken as the sum of the number of whole and half-cells within the frame. To speed up the counting process one commonly invokes a symmetry principle and counts all cells cut by two sides of the frame as whole cells and does not count those cells cut by the other two sides of the frame. As long as the number of boundary cells is small compared to the total number of whole cells within the frame, and cellular pleomorphism is not too great, this method can give reasonably accurate values for mean cell size. The size is obtained by dividing the cell count by the area of the frame and expressed as cell density in cells per mm^2. The area of the frame must be referred to the endothelium. This is accomplished by dividing the actual area of the frame by the square of the linear magnification of the specular microscope, and if the cells were counted from an enlargement of the negative, by the square of the linear magnification of the enlargement. One can also divide the area by the cell count and report mean cell area as well. In practice, except for very rough estimates of cell count, a minimum of 35 contiguous cells should lie within the counting frame, although for most studies it is preferable to have 50 to 100 cells within the counting frame. Otherwise the errors associated with the counting method itself will generally be too large to provide meaningful numbers.

Variable Frame Analysis of Cell Size

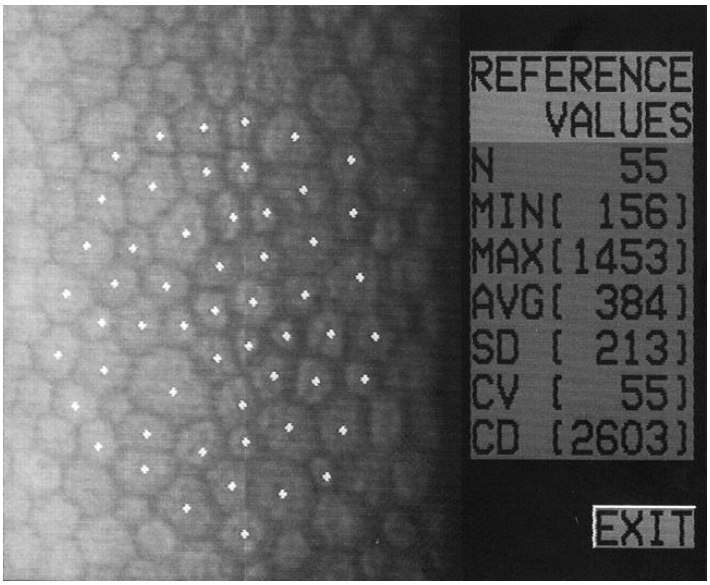
Variable frame analysis, originally proposed by Laing, is most conveniently done using a computer based analysis system. This method eliminates the problem of counting fractional cells along the boundary, thus providing a more accurate determination of mean cell size than fixed frame analysis, again assuming that cellular pleomorphism is not too great and that the cell sample is representative of the area under study.

In variable frame analysis, one first measures the variable area occupied by an integral number of cells by tracing around a contiguous group of cells with a mouse. The user then marks each cell by clicking it with the mouse. The computer then calculates the cell density by dividing the number of marked cells by the area of the frame. An equivalent value, the mean cell area, can also be obtained by dividing the frame area by the number of cells that have been circumscribed.

Using variable frame analysis, errors due to the counting method are very small as compared to fixed frame analysis so that many fewer cells must lie within the frame. Furthermore, with variable frame analysis, additional information can be obtained. If one traces around the smallest cell in the image and marks it, the computer calculates the cell density assuming that all cells are this small. If one then traces around the largest cell in the image and marks it, the computer calculates the cell density assuming that all cells are this large. These two numbers give the range of cell densities (or the range of

cell areas) that exist in this image providing more information regarding the endothelium than if only the mean cell density is obtained.

Topcon Specular microscope SP – 2000P



Center cell method - variable frame analysis

STERILE INTRAOCULAR IRRIGATING SOLUTIONS

The intraocular irrigating solutions available are

1. Lactated Ringer's solution
2. Balanced salt solution (BSS)
3. BSS plus

The major role of irrigating solutions during intraocular surgery, especially manual SICS are during cortical wash, removal of OVDs and formation of anterior chamber at the end of the surgery.

Ingredient	Human Aqueous Humor	Human Vitreous Humor	Hartman's Lactated Ringer's Solution	BSS PLUS® Intraocular Irrigating Solution	BSS® Intraocular Irrigating Solution
Sodium	162.9	144	102	160.0	155.7
Potassium	2.2-3.9	5.5	4	5.0	10.1
Calcium	1.8	1.6	3	1.0	3.3
Magnesium	1.1	1.3	-	1.0	1.5
Chloride	131.6	177.0	-	130.0	128.9
Bicarbonate	20.15	15.0	-	25.0	-
Phosphate	0.62	0.4	-	3.0	-
Lactate	2.5	7.8	28	-	-
Glucose	2.7-3.7	3.4	-	5.0	-
Ascorbate	1.06	2.0	-	-	-
Glutathione	0.0019	-	-	0.3	-
Citrate	-	-	-	-	5.8
Acetate	-	-	-	-	28.6
pH	7.38	-	6.0-7.2	7.4	7.6
Osmolality (mOsm)	304	-	277	305	298

Chemical composition of human aqueous humor, vitreous humor, BSS PLUS intraocular irrigating solution and BSS intraocular irrigating solution (all concentrations expressed in mmol/L or mEq/L solution)[24]

The primary purpose of an intraocular irrigating solution is to maintain both the anatomic and physiologic integrity of intraocular tissues. Many physiologic studies performed on the corneal endothelium have confirmed that a solution with a chemical composition similar to aqueous and vitreous humor provides the best protection for intraocular tissues.

Glutathione (GSH and GSSG) is one of the body's natural antioxidants. Free-radicals (by-products of oxidation) are capable of inflicting serious damage to the corneal endothelium and other delicate intraocular structures during ophthalmic surgery. There is evidence to suggest that glutathione is an effective free-radical scavenger. Glutathione also maintains the junctional complexes of corneal endothelial cells[25] and protects the integrity of the blood-aqueous barrier [26].The tissues within the eye are highly sensitive to depletion of cellular glutathione levels. Depletion of cellular glutathione can result in cell apoptosis.[27] **BSS PLUS** Solution has the ability to deliver a continuous supply of glutathione to the surgical site.

Sodium bicarbonate is the body's natural buffer. It is the major ion in the formation of aqueous humor and is vital for maintaining the blood-aqueous barrier. Sodium bicarbonate is also essential for maintaining retinal function. Studies have shown that omission of bicarbonate leads to significant alterations in both light-induced ERG potentials and metabolism of the isolated rat retina [28-29].

Glucose is the primary energy source of cellular function. Glucose helps maintain transparency of the cornea and lens and is critical for maintaining optimal retinal functions. Endothelial and retinal cells can rapidly become stressed or compromised when deprived of nourishment. Other intraocular irrigating solutions do not contain the ions, nutrients and substrates necessary to sustain healthy, normal functioning of ocular tissues. This is why a complete perfusion solution makes good sense in ocular procedures.

Thus BSS plus is considered an ideal intraocular irrigating solution. In developing countries, where economical constraints play a major role in provision of resources, Lactated Ringer's solution is still used as the intraocular irrigating solution.

RESEARCH BACKGROUND

Studies by Koskela et al have shown ascorbic acid, as an adaptive mechanism to protection by solar radiation [30].Ascorbic acid is known to exist in high concentration in the aqueous humor of the eye in many species. It has been observed that diurnal mammals have a very high concentration in aqueous humor whereas nocturnal mammals do not. It has been hypothesized that ascorbic acid protects the eye from the harmful effects of sunlight. The results of the study showed that of two closely related species of spiny mice, the diurnal species (Acomys russatus) had a concentration in aqueous humor that is 35 times higher than that of the nocturnal species (Acomys cahirinus). Studies of these two species may be fruitful to extend what is known about adaptation of the eye to protect itself from intense solar radiation.

Subsequent workers have shown that ascorbic acid is found in aqueous humor in concentrations 25 times that in plasma in other species, including humans[31-36]. Linner found that the ciliary body of rabbit cleared its plasma of ascorbic acid and pumped it into the posterior chamber along with newly formed aqueous humor[37]. This efficient process is carried out by sodium - ascorbate cotransporter in the ciliary body[38].

Rubowitz et al conducted an experiment to examine the role of ascorbic acid in reducing corneal endothelial cell loss secondary to high-energy ultrasound energy during phacoemulsification surgery[30]. Seventeen rabbit eyes were subjected to prolonged phacoemulsification within the anterior chamber, without manipulation or damage to other ocular structures.

In nine eyes, a balanced salt ophthalmic solution was used as the phacoemulsification irrigation solution, and in eight eyes the solution plus 0.001 M ascorbic acid was used, all other parameters being identical between the two groups. Specular microscopy was performed in all eyes before and 1 week after surgery. The animals were then killed, and the corneas were examined histologically.

Results showed no significant difference in preoperative endothelial cell counts between the two groups. Postoperative cell counts were reduced by 453.9 ± 233.3 (SEM) cells/mm^2 in the solution-alone group versus 123.2 ± 196.4 (SEM) cells/mm^2 in the solution-plus-ascorbic acid group, ($P = 0.011$). Corneal histology revealed a marked difference in endothelial cell morphology between the two groups. They concluded that the addition of ascorbic acid to the irrigation solution significantly reduced the amount of endothelial cell loss during phacoemulsification by approximately 70%. This was thought to be due to the free-radical scavenging properties of ascorbic acid. Further studies were warranted to find the optimal concentrations and combinations of free radical scavengers to be used in phacoemulsification irrigation solutions. It was previously found by the authors that the normal interexamination variation between endothelial cell counts was approximately ±5%, and postoperative results showing minor increases in endothelial cell counts were probably due to this variation.

Mathew et al determined endothelial cell loss and central corneal thickness in patients with and without diabetes after manual SICS[40]. There was a steady drop in the endothelial density in both the groups postoperatively, with the percentage of endothelial loss at 6 weeks and 3 months being 9.26 +/- 9.55 and 19.24 +/- 11.57, respectively, in patients with diabetes and 7.67 +/- 9.2 and 16.58 +/- 12.9, respectively, in controls. The percentage of loss between 6 weeks and 3 months was found to be of significant difference (P=0.023). In both the groups, an initial increase in CCT till the second postoperative week was followed by a reduction of CCT in the subsequent follow-up (sixth week) and a further reduction in the last follow-up (3 months). The change in CCT between the second and sixth weeks was significantly higher in the diabetic group (P = 0.045). It was concluded that the diabetic endothelium was found to be under greater metabolic stress and had less functional reserve after manual SICS than the normal corneal endothelium.

Studies by Ventura et al compared the corneal thickness and endothelial density before and after cataract surgery[41]. All patients had significant postoperative corneal swelling on the day after surgery; preoperative values were restored by 3 and 12 months, even though significant endothelial cell losses had occurred. No correlation was found to exist between central corneal thickness and central corneal endothelial cell numerical density. Measurements estimated by ultrasonic pachymetry were more variable and significantly higher than those determined by optical low coherence reflectometry. It was concluded that as long as the numerical

density of the corneal endothelial cells does not fall below the physiological threshold, a moderate decrease in this parameter does not compromise the pumping activity of the layer as a whole.

A healthy cornea is able to compensate rapidly for transient increases in central corneal thickness after cataract surgery, but such is not the case when the endothelium is diseased. In patients with endothelial polymegathism the return to preoperative values of corneal thickness is significantly slower than in those with a normal endothelium[42].

PART – II

AIM & OBJECTIVE OF THE STUDY

Aim of study:

To determine the role of ascorbic acid in corneal endothelial protection during manual small incision cataract surgery (SICS).

Primary objective:

To compare the corneal endothelial cell loss after manual SICS with and without ascorbic acid

Secondary objectives:

To compare the morphology of corneal endothelial cells before and after manual SICS, with and without ascorbic acid

To compare the central corneal thickness before and after manual SICS, with and without ascorbic acid

INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA :

1. Age – 40 to 80 years
2. Senile cortical cataract with grade I-II NS
3. Endothelial cell count > 1000
4. Clear cornea on slit lamp examination

EXCLUSION CRITERIA :

1. Age > 80 years
2. Endothelial cell count < 1000
3. Grade III-IV nuclear sclerosis
4. Diabetes
5. Herpetic scars
6. Elevated IOP
7. Prior trauma
8. Prior ocular surgeries
9. Corneal endothelial dystrophies

METHODOLOGY

(MATERIALS & METHODS)

MATERIALS USED:

1. Information sheet*
2. Consent form**
3. Slit lamp biomicroscopy for anterior segment examination and grading of cataract
4. Schiotz tonometer for intra ocular pressure measurement
5. Non-contact specular microscope (Topcon SP – 2000P) for corneal endothelial analysis and central corneal thickness measurement.
6. Biochemistry department of the Institute for blood glucose estimation
7. Parentral ascorbic acid (1 ampoule containing 100mg in 5 ml)
8. Ringer's lactate solution as sterile intraocular irrigating solution

*** - Appendix I**

**** - Appendix II**

METHODS:

After obtaining clearance from the Institution ethics committee, patients visiting the hospital for cataract surgery were selected for the study based on inclusion and exclusion criteria. Informed consent was obtained and patients were randomly allocated into the control and case groups.

PREOPERATIVE EVALUATION:

Patients selected based on inclusion and exclusion criteria were examined using the slit lamp biomicroscope to ensure a healthy anterior segment and for the purpose of cataract grading. Intra ocular pressure measurements were obtained using Schiotz tonometer. Patients diagnosed with diabetes mellitus were ruled out of the study. Subsequently, patients were examined with the non-contact specular microscope to assess endothelial cell count, cell morphology and central corneal thickness.

INTRA OPERATIVE PROCEDURE:

Ringer's lactate solution was used as the sterile intraocular irrigating solution during cataract surgery. 5ml containing 100 mg of parentral ascorbic acid was added to 500ml of ringer's lactate solution to obtain a final concentration of 0.2mg/ml which equals the concentration of ascorbic acid found in human aqueous humor.

The patients were operated upon by a single surgeon to rule out surgeon's factors contributing to endothelial cell loss during surgery. All

patients were subjected to manual small incision cataract surgery with standard posterior chamber intraocular lens implantation. Adequate use of ocular viscosurgical devices was ensured to prevent corneal endothelial cell loss as a result of instrumentation.

Ringer's lactate solution without ascorbic acid was used for controls and ringer's lactate solution with ascorbic acid was used for cases. As it was a single blinded study, the participants were unaware of whether they were subjected to the experimental drug or not. The irrigating solution was used during cortical wash, removal of OVD and anterior chamber formation at the end of surgery.

POST OPERATIVE FOLLOWUP:

All patients were prescribed same topical antibiotic/steroid eyedrops during the postoperative period. All eyes were examined on the first post-operative day and those with striate keratopathy were ruled out of the study. This was because striate keratopathy was a common complication of cataract surgery due to many other reasons in addition to corneal endothelial cell loss.

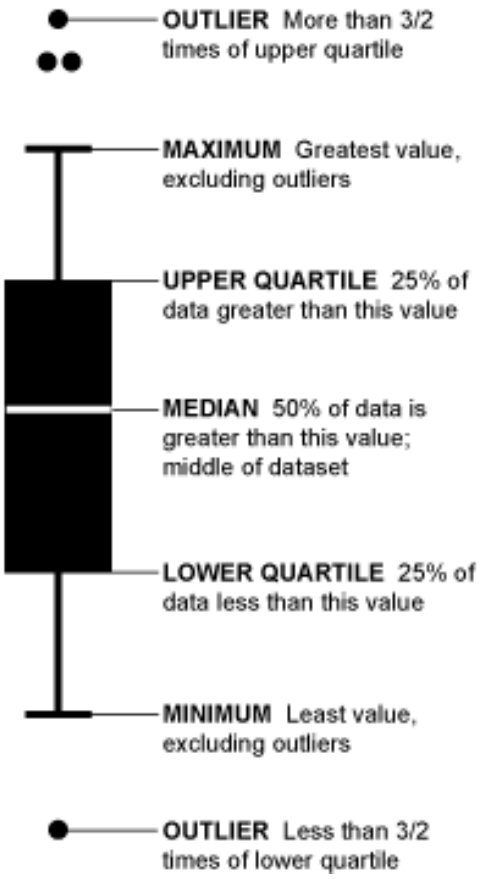
Post-operative measurements of corneal endothelial cell count, cell morphology and central corneal thickness were obtained on the first post-operative day and after one week during the first follow up.

STATISTICAL ANALYSIS:

The data collected during the course of the study was entered in an excel file and computed using a statistical software package (Graph pad Prism 5). The statistical analysis method used was unpaired t-test. Parameters studied were;

- Comparison of preoperative endothelial cell count between controls and cases
- Comparison of preoperative cell morphology between controls and cases
- Comparison of preoperative CCT between controls and cases
- Comparison of post operative endothelial cell count between cases and controls
- Comparison of post operative cell morphology between cases and controls
- Comparison of post operative CCT between cases and controls
- Comparison of follow up corneal endothelial cell count between cases and controls
- Comparison of follow up cell morphology between cases and controls
- Comparison of follow up CCT between cases and controls
- Comparison of pre and post operative reduction in endothelial cell count between cases and controls
- Comparison of variation in pre and post operative cell morphology between cases and controls
- Comparison of variation in pre and post operative CCT between cases and controls

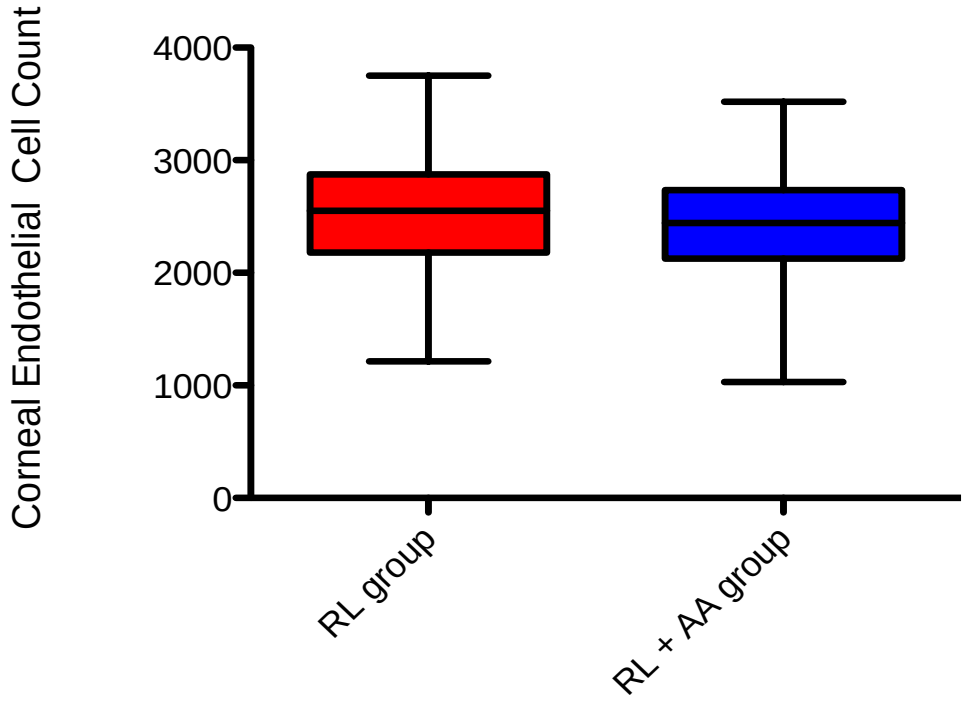
The analysed data was depicted using a box and whisker plot. In descriptive statistics, a **box plot** or **boxplot** (also known as a **box-and-whisker diagram** or **plot**) is a convenient way of graphically depicting groups of numerical data through their five-number summaries: the smallest observation (sample minimum), lower quartile (Q1), median (Q2), upper quartile (Q3), and largest observation (sample maximum). A boxplot may also indicate which observations, if any, might be considered outliers.



Boxplots display differences between populations without making any assumptions of the underlying statistical distribution: they are non-parametric. The spacings between the different parts of the box help indicate the degree of dispersion (spread) and skewness in the data, and identify outliers. Boxplots can be drawn either horizontally or vertically.

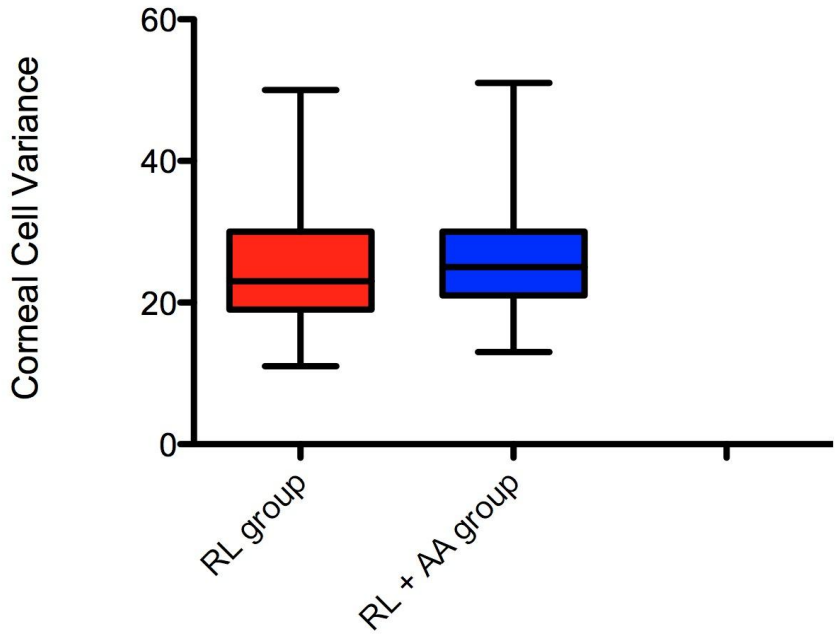
OBSERVATION & ANALYSIS

<u>Figure 1:</u> Comparison of preoperative endothelial cell count between controls and cases:



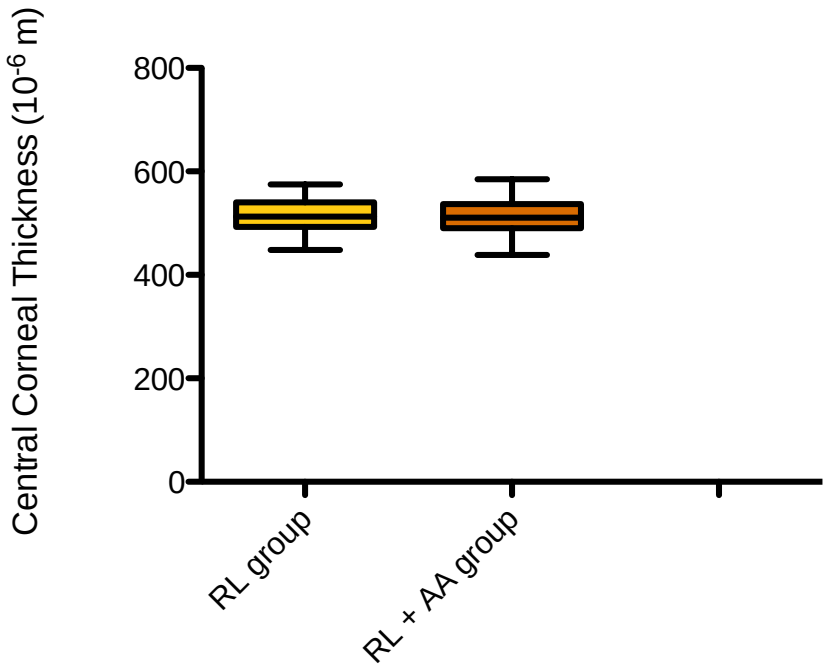
Mean ± SEM for RL group	**2531 ± 57.00**
Mean ± SEM for RL+AA group	**2420 ± 54.99**
Statistical test method	**Unpaired T-Test**
P-Value	**0.1617 (P > 0.05)**
Interpretation	**No statistical difference between groups**

<u>Figure 2:</u> Comparison of preoperative cell morphology (cell variance) between controls and cases:



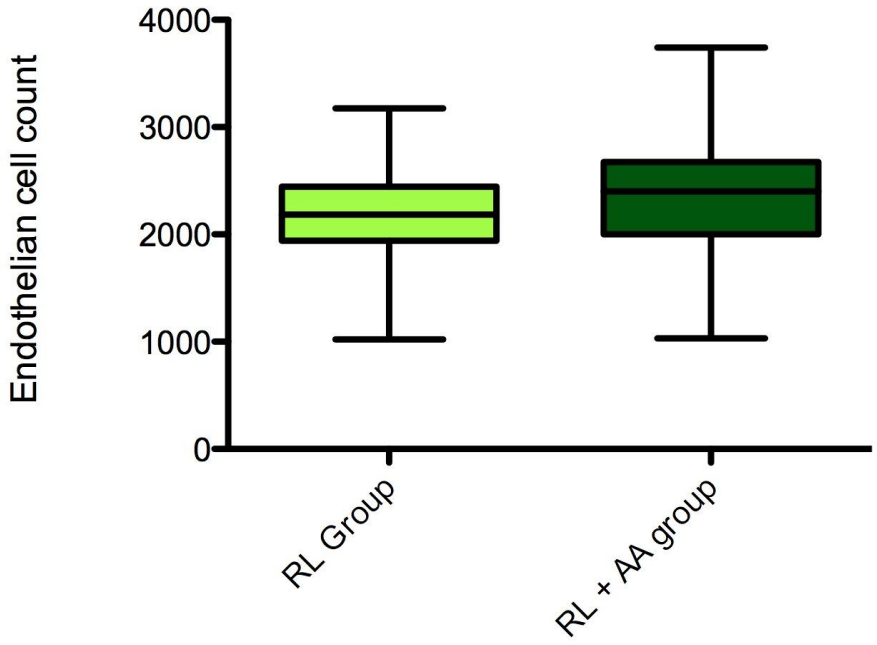
Mean ± SEM for RL group	**24.71 ± 0.95**
Mean ± SEM for RL+AA group	**26.56 ± 0.97**
Statistical test method	**Unpaired T-Test**
P-Value	**0.1737 (P > 0.05)**
Interpretation	**No statistical difference between groups**

Figure 3: Comparison of preoperative Central Corneal Thickness between controls and cases:



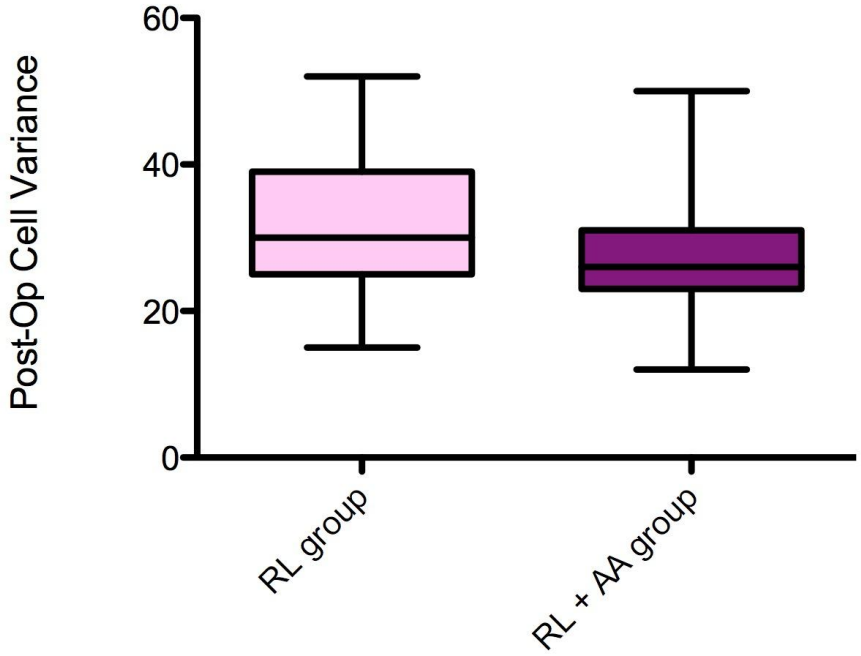
Mean ± SEM for RL group	**513.4 ± 3.527**
Mean ± SEM for RL+AA group	**512.6 ± 3.464**
Statistical test method	**Unpaired T-Test**
P-Value	**0.8759 (P > 0.05)**
Interpretation	**No statistical difference between groups**

<u>Figure 4:</u> Comparison of Post-Operative Corneal Endothelial Cell Count in Control and Cases



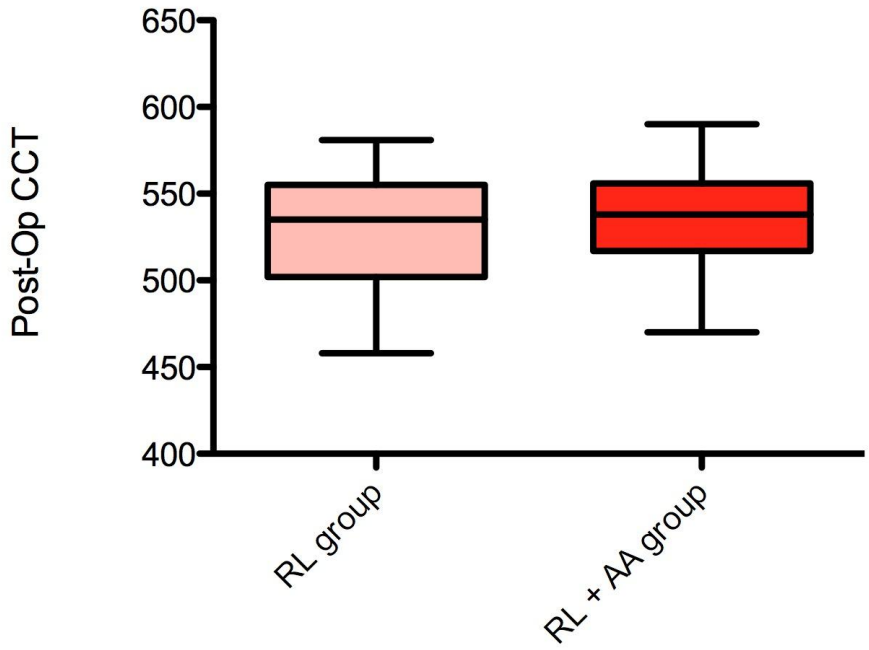
Mean ± SEM for Pre-Op	**2180 ± 49.56**
Mean ± SEM for Post-Op	**2341 ± 56.15**
Statistical test method	**Unpaired T-Test**
P-Value	**0.0339 (P < 0.05)**
Interpretation	**Significant statistical difference**

<u>Figure 5:</u> Comparison of Post-Operative Cell Variance between control and interventional group:



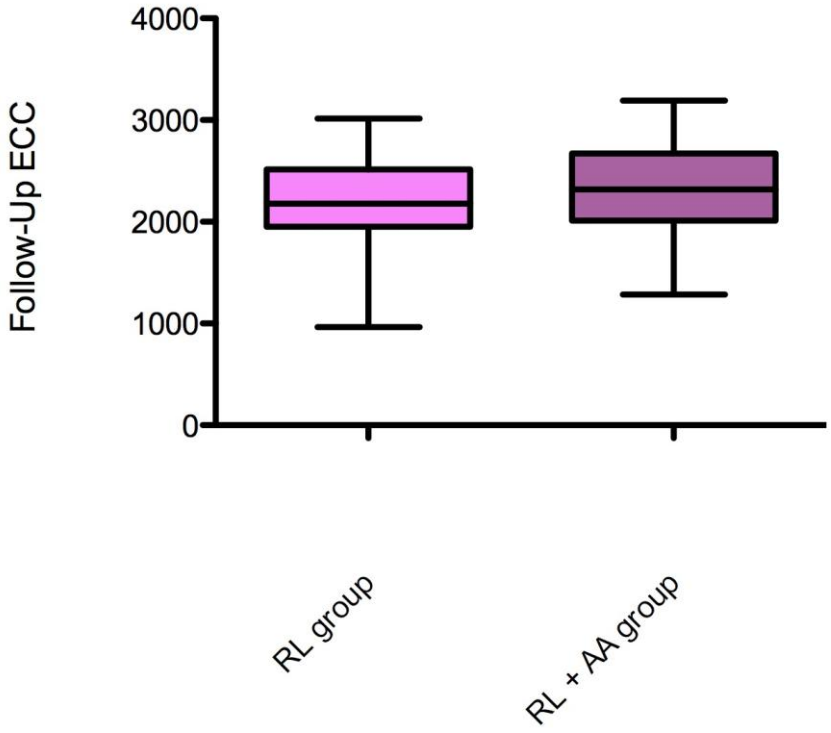
Mean ± SEM for Pre-Op	**32.27 ± 1.128**
Mean ± SEM for Post-Op	**27.79 ± 0.893**
Statistical test method	**Unpaired T-Test**
P-Value	**0.0022 (P <0.05)**
Interpretation	**Significant statistical difference**

Figure 6: Comparison of Post operative CCT between control and interventional groups:



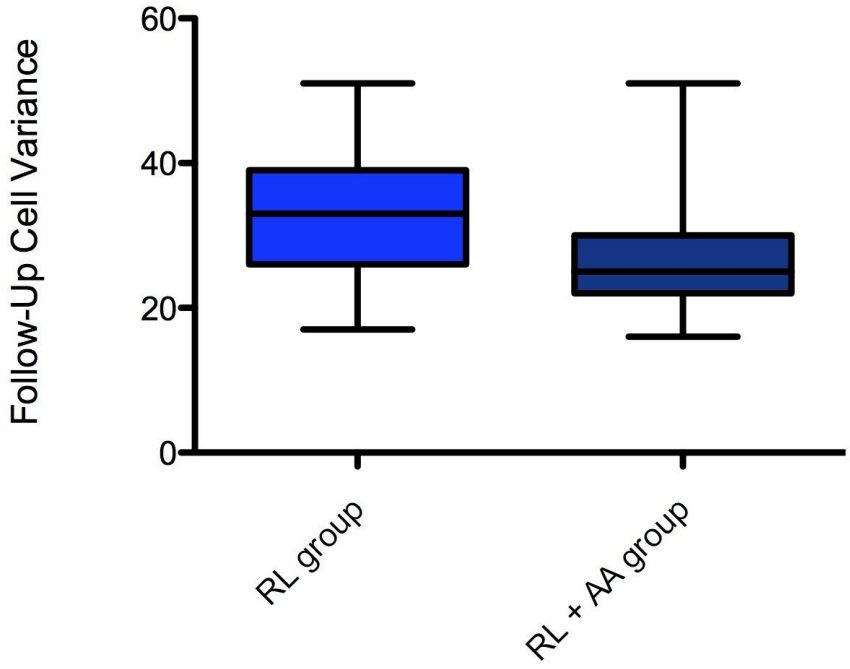
Mean ± SEM for Pre-Op	**529.5 ± 3.490**
Mean ± SEM for Post-Op	**534.5 ± 3.527**
Statistical test method	**Unpaired T-Test**
P-Value	**0.2941 (P > 0.05)**
Interpretation	**No Significant statistical difference**

<u>Figure 7:</u> Comparison of Follow-Up Corneal endothelial cell count between Control and Interventional groups:



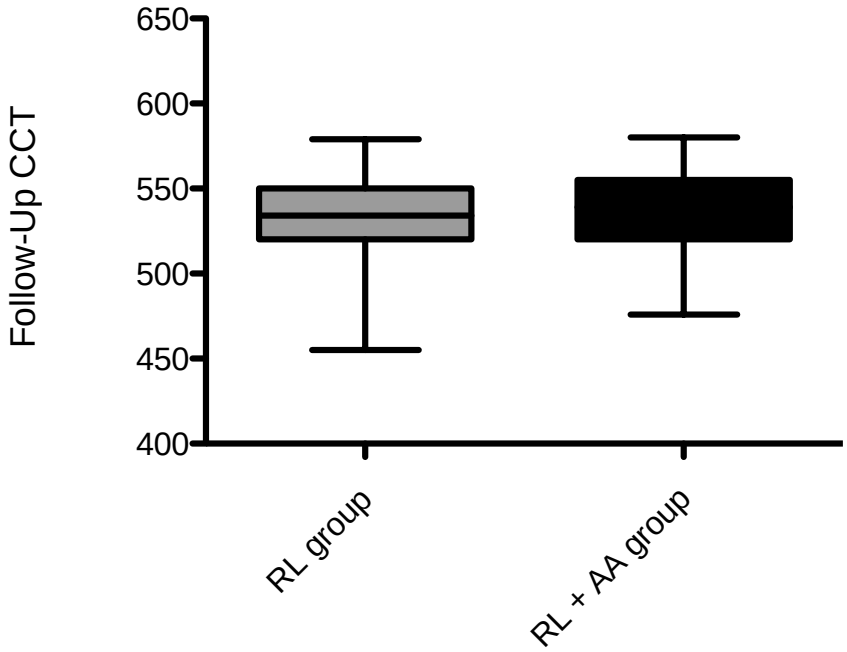
Mean ± SEM for Pre-Op	**2186 ± 48.21**
Mean ± SEM for Post-Op	**2337 ± 48.33**
Statistical test method	**Unpaired T-Test**
P-Value	**0.0286 (P < 0.05)**
Interpretation	**Significant statistical difference**

<u>Figure 8:</u> Comparison of Follow-Up Cell Variance between Control and Interventional Groups:



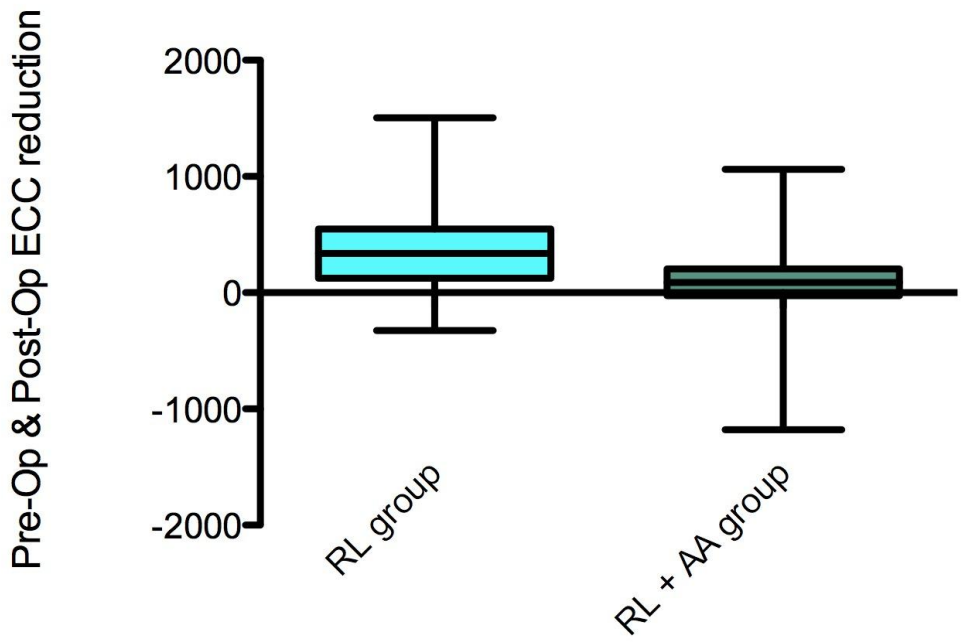
Mean ± SEM for Pre-Op	**33.28 ± 1.009**
Mean ± SEM for Post-Op	**27.08 ± 0.8276**
Statistical test method	**Unpaired T-Test**
P-Value	**0.0001 (P < 0.05)**
Interpretation	**Significant statistical difference**

<u>Figure 9:</u> Comparison of Follow-Up Central Corneal Thickness between Control and Interventional groups:



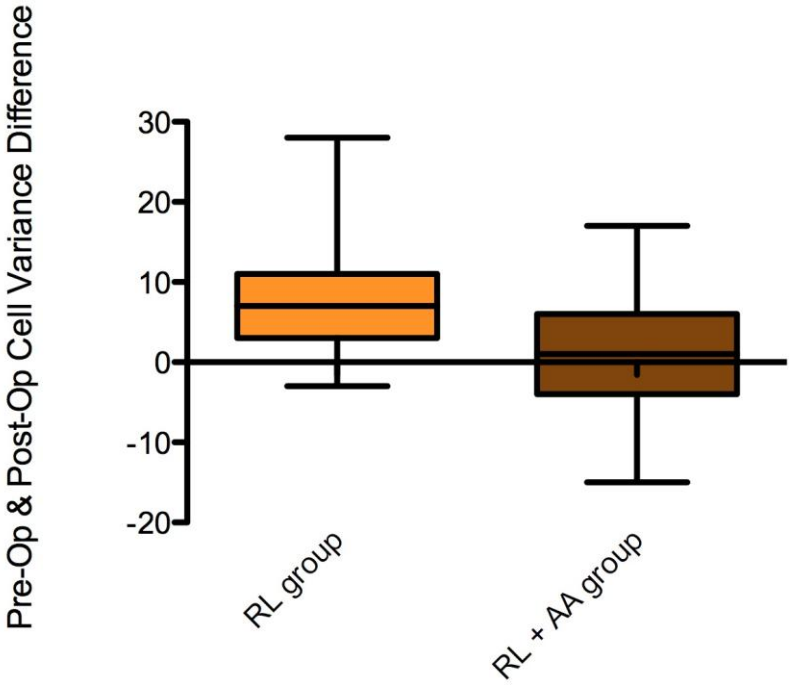
Mean ± SEM for Pre-Op	**532.2 ± 3.156**
Mean ± SEM for Post-Op	**534.4 ± 3.160**
Statistical test method	**Unpaired T-Test**
P-Value	**0.6357 (P > 0.05)**
Interpretation	**No significant statistical difference**

<u>Figure 10:</u> Comparison of Pre-Op and Post-Op Endothelial cell reduction between Control and interventional groups:



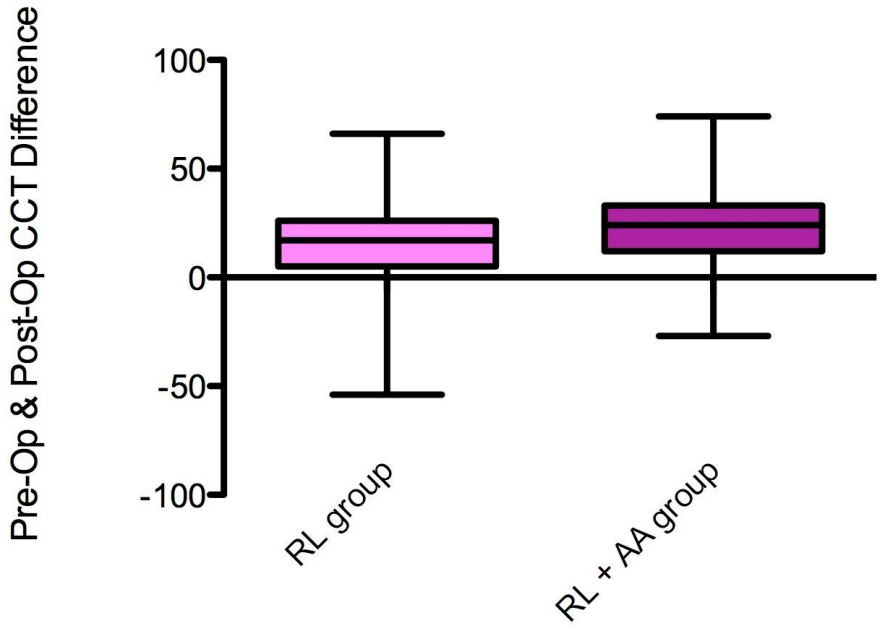
Mean ± SEM for RL group	**351 ± 37.63**
Mean ± SEM for RL+AA group	**79.19 ± 39.68**
Statistical test method	**Unpaired T-Test**
P-Value	**< 0.0001 (P < 0.5)**
Interpretation	**Significant statistical difference**

<u>Figure 11:</u> Comparison of Pre-Op and Post-Op Cell Variance difference between control and interventional groups:



Mean ± SEM for RL group	**7.560 ± 0.7457**
Mean ± SEM for RL+AA group	**1.227 ± 0.7497**
Statistical test method	**Unpaired T-Test**
P-Value	**< 0.0001 (P < 0.5)**
Interpretation	**Significant statistical difference**

<u>Figure 12:</u> Comparison of Pre-Op and Post-Op CCT difference between Control and Interventional groups:



Mean ± SEM for RL group	**16.07 ± 2.294**
Mean ± SEM for RL+AA group	**21.87 ± 1.919**
Statistical test method	**Unpaired T-Test**
P-Value	**0.0544 (P > 0.05)**
Interpretation	**No Significant statistical difference**

RESULTS

150 patients were enrolled in the study, of which 75 were enlisted to receive lactated ringer's solution without ascorbic acid (control group) and the remaining 75 to lactated ringer's solution with ascorbic acid (interventional group) as the intraocular irrigating solution during manual SICS. All patients were reviewed on the first post operative day and 1 week after surgery.

The preoperative corneal endothelial cell count, cell variance and CCT in both the groups were comparable. The endothelial cell count in control group was 2531 ± 57.00 vs 2420 ± 54.99 in the interventional group showing no statistical difference, p=0.1617 (fig.1). The cell variance based on morphology in the control group was 24.71 ± 0.95 vs 26.56 ± 0.97 in the interventional group with no significant statistical difference, p=0.1737 (fig.2). The CCT in the control group was 513.4 ± 3.527 vs 512.6 ± 3.464 in the interventional group with no significant statistical difference, p=0.8759 (fig.3). Thus both the groups were comparable with respect to the above parameters preoperatively.

The above parameters were measured on the first postoperative day in both the groups. Differences existed with regards to the cell count and cell variance, however the changes in CCT showed a similar pattern in both the groups. The endothelial cell count in the control group was 2180 ± 49.56 (13.8% reduction) vs 2341 ± 56.15 (3.26% reduction) in the interventional group. The above values were statistically significant (p=0.0339) (fig.4). Cell

variance in the control group was 32.27 ± 1.128 (30.59% increase) vs 27.79 ± 0.893 (4.63% increase) in the interventional group making the difference statistically significant (p=0.0022) (fig.5). The CCT measured was 529.5 ± 3.490 (3.04% increase) in the control group and 534.5 ± 3.527 (4.09% increase) in the interventional group, showing no statistically significant increase (p=0.2941) (fig.6).

All subjects were followed up one week after surgery. The results obtained during the first week of follow up were similar to those obtained on the first post operative day. There was no furthur reduction in the corneal endothelial cell count or increase in cell variance and CCT in both the control and interventional groups. However the difference in parameters between both the groups continued to exist. The endothelial cell count in the control group was 2186 ± 48.21 and interventional group was 2337 ± 48.33. Both groups showed a statistical difference in the cell count (p=0.0286) (fig.7). The cell variance in the control group was 33.28 ± 1.009 vs 27.08 ± 0.8276 in the interventional group, p=0.0001 (fig.8). The CCT values in the control and interventional group was 532.2 ± 3.156 and 534.4 ± 3.160 respectively, with no statistical difference (p=0.6357) (fig.9).

The reduction in the corneal endothelial cell count between the control group (351 ± 37.63) and interventional group (79.19 ± 39.68) was statistically significant, p<0.0001 (fig.10), implying that ascorbic acid had a role in the protection of corneal enodthelium during cataract surgery. The increase in cell variance in the control group was 7.560 ± 0.7457 vs 1.227 ± 0.7497 in the

interventional group ($p<0.0001$) (fig.11). This showed that addition of ascorbic acid reduced the degree of pleomorphism and polymegathism in the post operative period. However the CCT increase in both the groups showed no statistical difference, 16.07 ± 2.294 and 21.87 ± 1.919 for controls and cases respectively ($p=0.0544$) (fig.12). The CCT increase was similar in both the groups and ascorbic acid had no role in the reduction of postoperative corneal swelling.

DISCUSSION

L-Ascorbic acid, an antioxidant present abundantly in aqueous humor is thought to confer protection against the harmful effects of photochemical and oxidation reactions involving oxygen and its radicals. During manual SICS, this antioxidant is lost with aqueous humor and is replaced with irrigating solution which doesnot contain ascorbic acid thereby rendering the postoperative endothelium to greater oxidative stress and damage. The damage is increased further by the release of inflammatory mediators and exposure to sunlight following cataract surgery.

This study has shown that, although there is endothelial cell loss following cataract surgery in both the control and interventional groups, the amount of cell loss in the RL+AA group is much less when compared to RL only group. This can be attributed to the free radical scavenging activity of ascorbic acid, which prevents endothelial cell loss due to damage by reactive oxygen species during and following manual small incision cataract surgery.

Observation on changes in endothelial cell morphology has shown an increase in cell variance in both the groups. However, the increase in RL+AA group is less when compared to RL only group. This can be attributed to the reduced endothelial cell loss and hence the reduced requirement of the endothelial cells to undergo ‘cell slide’ and enlargement when subjected to ascorbic acid.

The central corneal thickness was found to increase in both the groups to the same extent and was not influenced by the addition of ascorbic acid. This might suggest that as long as the endothelial cell density is within the acceptable range, even significant changes in their number doesnot affect the occurence of postoperative corneal swelling.

The first follow up parameters recorded one week following surgery showed no significant change when compared with the parameters recorded on the first postoperative day. The endothelial cell count showed no further decrease and there was no change in the cell variance and central corneal thickness. This can be attributed to the active secretion of ascorbic acid into the anterior chamber following cataract surgery, hence preventing further loss of corneal endothelial cells. The endothelial cell count could not increase due to obvious reasons that the human corneal endothelium is incapable of undergoing mitosis.

CONCLUSION

Ascorbic acid has a protective effect over the human corneal endothelial cells during manual SICS. The protective effect is due to its free radical scavenging properties thus protecting the endothelium from oxidative damage.

Ascorbic acid was used in a constant concentration of 0.2mg/ml throughout the study. Further studies are warranted to find out the optimal and most effective concentration of ascorbic acid that could be combined with the intraocular irrigating solution.

The follow up period of this study was short. Further studies with long term follow up can establish the reversal of post operative corneal swelling and factors influencing it, in addition to the long term effects of oxidative damage on the corneal endothelium.

PART III

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APPENDIX I

INFORMATION SHEET

- We are conducting a study on the beneficial effect of ascorbic acid to the corneal endothelial cells during cataract surgery at the Regional institute of Ophthalmology, Chennai.

- We are selecting cases based on the eligibility criteria and if you are found to be eligible, your eyes may be subjected to the drug during cataract surgery.

- We will also be doing additional investigations before and after surgery to assess the effectiveness of intervention.

- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

- Taking part in the study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time, your decision willnot result in any loss of benefits to which you are otherwise entitled.

- The results of the study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in management or treatment.

Signature of investigator Signature of participant
Date:

APPENDIX II

CONSENT FORM

TITLE OF STUDY – Role of ascorbic acid in corneal endothelial protection during small incision cataract surgery

NAME : DATE :

AGE : IP NO :

SEX : STUDY NO :

- I have understood all the details clearly and I am willing to participate in the study.
- I am willing to undergo cataract surgery and I understand that during the surgery, I might be subjected to irrigating solution with ascorbic acid in it.
- I also agree to undergo the necessary investigations required for the study.
- I understand that I am at the risk of complications pertaining to cataract surgery as explained by the doctor, and still agree to undergo the surgery.
- As the adverse effects of ascorbic acid inside the eye have not been proven, I also understand that I am at the risk of developing new complications if any.
- I whole heartedly agree to participate in this study without compulsion from others and I am aware that I can withdraw from this study at any point of time and so doing will not affect my treatment in any way.
- Hence with total awareness and freedom of choice, I agree to participate in this study.

SIGNATURE

APPENDIX III

PROFORMA

RL ONLY / RL + AA

Name : OP number :

Age / Sex : IP number :

Date of surgery : Date of discharge :

Eye operated : Diagnosis :

Syst. Complications –

IOP (schiotz) -

		PRE OP	POST OP	1 WEEK
Snellen's chart	Vn with PH			
Specular microscopy	CCT			
	N			
	Min			
	Max			
	Avg			
	CV			
	CD			
Others (if any)				