OUTCOME OF CORNEAL COLLAGEN CROSS-LINKING IN KERATOCONUS
AT EAGLE EYE LASER CENTRE

DR. ANNE MUTHONI KARANU
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DECLARATION

I declare that this dissertation is my original work and to the best of my knowledge contains no materials previously published or written by another person in any other University.

PRINCIPLE INVESTIGATOR

Dr. Anne Muthoni Karanu

Registrar, Department of Ophthalmology, University of Nairobi

MBChB (UON)

Signature…………………… Date……………………
APPROVAL BY SUPERVISORS

This dissertation has been submitted with our approval as supervisors.

Dr. Kahaki Kimani

Senior Lecturer, Department of Ophthalmology, University of Nairobi.

MBChB (UoN), MMed (Ophth), MSc CEH (London), FEACO.

Signature……………………………… Date……………………………………

Professor Dunera Ilako

Associate Professor, Department of Ophthalmology, University of Nairobi.

Department of Ophthalmology, University of Nairobi.

MBChB (UoN), MMed (Ophth), MBA Health (Durban), FEACO

Signature……………………………… Date……………………………………
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LIST OF ABBREVIATIONS

**AST** - Astigmatism

**BCVA** – Best Corrected Visual Acuity

**BSCVA** – Best Spectacle Corrected Visual Acuity

**CCT** – Central corneal thickness

**CDVA** – Corrected distance visual acuity

**CKI** – Center keratoconus index

**CXL** – Corneal collagen cross-linking

**ECC** – Endothelial cell count

**EELC** – Eagle eye and Laser centre

**EI-CXL** – Epithelial Island crosslinking technique

**FDA** – US Food and Drug Administration

**FFKC** – Forme Fruste Keratoconus

**I-CXL** – Iontophoresis assisted trans-epithelial crosslinking

**IHA** – index of height asymmetry

**IHD** – index of height decentration

**ISV** – index of surface variance

**IVA** – index of vertical asymmetry

**KC** – Keratoconus

**KI** – keratoconus index

**Kmax** – Keratometry maximum
Kmean – Keratometry mean

MRSE – Mean Refractive Spherical Equivalent

OCT – Optical coherence tomography

RGP – Rigid Gas Permeable lenses

S-CXL – standard epi-off crosslinking

UCVA – uncorrected visual acuity

UDVA – Uncorrected distance visual acuity

UVA – Ultra-violet A
ABSTRACT

Study title: Outcomes of corneal collagen crosslinking in keratoconus at Eagle Eye Laser Centre

Study objective: To determine the outcomes of cross-linking in keratoconus patients at Eagle Eye Laser Centre, Nairobi from January 1st 2017 to June 30th 2018.

Methodology:

This study was a retrospective case series on the outcomes of corneal collagen crosslinking from 174 eyes that had undergone CXL at EELC for keratoconus and evaluated at 1 week, 1 month, 3 months, 6 months and 1 year. Postoperative results were categorised in terms of visual outcomes, topographic outcomes, complications and associations of the outcomes.

Results

Our study found that the UCVA at 3 months had improvement in 41.7% by 2.1 lines, at 6 months in 37.9% by 2.5 lines and at 12 months in 45.5% by 2.2 lines.

At 6 months Kmean flattening was seen in 15.2%, and remained the same in 75.8%, at 12 months Kmean flattening was seen in 10.7% and remained the same in 75.0%. At 6 months Kmax flattening was seen in 24.2% and remained the same in 48.5%, at 12 months Kmax flattening was seen in 28.6% and remained the same in 53.6%.

The complication rate was 13.2% with corneal haze the most common finding postoperatively which resolved after 3 months.

Progression was seen in 15.2% at 6 months and 14.3% at 12 months with no factors found significantly associated at 6 or 12 months.

Conclusion

CXL was shown to effectively halt the progression of keratoconus in 84.8% of eyes at 6 months and 85.7% at 12 months. It was also shown to improve or stabilise UCVA and BCVA in at least 50% of patients. The procedure had few complications all of which resolved after 3 months postoperatively. Even for eyes with TCT < 400µm CXL was found to be safe and effective.
1.0 INTRODUCTION AND BACKGROUND

Keratoconus (KC) originates from the Greek word Kerato meaning cornea and Konus which means cone, describing the cone-shaped protrusion seen in the disease. It is the commonest primary ectatic disorder and is characterised by clinical non-inflammatory corneal thinning, with abnormal corneal thickness distribution and posterior elevation\textsuperscript{1}. It leads to the paracentral or central cornea going through progressive thinning with steepening and apical scarring resulting in irregular astigmatism\textsuperscript{2}. The disease normally manifests itself during teenage years and early adulthood and tends to progress into the mid-30s and can lead to a severe visual impairment that may eventually result in blindness. However, the progression of the disease can halt at any stage.

Mechanism proposed in keratoconus pathophysiology, include alterations in enzymes that result in a breakdown of collagen found in the cornea were shown to cause a change in the collagen configuration leading to a progressive stromal thinning\textsuperscript{3}. Corneal collagen crosslinking (CXL) triggers the polymerisation (crosslinking) of the cornea providing biomechanical stiffening. Corneal collagen CXL has been employed as the treatment of progressive keratoconus for the past 20 years with the first patient treated in 1998, and the first clinical study done from 1999-2003 by Wollensak et al\textsuperscript{4}. Keratoconus is the leading indication of keratoplasty in Kenya at 48.8% due to the progression of the disease to blindness\textsuperscript{5}. Outcomes of crosslinking have repeatedly shown long-term stabilization of corneas after treatment of progressive keratoconus with corneal collagen crosslinking, proving it to be an effective therapeutic option\textsuperscript{6}. Crosslinking has been in existence in Kenya since 2010 and as yet there is no data on the outcomes of CXL. Analysis of the outcomes of crosslinking including stability and safety in our setting is important as well as increasing awareness, in order to reduce the demand for corneal transplant as this is the only known procedure that halts keratoconus progression.
2.0 LITERATURE REVIEW

2.1 Epidemiology of keratoconus
Keratoconus is described as a corneal ectactic disease characterized by a conical shape of the cornea due to progressive thinning with protrusion of the cornea that affects the male and female sex\(^1\). Commonly affecting adolescence and young adulthood, it is an uncommon diagnosis after the age of 35 years\(^7\).

Currently, the reported prevalence of the disease differs widely depending upon the diagnostic criteria, selected patients, and geographic location. In a comparative study carried out in the United States by Safarzadeh et al, Latinos and African-American were reported to have a higher risk of developing keratoconus, while Asian-Americans, people with diabetes and women were reported to have a lower risk with results showing that keratoconus occurred in 50 to 230 per 100,000 in the population\(^8\).

In Africa, De Smedt et al in Rwanda reported the prevalence of KC in a population of 121 primary school children with vernal keratoconjunctivitis (VKC) as 1.7\(^9\).

Mugho et al, reported the prevalence of keratoconus in patients with allergic conjunctivitis of 10.6\% by clinical diagnosis, 14.6\% by keratometry and 30.9\% by topography of 246 eyes of 123 patients with allergic conjunctivitis\(^10\). Studies in Getrude’s childrens hospital in Kenya on the characteristics of Keratoconus found the median age to be 20.97 years, with the youngest patient at 6 years and a range of up to 84 years with referral for keratoplasty in 16.5\%\(^11\)

Concerning sex preponderance results vary between studies. Overall KC is not considered to favour any one sex. In the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study, it was found to have similar rates of progression in both sexes\(^7\).

2.2 Etiology and risk factors of keratoconus
Relevant risk factors identified are atopy, ethnic factors like Latinos and Africans, sleep apnea, and Down’s syndrome\(^12\). The most important association is it’s relation to ocular allergic disorders\(^10\).
Eye rubbing, related to atopy.

Frequent eye rubbing in atopic individuals is induced by hot, dry climates with high levels of dust and may result in reports of asymmetric keratoconus due to asymmetric eye rubbing\textsuperscript{13}.

Sleep apnea

Woodward et al carried out a retrospective longitudinal cohort study in the United States to determine whether a relationship existed between common systemic diseases and keratoconus. These results show that sleep apnea was one of the conditions found to increase the likelihood of keratoconus\textsuperscript{12}.

Floppy Eyelid Syndrome

A case-control study conducted by Ezra et al in patients with floppy eyelid syndrome, showed that the syndrome was associated with keratoconus\textsuperscript{14}.

Poorly fitting contact lens

A history of poorly fitted contact lenses is believed to be one of the risk factors associated with the disease\textsuperscript{13}.

Genetic

In familial keratoconus, the majority is inherited in an autosomal dominant pattern. In populations with high consanguinity, an autosomal recessive pattern is documented. However, most cases of KC are sporadic\textsuperscript{13}.

Environmental

Geographical variation in KC prevalence is due to specific environmental agents fostering expression of genetic factors on ethnicity. Epigenetic mutations may be related to environmental factors such as toxins, environmental exposure, ultraviolet (UV) light. It is noted that keratoconic corneas have the deficiency in processing of reactive oxygen species. In the cornea, UV light increases the number of reactive oxygen species and in keratoconus this can lead to oxidative stress, cytotoxicity and corneal thinning\textsuperscript{13}. 

3
**Biochemical disorder**

In keratoconic corneas, at the level of the Bowman’s layer, there exist ruptures of varying degrees and sharply edged defects. Transmission electron microscope has shown that the thickness of corneal lamella is unaltered but the number of lamellae is reduced compared to normal corneas\textsuperscript{15}. Corneal strength gradually increases with age. Indirect evidence is provided by the fact that in younger populations, the manifestation of the disease shows greater severity and progression\textsuperscript{1}. Keratoconic corneas have upregulation of metalloproteinases, that favour collagen degradation and downregulation lysase-oxidase activity which is known to reduce CXL. Sharma et al, found alterations in enzymes that result in a breakdown of collagen in the cornea were shown to cause a change in the collagen configuration leading to a progressive stromal thinning\textsuperscript{3}.

It has still not been possible to trace changes in the keratoconic corneas back to primary causes. Extrapolating this data to disease mechanisms is complex and the absence of large effect contributions suggests a complex etiology or confluence of multiple disease pathways.

**2.3 Diagnosis of keratoconus**

The disease manifests itself early in adolescence and progresses into adulthood. It is important to search for a family history of the disease in close relatives, for a heritable pattern has been observed. The disease is also associated with symptoms of ocular allergy such as itchiness, discomfort, eye irritation with eye rubbing.

There is normally a history of refractive instability with frequent change in eyeglass prescription that does not correct the vision in a satisfactory manner. On examination, depending on the stage of the disease, different signs are present. In order to follow the impact of the disorder, visual acuity is central in evaluation. Diagnosis on pen torch examination include may reveal Munson sign and the Rizzuti’s sign\textsuperscript{10}.

Retinoscopy findings include, irregular astigmatism exists with features of a break/scissoring\textsuperscript{16}. With a dilated pupil, a honey or oil drop reflex is also seen (sign of Charleux)\textsuperscript{16}. On slit-lamp examination, corneal findings may include, cone protrusion,
thinning of the paracentral or central stroma more commonly in the central or inferior temporal cornea, with increased visibility of the corneal nerves, and presence of Vogt’s striae and Fleisher ring. With the placido disc, in keratoconus, the rings appear closer inferiorly and centrally.

Pachymetry is for the measurement of both central corneal thickness and peripheral corneal thickness, optical pachymeter or ultrasound pachymeter can be used. In keratoconic corneas, characteristics include inferior and central thinning. Keratometry involves the measurement of the curvature of the anterior surface of the cornea across a fixed spherical optical zone, usually the 4mm optical zone. It is based on the fact that the anterior surface of the cornea acts as a convex mirror. Its limitation is that it assumes the cornea is a spherical or spherocylindrical structure, but in reality, the cornea is aspheric. Hence loses accuracy when measuring very flat or very steep corneas. Automated keratometers are also available.

Corneal topography is a non-invasive exploratory technique used to create a three-dimensional map of the surface curvature of the cornea with the aim of producing a detailed depiction of the corneal morphology. Corneal topography has a role in early diagnosis of keratoconus, as well as topography for contact lens wear in KC. In KC, initial involvement is seen in the inferotemporal quadrant, thereafter, steepening spreads nasally, eventually, superotemporal cornea is last. Corneal topography can also assist to evaluate the dioptric change postoperatively created at the level of the cornea. In the cases where keratoconus has been established, the role of corneal topography is for monitoring its progression in order to perform a timely cross-linking.

Corneal tomography is a 3D reconstruction characterizing the front surface elevation, the back surface elevation as well as the pachymetry. In regard to keratoconus diagnosis, corneal tomography shows higher accuracy in comparison to derived topographic indices. Examples include the Orbscan which uses elevation based method for topography evaluation, the Orbscan II, where a placido based technique is in cooperated to measure the corneal curvature and the pentacam which uses rotating scheimpflug imaging and takes true images of the front and back of the cornea.
The ALLEGRO Oculyzer is mainly based on the pentacam technology and provides: corneal topography, pachymetry, cataract analyser, Scheimpflug image, 3D chamber analyser, densitometry advanced topography-guided treatments in combination with the WaveLight laser systems\textsuperscript{17}. Specular microscopy allows the study of the corneal endothelium. El-Agha et al, in Egypt compared the corneal endothelium changes which included endothelial cell count (ECC) and morphology at different stages of keratoconus and found that up to Amsler stage 3 keratoconus no changes were seen on specular microscopy\textsuperscript{18}.

Corneal confocal microscopy is a recent non-invasive imaging method that allows the examination of corneal cellular structure. Stromal keratocyte density in the anterior and posterior cornea was found to be lower than normal in keratoconic corneas. The stromal nerves were also found to be more tortuous in diameter in keratoconus\textsuperscript{19}.

2.4 Classification of keratoconus

No clinically adequate classification system for keratoconus currently exists. The first classification was proposed by Amsler and was based on disease evolution. Thereafter, with some modifications by Krumeich et al, the Amsler-Krumeich classification was established\textsuperscript{20}. This depended on the simulated keratometry (Sim-K), central corneal thickness (CCT) and clinical exam to grade severity from the least to the highest i.e. 1 to 4. However, this is limited as it does not reflect modern diagnostic methods.

The Alio-Shabayek classification added to the Amsler-Krumeich system with incorporation of corneal high order aberrations\textsuperscript{21}.

In the Ishii et al. classification, they added vertical asymmetry (VA), the index of surface variation(ISV), the anterior corneal surface minimum radius of curvature (Rmin) as well as six indices of; index of height decentration (IHD) which describes the decentration in elevation data in the vertical direction, center keratoconus index(CKI) describing the severity of the central KC, index of vertical asymmetry (IVA) which describes curvature symmetry, index of height asymmetry(IHA) which is more sensitive than IVA because it
is based on corneal elevation and the keratoconus index (KI) which also describes curvature symmetry. In the topographic keratoconus classification, it topographically classified keratoconus using parameters such as VA, retinoscopy and corneal findings with keratoconic screening indices such as the ISV, the KI which describes curvature symmetry and the Rmin. Belin et al. Categorization classified KC according to two criteria, progression and symptoms. This had 5 categories, Progressive symptomatic KC, non-progressing symptomatic KC, progressive asymptomatic KC, non-progressing asymptomatic KC and Keratoconus suspect. 

2.5 Keratoconus progression
The Global consensus on keratoconus and ectatic disease study defines progression in KC as a consistent change in at least two of the following parameters; 1. Progressing steeping of the anterior corneal surface 2. Progressive steepening of the posterior corneal surface 3. Progressive corneal thinning with or without an increase in the rate of thickness change from the periphery to the thinnest point. The changes need to be consistent and above the noise of the measurement system. A change in uncorrected visual acuity (UCVA) and best spectacle corrected visual acuity (BSCVA) is not required in the diagnosis of progression

2.6 Management of keratoconus
Keratoconus treatment normally depends on the symptoms of a patient and options include glasses, rigid gas-permeable (RGP) lenses, intacs, implantable collamer lenses (ICL), and keratoplasty. When the symptoms are mild, prescription eyeglasses are used to correct the vision. As the disease progresses, glasses cease to provide quality vision with the perception of ghost images “multiple images” known as monocular polyopia.
**Rigid gas permeable contact lenses**

For those with unsatisfactory correction with glasses, RGP lenses provide a safe alternative. In a retrospective study that was conducted by Ramdas and Vervaet in Netherlands, the rate of keratoconus progression in 82 patients wearing pancorneal RGP lenses was compared to those without. Corneal topography examination was carried out in all the patients. The results indicated that those wearing pancorneal RGP lenses saw their progression of keratoconus stabilizing more than those without\(^25\).

Hassani et al, looked at the outcomes of RPG contact lens in 28 eyes of patients with keratoconus, the results showed that the lens could improve visual acuity due to the corneal irregularities\(^26\).

However, with time the corneal steepening may render the RGP ineffective and other ways to treat the disease need to be considered.

Yildiz et al. in a research to determine the impact of RGP contact lenses and silicon hydrogel keratoconus lenses on the quality of life in keratoconus patients; found that the lens improved the visual acuity and provided a superior visual performance and a greater reduction of aberrations\(^27\).

**Intacs**

This is a minimally invasive surgical option designed for long-term vision correction. It flattens the cornea’s curve to improve vision with correction achieved with either one or two crescent-shaped intacs. They can be replaced with new rings of different sizes, with thicker rings providing more flattening of the cornea, which increases the level of vision correction achieved. This is useful if prescription changes.

Shetty et al conducted an investigation to evaluate the outcomes of intacs implantation in 14 eyes with progressive keratoconus. The findings showed that at 6 months, UCVA, BSCVA and mean refractive spherical equivalent (MRSE) improved. These results remained stable at 1 year. In addition, there were no complications, and 60% tolerated the intacs.\(^28\)
Amanzadeh et al carried out a comparable study focusing on the effects of intacs implantation on visual acuity on 42 eyes with progressive keratoconus, he found that the UCVA and BCVA improved, leading to a corneal shape that is less irregular\textsuperscript{29}.

**Implantable collamer lenses**

It is a single piece plate haptic (foot processes) lens which is designed to be implanted in the posterior chamber behind the iris with foot processes resting in the ciliary sulcus. They are ideal for keratoconus patients unfit for CXL and unable to tolerate spectacle correction.

Alfonso et al, evaluated keratoconic eyes that had implantation of toric intraocular collamer lenses at 1 month, 3 months, 6 months and 12 months. Results showed, at 12 months, all eyes were within ± 1.0D of the attempted refraction. No complications were reported\textsuperscript{30}.

**Keratoplasty**

With further progression of keratoconus and corneal scarring, keratoplasty is carried out to remove the damaged cornea and replace it with a healthy donor tissue. Its aim is to restore vision.

Either deep anterior lamellar keratoplasty is done which comprises replacing the central anterior cornea, leaving the endothelium of the patient intact, or penetrating keratoplasty which is a full thickness corneal transplant procedure\textsuperscript{31}.

Hydara et al carried out a retrospective case series, on the indications and outcomes of penetrating keratoplasty in Kenya and found that keratoconus was the most common indication with 48.8% followed by bullous keratopathy at 18.4%\textsuperscript{5}.

Djougarian and Zaidman carried out a retrospective study to investigate the outcome of corneal transplant in 37 eyes of 21 children under 18 years with progressive keratoconus. They also showed that 85% of the children with progressive keratoconus recovered their vision following corneal transplant surgery\textsuperscript{32}.

Patel et al, analysed the recurrence of keratoconus in donor corneas after penetrating keratoplasty, 36 eyes of 25 patients were analysed, and results showed that the average time for development of ectasia was 20 years after penetrating keratoplasty. With 15 eyes
regrafted for recurrent ectasia, and two eyes of 1 patient developing ectasia again and requiring a third grafting. The histopathological analysis of all excised grafts showed characteristic keratoconus changes.\textsuperscript{33}

2.7 Cross-linking

Cross-linking occurs physiologically with ageing through the lysyl oxidase and transglutaminase enzymatic pathways. The photochemical CXL of corneal collagen can be achieved by the collaboration between a photosensitiser such as riboflavin, and ultraviolet A (UVA), to create free radicals that will activate the lysyl oxidase pathway. Riboflavin also acts to absorb potentially cytotoxic UVA within the superficial corneal tissues protecting the endothelium, lens and retina.

2.7.1 History and discovery

Crosslinking technique was first developed by Professor Theo Seiler and Eberhard Spoerl in Germany in 1997 at the University of Dresden.\textsuperscript{34} Physiologically, the corneal collagen stiffens by crosslinking using the enzyme lysyl oxidase. Three main cross linkers were initially tested in corneas of porcine eyes; sugars, aldehydes and UV radiation and the stiffening effects were evaluated. At the time aldehydes were in use for tissue engineering for heart valves. Thus, the radiation of ultraviolet or blue light was preferred. UV light is known to harden polymers but it did not give such an effect in corneas and, it was dangerous.\textsuperscript{34}

A photosensitizer was proposed that would increase the effect of the UV radiation. Riboflavin was proposed as a photosensitiser due to its non-toxic nature being a vitamin. Several concentrations of riboflavin and their absorption in the cornea were tested. Two peaks were found in the absorption spectrum of riboflavin, 365–370 nm and 460 nm. The 365-370nm wavelength was preferred after comparing biochemical effects. The wavelength was produced by a mercury lamp and an interference filter 365nm ± 20nm. To introduce the UVA to the cornea a quartz light cable was proposed with an intensity of 2mW/cm\textsuperscript{2}. The preferred concentration was 0.1% riboflavin with epithelium-off to improve penetration. Soaking time for riboflavin of 5 min and irradiation time of 45min was initially used.\textsuperscript{34}
Bikbov and Surkova from Russia in a literature review described photochemical CXL as a biochemical effect that occurs due to a release of oxygen that promotes photochemical reaction\textsuperscript{35}. Kamaev et al supported this description stating that the oxygen that promotes photochemical reaction is as a result of a biochemical effect, leading to photochemical CXL\textsuperscript{36}.

Ambati et al in India focused on improving the physiochemical properties of collagen on the bases of hydrogels for the delivery of protein by photochemical CXL using vitamins as photochemical initiators. Their findings showed that the photochemical CXL improved the thermal stability of the collagen membrane\textsuperscript{37}.

In the year 2000, two UV light emitting diode (LED) lamps emitting 370nm wavelength at 3mW/cm\textsuperscript{2} were used to reduce treatment time to 30min, the so called “double diodes”. The same biochemical effect was seen with an irradiation time of 45minutes at 2mW/cm\textsuperscript{2}, thus the standard irradiation was then proposed to be 3mW/cm\textsuperscript{2}. In 1998 the first KC patient was treated with CXL and the first clinical studies were done in 1999 to 2003\textsuperscript{4}. The pilot study that enrolled 16 patients having progressive keratoconus saw their progression coming to a halt after CXL treatment. On top of this, the steep anterior corneal curvatures of 70\% of the patients flattened while 65\% showed a significant improvement in visual acuity. Of note, there were no reported complications\textsuperscript{4}.

A safety study conducted by Ashwin and McDonnell et al, showed that the endothelium was not damaged under the conditions that corneal thickness exceeded 400\mum and proper ultraviolet irradiance was maintained\textsuperscript{38}.

In the United States, clinical trials started in 2008. CXL using UV and riboflavin was approved by the food and drug administration (FDA) for the treatment of progressive keratoconus on April 18, 2016, and later on July 19, 2016, for corneal ectasia after refractive surgery\textsuperscript{39}.

In Kenya CXL was first started in Kenya at Laser centre Nairobi in 2010 by Dr Joshi and up to date there has been no data published concerning the techniques used or their outcomes. At Eagle Eye Laser Centre (EELC) crosslinking was started in 2011, the
Dresden protocol is often employed with some cases using modified epi-off technique for corneas <400µm with use of hypo-osmolar riboflavin.

2.7.2 Cross-linking techniques

Crosslinking incorporates three components, UVA, riboflavin, and oxygen. The combination of UVA and the riboflavin vitamin activates the polymerization cascade but requires oxygen to be successful.

Riboflavin is a hydrophilic molecule and has the ability to absorb UV photons. These excited riboflavin molecules pass on the energy to surrounding molecules such as oxygen. It is the preferred photoinitiator of the active forms of oxygen i.e. singlet oxygen and superoxide anion which trigger the formation of crosslinks in the corneal stroma.

For efficient distribution of riboflavin, drop by drop application is necessary and if the amount of riboflavin is limited, its concentration decreases. Riboflavin tends to diffuse in a homogenous fashion across the 8mm central cornea into the corneal layers and this is a time-dependent process taking several minutes.

It has been shown that the UV absorption of riboflavin is most effective in the anterior 400µm of the corneal stroma and there is a linear correlation with concentrations of up to 0.5% in the absorption coefficient of riboflavin\textsuperscript{40}.

A limitation of riboflavin is its hydrophilic property giving it poor diffusability across the corneal epithelium with development of the epi-off technique to allow diffusion of the riboflavin directly into the stroma. Newer methods have been developed that allow adequate diffusion through the epithelium barrier.

\textit{Epi-off technique}

\textit{Dresden protocol}\textsuperscript{41}

This was based on the diffusion behaviour of riboflavin across the tissues and entails epithelial debridement to achieve greater riboflavin absorption, which is hindered by epithelial tight junctions. The technique is as follows;
Topical anaesthetic e.g. proxymetacainhydrochloride 0.5% is applied and a lid speculum positioned. Thereafter, the central 8-10mm epithelium is debrided, using a spatula, followed by application of 0.1% riboflavin-5-phosphate in 20% dextran T-500 to the corneal surface at intervals of 5 minutes for 30 minutes before irradiation\textsuperscript{44}. After the absorption of riboflavin, the patient is positioned with UV light of a wavelength of 365nm to 370nm at a distance of about 1-5 cm from the apex of the corneal at an intensity of 3mW/cm\textsuperscript{2} and a dose of 5.6J/cm\textsuperscript{2} for about 30 minutes. Topical riboflavin is applied every five minutes during the UV light exposure. At the end of the procedure, topical antibiotics are applied and a bandage contact lens is placed on the cornea. Postoperatively, the patient is given drops of steroid-antibiotics to use 3-4 times every day for a week with postoperative removal of the bandage contact lens 5 days later.

Schumacher et al investigated the biochemical stability of porcine corneas when they were exposed to variable UV intensities ranging from 3mW/cm\textsuperscript{2} to 90mW/cm\textsuperscript{2} while maintaining the energy dose of 5.6 J/cm\textsuperscript{2}. The study found no difference in outcome with high intensity crosslinking suggesting that CXL is time-dependent if energy dose is kept constant\textsuperscript{42}.

Soeters et al investigated the outcome of epi-off corneal CXL in of 72 eyes 1 year after CXL. He divided the eyes into 3 pachymetry groups; group1 < 400µm, group 2 from 400-470µm and group 3 >470µm. Group 1 underwent crosslinking with hyposomolar riboflavin while group 2 and 3 used standard isotonic crosslinking. KC progression was halted in group 1 by 92% in group 2 by 97% and in group 3 by 86%. No significant difference was found in ECC. Kmax decreased in all groups and there difference between the 3 groups was not statistically significant\textsuperscript{43}.

Raiskup et al, evaluated the outcome of epi-off CXL technique in 32 eyes <400µm thickness with use of hypo-osmolar riboflavin after 1 year. Results showed no statistical difference in Kmax was seen after 1 year, with stability seen in all eyes and no complications in any of the eyes\textsuperscript{44}. 

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Drawbacks for the epi-off technique include, postoperative discomfort and pain and increased risk of complications like corneal haze, melting, infection and endothelial cell decompensation as well as a longer recovery time before resumption of normal activities.

**Epi-on technique**

The removal of the epithelium causes pain and discomfort to the patient, and methods for transepithelial CXL were researched to enhance the diffusion of riboflavin through the epithelium barrier.

This technique is considered to be non-invasive but allows transepithelial riboflavin penetration. The use of Benzalkonium chloride EDTA 0.01% as a chemical enhancer, functions to loosen epithelial tight junctions for the hydrophilic riboflavin molecules to pass through into the stroma. Topical anaesthetics have the same properties\(^\text{45}\).

Iontophoresis assisted transepithelial crosslinking (I-CXL), involves the use of an electric current to increase the imbibition of riboflavin through intact corneal epithelium and into the stroma. Riboflavin is lipophilic and is negatively charged making it appropriate for transepithelial iontophoresis. While iontophoresis yields better riboflavin concentration than conventional the epi-on technique it does not obtain the concentrations reached with epi-on technique.

In an experimental laboratory study carried out by Mastropasqua et al to determine differences in the concentration of riboflavin in the anterior, intermediate, and posterior stroma after CXL techniques of I-CXL, standard epi-off and conventional epi-on with 0.1% riboflavin. I-CXL showed deeper saturation of riboflavin with 15.0±5.1µg/g in relation to classical epi-on with7.2±3.7µg/g, but did not attain the high concentrations with relation to standard epi-off technique of 34.1±7.1µg/g\(^\text{46}\).

Administration of riboflavin through a femtosecond assisted intrastromal pocket is under investigation\(^\text{47}\).

Nawaz et al compared outcomes of epithelium-on and epithelium-off CXL technique and found no difference in corrected distance visual acuity (CDVA) and topographic changes but persistent stromal haze was noted in 10% of patients in the epithelium-off group.
Superior comfort post CXL was seen in the epithelium-off group. Return to contact lenses was a month postoperatively for the epi-off group when compared to a week for the epi-on group\textsuperscript{48}.

Cifariello et al compared the epi-off versus the epi-on technique in corneal collagen crosslinking of KC after a two-year follow-up. 20 eyes were treated with epi-off and 20 eyes were treated with epi-on. In both groups there was a statistically significant improvement in visual function and a statistically significant decrease in corneal thickness but the difference between the two groups was not significant. There was no change in the keratometry readings. Two eyes in the epi-off group developed complications of corneal haze and Vogt’s striae and 1 eye in the ep-on group developed a complication of Vogt’s striae at the apex. There was no progression in the two groups\textsuperscript{45}.

Epithelium-on technique has been shown to be tolerated well by all age groups and can be a better option in selected cases such as in young patients who tolerate it well with fast recovery. Epithelium-on technique when done correctly halts progression of KC with minimal vision loss, patient discomfort and pain.

\textit{Epithelial Island crosslinking technique}

Epithelial Island CXL technique (EI-CXL) may be used in isolation or in combination with hyposomolar riboflavin solutions. It is a midpoint where both epi-off and epi-on techniques can be used, where the epithelial island over the thinnest corneal area and intrastromal riboflavin create a shield for the endothelium below and the exposed stroma provides adequate riboflavin penetration\textsuperscript{49}.

In a randomized prospective study by Razmjoo et al., 44 eyes of 22 patients with keratoconus were examined. In the first group, corneal epithelium was completely removed while in the second one, the central 3 mm of epithelium was left intact and the periphery was removed. After six months, the difference between these groups was not statistically significant concerning postoperative refraction, corneal haziness, and visual acuity. The parameters of pre and postoperative surgery in each group showed that a total removal of the cornea led to statistically significant improvement of Q-value and k-max; whereas, in
the group of eyes with partial epithelium removal, they had greater improvement of corrected vision\textsuperscript{50}.

\textit{Athens protocol procedure}\textsuperscript{51}

Involves topography guided phototherapeutic keratectomy with same day CXL to not only halt progression but address the high amounts of irregular astigmatism found in KC.

The procedure of CXL causes corneal flattening and residual astigmatism presents persistent problems for patients postoperatively. The goal in the Athens protocol procedure is to regularise the corneal surface to improve the BCVA, therefore it is a therapeutic procedure not a refractive one, as patients may be myopic postoperatively but have smoother corneas with better BCVA outcomes. It involves 6.5mm phototherapeutic keratectomy removing 50µm of epithelium followed by partial topography guided partial photorefractive keratectomy. With application of 0.02\% mitomycin C for 20s and accelerated CXL procedure thereafter.

With the removal of epithelium and Bowman membrane the efficacy of CXL is shown to increase. The general consensus regarding this intervention shows it strengthens the cornea and helps prevent ectasia progression, improves corneal keratometry, refraction and visual acuity\textsuperscript{52}.

\textbf{2.7.3 Treatment parameters with respect to safety in crosslinking and prevention of corneal endothelium damage}

From 1999 to 2004 more studies were carried out and the threshold for keratocytes and endothelial cells was determined. Due to safety issues on endothelium cells, the minimum corneal thickness advised was 400µm.

According to Spoerl et al, there are parameters that are needed to ensure safety in CXL. They include: (a) 0.1\% riboflavin solution to be applied for 30 minutes before the exposure of ultraviolet; (b) corneal stroma minimal thickness to undergo CXL must be 400µm; (c) the ultraviolet-irradiance of 3mW/cm\textsuperscript{2} has to have a wavelength of 370 nm, and (d) epithelium should be removed to facilitate the diffusion of riboflavin\textsuperscript{53}.
Ashwin and McDonnell stated in their review that proper ultraviolet irradiance should be maintained and the corneal thickness should exceed 400μm. To achieve this, they advised that corneal edema should be induced with hypotonic riboflavin drops for cornea thickness that is less than 400μm\textsuperscript{38}.

Sehra et al, carried out a study on the microstructural changes of 25 eyes with the use of RGP contact lenses in patients with keratoconus following CXL. One group was offered contact lenses after 3 months after CXL while the other group was offered only spectacle correction. The results showed that RGP contact lenses use after CXL was related to a delay in the regeneration epithelial cell stress\textsuperscript{54}.

**Additional applications of cross-linking**

There are other beneficial impacts of cross-linking other than keratoconus on a series of corneal diseases. Other Indications of Cross-Linking include, pellucid marginal degeneration, terrien marginal degeneration, post-refractive surgery such as post-Laser in situ keratomileusis (LASIK) or Radial Keratotomy, ectasia, bullous keratopathy, and microbial keratitis/corneal ulceration\textsuperscript{55}.

**2.7.4 Outcomes of cross-linking**

A number of studies have been carried out to evaluate the efficacy of CXL in keratoconus. The first clinical research study conducted in human eyes was published in 2003. Conducted by a research group from Dresden University, the researchers examined the efficacy of CXL in 23 eyes with a follow-up period of 48 months. Their results showed that progression of KC was stopped in all treated eyes. Results also showed 16 eyes (70%) had reduction of maximum keratometry (Kmax) by 2.01D and refractive error by 1.14D. Since then, more clinical research studies have been conducted\textsuperscript{4}.

In Italy, Coporossi et al carried out the first open nonrandomized clinical trial from September 2004 to September 2008 on 363 eyes with progressive keratoconus using riboflavin and UVA. Results showed keratoconus stability in 44 eyes of minimum follow up 48 months showing reduction in cornea aberration improvement in UCVA by 2.7 Snellen lines and the mean BSCVA by 1.9 Snellen lines with significant reduction of mean
keratometry values by 1 year (-1.96D) and 4 years (-2.26D). No significant change in pachymetry, UCVA/BCVA or cylinder. Termed the Siena Eye Cross Study, it showed stability of KC after CXL without any relevant side effects\textsuperscript{56}.

In Australia, Witting-Silva et al conducted the first randomized prospective clinical trial on 46 eyes with keratoconus that were crosslinked and 48 controls and evaluated their outcomes after 3 years. The findings showed for the treated eyes, Kmax flattened at -0.72D at 12 months, -0.96D at 24 months and -1.03D at 36 months. The UCVA improved by 0.15 logMAR and the BSCVA improved by 0.09 logMAR by 36 months\textsuperscript{57}.

In a study conducted by Oltulu et al, the short term clinical and topographeral outcomes in patients with KC after CXL with dextran-free isotonic riboflavin were analysed. The BCVA were analysed at 1, 3 and 6 months follow up and the refractive and topographic findings were analysed at 6 months. Results showed, the difference between the preoperative and 6-month values was statistically significant, the mean spherical equivalent refraction decreased, the mean simulated keratometry decreased, Kmax decreased progressively and significantly from the preoperative value during follow-up and the central and minimal corneal thicknesses, including those of the epithelium. No intraoperative or postoperative complications were observed\textsuperscript{58}.

Jankov et al in Spain followed 25 eyes of 20 patients for six months and results at 6 months showed BSCVA increased from 0.41±0.27 to 0.49±0.29, Kmax decreased by more than 2D and refractive cylinder decreased by 0.5D and the progression halted in all patients. In addition, no eyes were found to have lost lines of the BCVA\textsuperscript{59}.

Hersh et al. in a 1 year study looking at the outcomes of CXL in 204 eyes with progressive keratoconus in the United States had results that showed the UCVA improved from 0.84 logMAR to 0.77 logMAR and a decrease in Kmax from baseline by 1.7 in the entire cohort\textsuperscript{60}.

Toosi et al. in Iran after a mean follow up of 48 months evaluated the outcomes of CXL in 132 eyes with progressive keratoconus patients at day 1, day 7, 1\textsuperscript{st} month, 3\textsuperscript{rd} month, 6 months, 12 months, then yearly for 4 years. Results showed an improvement in UCVA 12 months post CXL from preoperative 0.26 (Snellen decimal point) to 0.36. The Kmax
decreased by an average of 1.18D while the mean keratometry (Kmean) reduced from 46.18 ± 2.36 diopters before CXL to 45.6 ± 2.37 diopters. With follow up of 4 years showing decline in Kmean (-1.21 ± 0.61), spherical power (0.18 ± 1.14) and cylindrical power (0.50 ± 1.13) and success in halting of KC progression.

Padmanabhan et al in India, reported the long-term outcome of CXL in pediatric patients with progressive keratoconus at 3 months, 6 months and annually thereafter. In 85% of eyes stabilisation or flattening of Kmax was seen at 2 years and 76% at 4 years. 80.1% of the eyes after 2 years showed stabilisation and improvement in CDVA and 69.1% after 4 years. CXL remains an effective treatment option for stabilizing keratoconus for a period of over 2 years in pediatric patients.

Coskunseven et al evaluated the outcomes of CXL in keratoconus patients with treatment of the worse eye with CXL and the fellow eye used as a control with an average follow up of 9 months. Results showed a mean decrease in cylinder, the spherical equivalent refraction, as well as the maximum keratometry and the refractive cylinder and an improvement in both UCVA and BSCVA. Regarding CCT and ECC, no statistically significant difference was noted after CXL.

In a retrospective analysis of the outcomes in visual acuity and keratometry readings by Rowjee et al in South Africa following CXL in 41 eyes of 29 patients, results at 6 months showed UCVA improved in 39% of eyes, BCVA improved by 29%, with 56% showing average flattening of keratometry by 0.7D with 44% showing more steepening by 0.9D.

As such, the outcomes of CXL show that keratoconus can be effectively treated in small and large-scale numbers as well as in the short and long-term.

2.7.5 Complications of corneal collagen cross-linking
Crosslinking is a minimally invasive procedure with low complications and failure rates and is considered to be one of the best approaches for treating keratoconus. However, it may have complications due to primary factors like incorrect patient inclusion or secondary factors related to undiagnosed ocular surface disease, poor hygiene or therapeutic soft contact lens,
**Postoperative infection**

Debridement of the epithelium and use of a soft bandage contact lens postoperatively with topical steroids predisposes the cornea to microbial infection. The first case report of a rare postoperative complication was by Pollhammer et al, of a patient who developed bacterial keratitis 3 days after CXL which revealed an E. coli infection that was successfully treated but resulted in a corneal scar with permanent visual reduction. Acanthamoeba keratitis has also been reported in a patient after eye washing with tap water. Polymicrobial keratitis seen in poor patient contact lens hygiene has been reported.

**Corneal haze**

After CXL, corneal haze extends to about 60% depth into the anterior stroma. It is noted as a dust like change in the stroma with a mid-stromal demarcation line that may be as a result of back scattered and reflected light which decreases corneal transparency. In the anterior stroma the cornea develops a lacunar honeycomb pattern of hydration. This is due to the positions of apoptotic keratocytes which prevent interfibrillar crosslinking bonds as well as lacunar edema developing in the former positions of the apoptotic keratocytes. This also contributes to the elasticity of the crosslinked cornea and to the demarcation line viewed on biomicroscopy, showing the extent of the CXL thus making lacunar edema a sign of efficient crosslinking.

Confocal microscopy studies by Mazzota et al revealed activated keratocyte repopulating the cornea from the 2nd month post CXL to completion by 6 months. These activated keratocytes may also lead to the development of corneal haze post CXL. Raiskup et al in a study they conducted of 163 eyes with keratoconus, 8.6% of the 127 patients developed permanent corneal haze after a follow-up period of 1 year.

**Peripheral sterile infiltrates**

Sterile corneal stromal infiltrates may be due the enhanced cell mediated response to staphylococcal antigen deposition in areas of static tear pooling at the edge of the epithelial debridement zone beneath the bandage contact lens. While diffuse sterile infiltrates may be due to poor contact lens hygiene.
Cerman et al evaluated the risk factors leading to sterile corneal infiltrates after corneal collagen CXL which included 588 eyes of 459 patients treated with either epi-on technique or epi-off technique. Results showed no patients in the epi-on group developed sterile infiltrates, 19 patients in the epi-off group developed sterile infiltrates. Kmax, pachymetry, blepharitis and vernal conjunctivitis were not identified as risk factors. Postoperative use of NSAIDs was a statistically significant contributor with a four times increased chance.

**Herpes reactivation**

Among the risk factors to herpes reactivation, exposure to UV light is a potent trigger. Awad Al-Qarni and Mosa AlHarbi reported two cases, where neither had a history of herpetic keratitis or cold sores. The first case was of an 18-year-old male patient who developed herpetic keratitis 7 days post CXL of the second eye, which was then treated but central corneal opacity remained. The second patient was a 21 year old male, who developed herpetic keratitis 9 days post CXL after treatment a central corneal opacity remained.

**Endothelial damage**

The CXL standard irradiance protocol uses UVA of 3mW/cm² with 0.1% riboflavin for 30min resulting of an irradiance of the endothelium of 0.15mW/cm². The endothelium damage threshold is seen at an irradiance of 0.35mW/cm². Endothelium damage may also be due to corneal thickness of <400 because UVA.

Gokhale reported a case of endothelial cell damage with development of massive corneal edema 1 month post CXL in an eye with corneal thickness of >400um with ECC of 1776mm² post CXL compared with ECC of 2978mm² of the fellow eye.

**2.7.6 Factors affecting outcomes of cross-linking**

Outcomes of CXL may vary among patients therefore the ability to reliably forecast postoperative outcomes before the procedure will aid in guiding patient expectations and reducing less desirable outcomes.
Ibrahim et al conducted a retrospective study in Turkey focusing on the different factors that affect the outcomes of cross-linking. In the study which comprised of 96 eyes of 96 patients, the results showed that the sex, the preoperative Kmax and baseline topographic cone location of the patient did not affect the outcome of cross-linking (CXL). Those patients ≥ 30 years and baseline thinnest pachymetry of <450 lead to a more flattening effect in maximum keratometry and patients with CDVA of 20/40 Snellen equivalent or worse experienced greater visual improvement post-CXL\textsuperscript{75}.

Greenstein et al. in their cohort study, aimed to determine the characteristics of the preoperative patient that may predict visual acuity and topography outcomes of CXL. Eyes with preoperative CDVA of 20/50 or worse were 5.9 times more likely to improve by 2 Snellen lines while eyes with Kmax of ≥ 50D or more were 5.4 times more likely to have flattening of 2.0D or more\textsuperscript{76}.

In Egypt, Badawi et al. focused on the predictive factors that affect the outcome of CXL in 136 eyes of 84 adult patients with progressive keratoconus one year after treatment. The findings showed that with age ≥ 30 years there was more flattening of Kmax. Patients with worse baseline BCVA of ≥ 0.3logMAR had better improvement in post BCVA, pachymetry <450 was a good predictor of postoperative BCVA improvement. There was no significant difference in BCVA and Kmax seen in the male or female sex\textsuperscript{77}.

Koller et al showed increased risk of continued progression in patients whose preoperative Kmax value was >58D as well as more postoperative complications following epi-off CXL. CXL also had a higher rate of complications in patients aged over 35 years\textsuperscript{78}.

Dhawan et al in India in a review article on the CXL compilations of 117 eyes from 99 patients, found that poor hygiene on the part of the patient was one of the reasons for endothelial damage\textsuperscript{79}.

In CXL, a decrease of CCT is induced with the use of a standard isotonic riboflavin solution with dextran which in thin corneas can cause UV endothelial cell damage and stromal opacities. Swelling solutions of hypotonic riboflavin are recommended when CCT is below 400 \( \mu m \)\textsuperscript{43}.
In pregnancy, changes in hormone levels may alter biomechanics and trigger KC and is reported as a factor promoting ectasia progression. In a case report by Hafezi et al. a 33 year old woman who underwent LASIK with corneal thickness of 410 for the right eye and 400 for the left, developed keratectasia three years later during her pregnancy. CXL was done in both eyes with stability seen until four years later in her second pregnancy the patient developed progression of keratectasia in her right eye.\textsuperscript{80}
3.0 JUSTIFICATION

Keratoconus is a cause of visual impairment especially in the young who have many productive years ahead of them. Keratoconus is the leading indication of keratoplasty in Kenya at 48.8%\(^5\).

Crosslinking has been shown to halt the progression of the disease and reduce the demand for corneal transplant. Crosslinking has been in existence in Kenya since 2010 and as yet there is no data on the outcomes of CXL in Kenya. Our study aimed to provide information regarding the benefit of crosslinking in keratoconus in halting its progression, thus reducing demand for corneal transplantation in Kenya for the advanced keratoconus. The data collected will then provide an informed evidence-based approach to guidelines as regards to crosslinking, patient selection and follow up in our setting. In addition, it will act as an audit, pointing out factors that contribute to the surgery’s success or failure at the centre.
4.0 RESEARCH QUESTION

What is the outcome of cross-linking in patients with keratoconus at Eagle Eye Laser Centre?

4.1 Main objective
To determine the outcomes of corneal collagen crosslinking in patients with keratoconus at Eagle Eye Laser Centre.

4.2 Specific objectives
1. To assess the visual outcomes after crosslinking
2. To determine the topographic outcomes after crosslinking
3. To determine the factors associated with outcomes of crosslinking
4. To determine the postoperative complications following crosslinking

The primary outcome measures of cross-linking included the following
- Keratometry values; Kmax, Kmean, K1, K2
- Central corneal thickness
- Thinnest corneal thickness
- Uncorrected visual acuity
- Best corrected visual acuity
- Postoperative complications at 1 week, 1 month, 3 months and 6 months

The Secondary outcome measures
- Proportion of epithelium off to epithelium on technique

Success
- When no progression of keratoconus is seen after comparing the baseline pre-CXL values and 6month post CXL values of Kmax, Kmean, and pachymetry reading.
- Progression is defined as a change in any two parameters: an increase in Kmax by ≥1D, Kmean by ≥1D, and a decrease in pachymetry reading by ≥ 10%.
5.0 METHODS

5.1 Study design
This was a retrospective case series.

5.2 Study location
Eagle Eye Laser Centre is a free-standing outpatient surgical facility specialising in eye care and offering eye consultation services, an optical shop as well as all ophthalmological serving about 500 patients in a month, having two branches, one located in Nairobi, Lavington, off James Gichuru Rd and another branch on Ngong Road. Corneal collagen crosslinking is only performed at the Lavington branch, with about 100 eyes done in a year done by 4 different surgeons with the use of the CBM Vega X-Linker crosslinking machine with UVA 370nm, irradiance of 3-4mW/cm² and power of 5.4J/cm.

5.3 Study population
Patients at Eagle Eye and Laser Centre with keratoconus who underwent CXL within the study period of January 1st 2017 to June 30th 2018.

5.4 Criteria
5.4.1 Inclusion criteria
All patients with keratoconus who had undergone cross-linking within the study period.

5.4.2 Exclusion criteria
The exclusion criteria for patients in this study will include:

1. Patients who had undergone a previous eye surgery.
2. Patients who had repeat CXL
3. Patients who had no follow up at EELC
4. Patients that did not have preoperative topography analysis by the Allegro Oculyser Topography machine

5.5 Sample size
The following sample size determination formula for finite population correction (Wanga & Lemeshow, 1991) was used to estimate the proportion of population study size.
\[ n^1 = \frac{NZ^2 P(1 - P)}{d^2(N - 1) + Z^2 P(1 - P)} \]

Where

- \( n' \) = sample size with finite population correction;
- \( N \) = size of the target population = 216 (according to the registry book, there are 3 cases of cross linking per week seen in Eagle Eye Laser Centre, the study period was 18 months);
- \( Z \) = the value that specifies the level of confidence you want in your confidence interval when you analyse your data. Typical levels of confidence for surveys are 95\%, in which case \( z \) is set to 1.96;
- \( P = p \) is the estimated proportion of population value = 2.3\% is the prevalence rate for keratoconus at an eye clinic in a study carried out by Jonas et al\textsuperscript{81}, 2009 (closest regional study)
- \( d \) = margin of error = 2.1\%

\[
\begin{align*}
n^1 &= \frac{216 \times 3.8416 \times 0.023 \times (1 - 0.023)}{0.021^2 \times (216 - 1) + 1.96^2 \times 0.023 \times (1 - 0.023)} \\
n' &= 103 \text{ eyes}
\end{align*}
\]

5.6 Sampling procedure
The sample size has given the number of patients needed for the study to have power. All patients who fit the criteria and underwent crosslinking between the 1\textsuperscript{st} January 2017 and June 30\textsuperscript{th} 2018 are included in the study.

5.7 Study materials
Materials
- A pre-designed questionnaire was used
- Records of patients from Eagle Eye Laser Centre, Lavington
Personnel

- Principal researcher
- Records officers at Eagle Eye Laser Centre, Lavington
- Statistician

5.8 Data collection and procedure

All the patients who meet the inclusion criteria and underwent corneal collagen CXL treatment at the Eagle Eye Laser Centre, Lavington during the period of January 1st 2017 to June 30th 2018 were obtained from the theatre register.

The patient’s reference number was used to retrieve the patients file and obtain visual acuity and demographic details, such as sex, and age and the topographic data from the Allegro Oculyser Topography Machine, which includes pachymetry and keratometry readings. All the data related to the research study was collected and entered into a questionnaire. See the appendix 1 for the attached questionnaire.

All surgeries were performed by 4 surgeons (KK, DI, MG, JM) using the same technique for epi-on, epi-off and epithelial island. Preoperatively all patients had a preoperative topography done using the Allegro Oculyser Machine. On the day of surgery, patients were given oral vitamin C tablet, Valium 5mg tablet and ibuprofen 400mg all as a stat dose.

For the epithelium-off technique, intraoperatively, local anaesthetic gel was applied and 50% alcohol applied to the 9mm corneal epithelium demarcated by a trephine. This was washed out with normal saline after 15s and epithelium debrided using a hockey stick. Then for the next 30min, in corneas with TCT ≥ 400µm, riboflavin 0.1% in 20% Dextran was applied every 2min, xylocaine drops added every 5min and normal saline drops every 10minutes. Thereafter, an accelerated technique was applied using the CBM VEGA X linker which provides UVA of 370nm at irradiance of 3mW/cm² for 15mintutes. During the irradiation, the same concentration of riboflavin drops was applied every 2min, local anaesthetic is applied every 5 min and normal saline drops used to every 10min.
In the Epithelium-island technique, an island of epithelium is spared over the thinnest area of the cornea and appropriate riboflavin concentration is used instead.

For the epithelium-on technique, no debridement of epithelium is done, and hypotonic riboflavin 0.1% was used.

Postoperatively, patients were put on artificial eye gel drops, antibiotic and a BCL for 5 days and were evaluated at 1 week, 1 month, 3 months 6months and 1 year. UCVA is taken at all visits, BCVA was taken at 3 months, 6 months and 12 months.

**5.9 Data storage**

The soft copies of the data was encrypted and stored on a hard drive while the hard copies were locked away in a file cabinet at the Eagle Eye Laser Centre, Lavington. The data was retrieved only by agreed members taking part in the research.

**5.10 Data analysis**

The data was entered into the Microsoft excel 2013 package. It was then transferred to a computer statistical analysis software tool known as Statistical Package for Social Scientists (SPSS) version 23.0 and all statistical tests were conducted at 5% level of significance (p≤0.05). A paired t test was used to evaluate the difference in the visual and topographic values at baseline (pre-CXL) and post-CXL and was analysed to determine any statistical difference at 3 months or more. This was done to evaluate the effects of CXL, as well as to detect if keratoconus progression had stabilised. Description of the study population using socio-demographic characteristics was done by summarising the variables. The Categorical values were summarised by pie charts, bar charts and percentages while continuous variables were described by mean and standard deviation. The associations between the demographic characteristics and topographic variables with the outcomes of CXL was evaluated to determine any statistical significance using univariate regression analysis.
5.11 Ethical consideration

Ethical Approval
Approval for the research study was obtained from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee.

Institutional Permission: was obtained from Eagle Eye Laser Centre

Confidentiality
The confidentiality of information of patients was ensured by coding the names of all the patients with numbers and letters. This ensured the names of the patients could not be deduced by anyone apart from the people taking part in the research.
6.0 RESULTS
The study included 174 eyes of 108 subjects who underwent crosslinking at Eagle Eye Laser Centre, Lavington, Nairobi, between the dates of January 1st 2017 to June 30th 2018 who fit the inclusion criteria. There were 62 males (57.4%) and 46 females (42.6%). The Male: Female ratio was 1.3:1 (p=0.12). The mean age was 23.3 years (SD 9.1) and the median age was 21 years (IQR 16-31 years). There were 66 patients who had CXL in both eyes and 42 patients who had only one eye done. The median age for single eyes was 18 years and the median age for both eyes was 25.5 years.

Demographic characteristics

![Age and sex distribution](image)

**Figure 1: Age and sex distribution**

Range = 10 years to 44 years

Mean age males = 22.5 years (SD 9.2)  
Median age males = 19.5 (IQR 16-28.5)

Mean age females = 24.3 years (SD 8.9)  
Median age females = 25.5 (IQR 15.5 -31.5)

The age difference between the males and females was not statistically significant (p=0.89).
Table 1: Summary of pre-operative topographic characteristics (N=174)

<table>
<thead>
<tr>
<th></th>
<th>CCT(µm)</th>
<th>TCT(µm)</th>
<th>AST(D)</th>
<th>K1(D)</th>
<th>K2(D)</th>
<th>Kmean(D)</th>
<th>Kmax(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>320</td>
<td>319</td>
<td>0.4</td>
<td>39.1</td>
<td>41.6</td>
<td>40.3</td>
<td>42.4</td>
</tr>
<tr>
<td>Maximum</td>
<td>545</td>
<td>534</td>
<td>16.9</td>
<td>70.5</td>
<td>87.5</td>
<td>78.1</td>
<td>107.3</td>
</tr>
<tr>
<td>Mean</td>
<td>436.5</td>
<td>417.9</td>
<td>4.9</td>
<td>46.8</td>
<td>51.7</td>
<td>49.1</td>
<td>56.1</td>
</tr>
<tr>
<td>Median</td>
<td>438</td>
<td>421</td>
<td>4.3</td>
<td>45.9</td>
<td>50.8</td>
<td>48.0</td>
<td>55.0</td>
</tr>
</tbody>
</table>

Preoperative Thinnest cornea thickness (TCT)

![Bar chart showing percentage of eyes in different TCT categories]

**Figure 2: Pre CXL TCT (N=174)**

The lowest TCT was 319µm and the highest was 534µm.
6.1 Intra-operative data

![Pie chart showing proportion of eyes undergoing different CXL techniques (N=174)](image)

**Figure 3: Proportion of eyes that underwent different CXL techniques (N=174)**

![Pie chart showing proportion of eyes by different riboflavin preparations (N=174)](image)

**Figure 4: Proportion of eyes by different riboflavin preparations (N=174)**

In most of the eyes (63.8%) isotonic 0.1% riboflavin with Dextran was used while 10 eyes that underwent epithelium on and epithelial island technique had hypotonic 0.1% riboflavin (without dextran) used. Those with TCT less than 400µm (46 eyes) had hypotonic 0.1% riboflavin solution used.
6.2 Postoperative data

![Graph showing follow-up data](image)

**Figure 5:** Patients who reported for follow up (N=108 people)

![Graph showing UCVA comparison](image)

**Figure 6:** Comparison of pre and post-CXL UCVA at 3 months (n=72), 6 months (n=46) and at 12 months (n=11)
Figure 7: Post-CXL change in UCVA at 3 months (n=74), 6 months (n=77) and at 12 months (n=29)

For those that had improved, at 3 months it was by an average of 2.1 lines on the Snellen chart, at 6 months by 2.5 lines and at 12 months by 2.2 lines. For those that worsened it was by an average of 1.6 lines at 3 months, 1.4 lines at 6 months and 1.5 lines at 12 months. At 3 months the change in UCVA pre and post CXL was statistically significant (p=0.02) but was not significant at 6 months (p=0.89) and at 12 months (p=0.42).
Figure 8: Comparison of pre and post-CXL BCVA at 3 months (n=37), 6 months (n=37) and at 12 months (n=21)

Figure 9: Post-CXL change in BCVA at 3 months (n=74) 6 months (n=77) and 12 months (n=29)
For those that improved, at 3 months it was by an average of 2.5 lines on the Snellen chart, at 6 months by 1.7 lines and at 12 months by 2.9 lines. For those that worsened, at 3 months it was by an average of 1.2 Snellen lines, at 6 months 2.1 lines and at 12 months 2.0 lines. The change in BCVA pre and post-CXL was statistically significant at 3 months (p=0.00) but not at 6 months (p=0.99) or 12 months (p=0.69)

6.2.1 Topographic outcome analysis: Summary

Table 2: Kmean analysis at 6 months (n=33) and at 12 months (n=28)

<table>
<thead>
<tr>
<th>Range</th>
<th>Kmean at 6 months n (%)</th>
<th>Kmean at 12 months n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flattening</td>
<td>≤ -1D</td>
<td>5(15.2)</td>
</tr>
<tr>
<td>Same</td>
<td>&gt; -1 to &lt; 1</td>
<td>25(75.8)</td>
</tr>
<tr>
<td>Steepening</td>
<td>≥1D</td>
<td>3(9.1)</td>
</tr>
</tbody>
</table>

Figure 10: Post-operative change in K-mean at 6 months (n=33) and at 12 months (n=28)

At 6 months, there was an average flattening of 1.9D for the ones that flattened and an average steepening of 1.6D for the ones that steepened, overall there was a flattening of 0.1D. The change in Kmean pre and post-CXL was not statistically significant (p=0.79)
At 12 months there was an average flattening of 1.5D for those that flattened and an average steepening of 1.9D for those that steepened, overall there was a steepening of 0.1D. The change in Kmean pre and post-CXL was not statistically significant (p=0.70).

**Table 3: Kmax analysis at 6 months (n=33) and at 12 months (n=28)**

<table>
<thead>
<tr>
<th>Range</th>
<th>Kmax at 6 months n (%)</th>
<th>Kmax at 12 months n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flattening</td>
<td>≤ -1D</td>
<td>8 (24.2)</td>
</tr>
<tr>
<td>Same</td>
<td>&gt; -1 to &lt; 1</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>Steepening</td>
<td>≥ 1D</td>
<td>9 (27.3)</td>
</tr>
</tbody>
</table>

![Graph showing percentage of eyes with flattening, same, and steepening at 6 and 12 months](image)

**Figure 11: Post-operative change in Kmax at 6 months (n=33) and at 12 months (n=28)**

At 6 months, there was an average flattening of 2.6D for those that flattened and an average steepening of 1.9D for those that steepened, overall there was a flattening of 0.1D. The change in Kmax pre and post-CXL was not statistically significant (p=0.61).
At 12 months, there was an average flattening of 3.1D for those that flattened and an average steepening of 2.9D for those that steepened, overall there was a flattening of 0.3D. The change in Kmax pre and post-CXL was not statistically significant (p=0.44).

**Table 4: Astigmatism analysis at 6 months (n=33) and at 12 months (n=28)**

<table>
<thead>
<tr>
<th>Range</th>
<th>AST at 6 months n (%)</th>
<th>AST at 12 months n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased</td>
<td>≤ -1D</td>
<td>10(30.3)</td>
</tr>
<tr>
<td>Same</td>
<td>&gt; -1 to &lt; 1</td>
<td>19(57.6)</td>
</tr>
<tr>
<td>Increased</td>
<td>≥1D</td>
<td>4(12.1)</td>
</tr>
</tbody>
</table>

**Figure 12: Postoperative change in astigmatism at 6 months (33) and 12 months (n=28)**

At 6 months there was an average decrease of 2.6D for those that decreased, and an average increase of 1.9D for those that increased. Overall there was a decrease of 0.5D. The change in astigmatism pre and post-CXL was not statistically significant (p=0.07).
At 12 months, there was an average decrease of 2.2D for those that decreased and an average increase of 1.3D for those that increased. Overall there was a decrease of 0.4D.

The change in astigmatism pre an post-CXL was statistically significant (p=0.02)

### Table 5: CCT and TCT analysis at 6 months (n=33) and at 12 months (n=28)

<table>
<thead>
<tr>
<th>Range</th>
<th>CCT at 6 months</th>
<th>TCT at 6 months</th>
<th>CCT at 12 months</th>
<th>TCT at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Thickening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 10% thickening</td>
<td>0(0.0)</td>
<td>1(3.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Same</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; - 10% to &lt;10%</td>
<td>31(93.9)</td>
<td>28(84.8)</td>
<td>27(96.4)</td>
<td>25(89.3)</td>
</tr>
<tr>
<td>Thinning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ -10%</td>
<td>2(6.1)</td>
<td>4(12.1)</td>
<td>1(3.6)</td>
<td>3(10.7)</td>
</tr>
</tbody>
</table>

**Figure 13: Postoperative change in CCT and TCT at 6 months (n=33) and at 12 months (n=28)**

The change in CCT was statistically significant at 6 months (p=0.00) and at 12 months (p=0.01). The change in TCT was statistically significant at 6 months (p=0.00) and at 12 months (p=0.02).
6.2.2 Topographic outcome analysis: Analysis of Progression:

**Figure 14:** Progression in topographic parameters of Kmax, Kmean, CCT, TCT at 6 months (n=33)

At 6 months progression was stopped in 84.8%. There were 5 eyes (15.2%) that progressed.

**Figure 15:** Progression in topographic parameters Kmax, Kmean, CCT, TCT at 12 months (n=28)

At 12 months progression was stopped in 85.7%. There were 4 eyes (14.3%) that progressed.
6.2.3 Analysis of complications

There were no intraoperative complications

Figure 16: Postoperative corneal findings

At week 1 complications occurred in 23 eyes (13.2%). Corneal haze resolved after 3 months.
6.2.4. Factors associated with progression

The associations between the pre-operative values and the outcome of progression was analysed at 6 months and at 12 months.

Table 6: Univariate analysis: associations of progression at 6 months (n=33)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Progression</th>
<th>No Progression</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT &lt; 400µm</td>
<td>2</td>
<td>5</td>
<td>3.07</td>
<td>0.40 to 23.44</td>
<td>0.28</td>
</tr>
<tr>
<td>CCT ≥ 400µm</td>
<td>3</td>
<td>23</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCT &lt; 400µm</td>
<td>3</td>
<td>10</td>
<td>2.70</td>
<td>0.39 to 18.96</td>
<td>0.36</td>
</tr>
<tr>
<td>TCT ≥ 400µm</td>
<td>2</td>
<td>18</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kmean ≤ 58 D</td>
<td>4</td>
<td>27</td>
<td>0.15</td>
<td>0.01 to 2.87</td>
<td>0.28</td>
</tr>
<tr>
<td>Kmean &gt; 58 D</td>
<td>1</td>
<td>1</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kmax ≤ 58 D</td>
<td>3</td>
<td>19</td>
<td>0.71</td>
<td>0.10 to 5.03</td>
<td>1.00</td>
</tr>
<tr>
<td>Kmax &gt; 58 D</td>
<td>2</td>
<td>9</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astigmatism &lt; 4.3 D</td>
<td>1</td>
<td>9</td>
<td>0.53</td>
<td>0.05 to 5.43</td>
<td>1.00</td>
</tr>
<tr>
<td>Astigmatism ≥ 4.3 D</td>
<td>4</td>
<td>19</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Technique Epi-on</td>
<td>1</td>
<td>2</td>
<td>3.25</td>
<td>0.24 to 44.69</td>
<td>0.40</td>
</tr>
<tr>
<td>Technique Epi-off</td>
<td>4</td>
<td>26</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt; 18 years</td>
<td>3</td>
<td>11</td>
<td>2.32</td>
<td>0.33 to 16.19</td>
<td>0.63</td>
</tr>
<tr>
<td>Age &gt; 18 years</td>
<td>2</td>
<td>17</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex Male</td>
<td>4</td>
<td>15</td>
<td>3.47</td>
<td>0.34 to 35.06</td>
<td>0.37</td>
</tr>
<tr>
<td>Sex Female</td>
<td>1</td>
<td>13</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

At 6 months there were 5 eyes recorded with progression; however, there was no statistically significant association found when comparing these pre-operative categorical values with progression.
Table 7: Univariate analysis: associations of progression at 12 months (n= 28)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Progression</th>
<th>No Progression</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT &lt; 400µm</td>
<td>0</td>
<td>5</td>
<td>1.21</td>
<td>1.00 to 1.46</td>
<td>1.00</td>
</tr>
<tr>
<td>CCT ≥ 400µm</td>
<td>4</td>
<td>19</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCT &lt; 400µm</td>
<td>1</td>
<td>9</td>
<td>0.56</td>
<td>0.50 to 6.18</td>
<td>1.00</td>
</tr>
<tr>
<td>TCT ≥ 400µm</td>
<td>3</td>
<td>15</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kmean ≤ 58 D</td>
<td>3</td>
<td>23</td>
<td>0.13</td>
<td>0.01 to 2.68</td>
<td>0.27</td>
</tr>
<tr>
<td>Kmean &gt; 58 D</td>
<td>1</td>
<td>1</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kmax ≤ 58 D</td>
<td>2</td>
<td>12</td>
<td>1.00</td>
<td>0.12 to 8.31</td>
<td>1.00</td>
</tr>
<tr>
<td>Kmax &gt; 58 D</td>
<td>2</td>
<td>12</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astigmatism &lt; 4.3 D</td>
<td>1</td>
<td>7</td>
<td>0.81</td>
<td>0.07 to 9.18</td>
<td>1.00</td>
</tr>
<tr>
<td>Astigmatism ≥ 4.3 D</td>
<td>3</td>
<td>17</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt; 18 years</td>
<td>2</td>
<td>10</td>
<td>1.40</td>
<td>0.17 to 11.68</td>
<td>1.00</td>
</tr>
<tr>
<td>Age &gt; 18 years</td>
<td>2</td>
<td>14</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex Male</td>
<td>3</td>
<td>10</td>
<td>4.20</td>
<td>0.38 to 46.49</td>
<td>0.31</td>
</tr>
<tr>
<td>Sex Female</td>
<td>1</td>
<td>14</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the eyes that came for 12 months review had the epi-off technique and this variable was not analysed. At 12 months there were 4 eyes found to have progression, however, there was no statistically significant factors associated with the progression.
7.0 DISCUSSION

The outcomes of corneal collagen crosslinking in 174 eyes were evaluated at 1 week, 1 month, 3 months, 6 months and 12 months.

There were 108 patients that underwent surgery with a male preponderance of 57% with a male female ratio of 1.3:1 which was not statistically significant. Mugho et al did a study of prevalence of keratoconus in allergic conjunctivitis at KNH hospital, Kenya and found 51.2% were male and 48.8% females. Rowjee et al in South Africa found a prevalence of 69% males and 31% females.

The age of the patients ranged from 10 years to 44 years, with a positively skewed distribution with the median age at 21 years and a mode of 16 – 20 years. This is similar to the findings of Rowjee et al in South Africa with the age of the patients ranging from 18 years to 55 years with a median age of 29 years. In our study it was noted that the males had a median age of 19.5 years and the females 25.5 years. Although this age difference was not statistically significant it is reported in other studies that the males have earlier onset and more rapid progression of keratoconus. This can also result in a higher prevalence of keratoconus amongst males, although most studies report no sex preponderance.

There were 61.2% of patients who had CXL in both eyes and 38.8% of patients who had only one eye done. The median age for single eyes is 18 years and the median age for both eyes was 25.5 years. The proportions of those who develop bilateral KC increased with increasing age, similar findings were seen by Holland et al who followed up patients with unilateral keratoconus for 4 years and found the 2nd eye developed keratoconus within the period with only 1.8% remaining unilateral. Although keratoconus is bilateral, it is also asymmetrical in presentation. Advanced keratoconus can occur in one eye with progression, necessitating the need for early CXL, while for the other eye, keratoconus or its progression may not initially occur but can be noted on follow up visit.

The preoperative visual characteristics showed a normal distribution for UCVA with a mean of 6/36 and BCVA with a skewed distribution to the right with a median of 6/12. Vinay et al did a study in India on the characteristics of 274 keratoconus patients and found
56% had vision of 6/18 to 6/24, with over 75% having moderate to advanced keratoconus. Other studies also concluded that visual acuity is not a suitable parameter in diagnosis of severity of keratoconus.\textsuperscript{10}

The choice of crosslinking technique was determined by the TCT value. The most common technique used in crosslinking is the standard epithelium-off technique using the Dresden protocol for corneas with \text{TCT} \geq 400 \mu m. In those with \text{TCT} < 400 \mu m, hypotonic 0.1% riboflavin is used to cause swelling to thickness \geq 400 \mu m and proceeded to perform epithelium-off technique. Epithelium-on technique is found to have less postoperative pain and discomfort\textsuperscript{48}, with epithelial-island technique showing improved stromal concentrations of riboflavin\textsuperscript{49}. In our study, accelerated epithelium-off technique was used in \text{TCT} \geq 400 \mu m and epithelium-on or epithelial-island technique was performed in eyes with \text{TCT} < 400 \mu m, with the minimum \text{TCT} recorded as 319 \mu m. Hafezi et al performed corneal CXL on corneas with lowest \text{TCT} of 362 \mu m with post abrasion \text{TCT} measuring 327 \mu m and with the use of hyposomolar riboflavin resulted in all corneas having measured pre-operative \text{TCT} \geq 400 \mu m\textsuperscript{85}. Some studies reported no difference in outcomes when comparing techniques\textsuperscript{48,45}. Our study found that technique did not have any statistically significant association with progression. Bottos et al reported that the negative factor in corneal epithelium is its barrier to stromal riboflavin diffusion and not as a barrier to UVA\textsuperscript{86}.

The accelerated technique was done for all eyes. Wen et al did a meta-analysis of 11 studies comparing the standard epithelium-off technique with the accelerated technique and found the standard epi-off had more reduction in \text{Kmax}, but the decrease in \text{CCT} and endothelial cell density was less with the accelerated technique while for the other outcome measures there was no statistical difference\textsuperscript{87}. Of note, most studies did not report the exact crosslinking machine used and as of yet there are no studies on the factors affecting outcomes that looked specifically at the various crosslinking machines.

The follow-up after surgery is generally poor in Kenya, noted in our study and even for other conditions such as in cataract\textsuperscript{88} or glaucoma\textsuperscript{89} follow up after surgery.
The UCVA at 6 months, improved by 37.9% by an average of 2.5 lines on the Snellen chart and remained the same in 43.5% which is comparable to a study by Rowjee et al who found at 6 months, 39% improvement in UCVA by 1.3 Snellen lines and no change seen in 41% of eyes. In our study at 12 months there was an improvement in UCVA in 45.5% of eyes by an average of 2.2 Snellen lines which was better than the findings by Greenstein et al which showed an improvement of 2 or more Snellen lines by 25.4% of eyes⁶⁰.

In our study the change in BCVA pre and postoperatively at 6 months was not significant (p=0.99). Oltulu et al found the change in BCVA was significant at 6 months and reported an improvement in BCVA by 1.0 lines on the Snellen chart⁵⁸. In our study at 6 months improvement in BCVA is noted in 16.9% by 1.7 lines and no change in 9.1%. This is in slight contrast to Rowjee et al who found improvement in BCVA at 6 months by 29% and no change in BCVA by 44%⁶³. In our study at 12 months, the change in BCVA pre and post-operatively at 12 months was not significant (p=0.69). There was also improvement in BCVA in 27.6% of eyes by 2.9 lines on the Snellen chart. Asri et al reported BCVA stabilised in 47.6% of eyes and improved in 40%.

Many studies have shown that CXL stabilises or reduces the Kmean in patients with keratoconus⁶,⁵⁴,⁶¹. In our study, the change in Kmean when comparing pre and post-CXL values was not statistically significant, at 6months (p=0.79) and Kmean flattening occurred in 15.2% at 6months with an average flattening of 1.9D. Rowjee et al found the average Kmean flattening of 0.7D in 56% at 6 months with the change in Kmean pre and post-CXL not significant⁶³. In our study at 12 months, the change in Kmean pre and post-CXL was not significant (p=0.70) while the Kmean decreased in 10.7% at 12 months with an average flattening of 1.5D. Greenstein et al reported an average decrease in Kmean by 1.5D at 12 months⁶⁰. Coporossi et al in his study found that Kmean decreased significantly by 1.96D at 12 months⁵⁶. Raiskup et al noted a significant decrease in Kmean at 12 months by 2.68D⁹⁰.

The change in Kmax was not statistically significant at 6months (p=0.61), while Kmax flattening was seen in 24.2% with an average flattening of 2.6D. Bernado et al found at 6 months a decrease in Kmax in 54% by 1.29D which was statistically significant⁹¹. In our study, the change in Kmax was not significant at 12 months (p=0.44) while Kmax
flattening was seen in 28.6% with an average flattening of 3.1D. Greenstein et al reported the change at 12 months to be statistically significant and showed a flattening at 12 months in 21% by an average of 2.0D\textsuperscript{60}. The crosslinking machine however, was not mentioned in these studies.

The change in astigmatism was not statistically significant at 6 months (p=0.07). At 6 months there was a decrease in astigmatism in 30.3% of eyes by an average of 2.6D. The change was not significant at 12 months (p=0.02) but a decrease was found in 25.0% of eyes by an average of 2.2D. Coscunseven found an average decrease at 12 months by 1.04D\textsuperscript{62}. Ozgurhan et al found that at 6 and 12 months, the change in astigmatism was not statistically significant\textsuperscript{92}.

In our study, the change in CCT at 6 months (p=0.00) and at 12 months (p=0.01) was statistically significant. The change in TCT at 6 months (p=0.00) and at 12 months (p=0.02) was statistically significant. Oltulu et al in his study also found the CCT and TCT changes at 6 months to be statistically significant\textsuperscript{58}.

In our study progression at 6 months was seen in 15.2%, and at 12 months in 14.3%. Progression rate seen by Asri et al in France at 6 months was found to be similar at 15.3% but reported that progression was halted in 68.8% at 12 months\textsuperscript{93}. In our study the success in halting progression was seen at 6 months in 84.8% and at 12 months in 85.7%. Wen et al in his comparison between standard-CXL verses accelerated CXL, found that Standard CXL brought a greater reduction in Kmax but accelerated crosslinking brought about less thinning in CCT\textsuperscript{87}.

The complication rate was 13.2% at 1 week and the most common complication was corneal haze which resolved after 3 months. There were no complications noted after 3 months follow-up. Post crosslinking corneal haze extends about 60% into the anterior stroma, leaving a mid-stromal demarcation line. Due to its frequency, there has been a debate as to whether corneal haze is a complication or a normal finding\textsuperscript{79}. Greenstein et al studied the natural course of the cornea after crosslinking and found that corneal haze peaked at 1 month, plateaued from 1-3 months and decreased from 3 months to 12 months\textsuperscript{94}. 

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There was only one case (0.6%) of peripheral corneal infiltrate in the first week post-op in an eye that had epi-off technique this resolved within the 1st month post-op. Cerman et al found that peripheral sterile infiltrates developed in 3.2% of patients following corneal collagen crosslinking. This developed at about the 3\textsuperscript{rd} to 4\textsuperscript{th} day postoperatively. The associated risk factors he identified was epithelium-off technique and postoperative use of NSAIDS which increased the risk four fold\textsuperscript{71}. In our study, endothelial decompensation, Herpes reactivation and corneal infection did not occur unlike in other studies where Awad et al reported 2 cases of herpetic keratitis after crosslinking in which no prior history could be elicited\textsuperscript{72}. Pollhammer et al reported a case of bacterial keratitis post-CXL in a patient that left a permanent visually significant scar after its successful treatment\textsuperscript{64}. Gokhale et al reported a case of endothelial decompensation in 1 eye with TCT > 400µm, 1 month after crosslinking\textsuperscript{74}.

In our study, no factors were found to be associated with progression. Tuft et al in his study found that the number of eyes progressing was independently related to Kmean, Kmax, astigmatism and age, which were all statistically significant\textsuperscript{95}. Koller et al reported that the risk of progression was more in values of Kmax > 58D\textsuperscript{78}. Hafezi et al performed corneal CXL on corneas with lowest TCT of 362µm and found no complications at 6 months and no progression\textsuperscript{85}. 
8.0 LIMITATIONS, CONCLUSIONS, RECOMMENDATIONS

8.1 Study limitations
1. Some data records were incomplete.
2. The main limitation was that not all patients came back for the 6months and 12 months postoperative follow-up review.

8.2 Conclusions
1. CXL showed overall UCVA improvement in 26.6% of eyes worsened in 12.5% and remained stable in 25.9%. Overall BCVA improvement was seen in 24.3% of eyes with worsening in 21.1% and no change in 11.4%.
2. Corneal collagen crosslinking is an effective method to halt progression of keratoconus in our setting even in TCT of <400µm. Crosslinking halted progression in 84.8% at 6 months and 85.7% at 12 months.
3. In our study, the only two complications found were corneal haze in 12.6% and peripheral sterile infiltrates in 0.6% which cleared in all cases by 3 months.
4. There were no factors found to be significantly associated with progression.

8.3 Recommendations
1. Patients with keratoconus in our population should undergo CXL to halt progression.
2. A prospective study to assess the safety margin of CXL in cases of keratoconus with varying TCT in order to develop a minimum cut-off thickness for CXL in our population.
3. The same group of patients can be followed up for a longer period of time to determine the long term outcomes of crosslinking in our population.
4. Follow-up system for keratoconus patients who undergo CXL should be developed and implemented.
9.0 REFERENCES


10. Mugho. SN, The University of Nairobi. Prevalence of Keratoconus in Patients With Allergic Conjunctivitis Attending Kenyatta National Hospital Eye Clinic a
Dissertation Submitted in Partial Fulfillment for the Degree of Master of Medicine, in Ophthalmology At the University of Nairobi. 2016.


82. Fink BA, Wagner H, Steger-may K. Differences in Keratoconus as a Function of Gender. 2005;1–11.


88. NGÁNGA DMN. Outcome of age related cataract surgery at lighthouse for Christ Eye Centre Mombasa. 2014;


## 10.0 APPENDICES

### 10.1 Appendix I: Research budget

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10.2 Appendix II: Questionnaire

PART 1: DEMOGRAPHICS

1. Reference Number

________________________

2. Age

_____  

3. Sex  (1) Male  (2) Female

4. Laterality

(1) Right Eye  (2) Left Eye

PART 5: PRE-CROSSLINKING

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7. Date of topographic diagnosis; ____/_____/_____

8. Date of crosslinking; ____/_____/_____
9. Technique

(1) Epithelium-on

(2) Epithelium-off

**PART 10: POST-CROSSLINKING**

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## Part 12: POSTOPERATIVE COMPLICATIONS OF CROSSLINKING

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10.4 Appendix IV: Permission to access medical records at Eagle Eye Laser Centre

26th January 2019

To Whom It May Concern,

RE: PERMISSION TO ACCESS MEDICAL RECORDS AT EAGLE EYE LASER CENTRE

This is to confirm that Dr Anne Muthoni Karanu who is a postgraduate student at the department of ophthalmology, University of Nairobi, has been granted permission to access data on outcome of corneal collagen cross-linking in keratoconus in Kenya at Eagle Eye Laser Centre.

This is also to state that although the two supervisors of Dr Anne Muthoni Karanu are directors at Eagle Eye Laser Centre, they will in no way interfere with the collection, analysis and interpretation of the data.

[Signature]

Dr Kahaki Kimani
Director
10.5: Appendix V: Ethical approval KNH-UON ERC

Dear Dr. Karanu

RESEARCH PROPOSAL – OUTCOMES OF CORNEAL COLLAGEN CROSS-LINKING IN KERATOCONUS AT EAGLE EYE LASER CENTRE (P790/11/2016)

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 21st February 2019 – 20th February 2020.

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

Protect to discover
Yours sincerely,

[Signature]

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. Kahaki Kimani, Prof. Dunera Itako