A Study to Determine If the Iris Camera Can Be Used to Diagnose and Monitor Corneal Disease and Treatment Outcomes

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List of Abbreviations

(LSC) Limbal Stem Cells

(ECM) Extracellular Matrix

(KCN) Keratoconus

(RGP) Rigid Gas Permeable

(CXL) Corneal Crosslinking

(UV) Ultraviolet

(INTACS) Intrastromal Corneal Ring Segments

(NHSBT) National Health Service Blood and Transplant

(PK) Penetrating Keratoplasty

(DALK) Deep Anterior Lamellar Keratoplasty

(DSAEK) Descemet’s Stripping Endothelial Keratoplasty

(FSL) Femtosecond Laser

(SE) Spherical Equivalent

(IOP) Intraocular Pressure

(IR) Infrared

(OCT) Optical Coherence Tomography

(FTA) Failure to Acquire

(COM) Corneal Opacification Measurement

(MPS) Mucopolysaccharidoses

(BCVA) Best-Corrected Visual Acuity
(µm) Micron

(P-value) Probability Value

(SD) Standard Deviation

(VA) Visual Acuity

(MREH) Manchester Royal Eye Hospital

(NREC) National Office for Research and Ethics Committee

(UK) United Kingdom

(LOGMAR) Logarithm of Minimum Angle of Resolution

(D) Dioptre

(NHS) National Health Service

(M) Mean

(CM) Centimetre

(NM) Nanometre

(ACXL) Accelerated Crosslinking

(EBM) Epithelial Basement Membrane

(GSU) Grayscale Unit
Abstract

The cornea is the thin, transparent, and avascular connective tissue that lines the front of the eye. As the most anterior surface and a major refractive component of the eye, it allows light transmission to the rest of the eye. Corneal transparency is a general indicator of corneal health. Diseases and some corneal treatments can alter the clarity of the cornea. Assessment of corneal haze in healthy and diseased eyes will aid in the understanding of how diseases progress and the impact of treatment outcomes. Such information will be informative and useful in a clinical setting to assist disease prognosis for the best possible outcome for patients. The iris camera is a specialised device that employs near-infrared light to capture multiple images in seconds which can be analysed to generate a quantitative (objective) estimate of corneal haze.

The objective of this project was to investigate corneal haze and its impact on corneal clarity by evaluating changes that take place in health and disease. Specialised algorithms were used to analyse images from an iris camera. Firstly, we assessed changes in corneal transparency over time in a corneal disease with no treatment and compared outcomes with healthy individuals. Corneal diseases and treatments associated with haze were identified, and 163 participants were enrolled and followed up through treatment.

We found that corneal clarity changes between patients with the corneal disease (keratoconus) and healthy participants were significantly different. When we compared the outcomes between the two groups, we found that corneal clarity in the healthy participants remained the same with no change while clarity in patients with keratoconus deteriorated. When we compared the outcome of corneal cross-linking on corneal clarity in patients with keratoconus over a certain period, we discovered that the haze level changed (declined) over time, suggesting an improvement. Likewise, in advanced keratoconus, penetrating keratoplasty (PK) improves vision as the last resort when all other remedies prove ineffective against disease progression. The corneal clarity outcomes post-PK again showed improvement, with an observed decline in the haze over time.

The corneal response to several treatment methods and disease progression are dependent on a host of factors and will affect corneal clarity differently. With its image analysis software, the iris camera provides an objective means of monitoring corneal clarity in health, disease and assessing treatment modalities.
Declaration

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Thesis Format

This thesis includes five chapters which are arranged in a journal format. The second, third and fourth chapters have been published. The other chapters, except the sixth chapter (general discussion) one, had either been submitted or prepared for submission to peer-reviewed journals.
Dedication

I would like to dedicate this project to my family. I would like to appreciate my parents (Surv Emmanuel Duru and Prof Patricia Duru), for their undying support and motivation throughout the pursuit of my course. I would also love to appreciate my siblings, Oge, Chioma, Tochukwu and AK for their continued inspiration and support.
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Chapter 1

Introduction and Literature Review

1.1 Overview of the Corneal Structure

The cornea is the thin, transparent, avascular layer that lines the front of the eye (Ambekar, Toussaint and Wagoner Johnson, 2011; DelMonte and Kim, 2011). It appears deceptively simple macroscopically, although it is composed of a complex microstructural framework that combines with other ocular structures to make vision possible (Armitage, 2011; Niederer and McGhee, 2010). The cornea, when viewed externally, has the appearance and structure of an ellipsoid and forms an outer casing that protects the inner contents of the eye while playing a crucial role in vision. It is considered the major refractive component of the eye, transmitting more than 90% of the visible light received, making it responsible for two-thirds of the eyes refractive power (Takács et al., 2009). Besides providing the eye with a proper anterior refractive surface, the cornea also combines with the precorneal tear film and eyelids to protect the eye against injury, infection and desiccation (Sridhar, 2018).

Regarding its anatomy, the cornea has a central thickness of approximately 0.5mm and a diameter between 11-12mm (DelMonte and Kim, 2011). Corneal growth has been suggested to occur between 6months and 1 year after birth and attains adult size between 1 and 2 years (Beuerman and Pedroza, 1996). The average adult cornea is aspheric and convex in appearance due to an increase in thickness as it moves from its centre towards the edges or periphery, which is an almost perpendicular centre and a flatter peripheral surface (Sridhar, 2018; Tanaka et al., 2011). The curvature of the cornea surface itself is not constant but varies, being considerably greater at the centre and smallest at the limbus (Willoughby et al., 2010). In terms of nerve supply, the cornea is the most densely innervated tissue in the human body (300-400 times greater than the skin), receiving its fibres mainly from the trigeminal ganglion via an ophthalmic branch (Cholkar et al., 2012; Gambato et al., 2015). These nerve bundles or fibres reach the outer edges of the cornea and are arranged in the form of rays (like the radius of a circle) to form dark rings around the iris called the peri-limbal ring (the point where the sclera and the cornea meet) (Leiper et al., 2009). Within the cornea’s microstructure are five layers that can easily be distinguished and recognised in the order of positioning or layout. These are the superficial epithelium,
Bowman’s layer, the stroma, Descemet’s membrane and the endothelium (DelMonte and Kim, 2011; Willoughby et al., 2010).

1.1.1 The Corneal Epithelium

The first barrier to the outer environment is the corneal epithelium (Meek and Knupp, 2015). It is the outermost portion of the cornea comprising 4 to 6 stratified squamous layers of non-keratinized cells.
On average, the corneal epithelium measures about 40µm to 52µm in thickness, constitutes about ten per cent of the entire corneal density and is arranged uniformly from limbus to limbus. The corneal epithelium is said to develop from the ectoderm within the 5th and 6th week of foetal development, and by the 8th week, tissues differentiate to form a fully functional eye (Qazi et al., 2010). It primarily consists of 3 sheets of cells: the superficial (most anterior cell), the middle or wing cells, and the deeper-lying basal cell layer (Eghrari, Riazuddin and Gottsch, 2015). The corneal epithelial cells are about 40µm to 50µm in diameter, and 2 to 6µm thick, having a smooth surface with flat polygonal cells and is covered in the tear film. Although not part of the cornea, the tear film forms a symbiotic relationship with the corneal epithelium, creating an optically efficient interface extremely critical to vision (Beuerman and Pedroza, 1996; Sridhar, 2018). The middle wing cells or sub-basal cells are 2 to 3 layers thick with a diameter of 30 to 45µm, as its name indicates, assume a wing’s appearance due to the upward movement of new cells underneath, causing a change in shape (John and Thomas, 2013). These winged cells are firmly locked with each other forming tight junctions between each cell. The most posterior of the cells is the basal cell layer. It is a single cell layer of approximately 20µm arranged as columns (Efron et al., 2001). It is attached to the basement membrane by hemidesmosomes (tiny stud-like structures) which prevents the epithelium from detachment from underlying corneal layers (DelMonte and Kim, 2011). The basal cells are the only cells within the corneal epithelium capable of cell division (mitosis) and are responsible for forming the superficial and wing cells, thereby maintaining the corneal epithelium (Haddad and Faria-e-Sousa, 2014). Corneal epithelial cells undergo a process of programmed cell death (apoptosis) every 7 to 10 days. This process is maintained by the limbal stem cells (LSCs) and enables creation of new cells and the desquamation and shedding of old cells to sustain corneal integrity (Reinach and Pokorny, 2008).
1.1.2 Bowman’s Layer

The Bowman’s layer, typically referred to as a false membrane, is a second corneal layer located just beneath the epithelial basement membrane and directly above the anterior stroma. It has a thickness between 8µm to 15µm and is composed of closed interlinked and condensed acellular collagen fibrils and proteoglycans (mostly type I, III and V) (Knupp et al., 2009). Although the collagen fibrils of the Bowman’s layer bear similarity to that of the stroma, studies, however, have shown that its fibrils are only half to two-thirds in thickness that is 20µm to 30µm compared to 22.5µm to 35µm of the stroma (Germundsson et al., 2013; Wilson and Hong, 2000). Unlike the corneal epithelium, the Bowman’s layer is incapable of regeneration and tends to form a scar in the event of an injury or disruption to its surface (Eghrari, Riazuddin and Gottsch, 2015; Wilson, 2020a). The Bowman’s layer is said to thin with age, and its functionality has led to hypotheses that it plays a critical role in the maintenance of structural integrity of the cornea and acts as a barrier to the passage of macromolecules (Tong, van Dijk...
and Melles, 2019). However, this theory has been refuted by (Lagali, Germundsson and Fagerholm, 2009; Seiler et al., 1992) suggesting that the true function of the Bowman’s membrane is still unknown and that it might not actually play a significant role as ascribed to it.

1.1.3 The Stroma

The corneal stroma composes over 90% (about 500µm) of the entire corneal volume (Du et al., 2005; Eghrari, Riazuddin and Gottsch, 2015). The stroma stretches from the corneal epithelial basement membrane to the endothelial basement membrane directly beneath it (Espana and Birk, 2020). It is composed of extracellular matrix (ECM), water and a network of keratocytes (corneal fibroblasts) derived from the neural crest migration in the 8th week of gestation (Chen, Mienaltowski and Birk, 2015; Hassell and Birk, 2010). These keratocytes are responsible for synthesising the components of the extracellular matrix, which are required to maintain normal corneal structure and function (Kamma-Lorger et al., 2010). The extracellular matrix of the stroma tends to mix up with water and receives its hydration from the anterior chamber and the pre-corneal tear film through the endothelium and the epithelium individually (Kotecha, 2007). The stroma contains three primary non-aqueous components; collagens, proteoglycans and cells, and a concentration of specialised glycoproteins that vary with depth from the anterior to the posterior portions of the stroma (Eghrari, Riazuddin and Gottsch, 2015). At the microscopic level, collagen contains tough bundles of fibres arranged side by side in layers called fibrils (DelMonte and Kim, 2011). These fibrils are laid down with lamellae in a hydrated mix of proteoglycans and glycoproteins and can vary in density getting up to 0.2mm wide and 2µm in thickness in a typical human cornea (Du et al., 2005). An adult cornea contains some proteoglycans: lumican, keratocan, decorin and mimecan; however, lumican and keratocan are the major keratin sulfate proteoglycans in the stroma (Torricelli and Wilson, 2014). The fibrils are extremely regularly organised and form a rigid pattern of collagen fibrils with homogeneously small diameters that give it the characteristic appearance (Kotecha, 2007). A disruption to the efficient organisation would prompt an increase in light scatter within the tissue, affecting its optical properties (Phu et al., 2011). The collagen fibrils of the stroma are made out of a heterodimeric complex of type I, V and VI, although the latter occurs in lesser amounts (Hassell and Birk, 2010). Also found within the stroma are ions that play a
significant function in the arrangement of these fibrils, which keeps the cornea transparent (Kostyuk et al., 2002; Matthyssen et al., 2018; Regini, Elliott and Hodson, 2004). The pattern and structure of the stroma differ when moving from the anterior to posterior portions, with increased interfibrillar spacing seen within the anterior segments. In the human cornea, findings have suggested that the central stroma has approximately 200 to 250 lamellae stacked one each other at varying angles from zero degrees to ninety degrees, with a higher density observed anteriorly than posteriorly (Bergmanson et al., 2005). The anterior stroma can also be differentiated by the presence of thin sheets with broad interlacing in contrast to the posterior section, which exhibits broader and thicker lamellae that run from limbus to limbus without significant interlamellar attachments (Kotecha, 2007).

![Image of corneal cells](image)

*Figure 1-4: Microscopic images of corneal stromal cells (Kumar, Pandit and Zeugolis, 2016).*

1.1.4  **The Descemet’s Membrane**

This layer is also called the posterior limiting elastic lamina, and it is located just beneath the stroma integrating the posterior portion of the stroma with the basement membrane of the corneal endothelium (Espana and Birk, 2020). It is a thin, strong acellular layer that plays the role of protecting the cornea against infections and injuries due to its mitotic and regenerative capacities (Matthyssen et al., 2018). This layer is formed during foetal development from the 8th week and measures about 3μm in anterior
thickness but increases in density with ageing up to 10μm at adulthood (DelMonte and Kim, 2011; Sridhar, 2018). The Descemet’s membrane is secreted by the endothelium that lies below it. It consists of an anterior banded portion and an unbanded posterior section that forms the attachment to the underlying endothelium (de Oliveira and Wilson, 2020). Studies have suggested that this layer is an extracellular matrix composed of collagen IV and associated collagens VIII and XII, as well as laminin (heterotrimeric glycoproteins), and these matrixes combine to form hexagonal collagen networks (Hemmavanh et al., 2013). These networks formed between the collagen IV, laminin and other elements contribute to its stability (Haf kter et al., 2015). Additionally, the Descemet’s membrane acts as an adhesive to the monolayer of endothelial cells, which contributes to corneal integrity, which is essential for its hydration and transparency (Lwigale, 2015; Saikia et al., 2018). This posterior limiting membrane is suggested to play a role in endothelial cell proliferation and differentiation (Biswas et al., 2001; Hopfer et al., 2005).

1.1.5 The Corneal Endothelium

It is a single layer of metabolically active hexagonal squamous cells within the posterior end of the cornea. The endothelium is located beneath the Descemet’s layer and has the appearance of a honeycomb mosaic when viewed posteriorly, and its cells have dark cell borders (Patel and McGhee, 2007; Sridhar, 2018). After birth, the endothelial cell density is about 4000 cells/mm2. However, its density decreases progressively with age at an approximate rate of 0.6% decrease per year in healthy corneas (Bourne, Nelson and Hodge, 1997; Tananuvat and Khumchoo, 2020). These changes in its shape and density occur due to several factors such as age, inflammation, and trauma resulting from injury or surgical procedures (DelMonte and Kim, 2011). A review by DelMonte and Kim (2011) confirmed that the thickness of the endothelium declined from 3000 – 4000 cells/mm2 to approximately 2600 cells/mm2 between the 2nd and the 8th decade of life that is a drop from 75% to 60% of its entire density. Research has further attributed this decrease to the inability of the adult endothelium to regenerate and replace degenerated cells, thereby leading to the loss of endothelial cells (Joyce, 2003). The remaining cells flatten, expand and spread, taking up the spaces left by the lost cells by polymegathism and pleomorphism (Duman et al., 2016). The endothelium plays an important role in
maintaining corneal transparency by keeping the stroma relatively hydrated (78% deturgescence) through its tight junctions (Bonanno, 2012; Srinivas, 2010). These tight junctions prevent the over-accumulation of water through its anterior chamber into the stroma leading to corneal swelling or oedema (Bazzoni, 2006). The hydration mechanism of removing excess fluid by the endothelium is the pump-leak process (Bourne, 2010).

Figure 1-5: Specular microscopy of the endothelial cell layer (Sridhar, 2018).

1.2 Blood Circulation and Nerve Supply in the Cornea

The avascular nature of the cornea makes it reliant on the branches of the ophthalmic and facial arteries to supply it with blood (DelMonte and Kim, 2011). These tiny vessels supply nutrients and oxygen to the cornea via the aqueous humour and tear film. As one of the most densely innervated and sensitive tissues in the human body, the cornea has the richest supply of any ocular tissue (Oliveira-Soto and Efron, 2001). The cornea derives its sensation from the nasociliary branch of the trigeminal nerve's first division (Ophthalmic) (Shaheen, Bakir and Jain, 2014; Sridhar, 2018). At different levels within the stroma, broad and linear nerve trunks extend laterally and anteriorly to generate a network/plexus of fibres that thin as it progresses (He, Bazan and Bazan, 2010; Marfurt et al., 2010; Sridhar, 2018). These nerve fibres further enter the bowman’s layer and form a dense nerve plexus (tortuous and thin beaded) underneath the base.
1.3 Corneal Pathologies

1.3.1 Keratoconus (KCN)

This is an ectatic bilateral dystrophy of the cornea (Gomes et al., 2015). It is associated with biomechanical weakening, progressive thinning and eventual bulging of the corneal tissue giving it the semblance of a cone (Al-Mohaimed, 2019; Ambekar, Toussaint and Wagoner Johnson, 2011; Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010). As the disease progresses, there is an increase in visual aberrations in the form of irregular astigmatism, myopia and occasional scarring of the corneal tissue, resulting in a decline in vision (Jinabhai, O'Donnell and Radhakrishnan, 2010; J. H. Krachmer, R. S. Feder and M. W. Belin, 1984; Rabinowitz, 1998). Research has suggested that in a keratoconic cornea the stiffness is 40% less than that of a healthy one (Chen et al., 2015). The pathogenesis of KCN at a molecular level is still unknown despite decades of research; however, some findings have attributed its onset to a decrease in the collagen crosslinks and an elevation in the digestion of pepsin (Caporossi et al., 2010; Davidson et al., 2014). KCN has been firmly associated with environmental and genetic triggers although, vigorous and constant eye rubbing has been identified as a major risk factor in the development of the disease (McMonnies, 2009; Saad et al., 2020). KCN generally begins in adolescents and progresses between the second to fourth decade of life, where it usually stabilizes (from age 10 and 40 years) (Chang et al., 2020).

Figure 1-6: Slit-lamp photograph of an eye with keratoconus demonstrating increased corneal curvature (ectasia) (Vazirani and Basu, 2013).
The prevalence of KCN is widely varied, with studies demonstrating an incidence of 1 in every 2000 individuals within a given population, approximately 265 per 100,000 populace (Godefrooij et al., 2017). According to reports by Cozma, Atherley and James (2005), KCN has also been linked to ethnicity, although its prevalence rates vary among these racial groups. In a study in the United Kingdom, KCN was estimated to be 7.5% higher (4.4:1 incidence rate) in Asians than the Caucasian population (Romero-Jiménez et al., 2010; Weed et al., 2008) (Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010; Weed et al., 2008). Although Keratoconus is multifactorial in terms of prevalence and ethnic distribution, current evidence suggests that it is evenly distributed among males and females (Kuo et al., 2006; Mazzotta et al., 2008). A study by Ertan and Muftuoglu (2008) suggested an increased prevalence among males, while Gomes et al (2015) suggested its frequency was greater among women. However, Fink et al (2010) maintained that the rate of progression is similar among both sexes.

KCN presents various clinical signs and symptoms depending on the severity and progression of the disease. It presents mild to no subclinical or “forme fruste” features at its initial onset and tends to go unnoticed if it is visually asymptomatic (Vazirani and Basu, 2013). The protrusion of the cornea results in the manifestation of high irregular myopic astigmatism, which affects visual acuity (Wheeler et al., 2012). As the disease progresses from moderate to advanced cases, there is an accumulation of iron deposits from the tear onto the cornea as well as breaks in the Bowman’s layer to form a circle line or hemosiderin arc (Fleischer’s ring) around the base of the cone (Thota, Miller and Bergmanson, 2006). Also another characteristic feature is the compression of the Descemet’s membrane, which give rise to fine vertical lines known as Vogt’s Striae in the posterior stroma (Vazirani and Basu, 2013). As the cornea continues to protrude, the lower eyelid becomes deformed and assumes a V-shape (Munson’s sign) whenever the eye is in a downward gaze (Wheeler et al., 2012).

During KCN, the function of the keratocytes become abnormal with increased apoptosis, significant levels of endoplasmic reticulum and a movement into the Bowman’s membrane (Joseph, Srivastava and Pfister, 2011). As a result of these changes, especially the impact on the keratocytes, the corneal stroma becomes increasingly susceptible to external strain. The structural mutations that take place during KCN also alter the interfibrillar spacing as well as the proteoglycan contents of the cornea which play significant roles in the maintenance of corneal transparency (Mariam Lotfy Khaled et al., 2017).
In the early phases of the disease, corneal might be difficult to establish and diagnose as aforementioned, most cases are asymptomatic and only become more pronounced as the disease progresses.

In severe cases of KCN, acute hydrops occurs with sudden breaks in the Descemet's membrane and egress of aqueous and other fluid into the stroma. These breaks in the Descemet’s membrane affect the posterior stroma (Thota, Miller and Bergmanson, 2006). It takes approximately 3 months for corneal hydrops to resolve (wound healing events activated by the keratocytes), often leaving scar tissue on significant portions of the cornea which impact corneal transparency and impair vision (Fan Gaskin, Patel and McGhee, 2014). The ensuing sudden decline in vision is accompanied by acute stromal oedema, corneal clouding and significant pain (Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010; Thota, Miller and Bergmanson, 2006). As corneal transparency arises from the Nano architecture of the corneal constituents (particularly the stromal collagen fibrils/lamellar), therefore, the degradation of its precise organisation during KCN as well as other homeostatic assaults that occur in other layers of the cornea increases the backscatter and affects its overall transparency (Anayol et al., 2016; B. Lopes, I. Ramos and R. Ambrósio, 2014; Mathew et al., 2015). Corneal transplants are often the only viable alternatives to restore vision in cases of scaring (Jhanji, Sharma and Vajpayee, 2011).

Corneal transparency in KC is often affected by the degenerative processes in the integrity of the corneal tissues as well as well oedematous changes (Volatier, Figueiredo and Connon, 2020). Preliminary assessments of corneal haze or transparency in clinical settings is mostly done subjectively with a slit lamp biomicroscope in conjunction with arbitrary grading scales or systems. In recent clinical practice, when assessing KC, the clinician takes into account the topographic and biomechanical features of the cornea such as its axial length, tangential, elevation and pachymetry readings as well as other pathological findings and signs presented (Jiménez-García et al., 2021a). These topographic measurements allow the clinician assess inherent abnormalities, disease prognosis and infer appropriate diagnosis and management of the disease. Newer objective devices such as the Scheimpflug devices, confocal microscopy and Optical Coherence Tomography aid corneal transparency assessment based on data analysis approach giving characteristic and quantitative measurements of corneal haze. There are several grading systems or scales for KC, however, one of the most frequently used system is the Amsler-Krumeich (AK) classification. Amsler-Krumeich is one of the grading oldest and widely used
systems based on four (4) stages (1-4); transparency, mean corneal power, astigmatism, and the thickness of the cornea (Belin and Duncan, 2016; Belin et al., 2020). However, this system has been suggested to be a clinically inadequate system of assessing KC, as it focuses on the anterior surface (apical thickness and central anterior curvature) of the cornea without recognising any other changes (Gomes et al., 2015b).

Recently, newer systems of classification such as the ABCD have been integrated when grading KC and combine the anatomical (topographic parameters) features with functional performance (visual acuity) addressing the deficiencies of the Amsler-Krumeich (AK) classification (Balparda et al., 2020; Belin, Villavicencio and Ambrósio, 2014; Krolo et al., 2021; Sedaghat et al., 2018).

1.3.1.1 Contact Lens Use in Managing Keratoconus

Keratoconus in its early phase of development is generally asymptomatic or mild, and spectacles are employed to manage the refractive error. However, as the disease progresses with changes in the corneal shape, such remedies are no longer sufficient enough to combat irregular astigmatism and provide optimal vision (Rathi, Mandathara and Dumpati, 2013). Nowadays, contact lens represents the most frequent and familiar treatment option in managing early to moderate Keratoconus, avoiding the need for a corneal transplant in approximately 99% in most cases (Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010). Although soft and soft toric lenses made from hydrogel and silicone hydrogel are one of the initial lenses used in Keratoconus care, rigid gas permeable lens (RGP) is by far the most employed. This is due to its ability to create adequate tear exchange and correct high levels of irregular astigmatism, thereby improving visual acuity (Vazirani and Basu, 2013).

1.3.1.2 Management of KCN by Corneal Crosslinking procedure (CXL)

During KCN, the cornea loses its mechanical stability, rigidity and becomes increasingly fragile with increased hysteresis. This internal pressure within the cornea distorts its normal shape and becomes more prolate as the disease progresses. In recent decades, there has been advancement in surgical treatments in the management of KCN. Such minimally invasive techniques include the corneal collagen crosslinking (CXL), which act to increase and enhance the biomechanical stability of the
cornea using riboflavin (a photosensitizing agent) and ultraviolet light (UV) (Ambekar, Toussaint and Wagoner Johnson, 2011).

The technique of using ultraviolet light (UV-A) light and a photomediator to treat or manage KCN has been shown to slow or halt the progression of the ectasia by strengthening the cornea (Raiskup-Wolf et al., 2008; Wollensak, Spoerl and Seiler, 2003). Recently, several modifications have been made to existing crosslinking procedures to improve its efficacy as a current and future therapy for Keratoconus while minimizing unwanted side effects (Al-Mohaimeed, 2019; Kanellopoulos, 2009).

1.3.1.3 Management of KCN through intra Stromal Corneal Ring Segments (INTACS)

INTACS are two micro-thin segments in the shape of an arc made of inert material (polymethyl methacrylate and acrylic polymers). It was originally developed and applied in treating low degrees of myopia (less than 3Dioptres) and astigmatism. However, it’s been employed recently to manage mild to moderate cases of Keratoconus to reduce any ectasia. Importantly, segments do no halt the progression of the actual disease process (Jhanji, Sharma and Vajpayee, 2011). This is done by surgically inserting the rings or segments into the stroma to flatten its central surface, reshaping the corneal and consequently improving vision (Piñero and Alio, 2010; Rabinowitz, 2010). Although INTACS are indicated for milder forms of Keratoconus, it is suggested to be less effective in advanced disease cases (Ambekar, Toussaint and Wagoner Johnson, 2011).

1.4 Corneal Graft

Over the years, corneal graft surgery has steadily evolved, becoming one of the most successful forms of tissue transplantation amongst humans (Gain et al., 2016). Corneal transplantation is indicated in cases where corneal clarity or shape has been severely compromised as a result of disease or injury resulting in visual impairment (McColgan, 2009). Corneal disease has been shown to be responsible for a large proportion of blindness, being the 5th most prevalent cause of visual deterioration affecting approximately 4.5 million individuals worldwide (that is 12% of 39 million visually impaired persons) and a leading cause of blindness in emergent nations (Kong et al., 2012; Mathews et al., 2018). According to research findings, roughly about 185,000 corneal grafts are carried out each year globally (Alio et al., 2020; Gain et al., 2016). Reports from the National Health Service Blood and Transplant
(NHSBT) registry in the UK stated that approximately 3565 corneal transplants were performed between 2010 and 2011, while the Eye Bank of American reported that 46642 corneal transplants in 2010 alone (Donald Th Tan et al., 2012). Corneal transplants are usually indicated in severe pathologies or disorders where alternative forms of treatment have proved unsuccessful and could lead to blindness (George and Larkin, 2004). Such indications for surgical therapy include overly thinned and distorted corneas, scarring from infections or injury and oedema.

In recent years, corneal transplantation has evolved, introducing newer techniques with more improvements. However, despite these advances, corneal graft failures still occur and is a consistent concern despite successful corneal transplantation (Alio et al., 2020). These graft failures are largely due to immune-mediated rejection, which results in deterioration of the transplanted cornea (Panda et al., 2007). Approximately 60,000 corneal grafts are carried out annually across the globe, and about 5-7% of these grafts eventually result in failure and rejection (Panda et al., 2007; Price, Thompson and Price, 2003). The risk factors that constitute graft failure and rejection include neovascularization, inflammation of the corneal graft bed and trauma or injury (Bachmann, Taylor and Cursiefen, 2010). During corneal disorders, neovascularization is frequently induced by a wide range of associative factors such as inflammation, infections, injury as well as toxic disorders (Chang et al., 2001).
Fig 1-7: The five corneal layers and different procedures for corneal transplant (D. T. Tan et al., 2012).

Fig 1-8: Schematic overview of a healthy cornea and different corneal transplant procedures (Röck et al., 2017) (A) an intact cornea, (B) PK, (C) Boston Keratoprosthesis, (D) DALK, (E) DSAEK, (F) DMEK.
1.4.1 Penetrating Keratoplasty (PK)

Penetrating keratoplasty or full thickness graft as it is also called is the replacement of the entire 5 corneal layers with a full thickness graft (Mccolgan, 2009). It is one of the most frequent and successful corneal transplant procedures accounting for approximately 90% of corneal surgeries (Mathews et al., 2018). It is a treatment of choice for corneal opacification or haze. It is usually indicated in cases of deep-seated severe corneal infections, advanced Keratoconus (corneal hydrops) and trauma where visual acuity cannot be corrected by spectacles or contact lenses (D. T. Tan et al., 2012). Despite its frequent success, this procedure often results in increased astigmatism, graft rejection, and other suture-related problems. PK is typically performed under topical, local or general anaesthetic and usually last about 45 minutes. Routine post-operative lubrication and instillation of topical antibiotics and steroids are usually indicated for the grafted eye. Postoperatively, corneal grafts were assessed to monitor graft thickening to detect signs of failure, rejection and hypotonicity (Thompson et al., 2003).

![Image of PK at 2 years post-surgery](image_url)

**Fig 1-9: PK at 2 years post-surgery (Watson et al., 2004).**
1.4.2 Deep Anterior Lamellar Keratoplasty (DALK)

Deep Anterior Lamellar Keratoplasty (DALK), unlike PK, is a relatively newer surgical procedure where the anterior partial portion or graft of the affected/damaged cornea is removed and replaced with a healthy tissue or graft (Noble et al., 2007). The affected cornea is excised using a big-bubble technique where the Descemet’s membrane is separated from the stroma using a large injection of air (Anwar and Teichmann, 2002; Behrooz and Daneshgar, 2010). Most of the anterior layers are removed during the DALK procedure, while the Descemet’s and endothelium layers are retained. This process of retaining the deeper layers (the endothelium and Descemet) using DALK technique minimizes the risk of graft rejection or failure resulting from immunologic reactions during penetrating keratoplasty (Vajpayee et al., 2007). Reports have suggested that DALK preserves the endothelial cell density postoperatively and leads to significant improvements in visual acuity comparable to full-thickness grafts (Amayem and Anwar, 2000; Feizi, Javadi and Rastegarpour, 2010).
Figure 1-11: Intraoperative photograph during deep anterior lamellar keratoplasty (DALK) using big-bubble technique (Fontana, Parente and Tassinari, 2007).

Figure 1-12: Postoperative photo of DALK with sutures at 18 months follow-up (Zaki et al., 2015).

1.4.3 Descemet’s Stripping Endothelial Keratoplasty (DSAEK)

DSAEK is a partial thickness procedure to selectively transplant damaged or affected host Descemet’s membrane and dysfunctional corneal endothelium with healthy donated layers (Lee et al., 2009). It is a form of posterior lamella transplant where the graft is inserted into the anterior portion and pressed against the posterior segment of the host’s cornea using an air bubble (Maier, Reinhard and Cursiefen, 2013). This procedure is suggested to increase the tectonic stability of the cornea after transplantation, decrease the susceptibility to post-operative trauma and visual aberrations (induced astigmatism).
associated with surgical procedures such as PK (Price and Price, 2007). Although DSAEK offers quicker, best-corrected visual acuity, it can result in an overall loss of endothelial cells due to intraoperative graft manipulation. Specialised techniques are used to preserve the endothelium (Endosaver and Busin glide (Maier, Reinhard and Cursiefen, 2013; Rose et al., 2008).

1.5 Corneal Transparency

The cornea plays a significant role in the visual system being a major refractive element of the human eye (Knupp et al., 2009). Some of its functions range from protecting the ocular surface from debris and pathogens in the environment, intraocular injury down to its intricate and yet effective contribution in the refraction and transmission of light (Hsu and Sugar, 2017; Spadea et al., 2016). Its ultrastructure, physiological and optical properties combine to actively contribute to its overall integrity and transparency (Hassell and Birk, 2010). Although it is the most anterior portion of the eye, its capacity to transmit nearly all visible light without restriction is quite extraordinary and is down to its complex molecular arrangement as well as the co-association with surrounding structures (Knupp et al., 2009; Meek et al., 2003). These properties enable light from the visible spectrum to pass through the cornea unscathed and with minimal scatter, essential for clarity and good vision (O'Donnell and Wolffsohn, 2004; Parekh et al., 2014).

The cornea's transparency is an important indicator of corneal health and is maintained by the key contributions of its distinct layers to provide its physiological functions (Doughty and Jonuscheit, 2019; Nishida, 2008). The smoothness and deturgescence of its epithelium, the homogeneity and arrangement of the collagen bundles within its stroma, the renewability of the Descemet’s layer, its lack of blood vessels and the pump mechanism of the endothelium are critical to its biomechanical and optical properties (Hassell and Birk, 2010; Qazi et al., 2010). Therefore, any dysfunction or disruption in these capacities will greatly impair its ultrastructure, thereby constituting a defect (Meek and Knupp, 2015; Qazi et al., 2010).

Several theories have been propounded in an attempt to explain corneal clarity and factors associated with its transparency. A hypothesis by Maurice (1957) associated corneal transparency to the corneal stroma stating that its lucidity was achieved mainly by stroma as a result of the arrangement of its
collagen fibrils. Other findings support this theory implying that dissimilarities in the arrangement of the collagen fibrils correlated with light scattering and tended to be higher in the anterior stroma. Later findings proposed that the stroma alone wasn’t the only source of light scatter and corneal transparency but that the epithelium also played a part (Schoessler and Lowther, 1971). A study by Goldman and Benedek (1967) concluded that the arrangement of the collagen fibrils was not responsible for corneal transparency but rather was a consequence of its proteoglycan content as well as the interfibrillar spacing.

Corneal transparency has also been attributed to the keratocytes playing a significant role in scattering light (Jester et al., 1999). Several factors combine to maintain corneal transparency (Meek and Knupp, 2015). Some of such factors include its water content, the absence of blood vessels (which allows light to pass unimpeded) Meek et al (2003) and finally, the dense positioning and concentration of the keratocytes, collagen and extracellular matrix within the stromal matrix (Eghrari, Riazuddin and Gottsch, 2015). Maurice (1970) further indicated that when fibrils are aligned in a parallel form with equal thickness and patterned resembling a lattice, that these account for its transparency. The stromal collagen fibres combine and form patterned structures or packages called fibrils which runs continuously with each other but are arranged at right angles with adjoining lamellae (DelMonte and Kim, 2011). An understanding of the crystalline structure of the cornea and the nanoscopic arrangements of its substructure has provided more insight into the intricacies of its transparency (Freegard, 1997; Meek and Knupp, 2015). The various structures and components of the cornea play roles in decreasing variability in refractive indexes, thereby allowing more light to penetrate the cornea with minimal scatter, thus achieving clarity (Ellenberg et al., 2010; Knupp et al., 2009).

Although the structural basis of corneal transparency outlined above are widely accepted, other physiological factors simultaneously contribute to its lucidity (Edelhauser, 2006). One of such factors is the process by which the endothelium keeps the cornea relatively hydrated which plays a critical role in its optical transparency (Gandhi and Jain, 2015; Srinivas, 2010). Insight into this process can be explained by the pump-leak hypothesis which details how excessive fluid is removed from the corneal into its anterior structures as well as the provision of nutrition to its superficial layers (Bonanno, 2012).
Bonanno (2003) stated in his extensive review that transparency of the cornea is critically reliant on how well the stromal fluid is regulated by the corneal endothelium.

Under normal physiological conditions in a healthy cornea, fluid is generated via ion transport mechanisms which compensate for the cornea’s continuous loss of water (Bonanno, 2003). Glycosaminoglycans within the stroma that have a tendency to mix with water prompt the absorption pressure (imbibition pressure) by the stroma (60mmHg) which pushes fluid into the cornea (Bonanno, 2012; DelMonte and Kim, 2011). Imbibition pressure generated by the forces of the proteoglycans is the propulsion needed by the stroma to absorb water. (Maurice, 1962) termed the entire process “the pump-leak mechanism”. The Na⁺ and K⁺ ATPase sites (the basolateral membranes of the endothelium) are the two principal ion transport systems as well as the intracellular carbonic anhydrase pathway (Bonanno, 2012; DelMonte and Kim, 2011; Klyce, 2020). The corneal endothelium maintains stromal deturgescence (approximately 78% in humans) (Geroski et al., 1985; O'Donnell and Wolffsohn, 2004), therefore, when its density decreases as a result of injury, disease or ageing, the stroma due to its hydrophilic nature accumulates fluid and swells and oedema ensues which disrupts the organization of the collagen fibrils and impacting corneal transparency (Edelhauser, 2006; Klyce, 2020; Srinivas, 2010). It was suggested that when the collagen matrix is disrupted due to the swelling of the cornea, there is an increase in the scattering of light which decreases its transparency (O'Donnell and Wolffsohn, 2004).

Tight junctions comprising of molecules F-actin, ZO-1, α- and β-cadherin within the endothelium ensure this pump-leak system functions properly by acting as endothelial barriers (Edelhauser, 2006). These tight junctions are suggested to regulate corneal homeostasis through the diffusion of plasma proteins, fluids, ions as well as allowing leukocytes, lymphocytes and neutrophils to penetrate its paracellular barrier (Cong and Kong, 2020). Recently, these tight junctions have been shown to play a part in modulating cellular function through the process of signal transduction (Matter et al., 2005; Matter and Balda, 2003). The endothelium has limited or no proliferative capacity, therefore any dysfunction would result in corneal deterioration and consequently visual impairment (Zhang, Patel and McGhee, 2019).
Corneal opacification (haze), an uncharacteristic feature of a healthy cornea is usually induced by varying elements and degenerative changes taking place within the cornea. It is often a traditional indicator of the cornea’s current physiology and a potential identifier of subsequent dysfunction and deterioration of its integrity and optical function (O’Donnell and Wolffsohn, 2004). A decrease in corneal transparency is usually secondary to degenerative alterations occurring as a result of ageing, pathology and trauma (Meek et al., 2003). Refractive surgery has been shown to affect corneal transparency with associating decrease in contrast sensitivity, increased susceptibility to glare and consequent decline in visual acuity (Greenstein et al., 2010; Zarei-Ghanavati et al., 2017). The resultant haze is often a result of the wound healing process taking place due to the reorganization of the fibrils during the procedure (Alzahrani et al., 2018; Greenstein et al., 2010).

Ageing has been reported to influence corneal clarity with the suggestion that younger adults with healthy corneas showed higher clarity than the elderly (Cankaya et al., 2018). This decline in lucidity has been attributed to a 30% to 40% increase in corneal backscatter (particularly in the stroma) observed in older populations (Hillenaar et al., 2011). The decrease in corneal transparency has also been linked to endothelial dysfunction (loss of cells) as the cornea ages (Gipson, 2013; Peh et al., 2011).

In a typical clinical setting, corneal haze can be estimated via measuring the individual’s best acuity coupled with routine investigations or observations of the corneal media using a slit-lamp biomicroscope. Although these clinical evaluation techniques are commonly practised, they are subjective estimates and not a completely standardised grading system they could vary with clinical experience, creating possible bias (Aslam et al., 2012; Elflein et al., 2013). Therefore, standardised and objective forms of measuring haze are crucial to monitor corneal clarity and would represent a mainstay for treatment rather than conventional techniques. Since the advent of modern non-invasive imaging techniques, corneal transparency has been assessed in vivo using confocal microscopy to examine all corneal layers (Guthoff, Zhivov and Stachs, 2009; O’Donnell and Wolffsohn, 2004). This imaging device avoids issues encountered with conventional or standardized investigational methods that usually lead to the tissues shrinking and getting distorted (O’Donnell and Wolffsohn, 2004). It further assists in the early recognition of disruptions of the stromal matrix (being 90% of the cornea) as well as
the accumulation of deposits. Corneal clarity has also been assessed ex vivo using the confocal microscope (Berkowski et al., 2018; Radner and Mallinger, 2002).

In a healthy cornea, transparency is assessed by the relative ease with which light is absorbed or scattered as passes through the various layers. As the scattering increases, transparency decreases leading to opacification. Recently, certain custom scatterometers have been employed to measure the intensity of light that passes through unimpeded to a detector when the cornea is exposed to certain intensity (Dohlman, Yin and Dana, 2019).

Optical Coherence Tomography (OCT) is another non-invasive and non-contact imaging system used to evaluate the anterior cornea and create a 3 dimensional map (Huang et al., 1998; Wang, Simpson and Fonn, 2004). It is based on the assessment of the amount of time it takes infrared light to be reflected from tissue structures (Sridhar and Martin, 2018). The infrared light (low coherence, broad bandwidth light) permits proper penetration of tissues at various depths yielding high resolution images with no risk of radiation (Huang et al., 1991; Lim, 2015; Park et al., 2019). This device employs a Michelson Interferometer to create a reference beam and compares this to other beams of light reflected from various layers of tissue by a process known as interferometric detection (Gabriele et al., 2010; Sridhar and Martin, 2018). As the imaging progresses, series of multiple axial scans are produced and conglomerated to form composite cross-sectional images (B-scans) (Fercher, 2010; Ramos, Li and Huang, 2009). Images are produced by the OCT when the light dispersed or reflected back from tissues microstructures are detected (Javed, Aslam and Ashworth, 2016). Reports have suggested the use of OCTs in quantifying corneal haze in research (Boote et al., 2012; Dohlman, Yin and Dana, 2019; Rose et al., 2018). Besides its application in structural imaging, the OCT can also employed to permit functional imagery of tissues (e.g. visualizing the network of blood vessels) (Aumann et al., 2019).
Figure 1-13: The Optical Coherence Tomography. MREH, UK.

The Pentacam utilizes the Scheimpflug principle with a rotating lens/camera (that spins 360°) to detect and capture high-resolution (in-focus) images of the eye’s anterior and posterior segments over increased fields of depth (Dohlman, Yin and Dana, 2019; Javed, Aslam and Ashworth, 2016). The densitometry programme measures the amount of scattered light across different regions of the cornea (Bernardo Lopes, Isaac Ramos and Renato Ambrósio, 2014; Ni Dhubhghaill et al., 2014). This device presents a topographical and densitometric assessment of the cornea (Belin and Ambrósio, 2013; Koc et al., 2018). Slit images of 25 scans are acquired by the Scheimpflug rotating camera along the optical axis with a monochromatic slit-light source (Elflein et al., 2013). The Pentacam generates corneal optical density (COD) across 3 layers (anterior, central and posterior) and 4 annular zones (0-2mm, 2-6mm, 6-10mm and 10-12mm) by measuring the amount of backscattered light over a circumference of 12mm (Cankaya et al., 2018; Ozkan et al., 2021). This method of using corneal densitometry provides a quantifiable estimate of corneal transparency (Alzahrani et al., 2019; Cankaya et al., 2018). These numerical estimates provide objective quantifications of the level of corneal transparency and the overall health of the cornea (Alnawaiseh et al., 2016; Cankaya, Tekin and Inanc, 2016; Otri et al., 2012). Densitometry measurements are assigned scores from 0 (purely transparent) to 100 (total haze) depending on the level of backscattered light from the cornea and is expressed in grayscale units.
(GSU); which is the pixel luminance per unit volume in the Scheimpflug image (Ozkan et al., 2021). A map of corneal densitometry based on backscatter represents the level of corneal opacification or haze (Dohlman, Yin and Dana, 2019).

In the last decade, some studies have employed the Oculus Pentacam (densitometry programme) to assess corneal clarity by monitoring changes in opacity over time (Elflein et al., 2013; Takacs, Mihaltz and Nagy, 2011). Despite the acknowledgment of the Pentacam’s capacity in the quantification of haze in corneal diseases and corneal transparency, reports have suggested that the Pentacam can be unreliable in cases of extreme haze as well as in some forms of KC (Javed, Aslam and Ashworth, 2016; Javed et al., 2017; Jiménez-García et al., 2021a; Sornalingam et al., 2019). Faria-Correia and Ambrósio Júnior (2016) further stated that systems that operate using the Scheimpflug principle are often sensitive to opacities of the cornea due to the wavelength of light utilized (typically 470-475nm), which results in hyper-reflective images of a flawed outline. Also, without the addition of the densitometry function to the standard Pentacam, scatter analysis would not be possible and images would have to be analysed separately which would be time consuming (Dohlman, Yin and Dana, 2019).

![Figure 1-14: The Oculus Pentacam. Manchester Royal Eye Hospital (MREH), UK.](image)
Recently, the iris recognition camera technology has been used to objectively assess the level of corneal haze and monitor transparency over a certain duration (Javed et al., 2017; Somalingam et al., 2019).

1.5.1 **Principle of the Iris Camera Recognition System**

The iris camera is a non-invasive automatic imaging device that uses iris recognition software to acquire multiple images at high speed for analysis and identification. This technique exploited the application of infrared biometry (IR) to identify the distinct physical characteristics of the iris using interfaces and algorithms (Bolle, Ruud and M, 2004; Javed, Aslam and Ashworth, 2016; Nguyen et al., 2018). It employs the principle of using a two-dimensional wavelet to modify and convert iris images into iris codes or templates (Daugman, 2003; Daugman, 2007). Upon generation of the iris codes, hamming distance is calculated; (hamming distance being the sum of the bit variations between other templates to enable recognition or identification) (Bolle, Ruud and M, 2004). The iris camera has a dual focus horizontal mirror for visual feedback with a single wide-angle camera that acquires multiple images in under 5 seconds. Prior to image acquisition, the horizontal mirrors fitted in its anterior panel with the aid of multiple lights encourage eye-to-camera alignment through an intuitive interface which minimizes failure to acquire or capture rates (FTA).

![Figure 1-15: The setup of the modified iris recognition camera MREH, UK.](image-url)
Typically, the system of iris recognition usually entails four steps or modules that include image acquisition, detecting the eyes, selection of quality images and finally, the recognition of the iris (Eliza Du, 2006). During image acquisition, multiple images are acquired using the iris camera. Pre-processing the images isolates and extracts the iris patterns while detecting and excluding artefacts such as eyelids, eyelashes and reflections. Once the iris patterns have been extracted, the iris templates or codes are generated from it. Upon generation of the new codes, these are then compared with previously enrolled templates to detect a possible match (Matey et al., 2006). This principle of pattern recognition by the iris camera has also been employed at airports and other firms as a security measure or means for identification (Aslam, Tan and Dhillon, 2009; Trokielewicz, Czajka and Maciejewicz, 2015).

Although the iris camera technology has been employed in a vast array of applications, recently, it has been utilized in anterior segment imaging to quantify haze in corneal disorders (Sornalingam et al., 2019).

1.5.2 Iris Camera’s Testing algorithm in quantifying corneal haze

The iris camera as its name indicates was initially designed to take images of the iris which differentiates one person from another due to individual architecture of our irises being different. However, in over a decade, our team has fashioned the use of the iris camera to instead assess corneal images. Although

Figure 1-16: The modified iris recognition camera MREH, UK.
the device was programmed for iris images, the iris can only be seen if the cornea is clear. Similarly, the pupil area which is the particular area that the iris analysis works on would be black if the cornea was black. The Iris camera therefore focused on the pupillary plane and we found the region to be black (that is zero (0) on the images if the cornea was clear. However, when that cornea area was cloudy, that central area appears hazy and the iris camera picks this up.

In opacification of any area, as aforementioned, the pupillary areas appear less that black, therefore, the iris camera aggregates all the depths to generate the COM score. As the cornea involves several layers, some devices such as the Scheimpflug system captures a certain or particular layer, however, the iris camera doesn’t but rather assess all the light that passes through the pupillary area and gets an aggregate of the entire haze of any part of the cornea (i.e. the COM score is a measure of the complete transparency of all the layers of the cornea) – in effect, it is based on how clearly visible the pupil is and also takes into account the cornea and anterior chamber. Although the iris camera does not give a precise detail about depth, nevertheless, that was not the intent of this study but rather a collection (aggregation) was what we investigated for clinical purposes.

The iris camera takes images of the entire (complete) corneal area and gets a segment of the eye as shown in fig 1-17. Once this is done, the iris camera software separates out the internal pupillary area automatically. Although the rights to this software is restricted, we have utilized the software to take the central area defined by the iris camera and put it in a different software written with MATLAB detailed in this publication (Aslam et al., 2012). Unfortunately, the developer of this software was designed without the researcher and this thesis was merely describing its use for a different application.

The COM score is typically calculated by the mean score of the pixels in the central pupillary area of the cornea. Following image capture secondary to analysis, dual reference pinpoints are specifically selected on the pupil's edges, one superiorly and the other inferiorly (Fig 1-18). Once these designated spots have been selected, the computing algorithm automatically removes any flash artefacts by assessing extremely bright areas (as an extremely bright area shouldn’t exist) but rather should exist as dark single areas. Any bright area is often as a result of the flash. The iris camera captures the central area which is bright as well as surrounding areas. We discovered that the bright areas of the flash also infected some of the areas around it with a higher than expected intensity. Fig 1-19 shows the central
flash area and the processing that takes place to remove the flash region, as well as large areas affected by the flash artefacts. The iris camera then sums the intensity of the remaining pixels in the corneal area (that is it adds up the intensities of each of the individual pixels and divides it by the number of pixels thereby generating the average intensity. These calculations generate the corneal opacification measure (COM), giving an overall estimate of the cornea's level or degree of haze. This process is a partially automated procedure because the operator does the initial phase while the analysis software carries out subsequent computation. Artefacts such as eyelashes or epithelial erosions can affect the COM score measurement as the software may falsely include these artefacts during analysis which would impact the accuracy of the score of the level of corneal opacity (Javed, Aslam and Ashworth, 2016). Also, when a patient's eye is dilated, there is more retro-illumination which impacts the COM score. Additional factors that affect the COM score include extremely mobile and unsteady eyes which prevent capture of iris image and also partially closed lids prevent iris capture and analysis.

Fig 1-17: A typical image generated by the iris camera system software.
1.5.3  Repeatability and Reliability of the Iris Camera

The iris camera aforementioned is an automated device capable of acquiring images with less effort. Although it possesses pre-programmed functions, it is user-friendly, with minimal training required to manipulate or operate. This device can produce quantitative and qualitative information of anterior portions of the eye within limited assessment periods. The iris camera has indicated good repeatability.
as well as reliability in the quantification and measurements of haze in anterior segment disorders. According to (Aslam et al., 2012), the Corneal Opacification Measure (COM) generated by the modified image analysis algorithm of the Iris camera in the assessment of Mucopolysaccharidoses (MPS) syndrome demonstrated evidence of reliability and validity, having satisfactory measurements with no systemic bias and showing a narrow coefficient of repeatability of 1.21 (95% limits of agreement as well as an intraclass correlation coefficient (mixed model) for average measures of 0.997 (0.989-0.999). Their study further demonstrated a strong relationship between clinical grading and the COM score with a coefficient of 8.31 and p=0.0001 with an Adjusted R² of 0.69. A study conducted by Sornalingam et al (2019) concluded that the iris camera COM score detected levels of corneal clouding in patients with MPS with a certain degree of accuracy. The accuracy of the iris camera has been assessed in several studies. According to reports, the common occurrence of clinical haze in MPS has been assessed through the high precision of the iris camera (Javed, Aslam and Ashworth, 2016; Javed et al., 2017). The study by Javed et al (2017) further suggested that using an iris camera, clinically graded corneal haze or corneal clouding can be monitored with visual changes assessed over time.

Following corneal treatment, it is essential to have a device capable of producing repeatable and accurate measurements needed to monitor the disease progression or assessment of the corneal to determine visual improvements. Studies suggest that changes indicating cornea deterioration or improvement can be monitored effectively to indicate potential treatment outcomes (Alzahrani et al., 2019).

1.6 Aims and Rationales of the Project

1.6.1 Hypothesis of Research

This literature focuses mostly on corneal transparency. The area of interest in this project was using an iris camera to assess the cornea's clarity. Our project aims to assess the literature and address possible gaps in corneal clarity and corneal diseases. Corneal opacification measurement and visual acuity could be useful means of assessing corneal clarity objectively after CXL and corneal transplant. This study will determine the relationship between corneal clarity and visual acuity and how the management of
corneal diseases impact corneal clarity differently. Furthermore, we will investigate corneal clarity in healthy eyes and evaluate its relationship with corneal diseases.

1.6.2 Research Objectives

The fundamental reason for this study is to measure corneal clarity and explore changes in health and disease that impact its transparency. We will likewise ascertain if corneal opacification measurement will be helpful when monitoring corneal disease progression, evaluate therapy outcomes and help in disease management.

1.6.3 Keratoconus Study (KCN)

To assess the outcomes of Corneal Opacification Measure (COM) in treatment-naïve patients, look at the progression over time, and compare healthy eyes from controls.

To assess the relationship between Iris Camera Corneal Opacification Measure (COM), corneal densitometry measurement after CXL, look at the correlation with visual functions and compare outcomes in adult patients.

To assess the relationship between the Camera Corneal Opacification Measure (COM), corneal densitometry, BCVA after corneal transplant (PK).

A lack of a power calculation for our samples meant all our studies were feasibility studies as no one has ever used the iris camera on KCN.
Chapter 2

An exploration of a modified iris camera imaging technology in keratoconic patients and healthy controls

Contributions

I designed this study in collaboration with my supervisors. I enrolled all the participants, conducted all the experiments, and performed the imaging. I also completed all the experiments, analysed the study data, and wrote the manuscript. This work was realized by constant discussions, close collaboration, and repeated feedback on data analysis from my supervisors: Prof Tariq Aslam and Dr Chantal Hillarby.

Publication

The Chapter is prepared as a manuscript which will be submitted for publication.

EMMA-DURU, C., CARLEY, F., HILLARBY, C., ASLAM, T. An exploration of a modified iris camera imaging technology in keratoconic patients and healthy controls.

Conferences Presentations

The abstract was presented at the Manchester Optometry Meeting 2017 and at the Association for Research in Vision and Ophthalmology (ARVO), April 28 – May 2, 2019, Vancouver, Canada.

Acknowledgement

The authors thank the Manchester Royal Eye Hospital (corneal clinic) for supporting the enrolment of participants in this study.
2.1 Abstract

**Aims:** To assess levels of corneal haze using an iris camera and corneal opacification measure (COM) score in treatment-naïve adults with keratoconus and healthy control subject eyes over a period of 12 months.

**Methods:** This was a prospective observational study. Study participants were enrolled from the Manchester Royal Eye Hospital (MREH) cornea clinics. Participants underwent assessment of corneal clouding with Iris camera and corneal opacification measure (COM) score and best-corrected visual acuity (BCVA). All assessments were taken at a baseline visit and follow up 12 months later.

**Results:** 69 patients with keratoconus (47 males, 22 females) and 30 participants with healthy eyes (12 males, 18 females) were recruited. Analysis showed that baseline iris camera corneal clouding (COM) scores were significantly higher in keratoconic eyes than in healthy eyes with zero scores. The COM scores increased over time in keratoconic eyes as corresponding BCVA also worsened. COM scores and corresponding BCVA did not change in healthy controls.

**Conclusion:** The iris camera opacification measurements indicate worsening corneal clarity in keratoconic patients over 12 months. The results of the iris camera scores and change over time suggest the utility of this measure for quantification and monitoring cornea haze in keratoconus.
2.2 Introduction

Keratoconus (KCN) is an ectatic disorder of the cornea (Gomes et al., 2015a) that causes a weakening of its structure and composition as a result of the degenerations that take place (Ambekar, Toussaint and Wagoner Johnson, 2011). It is characterised by progressive thinning, steepening, and eventual protrusion or bulging of the corneal tissue making it resemble a cone (Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010). These degenerative changes occasionally result in scarring of the corneal tissue, leading to visual aberrations (irregular astigmatism and myopia) and, hence, distorted vision (Arnal et al., 2011; Jay H. Krachmer, Robert S. Feder and Michael W. Belin, 1984; Rabinowitz, 1998; Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010). This disease is progressive in nature and predominantly affects young adults and onsets from adolescence until the fourth decades of life (Olivares Jiménez et al., 1997; Yildiz et al., 2010). KCN is estimated to affect approximately 265 people in every 100,000 populations (Godefrooij et al., 2017). KCN is most generally bilateral (Jiménez-García et al., 2021b; Zadnik et al., 2002), although it is asymmetrical in presentation (Galletti et al., 2015; Goebels et al., 2017; Zadnik et al., 2002).

The cornea is a thin avascular tissue that combines with the sclera to form the outer shell of the eye. It is often referred to as the window of the eye and plays a physiological role in permitting the transmission of 90% of light into the eye (Hsu and Sugar, 2017; Spadea et al., 2016). Corneal transparency is a culmination of its anatomical and physiological (cellular) components, among other factors, to maintain healthy corneal homeostasis and appropriate refractive shape for optimum vision (Nishida, 2008). Any disruption to this intricate and delicate balance, either by injury or disease, would impair its function, thereby compromising transparency (Knupp et al., 2009; Meek and Knupp, 2015; Qazi et al., 2010). Corneal transparency is indeed one of the hallmarks used in clinical examinations to assess corneal health.

Subjective evaluation of corneal clarity entails the clinician using slit-lamp biomicroscopy to estimate transparency (Dohlman, Yin and Dana, 2019). Although easy and efficient, this system is inconsistent across clinicians and researchers, relies hugely on experience, and is prone to individual bias (Aslam et al., 2012; Dohlman, Yin and Dana, 2019). The need for standardised systems of evaluating haze has prompted the development of modern specialised equipment to aid the identification and objective
quantification of corneal haze with precision (Aslam et al., 2012; Consejo et al., 2020b; Hillenaar et al., 2011; Patel and McGhee, 2007; Patel et al., 2007; Uchino et al., 2011).

2.2.1 Iris Camera Imaging Technique

The iris camera is one such modality that can provide an objective score for corneal opacification. It can acquire multiple precise images of the eye within seconds. The iris camera utilises near-infrared light during imaging for automated high-speed capture of images (Aslam, Tan and Dhillon, 2009). Specially designed analysis algorithms then process the images and give quantitative measurements of haze known as a corneal opacification measurement (COM) score (Aslam et al., 2012; Javed, Aslam and Ashworth, 2016; Javed et al., 2017). Previous studies have been based on using the iris camera in investigating Mucopolysaccharidoses (Aslam et al., 2012; Javed, Aslam and Ashworth, 2016; Javed et al., 2017; Sornalingam, Aslam and Ashworth, 2018). However, this system has never been explored in the keratoconus.

In this study, we assessed the outcomes of the COM scores in keratoconic patients and looked at the progression over time and compared it to controls.
2.3 Participants and Methods

This explorative study was approved by the Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee. It was conducted in line with the tenets of the declaration of Helsinki, and informed consent was obtained from all participants prior to enrolment. Participants were recruited from the specialist Ophthalmology clinic at the Manchester Royal Eye Hospital (MREH). Inclusion criteria were patients over the age of 18 years with keratoconus and no previous treatment. Also, healthy volunteers with no history of ophthalmic condition or eye disease other than refractive error were recruited from attendees at the hospital or staff members of the hospital. All healthy participants were all above 18 years of age at the time of imaging. Exclusion criteria were concomitant ocular manifestations besides Keratoconus, ocular surgery. The iris camera (Iris model IG-AD100; Irisguard Ltd, Buckinghamshire, UK) was used to obtain corneal images at each visit under standard photopic lighting and no dilation eye drops. Bilateral imaging was performed on all eyes, but we selected the eye with the worse baseline vision for the purpose of the study. Recruited participants were put in two groups A and B. Group A comprised of patients with keratoconus, while participants in group B were controls with healthy eyes. The participants were assessed and imaged at baseline clinical appointments and again at follow-up visits one year later. Participants that were unable to fixate for imaging were further excluded from the study. Figure 2-1 represents the study flow chart. Data containing relevant information about the patients were extracted from the clinical notes. The patients also underwent clinical slit-lamp assessment, and corneal haze was denoted numerically as (0=absent), (1=mild), (2=moderate), (3=severe). Visual acuity measurements included the corrected distance visual acuity following the latest refraction performed by an optometrist. The examinations were carried out under the same conditions (i.e., same charts, same working distance and same rooms).
Statistical analyses

Data analyses were performed using IBM SPSS statistics V.25 (IBM Corporation, Armonk, NY, USA) for Windows. The test of normality between the two samples was assessed using Shapiro-Wilks tests. Pearson’s test was used to assess the correlation between subjective grading and COM measurement. All data were tabulated, and descriptive statistics were reported as median and interquartile range for baseline and follow-up. A $P$-value of <0.05 was regarded as a statistically significant result.
2.4 Results

2.4.1 Study Population

A total of 99 subjects (69 treatment-naïve patients with KCN (47 males, 22 females) and 30 healthy control subjects (12 males, 18 females) were included in this study. The mean age of the patients with keratoconus was 29.68±8.54 years (18-60), and that of the healthy control eyes was 31.9±8.49 years (22-59). A total of 69 keratoconic and 30 healthy eyes were used in this study. The medians and interquartile ranges of the COM scores and BCVA of both groups are summarised in Table 2-1. The findings from the slit-lamp assessments correlated with COM scores (coefficient of correlation r=0.891, p<0.005).

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Table 2-1: Test of normality for samples.

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Table 2-2: Demographics of the Subjects.
Figure 2-2: Distribution of corneal clouding in relation to COM score (Iris camera).

KCN COM score outcomes
The COM scores are displayed in Figure 2-2 for each of the clinical grading as the baseline. There was statistical significance between the COM scores in this group from baseline to follow up assessment (P=0.004). Figure 2-3 indicates the COM score outcomes on both visits.

Figure 2-3: Change in mean COM score on two visits for KCN eyes.
KCN Visual Acuity Outcomes

After 12 months from the baseline visit, the keratoconus group showed large variability in visual acuities across values, with the worst vision being 1.9 LogMAR. BCVA showed statistically significant differences (P=0.001) in visual acuity from baseline to follow up. Figure 2-4 shows the changes in BCVA over time.

Figure 2-4: BCVA on two different occasions in KCN.

Controls COM score outcomes

Objective assessments of all eyes in the healthy controls demonstrated that most of the participants showed COM scores of zero (0). In approximately (97%) of eyes, corneal clouding did not change between the period of baseline and follow-up. Changes in outcomes of baseline and follow-up appointments were statistically insignificant (P=1.000). These findings are represented in Figure 2-5.
Figure 2-5: COM score on two different occasions in healthy eyes.

**Controls Visual Acuity Outcomes**

The difference in BCVA for the study controls was also found to be statistically insignificant (P=0.32) between baseline visit and follow-up. Vision remained unchanged in most eyes across the group. **Figure 2-6** shows this distribution.

Figure 2-6: BCVA on two different visits in healthy eyes.
Keratoconic versus normal eyes

Finally, COM score measures of the keratoconus group were compared to measures of the normal control group. The eyes with KCN were found to have significantly higher levels of corneal clouding at both baselines and follow up compared with the normal control group (Figures 2-7 and 2-8).

Figure 2-7: Baseline COM scores between KCN and control eyes.

Figure 2-8: COM scores between KCN and control eyes at follow-up.
2.5 Discussion

In this study, we measured corneal clouding using the iris camera COM score and best-corrected visual acuity in patients with keratoconus at a baseline visit and a follow up 12 months later. Our findings indicate objective increases in the haze over this period in the eyes of keratoconus patients that had undergone no form of treatment and stability in corneal clarity among volunteers with healthy eyes.

The cornea is a transparent tissue with a major refractive surface and transmits most of the incident light entering the eye with minimal scatter. However, diseases such as KCN can affect the biomechanical structure of the cornea, thereby altering this balance and increasing the backscatter of light as the disease progresses and eventual opacification in advanced cases (Dohlman, Yin and Dana, 2019; Jiménez-García et al., 2021; Ní Dhubhghaill et al., 2014). Sherwin and Brookes (2004) stated that the abnormal changes in the cornea due to keratoconus are dependent on the extent of disease severity. In most cases, the structural remodelling that occurs during KCN often leads to haze and contributes to a decline in visual acuity (Alzahrani et al., 2017; Espandar and Meyer, 2010; Silverman et al., 2014). These subtle changes in the corneal haze from keratoconus were demonstrated in our study using the iris camera measurement.

According to our analysis, visual acuity was also seen to worsen in the eyes with KCN over the period. This finding was similar to other authors (Shehata et al., 2020) associating corneal changes with decreased vision. A possible explanation for the worsening vision could be that in KCN, as the disease progresses, the apex of the cornea is often displaced from the centre of the pupil (Sahin, Yildirim and Basmak, 2008). In other words, for every displacement that takes place in the cornea, vision worsens, increasing BCVA by almost 1.00 LogMAR (McAlinden and Moore, 2011). Findings have further suggested that the Fleischer’s ring in moderate and advanced cases of keratoconus has been shown to correlate with decrease visual acuity (Shehata et al., 2020). However, some studies have hypothesized that the decline in visual function may not be a true reflection of the disease severity, as the relationship between visual acuity and KC is disproportional but rather a measure of the higher order aberrations (HOA) and contrast sensitivity affecting the visual system (Shneor, Piñero and Doron, 2021). The COM score was seen to increase as the BCVA worsened in keratoconic eyes, whereas the healthy eyes remained the same at follow-up. Moreover, a study between a keratoconus group and a control group
showed that the mean BCVA of keratoconic eyes were worse when compared with healthy eyes (Aydin Kurna et al., 2014). This study represents the first time the relationship between corneal clouding and visual acuity has been reported in keratoconic and healthy eyes using an iris camera system. The COM score of healthy controls in recent studies by Aslam et al (2012) and Sornalingam et al (2019) gave corneal clouding estimates of zero, which was similar to our findings. Most of the normal eyes in our study had values within or close to that range (0 – 0.1). In a report, assessment of keratoconic corneas indicated greater optical densitometry (increased light scatter) compared to normal eyes (Anayol et al., 2016; Jabbarvand et al., 2021). Another student indicated that keratoconic eyes showed significantly increased intraocular light scatter compared with normal eyes (Jinabhai et al., 2012).

Our study demonstrated much greater COM scores in patients with keratoconus than healthy individuals. This finding was similar to a study by (Anayol et al., 2016; B. Lopes, I. Ramos and R. Ambrósio, 2014), where higher corneal densitometry measurements were seen in advanced cases of keratoconus.

The lack of corneal densitometry information should be regarded as a limitation as all conclusion was made on changes in corneal opacification measurement and changes in visual acuities. Another limitation is that our sample size was limited to patients between 18 and 60 years with treatment-naïve KCN, which may not represent the keratoconic population.

However, our findings suggest that the iris camera can provide objective estimates of corneal haze and document changes over time in patients with keratoconus. The results showed that the KCN worsened over the year whilst the healthy eyes remained with very low or zero values, indicating good corneal clarity. Further studies may be required to determine if corneal densitometry measurements will give similar information as the iris camera COM score regarding corneal clarity, haze and disease progression in keratoconus.
Chapter 3

One-year observational study of an Iris Camera Corneal Opacification Measurement and Pentacam Densitometry in Keratoconus after corneal crosslinking

Contributions

I designed this study in collaboration with my supervisors. I enrolled all the participants, conducted all the experiments and performed the imaging. I also completed all the experiments, analysed the study data and wrote the manuscript. This work was realised by constant discussions, close collaboration and repeated feedback on data analysis from my supervisors: Prof Tariq Aslam and Dr Chantal Hillarby.

Publication

The Chapter was prepared as a manuscript and submitted for publication.

EMMA-DURU, C., CARLEY, F., HILLARBY, C., ASLAM, T. An exploration of a modified iris camera imaging technology in keratoconic patients and healthy controls.

Acknowledgement

The authors thank the Manchester Royal Eye Hospital (corneal clinic) for supporting the enrolment of participants in this study.
3.1 Abstract

**Aim:** To investigate levels of corneal haze in adult patients with keratoconus treated by corneal crosslinking, using Iris camera and corneal densitometry imaging.

**Methods:** This was a prospective, exploratory, and observational study on patients attending the Manchester Royal Eye Hospital outpatient department. Iris camera, Pentacam densitometry and visual acuity measurements were collected from patients in two groups. In Group A, patients were assessed baseline (first visit) before crosslinking (CXL) and 12 months after, whereas in group B, measurements were made after their CXL had been performed and followed up after one year.

**Results:** Group A consisted of 13 eyes of 13 patients, and Group B consisted of 41 eyes of 41 patients.

In group A, the average age was 26.69 (7.19) years. Average time of measurements taken before CXL was 1.85 (0.80) months and after CXL 12.69 (0.63) months. When comparing values before CXL and after, a significantly decreased COM score (P=0.001) and Pentacam Corneal densitometry measurement (P=0.003) represented improved corneal clarity. There was a corresponding significant improvement in vision in all eyes in group A after the CXL procedure, and 100% of eyes reached a BCVA of ≤20/50 (≤0.4 LogMAR) (P=0.002). In group B, the mean first examination time was post-treatment 3.24 (1.48) months, and for the second examination, post-treatment 12.56 (0.81) months. Between these two visits, there was a significant decrease in COM score (P=0.002) and corneal densitometry (P=0.0004), as well as improvement in BCVA (P=0.0002). In this group, 100% of eyes reached a BCVA of ≤20/50 (≤0.4 LogMAR) by the time of their last follow up.

**Conclusion:** There is a significant improvement in corneal opacification measures using both Iris camera and densitometry when assessing before and after CXL and sequentially 3 and 12 months after the procedure. The iris camera represents a promising, portable and practical means of assessing and monitoring corneal clarity for this purpose.
3.2 Introduction

Keratoconus (KCN) is an asymmetric ectatic degeneration of the cornea characterised by a progressive thinning and an eventual steepening and protrusion of the thinned corneal tissue giving it the shape of a cone or a dome (Jay H. Krachmer, Robert S. Feder and Michael W. Belin, 1984; Rabinowitz, 1998; Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010). Consequences of this corneal degeneration include high myopia, irregular astigmatism, which, when combined with thinning and corneal scarring, leads to poor vision and a decline in the quality of life (Arnal et al., 2011; Davis et al., 2006; Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010; Zadnik et al., 1987).

KCN is progressive involving structural changes at a molecular level (M. L. Khaled et al., 2017; Meek et al., 2005; Volatier, Figueiredo and Connon, 2020). The management of KCN is dependent on the level of severity or the rate of advancement (Gore, Shortt and Allan, 2013; Subasinghe, Ogbuehi and Dias, 2018; Vazirani and Basu, 2013). At an early stage, optical corrections using spectacles and contact lenses are typically employed (Kloeck, Koppen and Kreps, 2021; Lim and Lim, 2020a). Corneal transplantation is often indicated in advanced cases of KCN where refractive corrections prove insufficient at managing visual the condition (Rabinowitz, 1998). Surgery may also be indicated for scarring of the cornea or rupture of the Descemet’s membrane. Approximately 12-20% of most keratoconic patients require corneal transplants (Jhanji, Sharma and Vajpayee, 2011).

An alternative treatment for several forms of corneal ectasia that has emerged over the last two decades is corneal crosslinking (CXL). Procedures inducing crosslinks have been identified to halt or limit disease progression in KCN (Mohammadpour, Heidari and Hashemi, 2018). CXL was first performed in 1998 at the University Eye Clinic in Dresden, Germany (Raiskup-Wolf et al., 2008; Wollensak, Hammer and Herrmann, 2008; Wollensak, Spoerl and Seiler, 2003). This promising, minimally invasive, the procedure has become an established treatment for progressive KCN- designed to slow down or halt its progression, thereby reducing the requirement for a transplant (Ashar and Vadavalli, 2010; Galvis et al., 2018; Godefrooij et al., 2016; Koppen et al., 2018). Over the years, this procedure has been performed predominantly on adults; however, findings suggest that it has been extended to
paediatric populations (Caporossi et al., 2012; Vinciguerra et al., 2012). CXL works by strengthening and stabilising the corneal stroma's collagen lamellae by creating new molecular chemical bonds (crosslinks) through a process known as localised photo-polymerisation (Hersh et al., 2017; Shalchi, Wang and Nanavaty, 2015). This treatment involves debriding the corneal epithelium then applying 0.1% riboflavin (Vitamin B2) and ultraviolet radiation (UVA) using an irradiation device that emits radiation between 360-380 microns in homologous sequential infusion with the riboflavin. The exposure to the UVA with the riboflavin acts as a synthesiser that produces reactive oxygen that eventually forms the covalent bonds within the stroma, thereby making the cornea stiffer.

There are two types of CXL procedures: the standard (Dresden) and the accelerated protocol. The standard protocol is the most conventional crosslinking technique using 3mW/cm2 UV intensity and 30-minute radiation (Deshmukh et al., 2019; Wollensak, Spoerl and Seiler, 2003). Although the standard Dresden protocol effectively treats KCN, its major drawback was its duration for the procedure (Choi et al., 2017). The Accelerated protocol emerged as an alternative technique to decrease the treatment time and employed the Bunson-Roscoe law, which stated that increasing the radiative dose with constant exposure of 5.47J/cm2 produced the same treatment effect (Ng, Chan and Cheng, 2016). After CXL, patients are examined post-operatively to ensure re-epithelialisation with subsequent follow up to assess response to treatment.

Due to its transparency and the role, it plays in the visual pathway, the cornea transmits 90% of light entering the eye (Hsu and Sugar, 2017; Spadea et al., 2016). Its transparency is an immeasurable pointer towards its health (optical quality and condition) or an indication of disease (Baratz et al., 2012; McLaren, Bourne and Patel, 2010; Qazi et al., 2010). Therefore, any alteration to its clarity either due to injury or disease would disrupt its biomechanical structure and constitute haze (Meek and Knupp, 2015).

In clinical practice, subjective assessment of corneal opacity or haze can be carried out using a slit-lamp (Dohlman, Yin and Dana, 2019; Martin, 2018). Over the years, the need for objective quantification of corneal haze as a baseline for disease severity has increased significantly, prompting additional methods.
of quantifying haze (Aslam et al., 2012). The severity of a disease or the corneal haze levels was previously categorised subjectively by a clinical observer as either healthy, mild, moderate or severely clouded based on the anterior chamber's visibility during clinical assessment. However, this individual evaluation is subject to bias and could lead to discrepancies in disease grading and classification (Elflein et al., 2013). The iris camera is a specially adapted device with a modified image acquisition software that takes quick, reliable images of the cornea (Aslam, Tan and Dhillon, 2009). It incorporates a 680,000-pixel sensor and near-infrared wavelength illumination (>740nm) to minimise or process out flash artefacts and determine disease patterns to generate a quantitative estimate of haze (Aslam et al., 2012). The estimate of this analysis by the iris camera’s adapted algorithm is known as the corneal opacification measurement (COM) (Aslam et al., 2012).

An alternative system of objective corneal clarity assessment involves Pentacam densitometry – this is a system that employs a rotating Scheimpflug camera to give a 3-dimensional cross-section of the cornea as other optic media (Elflein et al., 2013; Fujioka et al., 2007).

This study aimed to evaluate corneal clarity in patients with KCN and how this is affected by CXL. Measurements were taken using the iris camera and Pentacam in addition to BCVA.
3.3 Methodology and Participants

This was a prospective, exploratory, and observational study approved by the Manchester University NHS Foundation Trust, Manchester, UK, and NREC local ethics committee. All aspects of the research complied with the tenets of the Declaration of Helsinki. Participants were recruited from the Corneal Ophthalmology clinics at the Manchester Royal Eye Hospital (MREH), Manchester, United Kingdom, where they were treated and examined. All patients were informed about the study, and informed consent was obtained from them. All patients in this study had CXL treatment or were due for CXL treatment for progressive KCN.

3.3.1 Inclusion Criteria

All patients included in this study were 18–45 years, with a corneal thickness of 400μm or more (epithelium included) and were intolerant to rigid gas permeable contact lenses. Patients that had other corneal (besides KCN) or systemic disorders that affected the cornea were excluded from the study. Patients with an inability to fixate on the iris camera or Scheimpflug Pentacam during imaging were excluded as were patients unable to perform Scheimpflug examinations or return for review appointments. Patients were only assessed at existing appointments made for clinical reasons, and imaging was restricted to these occasions. Patients were recruited into two groups (A and B). Group A included patients examined before having CXL treatment and after. Patients in group B had already undergone the CXL procedure, so measurements were taken post-treatment at the baseline visit (3 months) and followed up 1 year from baseline. All subjects underwent the same CXL procedure (Mazzotta et al., 2014). Visual acuity values incorporated the best-corrected visual acuity distance measurements from the most recent manifest refraction performed by an optometrist. These assessments were completed under similar circumstances at each visit utilizing a similar chart, room and working distance.
3.3.2 Surgical Procedure (Accelerated CXL)

According to Mazzotta et al (2014), an accelerated corneal CXL was performed for all patients under sterile conditions as an outpatient procedure. Oxybuprocaine chlorhydrate 1.6mg/0.4 mL drops were used to anaesthetise the cornea before debridement. The KXL I system (Avedro, Waltham, MS, USA) was used to perform the 9Mw/5.4J/cm2 ACXL treatment. The topical anaesthesia was administered 10 minutes before the procedure following instillation of 2% pilocarpine 30mins before the procedure. For corneal debridement, a speculum was inserted, and a blunt spatula was used to remove about 9mm of epithelial thickness. Following the removal of the epithelium, 0.1% isotonic riboflavin was instilled in the cornea for 10 minutes (1 drop every minute to soak the cornea) before continuous light UV-A irradiation using UV-X 2000; IROC AG, Zurich, Switzerland) and UV diodes (365nm). The central and peripheral corneal thickness was measured and recorded. Riboflavin drops were applied (2 drops every 2.5 minutes) for a duration of 10 minutes of UV-A exposure at 9Mw/cm2 of UV-A power and standard Fluence of 5.4J/cm2. Saline solution was used for flushing the cornea. Postoperatively, all patients had antibiotics, dexamethasone and mydriatic eye drops (cyclopentolate) instilled. A soft
contact lens was used to bandage the eye for 4 days until re-epithelialisation was complete. Corticosteroids eye drops and preservative-free artificial tears were administered in the cross-linked eye for 4-8 weeks post-treatment.

3.3.3 Iris Camera imaging

Imaging taken included corneal opacification measure (COM) score from an iris recognition camera described in detail in previous publications (Aslam et al., 2012). We captured 6 corneal images (3 per eye) at each visit in a clinical room under photopic conditions and no pharmacological dilation. We calculated and recorded the mean COM score for patient images using image analysis algorithms MatLab © software.

Corneal haze in KCN following CXL tends to affect each eye differently and in both groups in our study, some patients had CXL done bilaterally. Therefore, we selected one eye at random for each eye cross-linked to be the study eye for analysis.

3.3.4 Pentacam Imaging

Additionally, to assess corneal transparency utilizing densitometry estimations, the Oculus Pentacam was used to assess changes. All measurements were performed by an experienced operator in a dark room. Only examinations that passed the device’s software quality check were utilized. Densitometry values were acquired at each visit (the baseline and follow-up appointment). For the purpose of analysis, the relative values were measured in the anterior layers of the cornea and concentric zones (0-2 mm) as this area demonstrates the highest densitometry readings following CXL procedure as it receives the maximum treatment.

The measurements from the Iris camera and Pentacam images were compared to visual acuity (BCVA in LogMAR) by the investigator via clinical notes together with any pertinent clinical history.

Statistical Analysis

Data analyses were performed using IBM SPSS Statistics for Mac, Version 25, NY: IBM Corp was used for statistical analysis. Shapiro-Wilks test was used to assess normality of data. Descriptive data were presented for each visit and follow-up as the median and interquartile range. Paired Wilcoxon-signed rank test was used to assess significance between baseline and follow-up. Spearman correlation
was used to assess correlation between COM score and Densitometry. A p-value <0.05 was considered statistically significant.
3.4 Results

3.4.1 Study Population

In this study, 13 patients were allocated to group A, and 41 patients to group B. Patients in group A had images acquired at (before CXL) while B patients included images acquired after CXL treatment. This means that in total, 54 patients were assigned to groups A and B (see Table 3-1). There was a baseline visit and a follow-up visit with an interval of 12 months between both visits to assess changes in COM, corneal densitometry and evaluating BCVA, as shown in Table 3-2. A correlation of the corneal densitometry and iris camera readings are represented in table 3-3 and table 3-4 respectively.

<table>
<thead>
<tr>
<th>Parameter (Mean ± SD)</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.69 ± 7.19</td>
<td>25.96 ± 5.96</td>
</tr>
<tr>
<td>Range</td>
<td>18 to 43</td>
<td>18 to 44</td>
</tr>
<tr>
<td>Male patients (n)</td>
<td>12/13</td>
<td>30/41</td>
</tr>
<tr>
<td>Female patient (n)</td>
<td>1/13</td>
<td>11/41</td>
</tr>
<tr>
<td>Mean time of baseline measurements (months) pre-CXL ± SD</td>
<td>1.85 ± 0.80</td>
<td>-</td>
</tr>
<tr>
<td>Mean time of measurements post-CXL ± SD</td>
<td>12.69 ± 0.63</td>
<td>3.24 ± 1.48</td>
</tr>
<tr>
<td>Mean time till last visit ± SD</td>
<td>-</td>
<td>12.56 ± 0.81</td>
</tr>
<tr>
<td>Total number of eye cross-linked</td>
<td>20</td>
<td>56</td>
</tr>
<tr>
<td>Total number of eye eligible</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Right eyes</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>Left eyes</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation, n = Number, CXL = Crosslinking

*Table 3-1: Patient baseline characteristics including details of patient visit times.*
Table 3-2: Test of Normality for samples.

<table>
<thead>
<tr>
<th></th>
<th>Kolmogorov-Smirnov&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
<td>df</td>
</tr>
<tr>
<td>Baseline visit COM score</td>
<td>.385</td>
<td>41</td>
</tr>
<tr>
<td>Follow-up visit COM score</td>
<td>.342</td>
<td>41</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lilliefors Significance Correction

Table 3-2: Summary of means and standard deviations of the COM score, densitometry and BCVA for both groups.

<table>
<thead>
<tr>
<th>Group A (Keratoconus)</th>
<th>Median Baseline measurement</th>
<th>Median Follow-up measurement</th>
<th>IQR Baseline measurement</th>
<th>IQR Follow-up measurement</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM Score</td>
<td>0.61</td>
<td>0.16</td>
<td>0.56</td>
<td>0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Visual Acuity (LogMAR)</td>
<td>0.50</td>
<td>0.20</td>
<td>0.56</td>
<td>0.25</td>
<td>0.003</td>
</tr>
<tr>
<td>Densitometry</td>
<td>18.40</td>
<td>17.20</td>
<td>3.10</td>
<td>1.35</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group B (Controls)</th>
<th>Median Baseline measurement</th>
<th>Median Follow-up measurement</th>
<th>IQR Baseline measurement</th>
<th>IQR Follow-up measurement</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM Score</td>
<td>0.46</td>
<td>0.18</td>
<td>0.59</td>
<td>0.31</td>
<td>0.002</td>
</tr>
<tr>
<td>Visual Acuity (LogMAR)</td>
<td>0.40</td>
<td>0.20</td>
<td>0.45</td>
<td>0.29</td>
<td>0.0004</td>
</tr>
<tr>
<td>Densitometry</td>
<td>17.90</td>
<td>16.60</td>
<td>1.65</td>
<td>1.90</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 3-3: A correlation of COM score and Corneal Densitometry in group A.

<table>
<thead>
<tr>
<th>Spearman’s rho</th>
<th>COM score</th>
<th>Densitometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient</td>
<td>1.000</td>
<td>.498</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.</td>
<td>.002</td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Densitometry</th>
<th>Correlation Coefficient</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient</td>
<td>.498</td>
<td>1.000</td>
<td>.</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.002</td>
<td>.</td>
<td>13</td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-4: A correlation of COM score and Corneal Densitometry in group B.

<table>
<thead>
<tr>
<th></th>
<th>COM score</th>
<th>Densitometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman’s rho</td>
<td>1.000</td>
<td>.250</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.005</td>
<td>.</td>
</tr>
<tr>
<td>N</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Densitometry</td>
<td>.250</td>
<td>1.000</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.005</td>
<td>.</td>
</tr>
<tr>
<td>N</td>
<td>41</td>
<td>41</td>
</tr>
</tbody>
</table>

Group A COM score

Group A eyes had lower COM scores for clinical corneal haze at the follow-up visit after CXL treatment than pre-treatment (P=0.001). There was a significant difference between COM scores one year after the baseline visit (Figure 3-2). The majority of the COM scores in these patient eyes were between (0) and 0.5.

Figure 3-2: COM scores before and after CXL.
Group A Densitometry Measurements

Corneal densitometry estimates in these patients also decreased one year after CXL treatment (see Figure 3-3). Statistically significant changes were found in corneal densitometry measurements between baseline (Pre-treatment) and follow-up visits over the period in the 0-2mm zone for anterior layers (P=0.003).

![Figure 3-3: Corneal Densitometry Pre and Post CXL](image)

Group A Visual acuity

The mean CXL-associated BCVA between baseline visit and follow-up indicated notable clinical improvements in vision (Figure 3-4). A bulk of these patients achieved postoperative visual acuity of 0.6 LogMAR (6/24) or better. The difference between both visit was statistically significant (P=0.002).
Group B COM scores

Analysis of the COM scores between the first postoperative visit and follow-up showed a decline in clinical corneal haze (see Figure 3-5). A comparison of the difference in mean COM scores at 3 months post-CXL and 1 year after baseline visit were statistically significant (P=0.002). A higher proportion of eyes had COM scores less than 1 at follow-up.
Group B Corneal Densitometry Measurements

Corneal densitometry values at the 0-2mm layers was higher at 3 months after CXL but declined in most eyes at follow-up measurements, indicating a reduction in corneal haze levels with scores between 14 and 18 (see Figure 3-6). Only two subjects had densitometry values greater than 20 at the follow-up visit. The difference in corneal densitometry at the third month and follow-up was statistically significant (P=0.0004).

![Figure 3-6: Corneal Densitometry after CXL.]

Group B Visual Acuities

Figure 3-7 shows the post-operative impact of CXL on the BCVA in category B. The mean BCVA improved significantly at the follow-up visit compared to measurements at the baseline (3 months post-CXL) (P=0.0002).
Figure 3-7: Visual acuities Post-CXL.
3.5 Discussion

In recent decades, CXL protocols and interventions as a treatment for KCN have steadily improved and has become an effective procedure of choice to manage early to moderate cases of KCN in an attempt to halt the progression and prevent continuous visual decline (Chang and Hersh, 2014; Wollensak, Spoerl and Seiler, 2003). The degenerative changes in corneal structure and composition as KCN progresses leads to a decline in vision secondary to significant visual aberrations associated with changes in optical properties (Ambekar, Toussaint and Wagoner Johnson, 2011). Although CXL produces satisfactory outcomes in the regression and stabilisation of KCN progression, it is often associated with a common postoperative complication; transient haze (Caporossi et al., 2012; Koller, Mrochen and Seiler, 2009; Mazzotta et al., 2007). The aetiology of corneal haze following CXL is not clear; however, it has been associated with a host of changes, including damage to the anterior stromal keratocytes as well as the transformation of the myofibroblasts (Dhawan, Rao and Natrajan, 2011; Koller et al., 2013; Mazzotta et al., 2007; Raiskup, Hoyer and Spoerl, 2009; Wollensak, Hammer and Herrmann, 2008). The formation of haze post-CXL generally decreases corneal clarity, which subsequently impacts vision (Asri et al., 2011; Kanellopoulos, 2009; Koller et al., 2013). The decrease in corneal transparency within the first few months after CXL procedure has also been attributed to changes in stromal optical density which induces an increase in light scatter (Greenstein et al., 2010). CXL-induced haze has been shown to differ in appearance from haze seen in other corneal procedures such as PRK, with the former having a dust-like appearance (Dhawan, Rao and Natrajan, 2011).

Following CXL treatment, complete recovery and healing can often last as long as a year (Beckman et al., 2019). This gradual improvement has been associated with the duration it takes for corneal haze to resolve and for the epithelium to be remodelled following CXL. During this period, there is an initial worsening in vision following by gradual improvements. In 91% of cases, the corneal haze clears up, and vision is improved. However, resolution takes longer on some occasions of dense haze, especially in advanced keratoconus (Raiskup, Hoyer and Spoerl, 2009).

The Pentacam Scheimpflug densitometry quantifies haze in KCN by measuring the amount of scattered light entering the eye, giving an objective estimate of its clarity (Cankaya et al., 2018; Ní Dhubhghaill
et al., 2014; Otri et al., 2012). The iris camera, unlike the Pentacam, utilises near-infrared light, which allows it to infiltrate severe haze or opacities and capture an image of the cornea to generate an estimate (Javed, Aslam and Ashworth, 2016). Although the two devices are based on different operating techniques and imaging principles, both devices demonstrated the capacity to give quantifying data as a marker for corneal clarity (Aslam et al., 2012; Consejo, Jiménez-García and Rozema, 2021).

Studies have validated the use of the iris camera to monitor disease progression and changes in corneal clouding over time in Mucopolysaccharidoses (MPS), a heterogeneous lysosomal storage disease that often results in visual impairment (Aslam et al., 2012; Javed, Aslam and Ashworth, 2016). Our study assessed the course of haze after CXL in patients with KCN using the iris camera and found significant changes in corneal haze. Our findings demonstrated that the COM score changed in both groups of patients between baseline (1-3 months) and follow-up visits (1 year after baseline/first visit). We also demonstrated these changes in corneal haze using the Pentacam Scheimpflug densitometry over the same period and found statistically significant difference between the baseline and follow-up visit. This increase in densitometry readings was predominantly in the anterior layers for the 0-2 mm corneal zones as this is the region that undergoes maximum treatment during CXL procedure. A similar finding by Pircher et al (2015) indicated that increase in densitometry was limited to the anterior portion predominantly. Our study also established that the COM scores and densitometry measurements were higher between 1-3 months and significantly decreased at postoperative follow-up visit. (Greenstein et al., 2010; Kim et al., 2016) also confirmed in their studies that corneal haze peaked within the first 3 months and subsequently declined showing decreased measurements year after CXL treatment. We found a correlation in CXL-associated measurements between the iris camera and the Pentacam as both devices recorded concomitant changes in corneal haze over the period.

Establishing the sensitivity of the iris camera may provide added advantages as it is designed to measure clinically valid opacification and is more portable, practical and affordable (Aslam et al., 2012; Sornalingam et al., 2019; Sornalingam, Aslam and Ashworth, 2018). This study simply describes its application in KC for clinical purposes. The iris camera is easier to use by examiners and, from our experience and quicker to acquire images on patients within seconds. This would require further
validation. It appears from our findings that values of change had higher values of significance than for the Pentacam comparing assessments before and after CXL.

Although the haze levels in our study using the COM scores as a baseline were predominantly mild, these values still showed a decrease within 12 months and were similar to findings by other authors using the Scheimpflug Pentacam to assess corneal haze (Greenstein et al., 2010). The subtle changes in densitometry measurements concur with these findings and evidence the iris camera's ability to produce an objective measurement of corneal clouding (Aslam et al., 2012; Javed et al., 2017). Our results also showed significant improvements in visual acuity at the follow-up visits, similar to reviews by (Chen et al., 2015), where some showed improvements in visual acuity while some did not. However, there is no consensus on VA improvement after CXL. These changes in COM scores and corneal densitometry coincided with improvement in vision indicating that corneal clouding after CXL eventually resolves with time (Subasinghe, Ogbuehi and Dias, 2018; Wittig-Silva et al., 2014). It is still unclear as to what factors influence the improvement of visual acuity following CXL procedure, however, some studies have suggested that higher order aberrations (HOA) are responsible for these changes in corneal clarity as the total corneal HOA is known to increase initially after the treatment but declines as time goes on (Zarei-Ghanavati et al., 2017). It could also be suggested that the more the CXL-related corneal haze (an adverse outcome of the procedure) due to greater CXL response, the lesser the visual function (Greenstein et al., 2010). Kim et al (2016) further suggested that although transient haze doesn’t necessarily correlate with changes in vision, the degree of haze, however, determined the level of visual acuity and could be due to the extent of disease severity. Despite these differences in findings, all patients showed visual improvements over time coinciding with a decline with corneal haze. A further study would be essential to elucidate the pathophysiology of this development.

In this study, both the Iris camera and Pentacam successfully demonstrated a capacity to monitor treatment through the change in corneal opacification in patients with KCN undergoing CXL therapy, validating their use as a useful tool in a clinical setting. This is the first time that the novel iris camera technique has been applied to objectively measure opacification changes over time in patients who have undergone CXL. We demonstrated that the iris camera and its image analysis software enable objective
quantification of haze in corneal diseases and could play an important role in evaluating, investigating, and monitoring corneal clouding in patients undergoing treatment.

A limitation to this study was the lack of repeated iris camera and Pentacam measurements between the baseline and final visit. After the first measurements at baseline, these patients were only followed-up at final visit due to Covid-19 and clinic restrictions. This meant that we were not able to follow-up measurements at 1month, 3months, 6months and 9months. Nonetheless, significant results were observed within the time span. A limitation to this study could be the lack of data from the 2-6, 6-10, and 10-12mm concentric corneal zones, as the information from the 0-2mm anterior zones might not be a true reflection on the outcome of CXL-induced haze on the cornea. Additionally, we did not assess some Pentacam parameters such as corneal thickness and other parameters as we were only comparing Pentacam densitometry findings to that of the iris camera’s COM scores to observe the relationship. These points may be investigated in prospective studies with well detailed follow-up and analysis. A lack of aberrometric readings could be a hindrance to this study as it denies us the opportunity to make profound statements on the impact of corneal haze on visual acuity as compared to higher order aberrations as stated in some studies with absolute certainty.
Chapter 4

Assessment of changes in corneal clarity in patients undergoing keratoplasty for advanced keratoconus: A one-year observational study

Contributions

This study was designed by me with the collaboration with my supervisors. I enrolled all the participants, conducted all the experiments, and performed the imaging. I also completed all the experiments, analysed the study data, and wrote the manuscript. This work was realized by constant discussions, close collaboration, and repeated feedback on data analysis from my supervisors: Prof Tariq Aslam and Dr Chantal Hillarby.

Publication

The Chapter was prepared as a manuscript and submitted for publication.

EMMA-DURU, C., CARLEY, F., HILLARBY, C., ASLAM, T. An exploration of a modified iris camera imaging technology in keratoconic patients and healthy controls.

Acknowledgement

The authors thank the Manchester Royal Eye Hospital (corneal clinic) for supporting the enrolment of participants in this study.
4.1 Abstract

**Aim:** To assess corneal haze in a group of adult patients with advanced keratoconus after penetrating keratoplasty (PK) using Iris camera and Pentacam densitometry imaging.

**Methods:** This was a prospective, observational study on patients attending outpatient appointments at the Cornea Clinics in Manchester Royal Eye Hospital. The data collected included measurements from the Iris camera and Pentacam densitometry, and best-corrected visual acuity (BCVA). After the penetrating keratoplasty procedure, all images were taken at a baseline and follow-up visit (one year after the initial appointment).

**Results:** 10 patients completed the study protocol. The average age was 31.1 ± 8.58 years at the baseline visit. The average timing of measurements was (0.25 ± 0.25) months after PK for the baseline and (13.35 ± 1.45) months for follow up. Analysis of eyes from these patients found a significant decrease in both iris camera corneal opacification score and Pentacam corneal densitometry measurement between baseline and follow up after PK. The postoperative BCVA was also seen to improve significantly between these time points (p=0.002), with 90% of these patients achieving visual acuities of ≤20/50 (≤0.4 LogMAR) or better at final follow-up.

**Conclusion:** Corneal opacification improved in the year of follow up after PK as measured by iris camera and Pentacam, with corresponding improvements in vision. The Iris camera has never been used for this precise purpose, and this study demonstrates its utility as a novel technological imaging device to quantify such corneal haze.
4.2 Introduction

Keratoconus (KCN) is a non-inflammatory, ectatic dystrophy marked by successive corneal thinning, steepening, irregular astigmatism, and in time severe visual impairment (Godefrooij et al., 2017; Torres Netto et al., 2018). Although it is usually bilateral, it often presents asymmetrically depending on the level of progression (Chan et al., 2021; Romero-Jiménez, Santodomingo-Rubido and Wolffsohn, 2010). It is the most prevalent of all corneal ectasias, and treatment modalities vary depending on its progression or level of severity (Gore, Shortt and Allan, 2013; Rabinowitz, 1998). In developing nations, KCN accounts for predominant cases that require transplants and is one of the principal indications for corneal transplants globally (Gain et al., 2016; Hashemi et al., 2020). Initial Therapeutic and management options include spectacles, contact lenses, corneal cross-linking, intracorneal rings and in severe cases, corneal transplants as a last resort when tissue transplantation is the only means of visual improvement (George and Larkin, 2004; Jhanji, Sharma and Vajpayee, 2011; Kanellopoulos, Krueger and Asimellis, 2015; Kloock, Koppen and Kreps, 2021; Lim and Lim, 2020b). PK is a full-thickness transplant used to replace diseased or abnormal corneal tissue. Although in the last decade and a half, advances in surgical techniques have gradually shifted towards less invasive procedures like deep anterior lamellar keratoplasty (DALK) to treat moderate to advanced-stage KCN, penetrating keratoplasty (PK) remains an established technique and an appropriate alternative/treatment option for acute as well as advanced cases of KCN (Bozkurt et al., 2017; Javadi et al., 2005). Indeed, PK for the management of KCN is a long-established and well-accepted procedure with high success rates and evidence of visual improvement within the first year of transplantation (Brierly, Izquierdo and Mannis, 2000; Buzard and Fundingsland, 1997; Coster et al., 2014; S. Pramanik et al., 2006).

Such improvements in visual function are reassuring, but more direct measures of the cornea may also be informative as psychophysical tests can be unreliable and affected by factors aside from corneal haze. Abnormalities such as corneal haze and the visibility of its anterior chamber frequently indicate the degenerative changes taking place within its structure and the level of disease severity and progression (Elflein et al., 2013; Qazi et al., 2010). The transparent nature of the cornea has often been
a baseline in understanding and assessing corneal health (Baratz et al., 2012; Klintworth, 2009; McLaren, Bourne and Patel, 2010; Meek et al., 2003).

Clinically, corneal haze can be assessed subjectively by observing corneal characteristics using a slit-lamp (Dohlman, Yin and Dana, 2019; Martin, 2018). Until recently, methods of assessing haze were mostly subjective examinations and prone to clinician bias (Aslam et al., 2012). Current imaging technologies offer objective estimations of haze levels with a degree of accuracy and validity (Patel et al., 2007). The Pentacam system is one of the most used commercially available imaging systems, employing corneal tomography and Scheimpflug camera principles to analyze the cornea three-dimensionally and determine its densitometry (Alzahrani et al., 2017; Motlagh et al., 2019). More recently, the iris recognition camera, a non-invasive portable device, has been shown to allow rapid imaging with quantitative, objective measurement of haze using specially adapted image analysis algorithms (Aslam et al., 2012; Javed et al., 2017). Analysis of corneal images using the iris camera generates an overall corneal opacification measurement (COM) (Aslam et al., 2012; Javed, Aslam and Ashworth, 2016).

This study aimed to assess how this characteristic (haze) and corneal clarity changed over time in patients that had undergone corneal transplant surgery for advanced keratoconus. In this study, we used objective measurements of corneal opacification from the iris recognition camera (COM score) and Pentacam Scheimpflug densitometry software to assess corneal clarity over a year after treatment with penetrating keratoplasty for keratoconus.
4.3 Participants and Methods

This was a prospective, observational study and was approved by the Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee. The study was performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent for study participation was obtained from the subjects after a description of the study's nature. We recruited patients with keratoconus who had previously undergone corneal transplantation (PK) and regularly attended the post-operative follow-up at the Corneal Service at the Manchester Royal Eye Hospital (MREH).

Images were taken with the Iris camera using standard lighting and no routine pharmacological dilation. All patients also underwent corneal tomography using Pentacam from which corneal densitometry measurements were obtained.

4.3.1 Inclusion Criteria

Inclusion criteria for the study were patients between 18-43 years and above who had undergone a penetrating keratoplasty for keratoconus. These patients had significant keratoconus (KCN) and could not tolerate contact lens wear. Patients with additional ocular pathologies or systemic conditions that affected the cornea other than KCN were excluded. Recruited patients had their corneal images taken by the iris camera (IrisGuard model IG-AD100, Irisguard, Buckinghamshire, UK). Corneal images were captured within three months of the grafting procedure and followed up one year from the baseline visit. Patients that had a loss of fixation during imaging with the iris camera were excluded from the study. When patients had PK done on both eyes, we selected the eye with the worst level of COM score for analysis.

Additionally, clinical notes were investigated at each appointment for descriptive analysis, and relevant information, including age, gender, corneal disease, type and date of surgery, was documented.
4.3.2 Surgical Technique

The surgical procedure was performed using a standard technique. A vacuum trephine (Barron Hessberg®, Barron Precision Instruments, MI, USA) or non-vacuum trephine (Coronet®, Network Medical, Ripon, North Yorkshire, UK) was used in all cases of the graft with diameters ranging from 8.00mm to 8.25mm. All tissues used during graft procedures were issued by NHS Blood and Transplant (UK) services and complied with the minimal standards for transplantation to include a minimum cell count of 2,200 cells/mm² and a central clear cornea of 9.00mm. Following the PK procedure, the patients are placed on a combination of topical antibiotics and steroids (tapered down). Generally, most sutures were removed within 1-2 years (12–24 months). The age difference between donor and recipient did not exceed 30 years. All subjects had the same procedure (PK) performed on them using the same surgical technique (Keenan et al., 2011; Lim, Pesudovs and Coster, 2000).
4.3.3 Iris Camera Imaging

The iris camera (Irisguard model IG-AD100®, Irisguard Ltd, Buckinghamshire, UK) uses near-infrared wavelength illumination (>740nm) to minimise artefacts from illumination. A specially adapted image acquisition software allows the analysis of corneal images and processes out aberrations before measuring the corneal clarity (COM score). The iris camera gives a numerical estimate of the clarity of the anterior portions of the cornea. The entire procedure is non-invasive, semi-automated and takes approximately 5 seconds to complete. Multiple images (3 each) of both eyes were taken of each patient at routine visits before other ocular examinations involving dilation. Imaging was performed in a lightened room under photopic conditions (luminance level 10 to 10^8 cd/m2) by a trained research team member. Quality standards were observed in the selection of all images according to the Iris camera protocol.

4.3.4 Pentacam Imaging

Prior to and after a transplant surgery, routine Pentacam images are taken to assess corneal layers and zones patients with advanced keratoconus. The Oculus Pentacam uses densitometry measurements to estimate of corneal clarity giving numeric estimates of all the corneal layers. The entire session is non-invasive and lasts approximately 5 minutes. Imaging sessions for all patients were performed at each visit (baseline and follow-up) under dim conditions (to eliminate ambient effects) by a well-trained operator preceding to the administration of drops or other eye examinations. Corneal densitometry information for the total cornea (anterior, central and posterior) at the 0-2 mm and 2-6 mm corneal zones were retrieved from the Pentacam database. The 6-10 mm and 10-12 mm zones were not analysed for backscatter as the peripheral zones are outside the optical zone and often contribute to artefacts scarring of the cornea between the host and donor tissue. Quality images were recorded following Pentacam analysis.

The measurements from the iris camera (COM score), Pentacam densitometry measurements and best-corrected visual acuity (BCVA) in LogMAR were analysed to determine the outcome of PK ove time. The VA values for all the patients were recorded from the latest refractive tests performed by the optometrist under the same conditions.
Statistical analysis

SPSS V.25 was used for statistical analyses. We assessed corneal haze as (Iris Camera and Pentacam values) as measurable estimates. As our sample size was small (<50) and the outcomes did not exhibit a normal distribution, we therefore assessed normality using the Shapiro-Wilks test. We employed a Paired Wilcoxon’s test to compare paired outcomes. The median and interquartile range were presented as descriptive statistics. Spearman’s correlation and regression line were used to look at correlation statistics between COM score and Corneal densitometry. Boxplots were used to represent our findings. A $P$-value of $<0.05$ was considered to be statistically significant.
4.4 Results

4.4.1 Study Population

A total of 11 eyes of 10 keratoconus patients underwent primary penetrating keratoplasty (PK) as a single procedure. In one case, PK was done bilaterally. We, therefore, used the worse eye as the study eye. In all, 10 eyes of 10 patients were enrolled and analysed. The patients were imaged after the first week of surgery and followed up 1 year from their first visit post-surgery range 12-14 months (13.35±1.45). The mean age of the patients was 31.1±8.58 years (range 20-43 years) at the first visit and 31.9±8.47 years (range 21-44 years). There were more males than females in this study group (6:4). Overall, 7 right and 3 left eyes were included in this study. There were more men than women in this study (6:4).

<table>
<thead>
<tr>
<th>Group A (Keratoconus)</th>
<th>Median Baseline measurement</th>
<th>Median Follow-up measurement</th>
<th>IQR Baseline measurement</th>
<th>IQR Follow-up measurement</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM Score</td>
<td>2.64</td>
<td>0.54</td>
<td>2.28</td>
<td>0.44</td>
<td>0.005</td>
</tr>
<tr>
<td>Visual Acuity (LogMAR)</td>
<td>0.90</td>
<td>0.10</td>
<td>0.91</td>
<td>0.35</td>
<td>0.002</td>
</tr>
<tr>
<td>Densitometry</td>
<td>25.10</td>
<td>17.80</td>
<td>7.45</td>
<td>2.75</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Imaging Time Point</th>
<th>Average time Baseline visit</th>
<th>Average time follow-up visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
<td>0.25±0.25</td>
<td>13.35±1.45</td>
</tr>
<tr>
<td>Weeks</td>
<td>1 week</td>
<td>58.01±6.32</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; p-value, probability value.

Table 4-1: Patient demographics of the study population.

<table>
<thead>
<tr>
<th>Tests of Normality</th>
<th>Kolmogorov-Smirnov a</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistic</td>
<td>df</td>
<td>Sig.</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>Follow-up Visit COM score</td>
<td>.296</td>
<td>10</td>
</tr>
</tbody>
</table>

a. Lilliefors Significance Correction

Table 4-2: Test of Normality for samples.
Table 4-3: A correlation of COM score and Corneal Densitometry.

<table>
<thead>
<tr>
<th>Spearman's rho</th>
<th>COM score</th>
<th>Correlation Coefficient</th>
<th>Densitometry</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.000</td>
<td>.539</td>
<td>.008</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 4-4: A summary of the post-operative densitometry in the 0-2mm and 2-6mm zones of the cornea.

<table>
<thead>
<tr>
<th>Corneal zones and layers</th>
<th>PK, (Median) (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 mm (Baseline Visit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>(27.37) (8.25)</td>
<td>0.006</td>
</tr>
<tr>
<td>Central</td>
<td>(25.33) (7.65)</td>
<td>0.001</td>
</tr>
<tr>
<td>Posterior</td>
<td>(22.66) (6.45)</td>
<td>0.001</td>
</tr>
<tr>
<td>2-6 mm (Baseline Visit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>(28.40) (8.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>Central</td>
<td>(25.39) (7.82)</td>
<td>0.005</td>
</tr>
<tr>
<td>Posterior</td>
<td>(21.78) (6.53)</td>
<td>0.006</td>
</tr>
<tr>
<td>0-2 mm (Follow-up Visit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>(20.01) (2.89)</td>
<td>0.006</td>
</tr>
<tr>
<td>Central</td>
<td>(17.82) (3.31)</td>
<td>0.001</td>
</tr>
<tr>
<td>Posterior</td>
<td>(15.60) (2.05)</td>
<td>0.001</td>
</tr>
<tr>
<td>2-6 mm (Follow-up Visit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>(20.82) (3.15)</td>
<td>0.005</td>
</tr>
<tr>
<td>Central</td>
<td>(16.90) (2.78)</td>
<td>0.005</td>
</tr>
<tr>
<td>Posterior</td>
<td>(14.71) (2.32)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Note:
Significant at p<0.01 and 0.05
Abbreviations: PK, penetrating keratoplasty; IQR, interquartile range.
Correlation between COM and Densitometry

A positive association between the COM score (Iris camera) and corneal densitometry (Pentacam) was observed. The results of the correlation are presented in Table 4-3. Figure 4-2 further depicts a scatter plot of the COM against densitometry measurements. The coefficient of correlation was R=0.52.

COM score

The post-operative COM scores demonstrated a marked decline in corneal opacification over the follow-up period (Figure 4-3). All study eyes showed an objective decrease in the haze levels, with most of these patients having values below 1. Improvements in visual acuity accompanied this. A statistically significant difference in mean COM scores was found between both visits surgery (P=0.005).
Corneal Densitometry

The changes in corneal densitometry measurements over one year were shown in Figure 4-3. Corneal densitometry was significantly lower at the final postoperative follow-up assessment than in the first visit. Of all the 10 patients, only one had a density unit greater than 20, while the others ranged from 16 to 19. The density units between both follow-ups were statistically significant (P=0.005). The densitometry values of the different zones and depths are presented in Table 4-3. There were significant differences between the anterior, central and posterior densitometry at 0 to 2 mm and 2 to 6 mm. In the first month after PK surgery, higher densitometry values were determined in the 0 to 2 mm and 2 to 6 mm in the anterior, central and posterior layers (p=0.006, p=0.001, p=0.001). At 13 months, the values in all layers were similar and were statistically significant (p<0.05).
Figure 4-4: Corneal Densitometry after PK.

Visual Acuity Outcomes

BCVA was at least 0.4 LogMAR or better at the final follow-up in over 90% of these patients. Visual outcomes of the difference between both visits post-PK are presented in (Figure 4-5). Gains in visual acuity were observed for postsurgical time points as BCVA improved between the 1-month and 13-month follow-ups. The improvement of postoperative BCVA between both visits was significantly different (p=0.002 Wilcoxon’s test). Postoperative BCVA were better at 13 months than at 1 month.

Figure 4-5: BCVA after PK.
4.5 Discussion

Following PK, the cornea often loses its transparency due to haze formation. This unwanted complication is often the side effect of the cornea responding to the traumatic impact and mechanical stress of the procedure, thereby triggering a wound healing response (Alzahrani et al., 2018). During the initial weeks following PK, the graft can remain swollen with folds in the Descemet’s membrane as well as stromal striae, and these morphological alterations affect corneal transparency (Acar, Vural and Acar, 2011; Szaflik et al., 2007). Subsequently, as the swelling recedes and the Descemet’s membrane folds resolve, coupled with the efficient endothelium pump mechanism, the cornea’s transparency improves. The subsequent cascade of events following surgery results from the cornea trying to re-establish normal function and restore its biomechanics to that of a healthy cornea as much as possible (Netto et al., 2005). This post-operative change was assessed in this study - In particular, we measured corneal clarity after penetrating keratoplasty in patients with keratoconus, using the Iris Camera COM score, Pentacam corneal densitometry and best-corrected visual acuity.

Our iris camera results indicated that the device was effective and able to demonstrate objective and significant improvement in corneal transparency after a year following PK, which was consistent with corresponding and significant improvements in visual acuity.

Corneal transparency is often interpreted as the extent to which light passes through the different layers of the cornea unimpeded or with minimal scatter. In the human eye, an increase in the scattering of light has an impact on the level of clarity and visual quality (Koh et al., 2014). Following penetrating keratoplasty, there is an increase in the degree of backscatter (Patel et al., 2008). This increase in backscatter is suggested to be related to several subclinical factors as a result of the wound healing process following the procedure (Alzahrani et al., 2018; Patel et al., 2007). Quantifying the degree of backscatter gives an indication of the level of corneal transparency (Spadea et al., 2016). In our study, the degree of backscatter (densitometry) correlated with the COM score. Our study attributed the changes in visual acuity to the level of transient haze over time, however, other studies have argued that such changes in vision is rather associated with postoperative higher order aberrations (HOA) during visual rehabilitation (Salvetat et al., 2013). Regardless, light scatter and its relationship and impact on
vision cannot be ruled out and does indeed have an effect on visual recovery (Patel et al., 2008; Pesudovs and Coster, 2006). Others have suggested that rather than backscatter affecting vision, it was forward scatter of light that has an impact on vision. In our study, the postoperative visual outcomes were shown to improve at follow-up visit comparable to findings by Patel et al (2008) which indicated improved visual acuity within 3 months after PK. Another study by Brierly, Izquierdo and Mannis, (2000) and Cung et al (2019) suggested that 12 months after PK treatment, over 80% of their patients achieved postoperative visual acuities of (0.3 LogMAR/20/40) or better which were similar to our data.

The Pentacam densitometry analyses on corneal haze showed similar results in this group of patients. This study showed that after PK, the densitometry measurements for these patients with KCN was higher at baseline compared to the values at the follow-up visit. In all corneal regions, the average densitometry measurements at the 0-2mm and 2-6mm were greater at initial visit than at 12 months after surgery. This indicates that the level of light scatter/haze was initially greater at initial visit but dissipated over time. The variations in corneal densitometry at all corneal zones and layers were statistically significant ($P<0.05$) in the 0-2mm and 2-6mm corneal zones. Our findings also indicate that the anterior layer displayed the higher densitometry values than the central and posterior layers comparable with results by (Ní Dhubhghaill et al., 2014).

Although the iris camera and Pentacam imaging systems employ different imaging principles, previous studies have validated the iris camera and Pentacam as accurate techniques in anterior segment imaging that provide quantitative estimates of corneal haze in Mucopolysaccharidoses (MPS); an inherited condition with potential visual impairment (Elflein et al., 2013; Javed et al., 2017). However, to our knowledge, there is limited understanding of the application of the iris camera in monitoring the progression of haze in keratoconus. This study is the first to provide objective assessments of changes in corneal opacification over time in patients with keratoconus that have been treated with penetrating keratoplasty. The correlation between our COM score findings and densitometry measurements were expected and demonstrated the validity of the iris camera (COM score), that is, it records higher readings when densitometry readings were elevated. Although the Pentacam densitometry software is capable of presenting standardized measurements of backscatter in different corneal layers (Ní
Dhubghaill et al., 2014), the iris camera on the other hand as indicated in aforementioned chapters, gives a complete estimate of the total corneal transparency (sum of all the layers) and not individual sections. The iris camera has previously been proven to generate mean values of the pixel intensities of segregated regions of the pupil to assess corneal clarity changes (Aslam et al., 2012); this correlates to recent findings where corneal transparency has been demonstrated in KCN using statistical analysis of pixel intensity distribution (Consejo et al., 2020a; Consejo et al., 2019). However, the iris camera is quite easy to manoeuvre and operate, is quite portable, reliable and is cheap unlike the Pentacam which requires the densitometry as an “add-on” to assess and analyse scatter (Dohlman, Yin and Dana, 2019).

Elflein and associates compared Pentacam densitometry values in patients with MPS using the Pentacam and noted mild, moderate and severe density units (Elflein et al., 2013). For comparison, our postoperative findings indicated moderate densitometry values that ranged from 20 to 35 at baseline visit and values less than 20 densitometry units at subsequent follow-up. In this study, a larger portion of our study population were men similar findings by other studies (Al-Mohaimed, 2013; Cung et al., 2019; Javadi et al., 2005). Various studies have reported a higher prevalence of KCN in males than females (Abu Ameerh, Al Refai and Al Bdour, 2012; Wagner, Barr and Zadnik, 2007). A possible explanations for this relative frequency among male subjects compared to females is that KCN is said to progress more quickly in men than women (Fan Gaskin et al., 2013).

Penetrating keratoplasty has for decades been the gold-standard procedure for the management and treatment of advanced keratoconus with improved post-operative visual outcomes (Han et al., 2009; S Pramanik et al., 2006). However, over the last two decades, there has been a significant decline in its frequency of application due to the introduction of more modern alternatives such as deep anterior lamellar keratoplasty (DALK) (Keenan et al., 2011). These newer therapeutic protocols have important advantages, including a reduced risk of graft rejection – a major cause of graft failure in unresponsive cases (Reinhart et al., 2011; Tan, Anshu and Mehta, 2009). Despite the decreasing rate of use of this surgical modality, PK remains an effective treatment for advanced KCN where there is significant corneal scarring, previous corneal hydrops or a failed DALK secondary to perforation (Brierly,
Izquierdo and Mannis, 2000; Yildiz et al., 2010) and these results should therefore be of relevance to modern clinical practice.

Limitations of this study included the small sample size which could impact the validity of our results particularly the cut-off values. Also, a lack of measurements at more frequent monthly intervals (1 month, 3 months, 6 months, and subsequent months) which could have allowed more comparable outcomes. A further limitation to this study was the non-inclusion of corneal thickness to assess its relationship to corneal opacity and other parameters following treatment. Also there was a 12-month difference between both visit, only the baseline visit and follow-up visits were evaluated as we could’ve obtained information at difference time points before the final visit. Further limitations include the non-inclusion of other Pentacam topographic and tomographic parameters such as the corneal thickness, K readings as well as readings from the which could have given additional information or influenced our outcomes. These points may be investigated in future prospective studies. Despite the limitations, both the iris camera and Pentacam densitometry measurements have successfully demonstrated precise levels of improved corneal clarity in patients who had undergone a penetrating keratoplasty for keratoconus.

In addition to documenting changes in corneal clarity, the study also demonstrated the utility of the iris camera in its use as a novel, portable and potentially diagnostic tool that provides quick and reliable means of quantifying corneal haze in eyes that have undergone penetrating keratoplasty. This device could play a future role in optimizing treatment modalities in a host of other anterior segment disorders besides keratoconus.

However, further studies are crucial to support these assumptions and may include studies to document changes in corneal clarity over time in keratoconus patients who wear contact lenses and those who have been treated with intra-corneal rings (Intacs) as well as measures at more time points and with larger groups of patients.
Chapter Five

Summary, Limitations, Future Works and Conclusion

5.1 Summary

This project aimed to explore the changes that occur when the cornea is healthy and diseased and its effect on corneal transparency based on an iris camera capture and analysis algorithm/software. Although subjective means of assessment such as the traditional slit-lamp relies on clinician assessment, this objective method of evaluating corneal clarity has shown to be more accurate and efficient. Subjective evaluation tends to be variable in the outcome and often generates a lack of reliability among clinical specialists who manage and frequently provide interventions for ocular defects (Dohlman, Yin and Dana, 2019; Grewal and Grewal, 2012). We have demonstrated that using the corneal opacification measurement on the iris camera gives useful information on corneal haze and serves as an additional clinical tool to monitor the progression of the disease and suggest treatment outcomes.

Although a few reports have looked at changes in corneal clarity and the measurement and assessment of corneal haze in patients with keratoconus using other imaging modalities, however, to our knowledge, there are no findings on the application of an iris camera to measure corneal transparency in KCN using its corneal opacification measurement (COM) (Anayol et al., 2016). Studies have applied the iris camera to explore corneal clouding outcomes in patients with Mucopolysaccharidoses (Aslam et al., 2012; Aslam, Tan and Dhillon, 2009; Javed, Aslam and Ashworth, 2016; Javed et al., 2017; Sornalingam et al., 2019; Sornalingam, Aslam and Ashworth, 2018). All these studies demonstrated changes in corneal clouding over time using the densitometry measurements and COM scores as objective means of measuring or quantifying haze. Our studies further validate and agree with these studies mentioned earlier on the idea that corneal transparency changes over certain periods. Our cohort of patients included adults with keratoconus between the ages of (18 – 60 years). KCN has never been assessed or investigated using an iris camera, we therefore, were the first to report outcomes on this topic. These findings have shown that the iris camera may be significant when quantifying haze, evaluating treatment modalities, and monitoring disease progression in corneal diseases.
This project investigated corneal clarity and haze in various keratoconic patients depending on the type of refractive procedure used to manage the disorder. Some of the remedies used to manage, halt or treat keratoconus, which we assessed, include corneal collagen cross-linking (CXL) and Penetrating Keratoplasty (PK); a form of corneal transplantation. We looked at how the iris camera’s corneal opacification measurement (COM score), corneal densitometry and visual acuity changed over one year following these treatments. Our studies found higher COM scores and corneal densitometry measurements at each baseline visit compared with follow-up appointments. We further collated COM scores between patients with untreated keratoconus and healthy controls with significantly higher values indicated in the keratoconic patients while the latter had negligible estimates. All our investigations demonstrated some degree of haze in keratoconic corneas with variations of values before, in-treatment and post-treatment. These findings also showed that these treatment modalities could have contributed to haze formation on the cornea due to wound healing response or inflammatory activities after the procedures. We, therefore, tried to associate these changes in haze levels to visual outcomes over time. Chapter 2 of this thesis investigated corneal clarity in treatment-naive KCN eyes and compared outcomes with healthy eyes. The outcomes of our investigation revealed that corneal haze in keratoconic eyes (from both iris camera and Pentacam) was significantly higher compared with age-matched controls with healthy eyes (Anayol et al., 2016; Bernardo Lopes, Isaac Ramos and Renato Ambrósio, 2014). A study by Lopes et al (2014) reported that when keratoconic eyes were compared with normal ones, the densitometry readings were higher in the former than in healthy eyes indicating that light absorption in healthy eyes were minimal and therefore, decreased light scatter (Bernardo Lopes, Isaac Ramos and Renato Ambrósio, 2014; Otri et al., 2012). Further findings have suggested that the measured difference between a healthy eye and a keratoconic one is a clinical indication or pointer towards the level of progression or severity of the disease (Kreps et al., 2020). Anayol and associates assessed the aggregate scatter (total densitometry) in different corneal annular zones among treatment-naïve keratoconus patients with various stages of the disease and compared these findings with data obtained from controls and indicated that notable variance existed between keratoconic eyes and normal eyes (Anayol et al., 2016). Their study further stated that even keratoconic patients with no clinically significant corneal haze still had higher densitometry measurements than that of a healthy eye.
comparable to findings by (Shen et al., 2016). Our BCVA measurements were found to worsen at one-year follow-up similar to results (Shehata et al., 2020). Reports explained that this decline in visual acuity is due to degenerative changes that alter the corneal structure and composition as KCN progresses (Espandar and Meyer, 2010; Silverman et al., 2014). This also supports our theory that KCN progression increases light backscatter in the cornea over time, which eventually leads to increased haze (Dohlman, Yin and Dana, 2019; Jiménez-García et al., 2021a; Ní Dhubhghaill et al., 2014).

Chapter 3 of this thesis investigated the outcomes of one of the most common techniques used to treat KCN; Corneal Collagen crosslinking (CXL). Numerous studies have reported CXL’s impact on stabilising the cornea, strengthening its structure, and halting the progression of KCN (Saad et al., 2020a; Wollensak, Spoerl and Seiler, 2003). Studies have also reported the formation of corneal haze following treatment of KCN with CXL. Some other studies have looked at postoperative CXL-induced corneal haze; assessing changes in corneal clarity over time, and concluding that corneal haze subsequently decreased, observing a significant reduction after one year of treatment (Greenstein et al., 2010; Kim et al., 2016). The study areas were the 0-2mm annulus of the anterior corneal layers where the effect of CXL has been observed to be highest with increased densitometry measurements (Pircher et al., 2015). Our study using the COM score and Pentacam Scheimpflug densitometry measurements agrees with these studies demonstrating that haze levels differed between baseline visit and follow-up. These same findings were seen in another investigation evaluation haze in standard and accelerated CXL procedures and indicated that clinically-reported haze had decreased to approximately 3.6% and 4.5% at the 1-year follow-up visit (Kandel et al., 2021). Shen and associates in a retrospective study assessed changes in corneal densitometry examined cross-linked KCN patients pre and postoperatively using the Pentacam observing them up to 12 months post-CXL demonstrating similar findings (Shen et al., 2016). Findings from their investigation in KCN patients after a year of CXL showed a notable decline in densitometry values comparable to our results. We showed that the COM score and densitometry measurements were elevated within the first three months following CXL but resolved over time with significantly lower measurements at the follow-up visit which concurred with reports by (Akkaya Turhan and Toker, 2017). This increase in COM score and densitometry is due to transient haze associated with the procedure (loss of keratocytes and oedema) (Koller, Mrochen and Seiler, 2009;
Mazzotta et al., 2007). Reports by Greenstein et al (2010); Seiler and Hafezi (2006) suggest that after CXL treatment, visual acuity often declines initially and improves afterwards; however, the consensus on the exact relationship between loss of corneal transparency and reduction in visual acuity is still unclear with various reasons proposed. While some reports indicated no significant relationship changes or correlation between haze and vision (Chang and Hersh, 2014), (Raiskup, Hoyer and Spoerl, 2009), however, indicated that roughly 8.6% of most eyes that undergo CXL procedure lose at least two or more lines of visual acuity. Shen et al suggested that the improvement noticed in visual acuity 12 months after CXL was linked to a decrease in corneal densitometry (Greenstein et al., 2010; Kim et al., 2016).

Greenstein et al (2010); Kim et al (2016) stated on the other hand, poorer visual acuity only corresponded with the manifest (absolute) degree of haze due to disease (KCN) severity. Moreover, Pircher and associates further suggested that the initial chain of events activated soon after the CXL procedure often induced loss of stromal transparency with a consequent increase in stray light which decreased visual acuity (Pircher et al., 2015).

In our study in chapter 4, we investigated postoperative outcomes of full-thickness transplants (PK). Overall, the COM scores and corneal Densitometry values were significantly less at follow-up (one year after baseline visit). Our data on BCVA at baseline (1 month) post-PK was poorer than follow-up (Patel et al., 2008). This would suggest that following PK, the corneal haze had some impact on visual acuity. Patel et al (2008) further associated the increased postoperative backscatter with elevated levels of forward light being scattered intraocularly with accompanying decline in VA. However, some reports have suggested that the postoperative decline in vision after PK resulted from uncorrected high-order aberrations and not a factor of haze (McLaren and Patel, 2012; Patel et al., 2007). Our study also demonstrated that the average postoperative BCVA was better at the follow-up visit and reached statistical significance (Henein and Nanavaty, 2017; Lim, Pesudovs and Coster, 2000). Our data indicated that scattered light (haze) was associated with visual degradation post-PK. Some reports have argued that the opacity seen in the cornea following PK was not necessarily induced by backscattering or forward scattering but rather a combination of subclinical elements such as keratocytes and endothelial cell density (Kobashi, Kamiya and Shimizu, 2018). Although the transparency of the cornea
is suggested to be a function of the degree of light scattered and absorbed (Dohlman, Yin and Dana, 2019), the issue of backward and forward scattering of light and haze in the cornea is unclearly correlated and its relationship with visual performance especially Post-PK is shrouded in controversies with various studies indicating different outcomes. These gaps would be worth investigating in future prospective studies to make conclusive remarks.

Over time, the concept of corneal transparency and its fundamental role in vision have been the subject of numerous discussions and intriguing reviews. Despite several theoretical and in-vivo investigations, the consensus suggests that the cornea's transparency is a direct consequence of the organisation of its structural and physiological components, particularly the corneal stroma (Knupp et al., 2009; Meek and Knupp, 2015). The stroma forms a significant portion (90%) of the cornea and constitutes predominantly keratocytes and type I and V collagen bundles rich in extracellular matrix (ECM) (Quantock and Young, 2008). Stromal keratocytes lie between the lamellae of the collagen bundles and are responsible for synthesising the ECM (Hassell and Birk, 2010). The development of the stroma and its unique properties are initiated by the keratocytes (Hassell and Birk, 2010; Jester et al., 1999).

Keratocytes are made of water, proteoglycans, glycoproteins, and inorganic salts. These keratocytes secrete crystallins which play critical roles in corneal transparency by minimising the amount of light scattered by the cornea (Jester et al., 2005). The anterior cornea is more populated with keratocytes than its portion (Bron, 2001). Dysfunction of the keratocytes, collagen fibres, ECM, proteoglycans, or abnormality in stromal hydration from the endothelial pump process would interfere with proper corneal function and lead to a loss of transparency (Bourne, 2003; Qazi et al., 2010). Several factors can alter the transparency of the cornea. Some of these factors could be ageing or degenerative effects of disease on corneal structure and function (Alzahrani et al., 2017; Garzón et al., 2017; Ní Dhubhghaill et al., 2014; Otri et al., 2012). Surgical procedures such as CXL and the process of wound healing following corneal treatment can also alter its transparency (Asri et al., 2011; Jester et al., 2012; Patel et al., 2008).

To preserve corneal integrity and transparency following surgical procedures, the corneal needs to heal properly via a cascade of related events and mechanisms to remodel the cornea (Ljubimov and Saghizadeh, 2015). According to Azar (2006) and Ellenberg et al (2010), following any corneal
procedure, the pattern, and differences by which wound healing occurs will be a crucial determinant of how corneal haze recedes how transparency is restored. This complex healing process is often activated by the keratocytes forming corneal fibroblasts close to the location of the injury, as well as entry of fibrocytes from blood vessels from the limbus (Wilson, 2020b).

Keratocytes apoptosis in the stroma is the first noticeable change identified following corneal injury (usually takes place within a few minutes) and often next to the injury site. It is suggested that the amount of keratocytes apoptosis is dependent on the extent of the injury (Mohan et al., 2003). Typically, in the absence of injury or disease, stromal apoptosis is strongly maintained via homeostasis. However, in an injury, the process becomes crucial to the wound healing response (Wilson, Chaurasia and Medeiros, 2007). The wound healing in the cornea is made up of complex interactions between growth factors, cytokines, and chemokines. The stroma's healing depends on the extent of injury, recovery of the epithelium and regeneration of the epithelium basement membrane (EBM) (Wilson, 2020a). Subsequent regeneration of the epithelial layer, restoration of the EBM and elimination of myofibroblasts antecedents lead to the maintenance or re-establishment of corneal transparency (Lassance et al., 2018; Marino et al., 2017; Torricelli et al., 2013). Eventually, the corneal stroma is repopulated with keratocytes, disorganised ECM is reabsorbed, and the restoration corneal transparency (Hassell and Birk, 2010; Torricelli et al., 2013). For corneal transparency to be maintained, the diameters of the collagen fibres need to be uniform, and the range of space connecting adjoining fibrils needs to be minimal.
5.2 Limitations of the Project

There are several limitations to this project, notably the sample size in some studies. This was due to patient availability and time constraints due to the pandemic (Covid-19) during recruitment in the clinic. A major limitation was the gap between the baseline measurements and follow-up measurements. Although significant findings were indicated, however, more accurate could’ve been gotten had these patients been imaged on a tri-monthly basis to see the changes in haze. Longer and repeated follow-up measurements would have been ideal to assess haze in these patients to reduce variability, and make the study efficient for more significant outcomes. Larger study sample sizes are recommended to achieve greater power, indicate better study effect (true effect), increase reliability and reduce bias. A further limitation to this project was the nature of some of the studies. We took an observational approach in the study investigating healthy control eyes against diseased eyes as there was no retrospective data on the iris camera’s COM score for these patients with KCN. It would have been useful to compare this data with corneal densitometry values to assess similarities, nevertheless, future studies could look into analysis. The selective use of information from particular concentric corneal zones could be a limitation as measurements from the anterior, central and posterior zones of the cornea might have altered the outcomes of our study. Inclusion of such data in future analysis could strengthen results. Also the absence of topographic, tomographic, topometric and aberrometric information from the Pentacam database were not included, examined or compared. Inclusion of these parameters, together with the densitometry measurements could give additional information on keratoconus both in treatment-naïve cases and with regards to surgical procedures. This could aid in the prediction and analysis of the natural history of corneal haze with reasonable accuracy. In light of these shortcomings, future studies could be aimed at incorporating these parameters with the iris camera COM score would provide much more detail and improve our knowledge on the cornea in ectatic disorders as well as other diseases. Future studies assessing iris camera and Pentacam imaging in keratoconus participants should take this into account and make the necessary adjustments accordingly. Also, vision testing and refraction was done by different individuals at each patient visit and the possibility of a specific individual conducting each visual test was not practical. this might have impact our vision data been different had it been performed by a delegated individual.
5.3 Future Studies

A further expansion of this work is recommended to understand better the factors that lead to corneal haze.Outlined below are summaries of potential approaches:

- The use of the iris camera’s COM score to investigate corneal dystrophies such as Fuchs Endothelial Dystrophy.
- Investigating haze in juvenile populations with KCN after CXL and making a comparison with adults to assess the difference. Larger sample sizes, power calculations for more significance and longer follow-ups to track the natural history of haze.
- Evaluating corneal haze in re-grafted patients with Pseudophakic Bullous Keratopathy (PBK) following cataract surgery and intraocular lens implantation (IOL).
- Investigating accelerated and Standard CXL protocols to know if both techniques have an effect on the amount of haze following treatment.
- An assessment of corneal clarity measurements in patients with keratoconus that have undergone DALK and comparison with PK to evaluate outcomes on visual performance.
- An investigation into pre-clinical, subclinical, form fruste and clinical KC using the iris camera and Pentacam measurements.
5.4 Conclusion

The results in this thesis include the repeatability of the COM score using the iris camera. In this thesis, the difference between the two measurements from both imaging devices were analysed and estimated in healthy eyes, treatment-naïve keratoconic patients as well as eyes that underwent treatment for KCN. The baseline values differed to follow-up values in group of participants with significant findings especially in the anterior layers. There is evidence to suggest based on our data that the iris camera’s COM score just like the Pentacam densitometry, is an objective method of quantifying corneal haze and estimating corneal clarity with relative accuracy, reliability, and precision. This technique of assessing haze can be very useful in clinical and scientific settings as an additional tool to identify, estimate haze and monitor progression in early and advanced diseases. Our project demonstrated that the COM score varied in both health and disease. Furthermore, different treatment procedures for corneal disorders will produce variations in results and can be assessed to estimate efficacy. However, these statements would need further verification. The feasibility and cost-effectiveness of integrating the iris camera into clinical assessments in the UK would need to be researched further.
References


Appendix

List of Publications

- Emma-Duru, C., Hillarby, C., Carley, F; Morley, D., Aslam, T. The Iris Camera Corneal Opacification Measurements may be a Useful Tool for Determining Disease Progression.

The Iris Camera Corneal Opacification Measurements may be a Useful Tool for Determining Disease Progression

Chimdi Emmanuel Emma-Duru; Chantal Hillarby; Fiona Carley; Debra Morley; Tariq Aslam

Author Affiliations & Notes
Investigative Ophthalmology & Visual Science July 2019, Vol.60, 2127. doi:

Abstract

Purpose: The iris camera is able to determine the clarity of the cornea and previous studies carried out in Manchester have used the iris camera to study a group of patients with Mucopolysaccharidoses; a rare metabolic disease that causes a dysfunction in the breakdown of glycosaminoglycan by the lysosomal enzymes which causes cloudiness of the cornea due to the accumulation of these proteins in ocular tissues. Normally the level of haze in these patients is hard to measure but the iris camera gave accurate and repeatable measurements. In this study we aimed to determine if the iris camera would be a useful tool to follow the progression and treatment outcome in other diseases where corneal clouding occurs.

Methods: In a clinical based study, 120 participants with keratoconus and corneal dystrophies (85 males and 35 females, aged 18-79) and 65 normal controls (45 males and 20 females, aged 18-50) were assessed in the corneal clinic at the Manchester Royal Eye Hospital. The examination of participants was performed under standard lighting with no form of dilation. During images analysis, two pinpoint areas on the pupil close to the limbal margins were selected, one superiorly and the other inferiorly. The result of the analysis was known as the corneal opacification measure (COM) score gives an estimate of the level of opacity of the cornea. The COM score was compared to visual acuity and corneal thickness to assess correlation.

Results: COM score was significantly increased in both keratoconus and dystrophy while controls had score of absolute zero (0). Although both keratoconus and dystrophy exhibited COM scores, the latter had significantly higher scores (12) compared to keratoconus (1.6). A significantly lesser correlation was observed in keratoconus between the COM score, visual acuity and corneal thickness compared to dystrophy which showed positive correlations between these corneal thickness and visual acuity with p-values <0.05.

Conclusions: All diseased groups were associated with increased levels of COM scores compared to controls. Thus, further indicating the capacity of the iris camera to estimate and measure haze levels in various corneal diseases similar to other imaging techniques, but, its low cost and ease of use could make it a preferable alternative to existing instruments.

This abstract was presented at the 2019 ARVO Annual Meeting, held in Vancouver, Canada, April 28 - May 2, 2019.
This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.
INVITATION TO PARTICIPATE IN RESEARCH

Dear Sir or Madam

A study to determine if the iris camera can be used to diagnose and monitor corneal disease and treatment outcomes

This is a letter of invitation to enquire if you would like to participate in a postgraduate research project regarding corneal diseases and corneal imaging in diseased patients in comparison to individuals with healthy eyes such as you.

Before you decide if you would like to take part it is important for you to understand why the project is being done and what it will involve. Please take time to carefully read the Participant Information Sheet attached to this letter and discuss it with others if you wish. Ask me if there is anything that is not clear, or if you would like more information. Participation in this study is completely voluntary. You will have 24 hours to consider the information and if you wish to participate in this study. If you decide to take part in this study, your participation will be kept confidential to the extent possible.

We have attached an information sheet that answers some of the questions that you might have. If you have any further questions about this work, you may contact the chief investigator by email: tariq.aslam@mft.nhs.uk or 01617014845 or the other investigator or the PhD student Chimdi.emma-duru@postgrad.manchester.ac.uk or by telephone 01612765502.

We look forward to your cooperation and hope you might find it an interesting experience.

Yours sincerely,

Chimdi Emma-Duru
On behalf of the study team

Participant Information Sheet (PIS)

Study title: A study to determine if the iris camera can be used to diagnose and monitor corneal disease and treatment outcomes

You are being invited to take part in a research study. The research is being performed in part fulfilment of a PhD degree. Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Who will conduct the research?

Chimdi Emma-Duru, Faculty of Biology, Medicine and Human science, University of Manchester and Manchester Royal Eye Hospital.

What is the purpose of the study?

The main purpose of this study is to find out if images taken of people’s eyes can be used to assess corneal clarity in patient with eye diseases.

Measuring corneal clarity is important because it could help when planning treatments or measuring how beneficial treatments have been. For example, in some diseases there is gradual reduction of corneal clarity. At present, when the cornea becomes very clouded there are no methods that gives numerical measurement of clarity which can be used along with other indicators such as visual acuity, contrast sensitivity and corneal thickness, of when surgical intervention would improve vision. Several different types of corneal surgeries are available and knowing the level of clarity would help the clinician select the best treatment for the patient. Being able to measure this reduction could help decide at what point a corneal graft may be beneficial. Also, by being able to measure corneal clarity before and after surgery, we would have another way of measuring how well the surgery has gone.

The images collected from patients will also be compared to the images taken from volunteers that do not have any eye diseases. This will help us to understand the normal changes that happen to corneal clarity as people get older.

Why have I been chosen?

You have been chosen to take part in this study because; you have a condition that can potentially affect the cornea (the window of the eye). The aim of this study is to explore the use of digital analysis of photographs of the eye to measure corneal clarity and assess whether this would be a useful diagnostic tool in routine assessment of similar eye condition to determine future treatments.
What would I be asked to do if I took part?

Digital images will be taken of each eye using the iris camera system. This procedure will be done as part of your routine assessment during your clinic visits to the corneal clinic of the Manchester Royal Eye Hospital. We are asking for permission to use these images with new software to measure the clarity of your cornea pre and post treatment.

What will happen to my personal information?
In order to undertake the research project, we will need to collect the following personal information/data about you: name and patient ID from your medical notes.

All data collected during the study such as your personal information as well as the corneal imaging data will be stored securely in the University of Manchester research office located within the eye hospital and looked after by the research team in line with the University’s policies and procedures. The research data will be kept for 10 years. This will include consent forms that contain personal data (e.g., your name and patient ID) and will be handed over to the university for archiving or storage after the study is over to be kept as long as is needed and will be destroyed as soon as they are no longer needed.

Some of the data collected during this study such as the corneal imaging data could be used to support other research studies in the future. With your permission, we would like to be able to use the anonymized data (your name and other identifiable data removed) as part of any research we carry out in the future. We would also like to be able to make this anonymized data available to other researchers working in this field. You will be able to indicate on the consent form if you are happy for us to do this.

[Only] the research team will have access to this information.

We are collecting and storing this personal information in accordance with the General Data Protection Regulation (GDPR) and Data Protection Act 2018 which legislate to protect your personal information. The legal basis upon which we are using your personal information is “public interest task” and “for research purposes” if sensitive information is collected. For more information about
the way we process your personal information and comply with data protection law please see our Privacy Notice for Research Participants.

The University of Manchester, as Data Controller for this project takes responsibility for the protection of the personal information that this study is collecting about you. In order to comply with the legal obligations to protect your personal data the University has safeguards in place such as policies and procedures. All researchers are appropriately trained and your data will be looked after in the following way:

The study team at the University of Manchester will have access to your personal identifiable information, that is data which could identify you, but they will anonymise it as soon as practical. However, your consent form as mentioned earlier will be stored securely at the University of Manchester research office within the eye hospital by the research team in line with the University’s policies and procedures. However, contact details and other personal details will be destroyed as soon as they are no longer needed.

All personal and identifiable data will be temporary stored on encrypted USBs and later transferred to the university servers for security of data. These identifiable data will however, be erased from the USBs. Hard copies will be safely filled in box system file stores and stowed away in private secure cabinets in the research office within the hospital with limited access.

You have a number of rights under data protection law regarding your personal information. For example, you can request a copy of the information we hold about you, images. This is known as a Subject Access Request. If you would like to know more about your different rights, please consult our privacy notice for research and if you wish to contact us about your data protection rights, please email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie Building, University of Manchester, Oxford Road, M13 9PL. at the University and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner’s Office, Tel 0303 123 1113.

Will my participation in the study be confidential?

Your participation in the study will be kept confidential to the study team and those with access to your personal information as listed above.

Everyone that takes part in the study will be allocated a study ID number. This ID number will be used on all the study documents and only the research team will be able to link the ID number to you. This means that only the research team will know who the data belongs to. Individuals from the University of Manchester, NHS Trust or regulatory authorities may need to access the information collected during the study to make sure the research is carried out as planned. With your permission, the data will include your name and other identifiable information. The individuals who may access the data during an audit or monitoring visit have a duty of confidentiality to you as a research participant.

What happens if I do not want to take part or if I change my mind?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still
free to withdraw at any time without giving a reason and without detriment to yourself. However, it will not be possible to remove your data from the project once it has been anonymised and forms part of the dataset as we will not be able to identify your specific data. This does not affect your data protection rights.

**Will my data be used for future research?**

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation. The future research should not be incompatible with this research project and will concern corneal research. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the [UK Policy Framework for Health and Social Care Research](#).

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you regarding any other matter or to affect your care. It will not be used to make decisions about future services available to you.

**Will I be paid for participating in the research?**

No, you will not be paid for taking part in the study.

**What is the duration of the research?**

The study and participant recruitment is expected to last for approximately 2 years (before and after treatment including follow up visits). The participant involvement is expected to take about 10 minutes which includes taking reading the information sheet and consenting to the study. Additional images taken at follow-up visits would be done whenever the patient attends the clinic until they no longer attend clinics or till recruitment is over.

**Where will the research be conducted?**

This study will be carried out at the corneal clinic of the Manchester Royal Eye Hospital.

**Will the outcomes of the research be published?**

The research is being performed in part fulfilment of a PhD degree and so some of the data will be included in the final thesis.

**Who has reviewed the research project?**

The project has been reviewed by the NHS REC/HRA Approval.

**Minor complaints**

If you have a minor complaint, then you need to contact the researcher(s) in the first instance.
MR CHIMDI EMMA-DURU, Faculty of Biology, Medicine and Human Science, Research office, Manchester Royal Eye Hospital, Oxford Road, Manchester, M13 9WL, by email: Chimdi.emma-duru@postgrad.manchester.ac.uk or by telephone 01612755271.

PROFESSOR TARIQ ASLAM (Chief Investigator), Manchester Royal Eye Hospital Division of Pharmacy and Optometry School of Health Sciences, Faculty of Biology, Medicine and Health, Manchester Royal Eye Hospital, Oxford Road, Manchester, M13 9WL, by email: tariq.aslam@mft.nhs.uk or by telephoning 01617014845.

Formal Complaints

If you wish to make a formal complaint or if you are not satisfied with the response you have gained from the researchers in the first instance, then please contact

The Research Governance and Integrity Manager, Research Office, Christie Building, University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674.

What Do I Do Now?

If you have any queries about the study or if you are interested in taking part, then please contact the researcher(s)

MR CHIMDI EMMA-DURU, Faculty of Biology, Medicine and Human Science, Research office, Manchester Royal Eye Hospital, Oxford Road, Manchester, M13 9WL, by email: Chimdi.emma-duru@postgrad.manchester.ac.uk or by telephone 01612755271.

PROFESSOR TARIQ ASLAM (Chief Investigator), Manchester Royal Eye Hospital Division of Pharmacy and Optometry School of Health Sciences, Faculty of Biology, Medicine and Health, Manchester Royal Eye Hospital, Oxford Road, Manchester, M13 9WL, by email: tariq.aslam@mft.nhs.uk or by telephoning 01617014845.
This Project has been approved by the NHS REC/HRA Approval

[ERM reference number]

Participant Consent Form for healthy volunteers

Title of Research

A study to determine if the iris camera can be used to diagnose and monitor corneal disease and treatment outcomes

If you are happy to participate, please complete and sign the consent form below

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<thead>
<tr>
<th>Activities</th>
<th>Initials</th>
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<tr>
<td>1  I confirm that I have read the attached participant information sheet (Version XX, Date XX-XX-XXXX for the above study and have had the opportunity to consider the information and ask questions and had these answered satisfactorily.</td>
<td></td>
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<tr>
<td>2  I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving a reason and without detriment to myself.</td>
<td></td>
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<tr>
<td>3  I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis.</td>
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<tr>
<td>4  I agree that the data collected, including corneal images, may be published in anonymous form in academic books, reports, or journals as part of this research project. I understand my identity will not be revealed in any publication.</td>
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<tr>
<td>5  I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.</td>
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<tr>
<td>6  I understand that data collected during the study may be looked at by individuals from the University of Manchester, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data.</td>
<td></td>
</tr>
<tr>
<td>7  I agree to take part in this study.</td>
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</table>

Data Protection

The personal information we collect and use to conduct this research will be processed in accordance with data protection law as explained in the Participant Information Sheet and the Privacy Notice for Research Participants.
(1 copy for participant, 1 for researcher, 1 for medical records)
Dear Dr Chantal Hillarby

9 November 2018

Dr Chantal Hillarby
Principal Research Scientist and Honorary Lecturer, Division of Pharmacy and Optometry
School of Health Sciences
Faculty of Biology, Medicine and Health Stopford Building, University of Manchester, Oxford Road M13 9PT

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above-referenced study, on the basis described in the application form, protocol,
supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the “*summary of assessment*” section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a ‘green light’ email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed [here](#).

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.
Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

**How should I work with participating non-NHS organisations?**

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

**What are my notification responsibilities during the study?**

The document “After Ethical Review – guidance for sponsors and investigators”, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics and is updated in the light of changes in reporting expectations or procedures.

**I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?**

You should work with the applicant and sponsor to complete any outstanding arrangements, so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Lynne Macrae
Tel: 0161 275 5436
Email: lynne.k.macrae@manchester.ac.uk
Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is 227987. Please quote this on all correspondence.

Yours sincerely

Michael Pate
Assessor
Email: hra.approval@nhs.net

Copy to: Ms Lynne Macrae – University of Manchester – Sponsor contact
Ms Elizabeth Mainwaring – Manchester University NHS Foundation T
Trust – Lead NHS R&D contact.
List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

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Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

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<tr>
<td>2.1</td>
<td>Participant information/consent documents and consent process</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>3.1</td>
<td>Allocation of responsibilities and rights are agreed and documented</td>
<td>Yes</td>
<td>A statement of activities has been submitted and the sponsor is not requesting and does not expect any other site agreement to be used.</td>
</tr>
<tr>
<td>4.2</td>
<td>Insurance/indemnity arrangements assessed</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>4.3</td>
<td>Financial arrangement assessed</td>
<td>Yes</td>
<td>No funding to site.</td>
</tr>
<tr>
<td>5.1</td>
<td>Compliance with the Data Protection Act and data security issues assessed</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>5.2</td>
<td>CTIMPS – Arrangements for compliance with the Clinical Trial Regulations assessed</td>
<td>Not Applicable</td>
<td>No comments</td>
</tr>
<tr>
<td>5.3</td>
<td>Compliance with any applicable laws or regulations</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>Section</td>
<td>Assessment Criteria</td>
<td>Compliant with Standards</td>
<td>Comments</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>6.1</td>
<td>NHS Research Ethics Committee favourable opinion received for applicable studies</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>6.1</td>
<td>CTIMPS – Clinical Trials Authorisation (CTA) letter received</td>
<td>Not Applicable</td>
<td>No comments</td>
</tr>
<tr>
<td>6.1</td>
<td>Devices – MHRA notice of no objection received</td>
<td>Not Applicable</td>
<td>No comments</td>
</tr>
<tr>
<td>6.4</td>
<td>Other regulatory approvals and authorisations received</td>
<td>Not Applicable</td>
<td>No comments</td>
</tr>
</tbody>
</table>

**Participating NHS Organisations in England and Wales**

*This provides detail on the types of participant NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.*

One participating NHS organisation; therefore, one site type.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.
## Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).

A local collaborator should be in place at site and this has been confirmed as Mr Tariq Aslam.

GCP training is not a generic training expectation, in line with the [HRA/HCRW/MHRA statement on training expectations](#).

## HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken.

For researchers not already holding a contract with the participating site, a Letter of Access would be expected to conduct the activities listed in IRAS.

Evidence of standard DBS and OH clearance would be expected.

## Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.

The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.