Corneal Imaging and Densitometry Measurements to Monitor Disease Progression or/and Treatments Outcome

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List of Abbreviations

(ANOVA) Analysis of variance

(ART) Ambrósio Relational Thickness

(BCVA) Best Corrected Visual Acuity

(BE) Back Elevation

(CL) Contact Lens

(CLEK) Collaborative Longitudinal Evaluation of Keratoconus

(CXL) Corneal Cross-Linking

(CD) Corneal Densitometry

(CCT) Central Corneal Thickness

(CKI) Central Keratoconus Index

(COR) repeatability coefficient

(DM) Descement's Membrane

(DALK) Deep Anterior Lamellar Keratoplasty

(DSAEK) Descemet's Stripping Endothelial Keratoplasty

(ECD) Endothelium Cell Density

(ECM) ExtraCellular Matrix

(FED) Fuchs Endothelial Dystrophy

(GAGs) Glycosaminoglycan's

(IOL) IntraOcular Lens

(INTACS) Intra Stromal Corneal Ring Segments

(ISV) Index of Symmetrical Variation

(IHA) Index of Height Asymmetry

(IHD) Index of Height Decentration

(KCN) Keratoconus

(KI) Keratoconus Index

(LASIK) Laser Assisted in Situ Keratomileusis

(LASEK) Laser-Assisted Sub-Epithelial Keratomileusis

(LSC) Limbal Stem Cells

(Log MAR) Logarithm of the Minimum Angle of Resolution

(μm) Micron

(MREH) Manchester Royal Eye Hospital

(M) Mean

(Rmin) Minimum Radius of Curvature

(NREC) National Research Ethics Committee

(NHS) National Health Services

(NHSBT) NHS Blood and Transplant, UK

(OCT) Optical Coherence Tomography

(PK) Penetrating Keratoplasty

(PRK) Photorefractive Keratectomy

(BFS) Posterior Best-Fit Sphere

(P) P-Value

Quality Statement (QS)

(RGP) Rigid Gas Permeable Contact Lens

(SD) Standard Deviation

(UV) Ultra-Violate

(VA) Visual Acuity

Glossary

Aberrometry: A technique used to measure refractive errors in much greater detail than is possible with traditional clinical refraction. Aberrometry measures the shape of a wavefront of light that has passed through the optical elements of the eye. Analysing the wavefront's shape determines the amount and type of refractive error present.

Analysis of variance: Analysis of variance is a collection of statistical models and their associated estimation procedures used to analyse the differences among group means in a sample

Apex: Geometric centre of the Pentacam's (or other devices) exam. This is the first Purkinje's reflex on the cornea while having the patient fixating on the fixation target.

ART: Ambrósio Relational Thickness, which considers the Thinnest Point (TP) in relation to the PPI. ART is calculated as the ratios between the TP and the maximal PPI meridian (ART Max = TP/PPI Max) and the average (ART Ave = TP/PPI Ave).

Astigmatic Surface: A surface that has two meridians of different curvature. The Pentacam reports the astigmatism of the corneal surface in the central 3 mm of the cornea.

Axis: Axis of corneal astigmatism (red for steep, blue for flat and user selectable).

BAD: An abbreviation for the Belin-Ambrósio Display, which is a refractive screening display incorporating both anterior and posterior elevation data and pachymetric data into one unified screening screen.

Best Corrected Visual Acuity: Best possible vision a person can achieve with corrective lenses.

Best-fit Shape: A reference shape or surface (sphere, ellipse or toric ellipse) mathematically generated by the elevation topographer of which corneal elevation is measured.

Center Keratoconus Index (CKI): Pachymetry based numerical index used to assess the likelihood of a cornea having keratoconus. Elevated especially in central keratoconus.

Corneal Thickness Spatial Profile (CTSP): A graphic display of the average of the thickness values along 22 imaginary circles centred on the thinnest point, with the diameter of the circles increasing in 0.4-mm steps.

Collaborative Longitudinal Evaluation of Keratoconus: multicentre, observational study of 1,209 keratoconus patients followed for 3 years at 15 clinical centres in USA.

Corneal Cross-Linking: Corneal collagen cross-linking with riboflavin and UV-A light is a surgical treatment for corneal ectasia such as keratoconus, PMD, and post-LASIK ectasia. It is used in attempt strength the cornea.

Descement's Membrane: is the basement membrane that lies between the corneal proper substance, also called stroma, and the endothelial layer of the cornea. It is composed of different kinds of collagen (Type IV and VIII) than the stroma.

Deep Anterior Lamellar Keratoplasty: a partial-thickness corneal transplant which involves only the donor stroma, leaving the recipient's own Descemet membrane and endothelium

Descemet's Stripping Endothelial Keratoplasty : a type of endothelial keratoplasty (EK) procedure where the host endothelium and Descemet's membrane (DM) are replaced by the donor endothelium and DM, along with a small amount of posterior stromal thickness

Ectatic Change: Progressive change in the cornea associated with increasing curvature, increased elevation and often associated thinning. Ectatic change is seen in keratoconus, pellucid marginal degeneration and post refractive ectasia.

Extracellular Matrix: a collection of extracellular molecules secreted by support cells that provides structural and biochemical support to the surrounding cells.

Elevation Topography: Method of imaging the corneal surface(s) that generates an X, Y, and Z coordinate systems (locates points in space) and creates maps of corneal surface compared to a reference surface (sphere, ellipse, toric ellipse). Curvature and pachymetry maps can be computed form this elevation data.

Enhanced Elevation Maps: These are corneal elevation maps where the reference surface has been calculated after excluding an area with potential corneal abnormalities. The resulting corneal elevation map will better highlight areas of abnormal corneal elevation.

Equivalent Keratometer Readings (EKR): Used for post refractive IOL calculations, this reading utilizes both the anterior and posterior corneal surfaces to produce a graphical and tabular representation of the "adjusted" post-surgical "K" readings at varying pupil sizes.

Fuchs Endothelial Dystrophy: a non-inflammatory, sporadic or autosomal dominant, dystrophy involving the endothelial layer of the cornea. With Fuchs' dystrophy the cornea begins to swell causing glare, halo, and reduced visual acuity

Forme Fruste Keratoconus: This refers to a mild or abated form of keratoconus with few clinical signs and subtle topographical changes. It has often been used incorrectly to describe "suspect" cases without any signs or symptoms of ectatic change.

Glycosaminoglycan's: a mucopolysaccharides are long unbranched polysaccharides consisting of a repeating disaccharide unit. The repeating unit (except for keratan) consists of an amino sugar (N-acetylglucosamine or N-acetylgalactosamine) along with a uranic sugar (glucuronic acid or iduronic acid) or galactose.

Grayscale: is one in which the value of each pixel is a single sample representing only an amount of light, that is, it carries only intensity information.

Intra Ocular Lens: Intraocular lens is a lens implanted in the eye as part of a treatment for cataracts or myopia. The most common type of IOL is the pseudophakic IOL. These are implanted during cataract surgery.

Index of Height Asymmetry (IHA): This index gives the degree of symmetry of height data with respect to the horizontal meridian. This index is analogous to the Index of Surface Variance (IVA), though it is sometimes more sensitive.

Index of Height Decentration (IHD): This index is calculated from Fourier analysis of corneal height and gives the degree of vertical decentration of the apex. This value is elevated in keratoconus.

Index of Surface Variance (ISV): Gives the deviation of individual corneal radii from the mean value. This index is elevated in all types of irregularity of the corneal surface (scars, astigmatism, deformities caused by contact lenses, keratoconus, etc.).

Index of Vertical Asymmetry (IVA): Gives the degree of symmetry of the corneal radii with respect to the horizontal meridian. This index is elevated in cases of oblique astigmatism, in keratoconus or in limbal ectasias.

Intrastromal corneal ring segment: is a small device implanted in the eye to correct vision either in people with short side or corneal ectasia.

Irregular Astigmatism: Type of astigmatism where the principal meridians are non-orthogonal. This type of astigmatism is not correctable fully by spectacles.

Keratoconus: is a progressive eye disease in which the normally round cornea thins and begins to bulge into a cone-like shape

Keratoconus-Index (KI): Compares measurements of the central and peripheral corneal thickness allowing quantification of corneal thinning. This index tends to be elevated in keratoconus.

Keratometer: Also known as an ophthalmometer, is a diagnostic device used for measuring corneal curvature at a defined and set optical zone.

KMax: Point of highest curvature on the axial or sagittal curvature map.

Logarithm of the Minimum Angle of Resolution: is a unit that allows quantifying the Visual in order to perform calculations statistics such as average, deviation of the Visual acuity in a population of eyes

Mean: refer to one measure of the central tendency either of a probability distribution or of the random variable characterized by that distribution.

National Research Ethics Committee: Research Ethics Committees (RECs) review research applications and give an opinion about whether the research is ethical.

National Health Services: The publicly funded national healthcare system for England

NHS Blood and Transplant, UK: it improves the supply of donated blood, organs and tissues, and raises the quality, effectiveness and efficiency of blood and transplant services.

Optical Coherence Tomography: Optical coherence tomography is an imaging technique that uses coherent light to capture micrometer-resolution, two- and three-dimensional images from within optical scattering media.

Orbscan (Bausch & Lomb): An early device employing slit scanning elevation topography combined with a Placido topographer which provides topographic maps of the anterior and posterior corneal surfaces and images of the anterior chamber.

Pellucid Marginal Degeneration (PMD): A bilateral, non-inflammatory, peripheral corneal thinning disorder characterized by a band of thinning of the peripheral inferior cornea.

Pentacam (Oculus): A rotating Scheimpflug elevation based corneal topographer which provides elevation and curvature maps of the anterior and posterior corneal surfaces, corneal thickness maps, anterior chamber dimensions and objective readings of lens densitometry.

Penetrating Keratoplasty: is a full-thickness transplant procedure, in which a trephine of an appropriate diameter is used to make a full-thickness resection of the patient's cornea, followed by placement of a full-thickness donor corneal graft.

Photorefractive Keratectomy: is a type of refractive surgery.

P-Value: the probability of finding the observed, or more extreme, results when the null hypothesis (H_0) of a study question is true – the definition of 'extreme' depends on how the hypothesis is being tested.

Percentage Thickness Increase (PTI): A graphical display showing the percentage increase in the average thickness along 22 imaginary circles centered on the thinnest point, with the diameter of the circles increasing in 0.4-mm steps.

Placido Disk: A planar keratoscope made of concentric rings that when reflected off the cornea, permit evaluation of the smoothness and an estimation of the curvature of the cornea.

Placido Topography: Curvature based corneal topography which uses a modified Placido disk, reflected off the corneal surface. The rings of the disk are digitally measured to create “topographic” maps of corneal curvature.

PPI or PI: Pachymetric progression indexes are calculated for all hemi-meridian over the entire 360 degrees of the cornea, starting from the TP. The average of all meridians is the (PPIAve) and the meridians with maximal (PPI Max) and minimal (PPI Min) pachymetric increase are noted along with their axes.

Q-value: Also known as asphericity, this is the corneal shape factor of the cornea.

Quality Statement (QS): This is a check on the Pentacam image acquisition quality. Here “OK” means the acquired image was of sufficient quality.

Radii Minimum (RMin): Gives the smallest radius of curvature over the corneal surface.

Reference Surface: A reference shape (sphere, ellipse or toric ellipse) mathematically generated of which corneal elevation is measured.

Repeatability coefficient: is a precision measure which represents the value below which the absolute difference between two repeated test results may be expected to lie with a probability of 95%. The standard deviation under repeatability conditions is part of precision and accuracy.

Rm/Km: Mean central radius, arithmetic average of the flat (Rf) and steep (Rs) radii of curvature of the cornea.

Rper: The mean radii of the peripheral zone between the 7mm and 9mm ring.

Sagittal Curvature: Also known as axial curvature, this is a measurement of curvature at different points on the corneal surface and assumes that the radius of curvature is equal to the distance from the corneal surface to the intersection with the line of sight (or measurement axis).

Scheimpflug Principle: Principle of photography where the lens plane is not parallel to the image plane. This technique is used to correct distortion and allows for accurate cross-sectional images of the cornea.

Standard Deviation: a quantity expressing by how much the members of a group differ from the mean value for the group.

Simulated Keratometry: Keratometry values as measured by a corneal topographer. Often these are reported as always orthogonal regardless of the corneal shape.

Tangential Curvature: Also called the instantaneous radius of curvature or local curvature, measures curvature of a single point by calculating the radius of a sphere which would intersect that point.

Tomography: The recreation of a three-dimensional object by computer imaging utilizing multiple images taken from different vantage points.

Topographical Keratoconus Classification (TKC): Based on anterior corneal data, this classifies keratoconus based on the Amsler/Muckenhirn staging. This classification ignores the degree of thinning and ectatic changes on the posterior surface. This classification would classify as normal eyes with significant posterior changes associated with thinning if the anterior surface was uninvolved and is also prone to false positives in corneas with a displaced apex.

Topography: Topography implies surface contour. This term is commonly applied (and incorrectly) to curvature maps which do not technically have true knowledge of surface topography.

Total Cornea Refractive Power: Ray tracing calculation of the corneal vergence power, considering the front and back elevation data along with corneal thickness.

TP: Thinnest Point or lowest pachymetric value on the map.

True Net Power: Corneal power calculation accounting for both anterior and posterior corneal surfaces and their respective optical performance. This power measurement should not be used in standard IOL calculation formulas.

Zernike Polynomials: In ophthalmology, Zernike polynomials are a mathematical way of representing the wavefront of the eye and help quantify ocular aberrations.

Abstract

The cornea is the most anterior layer of the eye and its transparent and avascular nature allows the transmission of light to the rest of the eye. Corneal clarity can be affected by a wide range of diseases or corneal treatments. Understanding the nature of corneal haze and its causes would give a better understanding of disease progression and disease treatment outcomes. This knowledge could be useful to the clinician when deciding which form of treatment is the best for the patients. The Oculus Pentacam is a device based on the schiempflug principle which can take 50 images in 2 seconds with the ability to give a numeric value to corneal densitometry.

This project aimed to investigate corneal clarity and get a better understanding of the changes taking place which lead to corneal haze in health and disease. Using corneal densitometry software to analyse Oculus Pentacam images, we first defined how corneal clarity changes with age in healthy individual. We then identified corneal diseases and treatments that are associated with cornea haze and recruited 238 patients and followed them through diagnosis and treatment.

We found corneal clarity changes in healthy participants with age which varies based on corneal layer and zone. When we compared the outcome of 2 different types of refractive laser surgery and found that corneal clarity appears to be localised to the wound healing processes in the area of injury. Likewise, in early keratoconus the Intra Stromal Corneal Ring Segments (INTACS) stabilize corneal clarity in comparison with patients managed by RGP contact lenses alone, suggesting haze is due to disease progressions and level of inflammation from the Rigid Gas Permeable (RGP) lenses. There are different corneal clarity outcomes post keratoplasty based on different transplant procedures and the disease been treated, again haze was related to the stromal interface, where inflammation and tissue repair was active.

Corneal clarity response to disease progression, and treatment type based on different factors involved. Juvenile patients seem to react to the corneal cross linking treatment differently than adults which open more questions about safety of some procedures. Dividing the cornea into zones and layers help to get precise information on affected areas, which could help in the understanding of corneal transparency and help to improve and develop technique and treatment for corneal disease. The Oculus Pentacam provides an objective tool to monitor the cornea at different stages in health and disease.

Declaration

That no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Thesis Format

This thesis includes nine chapters which are arranged in a journal format. The third and sixth chapters had been published. The seventh chapter has been accepted for publications. The other chapters, except the second (Pentacam methodology) and the ninth (general Discussion) one, had been either submitted or prepared for submission to peer-reviewed journals.

Dedication

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents (My father's **Saad Alzahrani** and My mother's **Saleha Alzahrani**), whose love and guidance are with me in whatever I pursue. They are the ultimate role models. Most importantly, I wish to thank my loving and supportive wife, **Alia,** and my five wonderful children, **Saad**, **Ghudi**, **Tala, Fahad** and **Farah**, who provide unending inspiration. Finally I expresses my thanks to my siblings for all there help and supports.

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Chapter 1

Introduction and Literature Review

1.1 Overview of Corneal Structure

The cornea is the most anterior layer of the eye and covers the iris and pupil. It consists of transparent and avascular tissue and is responsible for allowing transmission of light (Kolli et al., 2010, Eghrari et al., 2015). The cornea mainly contains connective stromal tissues and joins the sclera through the corneal limbus. Together, the cornea and the sclera form the outermost eyeball. The central cornea has an average thickness of about 500 μm (Courville et al., 2004), while the peripheral thickness is higher than the central (DelMonte and Kim, 2011) as it is found ranged from 551 to 565um at the centre of cornea and 612 to 640 um at peripheral (Feizi et al., 2014). The horizontal cornea has an average diameter of 10 mm in infants and 11 mm in adults, while vertical diameter measures 11 mm and 12 mm, respectively (Rufer et al., 2005, Hashemi et al., 2010). According to Rufer et al. (2005), the average corneal diameter is vary between 11 to 12 in male and 10.7 to 12.58 in female. The human cornea is comprised of five layers: the corneal epithelium, anterior limiting membrane (Bowman's membrane), corneal stroma, posterior limiting lamina (Descemet's membrane) and the corneal endothelium (Waring et al., 1982, Farjo et al., 2009). Figure 1-1 shows the structure of the eye and the five corneal layers.

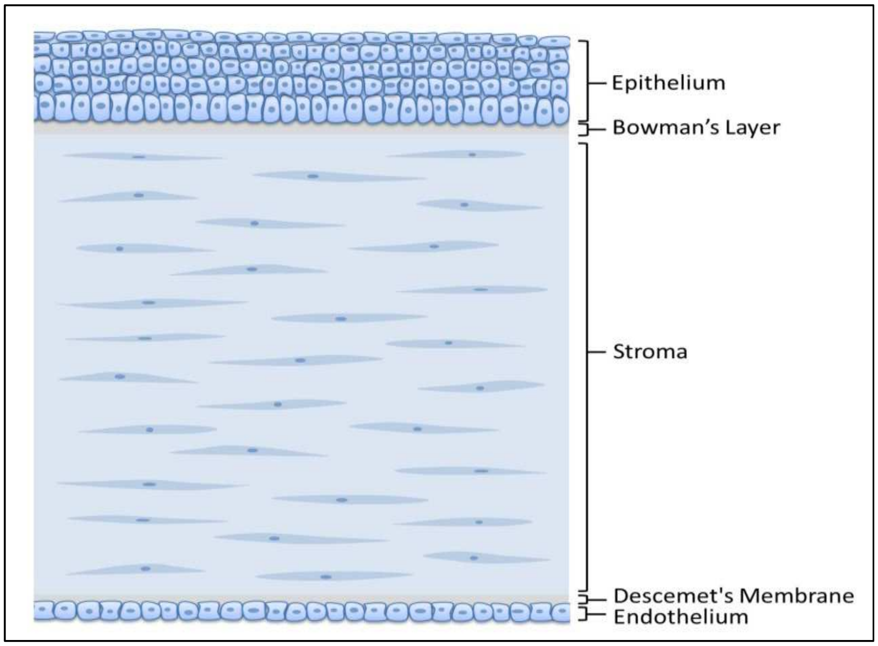


Figure 1-1:Eye structure and the five corneal layers.

1.1.1 Corneal Epithelium

Corneal epithelium is the outermost layer of the eye (Takács et al., 2009) and accounts for ten percent of the corneal tissues, measuring 50 µm to 60 µm on average, with four to six stratified squamous layers of non-keratinizing cells (Farjo et al., 2009). Its anterior surface has various microvilli that increase cellular surface area for exchange of oxygen and nutrients across the cells and is bathed by the tear film. Corneal epithelium consists of superficial cells and wing cells, each with two or three layers, and the basal cells, with one layer. The overall function of the corneal epithelium include blocking foreign material, including dust, water and bacteria from entering the eye. It also provides a smooth surface area across which oxygen and nutrients are absorbed from tears before they are distributed to the rest of the cornea (DelMonte and Kim, 2011, Dohlman, 1971).The ocular epithelium has the potential to regenerate every 7-14 days by limbal stem cells (LSC) located at the basal layer between the cornea and conjunctiva (Haddad, 2000).

1.1.2 Anterior Limiting Membrane

This layer is known as Bowman's membrane, and it is located directly beneath the corneal epithelium or between corneal epithelium and corneal stroma. Bowman's membrane has a thickness of about 15µm (DelMonte and Kim, 2011). It comprises of randomly condensed oriented collagen fibrils of both extracellular matrix as well as proteoglycans, most of which are type I collagens which is a protein encoded by the COL1A1 gene (Álvarez-Hernández et al., 2003) and type III collagens which is a protein encoded by COL3A1 gene (Janeczko and Ramirez, 1989). Both of them have smaller diameter of 20µm to 30µm compared to stromal fibres that measures 22.5µm to 35µm (Krachmer et al., 2005). Research shows that the Bowman's membrane lacks the ability to regenerate in the event of injury (DelMonte and Kim, 2011, Jacob and Naveen, 2016). The role of Bowman's membrane still remains unknown and it has been suggested that it may not have a useful function in the human cornea (Lagali et al., 2009).

1.1.3 Corneal Stroma

Corneal Stroma is the thickest layer of the cornea found beneath the Bowman's membrane. It is transparent, strong and accounts for 80% to 85% of the entire corneal thickness (DelMonte and Kim, 2011). Corneal Stroma has three different components; acellular, cellular and neurosensory. The stromal acellular component comprises of extracellular matrix collagen as well as glycosaminoglycans that mainly contain keratin sulphate and chondroitin. Although, stromal collagen fibrils occur in various types, the main type of collagen is type I and co-exists with proteins types III (encoded by COL3A1 gene) , V (encoded by COL5A1 gene)(Greenspan et al., 1992) , and VI (encoded by COL6A1 gene) in lesser amounts (Hassell and Birk, 2010).

The fibrils are organized into lamellae and they lie parallel to one another and also to the corneal surface. Research suggests that the central human corneal stroma has about 240 of lamellae (Bergmanson et al., 2005, Maurice, 1957, Naylor, 1953). Although the lamellae virtually lie at rotational angles throughout the corneal depth, there are indications for preferential orientations (Aghamohammadzadeh et al., 2004, Meek et al., 1987), a majority lie at anterior stroma which is approximately around 50% more than posterior stroma (Bergmanson et al., 2005). Surrounding the fibrils is the proteoglycans' interstitial matrix with no role in scattering light largely due to their small dimensions compared to light wavelength (Hart and Farrell, 1969). In the humans the central corneal collagen fibrils are highly homogeneous in diameter (about 32nm) with centre-to-centre spacing of inter fibrillar measuring 62 nm (Meek and Leonard, 1993).

The cellular component compresses of 2% to 3% modified fibroblasts lying between the lamellae of the stroma. The fibroblasts are thin, flat structured cells occurring in three dimensional matrixes, connected to each other by a gap junction through which they communicate. Most fibroblasts are located towards the anterior stroma. The densities, volume and their approximate sizes suggest that light passes across 100 layers fibroblasts as it travels through the cornea (Møller-Pedersen et al., 2000). Despite the fact that light propagation is likely to be affected by these cells (McCally and Farrell, 1990), there is evidence to show that intracellular crystalline proteins match the overall keratocytes' refractive index with that of stromal matrix. As a result of this delicate balance, the cell cytoplasm is kept transparent with the exception of the nuclei that contribute to specular scatter (Jester et al., 1999). It is evident that keratocytes are sparsely interspersed between stromal collagen sheets and they are responsible for maintaining as well as remodeling lying around them (West-Mays and Dwivedi, 2006), and they have the ability to scatter more light compared to the epithelium (Bonanno, 2012).

The neurosensory component has non-myelinated sensory nerves in the stroma which is important for maintaining corneal transparency. Due to organization of collagen lamella the area the anterior of stroma was found to have most of its nerves (Radner and Mallinger, 2002, Müller et al., 2001). An In-vivo study of corneal nerves using confocal microscopy has shown that the neurosensory component joins with the cornea through the stromal centre, where it forms a plexus of sub-epithelial nerves at the anterior stroma. Research suggests nerve innervation occurs in various nerve orientations, the vertical ones are the most common (Oliveira-Soto and Efron, 2001). In summary it is the extremely uniform arrangements of collagen fibrils ensure stromal transparency.

1.1.4 Posterior Limiting Lamina

This layer is also called Descement's Membrane (DM) and it is located beneath the stroma. DM is a thin, regenerating strong layer responsible for protecting the cornea against infection and injuries. The layer comprises of a basement endothelial membrane and measures about 3μm at birth and tends to increase gradually to about 10μm in thickness in adult (DelMonte and Kim, 2011, Murphy et al., 1984a). Research shows that it mainly comprises of type IV collagen, laminin as well as fibronectin (Krachmer et al., 2005), without nerve innervation. In their study, Forrester and his co- authors (Forrester et al., 2008) demonstrated that DM is tightly attached to the posterior stroma, and therefore easily affected by any change in stromal shape.

1.1.5 Corneal Endothelium

This is a single layer with identical hexagonal, squamous cells located at the posterior cornea (Komai and Ushiki, 1991). Bahn and co-workers reported (Bahn et al., 1986) that endothelium cell density (ECD) is about 6000 cells/mm^2 within a few months of birth and declines gradually between the rates of 0.5% and 0.6% annually (Bourne et al., 1997). However, one report (Forrester et al., 2008) showed ECD loss lower than 800 cells/mm^2 is likely to cause corneal stroma swelling. The tight junctions within corneal endothelium prevent the fluid from anterior chamber to leak into the stroma. McGowan and co-workers (McGowan et al., 2007) argue that the most important function of corneal endothelium is to maintain corneal hydration and transparency by pumping excess fluid outside the stroma through its active fluid pump mechanism. Corneal endothelium has active metabolic cells with large nuclei and numerous organelles such as mitochondria, endoplasmic reticulum and well-developed Golgi apparatus as well as ribosomes, indicating protein synthesis at high level.

1.2 Corneal Diseases

1.2.1 Keratoconus

Keratoconus (KCN) (Figure 1-2) is a corneal disease characterized by progressive corneal thinning and corneal protrusion, and it is usually bilateral with marked asymmetry (Krachmer et al., 1984, Mazzotta et al., 2016). As the disease progresses irregular astigmatism and high order aberration can led to reduced visual acuity (Jinabhai et al., 2010). The pathogenesis of KCN has yet to be established despite much debate and decades of research. The condition is strongly associated with atopy as well as eye rubbing (McMonnies, 2009). It has been proposed that the protrusion results from either stromal thinning or decreasing the mechanical stiffness that culminates in thinning. It is thought KCN usually presents in the teens or early 20s (Padmanabhan et al., 2014, Krachmer et al., 1984). However some find the disease can be presented earlier (Rabinowitz, 1998, Wollensak et al., 2003) or later in life (Shetty et al., 2015).

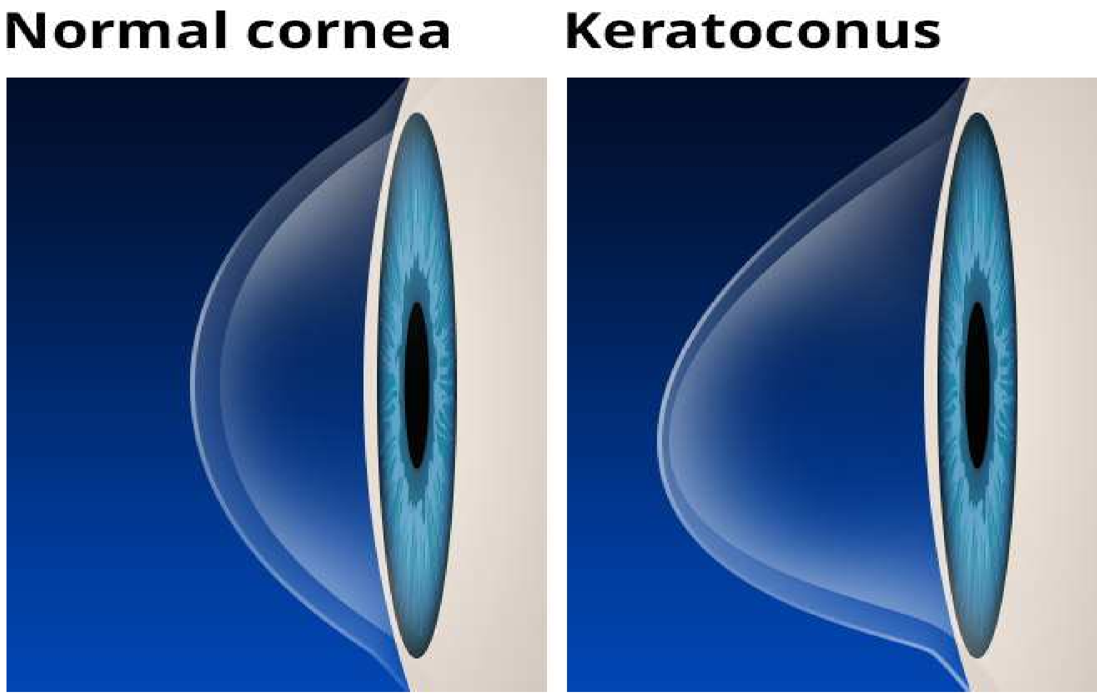


Figure 1-2:Difference between the Normal and Keratoconic Cornea (William Trattler, 2000).

KCN has a widely varied prevalence, this corneal ectatic disorder has found to be affecting approximately an average of 1 in 2000 individuals in any population, and it is equally distributed among men and women (Kuo et al., 2006, Mazzotta et al., 2008), ranging approximately from 50 to 230 per 100,000 (Kuo et al., 2006). A recent study on its prevalence in Saudi Arabia was found to be 1:21 patients (Netto et al., 2005a). It is thought to be more common in Asians than Caucasians (Vellara and Patel, 2015, De Sanctis et al., 2007) . KCN frequency in the UK proposed to be 32-65 per 100.000 of the population (Georgiou et al., 2004, Cozma et al., 2005). It is demonstrated that KCN has a higher rate in Asian (Pakistani-born) than those with Caucasian background and happened 700% in north –Pakistani born in comparison to Caucasian people (Georgiou et al., 2004) which is supported by other studies suggesting it is more prevalent in the Asian population (Pearson et al., 2000, Ziaei et al., 2012, Assiri et al., 2005). There has been controversy about which gender is more likely to be affected by the disease. One study found it is prevalent more in males (de Sanctis et al., 2008), one study suggested no difference (Mas-Aixala et al., 2016) and another study found females to be more affected by the disease (Krachmer et al., 1984).

KCN is linked to Bowman's membrane breakages and iron deposits within the epithelial basal layer known as (Fleischer rings) (Sherwin and Brookes, 2004). The protrusion of the cornea causes irregular astigmatism and/or myopia and affects visual acuity (Rabinowitz, 1998). Although its pathogenesis has yet to be established, KCN is thought to be a multifactorial disease with various contributing factors, including mechanical, genetic and environmental factors (Romero-Jiménez et al., 2010, Burdon and Vincent, 2013). Mechanical factors contributing to its development include eye rubbing, wearing contact lens, and atopy (Edwards et al., 2001). KCN mostly occurs as a sporadic disorder but some cases have autosomal recessive and autosomal dominant heritable patterns, suggesting a genetic influence to the disease (Kenney and Brown, 2003) . According to (Rabinowitz, 1998) 6 to 10% of the reported cases with KCN have a positive family history. Environmental factors could be contributing to the increase in prevalence in particular areas such as warmer countries (Gordon-Shaag et al., 2015).

It is well established that KCN can range from mild to severe and can eventually become an irreversible disability that cannot be corrected with either glasses or contact lenses. The most common classification system of KCN is the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study group (Zadnik et al., 1997, Zadnik et al., 1996a). It is an 8 years study which had taken place at malty centres in USA. The study recruited 1,209 patients who have KCN and is followed up annually aiming to prospectively categorise the changes in corneal curvature, corneal status, vision and quality of life (Zadnik et al., 1998, Wagner et al., 2007) . CLEK classified clinical cases based on keratometry readings. CLEK classified KCN severity to mild (<45D), moderate (45-52 D) and advance (>52 D) in addition to clinical slit-lamp finding as Vogts striea, fleschers ring and corneal scaring (Table 1.1).

Table 1-1: Classification of Keratoconus, as indicated by mean keratometric readings with eye data reported by percentage of the clinical finding by slit-lamp (Zadnik et al., 1996b). D = Dioptres

	Clinical finding by Slit-Lamp		
Severity of KCN	**Vogt's striae**	**Fleischer's ring**	**Corneal Scarring**
Mild (<45 D)	92/442 (21%)	151/446 (33.8%)	41/454 (9%)
Moderate (45-52 D)	428/1335 (40%)	709/1339 (53%)	290/1342 (22%)
Advanced (>52 D)	449/747 (60%)	503/745 (67%)	595/749 (67%)

1.2.1.1 Primary Managements of KCN with Contact Lens

The disease is usually asymptomatic during the early phase, and patients may only require eye spectacles or soft contact lens to correct their eyesight. With further progression, glasses are no longer effective, although rigid gas permeable contact lens (RGP) may provide improved visual acuity. Due to Contact lenses are considered the main treatment option at an early stage of KCN and it is reported to be widely used for disease management (Romero-Jiménez et al., 2010). RGP lenses are as the most typically used in KCN due to the advantages of improving VA and reducing the monochromatic, higher-order aberrations, via refractive properties of the tear-film that inevitably develops trapped underneath the lens posterior surface (Anayol et al., 2016). It was reported that close to 20 percent of KCN patients eventually require corneal transplantation (Mazzotta et al., 2008, Coskunseven et al., 2009), though more recently the introduction of a collagen cross linking procedure has proposed to reduce this number as it causes stabilization and BCVA improvement(Mohammadpour et al., 2017, Wollensak et al., 2003). According to (Sandvik et al., 2015) total KCN transplantations was reduced from 40.1% (period 2005-2006) to

11.3% (period 2013-2014) of total corneal transplant preformed at the Oslo University Hospital.

1.2.1.2 Managements of KCN with Corneal Cross-Linking

Keratoectasia, or bulging of the cornea, occurs because of increased fragility of the cornea. Thus far, however, no definite cause of this fragility has been identified. An attempt has been made in the last decade to decrease this fragility of the cornea, to increase its mechanical strength and enhance its biochemical characteristics by inducing cross-linking of corneal fibres using Ultra Violate (UV) light and riboflavin (Koller et al., 2009).

In patients who suffer from post-LASIK ectasia or keratoconus, corneal cross-linking (CXL) treatment with UVA and a photo-mediator has been successfully used to impart strength to the cornea and to halt the progression of ectasia (Toprak and Yildirim, 2013, Amsler, 1946, Koller et al., 2013). There have been positive case reports that demonstrate the mechanical benefits of cross-linking, as well as the beneficial effects of reduced visual morbidity and better corneal shape. An effort has been made in the last decade to improve the technique by making various modifications, and intensive testing has been done to minimize side effects so that the technique can be used with new patients (Kanellopoulos and Asimellis, 2014b).

1.2.1.3 Managements of KCN Intra Stromal Corneal Ring Segments (INTACS)

The main purpose of research into INTACS (Addition Technology, Fremont, California, USA) was to help patients with a low degree of near-sightedness (Figure 1-3). There is a general consensus in the scientific community that INTACS help in cases of myopia and that they can be safely removed from the eye if needed, but in cases where keratoconus is present, use of INTACS has led to flattening of the corneal surface and an decrease in visual morbidity in terms of the degree of refractive error (Kymionis et al., 2007, Wachler et al., 2003). Ectatic corneas are generally associated with steep central cones leading to a high degree of myopia, either unilaterally or bilaterally, thus further leading to unequal refractory error in both eyes. Generally, in such patients, contact lenses are not tolerated very well, and the best therapeutic option in such cases is lamellar transplant or keratoplasty (Moshirfar et al., 2006).

INTACS procedure performed under topical anaesthesia and corneal thickness should be measured and the area of the rings insertion should be 450 um at least and the keratometry reading maximum value at the steepest K not more than 58D. INTACS incision tunnels can be created either manually (TREPHINE) or via using Femtosecond Laser. INTACS segment is designed to be placed in the peripherals of the cornea which is outside the patient central optical zone at approximately tows -third depth at 6-9 mm zone which differs from Ferrara Ring Segment which is inserted at 4.40-5.60 mm (Ertan and Colin, 2007). The segment inserted typically temporal at the axis of positive cylinder through a small radial incision in the corneal stroma which may vary based on the cone position and keratoconus degree at the eye been treated (Colin et al., 2001, Siganos et al., 2003, Sansanayudh et al., 2010).

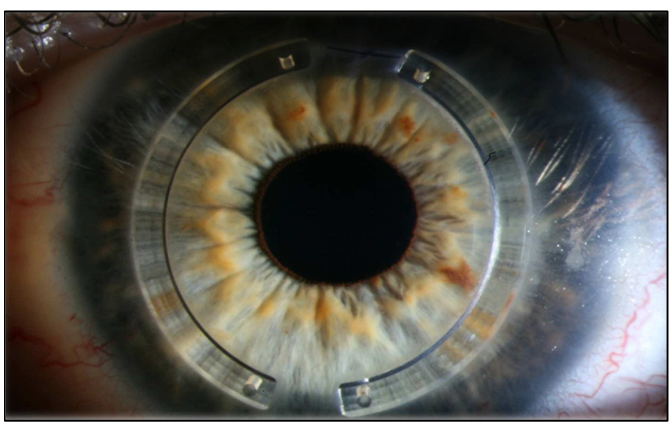


Figure 1-3:Intra stromal corneal ring segments (INTACS)(Zadnik and Lindsley, 2014).

1.2.2 Fuchs' Endothelial Dystrophy

Fuchs Endothelial Dystrophy (FED) is caused by corneal endothelial degeneration (Figure 1-4). This disorder is common in individuals aged 40 to 50 years, and usually, both eyes are affected (Eghrari and Gottsch, 2010). The prevalence of FED is difficult to estimate given its later onset, slow progression, and lack of its symptoms at its early stage. The prevalence of FED is varying in different areas in the world. Its suggested that it is effecting 4% of the population at the age over 40 in the USA and affecting females by 2.5 times more than males (Krachmer et al., 1978). In Europe 4.5%to 9% (Zoega et al., 2006) and Asia 3.8% to 8.5% (Kitagawa et al., 2002, Nagaki et al., 1996).

FED results in the development of asymptomatic corneal guttata within the Descemet's membrane (Klintworth, 2009, Schrems-Hoesl et al., 2013). The guttae are usually localized in the centre of the cornea during the early development stage of the disease and later spread towards the peripheral cornea as the disease progresses. This movement is accompanied by the loss of both hexagonal shape as well as endothelial cellular density (Elhalis et al., 2010, Schmedt et al., 2012). The degeneration of the endothelial layer results in sparse to absent cells. Moreover, both the number of Na+ and K+ ions, the sites of ATPase pump, and their ability to function are reduced (Geroski and Edelhauser, 1984, Sasaki et al., 1986) . The latter event triggers loss of iron transport activity by the endothelium, resulting in failure to ensure balance between stromal as well as epithelial edema, followed by a loss of corneal clarity (Elhalis et al., 2010, Bonanno, 2003).

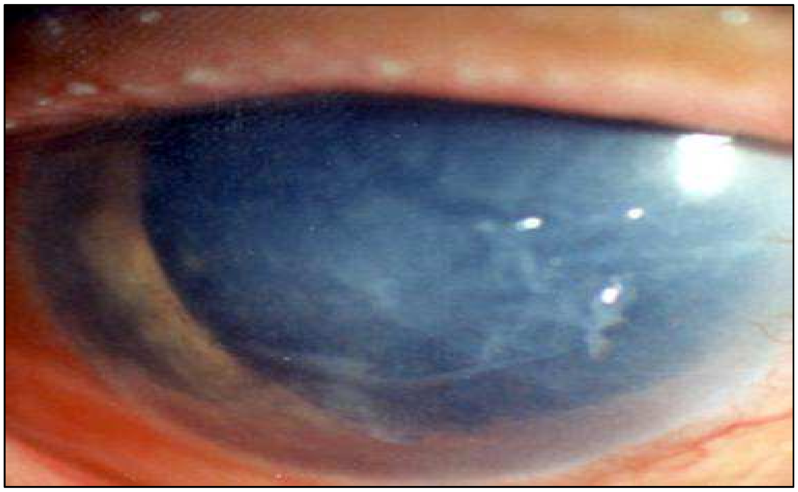


Figure 1-4:Fuchs endothelial dystrophy in a 65-year-old female (Singh, 1994).

Corneal dysfunction due to FED is associated with a reduction in progressive visual acuity and a likelihood of blindness (Klintworth, 2009). Despite the reported autosomal dominant pattern of inheritance associated with FED, in most cases, the mode of the disease progression is not precisely known (Iliff et al., 2012). FED is a multifactorial disease, and both genetic and environmental factors contribute towards its development (Elhalis et al., 2010). Although various genes and chromosomal loci are involved in the development of the disease, their precise role has yet to be established.

1.2.3 Pseudophakic Bullous Keratopathy

Cataract surgery and implantation of an intra-ocular lens is associated with an extremely painful non-treatable complication known as pseudophakic bullous keratopathy, which may develop in the post-operative period or may occur many years after surgery. It is a relatively new disease with incidence between 1% to 2% of post cataract surgery and 1.5% at patient with iris fixated IOL (Stark et al., 1983, Taylor et al., 1983).The basic pathology behind this condition is due to trauma during surgery, there is a loss of endothelial cells that causes the cornea to swell. This loss of endothelial cells leads to an asymmetrical increase in cell shape, further leading to polymorphism and polymegathism. In an eye with pseudophakic bullous keratopathy, there is also the presence of scar tissue, fibrillar deposition, and an increase in the number of collagen cells with a decrease in the number of keratocytes in the stromal layer (Liu et al., 2012).

1.3 Corneal Transplant

Corneal transplantation surgery is the most successful surgical tissue procedure in humans. It is indicated where corneal damage resulting from disease or traumatic injury culminates in vision loss. There is sufficient evidence to show that most blindness cases are caused by corneal scarring and active keratitis (Garg et al., 2005). Globally, the corneal transplant is among the most common procedures of transplantation. Although close to 100,000 procedures are performed annually, it is reported that about 10,000,000 people globally suffer from various disorders that could be treated with corneal transplantation. Typical examples of such cases include scarring, edema, and corneal thinning, as well as severe corneal distortions that have no alternative treatment except for corneal transplantation (Espana et al., 2003).

Despite the advances made in this technology, corneal graft failures are still observed, largely due to immune rejection, (Maurin and Cornand, 1989, Insler and Pechous, 1986). Inflammation of the corneal graft bed with attendant neovascularization constitutes the leading risk factor for graft rejection (Inoue et al., 2001, Rapuano et al., 1990). Neovascularization is commonly associated with various factors in corneal pathology, including trauma, inflammation, infections, and toxic insults (Kirkness et al., 1990). Various corneal transplantation techniques are described in Figure 1-5.

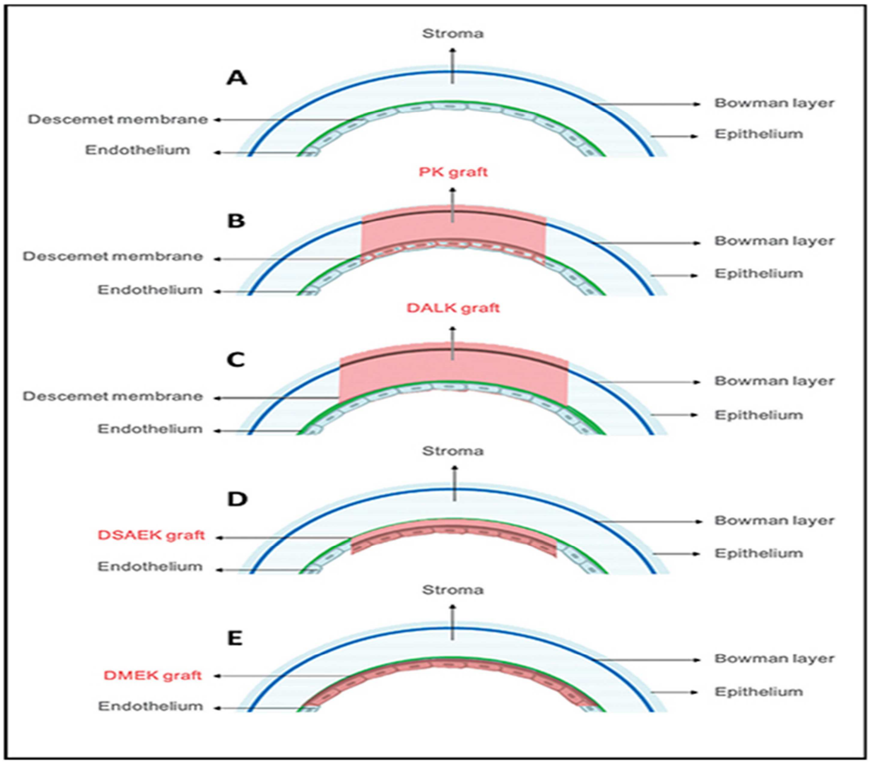


Figure 1-5:Healthy cornea and different procedures for corneal transplant (Korine van Dijk, 2014). PK (Penetrating Keratoplasty), DALK (Deep Anterior Lamellar Keratoplasty), DSAEK (Descemet Stripping Automated Endothelial Keratoplasty) ,and DMEK (Descemet Membrane Endothelial Keratoplasty).

1.3.1 Penetrating Keratoplasty (PK)

Full-thickness or penetrating keratoplasty is the most successful and most common corneal transplantation technique, which is why it makes up over ninety percent of all cornea surgeries in the U.S. (Pramanik et al., 2006). PK is indicated for various disorders, including corneal scars (central deep opacities), KCN, and disorders of the corneal endothelium that cause edema and loss of corneal clarity. It is also used in instances of reduced visual acuity that cannot be corrected by spectacles or contact lenses.

Recent reports suggest that cutting donor and recipient corneas using a femtosecond laser as opposed to traditional manual technique has various benefits, including significantly faster visual recovery because of reduced chances for induced astigmatism (Farid and Steinert, 2010). PK procedures can be performed after application of topical, local, or general anaesthesia. Lubrication and application of topical antibiotics and steroids are also provided to the patient preoperatively. Graft thickening should be monitored after the operation in order to detect the possibility of graft failure, rejection, or hypotonicity (Thompson et al., 2003, Hodge).

Suture status is vital for corneal graft visual rehabilitation. Although the PK graft may appear crystal clear, the patterns of suture may compress the graft significantly to form a highly astigmatic surface (Figure 1-6). A continuous suture is tolerable and well fixed for about 1 to 2 years, while loose sutures are likely to cause infection, irritation, and inflammation and must therefore be removed (Assil et al., 1992).

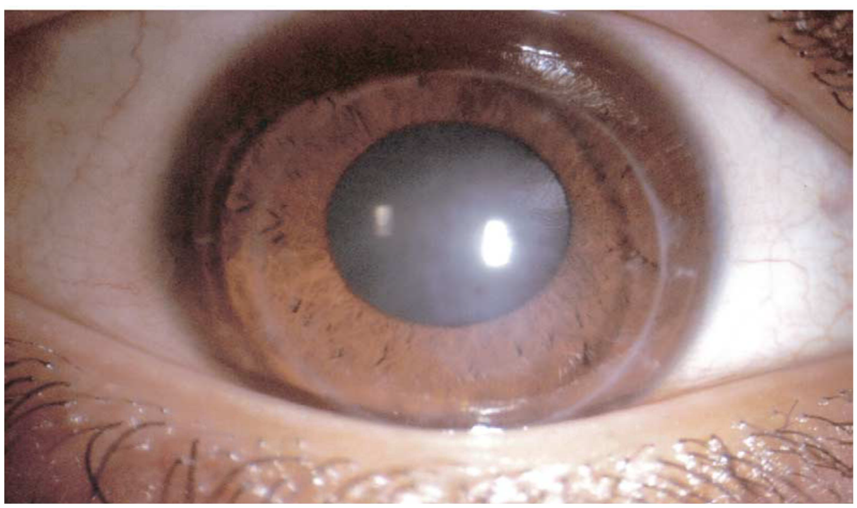


Figure 1-6:PK at 2 years post-surgery (Watson et al., 2004).

1.3.2 Deep Anterior Lamellar Keratoplasty (DALK)

Deep anterior lamellar keratoplasty (DALK) is a corneal surgical technique which entails making a partial-thickness incision up to D.M followed by dissecting the diseased cornea and replacing it with freshly donated or preserved tissue (Figure 1-7). The removal of the diseased stroma employs a big-bubble technique where the corneal stroma is dissected from the Descemet's membrane (DM) by injecting air (Anwar and Teichmann, 2002b). The method has merits, such as the early removal of sutures and decreased risk of rejection. Research shows that recovery of vision is quite faster than its improvement after PK (Borderie et al., 2009, Terry and Ousley, 2001, Yüksel et al., 2017). The graft from the donor is placed behind a deep dissection of stroma with the big-bubble technique. This also occurs when a femtosecond laser is used (Anwar and Teichmann, 2002a, Buzzonetti et al., 2010). The technique removes and replaces only the affected corneal stroma and not the healthy endothelium. Thus, the technique reduces the chances for endothelial graft rejection (Terry, 2000) and does not affect the number of endothelial cells (Watson et al., 2004, Morris et al., 1998, Panda et al., 1999). Compared to PK, DALK eliminates open system surgical complications including anterior synechia, expulsive hemorrhaging and endophthalmitis. In addition, the criteria for donor cornea selection does not have to be as strict for DALK as it does for PK (Shimazaki, 2000), excluding parametrise such as quality of the tissue (Feizi, 2014) .

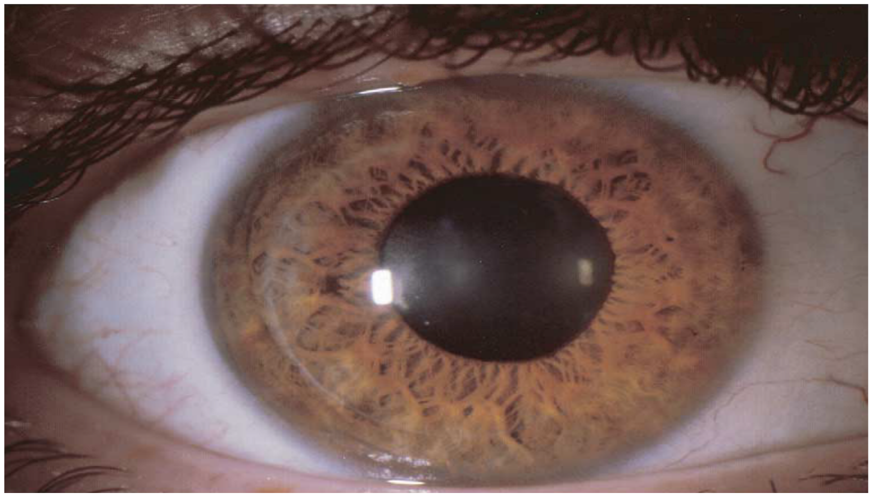


Figure 1-7:DALK at 2 years post-surgery (Watson et al., 2004).

1.3.3 Descemet's Stripping Endothelial Keratoplasty (DSAEK)

DSAEK is a type of posterior lamellar transplant technique that involves transplanting new and healthy DM and a donated endothelial layer. It is one of the more recent techniques, and it is similar to Descemet's membrane endothelial keratoplasty, both of which are associated with lower overall cellular loss in comparison with PK (Price et al., 2010, Rose et al., 2008). Moreover, the method has fewer complications than PK since it requires less recovery time, fewer refractive changes, fewer wounds and suture-related complications (Pramanik et al., 2006, Boutros and Insler, 1985, Barron et al., 1994). However, preparation of the donor disc, which is about 100 microns of tissue and some of the insertion techniques, can pose more complications (Goins, 2008).

1.4 Refractive Surgery

Refractive error refers to the disorder of the eye characterized with failure to focus on images found in the outside world clearly. The refractive surgical techniques are increasing evolving, particularly the discovery of flap-based femtosecond laser technology for laser in situ keratomileusis (LASIK) (Chen et al., 2012, Zhang et al., 2011). Such techniques have caused a paradigm shift in ophthalmic practice since most patients are able to enjoy the benefits of these technologies. For instance, changing corneal curvature to ensure controlled compensation of eye refractive errors has become more accurate (Munnerlyn et al., 1988).

The techniques have higher corneal ablation sub-micrometric precision, predictability and repeatability (Krueger and Trokel, 1985) with minimal side effects (Pettit et al., 1991). It has been shown that profiles for standard ablation employed in removing lenticules of convex-concave tissue with spherocylindrical are effective in compensating for primary refractive errors (Seiler et al., 1993). However, this procedure was accompanied with significant deterioration of visual quality, and in particular, under mesopic as well as low-contrast conditions due to increase of corneal aspherical aberration (Mastropasqua et al., 2006, Moreno-Barriuso et al., 2001). Moreover, the issue of optimizing corneal refractive procedures (Kirwan and O'Keefe, 2009) and the specific corneal layer to apply the procedures in order to derive maximum visual outcomes is still debatable.

1.4.1 Myopia

Myopia is described as a state of refraction where parallel light rays are focused in front of eyes retina (Curtin and Jampol, 1986). Despite the fact that this condition is sometimes considered a benign disorder, it currently a major health problem (Holden et al., 2015, Saxena et al., 2013) due to increasing prevalence observed in the past five to six decades (Pan et al., 2012). This has provoked a lot of attention to ensure better preventive measures. Although the condition has been linked to a genetic factor, recent studies have shown the role of environmental factors in its development. Factors such as intensive near work as well as a high educational level have been shown to augment myopic prevalence

(Morgan et al., 2012). In addition, delayed accommodation that causes retinal hyperopic defocus has been shown to accelerate axial elongation that eventually triggers onset of juvenile myopia (Berntsen et al., 2013).

Based on clinical entity, Amos (1987), classified myopia into 5 classes. The first is simple myopia, which results from development of very long axial length in the eye than optical power. Conversely, the condition occurs in the event that the optical power becomes stronger than the axial length. This myopic type generally has 6 Dioptres and below, and it may co-exist with astigmatism. Secondly is the nocturnal myopia, and it is also called night myopia. Nocturnal myopia develops as a result of increased accommodative response due to dim illumination. Thirdly is pseudo-myopia, and develops as result of excessive eye or ciliary spasm accommodative stimulation that eventually culminates into increased ocular refractive power. Patients with this condition may develop pseudo-myopia as a result of poor accommodation response. Fourth is the degenerative or pathologic myopia and it is characterized by high degree of myopia with concurrent posterior eye segment and degenerative changes such as retinal detachment. Research has shown that degenerative myopia causes serious public health issues arising from most cases that develop pathological signs (Morgan et al., 2012). Fifth is the induced myopia, also called acquired myopia, and it is usually a temporal, reversible condition that results from pharmaceutical exposures, unstable blood sugar or crystalline lens-nuclear sclerosis. Another classification by (Chaurasia et al., 2009) included congenital, youth, early adult and late adult myopia.

Myopic eyes have longer axial lengths as well as vitreous chamber depths in comparison to emmetropic eyes. Eyes with longer axial lengths usually have higher cup to disc ratio ratios, increased defects of optic nerve fiber layer and increased lamina cribrosa deformity and thereby more vulnerable to glaucomatous optic disc changes (Fong et al., 1990). Recent familial studies suggest a definite genetic and strong genetic basis for high and low myopia respectively (Young, 2009). It has also been established that environmental factors as well as near work which is also likely to be the main risk factor contributing to myopic development (Feldkämper and Schaeffel, 2003).

1.4.2 Hyperopia

This condition is also called farsightedness or long sightedness, and is a refractive eye disorder where parallel light rays entering the eye is cast behind the retina during relaxed accommodation, hence formation of blurred image (Azar, 2006).

Based on aetiology, hyperopia can be grouped as an Axial hyperopia which is the most common form that develops when eyeball axial length decrease. Curvatural hyperopia develops when corneal curvature, lens curvature or both flatten and reduce the eye refractive power, resulting in increased curvature radius by 1mm, and thereby causing 6D hyperopia. On the other hand, index hyperopia is caused by induced changes in lens refractive index due to aging while positional hyperopia is caused by posterior placement of crystalline lens, Aphakia, hence occurrence of high hyperopia (Azar, 2006, Alnawaiseh et al., 2017).

Based on clinical classifications, hyperopia is divided into simple hyperopia, caused by normal biological variation and can be axial as well as refractive. Pathological hyperopia results from abnormal ocular anatomy, which is caused by axial development and ocular disease or trauma as well. Functional hyperopia is caused by accommodation paralysis (Benjamin, 2006). Based on refractive error degree, hyperopia can be classified as low hyperopia, with refractive error of +2.00 D and below; moderate hyperopia, with refractive error ranging between +2.25 to +5.00 D; and high hyperopia, with refractive error above +5.00 D (Amos, 1987). Regarding accommodation, hyperopia can be grouped into facultative hyperopia, which is overcome by accommodation and absolute hyperopia, which cannot be compensated by accommodation (Gorovoy, 2006). Finally, hyperopia can be classified on the basis of non-cycloplegic or cyclopegic refractive outcome as manifest hyperopia, which occurs as either facultative or absolute manifest hyperopia that can be determined based on non-cycloplegic refraction. Latent hyperopia is diagnosed based on cyclopegia alone and it is accommodative compensated.

1.4.3 Astigmatism

Astigmatism refers to a refractive error that arise when parallel light rays incident to the non-accommodating eye does not converge at the retina. The condition is classified as regular and irregular astigmatism. Regular astigmatism is caused by the occurrence of the principal meridians positioned at 90^0 to one another (Elkington et al., 1999). Etiologically, regular astigmatism is further classified into corneal, lenticular and retinal astigmatism, of which corneal astigmatism is the most prevalent (Alnawaiseh et al., 2017). Based on the axial or angular intersection of two principal meridians, regular astigmatism is classified into With the Rule; corrective measure requires concave or convex cylinders with 180 +/- 20 degree or 90 +/- 20 degree respectively. Against the Rule, is in which corrective measure requires convex or concave cylinders with 180+/- 20^0 or 90 +/- 20^0 respectively. Research has shown that principal meridians due to oblique astigmatism are either at 45^0 and 135^0 or thereabouts (Alnawaiseh et al., 2017).

Regular astigmatism can further be classified based on the two focal lines' position and the retina. Simple astigmatism is where one ray is focused on the retina while another is focused in front of the retina (simple myopic astigmatism) or rather at behind of the retina (simple hyperopic astigmatism). In compound astigmatism, the two meridians are focused before the retina (compound myopic astigmatism) as well as both meridians are focused on the back of the retina (compound hyperopic astigmatism). For mixed astigmatism, one meridian is focused before the retina while the other is focused behind the retina (Katz and Kruger, 2009). Irregular astigmatism, on the other hand, occurs when the principal meridians do not occur at 90 degrees to one another and therefore cannot be corrected satisfactory using spectacles (Elkington et al., 1999).

1.4.4 Corneal Refractive Surgery

Refractive surgery has now become a popular procedure in adults. Although, the Food and Drug Administration has only granted approval for laser refractive surgery among people aged above 18 years, there are some instances where children are treated. Typical examples include children with conditions like bilateral high refractive error and unilateral severe anisometropia coupled with amblyopia whereby cases are unable to wear glasses or as well as contact lenses, hence refractive surgery becomes the last option (Stephenson, 2010).

1.4.5 Photorefractive Keratectomy (PRK)

Photorefractive keratectomy (PRK) (Figure 1-8) is an excimer laser technique used to reshape the cornea for treating refractive errors. PRK is usually indicated for patients with low myopia and hyperopia degrees. Follow up studies among low and moderate myopic patients have showed a one year refractive stability that was sustainable at 12 years (McAlinden, 2012, Vayr et al., 2007). Photorefractive keratectomy surgical technique is associated with complications such as under-correction and over correction, to poor night vision as well as corneal scarring (Seiler et al., 1994, Shojaei et al., 2009) . Permanent loss of vision is very rare. However, the method is typically for patients with stable refractive error, has no eye disease and is above 18 years. It is worth mentioning that the discovery of laser-assisted in situ keratomileusis (LASIK) was accompanied with reduced use of PRK (Duffey and Leaming, 2005).

1.4.6 Laser Assisted In Situ Keratomileusis (LASIK)

LASIK was first described by Pallikaris in 1990 a microkeratome was employed to cutting a hinged corneal flap to allow stromal bed ablation using an excimer laser before finally flap repositioning (Pallikaris et al., 1990, Pallikaris et al., 1991). Globally, this technique is currently the most common elective surgical procedure since it involves virtually painless surgical procedure recovery of vision within a short time compared to compared to PRK (Reinstein et al., 2012).

A hinged flap of cornea measuring 80 to 200μm in thickness, comprising the corneal epithelium, Bowman's membrane and anterior stroma, is created by using a microkeratome (Figure 1-8). The flap is made thin to prevent damage of surface tissue as well as limit the scarring response. At later stages, excimer laser is used to correct ametropia by altering the curvature of the cornea. Mechanical microkeratome creation of corneal flap creation increases intraocular pressure to more than 60 mmHg compared to that of femtosecond laser that has much lower increments of intraocular pressure and therefore reduced risk of optic nerve damage in the head and ischemia(Chaurasia et al., 2010). However, the later procedure requires more time besides the reported higher diffused lamellar keratitis incidences and other complications (Gil-Cazorla et al., 2008).

The merits of both PRK and LASIK have been documented, and highlight their similarities in the accuracy and safety in treating low to moderate myopia and cases where contemporary techniques were used, including wavefront-guided treatments as well as FSL flap creation (Shortt et al., 2013, Shortt and Allan, 2006, Ang et al., 2009). Nonetheless, there is sufficient evidence that deeper corneal cut employed in LASIK has significantly improved preoperative patients' screening by minimizing the risk due to ectasia (Randleman et al., 2003).

1.4.7 Laser Assisted Sub-Epithelial Keratectomy (LASEK)

Laser-assisted sub-epithelial keratomileusis (LASEK) is a surface ablation technique that is a modified form of photorefractive keratectomy (PRK) technique, and uses ethanol for creating epithelial flap which is usually repositioned after surgery. The technique result in reduced pain ,which promotes recovery of vision faster with less haze (Vinciguerra et al., 2003) since it provides stromal protective mechanical barrier against growth factors within the tear film (Lee et al., 2002) .

Litwak et al. (2002) contested the aforementioned advantage while the viability of the epithelial cell removal has raised questions such as the readhesion with the absence of the basement membrane and bowmen's layer on the stroma (Espana et al., 2003). LASEK is indicated for patients with thin corneas; those predisposed to trauma such as military personnel and as well as athletes (Azar et al., 2012). A Cochrane report suggested that LASEK was preferable to PRK (Li et al., 2012b) . In terms of postoperative visual outcomes together with the associated complication rates, (Kirwan and O'Keefe, 2009) reported LASEK induced less aberrations compared to LASIK, since the total high order aberration (HOA) together with vertical coma were far much greater and the likelihood of superior customized ablation (Dastjerdi and Soong, 2002).

In general, LASEK is currently a viable alternative of refractive surgery since it avoids complications related to flap as in LASIK as well as the recovery of vision in PRK. Furthermore, the technique has similar predictability, efficacy, safety and satisfaction of patients compared to PRK (Hashemi et al., 2004) and the use of LASIK (Kaya et al., 2004) in treating mild and moderate myopia. It is also reported that LASEK compares well with epi-LASIK in terms of effectiveness when used for correcting myopia (Bilgihan et al., 2008). In their study, Pirouzian and colleagues (Pirouzian et al., 2004) found no difference in patient satisfaction regarding postoperative visual acuity between PRK and as well as LASEK. On the contrary, half the patients showed preference of PRK over LASEK since the former required shorter surgical time while the other half showed preference for LASEK since it does not use epithelial scrubber as in the case of PRK procedure (Pirouzian et al., 2004) (Figure 1-8) shows the different steps for the above 3 refractive surgery.

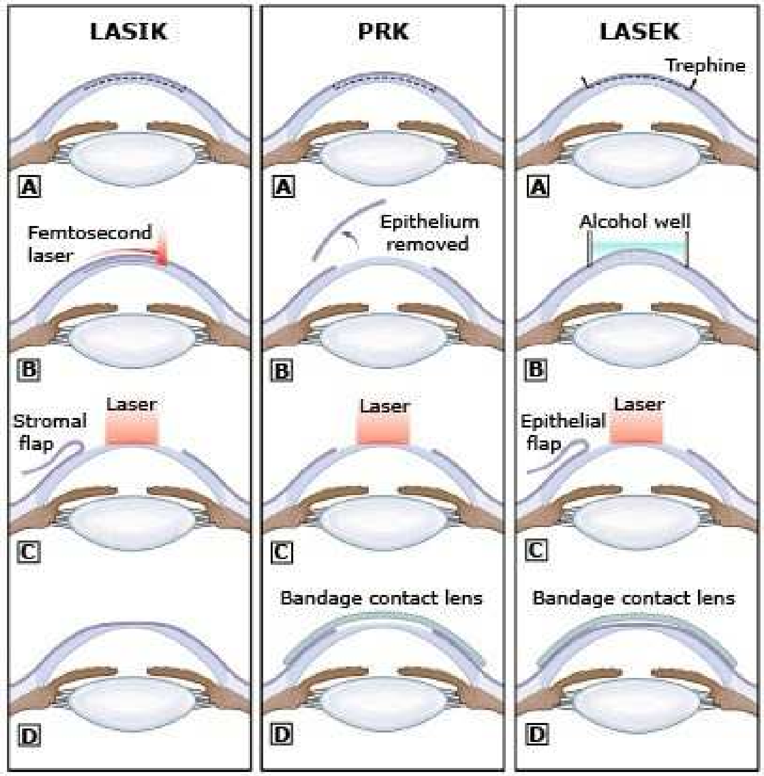


Figure 1-8:Different treatment steps for LASIK, PRK and LASEK procedures (Chandna, 2017).

In PRK (A) numbing drops (B) epithelium removed by chemical, mechanical or laser, (C) laser sculpting and (D) Bandag contact lens introduce to help in corneal surface healing. In LASIK, (A) numbing drops (B) flap creation using Femtosecond laser, (C) laser sculpting and (D) flap repositioning. In LASEK (A) numbing drops and Trephine (B) flab created using alcohol, (C) laser sculpting and (D) flap repositioned and bandage contact lens introduce to help in corneal surface healing.

Chapter 2

Oculus Pentacam Principle and Methodology

2.1 Corneal Clarity

For good vision, it is essential that the cornea should be clear and this clarity is achieved when almost no light scatters through the cornea. The entire physiology of the cornea is dedicated to maintaining its transparency. The characteristics of the smooth layer of the epithelium of the cornea; the pump located in the endothelium of the cornea (Krachmer et al., 2005); lack of blood vessels in cornea (Azar et al., 2012); the structural arrangement of collagen fibres in the corneal stroma (Maurice, 1957, Forrester et al., 2008) and the cells present in the cornea (Forrester et al., 2008, Hassell and Birk, 2010) all play an extremely important role in maintaining corneal transparency.

In 1968, Goldman et al. concluded that lattice arrangements are not so important for corneal transparency and proposed that it is due to a minimal relationship between fibrils and light which allow light transmission. Also only fibrils larger than one half of the wavelength of light that caused interference (Salgado et al., 2011). However, another theory by Maurice (1957) proposed that "for a tissue to be transparent it is necessary that its fibrils are parallel, equal in diameter and have their axes disposed in a lattice". To maintain corneal transparency molecular and cellular factors come together forming primarily stromal collagen arrangements. These are combined together in packages called fibrils which arranged in layer (lamina) in parallel form. The setting of fibrils in adjacent lamina perpendicularly (DelMonte and Kim, 2011, Komai and Ushiki, 1991) which give high level of transparency (Jester et al., 1999). The rules of corneal crystalline structure improved our understanding of corneal transparency further. It acts in reducing refractive index of larger macromolecules in their area such as keratocyte nuclei and other large structures and scatter less light to give more corneal clarity (Ellenberg et al., 2010).

Decrease in corneal clarity (haze) can be caused by different changes taking place in the cornea. In a healthy cornea, haze can be positively correlated to age or systemic illness and can appear after refractive surgery. Haze can also take place in a diseased cornea and during the progress of the disease and post treatment. Haze can worsen visual property by causing glare, reduced contrast sensitivity and a decrease in visual acuity (Zarei-Ghanavati et al., 2017). This encouraged diverse approaches using of enlarged corneal light backscatter which can assess several clinical conditions (Olsen, 1982). Traditionally, haze has been assessed clinically by a combination of measuring the patient's best visual acuity and grading how hazy the corneal tissue appears on slit lamp examination.

2.1.1 Slit lamp Biomicroscope

The slit lamp Biomicroscope is widely used in eye clinics as an essential diagnostic tool. It is composed from an illumination system containing a bright light source variable in width and height. The slit lamp has a binocular microscope with different magnification that provides a coincidental focus of the slit and microscope when correctly aligned. Assigning a camera to the slit lamp eye piece or inset beam splitter, in the observation pathway before the eye piece, can assist in taking images of the eye (Wolffsohn and Peterson, 2006). Fantes et al. (1990) introduced a grading system for corneal haze range from 0 (no haze) to 4 stage which was complete opacity of the cornea as in Table 2-1.

Table 2-1: Corneal Haze Grading System

Stage	Slit lamp image description
0	Complete clear cornea, no haze
0.5	Trace haze seen with carful oblique illumination
1	Haze not interfering with visibility of fine iris details
2	Mild obscuration of iris details
3	Moderate obscuration of the iris and lens
4	Complete opacification of the stroma in the area of the scar, anterior chamber is totally obscured

Although this method is simple there are some disadvantages in that it is subjective and not very reproducible (Olsen, 1982). In the assessment of disease progression and management it is important to measure haze accurately, therefore it is essential to have a robust objective method of evaluation and comparison.

2.1.2 Confocal Microscopy

Corneal clarity has been assessed ex-vivo (Radner and Mallinger, 2002) and in-vivo (Galvis et al., 2015, Müller et al., 2003) using confocal microscopy which overcomes the defocus light based on the confocal principle. The confocal microscope provides examination of the structure of five corneal layers with high magnification and resolution by simultaneously illuminating and imaging a single point of tissue (Lagali et al., 2013) . The point light source and the camera are in the same plane. Advances in confocal microscopes result in providing the ability to scan small areas of tissue, illuminating and imaging huge numbers of tissue points to create the final confocal image (Erie et al., 2009, Efron et al., 2001). By scanning different thickness levels of certain tissues in the anterior segment, significant information about structure and function at the cellular level can be gained. Confocal microscopy is considered to be the gold standard in haze quantification. The amount of back scatter light given in intensity-units or in intensity-thickness-units can be used to estimate corneal haze and monitor relative transparency of the corneal stroma (Nagel et al., 1995, Slowik et al., 1996, Møller-Pedersen et al., 1997). Based on estimation of haze of sub-epithelial peak on a light reflectivity curve , a valid haze estimation has a good correlation with the clinical haze grading system (Møller-Pedersen et al., 1997).

2.1.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is a non-invasive imaging tool able to establish cross-sectional images of tissue microstructure (Huang et al., 1991). It has been used to image the anterior and posterior eye based on various applied systems (Müller et al., 2001). Cross sectional imaging of the OCT allows ultra-high resolution images of the corneal layer to be obtained (Reiser et al., 2005). OCT for the anterior segment is similar to ultrasound, but it employs light waves instead of sound to produce extremely high resolution images of very small ocular structures. OCT uses dual scanning beams of light that are reflected off an eye structure and then detected and compared to a reference beam to create a cross-sectional image (Radhakrishnan et al., 2005, Radhakrishnan et al., 2001). OCT has been used to evaluate haze post refractive surgery (Eliaçik et al., 2015). OCT uses a longer wavelength with higher tissue penetration compared to the blue light of the Scheimpflug device so delivers a more detailed image of the corneal stroma (Doors et al., 2009).

2.1.4 X-ray

A biological and structural property of corneal collagen has been investigated using X-ray scattering designs. X-rays are scattered at different angles depending on their interaction with biological tissues (Meek and Quantock, 2001). This X-ray diffraction has been used to evaluate haze post PRK by investigating the inter-fibrillar spacing of collagen fibrils (Connon et al., 2003).

2.1.5 High Frequency Ultrasound

The conventional ultrasound which is used diagnostically for routine A and B scans utilizes a frequency of 7-15 MHz. High frequency ultrasound offers quantitative and qualitative data about changes that have taken place in the cornea (Allemann et al., 1993, Silverman et al., 2006).The high frequency ultrasound has a frequencies between 35 to 100 MHz (Pavlin and Foster, 1998) and has a high resolution between 150 to 200 um. Corneal tissues can be scanned by a frequency of 100 MHz offering tissue resolution up to 20um in thickness (Dada et al., 2007). It can used to analyse the corneal backscatter through power spectrum analysis (Reinstein et al., 2000).

Recently the non-invasive Oculus Pentacam has been used to investigate the anterior segment and corneal densitometry values at different areas and depth of the cornea have been obtained (Dhubhghaill et al., 2014, Lopes et al., 2014). This will be discussed in detail in the following sections.

2.2 The Principle of Scheimpflug and the Oculus Pentcam

Imaging of the cornea has developed rapidly since the beginning of this century with technology which has provided qualitative and quantitative analysis of the cornea. Historically, instruments employed Placido reflective image analysis to investigate corneal topography in the eye. This principle is based on multiple light concentric rings projected on the cornea and the reflective image is captured on a charge-coupled device camera (CCD) (Penna et al., 2017, Levene, 1965).

Another method used in corneal topography is a slit imaging technique. It used multiple slits to perform evaluation of the corneal surface by measuring the anterior and posterior surface and defines the spatial relationship between the two, thus giving a 3-dimensional image of the cornea. The most common instrument using this principle is Orbscan (Bausch

& Lomb) which used 40 slits projected on the corneal surface and gave 240 points on each slit (Liu et al., 1999).

Another principle, which was proposed by Schiempflug (1903) who aimed to establish an instrument with good quality, precise images of different contours and geometrical landscapes. However, slit imaging does not exhibit the same depth of field compared to those obtained by Scheimpflug systems.

The Scheimpflug principle is used to describe the photographic properties of an optical system in which the plane of focus and camera lens are tilted in a way so they are not parallel to each other (Brown, 1971). According to the principle, the plane with the slit beam and the plane of the image must intersect at a given point, with equal corresponding angles. The most important ability of scheimpflug based imaging systems is to measure the entire anterior segment of the eye and provide a cross-sectional view over all anterior ocular surfaces.

The most available devices which employ this principle are Pentacam (Pentacam; Oculus GmbH, Wetzlar, Germany), Sirius (CSO,Scandicci,Italy),and TMS-5 (Tomey, Corporation, Nagoya, Japan) and Gallili (Ziemer USA, Inc).

2.2.1 Oculus Pentacam

Pentacam (Pentacam; Oculus GmbH, Wetzlar, Germany) has grown in reputation to be used in eye clinics. It is a non-invasive device that employs the Scheimpflug principle to create high resolution images focused on the anterior segment of the eye. It has a 360 degree rotating camera that takes close to 50 slit-based photographs in less than 2 seconds. It was the first scheimpflug device with these features (Grewal et al., 2008). The Pentacam measured the cornea and anterior chambers but only captured images in the temporal side to avoid the shadow of the nose. Software based on computational logarithms is then used to develop a three dimensional image. A second camera is used to capture eye movement's eye and to make appropriate corrections. The collected data is used to calculate the topography of both anterior and posterior surfaces of the cornea, thickness of the cornea, depth of the anterior eye chamber, and opacity and thickness of the lens. The camera can take measurements of local points of elevation by fitting the shape of the cornea to a reference spherical surface of varying diameter or elliptical surface (Shankar et al., 2008, Mannion et al., 2011).

The anterior surface of the eye is not transparent but can be mapped by Pentacam. Based on light scattering properties of the pathological corneal tissue at different zones that become visible on the image (Jain and Grewal, 2009). The power of the anterior surface of the eye is calculated by finding the difference between refractive index of the air and corneal tissue. However, the posterior surface power is calculated by finding the difference between corneal tissue and aqueous humour. This elevation based system has been reported to have superiority over Placido based systems (Litoff et al., 1991). The advantage of Pentacam is the ability to calculate elevation data with accuracy even if the corneal tissue had changed as a result of disease or surgical treatment (Belin, 2007, Savini et al., 2001). This is done by measuring the elevation data compared to an aspheric surface and then using the outcome to find anterior and posterior curvature. This ability to measure cornea with severe irregularities in addition to pachymetry by subtractions of the front and back corneal elevation measurements with high resolution gives Pentacam superiority over other imaging devices (Byun et al., 2012). In addition corneal thickness (Miháltz et al., 2009), corneal aberration (Lackner et al., 2005), corneal densitometry (Garzón et al., 2016) and other parameters can be measured.

Oculus Pentacam uses a digital charged-coupled device camera for the image recording and its ultraviolet free blue light emitting diode (LED) and has a wavelength of 475µm as light source. The corrections of the image are highly important and done with algorithm software for automatic distortion correction (Dubbelman et al., 2002, Jain et al., 2007, Jain and Grewal, 2009).

2.2.2 Pentacam in clinical practise

Pentacam produces corneal measurements across all cornea diameters for up to 25,000 true elevation points (HR/AXL: 138.000). It can also provide 3D analysis of anterior chambers, pachymetry maps, topography maps (anterior and posterior), elevation map, anterior camper depth map, cataract analyser, Holladay reports, tomography, corneal densitometry and crystalline lens or intra ocular lens (IOL) opacity. These features have wide clinical applications for optimum analysis for corneal refractive surgeons (Ciolino and Belin, 2006), cataract surgeons (Cho et al., 2010) , glaucoma screening (Kurita et al., 2009) and contact lens fitting (Weber et al., 2016). The latest version of the Pentacam (Pentacam AXL) also has the ability to measure the axial length of the eye, utilizing partial coherence interferometry with actual rotating measurements (Shajari et al., 2017).

Pentacam has a feature of checking the quality of the scan generated as a report. Pentacam has a feature to define the quality of imaging data, called quality specification (QS). An acceptable image is indicated with an 'OK' on a white background otherwise the image needs to be repeated (Figure2-1). Those features can help with disease screening, track disease progression, planning treatment and evaluate disease management. Images taken by Pentacam during the examination can be transferred to the PC after being digitalized at the Pentacam unit. Therefore it is simple to use and the only difficulty would be where certain patients had problems in fixating.

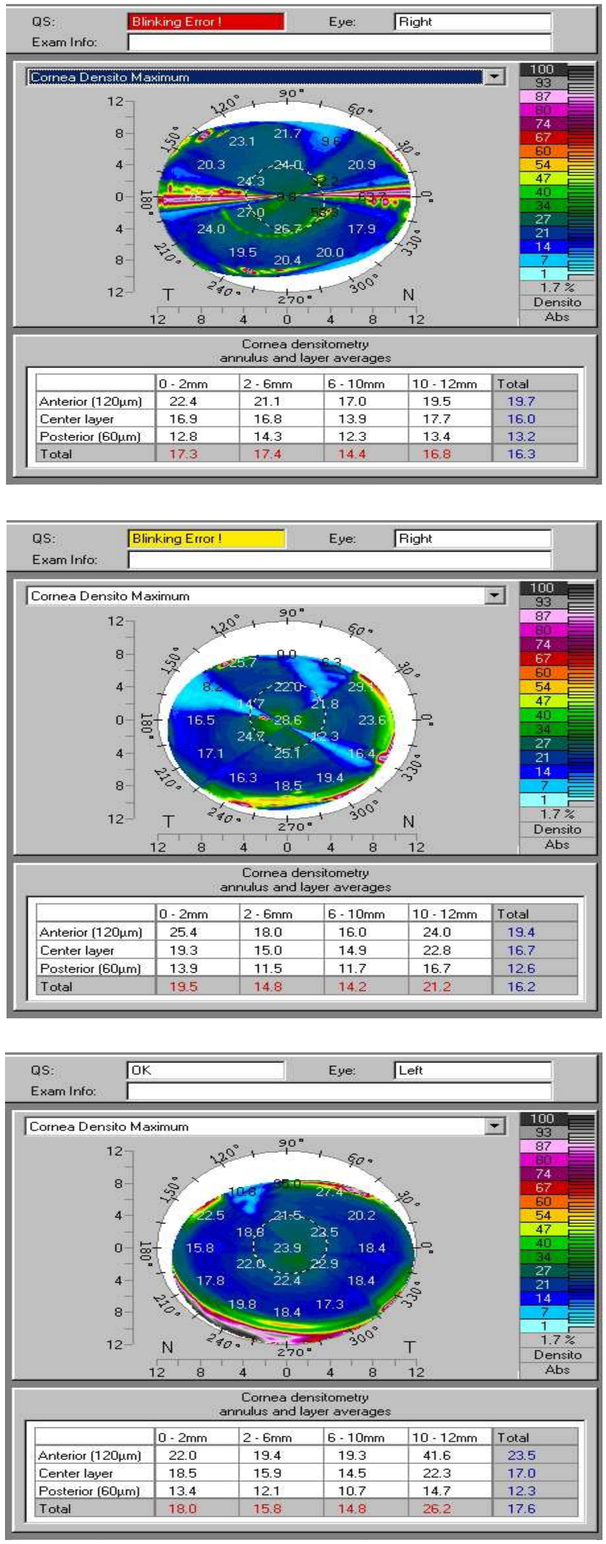


Figure 2-1:Assessment of imaging quality as generated by pentacam which shows the unaccepted images with red and yellow background and accepted images will be generated with white background and OK sign.

2.2.3 Pentacam examination

Imaging should take place in a dark room with uniform ambient light. This could be done using black shield provided by the manufacturer to standardize the light effect on corneal measurement. The Pentacam imaging protocol is performed by the participant being positioned in front of the Pentacam camera with the chin and forehead are resting on the frame. The image should be focused using the joystick adjustment until the corneal surface appears on the monitor, centring the pupil and corneal apex with the marker. The participant instructed to blink in order to maximise tears stability (Koh et al., 2006) and then with the eye open to look directly at a black fixation target centred in the scanning-slit light scan for 2 seconds duration. The device has to be used in automatic release mode to rule out confounding operator related variability. The accepted image indicated by the device is scanned by quality software in the device for further analysis (Chen and Lam, 2009).

Care must be taken regarding artefact which can affect the optical components of the slit illumination in the centre of the unit and lens in front of the camera. Those components are delicate and very sensitive to pressure and therefore should be prevented from being scratched. The lens needs to be carefully cleaned using a lint-free dray cloth. The slit illumination has to be cleaned using compressed air only. Pentacam is calibrated by the manufacturer as every Pentacam is provided with calibrating files, saved in pentacam CD-ROM and named Pentacam Calibration Data. These calibration CDs can only be used for the specified model. Pentacam must be surfaced every 2 years or after 25000 examinations according to the manufacturer's instructions and a test eye is also provided to check the instrument.

2.3 Corneal densitometry

The ability of any device to collect and quantify light scatter will provide an objective method of assessment. Light scatter has been assessed using confocal microscopy but has limited clinical use (Jester et al., 2001). Scatterometry has been used to evaluate corneal back scatter light (Braunstein et al., 1996) also stray light has been used by Harrison et al. (1995) to assess forward light scatter in post PRK but it was shown not to accurate. Sheimpflug imaging to assess corneal densitometry was first reported by Smith et al. (1990). Pentacam has been used to evaluate densitometry of the crystalline lens (Baradaran-Rafii et al., 2015, Ullrich and Pesudovs, 2012) and corneal densitometry (Gutiérrez et al., 2012, Lopes et al., 2014). Corneal densitometry is calculated automatically through a computer algorithm within Pentacam's add-on image analysis software. It is measured the straylight for the wavelength from the slit illumination. This is used to evaluate the level of optical (back- scattering) for each captured scheimpflug slit-like image. The level of backscattering measured by a scale ranging from 0 to 100 grey scale units (GSU) which is standardised by property software. The GSU scale is calibrated by means of property software. The relative density is the ratio of maximum level of grey divided by the observed level of grey. A value of 0 indicates transparency (no haze) while 100 represents complete opacification of the cornea (Lopes et al., 2014). This is a simple linear transformation from colour scale to densitometry scale.

Pentacam densitometry software is able to assess the location of the opacity by giving a numeric value of the cornea at different diameter zones (0-2, 2-6, 6-10 and 10-12). In addition, Pentacam can produce another numeric value for depth based on different layers. The "anterior layer" and the "posterior layer" represent the first 120 μm and the last 60 μm of the whole corneal thickness. The "central layer," which has no fixed thickness value, refers to the volume between the 2 boundary layers. The "Full depth" refers to the value of a cornea anterior 120um, central and, posterior 60um and full depth) (Figure 2-2).

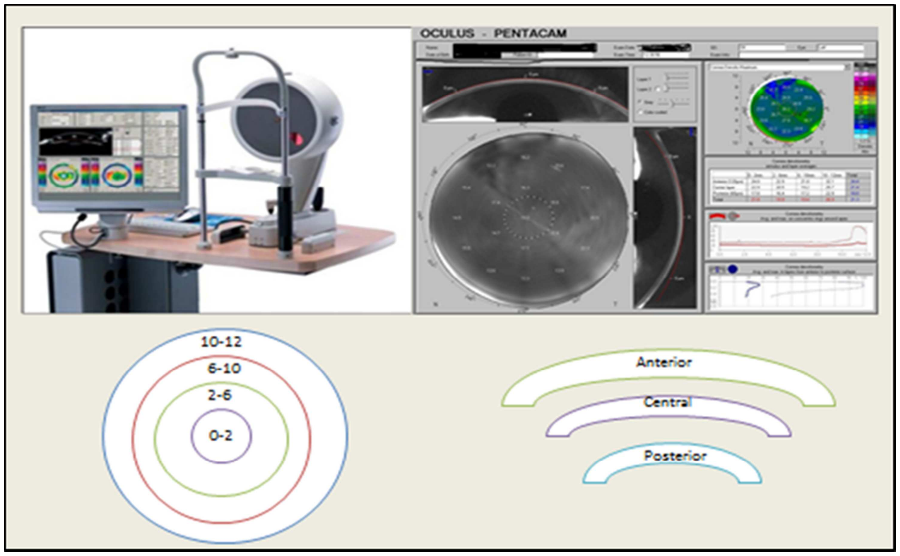


Figure 2-2: Oculus Pentacam , densitometry printed output and specific layer and zone.

Corneal densitometry has been used in haze assessment. Greenstein et al. (2010) used it to evaluate haze incident post CXL. Pentacam's corneal densitometry has been used in haze evaluation in relation to corneal dystrophy (Elflein et al., 2013, Chu et al., 2017), post refractive surgery (Rozema et al., 2011, Poyales et al., 2017, Lazaridis et al., 2017), post graft surgery (Takacs et al., 2011, Alnawaiseh et al., 2017, Alzahrani et al., 2018) ,infectious keratitis (Otri et al., 2012) ,KCN patients on CL (Lopes et al., 2014) and recently been used to evaluate some systemic diseases or the effects of treatment on corneal clarity(Alnawaiseh et al., 2016, Anayol et al., 2017).

2.4 Repeatability and Reliability of the Oculus Pentacam

The Oculus Pentacam requires less skill and training to use since it is automated. The device provides quantitative as well as qualitative data on the anterior eye segment within a very short examination time. There is sufficient evidence to suggest that the device has good repeatability or test retest reliability in measurements of the anterior segment (Jain et al., 2007). According to (Lackner et al., 2005), central corneal thickness (CCT) measurements generated by the Pentacam compare well with those generated with ultrasound pachymetry, with less variability, good reproducibility in cross observation by -6.0, 5.8 in comparison to -17.0, 22.0 of 95% limit of agreement for Ultrasound pachymetry, and satisfactory repeatability in measurement by -11.0, 13.0 in comparison to -6.7, 7.3 of 95% limit of agreement for ultrasound pachymetry. Amano et al. (2006) found Pentacam correlated significantly with ultrasonic pachymetry (r=0.908) and scanning –slit topography (r=0930). A study conducted by (Al-Mezaine et al., 2008) concluded that both Pentacam and ultrasound pachymetry provide a high degree of accuracy in corneal thickness measurements.

The accuracy of the Pentacam has been assessed in several studies. Any alterations observed in the elevation of the posterior corneal region after LASIK was not statistically different from PRK (Hernández-Quintela et al., 2001). According to the latest reports, the common occurrence of subclinical, post-LASIK ectasia have been controlled through higher precision of the Orbscan during the post-LASIK pachymetry. When it comes to the precision of the Pentacam in cases of post-LASIK pachymetry more information can be gathered from additional studies. In a study using the Pentacam topographer, the rate of occurrence of subclinical incidence of post-LASIK ectasia might be less (2.64 ± 4.95 μm) (Ciolino and Belin, 2006) than reported previously (40.9 ± 24.8 μm) (Baek et al., 2001).

After orthokeratology treatment or after refractive surgery, a repeatable tool is needed for monitoring the advanced changes occurring in the cornea longitudinally. Occurrence of myopic regression followed by refractive surgery is common. It is also suggested that posterior corneal change is a factor in myopic regression. Hence, after refractive surgery, the posterior corneal shift is frequently analyzed (Chayet et al., 1998, Kim et al., 1997). For this kind of analysis, and also in keratoconus the posterior elevation map can also be an efficient indicator, apart from relating the posterior best-fit sphere (BFS) (de Sanctis et al., 2008, Fam and Lim, 2006). Any difference in the actual position of the cornea and the

BFS, at any specific point, like the centre of the cornea, is marked as an elevation. As observed, when it comes to posterior corneal assessment, the Pentacam system offers superior intersession reproducibility as well as intersession repeatability for both elevation and BFS analysis. Longitudinal corneal alterations, like orthokeratology treatment and refractive surgery, can be monitored with the help of the Pentacam system (Chen and Lam, 2007).

In cases of keratoconic corneas, the measurements of the central thickness produced by a rotating Scheimpflug camera are more repeatable and reproducible when compared to the ones received through ultrasound pachymetry for a moderate stage (Uçakhan et al., 2006). Hence, the rotating Scheimpflug camera is ideal for staging and following disease progression and the measurements can repeated over time (and also by different examiners at different times). In measuring the keratoconic corneas' central thicknesses, on average, the rotating Scheimpflug camera offers slightly lower values compared to that of ultrasound pachymetry. This becomes evident particularly in cases with eyes at a severe stage of the condition, where the differences between the outcomes of the two techniques are larger (De Sanctis et al., 2007).

A newer version of the Oculus Pentacam called the Pentacam HR was recently launched, and it has a higher resolution as well as phakic intraocular lens (IOL) software that simulates the proposed lens position. The quality of data collected from the lens is dependent on the size of the pupil because that it is the only part of the lens that can be observed through the aperture of the pupillary (Emre et al., 2007, Ho et al., 2007, Nilforoushan et al., 2008, Quisling et al., 2006, Rufer et al., 2005).

Evaluation of the axial length, curvature of the corneal and anterior chamber depth measurement performed by the latest advanced Pentacam (Pentacam AXL) were found to be repeatable with coefficient of repeatability (COR = 0.055) with IOL Master 500 and COR=0.052 with IOL Master 700 which correlated well with both optical biometry for

IOL measurements (IOL Master)(Shajari et al., 2017) but it was thought not to be used interchangeably as pentacam AXL tended to produce results overestimated for IOL power (Muzyka-Woźniak and Oleszko, 2018).

Good, repeatable outcomes were achieved with the Pentacam HR and also previous model, which led to enhancements to be made of the standard model (McAlinden et al., 2011). The latest HR model is able to capture 5 times more data points compared to the basic model. The enhanced resolution of the HR model has assisted in studying the surface of the posterior cornea. The Pentacam HR offers precise measurements that are reproducible and repeatable across the range evaluated. There were also exceptions, which include pupil pachymetry, refractive power maps, axis estimates, front meridional and axial maps, and EKRs (Shankar et al., 2008). Amongst the three modes of measurement, the cornea fine scan was the most accurate. No big advantage was gained by the 2 second 50 picture scan compared to the 1 second 25 picture scan (McAlinden et al., 2011).

The Oculus Pentacam is repeatable in nature and it can also be employed effectively to assess corneal thickness in healthy eyes (Khoramnia et al., 2007, Barkana et al., 2005). However, in order to find the accuracy of Pentacam in cases of eyes with significant refractive problems, history of corneal surgery, or keratoconus, further investigations are necessary (Miranda et al., 2009). Corneal epithelial ingrowth in post LASIK was found to be detectable earlier by the Pentacam compared to the slit lamp (Jinabhai et al., 2010).

A study to determine if the Pentacam is capable of pinpointing differences in the aberrometric patterns of the anterior and posterior cornea in keratoconic and normal eyes, even with its limitations with regard to aberrometric classification, observed that coma significantly increased in early keratoconus stages at anterior (0.236 ± 0.103) and posterior (0.490 ± 0.302) in comparison to healthy eyes anterior (2.193 ± 0.822), and posterior (4.361 ± 1.778) corneal surface (Piñero et al., 2009).

Every parameter of the anterior and posterior surface of the cornea, including curvature, elevation, pachymetry, and wave front aberrations are received from the raw data of corneal elevation. Though this raw data has high variability, the Pentacam showed high repeatability in measuring the parameters of corneal curvature as well as thickness of the central cornea in post-operative and normal eyes and the measurement of thickness of the central cornea in healthy eyes (Shankar et al., 2008, Read et al., 2009). There are differences in the results of the different studies performed with the Pentacam. Hence, the Pentacam needs to be used cautiously in refractive surgery and keratoconus. The keratometry measurements were found to be less reproducible in advanced keratoconus (Flynn et al., 2015). In addition, the power readings of the cornea did not exactly match IOL power calculations for corneas after cataract surgery or after kerato-refractive surgery when the present IOL formula for power calculation was used (Oliveira et al., 2011). Furthermore, it was well established that Scheimpflug lens densitometry provides high intra-observer as well as inter-observer repeatability for both healthy eyes and those with cataracts (Kirkwood et al., 2009). Mingo-Botín et al. (2018) investigated the Repeatability and Intersession Reproducibility of Pentacam Corneal Thickness Maps in Fuchs Dystrophy and Endothelial Keratoplasty which shows good repeatability Intra Class Correlation (ICC) ranging from 0.960 to 0.994 for post-treatment, 0.965 to 0.993 pre-treatment and 0.985 to 0.989 for normal healthy eyes. The reproducibility with ICC ranged between 0.905 to 0.983 for post-treatment, 0.916 to 0.955 pre-treatment and 0.932 to 0.944 for normal eyes across corneal zones.

The most important quality of these objective methods over historical subjective methods of haze evaluation is repeatability (Wang et al., 2004). Densitometry measurements with the Pentacam were found to have good repeatability and reproducibility across all corneal zones with average repeatability of 3.3± 1.8% except the 10-12 mm zone (6.4%), which is in contact with limbus (Dhubhghaill et al., 2014). There was variation in repeatability measurements when used in post CXL treatment (7% to 15%) in comparison to healthy (2% to 4%) or progressed KCN (3% to 5%) in the coefficient of variation as the repeatability seems to be affected by disease progression and its treatment (Pahuja et al., 2016). McLaren et al. (2016) found the Pentacam was better than confocal microscopy in haze detection and was better in assessing disease progression. Its high quality images of the cornea give it an advantage in evaluating corneal clarity (Wacker et al., 2015). Pentacam has superiority in the ability to detect the corneal haze in normal or advanced corneal disease. The ability of Pentacam to detect the haze across the entire cornea in comparison to small areas by confocal microscopy is restricted by low level of depth resolution and difficulty to grad disease based on densitometry alone (McLaren et al., 2016). Penatacm has the ability to measure the anterior and posterior of the cornea and make further correction. However, due to the change of corneal refractive index due to disease and its progression in addition to considering the same refractive index change in anterior and posterior cornea that reduce precision of the result (Sideroudi et al., 2013). However, there is little in the literature about the repeatability or reliability of corneal densitometry analysis. Despite the higher resolution of the corneal structures based on confocal microscopy, Oculus Pentacam has able to distinguish any changes in corneal clarity (Gutiérrez et al., 2012). The Pentacam has mostly been found to be a robust device in terms of repeatable, reproducable results.

Recently many studies using corneal densitometry investigated the relationships between corneal clarity affected by age (Alzahrani et al., 2017b, Dhubhghaill et al., 2014, Garzón et al., 2016), ocular disease (Cankaya et al., 2016, Sekeroglu et al., 2016, Anayol et al., 2016) and post corneal treatment (Arnalich-Montiel et al., 2013, Pircher et al., 2015) and/or systemic disease (Calvo-Maroto et al., 2017, Anayol et al., 2017).

2.5 Study protocol

The study was approved by the Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee and was performed in accordance with the Declaration of Helsinki.

All participants were recruited at Manchester royal eye hospitals (MREH). All participants were provided both written and oral information before giving written consent to participate in the study.

Oculus Pentacam (OCULUS Optikgerate GmbH, Wetzlar, Germany, version 1.19r06) was used in all studies in this project. All patients were examined under the same conditions, same place, before being administrated any drugs or investigation to the eye rather than Visual Acuity. The automatic release mode of the Pentacam was used to minimize examiner-induced errors, and only Pentacam images of good quality were included.

The repeatability of the Pentacam used in this project was investigated at the beginning of this project. Three successive right eye measurements were obtained from 15 participants done by the same observer revealing an excellent repeatability over all measurements. The intra class correlation coefficient (ICC) was used to calculate the repeatability based on three measurements obtained from 15 participants. The highs repeatability was found for the central layer of zone 6-10 mm (0.991) and the lowest repeatability were found at thinnest area (0.848) as shown in table 2-2 . Bland and Altman were plotted for difference against mean to visualize the level of agreement within 95% between first and second reading measures and limits of agreement (LoA) were calculated (mean ± 1.96 standard deviation) as shown in Figure2-3, Figure 2-4 and Figure 2-5.

Our study investigation of for repeatability of corneal densitometry and data gained from previous literature, together with the unique quality assessment features of the scan which generated by Pentacam support the design to include only one successful scan for the analysis.

Table 2-2: Repeatability based on 3 measurements taking from 15 right eye participants.

	Intra class correlation	95% confidence interval	
Anterior 0-2 mm	0.968	0.923	0.988
Anterior 2-6 mm	0.973	0.937	0.990
Anterior 6-10mm	0.980	0.953	0.993
Central 0-2 mm	0.955	0.893	0.984
Central 2-6 mm	0.965	0.918	0.987
Central 6-10 mm	0.991	0.978	0.997
Posterior 0-2 mm	0.958	0.900	0.985
Posterior 2-6 mm	0.966	0.919	0.987
Posterior 6-10 mm	0.994	0.985	0.998
Full depth 0-2 mm	0.963	0.912	0.987
Full depth 2-6 mm	0.967	0.923	0.988
Full depth 6-10 mm	0.990	0.976	0.996
CCT	0.994	0.986	0.998
Thinnest area	0.848	0.643	0.945

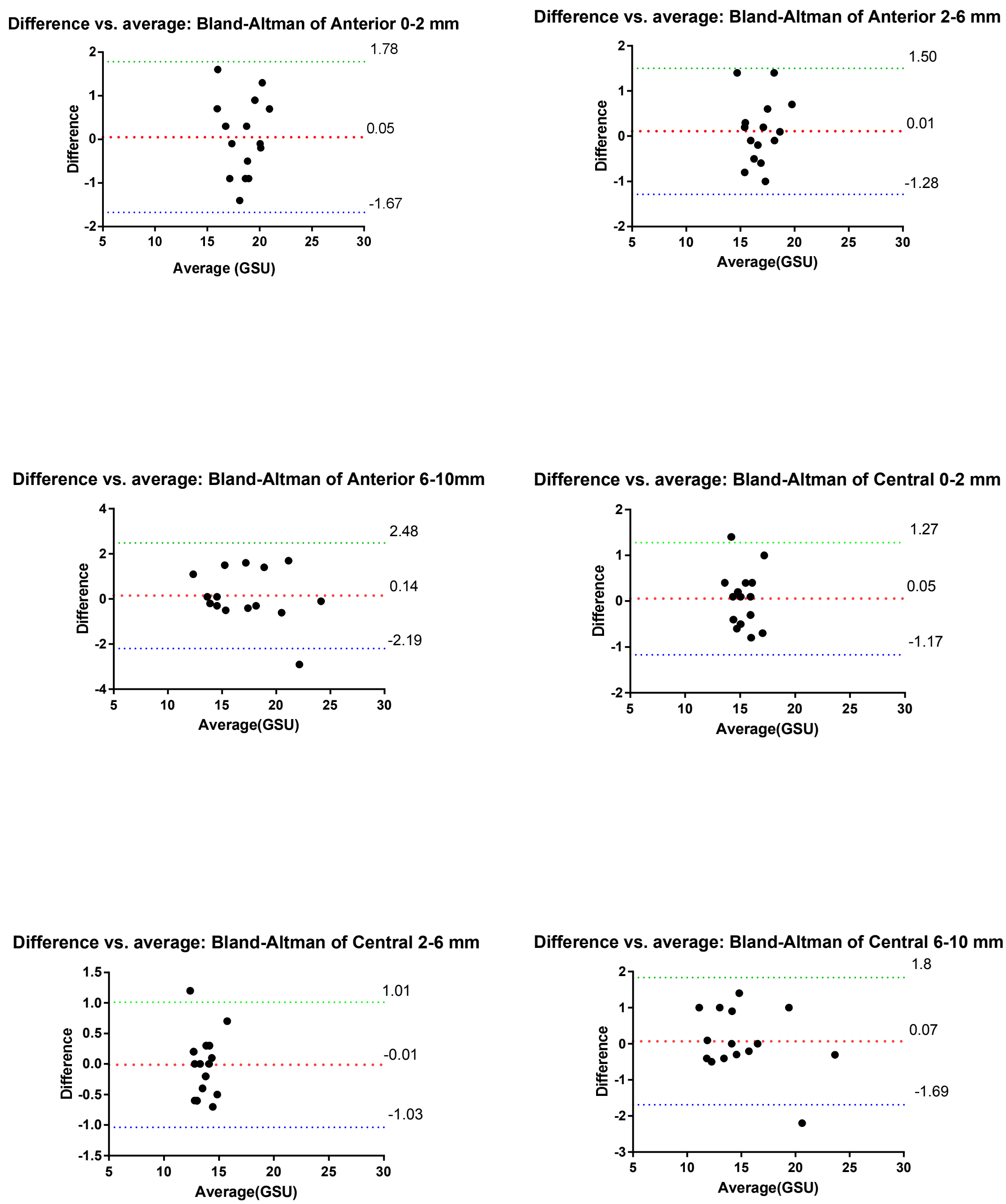


Figure 2-3: Bland and Altman plots show level of agreement between first 2 measurements for anterior and central layer for different corneal zone. Coloured lines represents, Bias (red), upper limit (green) and lower limit (blue).

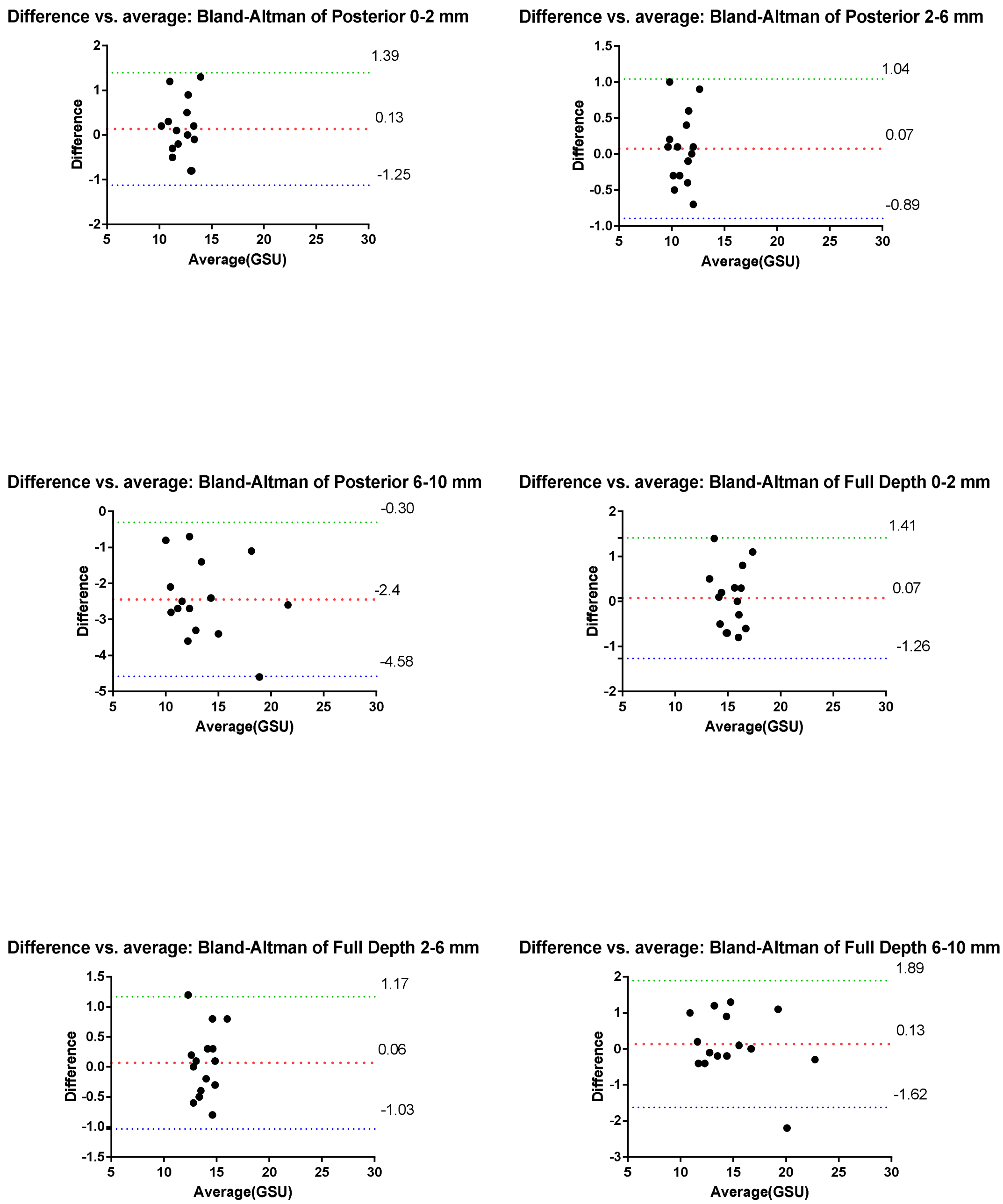
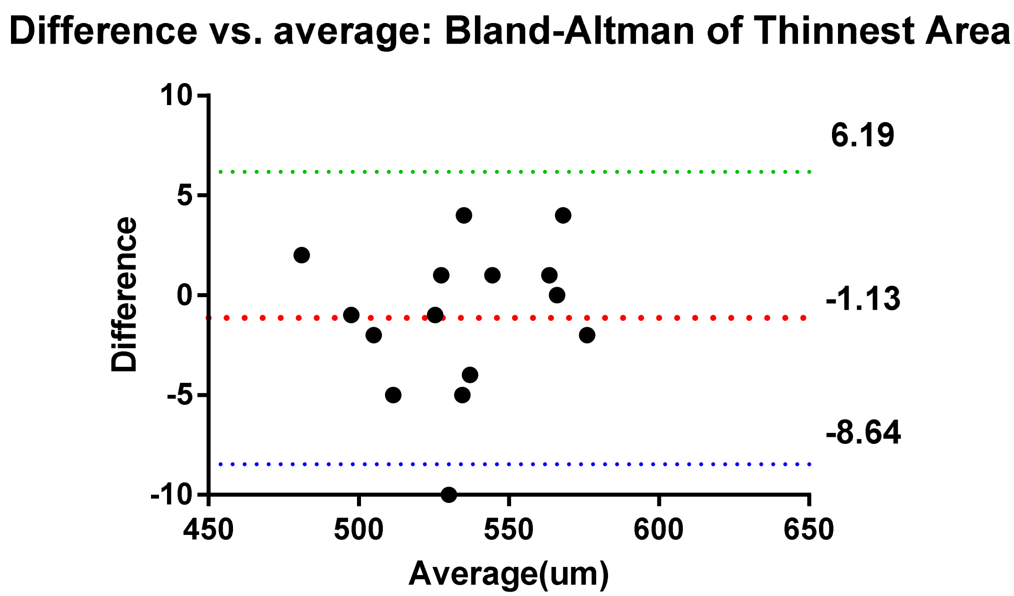


Figure 2-4:Bland and Altman plots show level of agreement between first 2 measurements for posterior and full depth layers at different corneal zone. Coloured lines represents, Bias (red), upper limit (green) and lower limit (blue).



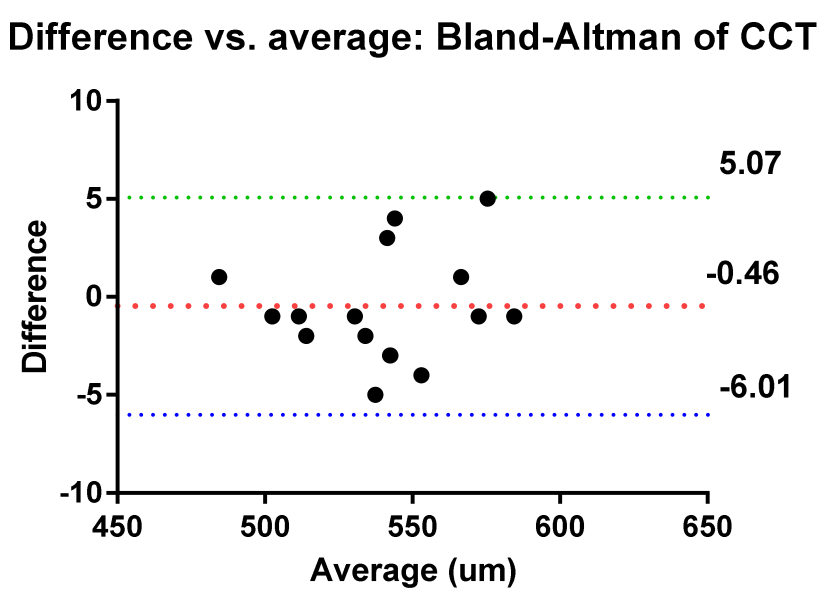


Figure 2-5: Bland and Altman plots show level of agreement between first 2 measurements for central corneal thickness and thinnest area. Coloured lines represents, Bias (red), upper limit (green) and lower limit (blue).

2.6 Rationales and Aims of the Project

2.6.1 Research Hypothesis

There is a great deal of research activity on corneal transparency In particular the use of the Oculus Pentacam to evaluate corneal clarity. This project aims to address some gaps in the literature about corneal clarity in health and disease. Corneal densitometry measurement together with visual acuity measurement may be useful tools to objectively assess corneal clarity after corneal transplant, corneal UV cross-linking and refractive surgery. The study will be looking at the correlation between corneal thicknesses, clarity, and visual acuity and also how corneal clarity differs based on the management of corneal disease. The intention is also to examine how age can effect clarity in healthy cornea and hence the effect of age on treatments for some corneal diseases. In addition the study investigates what is the nature of corneal haze when there is tissue to tissue interface or self-tissue interface.

2.6.2 Research Objectives

The main purpose of this study is to measure the clarity of the cornea and get more understanding of the changes taking place in its clarity in health and disease. We will also determine if densitometry can help in monitoring disease progression, the outcome of treatments and aid in the management of corneal diseases. The project specific aims are listed in the sections below.

2.6.3 Control Study

We wish to establish how the corneal clarity changes for different zone and layers of the cornea with age in a normal healthy eye and if all layers and zones have same trend of changes. The aim of this study is to standardise corneal clarity in healthy participants.

Additionally the study aims to investigate how corneal clarity is correlated with age and sex.

2.6.4 Refractive Surgery Study

This study will determine if corneal densitometry provides a valuable clinical measurement to assess treatment outcome of LASIK and LASEK. This will be done by measuring corneal densitometry at different corneal layers and zones of patients undergoing LASIK and LASEK preoperatively and postoperatively. The effect of the two different refractive surgical techniques will be compared to see which corneal zone and layers are most affected since each technique differs on flap thickness.

Investigation of corneal densitometry changes after LASIK and LASEK refractive surgery will also be carried out. We will determine any changes in after LASIK and LASEK is related to the corneal haze that takes place after refractive surgery.

2.6.5 Fuchs' Endothelial Dystrophy (FED) Study

Corneal transplant of the Fuchs as discussed earlier is done either partially DSAEK or full thickness (PK). DSAEK replaced diseased endothelial with new healthy endothelial obtained from donors. The new donor tissue contains Descemet's membrane and small amount or endothelial stroma. The interface between recipient tissues and donor tissue at the posterior cornea is affected by haze. This study aims to compare the corneal densitometry outcome between PK and DSAEK in patients with FED across all corneal layers and specific zones. In addition the study aims to observe the association between corneal densitometry and best corrected visual acuity (BCVA) after the corneal transplant in FED.

2.6.6 Keratoconus Study (KCN)

Corneal transplantation for Keratoconus can be done using traditional PK or an advanced technique, DALK. In the DALK procedures new healthy stroma from donors are used to replace diseased ones. The interface between both tissues can cause haze and decrease visual acuity. This study first aims to compare haze using corneal densitometry between PK and DALK in patients with keratoconus and also investigate for specific zones or layers prone to haze. A second aim of this study is to observe the association between corneal densitometry and best corrected visual acuity (BCVA) after corneal transplantation in patients with keratoconus. A third aim is to look at the correlation between BCVA, corneal densitometry, surgical type and central corneal thickness. The fourth aim is to assess the changes in corneal densitometry, BCVA and corneal haziness after corneal treatment.

Corneal cross linking (CXL) is considered to be an effective treatment for stabilizing KCN progression. One of the issues post treatment is corneal haze. Recently CXL been used for juvenile patients who have progressive KCN. Corneal remodelling is known to be more effective juveniles than for adults. The goal of KCN study for patients undergoing CXL is to assess the relationship between corneal densitometry after corneal CXL and specific layers and zones and also to compare that between adults and juveniles over 4 point times in a year. The study also aims to look at any correlation of corneal densitometry with visual function and compare outcomes between adult and juvenile patients.

The procedure of INTACS is used to manage KCN in patients who do not tolerate contact lenses (CL). This study aims to assess the difference in corneal densitometry between keratoconus patients using contact lenses or INTACS and assess the outcome and visual function.

Chapter 3

Corneal Imaging and Densitometry Measurements in Healthy Volunteers across Different Age Groups: Observational Study

Contributions

I designed the study in collaboration with my supervisors, conducted the experiments, recruited the participants, preformed the imaging investigation, completed the experiments, analysed the data, and wrote the manuscript. All this work was achieved with regular discussion, close collaboration and feedback on data analysis and writing from my supervisors: Dr Chantal Hillarby and Prof Fiona Carly.

Publication

ALZAHRANI, K., CARLEY, F., BRAHMA, A., MORLEY, D. & HILLARBY, M. C. 2017. Corneal clarity measurements in healthy volunteers across different age groups: Observational study. *Medicine,* 96**,** e8563.

Conferences Presentations

The abstract was presented at the Association for Research in Vision and Ophthalmology (ARVO), May 7–11, 2017, USA and at the Manchester Optometry Meeting 2017.

Acknowledgement

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3.1 Abstract

Aims: To standardize and investigate the changes in corneal clarity with age. Densitometry software for the Oculus Pentacam was used to examine corneal clarity at different age groups.

Methods and Materials: A total of 192 eyes from 97 healthy participants were included in this cohort comparative non-randomized cross-sectional study. An Oculus Pentcam was used to image the cornea of healthy participants grouped by age (between 10 and 70 years old). Data from the densitometry output has been used to determine clarity in concentric zones and different depths of the cornea.

Results: Corneal densitometry (CD) across all ages showed significant differences between groups when divided into the following layers: anterior, central and posterior or divided in to 0-2 mm, 2-6 mm, and 6-10 mm concentric zones ($p<0.05$). The most striking decrease in clarity occurred with age in all 3 layers of the periphery (6-10 mm) ($p<0.05$). Additionally, we showed that the 10-19 year age group had lower clarity than the 20-30 age group ($p<0.05$) and after 30 years the cornea shows a steady progression of increased or decreased clarity.

Conclusions: The values for CD, as well as for separate subdivisions based on layer and surface area, might provide a standard for use in further studies and clinical practice. This study established that relation between CD and age is differed when the cornea is divided into layers and zones. This study suggests that there are other factors that may play an essential role in corneal clarity as well as age.

3.2 **Introduction**

Corneal stromal transparency relies on the organised collagen fibrils arranged in parallel to form lamellae resulting in no or very minimal light scattering (Maurice, 1957). For both sclera and cornea, collagen is termed as the fundamental structural constituent. Its intense tensile strength is capable of facilitating the globe with a flexible, shielding coat. The stroma and Bowman's membrane essentially house the corneal collagen, with the former region comprising of 90% of a hydrated cornea's entire thickness. Going by the arguments stipulated (Maurice, 1988) the corneal stroma is composed of a vastly structured arrangement of small-diameter collagen fibrils supported apart by a proteoglycan matrix, which ensure uniformity in inter-fibrillar spacing.

The posterior corneal endothelium and anterior external corneal epithelial cell layer are the key light scattering sources (Maurice, 1957). The regular cornea disperses light primarily at the tear film-cornea interface and at the air-tear film, just as anticipated from an optically transparent tissue, where the light refraction's index change is presumably maximum with slight interior structures (cornea tissue) scattering, such as cell nuclei and nerves. Due to its uniform arrangement of collagen fibrils, the corneal stroma is able to sustain its clarity in a network-type formation in lamellar sheets (Freegard, 1997, Qazi et al., 2010, Maurice, 1957, Borcherding et al., 1975), this clarity is highly influenced by both the collagen fibrils' size and fibrils' spacing within the configuration (Borcherding et al., 1975).

In the endothelium, specific augmentation in cellular pleomorphism and polymegethism happens, with a consequent decrease of cell quantity with age. In addition to such age-affiliated variations is the loss of the cornea's initial regular hexagonal outline and no noteworthy vertical regional discrepancy or inconsistency among paired corneas in cellular pleomorphism or polymegethism exist in the typical corneas analysed (Yee et al., 1985).

According to Daxer et al. (1998), a corresponding increase in the fibrils' cross-sectional area is witnessed with increasing age owing to the progressive collagen deposition. Both functional and structural variations are produced in corneal aging. Such alterations can eventually influence the aptitude of the eye to repair itself, refract light and protect the internal structures (Faragher et al., 1997).

The human cornea is characterized by changeable thickness (CCT), aspheric curvature, as well as being anisotropic, meaning it is capable of exhibiting diverse physical qualities upon application of stress in various directions. Such properties are variable, varying with progressive age, corneal pathology and the degree of hydration whereby a loss of lamellae structuring leads to varied corneal biomechanics (Kotecha, 2007). Clarity failure is the innate reaction of cornea to an extensive variety of pathological problems such corneal dystrophies, infections, and degeneration. Analysis and assessment of the resultant corneal haze is hence a fundamental constituent of ophthalmological assessment. Medically, this is normally done by a typical slit-lamp assessment with documentation of results, which can be supplemented with an explanatory severity scale (Braunstein et al., 1996).

The Oculus Pentacam (OCULUS Optikgerate GmbH, Wetzlar, Germany) designed on the Schiempflug photography principle is a non-invasive camera, designed to capture images of the anterior segment of the eye and to produce a comprehensive analysis of the cornea and its densitometry. A better knowledge of corneal clarity in normal control subjects with no known eye disease will help us to understand the normal changes in corneal clarity with age. To date there is no such study which includes measurements from juvenile corneas. Such data may help when planning treatments or measuring how beneficial treatments have been in patients with corneal disease. This study compared the CD measurements in healthy volunteer participants within different age groups.

3.3 **Methods**

Participants and densitometry measurements: A prospective comparative and non-randomised cross-sectional study was approved by Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and Health Research Authority by NREC local ethics committee. The tenets of the Declaration of Helsinki were followed in this study. Written consent was taken from the subjects before collecting their data. All participants were healthy and had no ocular pathology other than refractive error and were all above 10 years of age at the time of imaging. Data collections started on 3rd of November 2015 till 1st of December 2017 for adult participants and juvenile by 2nd of June 2016 till December 2017.

The corneas of normal controls, with no known corneal disease, were photographed once with the Oculus Pentacam, In order to collect the images; participants were positioned in front of the Pentacam camera with their chin and forehead resting on the frame. CD was determined using the pentacam densitometry software. The data extracted from images of the controls' eyes was categorised in six age groups (10-19, 20-29, 30-39, 40-49, 50-59 and 60-69 age groups). For analysis corneas were split into three concentric zones 0-2 mm, 2-6 mm and 6-10 mm and also subdivided to anterior 120 um, central and posterior 60 um layers.

Statistics: A Data analysis was carried out using IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM Corp. Descriptive statistics were presented as the mean ± SD. Normality of data was examined using Kolmogorov-Smirnov test. Pearson correlation and regression liner model were used to look at correlation statistics between age and corneal densitometry. One way Analysis of Variance (ANOVA) was used for comparing more than 2 groups. A p value of < 0.05 was considered to be statistically significant. Power calculated using G*Power 3.1.9.2 retrospectively for full depth 0-2 mm zone was equal at 0.57.

3.4 **Results**

A total of 192 eyes (97 right eyes, 95 left eyes) from 97 healthy participants (36.08% male, 63.91% female) were included in this study that were distributed over six age groups. Participant's age varied between 10 to 69 years. The mean age of the controls was 36.15 years with a SD of 16.5. Female average age was 36.07 with SD 16.78 while the average of the male group was 36.12 with SD 17.45 .The demographics of the controls are summarised in Table 3-1 and shows there was no diversity between male and female participants.

Table 3-1: Demographics of the subjects.

Age Group	Male	Female	Age (MD, SD)
10-19	9(45%)	11(55%)	14(2.35)
20-29	4(23.5%)	13(76.5%)	24.8(2.7)
30-39	4(23.5%)	13(76.5%)	32.8(2.6)
40-49	8(47.1%)	9(52.9%)	46.23(2.4)
50-59	8(50%)	8(50%)	53.48(2.83)
60-69	2(20%)	8(80%)	62.68(1.83)
Total	35(36.08%)	62(63.9%)	36.15(16.5)

The mean CD results are summarised in Table 3-2. CD across all age groups differed significantly between groups when divided both into anterior, central or posterior layers or analysed in full depth. There were also significant differences when the cornea was divided into concentric zones of 0-2 mm, 2-6 mm, and 6-10 mm or when analysed as the total corneal diameter 0-10.

Table 3-2: Corneal densitometry Mean (SD) and P Value across the six age groups for different corneal zones and layers.

Corneal layer and zone	Age group						
Full depth	10-19	20-29	30-39	40-49	50-59	60-69	P Value
0-2	15.53(1.8)	14.49(2.3)	14.91(2.4)	16.09(1.8)	15.94(2.4)	15.37(1.5)	0.016
2-6	13.59(1.6)	13.03(1.7)	13.43(2.0	14.81(1.4)	14.89(2.2)	16.17(2.2)	<0.000
6-10	12.93(2.2)	12.87(1.7)	14.21(2.9)	18.44(3.2)	21.62(5.9)	31.49(7.6)	<0.000
0-10	14.01(1.5)	13.45(1.7)	14.19(2.2)	16.44(1.80	17.48(3.0)	21.01(3.3)	<0.000
Anterior (120 um)							
0-2	22.25(2.9)	18.74(5.1)	19.39(5.2)	21.54(3.8)	20.40(4.3)	18.31(2.9)	0.001
2-6	19.35(2.6)	16.99(3.9)	17.65(4.4)	19.71(3.1)	18.90(3.8)	18.93(3.5)	0.014
6-10	17.80(3.6)	16.14(3.3)	17.83(4.7)	22.85(4.3)	26.06(8.3)	36.25(10.3)	<0.000
0-10	19.81(2.5)	17.30(4.0)	18.29(4.5)	21.37(3.4)	21.79(4.9)	24.48(5.0)	<0.000
Centre							
0-2	13.34(1.4)	14.05(1.5)	14.31(1.3)	14.91(1.3)	15.21(1.9)	15.70(1.0)	<0.000
2-6	11.57(1.2)	12.40(1.1)	12.61(1.2)	13.61(1.2)	14.05(1.9)	16.46(1.8)	<0.000
6-10	11.12(1.9)	12.21(1.5)	13.37(2.8)	17.60(3.6)	21.01(6.1)	33.54(7.6)	<0.000
0-10	12.01(1.2)	12.89(1.2)	13.43(1.4)	15.38(1.8)	16.76(2.9)	21.90(3.1)	<0.000
Posterior (60 um)							
0-2	11.00(1.5)	10.70(1.1)	11.14(1.2)	11.79(1.2)	12.23(1.7)	12.09(1.2)	<0.000
2-6	9.88(1.2)	9.71(.9)	10.02(1.0)	11.11(1.0)	11.70(1.6)	13.05(1.9)	<0.000
6-10	9.88(1.5)	10.22(1.4)	11.39(2.3)	14.84(3.1)	17.80(4.9)	25.56(5.4)	<0.000
0-10	10.25(1.2)	10.21(.9)	10.85(1.2)	12.58(1.5)	13.91(2.3)	16.91(2.6)	<0.000

Corneal clarity in the anterior layer across all zones was found to be low in the 10-19 age groups. The clarity improved in the 20-29 age groups before it started to decrease in the 40-49 age groups, the most significant being the 0-2 mm and 2-6 mm zones which are clearer at 20-29 years before the densitometry values starting to be increased with age. The anterior layer at zone 0-2 mm has better clarity in the 60-69 age groups. CD in the central layer increases with age across all corneal zones. CD in the posterior layer also increases with age across all concentric zones. Once again the posterior 6-10 mm shows rapid increase with age.

A multiple liner regression model was carried out to investigate whether gender, eye and age could significantly predict participants' corneal densitometry for different layers and zones. The results of the regressions indicated that the models explained different percentage ranged between (2% to 61%) of the variance and that the model was a significant predictor of corneal densitometry ($P<0.0001$) except for the anterior 0-2 and 2-6 mm zones. Age contributes significantly to the predicted change in corneal densitometry across all corneal layers and zones ($P<0.0001$) except the anterior layer for zones 0-2, 2-6 mm and full depth for zone 0-2 mm ($p>0.05$). Eye and gender were found to not play a significant role in the model ($P>0.05$) Table 3-3.

Table 3-3: summarise multiple regression model for corneal densitometry and age, gender and eye predictor.

Layer	R Square	F	P Value	Age		Eye		Gender	
				B	P Value	B	P Value	B	P Value
Anterior 0-2	0.02	1.67	0.17	-0.03	0.75	0.23	0.7	-0.82	0.20
Anterior 2-6	0.01	0.755	0.52	0.013	0.42	-0.01	0.97	-0.69	0.203
Anterior 6-10	0.41	45.08	<0.0001	0.32	<0.0001	-0.73	0.42	-1.41	0.13
Central 0-2	0.21	17.24	<0.0001	0.045	<0.0001	-0.33	0.87	-0.28	0.18
Central 2-6	0.46	54.9	<0.0001	0.08	<0.0001	-0.22	0.28	-0.31	0.14
Central 6-10	0.61	97.09	<0.0001	0.363	<0.0001	-1.22	0.08	-0.11	0.88
Posterior 0-2	0.14	10.87	<0.0001	0.03	<0.0001	-0.1	0.58	-0.49	0.01
Posterior 2-6	0.39	40.34	<0.0001	0.06	<0.0001	-0.28	0.12	-0.47	0.01
Posterior 6-10	0.61	101.76	<0.0001	0.27	<0.0001	-1.33	0.01	0.12	0.81
Full depth 0-2	0.02	1.72	0.16	0.01	0.14	0.05	0.86	-0.54	0.08
Full depth 2-6	0.18	14.27	<0.0001	0.05	<0.0001	-0.17	0.52	-0.49	0.07
Full depth 6-10	0.57	83.05	<0.0001	0.31	<0.0001	-1.17	0.07	-0.50	0.46

Due to the high correlation between both eyes only right eye been selected for further analysis (Armstrong, 2013).

Age was found to be significantly correlated with CD at all layers zone when we look at all the total diameter of 0-10 mm. Additionally, significant correlation was found for all corneal zone and layers except the anterior 0-2 mm zone which were not statistically significant. Table 3-4 and Figure 3-1,3-2,3-3 and 3-4 give details of this relationship.

Table 3-4: Person correlation(r) between age and corneal densitometry in different corneal layer and zone for right eye only.

	0-2 mm		**2-6 mm**		**6-10 mm**		**0-10 mm**	
	r	P value	r	P value	r	P value	r	P value
Anterior	**-**.124	.11	.045	.33	.643	<0.000	.363	<0.000
Central	.480	<0.000	.649	<0.000	.785	<0.000	.789	<0.000
Posterior	.411	<0.000	.603	<0.000	.799	<0.000	.786	<0.000
Full depth	.128	.107	.423	<0.000	.769	<0.000	.681	<0.000

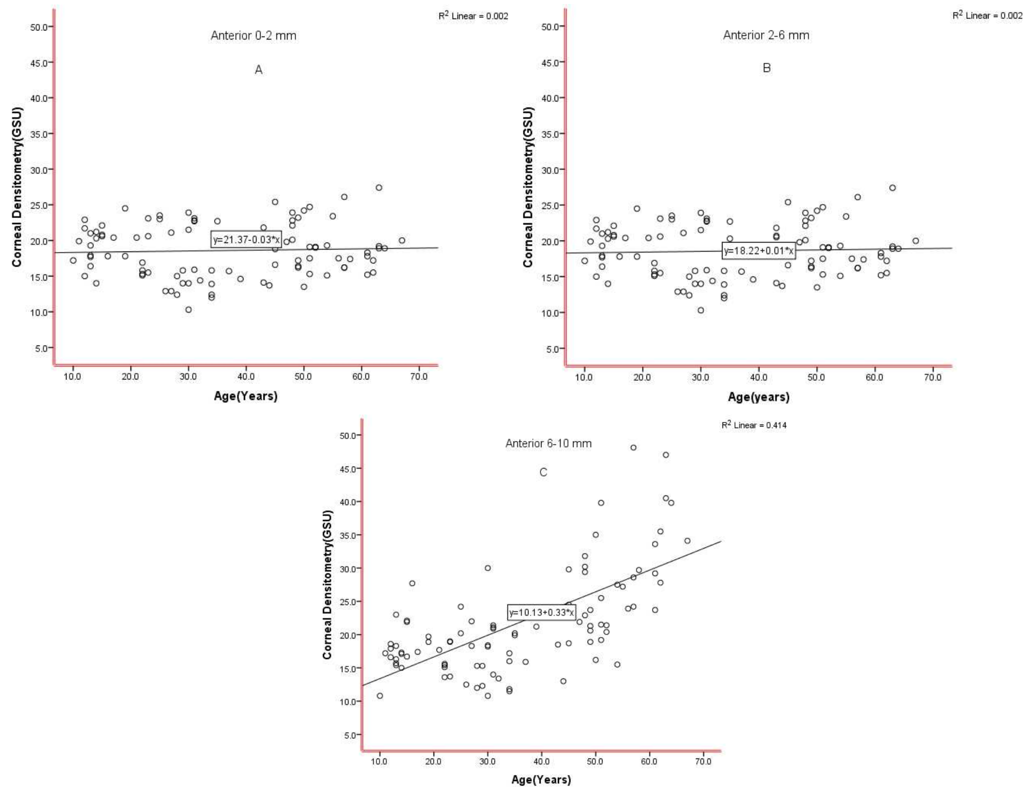

Figure 3-1: Relation between age and corneal densitometry at zones 0-2 (A) 2-6 (B) and 6-10 (C) for anterior corneal layer (right eye).

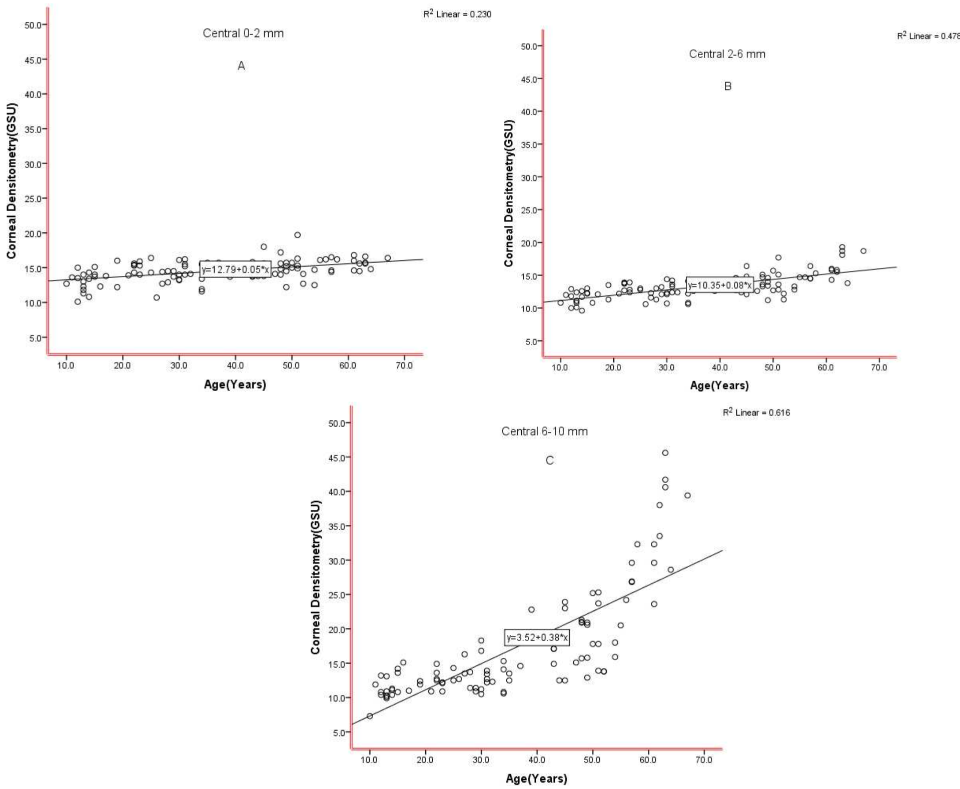

Figure 3-2: Relation between age and corneal densitometry at zones 0-2 (A) 2-6 (B) and 6-10 (C) for central corneal layer (right eye).

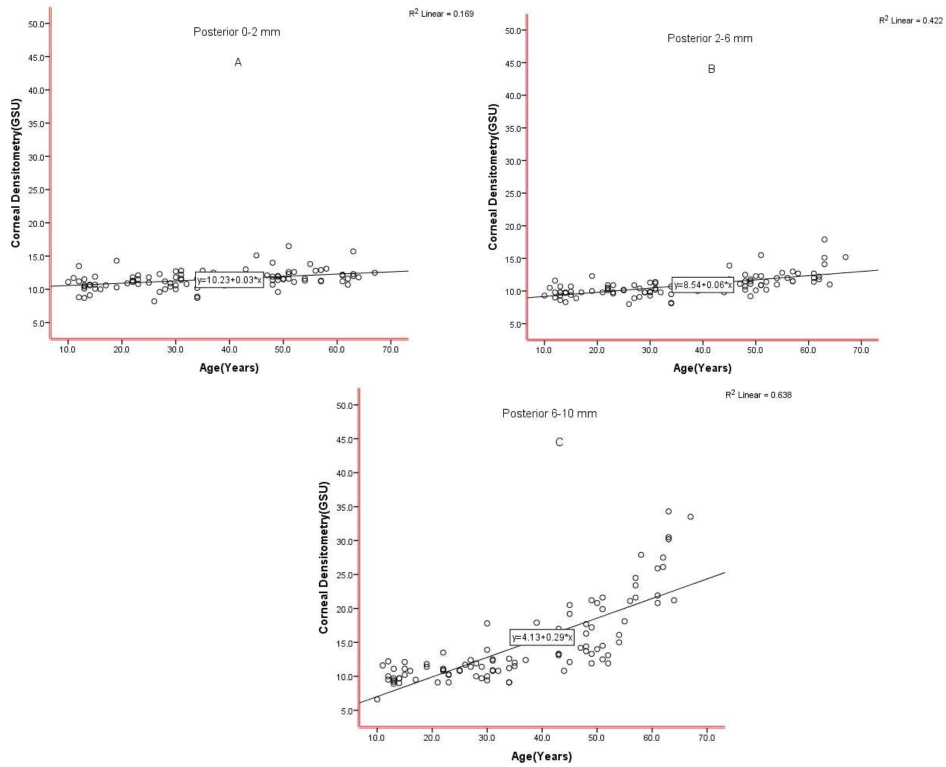

Figure 3-3: Relation between age and corneal densitometry at zones 0-2 (A) 2-6 (B) and 6-10 (C) for posterior corneal layer (right eye).

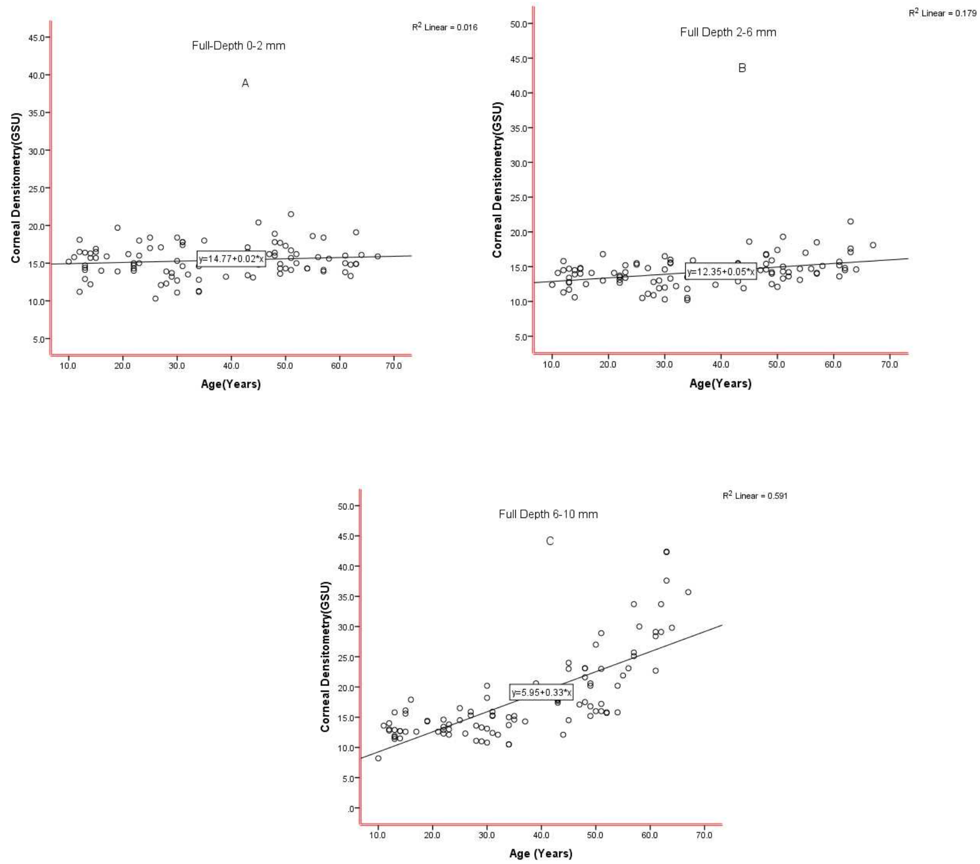

Figure 3-4: Relation between age and corneal densitometry at zones 0-2 (A) 2-6 (B) and 6-10 (C) for Full Depth corneal layer (right eye).

3.5 Discussion

This study demonstrates that corneal clarity decreases with age particularly in the 6-10mm concentric zone. One possible reason for this decrease in clarity may be changes that occur in the corneal endothelium. Endothelial changes related to age have been studied both experimentally and clinically. It has been demonstrated that between the ages of twenty and eighty, the yearly reduction in cell density of the corneal endothelium is about 0.6 percent, with concomitant increases in pleomorphism and polymegethism (Blatt et al., 1979, Bourne et al., 1997, Laule et al., 1978, Murphy et al., 1984b, Faragher et al., 1997). These age related changes to the endothelium lead to increases corneal thickness and may increase CD measurements.

Due to the fact that the cornea is the scaffold for the major refractive surface of the eye, any biological or mechanical response to injury will also affect optical performance. The same mechanisms responsible for preserving ocular integrity can, as a result, undermine the goals of accomplishing stable and predictable visual results after refractive procedures (Dupps and Wilson, 2006). One report (Cennamo et al., 2011) discovered a significant early rise in the mean anterior density readings (from 27.71 ± 4.39 to 37.812 ± 12.31) 3 months after photorefractive keratectomy (PRK) that subsequently reduced again by 12 months (26.291 ± 4.93). The high CD at 3 months is related to the wound healing process. Activated keratocyte apoptosis was found to be higher post PRK, these cell were located in the anterior stroma causing haze that improved over time (Mohan et al., 2003).

This study found a fluctuation of densitometry values in both 0-2 and 2-6 mm zone in the anterior layer at different age groups. 10-19 years and 40-49 years age groups were found to have higher CD. These two age groups are where significant hormonal changes can take place in the human body. CCT changes in women have been reported previously during pregnancy (Weinreb et al., 1988) and during the menstrual cycle (Leach et al., 1971). It has been reported that contact lens use during the premenstrual phase is easier than during the menstrual period (Guttridge, 1994, Serrander and Peek, 1993). This may be due to changes in CCT leading to changes of corneal curvature (Kiely et al., 1983) which can cause intolerance to contact lens(CL) wear. Additionally, the lack of sex hormones have been suggested as a possible reason for changes in the corneal biomechanics of individuals over the age of 40 (Goto et al., 2001). It is therefore possible that hormones may also play a role in the changes in corneal clarity that this study found with age.

Corneal remodelling reported to take place in keratoconus (KCN) (Silverman et al., 2014), post refractive ectasia (Rocha et al., 2013) and in injured stroma (Utsunomiya et al., 2014) all lead to increases in corneal haze. This remodelling has been suggested to play a role in the relationship between anterior cornea changes and visual acuity after corneal cross-linking (CXL) (Hafezi, 2012, El Saadany et al., 2015) or corneal trauma (Kanellopoulos and Asimellis, 2014a). Corneal remoulding activity may be able to explain why the teenage group in this study has higher levels of CD than those of the next age group (20-29) as the keratocyte activity is known to be higher in this younger group.

The cornea is the major physical covering of the eye. To contribute to the focusing ability of the eye, it has to be tough, transparent, and capable of maintaining a smooth and steady curvature. Controversies still exist over how transparency is maintained even with the advanced understanding of the structure and other properties of the cornea. One area of interest is to investigate the role of hydration, which is a major determinant of transparency and which can be manipulated within reasonable parameters under standardized conditions (Candia, 1980, Duane, 1949, Freegard, 1997). The study by Olsen (1982) of corneal backscatter established that there was a remarkable growth in total CD with advancing age. In contrast, it has also been shown that CD increases in the central 6mm of the cornea are minimal with respect to age. In the current study it was shown that, whereas there is no age-linked variation in the central 6 mm of the cornea, a significant rise is seen in the region next to the limbus. the study excluded the 10-12 mm area zone from analysis in our study because this area could be part of the limbus with various changes in the white-to-white thickness of the cornea (Cakmak et al., 2012), suggesting that some individuals have corneas with analysis parameters smaller than 12 mm. The Higher backscatter quantities therefore occur when sclera and parts of the limbus are involved in the assessment of the peripheral layers. Even slight variations in the situation of the limbus would physically influence the 10-12 mm CD outcomes. This, however, does not take place in the significantly dominant annuli. Owing to this factor, the substantial expansion in scatter in the 10-12 mm zone can neither be absolutely understood nor rightly correlated with age (Hillenaar et al., 2011).

All the measured limits were connected with each other, signifying that the scattering for every region of the cornea is constant with respect to other portions of the cornea. It is interesting, therefore, that a statistically substantial connection between the difference in visual acuity (VA), stromal scattering, and assessed interface was established. This indicates that the complete scattering capacities may not be as significant in influencing VA as is their change throughout the cornea (Srikumaran et al., 2014).

When this study looks at correlation between total CD and age we found it is correlated positively. An earlier studies (Patel et al., 2007, Patel et al., 2009) revealed that light scatter in the cornea is a phenomenon that is related to age and that the best preoperative forecaster of post-operative VA is age. Consequently, the ages of patients in the study might have an effect on the dimensions of the scattering that are determined from Pentacam images.

In Summary our study findings agree with other study (Dhubhghaill et al., 2014) as there was a considerable increase in CD with age, despite the fact that the increase was confined to the peripheral cornea. Conversely, this study finds a relationship between certain age groups and CD, notably within the teenage age group and those within the 40-49 age group. A possible explanation for this observation may be the hormonal changes known to occur at this time. Interestingly that teen-age group was found to be cloudier than 20-29 age groups, suggesting the additional factor of corneal remodelling with increased keratocyte activity may be relevant.

This study was limited by small sample size. However this study is first study to our knowledge that aims to standardized CD value in healthy control eyes to include those individuals aged between 10-19. This study suggests that additional factors other than age alone may have a role to play in corneal clarity. These values for CD dimensions, as well as subdivisions based on layer and surface area, might provide a standardized stage for use in further studies and clinical practice.

Chapter 4

Corneal Imaging and Densitometry Measurements in Patients with Myopia Undergoing Laser-Assisted In Situ Keratomileusis and Laser-Assisted Sub-Epithelial Keratectomy

Contributions

I designed the study in collaboration with my supervisors, conducted the experiments, recruited the participants, preformed the imaging investigation, completed the experiments, analysed the data, and wrote the manuscript. All this work was achieved with regular discussion, close collaboration and feedback on data analysis and writing from my supervisors: Dr Chantal Hillarby and Prof Fiona Carly.

Publication

The chapter is prepared as a manuscript and submitted for publication.

ALZAHRANI, K., CARLEY, F., BRAHMA, A., MORLEY, D. & HILLARBY, M. C. (2018). Corneal Imaging and Densitometry Measurements in Patients with Myopia Undergoing Laser-Assisted In Situ Keratomileusis and Laser-Assisted Sub-Epithelial Keratectomy. Target journal: *eye*.

Conferences Presentations

The abstract was presented at the 2017 European Association for Vision and Eye Research Conference and at Manchester optometry meeting 2017.

Acknowledgement

The authors thank the Manchester Royal Eye Hospital (corneal clinic) for supporting patient's recruitments for this study. Supported by Armed Forces Medical Services, Ministry of Defence, Riyadh, Saudi Arabia.

4.1 Abstract

Aim: This study aimed to compare the preoperative and postoperative corneal densitometry data of patients undergoing either laser-assisted in situ keratomileusis (LASIK) or laser-assisted sub-epithelial keratectomy (LASEK), and to determine the differences in surgical outcomes.

Methods: This comparative study was performed at Manchester Royal Eye Hospital, UK. Preoperative and postoperative corneal densitometry data were collected using Oculus Pentacam. The data were collected at three corneal depths (anterior, central and posterior) and at three corneal annulus ring diameter ranges (0-2 mm, 2-6 mm and 6-10 mm). Postoperative data were collected at 6 weeks and 3 and 6 months after treatment. The pre- and post-operative quality of vision was determined using best-corrected visual acuity (BCVA).

Results: Data was collected from 60 eyes of 16 patients who underwent LASIK and 14 who underwent LASEK only the 30 right eyes reported in the analysis, with mean ages of 37.06±10.0 and 37.7±6.6 years, respectively. A non-statistically significant increase in corneal densitometry values was observed in all concentric zones in all corneal layers at post LASIK treatment (P>0.0018). A statistically significant increase in densitometry values was observed after LASEK, but only in the central layer at all zones and posterior layers at 2-6 and 6-10 concentric zones (P<0.0018). Pre- and Post-operative BCVA did not significantly differ after LASIK but showed a statistically significant difference at 6 weeks after LASEK (P<0.016). Post-operative BCVA differed significantly between the groups at 6 weeks (P<0.01) and 3 months (P=0.02).

Conclusion: Corneal wound healing plays an important role in determining corneal transparency and may be responsible for an increase in corneal haze following refractive surgery. This response differs according to the procedure, as shown by the diversity of

increased haze after different procedure which corresponds to the region where the flap was cut and would lead to increased wound healing and inflammation. Corneal densitometry data obtained using Oculus Pentacam may help assess treatment outcomes after laser refractive surgery.

4.2 Introduction

Refractive error is an eye disorder which normally occurs when the eye cannot clearly focus images from the outside world. It occurs when light focuses incorrectly on the retina, and is determined by the balance of the optical power of the crystalline lens and cornea, and the axial length of the eye. It results in blurry vision and sometimes visual impairment (Sugar et al., 2002), thereby decreasing a person's quality of life (Coleman et al., 2006) and leading to an increase in depression (Owsley et al., 2007).

Refractive error can be corrected in many ways. Optical correction using spectacles or contact lenses is the simplest way (Shortt et al., 2013). Refractive surgery is another method to correct vision by adjusting the eye's focusing ability by reshaping the cornea or implanting a lens inside the eye. The most widely used medical procedure nowadays is laser refractive surgery which involves reshaping the corneal curvature by using an excimer laser, a cool ultraviolet (UV) beam of light (Juhasz et al., 1999). This photo-ablation procedure uses a far-UV (193-nm) argon fluoride excimer laser to remove corneal tissue precisely by vaporizing it and breaking up specific molecules with minimal adjacent corneal damage during laser refractive surgery (McDonald et al., 1991, Trokel et al., 1983). Each pulse of the excimer laser breaks the corneal collagen bond, ruptures the collagen polymer into small fragments and later expels a discrete volume of corneal tissue from the surface (Maurino and Nguyen, 2008).

Nowadays, several options of laser refractive surgery using excimer laser are available. Laser-assisted in situ keratomileusis (LASIK) was introduced in the 1990s (Pallikaris et al., 1990) and has become popular worldwide (Solomon et al., 2009). Photorefractive keratectomy (PRK), the latest variant of laser-assisted sub-epithelial keratectomy (LASEK), was introduced later in 1999 (Camellin, 1999).

Both LASIK and LASEK correct the refractive error by adjusting the corneal curvature using a cool excimer laser. Therefore, the transparency of the cornea may be affected after surgery. Oculus Pentacam (OCULUS Optikgerate GmbH, Wetzlar, Germany) is a non-invasive imaging device used to measure corneal pachymetry and topography. More recently, using a new analysis package which provides a numeric value of corneal clarity (Cho et al., 2010), this device has been found useful for evaluating outcomes after PRK (Zadnik et al., 1996a). An important area of study after refractive surgery is corneal haze formation. Studies suggest that corneal haze formation has an association with time (Chen et al., 2010, Amano and Shimizu, 1995, Siganos et al., 1999, Celik et al., 2014, Ang et al., 2016).

In this study, we aimed to measure the pre- and post-operative corneal densitometry data of patients undergoing LASIK and LASEK to investigate how corneal densitometry values and haze change after laser refractive surgery, to determine the differences in corneal densitometry values between both procedures and to identify the region of the cornea where these differences occur.

4.3 Methodology and Participants

This comparative and non-randomised cross-sectional study was approved by the Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK, and the NREC local ethics committee no 15/NE/0363. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature of the study. Data collections started on 3rd of November 2015 till 1st of December 2017. Pre- and post-operative corneal densitometry and best-corrected visual acuity (BCVA) LogMar based on monocular subjective refraction data recorded at 6 weeks and 3 and 6 months were recorded.

4.3.1 Inclusion Criteria

Participants were patients who had undergone LASIK and LASEK at the Laser Vision Clinic in Manchester Royal Eye Hospital (MREH) and were selected by convenient sampling. Patients included had a healthy eye without any diseases at treatment date or any previous eye surgery. Patients aged 18 years and below were excluded as their eye power was not yet stable, and so were patients aged above 50 years. Only patients with myopia and a BCVA of 6/9 or better were included.

4.3.2 Corneal Imaging

Oculus Pentacam is a valuable instrument in planning and monitoring refractive surgery. In this study, we used the densitometry software for Pentacam. Densitometry data which was collected during routine pre- and post-operative visits at MREH was analysed in this study. LASIK and LASEK were performed after complete examination, and the patients' suitability for any procedure was determined by the surgeon.

4.3.3 Surgical Procedure

The clinical notes of each patient enrolled into this study were retrieved to collect data on pre- and post-operative BCVA and refractive power. BCVA was assessed using a LogMAR chart. Prior to surgery, each patient was examined thoroughly to ensure their eyes were healthy and to determine their suitability for laser refractive surgery. After the surgery, the patients were advised to return for follow-up to monitor their recovery. After LASIK, the patients were normally monitored for up to 3-6 months after surgery and for LASEK, they were monitored for up to 6-9 months. During the pre- and post-operative visits, refractive power and BCVA were routinely recorded.

All surgeries were performed under topical anaesthesia. WaveLight Allegretto Wave Eye-Q excimer laser was used to ablate the corneal curvature in all patients. For LASIK, a

130±30-μm-thick flap with a diameter of 8.5-9.0 mm was cut using an oscillating microkeratome. The flap hinge was opened superiorly before the excimer laser was used on the cornea for ablation (ablation zone diameter varied from 6.0 to 7.0 mm). For LASEK, ethanol was used to soften the epithelial layer for less than 20 seconds before peeling it off or cutting open superiorly. The thickness of the epithelial flap was about 50-60 μm. The excimer laser was then used on the cornea for ablation (ablation zone diameter varied from 6.0 to 7.0 mm).

For the purpose of analysis, corneal densitometry data were documented in concentric radial zones with diameter ranges of 0-2 mm, 2-6 mm and 6-10 mm at three different depths from the anterior (120 μm), central and posterior (60 μm) cornea.

4.3.4 Statistical Analysis

The data were analysed using IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM Corp. Independent t-test was used to compare the LASIK and LASEK groups. Paired t-test was used to compare the pre- and post-operative densitometry and BCVA data in each group after adjusted P value with Bonferroni correction. For the purpose of these comparisons, the pre- and post-operative data at 6 weeks and 3 and 6 months were collected. To observe the patterns of densitometry changes over time, graphs were plotted for each case of LASEK and LASIK. Power calculated using G*Power 3.1.9.2 retrospectively for anterior 0-2 mm zone at 6 months post-treatment was equal to 0.37.

4.4 Results

Data from 60 eyes of 30 patients (17 women and 13 men; 32 eyes of patients who underwent LASIK and 28 eyes of patients who underwent LASEK) were analysed in this study and only data from 30 right eyes was used in further analysis due to the high correlation between both eyes (Armstrong, 2013). The mean age and standard deviation of the LASIK group was 37.06±10.0 years and that for the LASEK group was 37.7±6.6 years, which was not statistically significant (P=0.77).

4.4.1 LASEK Group

Pre- and post-operative analyses in the LASEK group showed statistically significant differences at corneal central layer at all zones and zones of 2-6 and 6-10 at posterior layer at 6 weeks post treatment (P<0.0018). At 3 months, corneal densitometry values showed statistically significant differences from the pre-operative values in the zone of 6-10 mm at posterior layer (P<0.0018). Corneal densitometry values at 6 months showed statistically significant differences from the pre-operative values in posterior layer at zones 2-6 mm and 6-10 mm (P<0.0018), (Table 4-1).

Table 4-1: Mean and SD for corneal densitometry at pre-treatment and post treatment at 6 weeks, 3 and 6, months for LASEK with statistical value differences from Pre-treatment.* Represent statistical significant at level (P<0.0018).

Layer	Zone	Pre treatment Mean±SD	Post treatment Mean±SD, P value from baseline		
			6 weeks	3 months	6 months
Anterior	0-2 mm	19.36±1.1	22.3±2.8, P= 0.003	21.3±4.9, P=0.13	21.5±4.4,P=0.04
	2-6 mm	17.6±1.1	20.02±3.1,P=0.011	19.3±4.02, P=0.1	20.2±5.9,P=0.08
	6-10mm	18.5±3.5	20.1±4.5,P=0.03	18.6±3.4, P=0.36	19.4±4.7,P=0.37
Central	0-2 mm	11.9±0.6	13.1±1.3,P=0.001*	12.9±1.6, P=0.01	13.3±1.9, P=0.010
	2-6 mm	10.8± 0.7	11.9±1.2,P=0.001*	11.8±1.4, P=0.01	12.1±1.7, P=0.003
	6-10 mm	12.1±2.4	13.5±2.8,P=0.001*	13.2±2.4, P=0.005	14.0±3.3, P=0.003
Posterior	0-2 mm	9.9±0.7	10.9±1.4,P=0.006	10.8±1.4, P=0.02	11.3±1.3, P=0.002
	2-6 mm	9.3±0.5	10.5±1.2,P<0.001*	10.4±1.2, P=0.007	10.8±1.2,P<0.001*
	6-10 mm	11.4±2.2	12.96±2.7,P<0.001*	12.9±2.2,P<0.001*	13.9±3.1,P<0.001*

4.4.2 LASIK Group

Pre- and post-operative corneal densitometry values showed no statistically significant differences at 6 weeks 3 months and 6 months post treatment in all corneal layers and zones (P>0.0018), (Table 4-2).

Table 4-2:Mean and SD for corneal densitometry at pre-treatment and post treatment at 6 weeks, 3 and 6 months for LASIK with statistical value differences from Pre-treatment.* Represent statistical significant at level (P<0.0018).

Layer	Zone	Pre treatment Mean±SD	Post treatment Mean±SD, P value from baseline		
			6 weeks	3 months	6 months
Anterior	0-2 mm	18.9±1.5	20.1±1.7,P=0.03	19.4±2.8,P=0.75	19.5±1.5,P=0.48
	2-6 mm	17.2±1.5	18.6±1.8,P=0.004	18.1±2.7,P=0.4	18.3±1.6,P=0. 9
	6-10mm	17.0±2.5	18.5±3.2,P=0.05	18.9±4.7,P=0.51	19.3±3.5,P=0.25
Central	0-2 mm	11.5±0.8	12.3±1.2,P=0.14	12.0±1.6,P=0.6	12.2±1.1,P=0.24
	2-6 mm	10.4±0.9	11.4±1.2,P=0.003	11.2±1.5,P=0.3	11.4±1.2,P=0.03
	6-10 mm	11.9±2.3	12.9±2.6,P=0.02	13.3±3.5,P=0.4	13.6±2.9,P=0.13
Posterior	0-2 mm	9.96±0.8	10.3±1.1,P=0.3	10.1±1.2,P=0.9	10.1±1.1,P=0.92
	2-6 mm	9.3±0.96	9.99±1.1,P=0.04	9.9±1.2,P=0.29	9.9±1.2,P=0.23
	6-10 mm	11.7±2.6	12.6±2.5,P=0.016	13.05±2.9,P=0.23	13.2±2.5,P=0.07

4.4.3 Differences between the Two Groups

A comparison between the pre-operative LASIK and LASEK corneal densitometry values revealed no significant differences in any of the layers and corneal zones (P>0.05). However, statistically significant intergroup differences in corneal densitometry values in the anterior layer at zones 0-2 mm (P=0.01) were found at 6 weeks post treatment. At 3 months, no statistically significant intergroup difference was found in corneal densitometry values (P>0.05).

At 6 months, no statistically significant intergroup differences were found in the anterior and central layers at all zones (P>0.05). Posterior-layer densitometry revealed statistically significant intergroup differences at zones 0-2 mm (P=0.01).Figure 4-1, 4-2 and 4-3 represent change from base line difference between both groups.

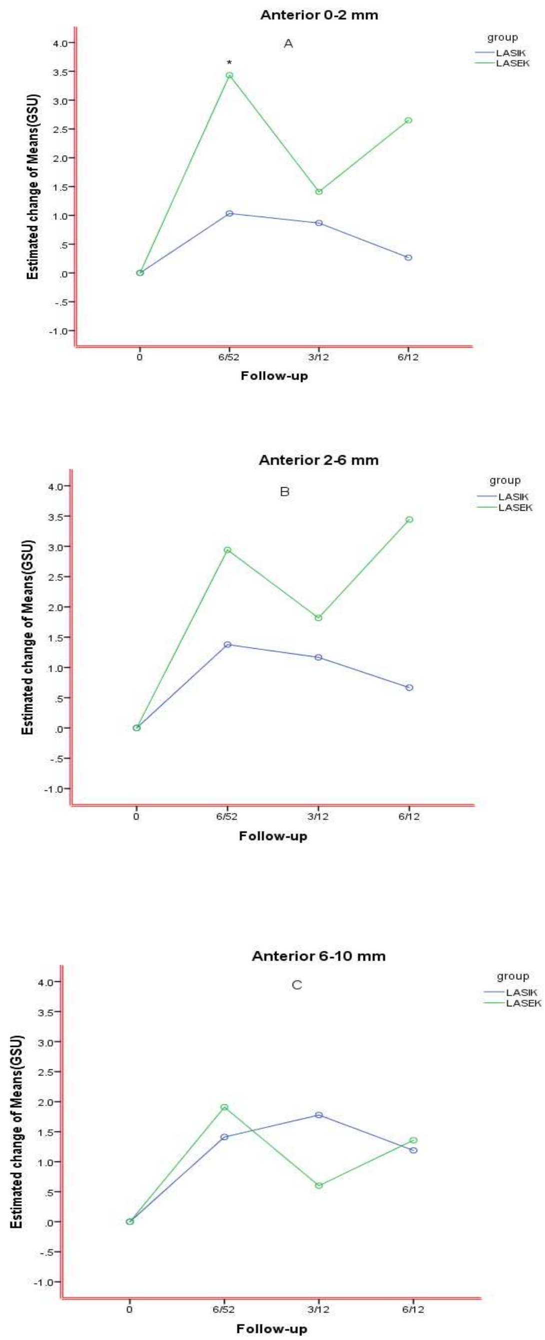

Figure 4-1: Mean of corneal densitometry change from baseline of the corneal densitometry for both groups at anterior 0-2 mm (A), anterior 2-6 mm (B) and anterior 6-10 mm(C) layers.

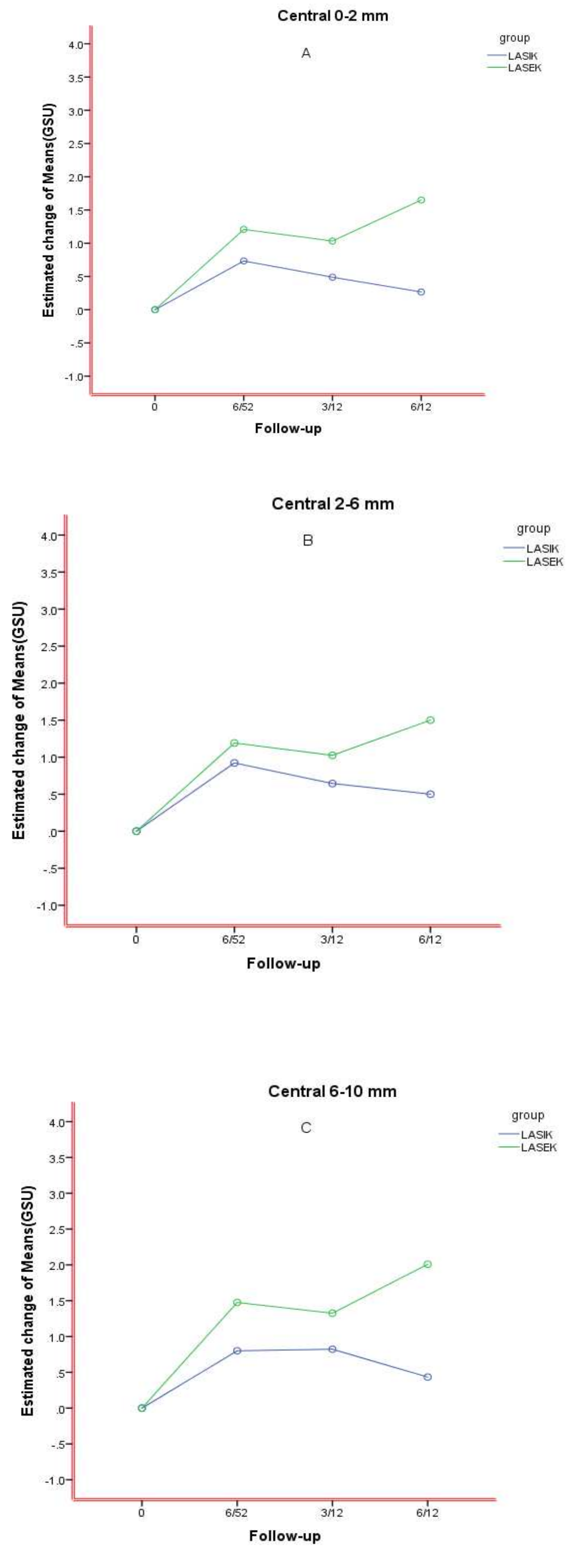

Figure 4-2: Mean of corneal densitometry change from baseline of the corneal densitometry for both groups at central 0-2 mm (A), central 2-6 mm (B) and central 6-10 mm(C) layers.

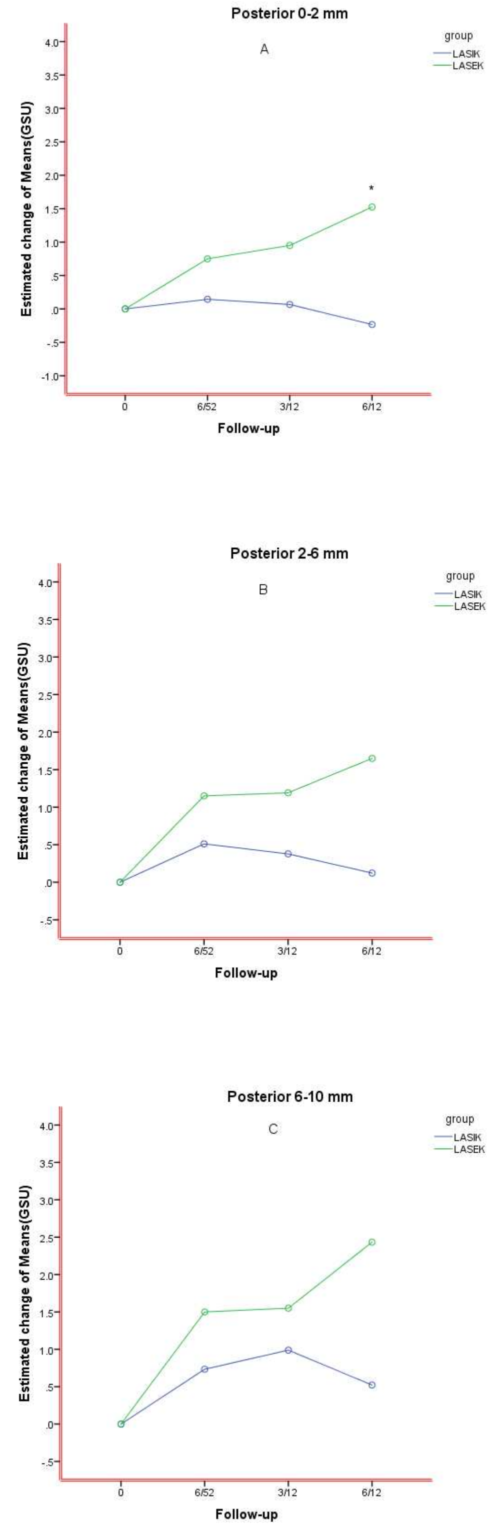

Figure 4-3:Mean of corneal densitometry change from baseline of the corneal densitometry for both groups at posterior 0-2 mm (A), posterior 2-6 mm (B) and posterior 6-10 mm(C) layers.

4.4.4 Best-Corrected Visual Acuity

In the LASEK group, the 6-week values showed none statistically significant differences from the baseline values (P=0.006), but not the values at 3 months (P=0.02) and 6 months (P=0.06). In the LASIK group, none of the post-operative BCVA values showed any statistically significant differences from the baseline values as at 6 weeks (P=0.09), 3 months (P=0.4) and 6 months(P= 0.4) . Looking at comparison between both groups neither group showed statistical differences before surgery. However, the 6-week BCVA values showed statistically significant intergroup differences (P<0.001). Whereby, the 3 month BCVA values also showed statistically significant intergroup differences. Nevertheless, the 6-month BCVA values showed no significant intergroup differences (P>0.05) (Table 4-3).

Table 4-3:Mean±SD and P value for BCVA at pre-treatment and post treatment at 6 weeks, 3 and 6 months post-surgery for both LASIK and LASEK groups (* represent significant from pre-treatment for each groups).

	LASIK Mean±SD	**LASEK** Mean±SD	**P Value**
Pre treatment	-0.04±0.06	-0.04±0.07	0.99
Post treatment 6 weeks	-0.01±0.08	0.07±0.07*	0.01
Post treatment3 months	-0.029±0.07	0.09±0.17	0.02
Post treatment 6 months	-0.026±0.11	-0.01±0.07	0.64

4.5 Discussion

Corneal transparency can be affected by many pathological conditions including infections, dystrophies, degeneration and tissue injury. Adjusting the corneal curvature to correct refractive errors by using a laser causes tissue injury on the cornea. LASEK and LASIK have been proven safe and efficient in myopia correction (Kim et al., 2004, Hashemian et al., 2012). Corneal haze, which is considered an undesirable complication, can develop during wound healing. Using advanced imaging systems helps us understand the healing process and the effect of LASIK and LASEK on the corneal structure during the wound healing response.

Our study found that corneal densitometry values after LASEK were higher at 6 weeks, 3 and 6 months than the pre-operative values. Haze at 6 weeks post LASEK was found in central corneal layer at all zones and posterior layer at zone 2-6 mm and 6-10 mm. only posterior layer at zone 6-10 mm as shows an increase haze at 3 months post LASEK . At 6 months post LASEK haze showed an increase at the posterior layer of the zones 2-6 mm and 6-10 mm. However, after LASIK none statistical significant haze was found at all post treatment period.

This diversity in haze formation and improvement between the two groups suggested that the corneal wound healing response differed after each procedure (Lazaridis et al., 2017, Litwak et al., 2002). Haze formation is a well-known high-risk factor after LASEK rather than LASIK. Corneal wound healing occurs during compound stages, and the cascade results in corneal stability. Biochemical, histochemical and histological changes are the mainstays of these stages (Sappino et al., 1990). Keratocytes play an important role in maintaining corneal transparency, wound healing and synthesising components during normal tissue homeostasis. Keratocytes are known to be activated during the first months after LASIK (Avunduk et al., 2004). Cytokine induction after LASIK leads to the activation of myofibroblast transformation and epithelial keratocyte apoptosis. Keratocyte density varies across the stromal layer; it is estimated to be 10% lesser in the central and posterior layers than in the anterior layer (Patel et al., 2001). Keratocyte density has been reported to increase between the first week and 3 months after surgery, and to reach its normal value within 6 months (Pisella et al., 2001, Mitooka et al., 2002). In LASIK, the apoptotic cells are located anterior and posterior to the lamellar interface created by the microkeratome cut (Mohan et al., 2003). Subsequent to keratocyte apoptosis, the necrotic cells activate stromal keratocytes in deeper layers (Alio and Javaloy, 2013).

The wound healing process is similar in surface ablation such as LASEK and LASIK, but differs in its intensity and location of wound repair. In LASIK, keratocyte apoptosis and proliferation occur in the posterior flap, anterior retro-ablation layer and regions adjacent to the lamellar flap within the stroma. In our study, we cut a 130±30-μm-thick flap with an 8.5-mm diameter, and the ablation zone had a diameter of 6.0-7.0 mm for LASIK; the changes to the keratocyte populations occurred within this area. Our results showed that at 6 weeks after LASIK, corneal densitometry values increased compared to their pre-operative levels in all corneal layers within all the corneal annulus ring diameters. Since the ablation zone for LASIK had a diameter of about 6.0-7.0 mm in our study, the changes in corneal transparency were notable in all layers within that diameter. May be the lamellar interface is still a site of repair and inflammation Moreover, no myofibroblast generation was observed in the central stroma as seen in rabbits undergoing LASIK (Alio and Javaloy, 2013). This could be due to the intact basement membrane in the central cornea after LASIK. The basement membrane is believed to act as a barrier to signalling molecules from the epithelium. A pervious study reported only mild stromal cell apoptosis after LASIK (Ying et al., 2006).

Our results showed that corneal densitometry values were significantly higher after LASEK than before surgery in all corneal layers and zones at all three times points but statistically at all zones of central layer and 2-6, 6-10 of posterior layer at 6 weeks, 6-10 mm posterior layer at 3 months and zone 2-6 mm ,6-10 mm at 6 months . The epithelial layer was peeled off at a thickness of 50-60 μm; hence, keratocyte apoptosis occurred in the superficial cornea and keratocyte proliferation occurred in the posterior and peripheral stroma (Netto et al., 2005b). The wound healing response was significantly better after LASEK because keratocyte density expected to be higher in the superficial anterior stroma. The keratocytes have great capability to be activated and transformed into myofibroblasts, and this affects corneal clarity by reducing crystalline synthesis. Moreover, disruption of the epithelial basement membrane and Bowman's membrane over the central cornea implies a higher rate of corneal haze. Our findings showed a significant increase in corneal densitometry values after the surgery until 6 months after LASEK. This finding is in agreement with those of studies that conclude haze formation is highly likely to occur within 6 months after LASEK (Kim et al., 2004, Lin et al., 2004). Surprisingly, the anterior cornea shows insignificant differences in transparency at 6 months after both procedures. This could be due to the rapid restoration of the epithelial layer to its normal pre-operative state immediately after surgery. Our result indicated that the LASEK procedure has a short come in term of quick recovery (Claringbold, 2002).

Our findings based on BCVA assessments also showed that LASIK was superior to LASEK in terms of speedy visual acuity recovery as no significant pre-treatment versus post-treatment noted overall 6/12 follow-up. This could be attributed to high corneal haze formation in addition to rapid changes in refraction after LASEK (Kim et al., 2004). However, at 6 months after surgery, no significant intergroup differences were observed in visual acuity similarly observed previously (Zhao et al., 2014).

This study was limited by a small sample size. Another limitation was missed analysis of the haze around individual ablation zones, as differences in the exact location between 2-6 and 6-10 mm zone are possible. Also despite the repeatability of the Pentacam but it is not correlated will with VA so not including a qualitative assessment of VA was another limitation. It is important to include patient subjective compline in the assessment. Also assuming constant refractive index of the cornea by Pentacam algorithm and not account for changes take place is another limitations.

On the basis of these findings, we conclude that differences in the wound healing response between LASEK and LASIK lead to differences in the presence and duration of corneal haze. Visual acuity found to be have same phase in improvement with change on corneal densitometry. We present our objective findings obtained using Oculus Pentacam on how corneal clarity changes after LASEK and LASIK in all corneal stromal layers.

Chapter 5

Corneal Imaging and Densitometry Measurements in Patients with Fuchs' Dystrophy Undergoing Penetrating Keratoplasty and Descemet's Striping Automated Endothelial Keratoplasty

Contributions

I designed the study in collaboration with my supervisors, conducted the experiments, reviewed surgical data base, completed the experiments, analysed the data, and wrote the manuscript. All this work was achieved with regular discussion, close collaboration and feedback on data analysis and writing from my supervisors: Dr Chantal Hillarby and Prof Fiona Carly.

Publication

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

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Conferences Presentations

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5.1 Abstract

Aims: In the present study, we used the densitometry software from the Oculus Pentacam to compare postoperative corneal clarity between penetrating keratoplasty (PK) and Descemet's stripping endothelial keratoplasty (DSAEK) in patients with Fuchs' dystrophy.

Methods: A retrospective comparative study was carried out at Manchester Royal Eye Hospital. In 28 patients with Fuchs' dystrophy, corneal densitometry measurements were performed 12–18 months after corneal transplantation. The correlations of the densitometry measurements with the best corrected visual acuity (BCVA) and central corneal thickness (CCT) were analysed and compared between eyes that underwent PK and those that underwent DSAEK.

Results: Corneal densitometry measurements in the 33 eyes showed no significant differences between the PK and DSAEK post-surgery groups. There was no significant correlation between CCT and corneal densitometry measurements in either group ($P>0.05$ in both cases). After DSAEK, corneal densitometry measurements were significantly correlated with BCVA in the central ($P=0.01$), posterior ($P=0.007$), and full-depth ($P=0.008$) 0–2 mm zones of the cornea but not in PK group. The postoperative CCT was significantly different between the two groups ($P<0.01$).

Conclusion: The two types of corneal transplantation resulted in different outcomes in terms of corneal densitometry measurements. Improved visual acuity after DSAEK was found to positively correlate with improvements in corneal clarity. Oculus Pentacam provides an objective evaluation tool to monitor corneal status after surgery.

5.2 Introduction

Fuchs' endothelial dystrophy (FED) is normally treated using corneal transplantation (Kang et al., 2005, Weisenthal and Streeten, 1997), which restores healthy endothelial function (Dapena et al., 2009). The first description of FED was published in 1900 by Ernst Fuchs, who described deposits known as guttata on the Descemet's membrane beneath the corneal endothelium; an abnormal extracellular matrix and endothelial cell loss were also apparent (Bruinsma et al., 2013).

During the initial phase of the FED, epithelial oedema is absent. However, it builds up progressively in later stages. The degree of epithelial oedema is estimated by measuring the central corneal thickness (CCT). Corneal oedema begins within the central optical area and is a key cause of reduced vision in FED (Eghrari and Gottsch, 2010). This is particularly severe in the morning due to reduced tear evaporation during sleep (Fuchs, 1910). As the cornea becomes more oedematous, discomfort occurs, followed by extreme pain and photophobia. The eventual effects of extreme endothelial cell loss include stromal swelling, creation of connective tissue in the stroma and epithelium, corneal oedema, and a reduction in clear vision.

In 1900, the first successful full-thickness keratoplasty was performed by Edward Zirm. Since then, this approach has seen considerable alteration and progress. Currently, over 3,000 corneal transplants are conducted annually in the United Kingdom alone (Tan et al., 2012). FED transplants account for 25.8% from overall proposed number for corneal transplant(Gaum et al., 2012) .Historically, FED was treated using full thickness (penetrating) keratoplasty (PK), which replaces all five corneal layers, However, as only the endothelial layer is diseased in FED, it is possible to use Descemet's stripping automated endothelial keratoplasty (DSAEK), whereby only the posterior stroma and endothelium are replaced (Espandar and Carlson, 2013).

The success of these surgical procedures is evaluated in terms of best corrected visual acuity (BCVA) and CCT. Clinically, Pentacam (OCULUS Optikgerate GmbH, Wetzlar, Germany) images are routinely used to assess patients undergoing transplant surgery. However, an update in computer software now allows corneal clarity to be measured in the same image. In the present study, we aimed to evaluate the use of corneal densitometry using Pentacam to assess the outcome of PK and DSAEK.

5.3 Methods

5.3.1 Participants

This was a, retrospective, comparative, non-randomised cross-sectional study. It was approved by the Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, and by the local national research ethics committee. The study followed the tenets of the Declaration of Helsinki, and written, informed consent was obtained from all subjects after the nature of the study had been explained to them. The study included patients with FED who had been visiting the Cornea Clinic of the Manchester Royal Eye Hospital. Patients were recruited from the corneal transplantation database which started on 3rd of November 2015 till 1st of December 2017 for adult participants and juvenile by 2nd of June 2016 till December 2017.

Clinical notes were examined, and relevant information such as age, sex, date of surgery, and type of surgery was recorded for descriptive data analysis, as were post-operative parameters, including BCVA (in LogMAR) and clinical details..

5.3.2 Pentacam Imaging

All imaging was performed by the same trained staff in a dark room 12–16 months after surgery. A single image that met the quality requirements of Pentacam analysis was taken from each patient. The inclusion criteria for this study were: age ≥ 18 years, history of FED corneal transplantation (PK or DSAEK), completion of a 1-year post-treatment follow-up, availability of a Pentacam image from that time.

5.3.3 Surgical Procedures

The surgical procedures (DSAEK and PK) were each performed by one of two surgeons; they used identical techniques during the respective procedures to minimise variation (Keenan et al., 2011). PK was performed using a standard technique that employs a Hessburg-Barron trephine (JedMed Instrument Co., St. Louis, MO) (Lim et al., 2000). DSAEK was conducted according to standard techniques (Gorovoy and Price, 2005, Pramanik et al., 2006, Gorovoy, 2006). As FED is a corneal disease associated with ageing, a triple procedure of cataract extraction, intraocular lens implantation, and corneal transplantation was performed in that order when necessary.

5.3.4 Statistical Analysis

Data analyses were carried out using SPSS V22.0 statistics software package for Windows. Descriptive statistics were presented as means ± standard deviation. The normality of the data was examined using the Shapiro–Wilk test, which is more appropriate for small sample sizes (N < 50). When parametric analysis was possible, the Student’s t-test for two independent samples was used. All P-Values less than 0.05 were considered statistically significant. Retrospective Power was calculated using G*Power 3.1.9.2 for posterior 0-2 mm zone at 12 months post-treatment was equal to 0.06.

5.4 Results

A total of 33 eyes of 28 patients were studied. The sample characteristics were comparable between the groups; however, there were more women than men in both groups (PK, 11:2;

DSAEK, 15:5); importantly, this did not constitute a significant difference between the groups (P=0.96). The mean age of patients in the PK group was 70.0 ± 10.0 years, while that in the DSAEK group was 69.9 ± 9.84 years (P=0.96).

In the PK group, seven eyes (53.8%) achieved a post-operative BCVA of 0.3 LogMAR or better, while in the DSAEK group, 15 eyes (75%) achieved a post-operative BCVA of 0.3 LogMAR or better. The mean post-operative BCVA in the DSAEK group was 0.19 ± 0.12 LogMAR, which was better than that in the PK group (0.37 ± 0.22 LogMAR; P= 0.01).

Densitometry measurements showed that DSAEK causes less light scattering than PK in all layers of both the inner 0–2 mm zone of the cornea and the surrounding 2–6 mm annulus zone. However, further statistical analysis showed that none of the differences in densitometry measurements were significant (P>0.05 in all cases; Table 5-1).

Table 5-1: Post-operative densitometry outcome in both central 0-2 mm and surrounding 2-6 mm of the corneal for anterior, middle, posterior and full depth layers for both PK and DSAEK.

Zones	Layers	PK (M±SD)	DSAEK (M±SD)	P Value
0-2 mm	Anterior	26.05±3.16	25.26±5.33	0.63
	Central	23.19±3.05	21.59±3.43	0.18
	Posterior	18.34±2.54	17.87±4.26	0.72
	Full Depth	22.53±2.75	21.56±3.77	0.43
2-6 mm	Anterior	29.13±4.34	26.44±5.8	0.16
	Central	26.03±5.15	22.55±5.68	0.08
	Posterior	20.06±3.8	18.38±4.66	0.28
	Full Depth	25.07±4.31	22.44±5.01	0.11

The mean CCT 1 year after PK treatment was 510.8 ± 54.9 μm, while that 1 year after DSAEK treatment was 639.55 ± 59.0 μm. Both CCT and the thickness of the thinnest corneal area were significantly different between the PK and DSAEK groups (P<0.05) (Table 5-2).

Table 5-2: Post-operative central corneal thickness and thinnest area in both PK and DSAEK.

	PK (M±SD)	DSAEK (M±SD)	P Value
CCT	510.8±54.9	639.55±59.0	< 0.01*
Thinnest Area	493.5±60.7	605.2±53.5	< 0.01*

In the PK group, post-operative BCVA was not significantly correlated with the densitometry measurements and CCT after surgery. However, in the DSAEK group, both correlations were significant (P<0.05; correlation between post-operative BCVA and densitometry measurement at the full-depth 0–2 mm: r = 0.57, P=0.008; at the middle 0–2 mm: r = 0.53, P=0.01; at the posterior 0–2 mm: r = 5.80, P=0.007). Neither group showed significant correlation between CCT and the densitometry measurements in any of the corneal layers and any of the zones (P>0.05). Figures 5-1 shows a linear regression comparison between the two groups at those zones found significant correlation in DSAEK.

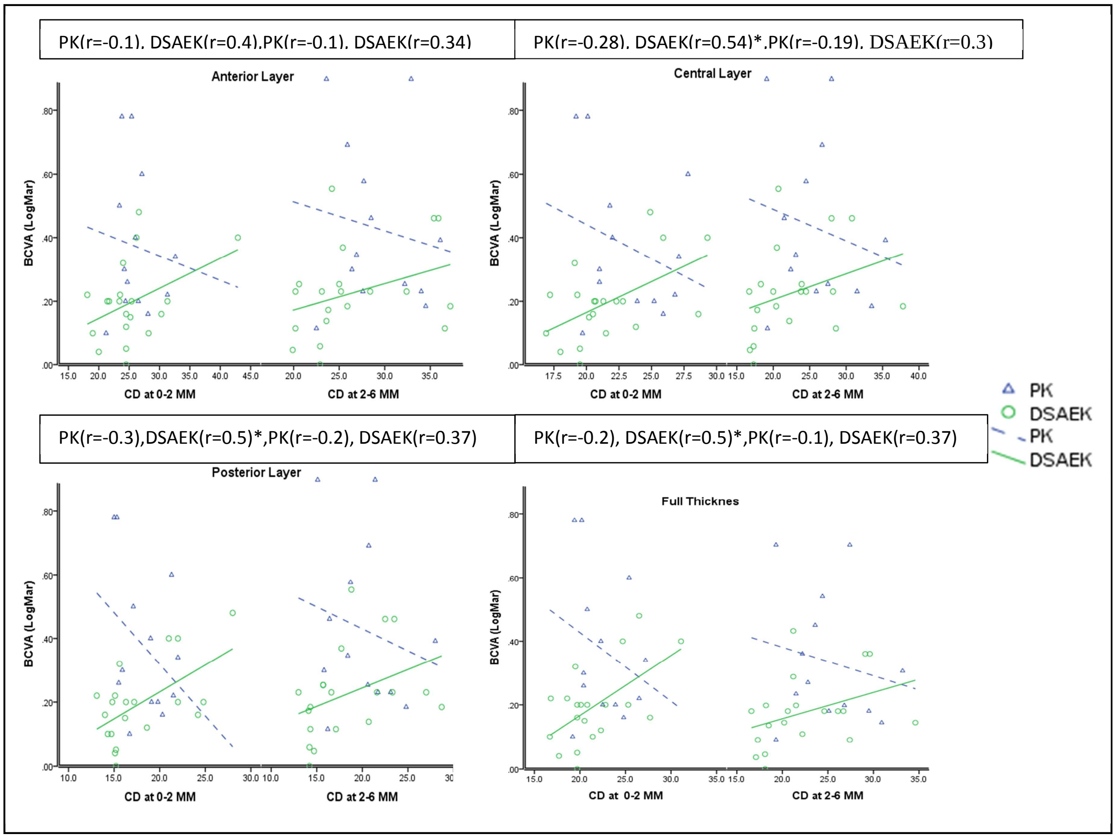

Figure 5-1: The correlation between BCVA and Corneal densitometry in PK and DSAEK (* represent significant at P-value of <0.05).

5.5 Discussion

DSAEK is a comparatively new method for transplanting the endothelium from a donor cornea, and it is now the primary surgical treatment of FED (Keenan et al., 2011, Price et al., 2011, Terry et al., 2009b). DSAEK has a number of advantages over PK in patients with endothelial dystrophy; it is associated with fewer complications and a shorter recovery time (Nanavaty et al., 2014, Nanavaty and Shortt, 2011, Keenan et al., 2011). Several studies have reported that PK and DSAEK differ in a number of areas (Nanavaty and Shortt, 2011), including rates of rejection and post-operation BCVA. Updates from

Nanavaty have reported similar findings (Nanavaty et al., 2014). However, to our knowledge, the present study was the first to find that postoperative densitometry measurements differed between DSAEK and PK in patients with FED which show significant correlation to BCVA in the DSAEK group.

Scheimpflug imaging has numerous applications in corneal assessment. Moreover, with the updated software, it may be possible to measure the amount of backscattered light in diverse regions of the cornea (Lopes et al., 2014, Alnawaiseh et al., 2015). In this regard, corneal transparency is attributable to complex mechanisms, including size, regularity and arrangement of collagen fibrils (Maurice, 1957).

The present study established that, 12–18 months after surgery, the mean corneal densitometry in FED patients was somewhat higher than the normative value quantified in a previously published age-matched set (Dhubhghaill et al., 2014, Alzahrani et al., 2017b). Patients who undergo PK still receive sutures that must be extracted, while patients who undergo DSAEK do not. Overall, in patients with PK, the corneal graft/host intersection requires 12–24 months of recovery, as well as entire suture extraction. Moreover, as PK involves complete thickness grafting, it contravenes the structural and immunological integrity of the eyes and can cause greater harm than DSAEK. Indeed, PK causes greater damage to corneal tissue, so it is likely that greater activation of keratocytes occurs as a reaction to trauma, leading to raised corneal densitometry measurements.

The average endothelial cell density loss per year after successful PK ranges from 7.8% between 3 and 5 years after surgery (Bourne et al., 1994) to 4.2% between 5 and 10 years after surgery (Ing et al., 1998). This is contrasted with a remarkable 50% loss of endothelial cells just 6 months after successful DSAEK (Koenig et al., 2007). Because the present study involved only a small sample and was retrospective in nature, we had limited ability to research this matter. Therefore, future research must conduct a well-planned study that explores the function of the endothelium pump or alternative factors contributing to enhanced corneal transparency in FED following DSAEK.

In addition, the present research confirmed that post-operative BCVA is significantly better after DSAEK than after PK, probably because partial suture extraction is necessary during PK. Regarding post-operative BCVA measurement, comparisons between DSAEK and PK are best carried out after entire suture extraction; an additional review of each patient's clinical notes revealed that post-operative BCVA is improved after entire suture extraction. The present study also found a considerable association between post-operative BCVA and densitometry quantification in the DSAEK group, corroborating previous studies (Koh et al., 2012, Ivarsen and Hjortdal, 2013). However, this correlation did not occur in the PK group. Positive correlations were also found in the central, posterior, and full depth layers.

We should clarify that we excluded patients from our data analysis if they experienced complications during or after surgery. DSAEK is renowned for providing swifter visual rehabilitation than PK. Nonetheless, this has to be confirmed through additional research employing signals that are more dependable than visual results (e.g. contrast susceptibility and higher order aberration HOA steps following procedures), as well as larger sample sizes.

After surgery, improvements in visual acuity can take several months or even years (Price and Price, 2006, Koenig et al., 2007, Li et al., 2012a). In this regard, visual acuity can be affected by the thickness of the lamellar graft and the interfere optical quality of the graft/host interface (Neff et al., 2011, Busin et al., 2013, Ivarsen and Hjortdal, 2013). However, in the current study, there was no correlation between BCVA and corneal thickness comparable to previous study (Mencucci et al., 2015).

Increased corneal densitometry was found in the stroma, the anterior cornea; overall, corneal thickness was also improved. Therefore, the changes detected using densitometry appeared to affect the whole stroma and consisted of more than simply an increase in light scatter as a result of sub-epithelial fibrosis or augmented scatter from the graft/host interface, although both components still can cause light scattering in post-DSAEK patients.

Recently, a significant relationship between visual outcome and corneal densitometry was discovered after DSAEK (Koh et al., 2012), corroborating observations of the present study. To some extent, Koh et al. reported higher values than we did. However, their study did not clearly define which thickness and diameter the densitometry measurements were acquired from. For this reason, the measurements are not directly comparable with those of the present study.

Poor graft interface can lead to graft failure. This illustrates the importance of maintaining a pristine graft interface and avoiding wrinkles in the graft across the pupillary area. In this regard, greater curvature disparities between recipient and donor corneas may promote wrinkle development (Price et al., 2009, Price et al., 2011).

Gradual improvements in visual acuity after surgery are associated with increases in optical aberrations and corneal backscatter, which signify that the cornea is steadily restored after corneal oedema, with an average total corneal breadth of about 700 mm. In fact, reduced BCVA after DSAEK is sometimes believed to be caused by corneal backscatter (haze) (Anshu et al., 2012, Hindman et al., 2013, Mencucci et al., 2015). However, backscatter cannot influence vision since it is not distributed around the retina. In contrast, forward scatter does alter vision, but it naturally causes glare rather than diminished visual acuity (Unterlauft et al., 2015, Terry et al., 2009a, Terry et al., 2012, Wacker et al., 2016).

The present study found that the patients’ average CCT was 510.8 ± 54.9 μm 1 year after PK and 639.55 ± 59.0 μm 1 year after DSAEK. This constituted a significant difference

(P=0.0001) and is similar to the results of (Verdier et al., 2013) who documented an average CCT of 535.0 ± 45.0 μm 6 months after PK; this had increased to 580.0 ± 59 μm 5 years later. Nonetheless, the present study found no statistical correlation between BCVA and CCT in either group. Furthermore, CCT varies greatly among normal eyes.

The results of the present study agree with those of Terry et al. with regards to DSAEK graft thickness. They stated the following: ‘graft thickness may have a small effect on visual outcomes in the extremes of thickness, but not in the common range of 100 mm to 200 mm’, and ‘donor thickness has a tenuous relationship with visual outcomes, accounting for only 5% of the variance in vision between patients, and should play a minimal role in surgical planning’ (Terry et al., 2012).

The present study was limited by its relatively small sample size and the possibility that suture removal may have affected the outcome, especially with respect to BCVA.

In conclusion, the present study showed that corneal densitometry can be used to detect subtle changes that occur in the cornea after transplantation, as well as to monitor recovery. Densitometry may also further our understanding of the cornea in both normal and diseased conditions.

Chapter 6

Corneal Clarity Measurements in Patients with Keratoconus Undergoing either Penetrating or Deep Anterior Lamellar Keratoplasty

Contributions

I designed the study in collaboration with my supervisors, conducted the experiments, reviewed the surgical data base, completed the experiments, analysed the data, and wrote the manuscript. All this work was achieved with regular discussion, close collaboration and feedback on data analysis and writing from my supervisors: Dr Chantal Hillarby and Prof Fiona Carly.

Publication

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Conferences Presentations

The abstract was presented at University of Manchester show case 2016.

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6.1 Abstract

Purpose: To compare the corneal clarity measurement between Penetrating Keratoplasty (PK) and Deep Anterior Lamellar Keratoplasty (DALK) in patients with keratoconus, using densitometry software for the Oculus Pentacam.

Patients and methods: A retrospective comparative study was carried out at Manchester Royal Eye Hospital. Data was collected 12-18 months after corneal transplantation for keratoconus including; post-operative corneal densitometry, best corrected visual acuity (BCVA), central corneal thickness (CCT) and other relevant clinical details.

Results: Analysis of 37 keratoconus eyes from 36 patients found there was a significantly higher corneal densitometry measurement after DALK than PK. This was predominantly in the posterior layer of the concentric zone 0-2mm of the cornea (P=0.0004). A significant correlation was found between post-operative BCVA and corneal densitometry in DALK groups at full thickness (P=0.03). This correlation was seen in the central 0-2 mm (P=0.03) and posterior 0-2mm (P=0.04) zones. In addition, within the DALK group, a correlation was found between central corneal thickness and densitometry at full thickness 2-6 mm (P=0.007), central 0-2(P=0.04), central 2-6 mm (P=0.01) and at posterior 2-6 mm (P=0.01) zones.

Conclusion: This study showed that corneal densitometry measurement differs depending on the type of corneal transplantation used to treat keratoconus patients. Densitometry may have an important role to play in the final BCVA achieved by patients undergoing corneal transplantation for keratoconus. Analysis of Oculus Pentacam images provides an objective evaluation to monitor the cornea status after the surgery.

6.2 **Introduction**

Keratoconus (KCN) is a non-inflammatory ectatic dystrophy characterised by successive thinning, steepening and apical conical projection of the cornea. Irregular astigmatism and myopic changes result in progressive visual impairment which arise due to shifts in the shape of the cornea (Krachmer et al., 1984). In KCN, vision is improved with spectacle correction and/or soft contact lenses until irregular astigmatism demands the use of rigid gas permeable (RGB) contact lenses (Smiddy et al., 1988, Atebara et al., 2009). In severe cases, a corneal transplant may be necessary for visual improvement (Kanellopoulos et al., 2015).

When a corneal transplant is deemed necessary, a full thickness penetrating transplant (PK) (Lass et al., 1990) has historically been the treatment of choice. However, advances in surgical techniques over the last decade has led to less-invasive procedures such as Deep Anterior Lamellar Keratoplasty (DALK) becoming more popular (Jhanji et al., 2011b).

PK involves the complete replacement of the affected (8-9mm) cornea with healthy donor corneal tissue. However, it is now the trend to perform lamellar transplants, aimed at replacing only abnormal corneal tissue (Keenan et al., 2011). DALK is now used to treat KCN and other diseases that may cause anterior scarring or ectasia. This procedure involves the replacement of only the affected corneal stroma and overlying epithelium, thereby preserving the patient’s endothelial layer and Descemet’s membrane. This established technique confers a number of advantages. Throughout this surgical procedure, the eye remains “closed”, reducing the potential risk of intraocular infection (endophthalmitis) and choroidal haemorrhage. As the patient’s own endothelial layer is preserved, the risk of rejection is significantly reduced (Jonuscheit and Doughty, 2013). Structural integrity is preserved, thereby reducing the risk of traumatic graft dehiscence (McKee et al., 2012). One concern of this technique however has been the loss of BCVA thought to be due to the interface between the donor and recipient tissue (Reinhart et al., 2011).

The healthy cornea does not absorb discernible light and the dispersal of light is minimal. Therefore, densitometry can provide a measurement of the level of the transparency of the cornea. Densitometry of the whole cornea can be measured in a single scan using the Pentacam. The standardization of the density begins from 0 to 100. As a result, 0 implies that the cornea demonstrates no blurring while 100 signifies that the cornea is totally obscure (Cho et al., 2010). The Pentacam (Oculus Inc., Wetzlar, Germany) facilitates assessment of the whole anterior section from the anterior corneal surface, through to the surface of posterior lens by use of a rotary Scheimpflug camera (Swartz et al., 2007).

Despite the large number of transplants performed for keratoconus, no corneal densitometry measurements have been reported. Historically, post-surgery analyses for patients recovering from corneal grafts are conducted by use of qualitative analysis of slit-lamp bio microscopy through subjective grading system (Tabibian and Hafezi, 2015, Chatzis and Hafezi, 2012a, Sporl et al., 1997). This is possibly inadequate because it appears to differ from visit to visit and from clinician to clinician.

In this study we have used densitometry to assess and compare the outcome of both PK and DALK grafts in keratoconus patients.

6.3 Methods

6.3.1 Participants

This is a retrospective, comparative and non-randomised cross-sectional study and was approved by Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee. The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from the subjects after explanation of the nature of the study. Patients with keratoconus attending the Manchester Royal Eye Hospital (MREH) cornea clinic were selected if they had undergone corneal transplantation either DALK or PK. Inclusion criteria for the study were patients 18 years or above who had a postoperative follow up between 12-18 months where Pentacam images had been taken. Data collections started on 3rd of November 2015 till 1st of December 2017.

Clinical notes were examined where relevant information including patient age, gender, corneal disease, date of surgery and type of surgery were recorded for descriptive data analysis. Post-operative parameters including best-corrected visual acuity in LogMAR (BCVA) and clinical details were also extracted from the notes for data analysis.

6.3.2 Surgical Technique

The surgical procedures (DALK, PK) were performed by one of three surgeons who used the same surgical technique (Keenan et al., 2011). Penetrating keratoplasty was performed with a standard technique using a Hessburg-Barron trephine (JedMed Instrument Co., St. Louis, MO) (Lim et al., 2000). All DALK procedures were performed using Anwar's big-bubble technique (Anwar and Teichmann, 2002a, Jhanji et al., 2011a, Melles et al., 2000), with the aim to obtain a type 1 big-bubble (McKee et al., 2012).

In all cases, the graft diameter was between 8.00-8.25mm. Corneal tissue was issued by NHSBT (NHS Blood and Transplant, UK) services. All tissue met or exceeded the minimum standard for transplantation to include a minimum cell count of 2200 cells/mm^2 and a central clear cornea of 9.00mm. All recipients received tissue within a 30 years age difference. Wherever possible, all sutures had been removed before the time of imaging and BCVA measurements.

6.3.3 Pentacam Imaging

Pentacam images are routinely used clinically to assess patients undergoing transplant surgery. A new update in the computer software now allows the corneal clarity to be measured from the same image across different corneal zones and layers. Pentcam gives numeric value of the clarity at anterior, central, posterior and total depth layers. In addition, similar numeric values are given by Pentacam at 0-2, 2-6, 6-10 and 10-12 mm of the concentric corneal zone, and for the total corneal diameter (Cho et al., 2010, Lopes et al., 2014) . The protocol takes 5 minutes and is non-invasive. Patients were imaged prior to any other eye examination or drug administration. A single image of the cornea was taken at each visit. All images were taken by a trained member of staff in a darkened room to minimise the effect of ambient lighting on the corneal measurements. All images selected met the quality requirement as determined by Pentacam analysis.

Post-operative data of corneal densitometry for all layers at zones 0-2 mm and 2-6 mm in addition to central corneal thickness (CCT) were retrieved from the Oculus Pentacam database. The measurements from the Pentacam images were compared with BCVA and CCT to determine if these quick and easy measurements could provide additional information on the surgical outcome of corneal transplants using different techniques.

6.3.4 Statistical Analysis

Data analyses were performed using IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM Corp and Graph pad prism7 for Windows. Descriptive statistics were presented as the Mean ± SD. Normality of data was examined using the Shapiro-Wilk test, which is more appropriate for small sample sizes (<50 samples). Student's t-test for two independent samples was used when parametric analysis was possible. Pearson correlation (r) was used when look at relation between surgical outcome of study. A p value of <0.05

was considered to be statistically significant. Power calculated using G*Power 3.1.9.2 retrospectively for posterior 2-6 mm zone at 12 months post-treatment to be equal 0.23.

6.4 Results

A total of 37 eyes from 36 patients were studied in the keratoconus group. The sample characteristics are comparable between both groups. However there were more male than female in this study (14:7 PK and 11:5 DALK). Both the PK and DALK groups showed good similarity in their demographic characteristics and demonstrated no statistical difference in the age of patients (P=0.43) (Table 6-1).

Table 6-1: Mean and SD of Age, Time of the Densitometry and BCVA in both PK and DALK.

	PK M(SD)	DALK M(SD)	P Value
Age	30.14(9.67)	32.56(8.73)	0.43
Time of imaging post-surgery (months)	14.9(3.59)	14.62(4.80)	0.84
Time of taking BCVA post-surgery (months)	13.95(2.74)	14.87(4.03)	0.41
BCVA	0.33(0.21)	0.25(0.22)	0.28

The mean period of time between surgery and BCVA measurement in this study was comparable and showing no statistical significant between both groups (P=0.80). In the PK group, 66% of eyes (n=14) achieved post-operative BCVA of 0.3LogMAR (6/12) or better, while in the DALK group, 81% of eyes (n = 13) achieved post-operative BCVA of 0.3LogMAR (6/12) or better.

There was no statistical significance between the mean period of time between surgery and densitometry measurements between the two groups. Table 5-2 summarises the demographic data. Densitometry measurements were taken for both the central 0-2mm and the surrounding in 2-6 mm concentric zone, results are summarised in Table 6-2. The measurements showed that there is a significant statistical difference between the two groups when the full depth cornea was examined at 0-2 mm concentric zone (P=0.02). However, further statistical analysis showed a higher corneal densitometry measurement in the posterior layer after DALK which was statistically significant for the 0-2 mm of the cornea zone (P=0.0004).

Table 6-2: Summary of the post-operative densitometry in the surrounding 2-6 mm annulus zone of the cornea.

		PK M(SD)	DALK M(SD)	P Value
0-2 mm	Anterior	23.57(3.69)	24.62(3.1)	0.36
	Central	19.4(2.02)	20.62(1.68)	0.07
	Posterior	14.6(1.97)	17.4(2.4)	0.0004**
	Full depth	19.2(2.2)	20.8(1.9)	0.02*
2-6 mm	Anterior	24.83(4.6)	25.09(3.3)	0.8
	Central	20.19(3.6	19.88(2.07)	0.3
	Posterior	14.89(2.8)	16.08(3.09)	0.2
	Full depth	19.97(3.56)	20.36(2.47)	0.7

There was no statistical difference in post-operative central corneal thickness between the 2 groups (Table 6-3).

Table 6-3: Corneal thickness post-surgery in both groups.

	PK (M±SD)	DALK (M±SD)	P Value
CCT	541(32.5)	556(41.9)	0.2
Thinnest Area	513(50.5)	539(34.7)	0.07

There was correlation but it did not reach significance between post-operative BCVA and the densitometry measurement after the surgery in the keratoconus group for PK except with posterior layer at zone 2-6 mm of the cornea which statistically significant P<0.05 (Figure 6-1).

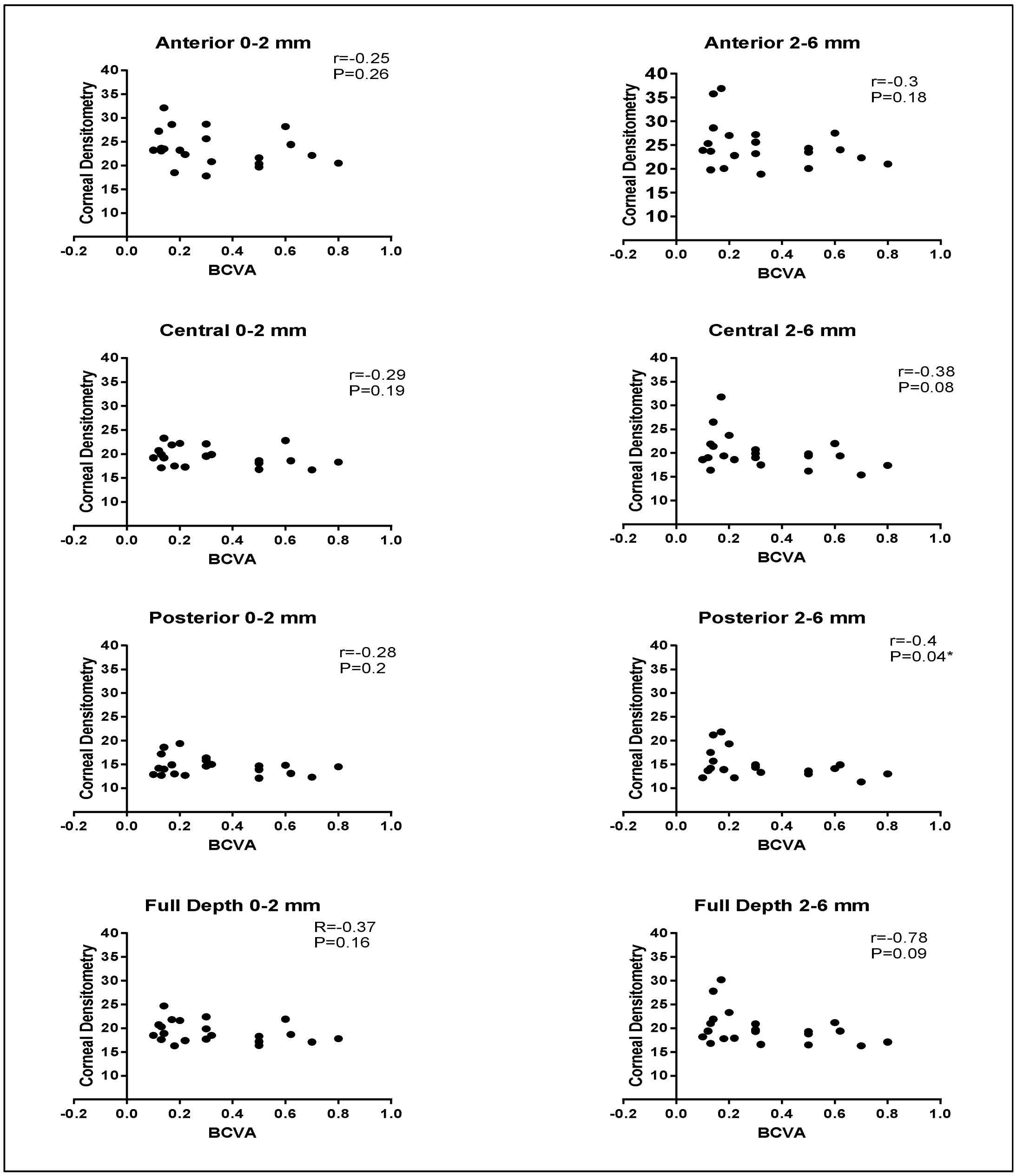

Figure 6-1: Correlation between BCVA and corneal densitometry in different corneal zones and layers in the PK group. (r = correlations coefficient).

In the DALK group a correlation was shown between BCVA and the densitometry measurement after the surgery. These correlations were found at central zone at 0-2 mm corneal concentric zone. Correlation with BCVA was found also in the posterior layer of corneal zone at 0-2 mm and at the full depth at corneal zone of 0-2 mm P<0.05 (Figure 6-2).

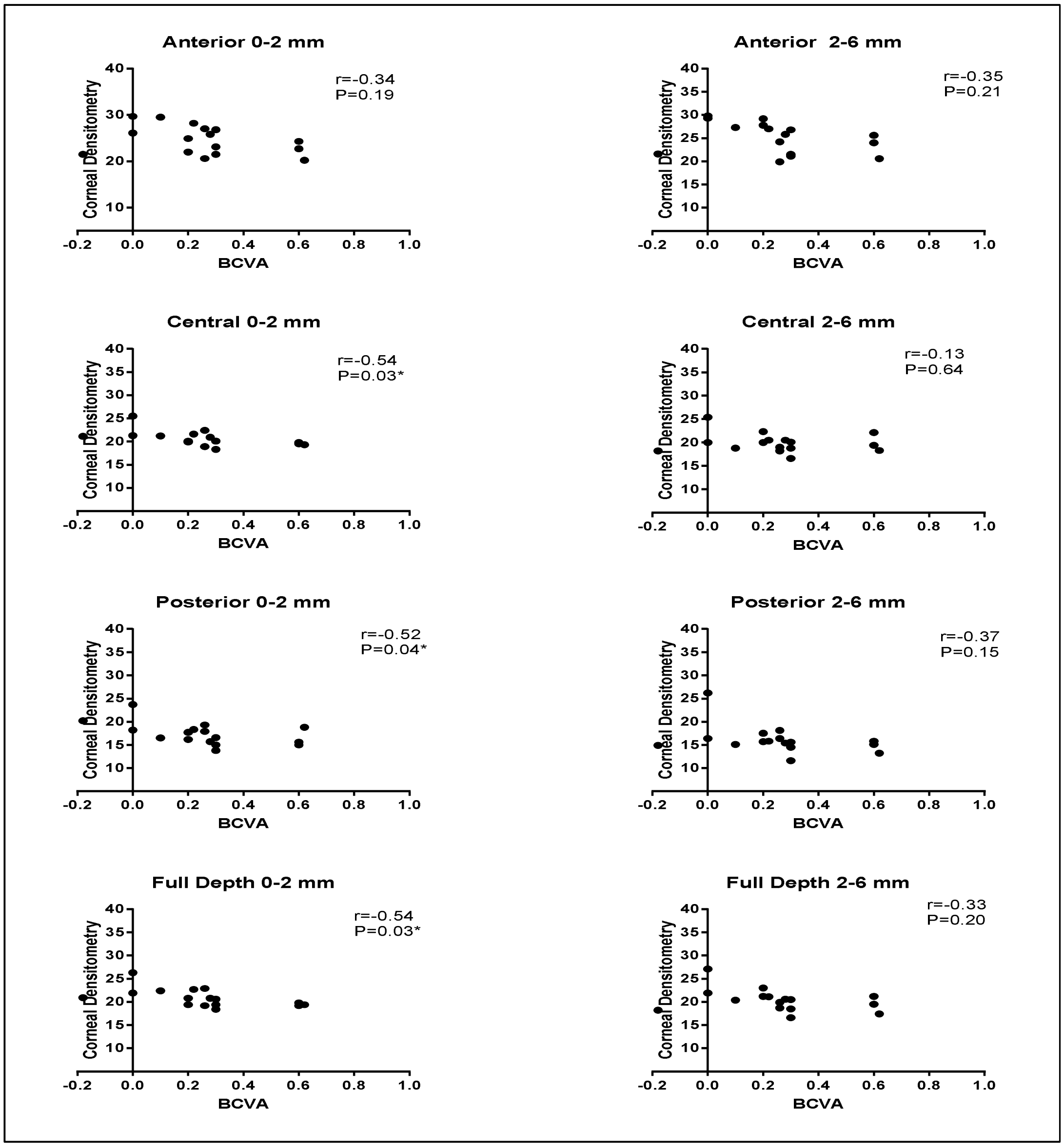

Figure 6-2: Correlation between BCVA and corneal densitometry in different corneal zones and layers for the DALK groups. (r = correlations coefficient).

Central corneal thickness was found to be correlated with corneal densitometry in the DALK group only. These correlations were found to take place at full depth 2-6 mm, posterior 2-6 mm and at both central 0-2 mm and 2-6 mm, P<0.05 (Figure 6-3).

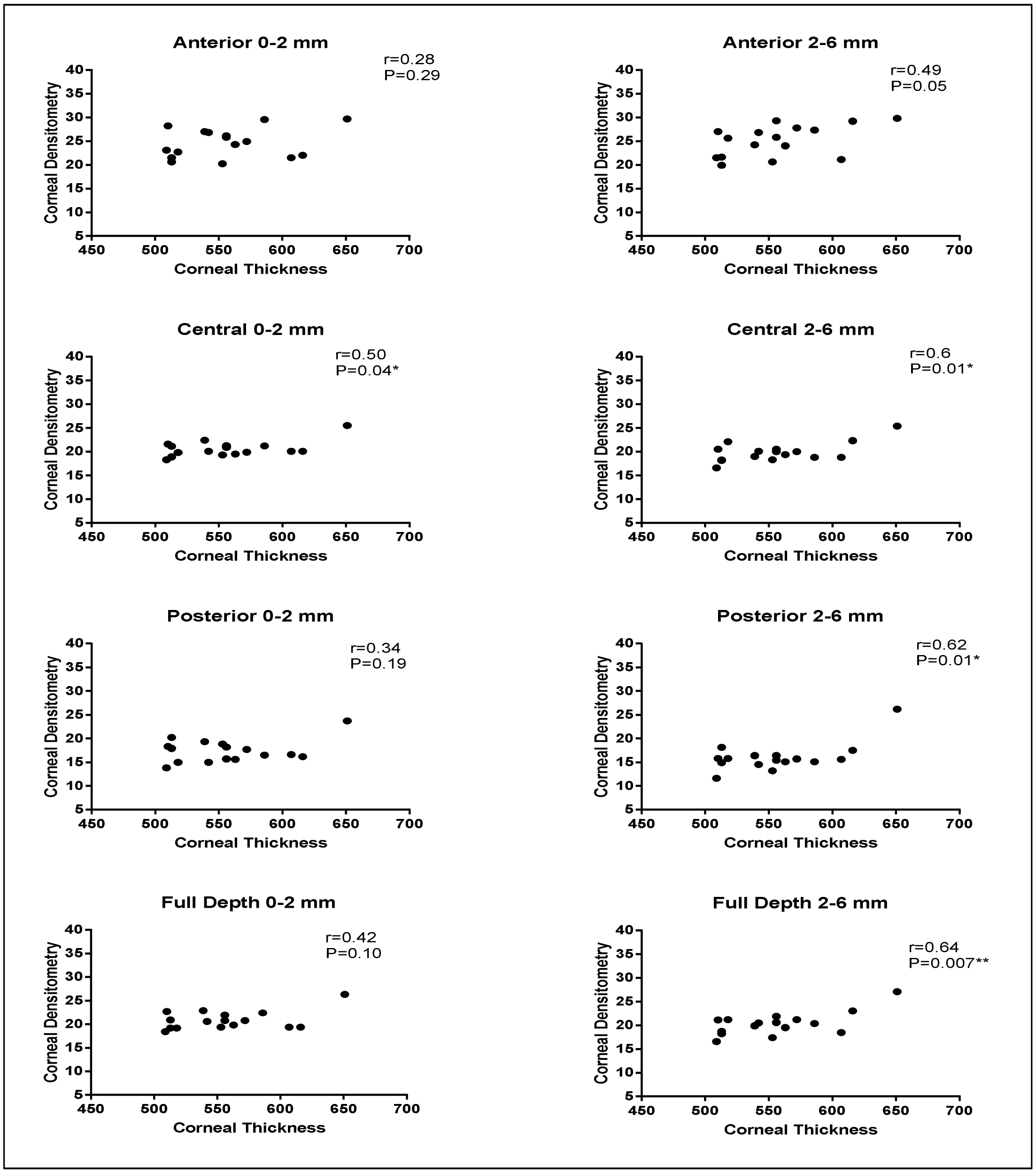

Figure 6-3: Correlation between central corneal thickness and corneal densitometry in different corneal zones and layers in the DALK group. (r = correlations coefficient) (* represent significant at P value <0.05 ** represent significant at P value <0.01).

6.5 Discussion

DALK is becoming the procedure of choice when corneal transplantation is needed to treat advanced keratoconus. It has several advantages compared to PK as it reduces the number of risks factors related to intra-ocular surgery, results in fewer allograft rejections and other long term problems (Olson et al., 2012).

This study has shown that between 12-18 months after surgery, the mean corneal densitometry for patients with KCN, having had either PK or DALK, was still higher compared to a normal control value within a matched-age group, as illustrated by Dhubhghaill et al. (2014) and Alzahrani et al. (2017b). At all corneal regions apart from the central area at 2-6 mm annulus, the average corneal densitometry was greater in DALK than PK. This suggests that a greater scattering of light was provided by DALK as opposed to PK. Variations in corneal densitometry quantification at the posterior layer were statistically significant ($P<0.05$) in the (0-2 mm) corneal zone. This result in the DALK group suggests the host/donor interface reduces clarity and could have an effect on vision (Funnell et al., 2006).

The corneal densitometry was quantified using the manufactures pre-set for the posterior 60 μm of the cornea. In DALK, the quantification of densitometry may be even higher if the quantification marker had been customized to concentrate on the host/donor interface of the region. The same pre-set was applied for all layers, the host/donor interface morphology, which results in considerably greater quantification of densitometry in the posterior layer following DALK, is best described by research employing confocal microscopy. When employing a confocal microscope after DALK, researchers (Feizi et al., 2010) discovered keratocyte configuration that was uneven and minimized as compared to PK in keratoconus. Several contributory elements were considered by Feizi and co-workers to clarify their results, including mechanical injury of the host and donor sides during surgery, and transient lack of an endothelium pump resulting in an influx of fluid at the interface (Marchini et al., 2006).

A subsequent wound healing response may also cause undesirable complications like corneal haze, which may reduce corneal clarity. Remodelling of the extracellular matrix and re-population of keratocytes following the surgical induced injury of DALK can influence the corneal transparency resulting from reorganization of the corneal fibrils. Netto et al, showed that significant apoptosis has been detected within four hours after corneal injury, (Netto et al., 2005b). Following keratocyte apoptosis, the cells undergo secondary necrosis and later activate other keratocytes at deeper layers of the stroma. In addition, there is an activation of inflammatory cells to invade the stromal layer and engulf the resulting dead cells (Zieske et al., 2001). Keratocytes that die in the anterior stroma are replenished in just 2-4 days by proliferation and migration of neighbouring keratocytes (Alio and Javaloy, 2013). The proliferation and migration of residually activated keratocytes begins 12 to 24 hours after epithelial injury. These activated cells may start synthesizing matrix metalloproteinases for tissue remodelling. The replenishing cells in the anterior keratocytes are the activated myofibroblast cells between 1-2 weeks post injury which produce collagen, hyaluronic acid, growth factors modulating epithelial healing, and more (Netto et al., 2005b). The role of myofibroblast cells is very important in remodelling the extracellular matrix and stroma.

The whole process of keratocytes apoptosis, secondary necrosis, activation, proliferation, migration, and transformation regulates corneal wound healing and consequently causes haze formation (Abdelkader et al., 2010). As discussed above the raised densitometry quantification in DALK could be the result of mechanical injury from the surgical technique used within this study. It would be interesting to contrast the densitometry result in different DALK procedures to include those performed using Big-Bubble type 1, Big-Bubble type 2 and manual dissection.

It is debateable if reduced endothelial cell density (ECD) can also contribute to our results, as the endothelium pump is crucial for sustaining the transparency of the cornea. It was established (Cheng et al., 2011) that if there was no perforation of DM in surgery, there was considerably less attrition of endothelial cells following DALK as opposed to PK (Reinhart et al., 2011, Kubaloglu et al., 2011, Zotta et al., 2012). This raises the question that, as the host's own endothelium is maintained by DALK, is the reduction in the transparency of the cornea in DALK entirely due to the host/donor interface following this technique when ECD is higher as opposed to PK.

Our study demonstrated that the average post-operative BCVA result employing the Log MAR chart in DALK is better than that of PK, although this does not reach statistical significance. This was interesting but unexpected, given the raised corneal densitometry quantified in the DALK patients. There are uncertainties surrounding the quality of vision after DALK. There is conflicting published evidence on BCVA, with some studies demonstrating similarity between PK and DALK (Coombes et al., 2001, Shimazaki et al., 2002, Sugita and Kondo, 1997), whilst others have reported decreased vision in eyes after DALK (Watson et al., 2004, Panda et al., 1999, Funnell et al., 2006). However, a numbers of studies have concluded that DALK provides better BCVA (Watson et al., 2004, Tan and Mehta, 2007, Fontana et al., 2007, Borderie et al., 2008) in keeping with our own findings. Researchers have proposed that, after lamellar graft surgery, unevenness at the host/donor interface could reduce vision in some eyes (Saini et al., 2003). The almost complete exposure of DM by using the big-bubble technique most likely has a role in the improved post-operative BCVA in DALK as opposed to PK (Sugita and Kondo, 1997). Also the grade classifications of how advance is the disease plays an important role in final visual acuity outcome and the which suitable surgical technique (Parker et al., 2015, Arnalich-Montiel et al., 2016).

The limitation in this study was its retrospective nature and the relatively small sample size for each group that could impede statistical analysis hence a larger sample size would increase the strength of any further analysis.

In conclusion, this study has found increased densitometry measurements in DALK compared to PK primarily at posterior layer within 0-2 mm zone. This doesn't appear to have an effect on BCVA when compared with those patients undergoing PK for KCN. We have discussed a number of factors which may have a contributed to the raised densitometry result.

This is the first study to demonstrate raised densitometry in patient undergoing DALK and provides further information on this lamellar technique. In the future, other forms of corneal imaging to include SD-OCT and confocal microscopy may help provide greater understanding on this important surgical zone. Corneal densitometry measurements provided by the Oculus Pentacam provides an objective evaluation to further monitor the treatment outcome of corneal transplants surgery in KCN. It provides a non-invasive technique which is safe to use and useful in planning the management of surgery.

Chapter 7

Corneal Imaging and Densitometry Measurements in Keratoconus Patients to Monitor Disease Progression and Treatment Outcomes after Contact Lens or INTACS Treatment

Contributions

I designed the study in collaboration with my supervisors, conducted the experiments, recruited the participants, preformed the imaging investigation, completed the experiments, analysed the data, and wrote the manuscript. All this work was achieved with regular discussion, close collaboration and feedback on data analysis and writing from my supervisors: Dr Chantal Hillarby and Prof Fiona Carly.

Publication

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

ALZAHRANI, K., CRISTIAN, C., HARPER, S., CARLEY, F., BRAHMA, A., MORLEY, D. & HILLARBY, M. C. (2018). Corneal Imaging and Densitometry Measurements in Keratoconus Patients to Monitor Disease Progression and Treatment Outcomes after Contac Lens or INTACS Treatment. Target journal: *clinical ophthalmology*

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7.1 Abstract

Aims: To compare the pre and post treatment corneal densitometry values of keratoconic (KCN) patients managed via contact lenses or by contact lens with intra stromal corneal rings.

Methods: Prospective study was performed at Manchester Royal Eye Hospital UK. Patients were recruited before treatment and followed up for 12 months. Data of corneal densitometry and corneal thickness was collected using the Oculus Pentacam at pre-treatment visit and at the post treatment visit at 12 months.

Results: Corneal clarity significantly differs between both groups at pre-treatment at zone 0-2 mm for anterior layer (P=0.002). The same diversity is present at zone 2-6 mm for anterior layer (P=0.003) and posterior layer (P=0.008). The corneal clarity diversity found was not statistical significant at 12 months post treatment (P>0.05). Corneal thickness was found to be statistically significantly different between pre-treatment and post treatment for contact lens group at CCT and thinnest area (P=0.01, P=0.02) respectively.

Discussion This study shows that KCN management with INTACS was found to be effective in maintaining corneal clarity for a longer time than contact lens alone. On the other hand corneal clarity reduces with disease progression in cases managed with contact lenses only. Analysis of Oculus Pentacam images provides an objective evaluation to monitor the corneal status after these different management's pathways.

7.2 Introduction

Keratoconus (KCN) is non-inflammatory, progressive bilateral dystrophy with corneal stromal thinning (Krachmer et al., 1984). Spectacles and/or contact lenses are the optional managements available at early stage of the disease. KCN is a progressive disease, when the advanced stage is reached the only treatment option is corneal transplantation (Kymionis et al., 2007, Romero-Jiménez et al., 2010). In some cases when the cornea is considered clear and the only reasons for corneal transplantation are contact lens intolerance or decreased visual acuity, using alternative treatment may be in the best interest of both surgeon and patients (Colin, 2006).

Contact lenses have been used for long time for improve visual acuity in KCN even at an advanced stage (Sudharman et al., 2010, Kok and Van Mil, 1993, Barnett and Mannis, 2011, Yeung et al., 1995). Improving visual acuity without causing any damage to the corneal structures is the most important aim (Barr et al., 2000). Rigid gas permeable (RGP) lenses are the most widely used lens for keratoconic management at the early stage of the disease (Anayol et al., 2016). The three-point-touch fitting method is the most commonly used for RGP lenses (Mofty et al., 2017).

Intra Stromal Corneal Rings INTACS (Addition Technology, Fremont, California, USA) as keratoconus treatment was firstly performed by Colin in 1997 (Rabinowitz, 2006). INTACS have proved to improve the topographic regularity and reduce contact lens intolerance both leading to better visual acuity (Colin et al., 2001, Siganos et al., 2003, Wachler et al., 2003). INTACS work via shortening the (arc) and flattening the central cornea along with providing a biomechanical strengthening for the corneal ectatic thinning (Tabibian et al., 2015). However, an INTAC may give a good result in early and moderate stages of the disease but the INTAC is considered poor in advanced stages of keratoconus (Alió et al., 2006).

Corneal clarity in KCN is stated to be at a high level when compared with normal, healthy eyes (Lopes et al., 2014). However, most of keratoconics report increased corneal haze to differing levels. Less attention has been given to the effect of contact lenses and INTACS on formations of corneal haze.

This study aims to use the Oculus Pentacam (Oculus Inc., Wetzlar, Germany) to assess the corneal densitometry level changes in a keratoconic eye before and after fitting with a contact lens. Secondly to compare changes that takes place in corneal densitometry before and after implanting INTACS in the eye for KCN keratoconic management. Finally to assess the corneal clarity changes between both treatment procedure at pre and post treatments.

7.3 Participants and Methods

This is a prospective, comparative and non-randomised cross-sectional study and was approved by Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee (ref:15/NE/0363). The tenets of the Declaration of Helsinki were followed in this study. Data collections started on 3rd of November 2015 till 1st of December 2017 for adult participants and juvenile by 2nd of June 2016 till December 2017.

Patients who were aged over 13 years at the contact lens fitting or INTACS and contact lens placements and have a one year follow up post treatment were selected in this study. Those patients were identified and then recruited following a clinic visit. Clinical notes were examined, where relevant information including patient age, gender, date of procedures were recorded for descriptive data analysis. Post-treatment parameters including monocular best corrected visual acuity in logMAR (BCVA), subjective refraction and relevant clinical details were extracted from the clinical notes for data analysis. Pre and Post-treatment data of corneal densitometry and central corneal thickness (CCT) were retrieved from the Oculus Pentacam image with the densitometry software.

7.3.1 Contact Lens Fitting

All patients were fitted with RGP contact lenses by an optometrist at MREH. The fitting included analysis of the topography results and slit lamp examination. The fitting procedures are following standard methods for keratoconic patients (Leung, 1999, Lee and Kim, 2004, Das et al., 2015).

7.3.2 INTACS Procedure

INTACS fitting were performed by one surgeon for all subjects in this study. The procedure was done under local anaesthesia. Mean corneal thickness was ranged between 425um to 457um for all patients and more than 450 um at proposed incision location. Keratometry reading with less than 58D with mean (50) and standard deviation (5.27) for sim k1 and Mean (52.77) and standard deviation (8.34) for Sim k2 with clear central cornea optical zone. Endothelial cell count was more than 1000 cells/mm^2. Two INTACS segments of 150-350 um thickness for each eye were used to embrace the steepest keratoconic meridian at depth between (300-400um). The aim was to achieve maximum flatting of the cornea (Armitage et al., 2006).

7.3.3 Pentacam Imaging

Pentacam topography images are routinely used clinically to assess patients mostly as a corneal diagnostic tool. Densitometry software allows the corneal clarity to be calculated from the same image. The protocol takes only 5 minutes and it is none invasive so carries no risks to the patient (Cho et al., 2010, Lopes et al., 2014). A good quality single image of the cornea is taken at each visit. The measurements from the Pentacam images are compared for pre-treatment and post treatment of both groups at 12 months. The same measurements are compared between groups at one year post treatment.

7.3.4 Statistical Analysis

IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM Corp and Graph pad prism7 for Windows .Normality of data was assessed with the Shapiro-Wilk-test. Data was presented in each follow-up as the mean (M) ± standard deviation (SD) at baseline, and 12 months. Mann-Whitney U test was used to analyse to compare both groups. Wilcoxon signed-rank test was used to analyse follow ups as compared to the baseline. A P value <0.05 was considered statistically significant result. Power calculated using G*Power 3.1.9.2 retrospectively for anterior 0-2 mm zone at 12 months post-treatment was equal to 0.35.

7.4 Results

Twenty seven eyes from 16 patients met our criteria at 12 months post treatment follow up. Those were 7 patients (9 eyes) for INTAC treatment with a mean and standard deviation age of 32.04±8.38 and 9 patients (18 eyes) for contact lens treatment with mean age and standard deviation of 30.22±7.27 which is not statistically significant (P>0.05). The two groups show similar demographic data.

At pre-treatment the mean (SD) of BCVA logMar was found to be 0.11 (0.14) for the contact lens group whereas it was 0.42 (0.25) for the INTACS group. At post treatment BCVA logMAR mean (SD) was found to be 0.16 (0.35) for contact lens group and 0.24 (0.35) for INTACS group. When compared both groups in terms of BCVA a significant difference was found in pre-treatment (P<0.001). In contrast at 12 months post treatment no statistical significant found between both groups (P>0.05).

The study found a statistically significant difference for the contact lens group for mean corneal densitometry only in the anterior layer of zone 0-2 mm between pre-treatment and

12 months post treatment (P<0.05) (Table 7-1). However, the INTACS group showed no statistical difference between corneal densitometry at pre-treatment and at 12 months post treatment (P>0.05) (Table 7-2).

Table 7-1: Mean and SD for corneal densitometry at pre-treatment and post treatment for the contact lens group. (* represent significant at P value <0.05).

Zone	Layers	Pre-treatment Mean (SD)	Post-treatment Mean (SD)	P value
0-2 mm	Anterior	20.6 (1.78)	21.2 (1.6)	0.03*
	Central	17.6 (1.7)	17.2 (1.09)	0.27
	Posterior	12.9 (1.07)	12.5 (1.1)	0.19
2-6 mm	Anterior	17.96 (1.04)	18.24 (1.1)	0.19
	Central	15.07 (0.98)	15.1 (0.63)	0.82
	Posterior	11.8 (0.88)	11.9 (0.48)	0.65
6-10 mm	Anterior	18.27 (2.3)	17.83 (1.95)	0.4
	Central	15.3 (2.1)	15.7 (2.3)	0.4
	Posterior	12.95 (2.06)	12.97 (2.01)	0.96

Table 7-2: Mean and SD for corneal densitometry at pre-treatment and post treatment for the INTACS group.

Zone	Layers	Pre-treatment Mean (SD)	Post-treatment Mean (SD)	P value
0-2 mm	Anterior	25.02 (3.23)	23.14 (3.9)	0.06
	Central	19.47 (3.3)	17.03 (4.4)	0.13
	Posterior	14.40 (2.78)	12.31 (3.36)	0.09
2-6 mm	Anterior	21.48 (2.89)	22.10 (5.1)	0.59
	Central	16.6 (2.9)	17.95 (5.07)	0.50
	Posterior	13.81 (1.9)	15.27 (4.9)	0.40
6-10 mm	Anterior	19.27 (2.3)	18.88 (5.59)	0.80
	Central	15.14 (2.43)	15.32 (5.32)	0.92
	Posterior	13.63 (2.05)	13.10 (4.38)	0.69

Corneal densitometry at pre-treatment comparison between both groups shows a difference at zone 0-2 mm for the anterior layer (P=0.0001) A significant difference was also shown in the 2-6 mm zone for anterior layer (P=0.006) and the posterior layer (P=0.01).

Comparing the mean corneal densitometry values between both groups at 12 month post treatment reveals no difference in any zones or layers (P>0.05) (Figure 7-1).

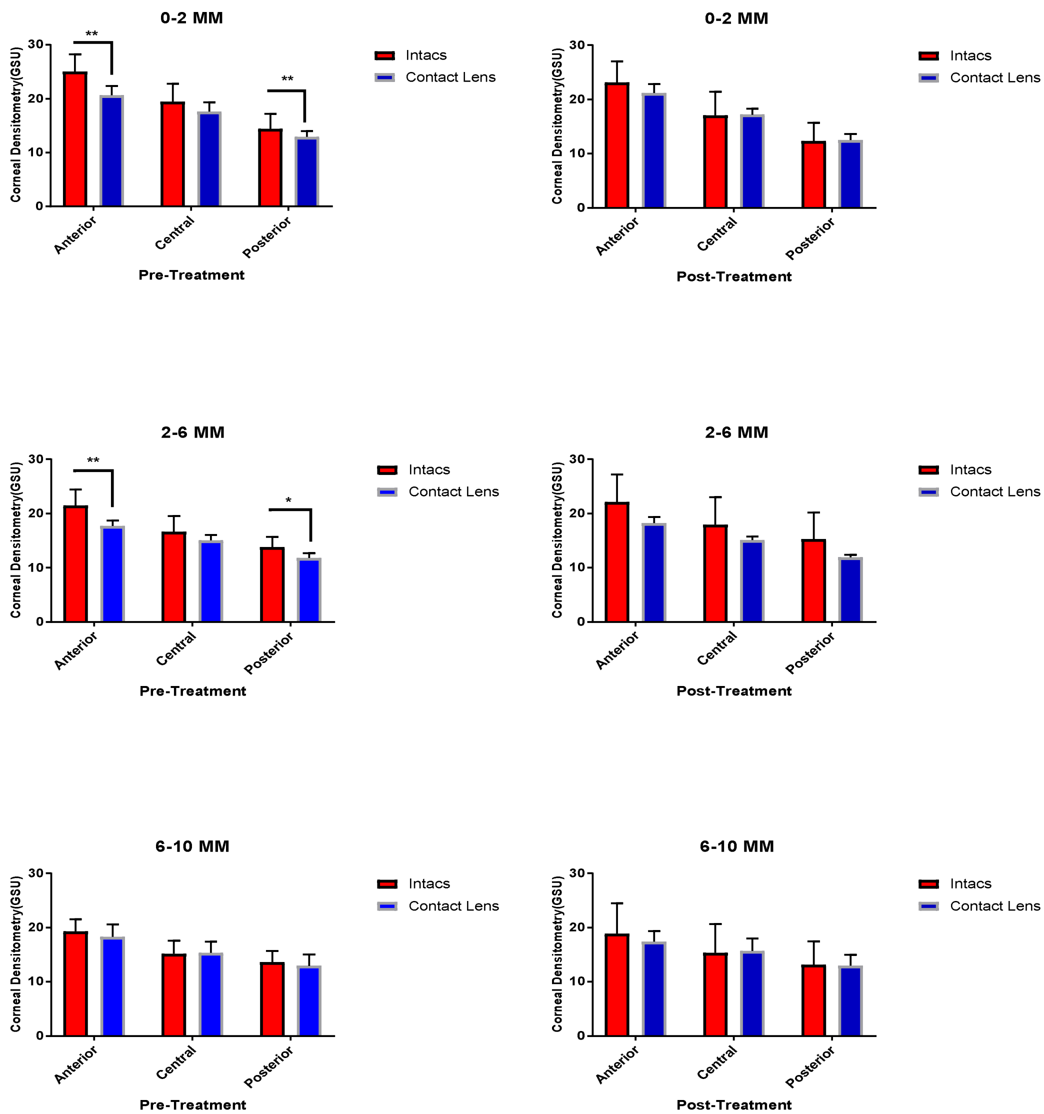

Figure 7-1:A comparison of the pre-treatment and post-treatment corneal densitometry for both groups at different corneal zones and layers. (* represent significance at P value <0.05 ** represent significance at P value <0.01).

Corneal thickness was found to be statistically significantly different between pre- and post-treatment for the contact lens group in both CCT and the thinnest area (P=0.02, P=0.04) respectively. This difference was not shown for INTACS group (P>0.05) (Table 6-3). Additionally there was significant difference between both groups at pre-treatment for CCT (P=0.02) and thinnest area (P=0.02). Similar differences were found at 12 month post treatment in CCT (P=0.3) and thinnest area (P=0.2) (Table 7-3).

Table 7-3:Mean and SD for corneal thickness at pre-treatment and post treatment at 12 months for CLs and INTACS groups.

	Treatment type	Pre treatment Mean (SD)	Post treatment Mean (SD)	P Value
CCT	**CL**	502.1(57.3)	480.14(54.0)	0.01
	INTACS	457.5(32.1)	449.4(31.0)	0.27
Thinnest Area	**CL**	478.7(58.0)	460.3(49.8)	0.02
	INTACS	430.4(39.8)	425.2(39.9)	0.54

7.5 Discussion

Corneal clarity change in KCN has been reported in several recent studies (Anayol et al., 2016, Alzahrani et al., 2017a). Factors that can alter corneal clarity include age (Dhubhghaill et al., 2014, Garzón et al., 2016, Alzahrani et al., 2017b). Viral corneal disease as well as keratoconus and Fuchs disease (FED). KCN progression shows decreased corneal clarity (Lopes et al., 2014, Mofty et al., 2017) . Management of KCN by CXL was found to have an effect on corneal clarity and increase corneal haze (Gutiérrez et al., 2012, Greenstein et al., 2010). However no reports about the effect of contact lenses or INTACS on corneal clarity, using Pentacam, have yet been published.

Contact lens management of KCN is a well-documented evidence based option for patients. This research did not find any statistical differences in corneal clarity between baseline and 12 months post fitting of contact lens except at the anterior layer of zone 0-2 mm.

KCN management can involve the INTACS procedure for some advance cases were patients have difficulty using RGP lenses. This study found no significant change in corneal clarity after 12 months after INTACS are fitted compare to the baseline.

The results for both the INTACS and CK groups showed increased corneal haze than published date for age matched healthy controls at both pre and post treatment imagining (Alzahrani et al., 2017b). Both treatment groups showed significant differences pre-treatment clarity with that at 12 month post treatment, however, there was no difference between the two treatment groups. Other studies of KCN management show a difference between pre-treatment and post treatment of corneal clarity for example Collagen Cross linking where corneal clarity is increased (CXL) (Greenstein et al., 2010, Herrmann et al., 2008).

Damage of keratocytes has been associated with haze development post CXL in many reports (Mazzotta et al., 2007, Dhaliwal and Kaufman, 2009). It has been reported that corneal morphology can be altered due to a long period wearing of contact lenses (Carlson and Bourne, 1988). However keratocyte density does not seem to be effected by wearing daily contact lenses for a long period (Patel et al., 2002). Correspondingly endothelial cell density was found to be unaffected in keratoconus for those patients who were not wearing contact lenses (Timucin et al.). However, there are conflicting reports on changes in endothelial density, either increasing (Hollingsworth et al., 2005), no change (Yeniad et al., 2010) or decreasing (Niederer et al., 2008) for long time wearing contact lens. On the other hand INTACS placed in the cornea lead to a decrease in irregular astigmatism caused by KCN without attacking the centre of the cornea and its tissues (Colin, 2006, Ertan and

Kamburoğlu, 2008). It is commonly believed that lipid deposits will accumulate after INTAC insertion at the area of tunnel (Twa et al., 2004) . Lipids effect the extracellular matrix in the cornea due to change on the biomechanics (Ly et al., 2006) and this may change corneal clarity. However we showed clarity was increased 12 month after INTACS were fitted.

Contact lenses may cause alteration of corneal thickness (Liu and Pflugfelder, 2000). This could be linked to structural alteration in the cornea due to reduced basal epithelial cells (Bitirgen et al., 2013). This study result shows statistically significant change between pre- and post-treatment which increase the central corneal thickness (Romero-Jiménez et al., 2015). These changes in corneal thickness could be due to the expected inflammation caused by contact lens wear or induced by an increase in pro-inflammatory cytokines in tears (Lema and Durán, 2005). which increased with wearing contact lenses (Lema et al., 2008). However this study did not find any effect of the INTACS procedure for corneal thickness. BCVA was significantly better for contact lens group versus INTACS group at pre-treatment while there was no significant difference at post-treatment period that's could be linked to improved corneal surface regularity after INTACS(Rabinowitz, 2006).

This study was limited by a small sample size therefore result is preliminary and needs to be replicated in a large study group. Another limitation was the average age of the participants which may reflect a stable stage of keratoconic disease progression. The change of the corneal refractive index affect which not corrected for by pentacam algorithm is another limitation.

In conclusion, corneal clarity seems not be affected by management of the disease progression either by contact lens alone or INTACS and contact lens alone. However treatment INTACS seem to stabilize the corneal clarity in comparison with contact lenses alone. INTACS slow down the decrease in clarity associated with CL treatment alone. Possibly there is less irritation and hence less inflammation after the INTACS are fitted

.This study represents the first report, to our knowledge about change in clarity measured using the Pentacam in keratoconic patients under management by contact lenses alone or with INTACS and contact lens Analysis of Oculus Pentacam images provided an objective evaluation to monitor the corneal status after these treatments.

Chapter 8

Corneal Imaging and Densitometry Measurements in Juvenile and Adult Keratoconus Patients to Monitor Disease Progression and Treatment Outcomes after Corneal Cross-Linking

Contributions

I designed the study in collaboration with my supervisors, conducted the experiments, recruited the participants, preformed the imaging investigation, completed the experiments, analysed the data, and wrote the manuscript. All this work was achieved with regular discussion, close collaboration and feedback on data analysis and writing from my supervisors: Dr Chantal Hillarby and Prof Fiona Carly.

Publication

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

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Conferences Presentations

The abstract was presented at the 2017 European Association for Vision and Eye Research Conference. Part of the data has been presented at International CXL Experts Meeting 2016 and at 21st ESCRS Winter Meeting, Maastricht 2017.

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8.1 Abstract

Purpose: In this study densitometry software for the Oculus Pentacam was used to investigate the treatment outcomes of corneal cross linking (CXL) in adult and juvenile keratoconus (KCN) patients. Densitometry measurements were taken before and after treatment and followed up for one year.

Methods: A comparative study was carried out at Manchester Royal Eye Hospital. Corneal densitometry measurements collected before and after CXL treatment for 32 eyes from KC patients, aged between 12 and 39, were divided to 2 groups 13-18 years (juvenile group) and 19-39 years (adult group) and analysed and compared to pre and post treatment at 3, 6 and 12 months for each group and between both groups.

Results: Analysis of densitometry measurements found higher corneal densitometry after CXL which peaks at three months post treatment in both groups. There was significant diversity in corneal densitometry measurements in the stromal zone 0-2 and 2-6 mm for all layers except the posterior layer for both groups ($P<0.05$). Significantly increased densitometry value was found higher in the juvenile group at six months in the central ($P=0.006$) and posterior ($P=0.004$) layers for zone 0-2 mm. The same layers differed significantly also in the 2-6 mm zone in all layers ($P=0.01$). One year post treatment the same significant increased densitometry level was seen in the juvenile group in the 0-2 mm zone of the central ($P=0.007$) and posterior layers ($P=0.01$), as was the 2-6 mm zone ($P=0.04$). However, no significant difference was found between pre and post treatment for best corrected visual acuity (BCVA), central corneal thickness (CCT) and thinnest area between both groups. A significant difference was found between pre and post treatment for best corrected visual acuity (BCVA), in the adult group at 6 and 12 months post-treatment from pre-treatment ($P=0.02$, $P=0.16$) respectively.

Conclusion: Corneal clarity post CXL treatment in the juvenile group differed significantly from the adult group. Both groups showed increased haze at 3 months post treatment but the adults showed improvement over the next 9 months. In contrast the juvenile group showed higher densitometry readings at both 6 and 12 month post treatment in comparison to adult group. The reasons for this remain unclear.

8.2 Introduction

Collagen cross-linking (CXL) is one of the treatment options that has led to a reduction in corneal transplantations for keratoconus (KCN) (Wollensak, 2006). Simplicity and lower cost along with its good results makes CXL the most popular treatment for stabilizing disease progression. The key to success is a lasting stiffening effect and a halt to further thinning as well as preventing long-lasting side effects (Raiskup-Wolf et al., 2008). CXL is an approach with the objective of elevating corneal biomechanical stability and rigidity (Wollensak et al., 2003, Spörl et al., 1997). The process entails debriding the corneal epithelium within a central zone diameter of 6–7mm followed by application of riboflavin 0.1% solution plus corneal radiation at 370 nm of ultraviolet-A light. This radiation triggers riboflavin, hence producing reactive oxygen species that enables the formation of extra covalent bonds (situated amid collagen fibrils) in the cornea stroma. The level of irradiation penetrating the corneal endothelium, retina, and lens is considerably less than the harmful threshold (Spoerl et al., 2007).

The cornea's mechanical strength is reduced in keratoconus. In addition to load values, this is valid equally for stress values, typically the load values that are cross sectional area segmented. A distinctive corneal haze is often detected on clinical evaluations after CXL (Greenstein et al., 2010). The literature reveals how CXL's depth is capable of being viewed by simply employing the separation line observed in the corneal stroma (Spoerl et al., 2004) or via ranking slit lamp's cornea haze (Koller et al., 2009). Additionally, further confirmation of cornea haze after CXL has been delineated utilizing confocal microscopy (Caporossi et al., 2010, Herrmann et al., 2008).

The healthy cornea does not absorb discernible light and the dispersal of light is minimal. Therefore, densitometry can provide a measurement of the level of the transparency of the cornea. Densitometry of the whole cornea can be is measured in a single scan using the

Oculus Pentacam(Oculus Inc., Wetzlar, Germany), a device that employs the Scheimpflug principle to focus images at the anterior segment. It has a rotating camera that takes close to 50 anterior segment images in 2 seconds thus providing a quantifiable measurement of corneal clarity. This study aims to re-evaluate cross-linking procedure outcome by using corneal densitometry to quantify haze in both adult and juvenile keratoconus.

8.3 Participants and Methods

This is a comparative and non-randomised cross-sectional study and was approved by Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee. The tenets of the Declaration of Helsinki were followed in this study. Data collections started on 3rd of November 2015 till 1st of December 2017 for adult participants and juvenile by 2nd of June 2016 till December 2017.

Patients who were more than 12 years old at the time of CXL treatment and had a one year follow up post treatment were selected in this study. Clinical notes were examined where relevant information including patient age and gender, were recorded for descriptive data analysis. Post-treatment parameters including best-corrected visual acuity in LogMAR (BCVA), refraction and relevant clinical details were extracted from the clinical notes for data analysis. Post-operative data of corneal densitometry and central corneal thickness (CCT) were measured by the Oculus Pentacam.

8.3.1 CXL Treatment Procedures

A standard protocol for corneal CXL technique was carried out for all patients following (Wollensak et al., 2003). Topical anaesthesia of the cornea was obtained using Benoxinate 0.4%. After a lid speculum was inserted, 20% alcohol was applied for 20 seconds to debride the epithelial layer. Ultrasound pachymetry was used to check central corneal thickness after debridement. If CCT was more that 400um the process continued to be applied by one drop of 0.4% hypotonic riboflavin applied to the cornea every minutes for 20 minutes. The centre of the cornea was then irradiated with ultraviolet-A (9.86mW-UVA) for 10 minutes (UVX 1000 system; IROC Innocross AG, Zurich, Switzerland). Following the CXL procedure all patients receive a topical antibiotic with appropriate pain relief and dexamethasone 1% for period of 4-8 weeks post treatment.

8.3.2 Pentacam Imaging

Pentacam images are routinely used clinically as a corneal diagnostic measurement to assess disease progression in keratoconus patients. Densitometry software allows the corneal clarity to be measured from the same image. The protocol takes only 5 minutes and it is non- invasive so carries no risks to the patient. A good single image of the cornea was taken at each visit both pre- and post-treatment at routine clinic visits at 3, 6 and 12 months post treatment. For the purpose of analysis densitometry measurements, the cornea was divided in to 3 layers (anterior, central and posterior) and 3 concentric zones (0-2, 2-6 and 6-10mm).

The measurements from the Pentacam images were compared to other measurements such as visual acuity to determine if these quick and each measurement give useful information on the progression of the disease and the subsequent improvement after CXL.

8.3.3 Statistical Analysis

Data analyses were performed using IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM Corp and Graph pad prism7 for Windows) and Graph Pad was used for statistical analysis. Normality of data was assessed with the Shapiro-Wilks test. Data was presented for each follow-up as the mean ± standard deviation (SD) at baseline, 3 month, 6 months, and 12 months. Mann-Whitney test was used to analyse to compare both groups. Paired Wilcoxon-signed rank was used to analyse follow ups as compared to the baseline.

A P value <0.05 considered statistically significance. Power calculated using G*Power 3.1.9.2 retrospectively for anterior 0-2 mm zone at 12 months post-treatment was equal to 0.61.

8.4 Results

A total numbers of 32 eyes from 31 patients were included (14 eyes in the juvenile group and 18 eyes in the adult group). Male to female ratio were 20:12 and 17 were right eyes and 15 left eyes. The mean and standard deviation of the age at treatment time was 16.0 (1.79) for the juvenile group and 26.2 (7.67) for the adult group (p<0.0001). Mean and SD for different layer and zone for both groups have been summarized in Tables 8-1 and 8-2.

Table 8-1: Mean and SD for corneal densitometry at pre-treatment and post treatment at 3, 6, 12 months for adult group.

Zone	Layers	Pre CXL, Mean(SD)	Post CXL, Mean(SD)		
			3 Months	6 Months	12 Months
0-2 mm	Anterior	24.7(8.3)	29.5(7.1)	25.9 (7.7)	24.9(3.1)
	Central	18.4(2.8)	20.9(3.3)	18.3 (2.4)	18.5(1.8)
	Posterior	12.6(0.7)	13.1(1.8)	12.5(1.2)	12.7(1.4)
2-6 mm	Anterior	20.1(6.1)	24.3(6.5)	21.4(6.8)	19.8(2.0)
	Central	16.0(2.8)	15.8(2.4)	16.0(1.7)	18.3(2.7)
	Posterior	12.2(0.5)	12.6(1.1)	11.8(1.1)	12.2(1.1)
6-10 mm	Anterior	18.5(4.8)	22.0(6.4)	19.9(6.6)	18.5(2.9)
	Central	15.0(2.2)	16.5(2.6)	15.4(2.6)	15.8(2.1)
	Posterior	12.9(1.4)	13.4(2.0)	13.1(1.8)	13.3(1.8)

Table 8-2: Mean and SD for corneal densitometry at pre-treatment and post treatment at 3, 6, and 12 months for juvenile group.

Zone	Layers	Pre CXL, Mean(SD)	Post CXL, Mean(SD)		
			3 Months	6 Months	12 Months
0-2 mm	Anterior	24.2(0.8)	39.7(20.8)	28.5(5.4)	31.5(14.2)
	Central	19.3(0.6)	32.9(22.2)	21.5(3.0)	24.4(11.1)
	Posterior	13.8(0.6)	20.3(14.6)	14.3(1.2)	16.2(6.8)
2-6 mm	Anterior	19.8(0.8)	33.8. (20.6)	23.5(4.4)	27.8(16.4)
	Central	16.2(0.7)	25.7(16.2)	17.8(1.7)	21.0(10.7)
	Posterior	12.6(0.8)	17.4(11.4)	12.9(1.1)	14.4(5.3)
6-10 mm	Anterior	18.5(1.9)	23.3(6.4)	19.1(2.9)	26.09(11.0)
	Central	14.9(1.3)	18.2(7.0)	14.6(1.7)	16.7(5.2)
	Posterior	12.2(1.2)	14.3(5.4)	12.0(1.7)	12.7(2.6)

8.4.1 Adult Group

Figure 8-1A illustrates the differences found between post treatment measurements and the different zones of the cornea. A significant statistical difference in corneal densitometry was found between pre-treatment and 3 months post treatment period. That was found at layers of the anterior (Z=-2.48, P=0.01) and central (Z=-2.20, P=0.02) of the corneal zone 0-2. Another statistical significant was found also at 2-6 mm zone at anterior layer (Z=-2.48, P=0.003), and central layer (Z=-2.20, P=0.02). However, the difference between pre-treatment and post treatment at 6 and 12 months found no statistically significant (P>0.05).

When we compared the result of post treatment at 3 months with post treatment at 6 months statistical significant was found in zone 0-2 mm of the anterior layer (Z=-2.39, P=0.03) central (Z=-2.10, P=0.03). For the 2-6 mm zone significant statistical was found in the anterior (Z=-2.03, P=0.04) and central (Z=-2.52, P=0.008). Also at zone 6-10 mm the study found statistical difference in corneal densitometry for anterior (Z=-2.52, P=0.01) and central (Z=-2.03, P=0.04). Compering result between 3 months and 12 months found densitometry statistically differed between both groups at 0-2 mm zone for the central layer (Z=-2.38, P=0.01). In the 2-6 mm zone of the anterior (Z=-2.24, P=0.02) and central (Z=-2.52, P=0.01). In the 6-10 mm zone anterior (Z=-2.38, P=0.01) and central (Z=-2.03, P=0.04). No statistical differences take place when we compared densitometry value between 6 and 12 months post treatment.

8.4.2 Juvenile Group

Corneal densitometry found differences at 3 months post treatment from the pre-treatment value. Statistical differences were found in the 0-2 mm zone for anterior (Z=-2.93, P=0.01) central and (Z=-2.29, P=0.02). At zone 2-6 mm for anterior (Z=-2.59, P=0.009), and central (Z=-2.80, P=0.009).

Comparing post treatment at 6 months to pre-treatment result found statically significant in the 0-2 mm zone for the anterior (Z=-2.09, P=0.03) ,Central (Z=-2.66, P=0.008) and posterior (Z=-2.14, P=0.03) layers. In the 2-6 mm zone for the anterior (Z=-2.59, P=0.009) and central (Z=-2.59, P=0.009).

At 12 months post treatment the value of densitometry found differed statistically from pre-treatment in the 0-2 mm zone for the anterior layer (Z=-2.13, P=0.03). In the 2-6 mm zone for anterior (Z=-2.49, P=0.01) and central (Z=-2.49, P=0.01).

At 12 months post treatment the value of densitometry found differed statistically from post treatment at 3 months in the 2-6 mm zone for the central layer (Z=-2.02, P=0.04). At 6 months post treatment the value of densitometry found not differences statistically from the post treatment at 3 months and 12 months in all corneal zones and layers (Figure 8-1B).

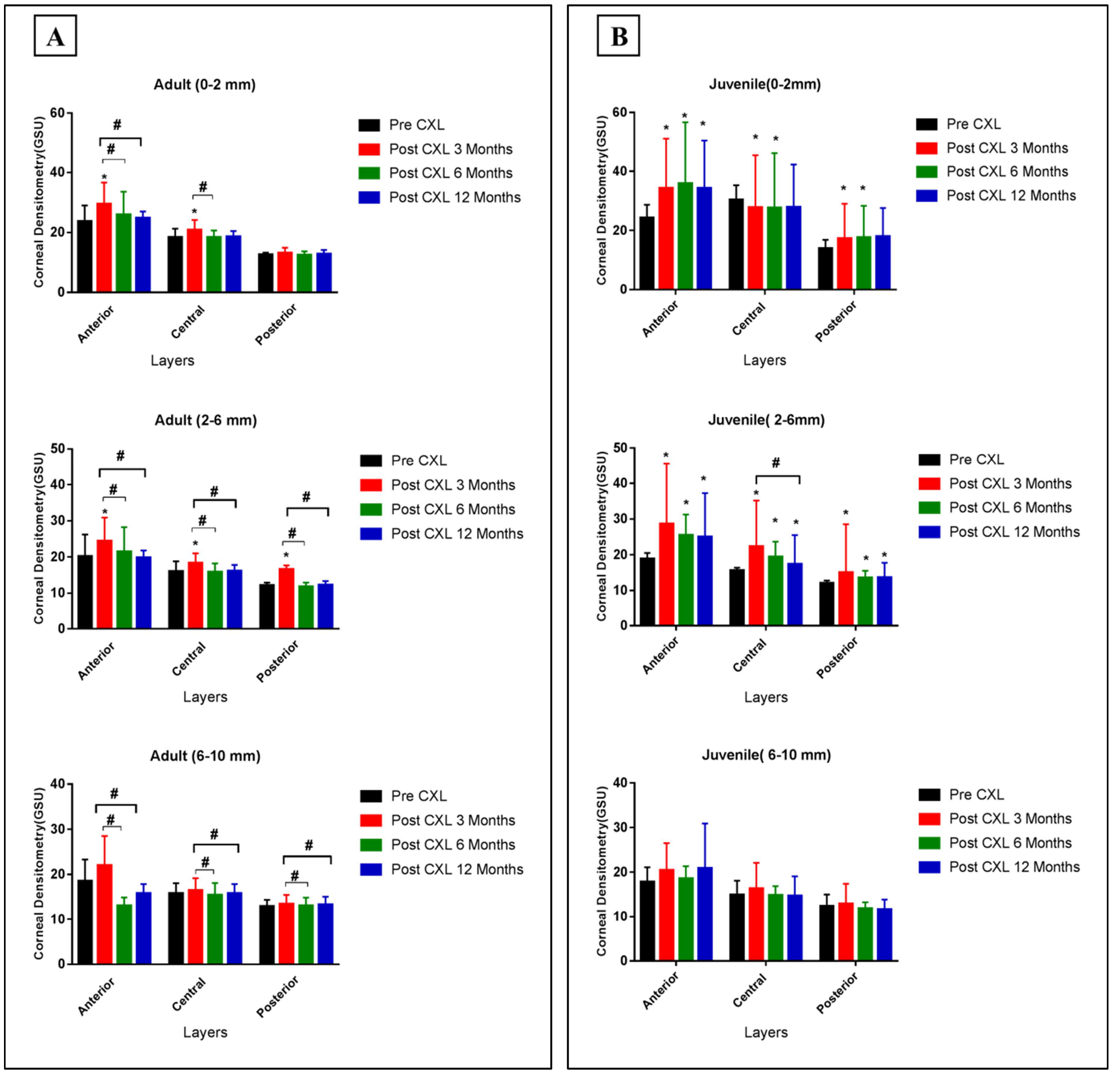


Figure 8-1:Corneal densitometry diversity at 3, 6 and 12 months post treatment for both groups (A=adult and B=juvenile) in the different corneal zones and layers, (* significant from pre-treatment and # represent significant from 3 months post treatment) at level (P<0.05).

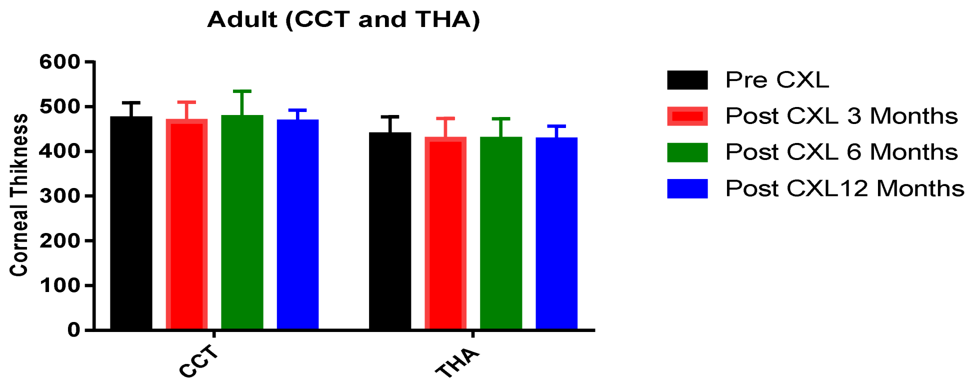
8.4.3 Compressions of Both Groups

The results were compared between the adult and the juvenile groups at each time point and each corneal zone or layer. At pre-treatment a statistical significant was found in the 0-2 mm concentric zone for the central layer (Z=-2.26, P=0.022), posterior layer (Z=-2.45, P=0.013) and at full depth (Z=-1.95, P=0.04). However, at three months post treatment there were non-statistical differences between the two groups.

A significant statistical differences was noted at six months post treatment in the 0-2 mm zone for the central layer (Z=-2.69, P=0.006), posterior layer (Z=-2.84, P=0.004) and full depth (Z=-2.20, P=0.026). Also zone of 2-6 mm found statistically significant in the anterior layer (Z=-2.37, P=0.017), central layer (Z=-2.49, P=0.013), posterior layer (Z=-2.43, P=0.013) and at full depth (Z=-2.52, P=0.011).

One year post treatment comparison found a statistical significant between both groups in the corneal zone 0-2 mm for the central layer (Z=-2.71, P=0.007), posterior layer (Z=-2.50, P=0.01) and at full depth (Z=-2.07, P=0.036). Another statistical significant found in the same post treatment time at 2-6 mm zone for the central layer (Z=-2.01, P=0.04).

No statistical differences was noted in CCT and thinnest area between pre-treatment and post-treatment at different measurement times within the groups and between both groups (P>0.05) (Figure 8-2).



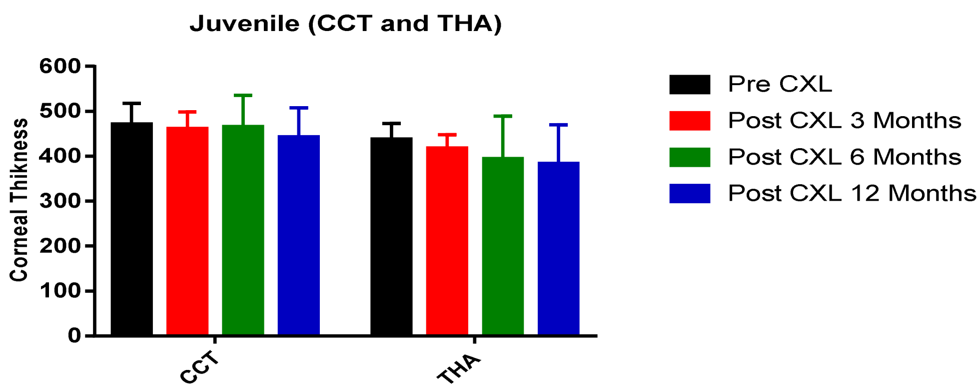


Figure 8-2: Corneal thickness (CCT) and thinnest area (THA) diversity between pre-treatment and all post treatment period at each group.

There were no significant differences in BCVA between pre-treatment and all post treatment periods in the juvenile group (P<0.05) but were significant in the adult group at 6 months post treatment (P=0.02) and at 12 months post treatment (P=0.016) (Figure 8-3).

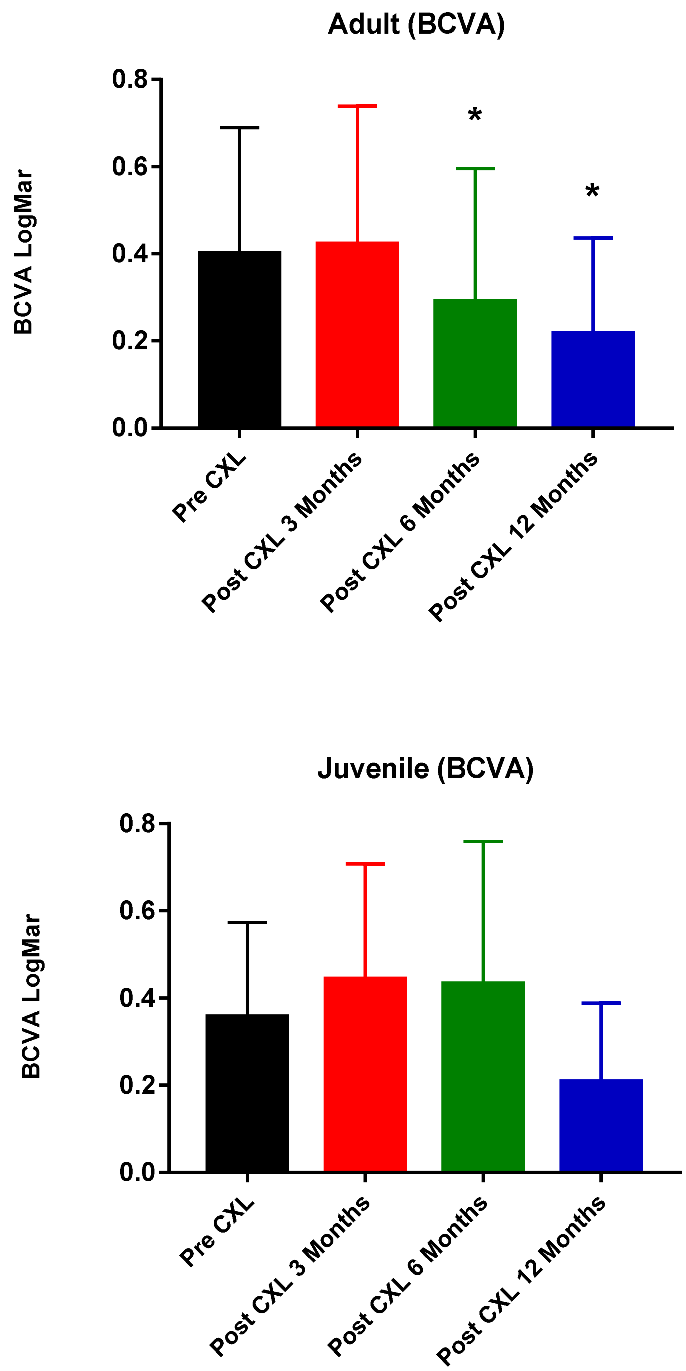


Figure 8-3: Diversity on BCVA between pre-treatment and post treatment both the adult and juvenile groups (* significant from pre-treatment at (P<0.05).

8.5 Discussion

One of the most common problems post- CXL in keratoconus is a significant increase in transient corneal haze. This haze formation can effectively restrict the treatment benefits by reducing corneal clarity which will ultimately affect BCVA outcome (Kanellopoulos, 2009, Alfonso et al., 2010). This study has investigated the nature of the haze objectively by using Pentacam Scheimpflug densitometry. This objective method has superiority over the clinical judging of the level of haze grade (McLaren et al., 2016) and may be useful in clarifying the role of post CXL haze. The aetiology of haze formation post CXL treatment is not fully understood and there are a few discrepancies in the literature. Haze formation after CXL is well known to differ from the haze seen after other procedures on the cornea include refractive surgery (Møller-Pedersen et al., 2000). Formation of new molecular bonds among collagen lamellae post treatment can cause disturbance of the structural organisation in the stroma which effects corneal clarity (Gutiérrez et al., 2012, Wollensak et al., 2004b). An early report has shown that between 3 and 12 months post CXL treatment there is keratocytes repopulation and nerve plexuses renewal along with lamellar compaction within the corneal stroma (Anayol et al., 2016). Additionally damage of keratocytes post CXL (Ringvold and Davanger, 1985) been linked to haze formation in many studies (Mazzotta et al., 2007).

In this study we found a statistically significant increase in corneal densitometry in both groups between pre-treatment and 3 months post treatment. This increase on densitometry value was in the anterior and central layers for both corneal zone 0-2 mm and 2-6 mm ($P<0.05$). This is as expected as the CXL treatment only reaches these areas of the cornea.

In the adult group corneal clarity significantly improved at 6 months and 12 months when compared with corneal clarity at 3 months post treatment in the anterior and central layers for all 3 corneal zones ($P<0.05$). However in the juvenile group statistical significant increases in corneal densitometry were found at 6 months post treatment compared to pre-treatment for all corneal layers in the 0-2 mm zone and in the anterior and central layer at 2-6 mm zone (P<0.05). At 12 months post treatment compared to pre-treatment statistical significant high densitometry was found in the anterior layer of 0-2 zone and anterior and central in zone 2-6 mm (P<0.05). A statistical significant improvement in corneal clarity was noticed at 12 months post treatment compared to 3 months post treatment at central layer of zone2-6mm (P<0.05). It have been proposed by (Greenstein et al., 2010) that corneal densitometry elevates between 1 and 3 months and then from 3rd months through the 6th months, clarity improvement was witnessed and CXL-correlated corneal haze appeared to decrease significantly. Our study agrees with their results but only for adult groups not the juvenile patients.

Our results show that comparative analysis of both groups reveals that two groups have significantly different corneal haze profiles post CXL. This was evident at six months post treatment for both the central and posterior layers in the 0-2 mm zone and for all layers in the 2-6 mm zone (P<0.05). One year post treatment significant differences in corneal clarity between both groups found in both central and posterior layers in the 0-2mm zone and in the central layer in the 2-6 mm zone (P<0.05). Diversity between the adult and juveniles keratoconus groups could be an effect of age as reports show that densitometry in healthy eyes increased with age in adults (Dhubhghaill et al., 2014, Alzahrani et al., 2017b). CXL outcomes report that adults patients have better outcome of functional and morphological criterion than juvenile patients (Vinciguerra et al., 2013). This diversity could be linked to the possible differing activation pathways on keratocytes post-treatment (Gardner et al., 2015, Meek et al., 2003) which could last for different times in the two groups. Diversity of improvement between adult and juvenile groups could be influenced by corneal remodelling and wound healing process both of which play role (Wollensak et al., 2004a, Wilson and Kim, 1998).

Our result showed that maximum effect of haze following CXL treatment was in the corneal centre 0-6mm rather than in the periphery which agrees with earlier reported studies on corneal densitometry in keratoconus (Lopes et al., 2014). The anterior layer in 0-2 mm zone has the highest densitometry level after the treatment because it received maximum treatment (Pircher et al., 2015). In addition the age of the participants could have an impact in corneal clarity changes (Alzahrani et al., 2017b, Buzzonetti and Petrocelli, 2012). However, despite the common belief that CXL is a safe technique in children the positive effect of the treatment does not last as long (24-26 months) (Chatzis and Hafezi, 2012b).

This study did not find any significant differences in the BCVA for pre and post treatment for either group. These results supported earlier reports of a poor correlation between haze and visual acuity (Greenstein et al., 2010). However a study looking at the changes in BCVA between pre-treatment and post-treatment in adult keratoconus showed BCVA improved significantly between 6 (Raiskup-Wolf et al., 2008) and 12 months post-treatment (Caporossi et al., 2010). In the juvenile group there was no significant improvement or diversity of BCVA at 12 months post-treatment from pre-treatment (Efron and Hollingsworth, 2008) .This study could not found any diversity in corneal thickness and thinnest area for both groups and for each group which does agree with (Shen et al., 2016).

In conclusion, haze levels increase after CXL differing in severity in different corneal zones and between the juvenile and adult keratoconus. The 0-2 mm and 2-6 mm zones were found to be the most affected area post treatment. Corneal haze reached its maximum level at three months after treatment then appears to differ significantly in improvement level between adult and juvenile groups, with the latter not returning to pre-treatment clarity in the most anteriorly central zone in juvenile group by 12 months. Longer follow up may need to evaluate if the effect is permanent.

Chapter 9

Summary, Limitations and Future work

9.1 Summary

This project aimed to investigate and improve understanding of the changes taking place in corneal clarity in health and disease based on Oculus Pentacam corneal densitometry software. This objective method of assessing corneal clarity was found to be more accurate, repeatable and efficient rather than the traditional slit lamp assessment which depends on clinician assessment. This subjective evaluation shows variability in results between clinicians (Braunstein et al., 1996, Grewal and Grewal, 2012). We have shown that using the densitometry software on the Pentacam can define the location and severity of corneal haze and may be a good tool to monitor disease progression and treatment outcomes.

The control study (Chapter 3) observed corneal clarity value at different age between 10-69 years divided to 6 equal decade groups. Few reports have looked at relation between age and changes in corneal clarity (Dhubhghaill et al., 2014, Garzón et al., 2017). However those studies conclude that corneal clarity has correlated with age when they look at total corneal diameter. Our study agrees with the earlier studies corneal clarity changes with age. However, our study include a juvenile group (10-19 years) not previously studied; therefore we were the first to report that Juveniles have hazer corneas than the 20-30 age group. This new finding may be of significance when treating younger patients with keratoconus. The safety of some treatments at certain age groups for example CXL in juvenile is open to question.

In this project corneal clarity has been investigated in a range of conditions patients undergo different forms of refractive procedure and both full or partial depth corneal transplantation in FED and KCN. In Addition to transplant for KCN we have looked at the alternative treatments now available to these patients including CXL in juvenile and adult at one year post-treatment. Likewise, how corneal densitometry differed between cases undergo INTACS and those treated with contact lens alone for one year. The corneal densitometry measurement for all above investigation found a higher densitometry value compared to age matched healthy controls (Alzahrani et al., 2017b) suggesting that all treatments for KCN involve a degree of haze with in the first 12 months post- treatment. All these treatments involve a degree of either inflammation or wound healing; this suggested that stromal repair may be involved in this post treatment haze. This was further investigated by looking at haze levels after other treatments that include tissue damage and repair.

Refractive error correction using laser treatment is recognised to cause stromal damage and repair. Chapter 4 of this thesis investigates the different outcomes of the two most commonly used refractive surgery techniques, LASEK and LASIK. The pre-treatment value of corneal densitometry shows high level of comparison to age match group in our previous work of corneal clarity in healthy eyes (Alzahrani et al., 2017b). However, at 6 weeks post treatment there is significant diversity of corneal clarity from base line in LASEK group. Post LASIK there is a rapid improvement in corneal haze more so than LASEK. Our results from this study confirmed that tissue repair has an effect of the final surgical outcome and recovery duration which give LASIK procedure superiority (Ambrósio and Wilson, 2003) specially in haze improvement although the area around the flap show a haze at the last follow up. This led us to ask the question, is haze present after other types of corneal treatment where there is a stromal interface? To study this further we look at corneal transplant techniques that only replace the diseased tissue, such as DASEK or DALK, which create a stromal interface between donor and recipient tissue.

When we compared PK or DALK treatments in KCN (Chapter 6) patients we showed that found that's corneal haze after one year post surgery was higher than age match control as proposed by Dhubhghaill et al. (2014) and Alzahrani et al. (2017b). Moreover, the densitometry values in the posterior layer after DALK were significantly higher than in PK in the 0-2 mm zone. This corresponds to the interface between the donor and recipient tissue. The big bubble technique was used, however, in many patients the Descement's membrane does not fully separate from the stroma, so some recipient stroma remains in this zone creating the interface. Again this supports the theory that creating an interphase in the stroma leads to increased haze.

In our FED study (chapter 5) comparing one year post treatment results of full thickness transplants and partial thickness transplant shows that corneal densitometry was higher than age match controls. Despite the overall higher corneal densitometry values of the PK group compared to the DASEK group, this was not statistically significant. Our one year densitometry value found to be less than the 6 months value proposed by Alnawaiseh et al. (2017) but did not support our theory that the stromal interface created in DASEK would cause corneal haze.

Throughout our study we found Keratoconus (KCN) and its treatments had high levels of associated corneal haze. Corneal haze was found to be higher in KCN when compared with age matched healthy control corneas (Lopes et al., 2014, Anayol et al., 2016). In this we showed that patients with early stages of KCN who had INTACS treatment had lower levels of corneal haze than patients who were managed with CLs alone (chapter 7). It appears that INTACS stabilize corneal clarity whereas the patients with CLs alone had increasing haze which followed disease progression (Mofty et al., 2017). Similarly when comparing the pre-treatment and post treatment in both procedure to age matched control

(Alzahrani et al., 2017b) found both corneal densitometry value show higher level than healthy control proposed by our study. This may be due to inflammation from the irritation of the CLs (Efron and Morgan, 2006, Efron, 2017) in the patients without INTACS. The inflammation would cause tissue disturbance similar to that found in corneal repair and remodelling and hence corneal haze.

Haze formation after KCN treatment using CXL is reported in many studies. It is well documented that CXL is safe and produces a good results in corneal strengthening and stabilization of KCN (Spörl et al., 1997). Due to the good results achieved in adult KCN and the increased progression rate in juvenile KCN it has been suggested that CXL should be used at an early stage of disease in younger patients rather than wait for more progression (Chatzis and Hafezi, 2012a). Some studies on juvenile KCN looking at disease progression compared to the adult KCN post CXL treatment conclude that’s it is safe and can be applied to children (Zotta et al., 2012). On the other hand some studies conclude that CXL in children does not effectively halt disease progression (Buzzonetti and Petrocelli, 2012). Our study agrees with the second study that haze improvement were differed between adult and Juveniles. We showed that adults’ densitometry measurements return to base line levels after 3 months and juveniles shows a low rate of improvement even at 1 year post treatment. The level of corneal remodelling which was reported to be higher in juveniles in comparison to the adult could explain disparity in haze improvement between both groups(Wilson and Kim, 1998). This diversity can also be associated with potential differing activation pathways on keratocytes during wound healing post-treatment (Gardner et al., 2015, Meek et al., 2003). These diversity could be indicted about how corneal stroma responds to the treatment and about the depth effect of the treatment (Rehnman et al., 2011).

The data on the effect of decreased corneal clarity on BCVA following corneal treatment such as CXL is unclear. An early study reports increased corneal haze after some procedure reaches a maximum at 3 months, which reproduce the wound healing reaction rather than a significant effect of visual acuity (Agca et al., 2014). However another report suggests that corneal haze has a sign effect on the visual acuity post PK (Patel et al., 2008), however, another report suggest that increase high order aberration (McLaren et al., 2011) most likely effect the visual acuity rather than haze itself (Patel et al., 2007). One study concludes that age of the FED patients undergoing DASEK procedure play an important role for haze improvement which affect the visual acuity (Baratz et al., 2012). Our data shows little correlation between BCVA and haze in patients of any age.

The full story of corneal transparency still not fully understood. Several factors play a role including the tightly controlled structure of the stroma which contains type I and V collagens fibres that known as lamella (Fini and Stramer, 2005). This structure is maintained by keratocytes and the anterior cornea has more keratocytes than the posterior. Keratocytes in the anterior have a crystalline structure which plays a role in corneal clarity (Jester et al., 1999). Loss of this crystalline nature (Jester et al., 1999) and other factors such as a reduced stromal dehydration, which is maintained through the corneal endothelium pumping process (Bourne, 2003) led to decrease in corneal clarity. This could be related to aging, an abnormal remodelling process in diseases KCN or wound healing process that take place after refractive surgery or corneal disease treatments such as CXL. The differences in the wound healing after each procedure play an important role in corneal haze improvement (Azar, 2006, Ellenberg et al., 2010). According to (Netto et al., 2005b) the haze formation is passed through a different cascade related to the surgical injury. A variety of molecular growth factors, cytokines, and chemokines interaction, that's endorsing renewal as an alternative of fibrosis after corneal injury. Stromal wound healing depends on epithelial cells and how they interact together with keratocytes and subsequently lead myofiproblasts (Chaurasia et al., 2009) that's produced collagen and extracellular matrix.

The type and occurrence of keratocytes apoptosis varies following different types corneal injury. Epithelial injury levels and distribution of keratocytes and the following repopulation by activating stromal keratocytes may have an important role in modulating apoptosis of keratocytes and wound healing response (Helena et al., 1998).

Corneal organisation is multifaceted and its shape, biomechanical behaviour and clarity are based on collagen fibrils, arrangement of proteoglycans around and between fibrils and the accompanying crystalline stromal cells. Corneal clarify during the disease process, post-treatments is controlled by many of diverse structural changes (Meek and Knupp, 2015).

9.2 **Limitations of the Project**

The project has several limitations, the notable being, and the small sample size in some studies. This was unavoidable due to time constraints and the availability of suitable patients. The small number of patients available for recruitment allowing a reasonable follow was also a major issue. Many of the studies would have benefited from a longer follow up. This would have been particularly useful in the juvenile KCN patients as corneal haze had started to increase at 12 months when the study finished. So it is recommended to be repeated with bigger sample size and longer follow-ups.

Another limiting issue is the nature of the retrospective approach taken in some studies. In the Fuch's dystrophy study there was no Pentacam data available prior the transplant. It would have been nice to look at haze in these patients at the early stages of the disease and see how this changed after transplant especially in the DASEK patients who retained their own corneal stromal tissue.

Even with high repeatability, Pentacam algorithm assumes that the corneal refractive index is the same at the posterior and anterior of the cornea and constant without taking in the account that could be change with the disease is another limitation for Pentacam outcome.

9.3 **Future Studies**

This work can be further expanded in several ways that may lead to a better understanding of the causes of corneal haze; the following is a brief summary of possible approaches:

- The addition of contrast sensitivity may clarify some of the discrepancies between visual acuity and corneal clarity. The qualitative assessment of quality of vision needs to be included in pre-treatment and post-treatment. It is important to include patient's subjective complaints of haze to the investigations and its diurnal variation.
- The use of OCT-SD to look at the area healing after corneal transplants to further understand the changes which take place.
- Using corneal confocal microscopy to investigate the role different cells play in the haze process Macrophages are known to play a role in wound healing and their presence, location and activation status may be important in corneal remodelling in disease and treatments within the stroma during haze formation.
- The levels of corneal haze seem in healthy controls at differing ages could be investigated further. The increase in haze between the ages of 13 to 20 may be due to hormonal changes. Likewise, the increase in corneal haze in the peripheral cornea after the age of fifty may be influenced by the use of statins which are known to affect the formation of new blood vessels. The effect of certain systemic diseases associated with aging such as diabetes or high cholesterol on corneal clarity may also be worth considering.
- The significantly lower corneal densitometry in KCN compared to FED after PK raises the question of other mechanisms involved, especially at a cellular level or genetic involvement. A genetic or detailed cellular study could possibly offer answers. In addition, more endothelial deficiencies diseases could be included in this type a study, such as Posterior Polymorphous Dystrophy (PPD).

- Significant correlation between corneal densitometry and post-operative BCVA in DSAEK but none in others groups studied requires a further investigation with a larger sample size to fully establish this relationship and to understand common dissatisfaction on post-operative BCVA among patients.
- It is important to establish a comparison of Pentacam automated measurements to its manual corneal densitometry data in order to investigate the exact effect of treatment methods as it varies sometimes between individuals.
- The significant variety in haze improvement between juvenile and adult open a question about the safety and efficacy of CXL across different age of patients. Longer follow up time with a bigger sample size and employing other imaging system such as confocal microscopy may improve the understanding of haze and its nature after CXL.

9.4 Conclusion

Densitometry remains to be recognized as an objective, effective and reliable measure of corneal haze and as such should be used to understand mechanisms of corneal clarity and haze. It can assist both the clinician and scientist in identifying the early change and in monitoring diseases progression. Our project revel that corneal densitometry differed in health and in disease. Corneal densitometry is affected by age, diseased type and disease management procedures. Evaluating cornea based on layers and zone could help improve our understanding of corneal transparency or the reduction of clarity in the disease process.

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Appendix

List of Publications

- **Alzahrani, K. , Carley, F. , Brahma, A. , Morley, D. and Hillarby, M. (2016),** Corneal imaging and densitometry measurements to monitor fuchs progression and treatments outcomes. Acta Ophthalmol, 94: (Published abstract).
- **Alzahrani, K ,Carley, F., Brahma, A., Morley, D. and Hillarby, M.C., 2017**. Corneal Imaging and Densitometry Measurements in Healthy Volunteers Across Different Age Groups. *Investigative Ophthalmology & Visual Science*, *58*(8), pp.3546-3546. (Published abstract).
- **Mofty, H., Alzahrani, K., Carley, F., Harper, S., Brahma, A., Au, L., Morley, D. and Hillarby, M.C., 2017.** Evaluation of corneal symmetry after UV corneal cross-linking for keratoconus Jun 2017 *Investigative Ophthalmology & Visual Science: IOVS.* (Published abstract).
- **Alzahrani, K., Carley, F., Brahma, A., Morley, D. and Hillarby, M.C., 2017**. Corneal clarity measurements in healthy volunteers across different age groups: Observational study. *Medicine*, *96*(46). (Published articles).
- **Alzahrani, K., Din, N., Brahma, A., Carley, F. and Hillarby, M.C., 2017**. Corneal clarity measurements in patients with myopia undergoing laser assisted in situ keratomileusis and laser assisted sub-epithelial keratectomy. *Acta Ophthalmologica*, *95*(S259). (Published abstract).

- **Alzahrani, K., Carley, F., Brahma, A., Mofty, H., Biswas, S., Lin, Y., Morley, D. and Hillarby, C., 2017**. Corneal haze in juvenile and adult keratoconus patients after corneal cross-linking. *Acta Ophthalmologica*, *95*(S259). (Published abstract).
- **Mofty, H., Alzahrani, K., Carley, F., Harper, S., Brahma, A., Au, L., Morley, D. and Hillarby, M.C., 2017.** Evaluation of corneal symmetry after UV corneal cross-linking for keratoconus. *Clinical Ophthalmology (Auckland, NZ)*, *11*, p.2043. (Published articles).
- **Alzahrani K, Dardin SF, Carley F, Brahma A, Morley D, Hillarby MC., 2018.** Corneal clarity measurements in patients with keratoconus undergoing either penetrating or deep anterior lamellar keratoplasty. *Clinical Ophthalmology*. (Published articles).

Observational Study

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Corneal clarity measurements in healthy volunteers across different age groups

Observational study

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Abstract

The aim of this study was to standardize and investigate the changes in corneal clarity with age. Densitometry software for the Oculus Pentacam was used to examine corneal clarity at different age groups.

A total of 192 eyes from 97 healthy participants were included in this cohort comparative nonrandomized, cross-sectional study. An Oculus Pentcam was used to image the cornea of healthy participants grouped by age (between 10 and 70 years old). Data from the densitometry output have been used to determine clarity in concentric zones and different depths of the cornea.

Corneal densitometry (CD) across all ages showed significant differences between groups when divided into the following layers: anterior, central, and posterior or divided into 0 to 2, 2 to 6, and 6 to 10 mm concentric zones ($P<.05$). The most striking decrease in clarity occurred with age in all 3 layers of the periphery (6–10 mm) ($P<.05$). In addition, we showed that the 10 to 19-year age group had lower clarity than the 20 to 30-age group ($P<.05$), and after 30 years, the cornea shows a steady progression of increased or decreased clarity.

The values for CD, as well as for separate subdivisions based on layer and surface area, might provide a standard for use in further studies and clinical practice. This study established that relation between CD and age is differed when the cornea is divided into layers and zones. This study suggests that there are other factors that may play an essential role in corneal clarity as well as age.

Abbreviations: CCT = corneal thickness, CD = corneal densitometry, CL = contact lens, CXL = corneal crosslinking, KCN = keratoconus, PRK = photorefractive keratectomy, VA = visual acuity.

Keywords: apoptosis, cornea, densitometry, keratocyte, stroma

1. Introduction

Corneal stromal transparency relies on the organized collagen fibrils arranged in parallel to form lamellae resulting in no or very minimal light scattering.[1] For both sclera and cornea, collagen is termed as the fundamental structural constituent. Its intense tensile strength is capable of facilitating the globe with a flexible, shielding coat. The stroma and Bowman layer essentially house the corneal collagen, with the former region comprising of 90% of a hydrated cornea's entire thickness. Going by the arguments stipulated,[2] the corneal stroma is composed of a vastly structured arrangement of small-diameter collagen fibrils supported apart by a proteoglycan matrix, which ensure uniformity in inter-fibrillar spacing.

The posterior corneal endothelium and anterior external corneal epithelial cell layer are the key light scattering sources.[1] The regular cornea disperses light primarily at the tear film-cornea interface and at the air-tear film, just as anticipated from an optically transparent tissue, where the light refraction's index change is presumably maximum with slight interior structures (cornea tissue) scattering, such as cell nuclei and nerves. Due to its uniform arrangement of collagen fibrils, the corneal stroma is able to sustain its clarity in a network-type formation in lamellar sheets[1,3–5]; this clarity is highly influenced by both the collagen fibrils' size and fibrils' spacing within the configuration.[5]

In the endothelium, specific augmentation in cellular pleomorphism and polymegethism happens, with a consequent decrease of cell quantity with age. In addition to such age-affiliated variations is the loss of the cornea's initial regular hexagonal outline and no noteworthy vertical regional discrepancy or inconsistency among paired corneas in cellular pleomorphism or polymegethism exist in the typical corneas analyzed.[6]

A corresponding increase in the fibrils' cross-sectional area is witnessed with increasing age owing to the progressive collagen deposition.[7] Both functional and structural variations are produced in corneal aging. Such alterations can eventually influence the aptitude of the eye to repair itself, refract light, and protect the internal structures.[8]

The human cornea is characterized by changeable thickness (CCT), aspheric curvature, as well as being anisotropic, meaning it is capable of exhibiting diverse physical qualities upon application of stress in various directions. Such properties are variable, varying with progressive age, corneal pathology, and

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the degree of hydration whereby a loss of lamellae structuring leads to varied corneal biomechanics.[9] Clarity failure is the innate reaction of cornea to an extensive variety of pathological problems such corneal dystrophies, infections, and degeneration. Analysis and assessment of the resultant corneal haze is hence a fundamental constituent of ophthalmological assessment. Medically, this is normally done by a typical slit-lamp assessment with documentation of results, which can be supplemented with an explanatory severity scale.[10]

The Oculus Pentacam (OCULUS Optikgerate GmbH, Wetzlar, Germany) designed on the Schiempflug photography principle is a noninvasive camera, designed to capture images of the anterior segment of the eye and to produce a comprehensive analysis of the cornea and its densitometry. A better knowledge of corneal clarity in normal control subjects with no known eye disease will help us to understand the normal changes in corneal clarity with age. To date, there is no such study that includes measurements from juvenile corneas. Such data may help when planning treatments or measuring how beneficial treatments have been in patients with corneal disease. This study compared the corneal densitometry (CD) measurements in healthy volunteer participants within different age groups.

2. Methods

2.1. Participants and densitometry measurements

A prospective comparative and nonrandomized cross-sectional study was approved by Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK, and Health Research Authority by NREC local ethics committee. Written consent was taken from the subjects before collecting their data. All participants were healthy and had no ocular pathology other than refractive error and were all above 10 years of age at the time of imaging.

The corneas of normal controls, with no known corneal disease, were photographed with the Oculus Pentacam. In order to collect the images, participants were positioned in front of the Pentacam camera with their chin and forehead resting on the frame. CD was determined using the pentacam densitometry software. The data extracted from images of the controls' eyes were categorized into 6 age groups (10–19, 20–29, 30–39, 40–49, 50–59, and 60–69 age groups). For analysis, corneas were split into 3 concentric zones 0 to 2, 2 to 6, and 6 to 10 mm and also subdivided into anterior 120 μ m, central, and posterior 60 μm layers.

2.2. Statistics

A data analysis was carried out using IBM SPSS statistics software package v.23 for MAC; Armonk, NY: IBM Corp. Descriptive statistics were presented as the mean ± SD. Normality of data was examined using Kolmogorov–Smirnov test. Student *t* test for 2 independent samples was used when parametric analysis was possible and the nonparametric of Mann–Whitney *U* test was applied when parametric analysis was not possible. Person correlation and regression line were used to look at correlation statistics between age and CD. One-way analysis of variance (ANOVA) was used for comparing more than 2 groups. A P value of $< .05$ was considered to be statistically significant.

IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY: IBM Corp

3. Results

A total of 192 eyes (97 right eyes, 95 left eyes) from 97 healthy participants (36.08% male, 63.91% female) were included in this study that were distributed over 6 age groups. Participant's age varied between 10 and 69 years. The mean age of the controls was 36.15 years with a SD of 16.5. Female average age was 36.07 years with SD 16.78, while the average age of the male group was 36.12 years with SD 17.45. The demographics of the controls are summarized in Table 1 and shows that there was no diversity between male and female participants.

Table 1

Demographics of the subjects.

Age group	Male	Female	Age (MD, SD)
10–19	9 (45%)	11 (55%)	14 (2.35)
20–29	4 (23.5%)	13 (76.5%)	24.8 (2.7)
30–39	4 (23.5%)	13 (76.5%)	32.8 (2.6)
40–49	8 (47.1%)	9 (52.9%)	46.23 (2.4)
50–59	8 (50%)	8 (50%)	53.48 (2.83)
60–69	2 (20%)	8 (80%)	62.68 (1.83)
Total	35 (36.08%)	62 (63.9%)	36.15 (16.5)

The mean CD results are summarized in Table 2. CD across all age groups differed significantly between groups when divided both into anterior, central, or posterior layers or analyzed in full depth. There were also significant differences when the cornea was divided into concentric zones of 0 to 2, 2 to 6, and 6 to 10 mm or when analyzed as the total corneal diameter 0 to 10.

Corneal clarity in the anterior layer across all zones was found to be low in the 10 to 19 age groups. The clarity improved in the 20 to 29 age groups before it started to decrease in the 40 to 49 age groups, the most significant being the 0 to 2 mm and 2 to 6 mm zones, which are clearer at 20 to 29 years before the densitometry values starting to be increased with age. The anterior layer at zone 0 to 2 mm has better clarity in the 60 to 69 age groups. CD in the central layer increases with age across all corneal zones. CD in the posterior layer also increases with age across all concentric zones. Once again, the posterior 6 to 10 mm shows a rapid increase with age. Figure 1 shows the changes over each layer across different corneal layer zones.

Age was found to be significantly correlated with CD at all layers zone when we look at all the total diameter of 0 to 10 mm. In addition, a significant correlation was found for all corneal zone and layers except the anterior 0 to 2 mm, zone which were not statistically significant. Table 3 and Fig. 2 give details of this relationship.

4. Discussion

This study demonstrates that corneal clarity decreases with age particularly in the 6 to 10 mm concentric zone. One possible reason for this decrease in clarity may be changes that occur in the corneal endothelium. Endothelial changes related to age have been studied both experimentally and clinically. It has been demonstrated that between the ages of 20 and 80, the yearly reduction in cell density of the corneal endothelium is about 0.6%, with concomitant increases in pleomorphism and polymegethism.[8,11–14] These age-related changes to the endothelium lead to increases in corneal thickness and may increase CD measurements.

Due to the fact that the cornea is the scaffold for the major refractive surface of the eye, any biological or mechanical response to injury will also affect optical performance. The same mechanisms responsible for preserving ocular integrity can, as a result, undermine the goals of accomplishing stable and

Table 2

Corneal densitometry mean (SD) and *P* value across the 6 age groups for different corneal zones and layers.

Corneal layer and zone	Age group						
Full depth	10–19	20–29	30–39	40–49	50–59	60–69	*P*
0–2	15.53 (1.8)	14.49 (2.3)	14.91 (2.4)	16.09 (1.8)	15.94 (2.4)	15.37 (1.5)	.016
2–6	13.59 (1.6)	13.03 (1.7)	13.43 (2.0	14.81 (1.4)	14.89 (2.2)	16.17 (2.2)	<.000
6–10	12.93 (2.2)	12.87 (1.7)	14.21 (2.9)	18.44 (3.2)	21.62 (5.9)	31.49 (7.6)	<.000
0–10	14.01 (1.5)	13.45 (1.7)	14.19 (2.2)	16.44 (1.80	17.48 (3.0)	21.01 (3.3)	<.000
Anterior							
0–2	22.25 (2.9)	18.74 (5.1)	19.39 (5.2)	21.54 (3.8)	20.40 (4.3)	18.31 (2.9)	.001
2–6	19.35 (2.6)	16.99 (3.9)	17.65 (4.4)	19.71 (3.1)	18.90 (3.8)	18.93 (3.5)	.014
6–10	17.80 (3.6)	16.14 (3.3)	17.83 (4.7)	22.85 (4.3)	26.06 (8.3)	36.25 (10.3)	<.000
0–10	19.81 (2.5)	17.30 (4.0)	18.29 (4.5)	21.37 (3.4)	21.79 (4.9)	24.48 (5.0)	<.000
Center							
0–2	13.34 (1.4)	14.05 (1.5)	14.31 (1.3)	14.91 (1.3)	15.21 (1.9)	15.70 (1.0)	<.000
2–6	11.57 (1.2)	12.40 (1.1)	12.61 (1.2)	13.61 (1.2)	14.05 (1.9)	16.46 (1.8)	<.000
6–10	11.12 (1.9)	12.21 (1.5)	13.37 (2.8)	17.60 (3.6)	21.01 (6.1)	33.54 (7.6)	<.000
0–10	12.01 (1.2)	12.89 (1.2)	13.43 (1.4)	15.38 (1.8)	16.76 (2.9)	21.90 (3.1)	<.000
Posterior							
0–2	11.00 (1.5)	10.70 (1.1)	11.14 (1.2)	11.79 (1.2)	12.23 (1.7)	12.09 (1.2)	<.000
2–6	9.88 (1.2)	9.71 (0.9)	10.02 (1.0)	11.11 (1.0)	11.70 (1.6)	13.05 (1.9)	<.000
6–10	9.88 (1.5)	10.22 (1.4)	11.39 (2.3)	14.84 (3.1)	17.80 (4.9)	25.56 (5.4)	<.000
0–10	10.25 (1.2)	10.21 (0.9)	10.85 (1.2)	12.58 (1.5)	13.91 (2.3)	16.91 (2.6)	<.000

predictable visual results after refractive procedures.[15] One report[16] discovered a significant early rise in the mean anterior density readings (from 27.71 ± 4.39 to 37.812 ± 12.31) 3 months after photorefractive keratectomy (PRK) that subsequently reduced again by 12 months (26.291 ± 4.93). The high CD at 3 months is related to the wound healing process. Activated keratocyte apoptosis was found to be higher post-PRK; these cells were located in the anterior stroma causing haze that improved over time.[17]

This study found a fluctuation of densitometry values in both 0 to 2 and 2 to 6 mm zone in the anterior layer at different age groups. Ten to 19 years and 40 to 49 years age groups were found to have higher CD. These 2 age groups are where significant hormonal changes can take place in the human body. CCT changes in women have been reported previously during pregnancy[18] and during the menstrual cycle. [19] It has been reported that contact lens (CL) use during the premenstrual phase is easier than during the menstrual period.[20,21] This may be due to changes in CCT leading to changes of corneal curvature,[22] which can cause intolerance to CL wear. In addition, the lack of sex hormones has been suggested as a possible reason for changes in the corneal biomechanics of individuals over the age of 40.[23] It is therefore possible that hormones may also play a role in the changes in corneal clarity that this study found with age.

Corneal remodeling reported to take place in keratoconus (KCN),[24] postrefractive ectasia,[25] and in injured stroma[26] all lead to increases in corneal haze. This remodeling has been suggested to play a role in the relationship between anterior cornea changes and visual acuity (VA) after corneal crosslinking (CXL)[27,28] or corneal trauma.[29] Corneal remolding activity may be able to explain why the teenage group in this study has higher levels of CD than those of the next age group (20–29), as the keratocyte activity is known to be higher in this younger group.

The cornea is the major physical covering of the eye. To contribute to the focusing ability of the eye, it has to be tough, transparent, and capable of maintaining a smooth and steady curvature. Controversies still exist over how transparency is maintained even with the advanced understanding of the structure and other properties of the cornea. One area of interest is to investigate the role of hydration, which is a major determinant of transparency and which can be manipulated within reasonable parameters under standardized conditions.[3,30,31] The studies[32] of corneal backscatter established that there was a remarkable growth in total CD with advancing age. In contrast, it has also been shown that CD increases in the central 6 mm of the cornea are minimal with respect to age. In the current study, it was shown that, whereas there is no age-linked variation in the central 6 mm of the cornea, a significant rise is seen in the region next to the limbus. The study excluded the 10 to 12 mm area zone from analysis in our study because this area could be part of the limbus with various changes in the white-to-white thickness of the cornea,[33] suggesting that some individuals have corneas with analysis parameters smaller than 12 mm. The higher backscatter quantities therefore occur when sclera and parts of the limbus are involved in the assessment of the peripheral layers. Even slight variations in the situation of the limbus would physically influence the 10 to 12 mm CD outcomes. This, however, does not take place in the significantly dominant annuli. Owing to this factor, the substantial expansion in scatter in the 10 to 12 mm zone can neither be absolutely understood nor rightly correlated with age.[34]

All the measured limits were connected with each other, signifying that the scattering for every region of the cornea is constant with respect to other portions of the cornea. It is interesting, therefore, that a statistically substantial connection between the difference in VA, stromal scattering, and assessed interface was established. This indicates that the complete scattering capacities may not be as significant in influencing VA as is their change throughout the cornea.[35]

When this study looks at correlation between total CD and age, we found it is correlated positively. Earlier studies[36,37] revealed that light scatter in the cornea is a phenomenon that is related to age and that the best preoperative forecaster of postoperative VA is age. Consequently, the ages of patients in the study might have

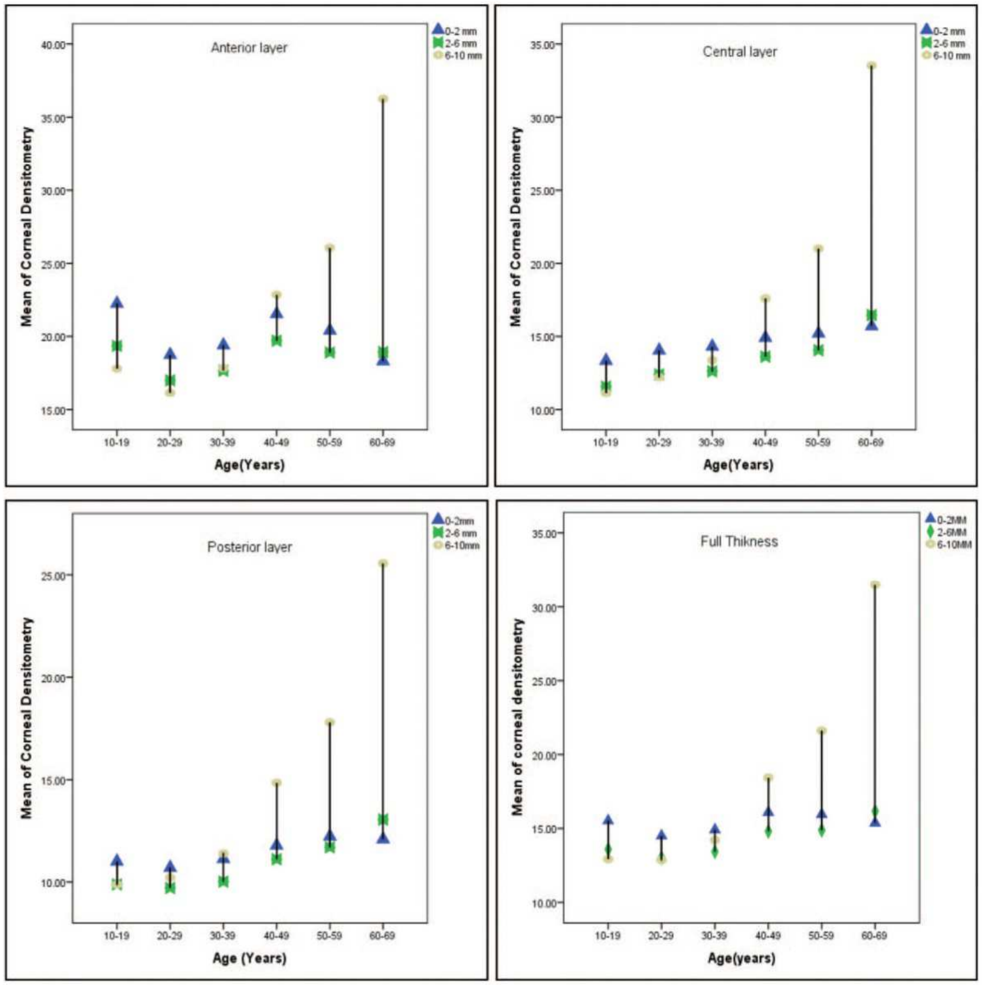

Figure 1. Diversity in corneal densitometry take place in different corneal layers at different age groups.

an effect on the dimensions of the scattering that are determined from Pentacam images.

In summary, our study findings agree with other study,[38] as there was a considerable increase in CD with age, despite the fact that the increase was confined to the peripheral cornea. Conversely, this study finds a relationship between certain age groups and CD, notably within the teenage age group and those within the 40 to 49 age group. A possible explanation for this observation may be the hormonal changes known to occur at this time. Interestingly, that teen-age group was found to be cloudier than 20 to 29 age groups, suggesting that the additional factor of corneal remodeling with increased keratocyte activity may be relevant.

Table 3

Person correlation between age and corneal densitometry in different corneal layer and zone.

	0–2 mm		2–6 mm		6–10 mm		0–10 mm	
	r	*P*	*R*	*P*	*r*	*P*	*r*	*P*
Anterior	−0.130	.073	0.058	.428	0.640	<.000	0.361	<.000
Central	0.456	<.000	0.677	<.000	0.775	<.000	0.774	<.000
Posterior	0.344	<.000	0.603	<.000	0.778	<.000	0.745	<.000
Full depth	0.106	.143	0.412	<.000	0.749	<.000	0.656	<.000

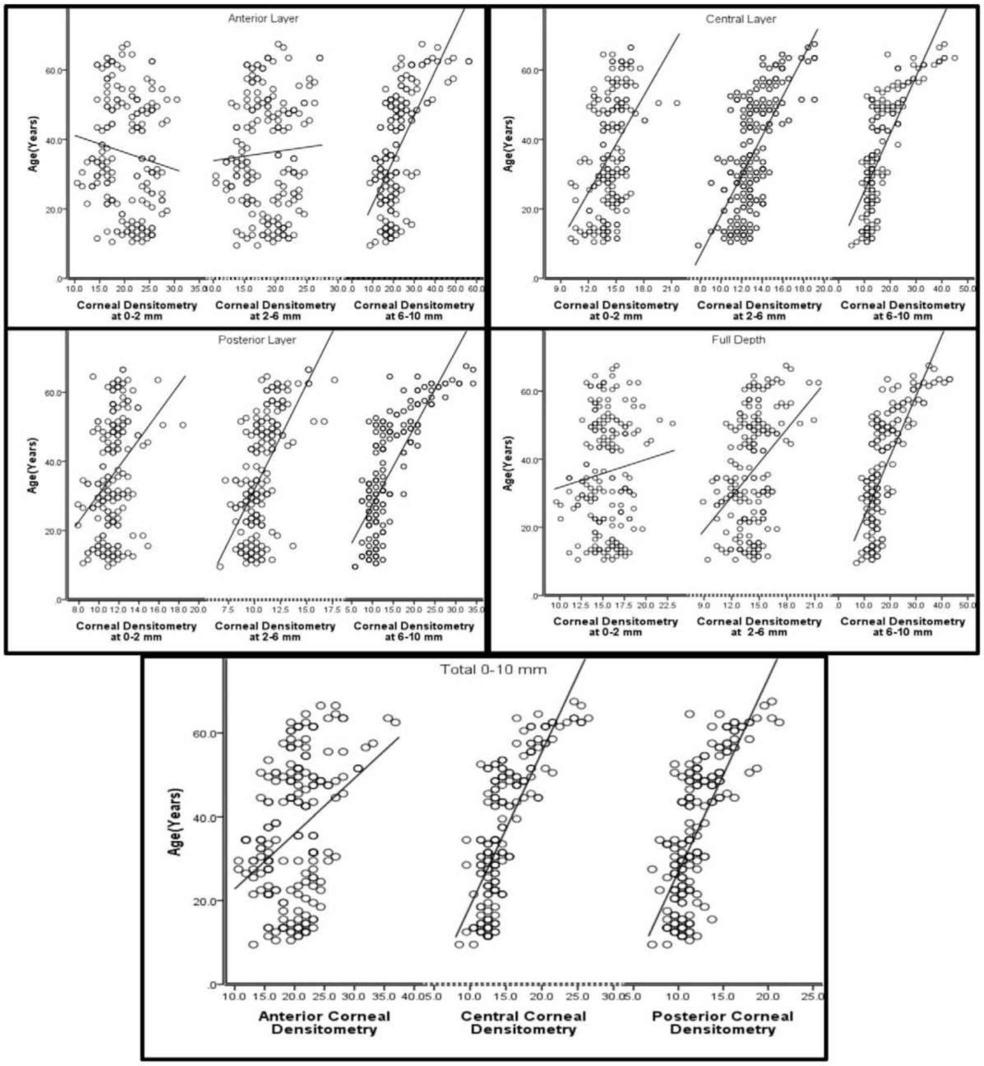

Figure 2. Relation between age and corneal densitometry in different layers and zones.

This study was limited by small sample size. However, this study is first study to our knowledge that aims to standardized CD value in healthy control eyes to include those individuals aged between 10 and 19 years. This study suggests that additional factors other than age alone may have a role to play in corneal clarity. These values for CD dimensions, as well as subdivisions based on layer and surface area, might provide a standardized stage for use in further studies and clinical practice.

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ORIGINAL RESEARCH

Corneal clarity measurements in patients with keratoconus undergoing either penetrating or deep anterior lamellar keratoplasty

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Purpose: To compare the corneal clarity measurement between penetrating keratoplasty (PK) and deep anterior lamellar keratoplasty (DALK) in patients with keratoconus, using densitometry software for the Oculus Pentacam.

Methods: A retrospective comparative study was carried out at Manchester Royal Eye Hospital. Data were collected 12–18 months after corneal transplantation for keratoconus, including post-operative corneal densitometry, best corrected visual acuity (BCVA), central corneal thickness (CCT), and other relevant clinical details.

Results: Analysis of 37 keratoconus eyes from 36 patients found there was a significantly higher corneal densitometry measurement after DALK than PK. This was predominantly in the posterior layer of the concentric zone 0–2 mm of the cornea (P=0.0004). A significant correlation was found between postoperative BCVA and corneal densitometry in DALK groups at full thickness (P=0.03). This correlation was seen in the central 0–2 mm (P=0.03) and posterior 0–2 mm (P=0.04) zones. In addition, within the DALK group, a correlation was found between central corneal thickness and densitometry at full thickness 2–6 mm (P=0.007), central 0–2 (P=0.04), central 2–6 mm (P=0.01), and at posterior 2–6 mm (P=0.01) zones.

Conclusion: This study showed that corneal densitometry measurement differs depending on the type of corneal transplantation used to treat keratoconus patients. Densitometry may have an important role to play in the final BCVA achieved by patients undergoing corneal transplantation for keratoconus. Analysis of Oculus Pentacam images provides an objective evaluation to monitor the cornea status after the surgery.

Keywords: keratocytes, corneal transplantation, corneal wound healing, keratoconus, transparency

Introduction

Keratoconus (KCN) is a non-inflammatory ectatic dystrophy characterized by successive thinning, steepening, and apical conical projection of the cornea. Irregular astigmatism and myopic changes result in progressive visual impairment, which arises due to shifts in the shape of the cornea.[1] In KCN, vision is improved with spectacle correction and/or soft contact lenses until irregular astigmatism demands the use of rigid gas permeable (RGB) contact lenses.[2,3] In severe cases, a corneal transplant may be necessary for visual improvement.[4]

When a corneal transplant is deemed necessary, a full thickness penetrating keratoplasty (PK)[5] has historically been the treatment of choice. However, advances in surgical techniques over the last decade have led to less invasive procedures such as deep anterior lamellar keratoplasty (DALK) becoming more popular.[6]

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PK involves the complete replacement of the affected (8–9 mm) cornea with healthy donor corneal tissue. However, it is now the trend to perform lamellar transplants, aimed at replacing only abnormal corneal tissue.[7] DALK is now used to treat KCN and other diseases that may cause anterior scarring or ectasia. This procedure involves the replacement of only the affected corneal stroma and overlying epithelium, thereby preserving the patient's endothelial layer and Descemet's membrane. This established technique confers a number of advantages. Throughout this surgical procedure, the eye remains "closed", reducing the potential risk of intraocular infection (endophthalmitis) and choroidal hemorrhage. As the patient's own endothelial layer is preserved, the risk of rejection is significantly reduced.[8] Structural integrity is preserved, thereby reducing the risk of traumatic graft dehiscence.[9] One concern of this technique, however, has been the loss of best corrected visual acuity (BCVA), thought to be due to the interface between the donor and recipient tissue.[10]

The healthy cornea does not absorb discernible light, and the dispersal of light is minimal. Therefore, densitometry can provide a measurement of the level of the transparency of the cornea. Densitometry of the whole cornea can be measured in a single scan using the Pentacam. The standardization of the density begins from 0 to 100. As a result, 0 implies that the cornea demonstrates no blurring, while 100 signifies that the cornea is totally obscure.[11] The Pentacam (Oculus Inc., Wetzlar, Germany) facilitates assessment of the whole anterior section from the anterior corneal surface, through to the surface of the posterior lens by use of a rotary Scheimpflug camera.[12]

Despite the large number of transplants performed for keratoconus, no corneal densitometry measurements have been reported. Historically, post-surgery analyses for patients recovering from corneal grafts are conducted by use of qualitative analysis of slit-lamp bio microscopy.[13–15] This is possibly inadequate, because it appears to differ from visit to visit and from clinician to clinician.

In this study we have used densitometry to assess and compare the outcome of both PK and DALK grafts in keratoconus patients.

Methods

Participants

This was a retrospective, comparative, and non-randomized cross-sectional study and was approved by Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee. The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from the subjects after explanation of the nature of the study. Patients with keratoconus attending the Manchester Royal Eye Hospital (MREH) cornea clinic were selected if they had undergone corneal transplantation, either DALK or PK. Inclusion criteria for the study were patients 18 years or above who had a postoperative follow-up between 12 and18 months where Pentacam images had been taken.

Clinical notes were examined where relevant information, including patient age, gender, corneal disease, date of surgery, and type of surgery, were recorded for descriptive data analysis. Postoperative parameters including BCVA in logMAR, and clinical details were also extracted from the notes for data analysis.

Surgical technique

The surgical procedures (DALK and PK) were performed by one of three surgeons, who used the same surgical technique.[7] PK was performed with a standard technique using a Hessburg–Barron trephine (JedMed Instrument Co., St Louis, MO).[16] All DALK procedures were performed using Anwar's big-bubble technique,[17–19] with the aim to obtain a type 1 big-bubble.[9]

In all cases, the graft diameter was between 8.00 and 8.25 mm. Corneal tissue was issued by NHS Blood and Transplant (UK) services. All tissue met or exceeded the minimum standard for transplantation to include a minimum cell count of 2,200 cells/mm^2 and a central clear cornea of 9.00 mm. All recipients received tissue within a 30 years age difference. Wherever possible, all sutures had been removed before the time of imaging and BCVA measurements.

Pentacam imaging

Pentacam images are routinely used clinically to assess patients undergoing transplant surgery. A new update in the computer software now allows the corneal clarity to be measured from the same image across different corneal zones and layers. Pentcam gives numeric value of the clarity at anterior, central, posterior, and total depth layers. In addition, similar numeric values are given by Pentacam at 0–2, 2–6, 6–10, and 10–12 mm of the concentric corneal zone, and for the total corneal dimeter.[11] The protocol takes 5 min and is non-invasive. Patients were imaged prior to any other eye examination or drop administration. A single image of the cornea was taken at each visit. All images were taken by a trained member of staff in a darkened room to minimize the effect of ambient lighting on the corneal measurements.

All images selected met the quality requirement, as determined by Pentacam analysis.

Postoperative data of corneal densitometry for all layers at zones 0–2 mm and 2–6 mm, in addition to central corneal thickness (CCT) were retrieved from the Oculus Pentacam database. The measurements from the Pentacam images were compared with BCVA and CCT to determine if these quick and easy measurements could provide additional information on the surgical outcome of corneal transplants using different techniques.

Statistical analysis

Data analyses were performed using IBM SPSS Statistics for Mac, Version 23.0 (IBM Corp, Armonk, NY), and Graph pad prism7 for Windows. Descriptive statistics were presented as the mean ± SD. Normality of data was examined using the Shapiro–Wilk test, which is more appropriate for small sample sizes (<50 samples). Student's *t*-test for two independent samples was used when parametric analysis was possible. Pearson correlation (r) was used when looking at the relationship between surgical outcomes of the study. A *P*-value of <0.05 was considered to be statistically significant.

Results

A total of 37 eyes from 36 patients were studied in the keratoconus group. The sample characteristics are comparable between both groups. However, there were more males than females in this study (14:7 PK and 11:5 DALK). Both the PK and DALK groups showed good similarity in their demographic characteristics and demonstrated no statistical difference in the age of patients (*P*=0.43) (Table 1). The mean period of time between surgery and BCVA measurement in this study was comparable, and showed no statistical significant between both groups (*P*=0.80). In the PK group, 66% of eyes (n=14) achieved postoperative BCVA of 0.3 LogMAR (6/12) or better, while in the DALK group, 81% of eyes (n=13) achieved postoperative BCVA of 0.3 LogMAR (6/12) or better.

Table 1 Mean and SD of age, time of the densitometry, and BCVA in both PK and DALK

	PK, M (SD)	DALK, M (SD)	*P*-value
Age	30.14 (9.67)	32.56 (8.73)	0.43
Time of imaging postsurgery (months)	14.90 (3.59)	14.62 (4.80)	0.84
Time of taking BCVA postsurgery (months)	13.95 (2.74)	14.87 (4.03)	0.41
BCVA	0.33 (0.21)	0.25 (0.22)	0.28

Abbreviations: BCVA, best corrected visual acuity; PK, penetrating keratoplasty; DALK, deep anterior lamellar keratoplasty; M, mean.

Table 2 Summary of the postoperative densitometry in the surrounding 0–2 and 2–6 mm annulus zone of the cornea

Corneal zones and layers	PK, M (SD)	DALK, M (SD)	*P*-value
0–2 mm			
Anterior	23.57 (3.69)	24.62 (3.10)	0.36
Central	19.40 (2.02)	20.62 (1.68)	0.07
Posterior	14.60 (1.97)	17.40 (2.40)	0.0004**
Full depth	19.20 (2.20)	20.80 (1.90)	0.02*
2–6 mm			
Anterior	24.83 (4.60)	25.09 (3.30)	0.8
Central	20.19 (3.60)	19.88 (2.07)	0.3
Posterior	14.89 (2.80)	16.08 (3.09)	0.2
Full depth	19.97 (3.56)	20.36 (2.47)	0.7

Note: **Signficant at $p<0.01$ and *signfcant at $p<0.05$.
Abbreviations: PK, penetrating keratoplasty; DALK, deep anterior lamellar keratoplasty.

There was no statistical significance between the mean period of time between surgery and densitometry measurements between the two groups. Table 1 summarizes the demographic data. Densitometry measurements were taken for both the central 0–2 mm and the surrounding 2–6 mm concentric zone; results are summarized in Table 2. The measurements showed that there is a significant statistical difference between the two groups when the full depth cornea was examined at 0–2 mm concentric zone (*P*=0.02). However, further statistical analysis showed a higher corneal densitometry measurement in the posterior layer after DALK, which was statistically significant for the 0–2 mm of the cornea zone (*P*=0.0004). There was no statistical difference in postoperative central corneal thickness between the two groups (Table 3).

There was a correlation, but it did not reach significance, between postoperative BCVA and the densitometry measurement after the surgery in the keratoconus group for PK, except with the posterior layer at zone 2–6 mm of the cornea, which was statistically significant at *P*<0.05 (Figure 1).

In the DALK group, a correlation was shown between BCVA and the densitometry measurement after the surgery. These correlations were found at central zone at 0–2 mm corneal concentric zone. Correlation with BCVA was found also in the posterior layer of the corneal zone at 0–2 mm

Table 3 Corneal thickness postsurgery in both groups

	PK, M (SD)	DALK, M (SD)	*P*-value
CCT	541 (32.5)	556 (41.9)	0.20
Thinnest area	513 (50.5)	539 (34.7)	0.07

Abbreviations: PK, penetrating keratoplasty; DALK, deep anterior lamellar keratoplasty; M, mean; CCT, central corneal thickness.

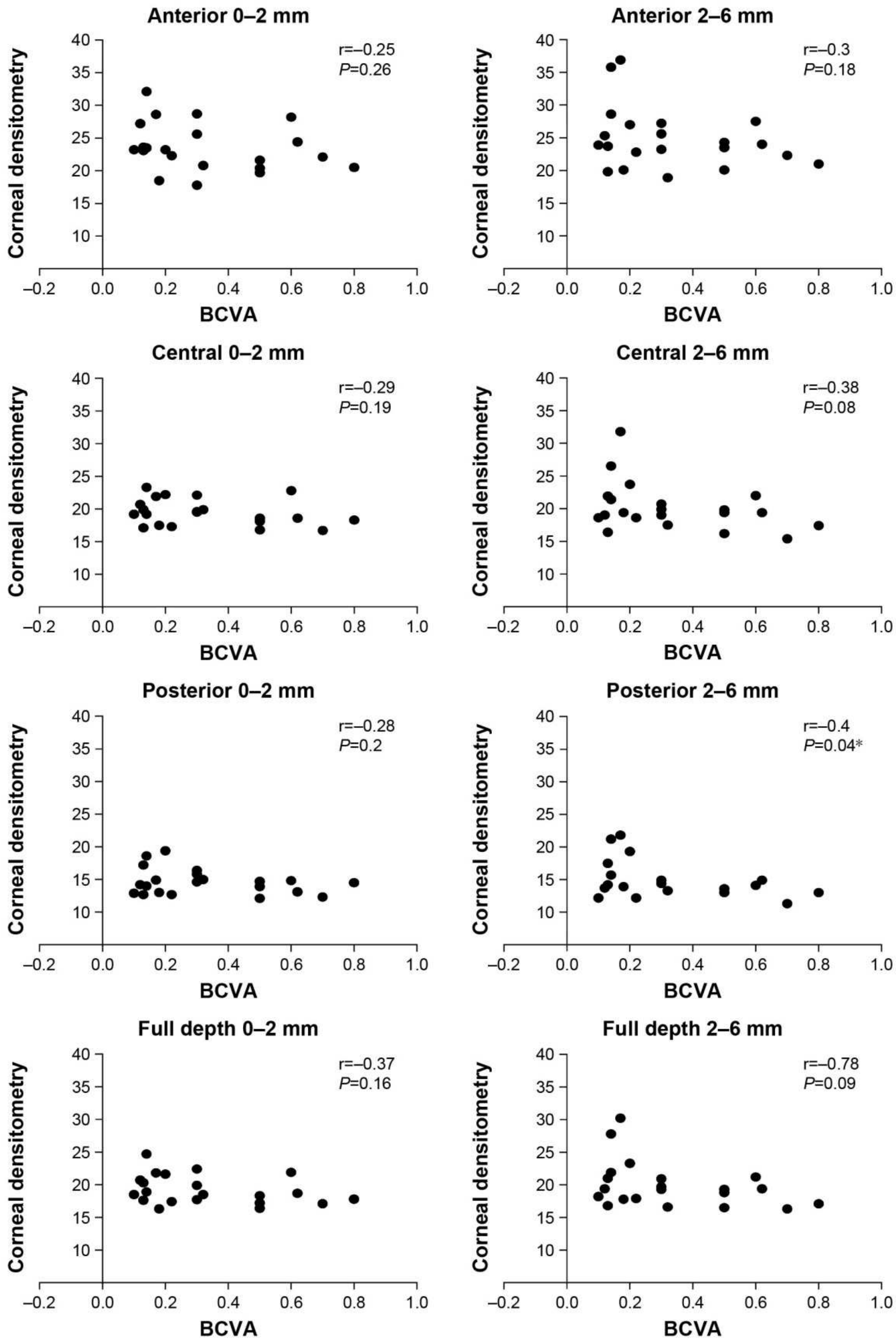

Figure 1 Correlation between BCVA and corneal densitometry in different corneal zones and layers in the PK group (r = correlations coefficient).
Note: *Significant at $P<0.05$.
Abbreviations: BCVA, best corrected visual acuity; PK, penetrating keratoplasty.

and at the full depth of corneal zone at 0–2 mm, $P<0.05$ (Figure 2).

Central corneal thickness was found to be correlated with corneal densitometry in the DALK group only. These correlations were found to take place at full depth 2–6 mm, posterior 2–6 mm, and at both central 0–2 mm and 2–6 mm, $P<0.05$ (Figure 3).

Discussion

DALK is becoming the procedure of choice when corneal transplantation is needed to treat advanced keratoconus. It has several advantages compared to PK as it reduces the number of risk factors related to intraocular surgery, resulting in fewer allograft rejections and other long-term problems.[20]

This study has shown that, between 12 and 18 months after surgery, the mean corneal densitometry for patients with KCN, having had either PK or DALK, was still higher compared to a normal control value within a matched-age group, as illustrated by Dhubhghaill et al[21] and Alzahrani et al.[22] At all corneal regions, apart from the central area at 2–6 mm annulus, the average corneal densitometry was greater in DALK than PK. This suggests that a greater scattering of light was provided by DALK as opposed to PK. Variations in corneal densitometry quantification at the posterior layer were statistically significant ($P<0.05$) in the 0–2 mm corneal zone. This result in the DALK group suggests the host/donor interface reduces clarity, and could have an effect on vision.[23]

The corneal densitometry was quantified using the manufactures pre-set for the posterior 60 μm of the cornea. In DALK, the quantification of densitometry may be even higher if the quantification marker has been customized to concentrate on the host/donor interface of the region. The same pre-set was applied for all layers, the host/donor interface morphology, which results in considerably greater quantification of densitometry in the posterior layer following DALK, which is best described by research employing confocal microscopy. When employing a confocal microscope after DALK, researchers[24] discovered a keratocyte configuration that was uneven and minimized as compared to PK in keratoconus. Several contributory elements were considered by Feizi et al[24] to clarify their results, including mechanical injury of the host and donor sides during surgery, and transient lack of an endothelium pump, resulting in an influx of fluid at the interface (Marchini et al).[25]

A subsequent wound healing response may also cause undesirable complications like corneal haze, which may reduce corneal clarity. Remodeling of the extracellular matrix and repopulation of keratocytes following the surgical induced injury of DALK can influence the corneal transparency resulting from reorganization of the corneal fibrils. Netto et al,[26] showed that significant apoptosis has been detected within 4 h after corneal injury. Following keratocyteapoptosis, the cells undergo secondary necrosis and later activate other keratocytes at deeper layers of the stroma. In addition, there is an activation of inflammatory cells to invade the stromal layer and engulf the resulting dead cells.[27] Keratocytes that die in the anterior stroma are replenished in just 2–4 days by proliferation and migration of neighboring keratocytes.[28] The proliferation and migration of residually activated keratocytes begins 12–24 h after epithelial injury. These activated cells may start synthesizing matrix metalloproteinases for tissue remodeling. The replenishing cells in the anterior keratocytes are the activated myofibroblast cells between 1 and 2 weeks postinjury, which produce collagen, hyaluronic acid, growth factors modulating epithelial healing, and more.[26] The role of myofibroblast cells is very important in remodeling the extracellular matrix and stroma.

The whole process of keratocytes apoptosis, secondary necrosis, activation, proliferation, migration, and transformation regulates corneal wound healing and, consequently, causes haze formation.[29] As discussed above, the raised densitometry quantification in DALK could be the result of mechanical injury from the surgical technique used within this study. It would be interesting to contrast the densitometry result in different DALK procedures to include those performed using Big-Bubble type 1, Big-Bubble type 2, and manual dissection.

It is debateable if reduced endothelial cell density (ECD) can also contribute to our results, as the endothelium pump is crucial for sustaining the transparency of the cornea. It was established[30] that, if there was no perforation of Descement's membrane (DM) in surgery, there was considerably less attrition of endothelial cells following DALK as opposed to PK.[10,31,32] This raises the question that, as the host's own endothelium is maintained by DALK, is the reduction in the transparency of the cornea in DALK entirely due to the host/donor interface following this technique when ECD is higher as opposed to PK.

Our study demonstrated that the average postoperative BCVA result employing the LogMAR chart in DALK is better than that of PK, although this does not reach statistical significance. This was interesting but unexpected, given the raised corneal densitometry quantified in the DALK patients. There are uncertainties surrounding the quality of vision after DALK. There is conflicting published evidence on BCVA, with some studies demonstrating similarity between PK and DALK,[33–35] whilst others have reported decreased vision in eyes after DALK.[23,36,37] However, a numbers of studies have

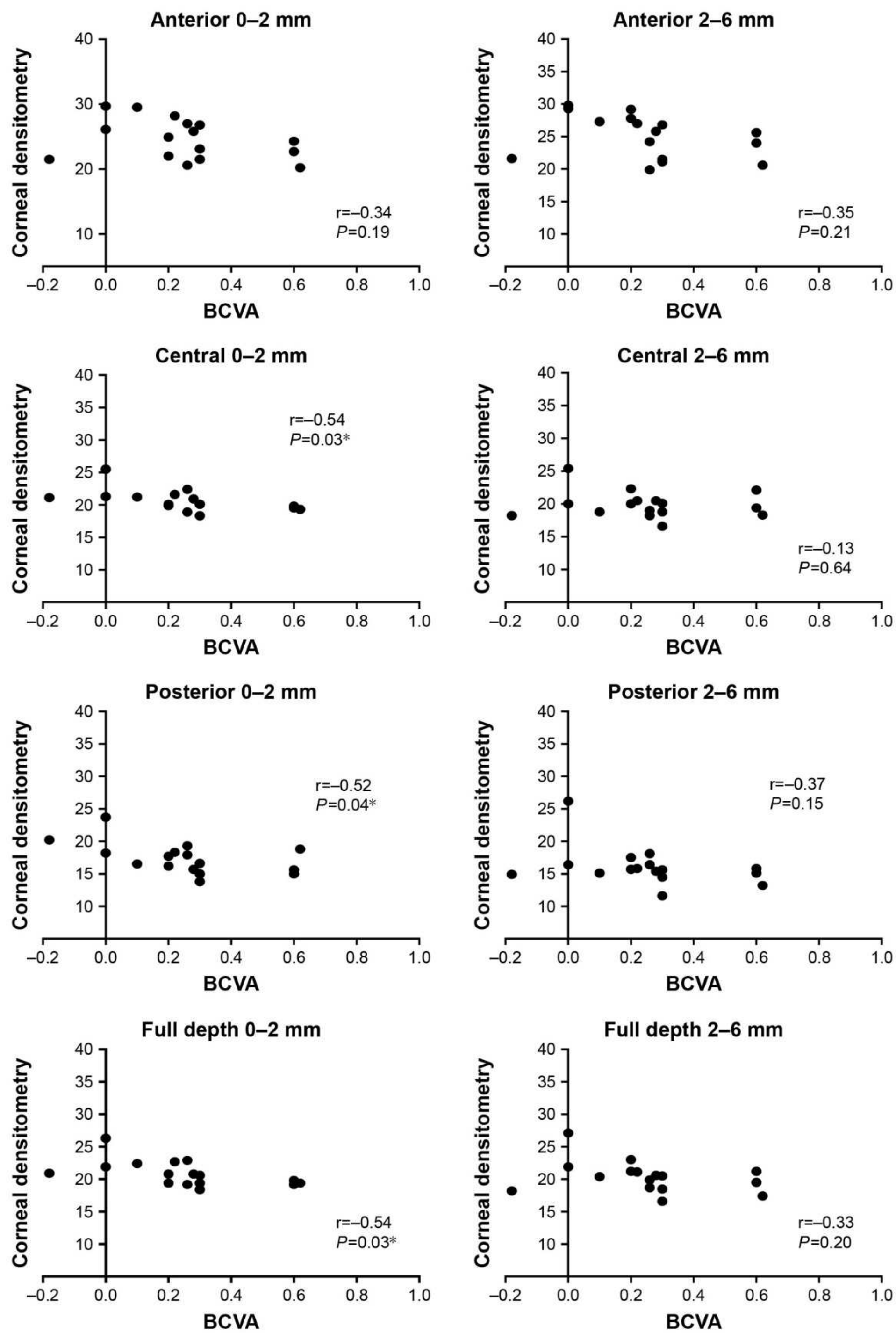

Figure 2 Correlation between BCVA and corneal densitometry in different corneal zones and layers for the DALK groups. (r = correlations coefficient).
Note: *Significant at $P<0.05$.
Abbreviations: BCVA, best corrected visual acuity; DALK, deep anterior lamellar keratoplasty.

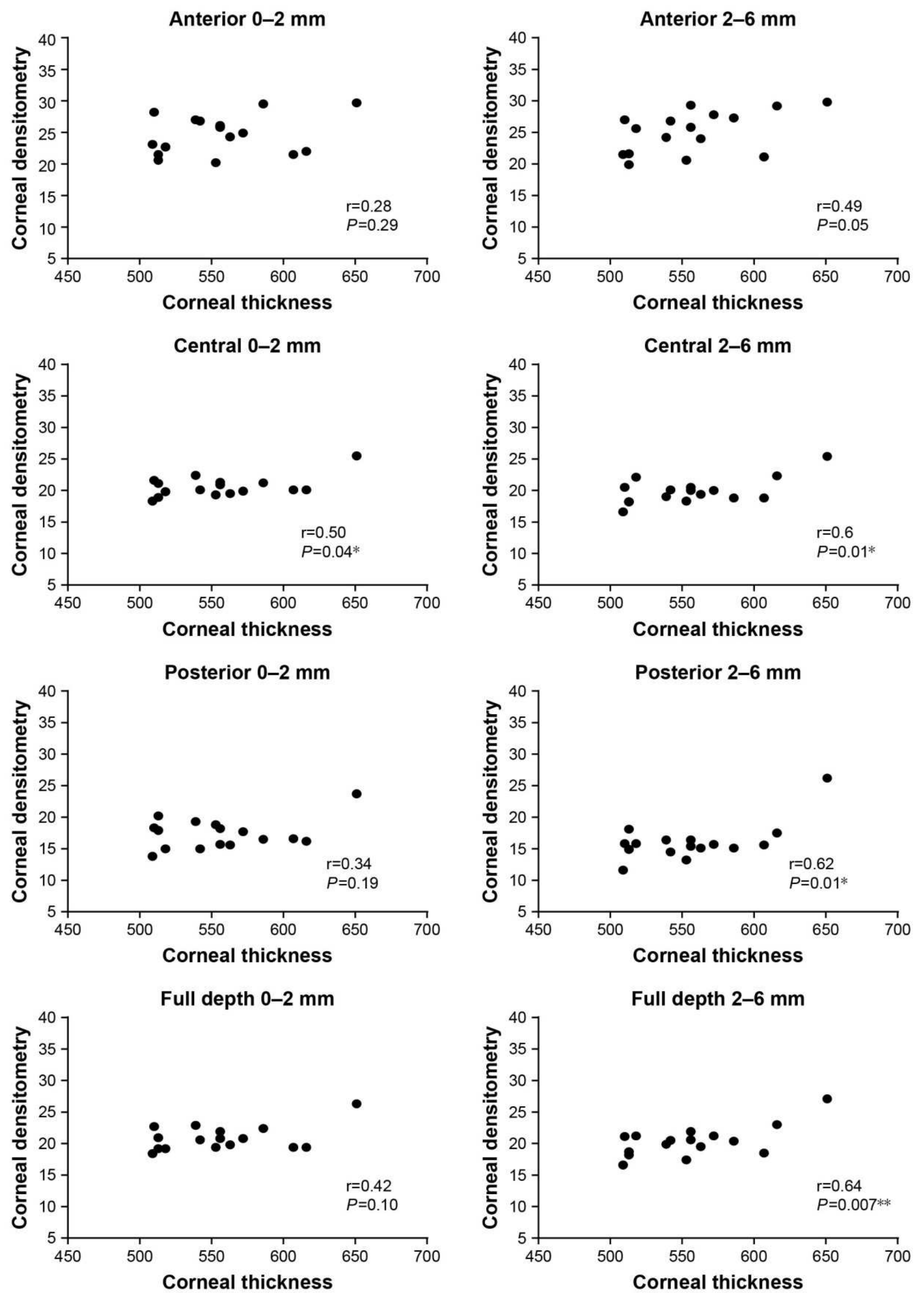

Figure 3 Correlation between central corneal thickness and corneal densitometry in different corneal zones and layers in the DALK group (r = correlations coefficient).
Note: *Significant at $P<0.05$ and $^{**}P<0.01$.
Abbreviation: DALK, deep anterior lamellar keratoplasty.

concluded that DALK provides better BCVA,[36,38–40] in keeping with our own findings. Researchers have proposed that, after lamellar graft surgery, unevenness at the host/donor interface could reduce vision in some eyes.[41] The almost complete exposure of DM by using the big-bubble technique most likely has a role in the improved postoperative BCVA in DALK, as opposed to PK.[35]

The limitation in this study was its retrospective nature and the relatively small sample size for each group that could impede statistical analysis, hence a larger sample size would increase the strength of any further analysis.

In conclusion, this study has found increased densitometry measurements in DALK compared to PK, primarily at the posterior layer within the 0–2 mm zone. This doesn't appear to have an effect on BCVA when compared with those patients undergoing PK for KCN. We have discussed a number of factors which may have contributed to the raised densitometry result.

This is the first study to demonstrate raised densitometry in patients undergoing DALK and provides further information on this lamellar technique. In the future, other forms of corneal imaging to include spectral domain optical coherence tomography and confocal microscopy may help provide greater understanding on this important surgical zone. Corneal densitometry measurements provided by the Oculus Pentacam provide an objective evaluation to further monitor the treatment outcome of corneal transplants surgery in KCN. It provides a non-invasive technique which is safe to use and useful in planning the management of surgery.

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Disclosure

The authors report no conflicts of interest in this work.

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Evaluation of corneal symmetry after UV corneal crosslinking for keratoconus

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Purpose: The purpose of this study was to assess UV corneal crosslinking (CXL) treatment outcomes for keratoconus by evaluating the corneal regularity in patients through follow-up using the Oculus Pentacam.

Patients and methods: A total of 18 eyes from CXL patients with keratoconus were studied before and after CXL treatment, and six eyes from six patients who were not treated with CXL served as controls. Treated patients had Pentacam images taken before CXL treatment and regularly 3 months post treatment up to the 12th month. Controls were imaged during their first appointment and after 12 months. Symmetry and asphericity were evaluated and correlated with both best-corrected visual acuity (BCVA) and maximum *K*-readings.

Results: In the CXL-treated group, there was a significant improvement in the index of symmetrical variation (ISV) and keratoconus index (KI) at 3 months and in the index of height asymmetry (IHA) and minimum radius of curvature (R_{min}) at 9 months post treatment. On the contrary, the untreated group's indices showed some significant worsening in ISV, KI, central keratoconus index (CKI), and R_{min}. A novel finding in our study was a slight positive shift of anterior asphericity in the 6 mm, 7 mm, and 8 mm 3 months after treatment, which had a correlation with BCVA (R^2=0.390, p=0.053) and a strong correlation with maximum *K*-reading (R^2=0.690, p=0.005). However, the untreated group had no significant changes after 1 year.

Conclusion: The corneal asymmetrical shape is associated with the spherical aberration alteration influenced by temporal evolution of surface ablation and increased corneal haze. However, insignificant changes in symmetry attest the stabilization effect on cornea postoperatively as compared with controls.

Keywords: keratoconus, crosslinking, topography, corneal haze, asphericity

Introduction

Keratoconus is a bilateral corneal disorder that starts with anterior corneal deterioration and proceeds by affecting the posterior layers.[1] Onset is usually at puberty and, in many cases, progresses until the third to fourth decade of life.[2] Asymmetrical corneal thinning, irregular astigmatism, and conical shape reflect an early stage, while a progressive protrusion shows an advanced progression. Krachmer et al[2] suggest that keratoconus thinning is central, while others argued that it can also start with the peripheral area.[3] More recently, keratoconus has been demonstrated to be associated with inflammatory cytokines, especially in the tear film where there is increased proteolytic activity and overexpressed matrix metalloproteinases (MMPs).[4] Histologically, it is important to understand that the contribution of other stromal cells with the light transmission is essential where keratocytes play a major role to maintain corneal transparency by producing crystalline molecules, which remain in their collagen matrix and decrease scattering.[5] Therefore, the reduction of basal epithelium density affects

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the hemidesmosomes and anchoring fibrils with Bowman's layer accompanied by the down growth of epithelial cells. This weakness allows proteolytic enzymes to be released, which may cause instability of Bowman's layer.[6] As a consequence, stromal collagen lamellae may break down the crosslinks between themselves and grow up to the anterior surface.[6] However, the posterior stroma in progressive stages is more suspect of increasing the level of corneal hydration and more likely to increase light scatter.[5,7] In many early cases, both epithelial nuclei and keratocyte nuclei from the anterior stroma appeared, using corneal confocal microscopy, to be in the same plane as Bowman's layer.[8]

Recently, corneal UV crosslinking (CXL) has been established as an effective treatment of keratoconus.[9–11] This simple technique increases the corneal rigidity and improves the biomechanical properties of the cornea by strengthening the crosslinks between collage lamellae and stabilizes the corneal tissue.[12,13] In the 1990s, the first study on photobiology began with attempts to detect the biological glues that could increase the resistance of stromal collage.[14] A standard approach treating keratoconus was first used in Germany at Dresden Technical University.[14] Many studies, thereafter, provide an explicit description of the treatments, various procedures, and how it is very necessary to follow specific criteria to prevent any complications. However, these procedures need further investigations to determine if they are adequate to improve corneal rigidity and its biomechanical properties.[15]

For this reason, assessing UV corneal CXL is crucial, especially where CXL treatment has the potential to be an alternative procedure in reducing the progression in forme fruste or preclinical keratoconus. This can be achieved by some new imaging devices that have become more sophisticated by using different imaging principles and techniques. Accordingly, differences in cell density and morphology help contour the corneal layers more accurately. Moreover, the ability of these devices to measure parameters such as thickness, elevation, and biomechanical properties combined with refractive power is used to clarify the disease progression in in vivo cornea as compared to the actual alterations described by laboratory ex vivo studies.[16] Topographic and tomographic measurements are essential to estimate the capacity of these parameters to assess the treatment effectiveness. This finding confirms the efficacy of corneal biomechanical changes and collagen lamellae organization.

Pachymetry and elevation parameters show high repeatability and reproducibility in several studies[16] where posterior elevation measurements are more accurate in detecting keratoconus.[17] Recently, the most common consideration is the accuracy of quantifying surface sphericity, which indicates the maintenance of the corneal structure.[18] Therefore, descriptors of corneal asymmetry parameters should emphasize and refer to the clinical assessment correctly to classify, monitor, and evaluate the crosslinked cornea. There are many separate elements available in the Oculus Pentacam software for assessing corneal asymmetry. These elements have been found to be more valuable in monitoring the normalization of the cornea. These include the index of symmetrical variation (ISV), keratoconus index (KI), central keratoconus index (CKI), index of height asymmetry (IHA), index of vertical asymmetry (IVA), index of height decentration (IHD), and minimum radius of curvature (R_{min}). Previous studies had reported that the cornea becomes more optically regular after CXL using the same indices on patients who were analyzed after 1 year of treatment.[19,20] However, data from these studies did not assess the posterior corneal elevation changes and peripheral corneal asphericity to show reliable improvement in corneal shape after CXL.

In this study, the corneal asymmetry indices were measured by Oculus Pentacam before and after CXL for keratoconus patients in conjunction with the back elevation (BE) map. Peripheral corneal asphericity (6–8 mm) was also evaluated to to understand the cumulative effect of corneal haze after treatment.

Patients and methods

This preliminary investigation was a retrospective nonrandomized comparative study. All subjects were recruited from Manchester Royal Eye Hospital (MREH) from August 2015 to August 2016. The study protocol was approved by Central Manchester University Hospital NHS Foundation Trust, Manchester, UK, and Research Ethics Committees in adherence to the guidance of the Declaration of Helsinki. All patients provided informed consent after receiving a detailed description of the nature of the study. A total of 24 eyes from 24 patients were included in this study. The exclusion criteria included patients younger than 18 years or patients with other ocular or systemic disease associated with keratoconus, contact lens wearers, history of eye surgery, scarring, and current topical medication users. Changes in the corneal symmetry in 18 eyes from patients with keratoconus treated with CXL were measured before corneal CXL treatment and 1 month, 3 months, 6 months, and 12 months after treatment. A matched control group of patients with keratoconus who did not have corneal CXL was seen on their first clinical visit and 12 months later. Table 1 summarizes

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Table 1 Demographic data of both groups, including sample size, age, gender, and Amsler–Krumeich classification of grading keratoconus

	Treated group	Untreated group
Sample size	n=18	n=6
Age (years)	26.35±6.49	29.83±7.03
Gender (ratio M:F)	Male 15/female 3 (5:1)	Male 5/female 1 (5:1)
Classification of keratoconus	2.063±0.5	2.15±0.4

Note: Data shown as mean ± SD and *p*-value.
Abbreviations: SD, standard deviation; M, male; F, female.

the demographic data of both groups, including sample size, age, gender, and Amsler–Krumeich classification of grading keratoconus. Patients who underwent corneal CXL treatment were selected from the hospital clinic database. Relevant information including their age, gender, best-corrected visual acuity (BCVA), and date of surgery were recorded for descriptive data analysis. Corneal tomography was evaluated with the Pentacam (OCULUS Optikgeräte Gmbh, Wetzlar, Germany). The diagnosis of keratoconus stages was based on the interpretation of corneal curvature and relative thickness as discussed in the Amsler–Krumeich classification.[21]

Clinical signs such as stromal thinning, corneal steeping, Fleischer ring, Vogt's striae, scissoring reflex, applanation tonometry, dilated funduscopic examination, and abnormal corneal astigmatism were also recorded. Patients following the standard surgical protocol of CXL (0.4% hypotonic riboflavin every 5 min for 20 min/ultraviolet-A [UVA; 9.86 mW] for 10 min) were chosen. Postoperative measurements including corneal tomography and BCVA were also recorded. To minimize the effect of diurnal variation in the corneal swelling, all measurements were performed at the same time between 10:00 and 3:00 PM. Postoperative data of corneal asymmetry indices were retrieved from the Oculus Pentacam database stored in the Oculus Pentacam instrument located in MREH.

Statistical analysis

Data were analyzed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA). Normality of data was assessed with the Shapiro–Wilk *W*-test. Two groups were analyzed: the untreated keratoconus group and the treated subgroup. Data of the treated group were presented in each follow-up as the mean ± standard deviation (SD) at baseline, 1–3 months, 4–6 months, 7–9 months, and 10–12 months. The same analysis was performed for the untreated group for two periods of time: first visit and follow-up a year after. A paired two-tailed *t*-test was used to analyze changes in follow-ups as compared to the baseline. An independent *t*-test was used to compare postoperative changes in Pentacam parameters between patients who received corneal CXL and those who did not have CXL after 1 year. Pearson correlation coefficient was used to analyze the possible correlation between BCVA and both asphericity and densitometry. A p-value <0.05 was used to determine statistical significance.

Results

A total number of 18 eyes from 18 patients with keratoconus were studied before and after corneal CXL treatment, and an additional six eyes from six patients with keratoconus who were not treated were used as controls. Treated patients were seen throughout their follow-ups at 1–3 months, 4–6 months, 7–9 months, and 10–12 months. For the purpose of analysis, the pre-CXL treatment measurements were considered the baseline. The control group was seen twice: once during their first clinic visit (baseline) and after 1 year.

In the treated group, the initial measurement after CXL shows that central corneal thickness (CCT) has been stabilized as there was no significant change compared to the baseline (440.47±43.22 µm to 439.57±29.68 µm, p=0.313). However, the maximum *K*-reading value significantly worsened after 3 months (57.06±7.71 D to 54.51±5.47 D, p=0.026) and returned with a significant improvement at 9 months (58.65±8.77 D, p=0.035). Moreover, BCVA has continuously improved over time, where it showed a significant improvement at 6 months after CXL. However, the untreated group showed a significant progression with a reduction in CCT (494.83±46.39 µm to 480.50±46.89 µm, p=0.038) and a steeping of the maximum keratometric reading after 1 year. Table 2 shows the initial parameters for both groups at scheduled visits.

In the CXL-treated group, there were significant changes in some of the corneal asymmetry indices such as the ISV and KI at 3 months after treatment and significant changes in IHA and R_{min} at 9 months after treatment, but all indices showed no significant changes 1 year after CXL compared with the baseline. However, the control group had significant changes in ISV, KI, CKI, and R_{min}. Surprisingly, maximum posterior elevation (BE) showed continued elevation after 1 year. There were no significant differences between the treated group and the untreated group after 1 year of follow-up in any of the topographic indices.

The mean value of the anterior corneal surface asphericity in the treated group decreased significantly 3 months after treatment in all peripheral rings: 6 mm, 7 mm, and 8 mm (p=0.027, p=0.018, and p=0.005, respectively). Conversely, there was a significant negative shift in asphericity 9 months

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Table 2 Comparison of the corneal symmetry indices and asphericity measurement in both treated and untreated group through their scheduled visits

	Treated group					Untreated group	
	Baseline (n=18)	1–3 months (n=9)	4–6 months (n=8)	7–9 months (n=6)	10–12 months (n=7)	Baseline (n=6)	1 year follow-up (n=6)
CCT (μm)	440.47±43.22	441.11±46.37	436.50±53.21	440±48.19	439.57±29.68	494.83±46.39	480.50±46.89
Maximum *K*-reading (D)	57.06±7.71	54.51±5.47*	60.07±8.30	58.65±8.77*	55.36±6.05	49.95±2.25	51.97±3.83*
Minimum *K*-reading (D)	52.54±5.90	50.84±4.39	55.43±8.38	53.57±5.86	51.46±4.97	47.46±2.14	49.41±4.04*
BCVA	0.39±0.39	0.41±0.23	0.21±0.21*	0.03±0.03	0.04±0.18	–	–
ISV	138.47±36.71	126.33±31.35*	157.77±40.44	154.83±45.78	121±30.08	97.16±29.10	107.83±31.85*
IVA	1.23±0.43	1.18±0.19	1.32±0.38	1.26±0.39	1.05±0.314	1.05±0.43	1.07±0.41
KI	1.37±0.12	1.35±0.13*	1.44±0.14	1.37±0.15	1.32±0.14	1.25±0.12	1.29±0.13*
CKI	1.16±0.09	1.13±0.05	1.20±0.10	1.23±0.10*	1.15±0.06	1.09±0.03	1.12±0.05*
IHA	52.69±35.21	42.87±37.22	39.95±35.25	18.46±12.30*	42.51±16.99	34.75±22.05	25.30±16.19
IHD	0.22±0.07	0.18±0.05	0.24±0.07	0.22±0.08	0.19±0.06	0.16±0.06	0.17±0.06
R_{min} (mm)	5.14±0.71	5.36±0.56	4.83±0.73	4.82±0.86*	5.26±0.67	5.80±0.54	5.55±0.71*
BFS (mm)	5.89±0.32	6.00±0.22	5.80±0.32	5.90±0.31	5.96±0.11	6.13±0.26	6.05±0.29
BE (mm)	69.70±35.10	61.44±29.97	83.00±43.37	87.66±39.98	88.28±31.32*	40.17±15.30	52.17±24.54*
Asphericity 6 mm (*Q*-value)	−1.91±0.85	−1.78±0.74*	−2.30±0.94	−2.52±0.96*	−1.86±0.63*	−1.13±0.45	−1.45±0.67
Asphericity 7 mm (*Q*-value)	−1.68±0.76	−1.58±0.66*	−2.06±0.86	−2.16±0.87	−1.61±0.55*	−1.05±0.38	−1.36±0.53
Asphericity 8 mm (*Q*-value)	−1.50±0.65	−1.40±0.56*	−1.82±0.79	−1.87±0.77	−1.43±0.47	−0.87±0.32	−1.10±0.44

Notes: *Statistically significant compared to baseline ($p<0.05$). Data presented as mean ± SD and *p*-value.
Abbreviations: SD, standard deviation; CCT, central corneal thickness; BCVA, best-corrected visual acuity; ISV, index of symmetrical variation; IVA, index of vertical asymmetry; KI, keratoconus index; CKI, central keratoconus index; IHA, index of height asymmetry; IHD, index of height decentration; R_{min}, minimum radius of curvature; BFS, best-fit sphere; BE, back elevation; *Q*-value, anterior corneal asphericity.

after surgery in all peripheral rings (6 mm, 7 mm, and 8 mm), where it was only significant in the 6 mm ring with $p=0.048$. After that, all peripheral rings turned with a positive shift 1 year postoperatively ($p=0.031$, $p=0.047$, and $p=0.105$) as shown in Figure 1. The untreated group showed no significant changes after 1 year of follow-up, although there was a slight increase in their measurements. Moreover, there were no significant differences between the treated group and the untreated group in the mean value after 1 year of follow-up in all topographic indices ($p>0.05$).

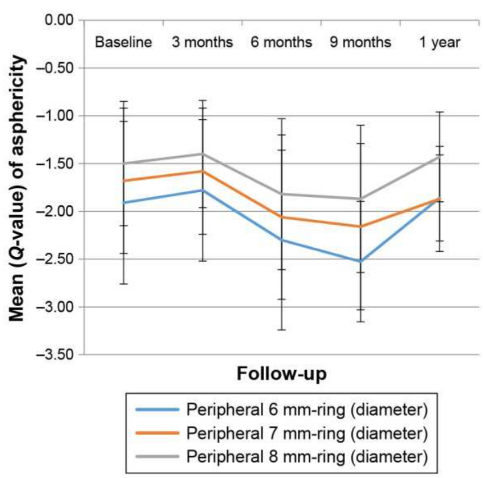

Figure 1 Changes in the anterior corneal surface asphericity over time in the treated group.

Within the CXL group, the absolute measurement of the peripheral 6 mm ring of the anterior corneal asphericity at 3 months indicates an insignificant negative correlation with BCVA ($R^2=0.390$, $p=0.053$), but maximum *K*-reading shows a strong negative correlation with all zones at 3 months (6 mm: $R^2=0.704$, $p=0.005$; 7 mm: $R^2=0.676$, $p=0.007$; 8 mm: $R^2=0.682$, $p=0.006$; Figures 2 and 3). However, both show no correlation with asphericity changes at 6 months, 9 months, and 12 months after treatment in all peripheral rings.

Discussion

Collagen CXL treatment is widely used for keratoconus patients to improve corneal rigidity and visual outcome. According to the significant improvement in distance vision and evidential ability to stabilize progression of this disease, this treatment has been used in many cases as a first-line treatment option for early keratoconus stages. On the other hand, the alteration in the stromal histological structure induces a postoperative visual acuity reduction within the first few months after treatment. Moreover, the collagen fibers will vary in their diameter and regularity, which increase light scatters and affect corneal transparency. As a consequence, continuous corneal haze symptoms have been observed in most cases after CXL.[22]

The first part of this study demonstrates evaluating tissue disturbances by measuring corneal symmetry, which

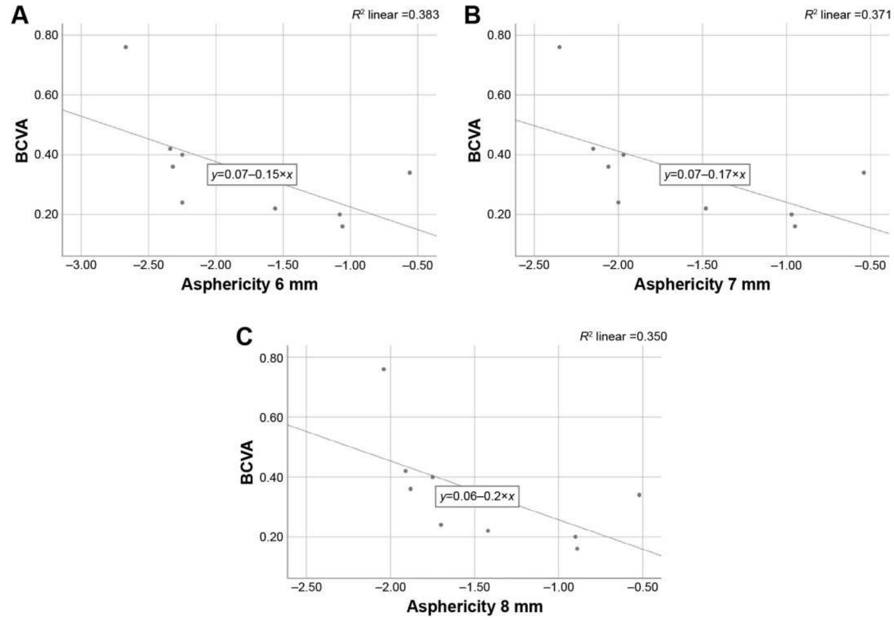

Figure 2 Correlation between BCVA and asphericity at 3 months in (**A**) in 6 mm ring, (**B**) in 7 mm ring and (**C**) in 8 mm ring.
Abbreviation: BCVA, best-corrected visual acuity.

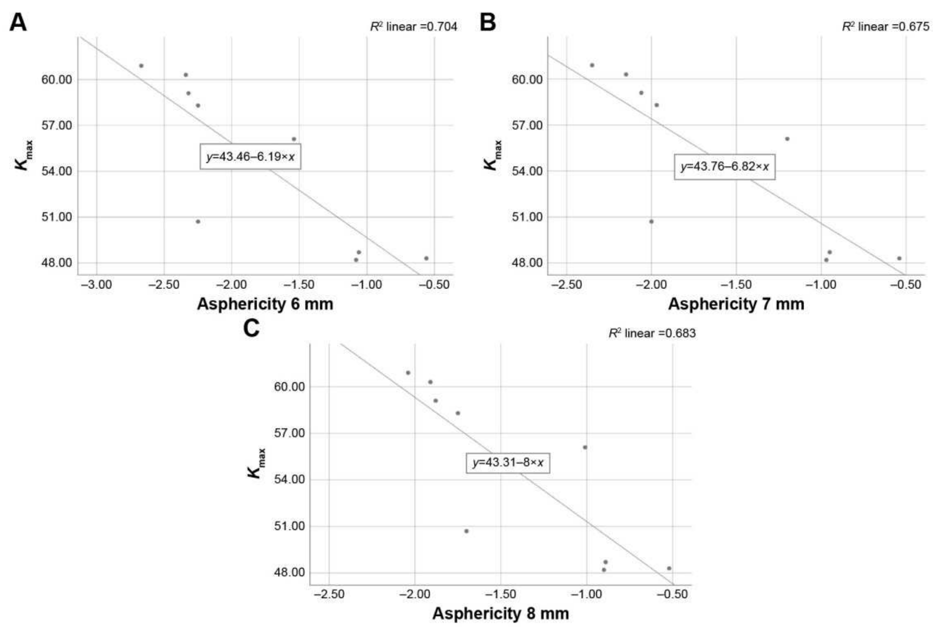

Figure 3 Correlation between K_{max} and asphericity at 3 months in (**A**) in 6 mm ring, (**B**) in 7 mm ring and (**C**) in 8 mm ring.
Abbreviation: K_{max}, maximum *K*-reading.

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indicates the recovery of corneal asymmetrical shape and the reduction in the biomechanical deformation. The Oculus Pentacam software provides abnormal values in topographic measurement of symmetrical indices in keratoconus. Therefore, these parameters may be useful in following the progressive changes in keratoconus patients and increase our comprehension of the restoration of the corneal shape after CXL treatment.

Our finding for the treated group shows a significant reduction in the ISV and KI after 3 months and a significant reduction in IHA and R_{min} 9 months after treatment, whereas other indices show no significant changes. Based on this finding, we suggested that the cornea has been flattened as a result of CXL treatment.

To our knowledge, the study of Gutiérrez et al[23] is the only study measuring index changes over time and, in contrast to our results, found a significant reduction in the IVA and the IHA at 3 months ($p<0.05$) which returned to baseline values after 6 months.

Our study also showed that there were no significant changes in all indices 1 year after treatment, and this finding has good agreement with the studies of Gutiérrez et al and Razmjoo et al. In contrary to our findings, Toprak et al reported an improvement in the corneal regularity by significant reduction in ISV, CKI, and R_{min}. In their study, patients were treated with isotonic riboflavin solution and the baseline of maximum K-reading value was lower than that of our treated group (53.83±64.43 D).[19,23,24]

Koller et al also found significant improvements in CKI, R_{min}, KI, and IHA indices 1 year after CXL in patients with progressive keratectasia. However, their crosslinked group had an average corneal thickness of 456 μm, while in our study, the group has been treated with further reduction in CCT of 440 μm. This may reflect that both Toprak et al and Koller et al treated their patients in earlier stages of keratoconus than in our patients. Changes in the refractive index of the crosslinked stromal layer also might be an explanation leading to a false thinner result of optical ray tracing.[20]

Moreover, we have detected changes in some indices in the untreated group where ISV, KI, CKI, and R_{min} were significantly progressed, which was similar to the control group of Koller et al. This finding supports the idea of the effectiveness of CXL in declining progression and enhancing corneal regularity in the treated group.

Surprisingly, we have found a significant continuous progression of the posterior cornea after 1 year in both groups. The same trend was seen in the study of Gutiérrez et al, although insignificant changes were observed. Our assumption of this continuous elevation is related to intrastromal shrinking processes of the anterior part of chemical bonds induced by the radicals formed during CXL, causing a secondary distortion of the posterior layers.[20]

A novel finding in our study was a significant positive shift of anterior corneal asphericity in the 6 mm, 7 mm, and 8 mm peripheral rings 3 months after treatment. This has a weak correlation with visual acuity reduction (R^2=0.390, p=0.053) but a strong correlation with the maximum K-reading (R^2=0.704, p=0.005). This change might be explained by pointing out that the cause of spherical aberration is influenced by the alteration in the corneal curvature. Subsequent measurements resulted in a significant negative direction after 6 months, which may be due to the temporal evolution of surface ablation. At 12 months, there was another significant positive change in asphericity in the 6 mm ring diameter, while there were no changes verified at 7 mm and 8 mm. This finding is similar to the finding of Koller et al where they reported that Q_{ant} was calculated within a circular area of 8 mm. As compared to the treated group, the untreated group showed an insignificant negative shift at the 1 year follow-up. This finding contrasted to that of Kovács et al where they reported on those who had relevant negative posterior Q-values because of corneal protrusion. However, they used a different way of measuring asphericity by measuring Q-value at the sagittal angle ring at 30° centered on the apex, which showed high accuracy (90% of specificity) and moderate sensitivity (60%).[25] This difference could be attributed to the size of our untreated group, which was too small as compared to their study. The limitation of this study was because of a small sample size which had some impact on the statistical results due to a small number of non-contact lens wearers in this stage of keratoconus. Another problem was the loss of patients during their follow-up, which is an important factor in the strength of our conclusions about each period. For instance, for precise results, the same methodology in previous studies was chosen for our research and measurements were taken after a 3-month period of time subsequently.[23] However, due to the high demand and overloading of hospital appointments, there may have been inability of patients to visit clinics at a required time. Moreover, extending and expanding follow-up assessment are required to understand the nature of the corneal haze. In doing so, finding a correlation between corneal shape objectively and contrast sensitivity subjectively after treatment is helpful to investigate the relationship between visual improvement, spherical aberration reduction, and haze discomfort.[26]

Conclusion

This study reveals the impact of CXL treatment in corneal symmetrical indices and asphericity measurements during

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consecutive periods of follow-ups. The strong correlation between asphericity and keratometric reading 3 months after CXL treatment reflects the tissue disturbance influenced by the temporal ablation of the cornea. This contributes to some complaints such as visual reduction and the corneal haze. Interestingly, our study shows insignificant asymmetrical alteration in the corneal shape 1 year after treatment, which explains the structural constancy and confirms the tissue recovery. This also emphasizes the aspect that these objective measurements are able to interpret the progression of keratoconus.

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Disclosure

The authors report no conflicts of interest in this work.

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Abstracts from the 2016 European Association for Vision and Eye Research Conference

Corneal imaging and densitometry measurements to monitor fuchs progression and treatments outcomes

K. Alzahrani, F. Carley, A. Brahma, D. Morley, M.C. Hillarby

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Manchester University

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Abstract

Purpose

Fuchs' dystrophy is a degenerative disease of the corneal endothelium leading to corneal edema and eventually to loss of vision. There are several treatment options including corneal transplantation, which can be full (Penetrating Keratoplasty) or partial (Descemet's stripping automated endothelial keratoplasty) thickness. Disease progression and treatment out comes are normally monitored by corneal thickness and visual acuity. In this study, we have used the new densitometry software for the Oculus Pentacam to compare the corneal clarity measurement between Penetrating Keratoplasty (PK) and Descemet's Stripping Endothelial Keratoplasty (DSAEK) in patients with Fuchs dystrophy

Methods

A retrospective comparative study was carried out at Manchester Royal Eye Hospital. Data collection of one year after the corneal transplantation for 23 Fuchs dystrophy patients, including; best corrected visual acuity (BCVA), corneal densitometry, central corneal thickness (CCT) was analysed

Results

Analysis of densitometry measurements found higher corneal densitometry after PK than DSAEK in the posterior layer between 2 and 6 mm from the centre but it was not significant. There were no significant correlation between CCT and corneal densitometry. Corneal densitometry was found to be significantly correlated with BCVA inthe central 0–2 mm zone. This correlation differed in corneal depth, in PK it was with the posterior layer but in DSAEK it was with the anterior stroma These difference were found to be significant between the two group (p<005.).

Conclusions

There were different outcomes in the corneal densitometry measurement after different type of corneal transplantation. Oculus Pentacam provides an objective evaluation to monitor the cornea status. Further investigation with prospective design, a longer study period and larger sample size are now underway.

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ARVO Annual Meeting Abstract | June 2017

Corneal Imaging and Densitometry Measurements in Healthy Volunteers Across Different Age Groups

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Investigative Ophthalmology & Visual Science June 2017, Vol.58, 3546. doi:

Abstract

Purpose : Measuring corneal clarity in healthy eyes is important because it could help when planning treatments. This study aims to standardize and investigate the changes in corneal clarity with age. Densitometry software for the Oculus Pentacam was used to examine corneal clarity at different age groups.

Methods : A total of 192 eyes of 97 healthy participants were included in this cohort comparative non-randomized cross-sectional study. Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK and NREC local ethics committee approved this Study. An Oculus Pentcam was used to image cornea for healthy participants grouped by sex and age (between 10 and 70 years old). Data from the densitometry output has been used to determine the clarity in concentric zones and different depths of the cornea.

Results : Corneal densitometry across all age groups showed significant differences between groups when we divided the corneal into layers: anterior, central and posterior ($p<0.05$). We also showed significant differences when the cornea was divided in to 0-2 mm, 2-6 mm, and 6-10 mm concentric zones ($p<0.05$). The most striking increase in densitometry values occurred with age in all 3 layers was in the periphery (6-10 mm) ($p<0.05$). Additionally, we showed that the 10-20 yr age group had lower clarity than the 20-30-age group ($p<0.05$) and after 30 years the cornea shows a steady progression of increased densitometry values. Densitometry of the anterior and posterior layer was shown to be the least stable with aging.

Conclusions : This study is the first study to our knowledge that has standardized corneal densitometry value in healthy control eyes, which include teenage densitometry values. These values for corneal densitometry, as well as subdivisions based on layer and surface area, might provide a standardized stage for use in further studies and clinical practice. This study established that relation between corneal densitometry and age is differed when cornea divided by layers and zone. This study suggested that there are other factors play an essential role in corneal densitometry level as well as age.

This is an abstract that was submitted for the 2017 ARVO Annual Meeting, held in Baltimore, MD, May 7-11, 2017.

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ARVO Annual Meeting Abstract | June 2017

Evaluation of corneal symmetry after UV corneal crosslinking for keratoconus

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Abstract

Purpose : To assess UV corneal crosslinking (CXL) treatment for keratoconus by evaluating the corneal regularity through patients' follow-up using Oculus Pentacam. This could provide relevant information about secondary haze which is commonly present treatment.

Methods : : A total of eighteen eyes from eighteen CXL keratoconic patients were studied before and after treatment and six eyes from six keratoconic patients, who were not treated with CXL were used as controls. Treated patients were seen at 1-3 months, 4-6 months, 7-9 months, 10-12 months afetr treatment and analysis was compared to an untreated baseline. Symmetry and asphericity were evaluated and correlated with BCVA and with maximum k-readings.

Results : In the CXL treated group, there were significant changes in the ISV and KI at 3 months after treatment and in IHA and Rmin at 9 months after treatment. After one year, all indices show no significant changes from the baseline but there were significant changes in ISV, KI, CKI and Rmin in the untreated group. A novel finding in our study was a slight positive shift of anterior asphericity in 6mm, 7mm and 8mm three months after treatment which had a correlation with BCVA ($R2= 0.390$, $P=0.05$) and strong correlation with maximum k-reading ($R2= 0.690$, $P=0.005$).

Conclusions : transient corneal haze is associated with the spherical aberration influenced by temporal evolution of surface ablation and increases of cloudiness. However, Insignificant changes in symmetry readings after one year leads to support the idea that CXL stabilizes the cornea structure and normalizes corneal shape postoperatively when compared to untreated patients.

This is an abstract that was submitted for the 2017 ARVO Annual Meeting, held in Baltimore, MD, May 7-11, 2017.

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Abstracts from the 2017 European Association for Vision and Eye Research Conference

Corneal clarity measurements in patients with myopia undergoing laser assisted in situ keratomileusis and laser assisted sub-epithelial keratectomy

K. Alzahrani, N. Din, A. Brahma, F. Carley, M.C. Hillarby

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Manchester University

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Abstract

Purpose

To compare the preoperative and postoperative corneal densitometry of patients undergoing Laser Assisted in Situ Keratomileusis (LASIK) and Laser Assisted Sub-epithelial Keratectomy (LASAK) and to determine how result differed between the 2 types of surgery.

Methods

A retrospective and comparative study was performed at Manchester Royal Eye Hospital,UK. Preoperative and postoperative corneal densitometry data were collected from the Oculus Pentacam. The data were taken at 3 corneal depths (anterior,centre and posterior) and at 3 corneal annulus ring diameter (0-2 mm, 2-6 mm and 6-10 mm). Postoperative data were collected at the time 6 weeks, 3 and 6 months post treatment.Quality of vision pre and post-surgery was determined by BCVA.

Results

Data of 60 eyes from 16 LASIK and 14 LASEK patients were collected with a mean age of 37.06 ± 10.0 and 37.7 ± 6.6 respectively. There was statistically significant increase of corneal densitometry in all concentric zones in all corneal layers after 6 weeks in the LASIK treatment group ($p < 0.05$). There was a statistically significant increase in densitometry after LASEK but only in the central and posterior layers at all concentric zones ($p < 0.05$). Pre and Postoperative BCVA did not significantly differ after LASIK but found statistically difference at 6 weeks and 3 months in post LASEK ($p < 0.05$).BCVA post treatment was significantly different between groups at 6 weeks ($p < 0.0001$) and 3 months ($p = 0.001$).

Conclusions

Corneal wound healing plays an important role in determining corneal transparency and may be responsible for an increase in corneal haze following refractive surgery.This response differs based on the type of procedure used shown by the increased haze in the anterior layer of the LASIK group which corresponds to the region where the flap was cut which will lead to increased would healing and inflammation.

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Acta Ophthalmologica / Volume 95, Issue S259
Abstracts from the 2017 European Association for Vision and Eye Research Conference

Corneal haze in juvenile and adult keratoconus patients after corneal cross-linking

K. Alzahrani, F. Carley, A. Brahma, H. Mofty, S. Biswas, Y. Lin, D. Morley, C. Hillarby

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Manchester University

About Tools Share

Abstract

Purpose

Densitometry software for the Oculus Pentacam was used to investigate the treatment outcomes of corneal cross linking (CXL) in adult and juvenile keratoconus patients.

Methods

A retrospective comparative study was carried out at Manchester Royal Eye Hospital. Corneal densitometry measurements collected before and after CXL treatment for 32 eyes from KC patients, aged between 13 and 39, were divided to 2 groups 13-18 years(juvenile group) and 19-39 years (adult group) was compared to pre and post treatment at 3, 6 and 12 months for each group and between both groups.

Results

Analysis of densitometry measurements found higher corneal densitometry after CXL which peaks at 3 months post treatment in both groups. There was significant diversity in corneal densitometry measurements at stromal zone 0-2 and 2-6 mm for all layer except the posterior layer for each group $p<0.05$). Significant differences were found between both groups at six months in the central ($p=0.006$), posterior ($p=0.004$) and full depth ($p=0.02$) layers for zone 0-2 mm.The same layers differed significantly in the 2-6 mm zone in all layers ($p=0.01$). One year post treatment significant differences were shown in corneal densitometry level between both groups in the 0-2 mm zone of the central layer ($p=0.007$), posterior layer ($p=0.01$) and full depth ($p=0.03$).The central layer in zone 2-6 mm was significantly different between both groups ($p=0.04$).

Conclusions

Haze levels post CXL differing in severity in different corneal zones and between both groups.The 0-2 mm and 2-6 mm zones were found to be the most affected area post treatment.Haze reached its maximum level at three months post treatment then appears to differ significantly in improvement level between adult and juvenile group, with the later never returning to pre-treatment clarity in the most anteriorly central zone in juvenile group

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