CORNEAL STRUCTURE AND BIOMECHANICS: RELATIONSHIP TO DIAGNOSIS AND TREATMENT OF GLAUCOMA AND KERATOCONUS

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DECLARATION OF ORIGINALITY

I hereby declare that this thesis is my original work, as supported by chapters either published or to be submitted to peer reviewed journals. To the best of my knowledge, this thesis does not contain material that has been accepted for the award of any other degree or diploma at the University or any other tertiary institution. Data collection included in this thesis was performed at the Eye clinic, Australian School of Advanced Medicine, Macquarie University, The Eye Associates and at the Sydney Cornea Clinic. I give consent for the thesis to be made available for photocopying and loan if accepted for the award of the degree.

Deepa Viswanathan

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PUBLICATIONS AND PRESENTATIONS RELATED TO THIS THESIS

Peer reviewed journal articles:

1. Viswanathan D, Goldberg I, Graham SL. Longitudinal effect of topical antiglaucoma medications on central corneal thickness. Clin Experiment Ophthalmol. 2013;41(4):348-54*

2. Viswanathan D, Males J. Prospective longitudinal study of corneal collagen crosslinking in progressive keratoconus. Clin Experiment Ophthalmol. 2013;41(6):531-6*

3. Viswanathan D, Goldberg I, Graham SL. Relationship of change in central corneal thickness to visual field progression in eyes with Glaucoma. Graefes Arch Clin Exp Ophthalmol. 2013;251(6):1593-9*

4. Viswanathan D, Kumar NL, Males JJ. Outcome of Corneal Collagen Crosslinking for progressive Keratoconus in paediatric patients. BioMed Research International.2014 http://dx.doi.org/10.1155/2014/140461*

5. Viswanathan D, Kumar NL, Males JJ, Graham SL. Comparative analysis of corneal measurements obtained from a Scheimpflug camera and an integrated Placido-optical coherence tomography device in normal and keratoconic eyes. Acta Ophthalmol. 2014 Dec 14. doi: 10.1111/aos.12622 *

6. Viswanathan D, Kumar NL, Males JJ, Graham SL. Relationship of Structural Characteristics to Biomechanical Profile in Normal, Keratoconic, and Crosslinked Eyes. Cornea. 2015 Jul;34(7):791-6 *

[*] In all of these publications, the contribution of each author is as the following:

Viswanathan D: Study design, subject recruitment, data collection and analysis, manuscript draft

Goldberg I: Academic advisory, subject recruitment, manuscript revision

Graham SL: Study design, Academic advisory, subject recruitment, manuscript revision

Males JJ: Academic advisory, subject recruitment, manuscript revision

Kumar NL: Academic advisory, subject recruitment, manuscript revision

Published abstracts (conference presentations):

1. Viswanathan D, Goldberg I, Graham SL. "Longitudinal effect of topical antiglaucoma medications on central corneal thickness". RANZCO Annual meeting, November 2011, Canberra, Australia.

2. Viswanathan D, Goldberg I, Graham SL. "Relationship of central corneal thickness to visual field progression". 20th international visual field and imaging symposium, January 2012, Melbourne, Australia.

3. Viswanathan D, Goldberg I, Graham SL. "Longitudinal variation in central corneal thickness and its relationship to visual field progression in eyes with glaucoma". ARVO Annual meeting, May 2012, Fort Lauderdale, FL, USA.

4. Viswanathan D, Males J. " Prospective longitudinal outcome of corneal collagen crosslinking in progressive keratoconus". RANZCO Annual meeting, November 2012, Melbourne, Australia.

5. Viswanathan D, Kumar NL, Males JJ, Graham SL. "Comparison of corneal parameters between the Pentacam and Visante OMNI and their relationship to Ocular reponse analyser biomechanical measurements". European Union Cornea society (EU Cornea) meeting, October 2013, Amsterdam, The Netherlands.

6. Viswanathan D, Kumar NL, Males JJ. "Outcome of Corneal Collagen Crosslinking for progressive Keratoconus in paediatric patients". RANZCO Annual meeting, November 2013, Hobart, Australia.

7. Viswanathan D, Kumar NL, Males JJ, Graham SL. Sensitivity and specificity of novel biomechanical waveform parameters in detecting forme fruste keratoconus and keratoconus. EU Cornea, September 2014, London.

8. Viswanathan D, Kumar NL, Males JJ, Graham SL. Relationship of corneal biomechanical profile to structural charecteristics in normal, forme fruste keratoconic, keratoconic and crosslinked eyes. RANZCO Annual meeting, November 2014, Brisbane, Australia.

THESIS SUMMARY

This thesis extensively investigates the relationship of corneal structure and biomechanical profile to the diagnosis and treatment of two ocular disorders: glaucoma and keratoconus. The research papers arising out of these studies have been compiled to form this thesis by publication. The main hypotheses addressed by this PhD thesis include:

1. Baseline central corneal thickness could be a predictive factor of glaucoma progression
2. Ongoing change in corneal thickness may occur in glaucoma subjects and this might be related to glaucoma progression.
3. Altering the dynamic properties of the cornea by collagen crosslinking can affect the progression of disease in keratoconic subjects.

Despite having different mechanisms, both conditions are significant causes of preventable blindness. Glaucoma is more prevelant in the older population whereas keratoconus tends to affect younger subjects. Interestingly, thinner corneas and altered biomechanics are related to glaucoma risk and also characteristic of keratoconus. The aim of the glaucoma studies was to identify longitudinal variation in corneal thickness and its relationship to antiglaucoma medications and visual field progression. This could provide some insight on the clinical relevance of repeat corneal thickness measurements in glaucoma practice. Our findings indicate that corneal thickness reduces slightly over time in eyes on topical prostaglandin therapy and that thinner corneas may be associated with an increased risk of visual field progression.

The keratoconus studies aimed to explore the relationship between corneal structural and biomechanical characteristics, compare corneal imaging devices, analyse the sensitivity and specificity of biomechanical parameters in keratoconus detection and study the outcome of collagen crosslinking in adult and paediatric progressive keratoconic eyes. Knowledge from these studies would allow clinicians to identify and manage keratoconus more efficiently. Our results indicate significant correlations between corneal structural and biomechanical characteristics. We also demonstrated that some commercially available corneal imaging devices are not interchangeable in clinical practice and that newer biomechanical parameters are sensitive in detecting corneal ectasia. Furthermore, we validated the efficacy of the corneal collagen crosslinking procedure in adult and paediatric progressive keratoconic eyes.

THESIS OUTLINE

Chapter 1: Introduction and review of literature

This introductory chapter comprises a broad overview of the corneal structural and biomechanical charecteristics with emphasis on two ocular disorders: Glaucoma and keratoconus. As an initial background, the anatomy and physiology of the human cornea are explained. The various diagnostic tests used to assess corneal structural and biomechanical parameters are also mentioned. Thereafter, the clinical features, diagnosis and treatment options of glaucoma and keratoconus are described in detail with reference to previous research studies conducted on these disorders.

Chapter 2: Materials and methods

This chapter describes in detail, the conduct of clinical studies including operational techniques of each of the devices used. The statistical analyses employed for various studies are also mentioned.

Chapter 3: Longitudinal effect of topical antiglaucoma medications on central corneal thickness

This chapter is a study that was performed to assess the longitudinal variation in central corneal thickness. Glaucomatous eyes on long-term topical antiglaucoma medications were compared to a group of control glaucoma suspect eyes that were not on any topical treatment. Results have been discussed in detail in comparison to previous studies.

Chapter 4: Relationship of change in central corneal thickness to visual field progression in eyes with glaucoma

This study is a logical sequence to the previous study and analyses whether baseline central corneal thickness or change in corneal thickness functions as a risk factor for visual field progression in eyes with glaucoma.

Chapter 5: Relationship of biomechanical profile to structural characteristics in normal, forme fruste keratoconic, keratoconic and crosslinked eyes

This chapter describes in detail the influence of corneal structural characteristics on biomechanical behaviour in normal, forme fruste keratoconic, keratoconic and crosslinked keratoconic eyes. Results of this study have been compared to previous studies and discussed in detail.

Chapter 6: Comparative analysis of corneal measurements obtained from a scheimpflug camera and an integrated placido-optical coherence tomography device in normal and keratoconic eyes

This study was performed to compare corneal measurements in normal and keratoconic between two commercially available imaging devices that use different technologies. Furthermore, the intra-operator repeatability and inter-operator reproducibility for both devices were assessed. Results of this study have been discussed in detail in this chapter.

Chapter 7: Sensitivity and specificity of novel biomechanical waveform parameters in detecting forme fruste keratoconus and keratoconus

This chapter studies the accuracy of newer biomechanical waveform parameters in diagnosing Forme fruste and manifest keratoconus and determines whether these are superior to conventional Ocular response analyser parameters. There are very few studies conducted on this topic and they have been discussed.

Chapter 8 : Prospective longitudinal study of corneal collagen cross-linking in progressive keratoconus

This chapter is a prospective study that analyses the long-term outcome of the corneal collagen crosslinking procedure in progressive keratoconic eyes and compares treated eyes to untreated fellow keratoconic eyes.

Chapter 9 : Outcome of corneal collagen crosslinking for progressive keratoconus in paediatric patients

This chapter studies in detail the effect of corneal collagen crosslinking procedure in paediatric patients with progressive keratoconus and compares results with previous case reports.

Chapter 10 :

10 A: Discussion and summary

An overarching discussion that outlines the evolution of this PhD thesis and amalgamates the findings of the various studies conducted as part of this research project.

10 B: Conclusions and future directions

The results of the studies conducted as part of this thesis have been summarised in this chapter. Future perspectives for research studies have also been discussed.

LIST OF ABBREVIATIONS

1. AA	Alpha agonists
2. ANOVA	Analysis of Variance
3. AUC	Area under curve
4. BB	Beta blockers
5. CAI	Carbonic anhydrase inhibitors
6. CCT	Central corneal thickness
7. CH	Corneal Hysteresis
8. CI	Confidence interval
9. CRF	Corneal Resistance Factor
10. CXL	Collagen crosslinking
11. D	Dioptres
12. EGPS	European Glaucoma Prevention Study
13. FFKC	Forme Fruste Keratoconus
14. GAT	Goldmann applanation tonometry
15. GPA	Guided Progression Analysis
16. HFA	Humphrey Field Analyser
17. HRT	Heidelberg Retina Tomograph
18. ICC	Intra Class Coefficient
19. IOP	Intra Ocular Pressure
20. IOP_{CC}	Intra Ocular Pressure Corneal-compensated
21. IOP_G	Intra Ocular Pressure Goldmann-correlated
22. ISV	Index of Surface Variance
23. IHA	Index of Height Asymmetry

24. IRB	Institutional Review Board
25. KC	Keratoconus
26. KMI	Keratoconus Match index
27. KMP	Keratoconus match probability
28. MD	Mean Deviation
29. MMPs	Matrix Metalloproteases
30. NTG	Normal tension glaucoma
31. OCT	Optical Coherence Tomography
32. OHTS	Ocular Hypertension Treatment Study
33. ORA	Ocular Response Analyzer
34. PG	Prostaglandins
35. POAG	Primary Open Angle Glaucoma
36. PACG	Primary Angle Closure Glaucoma
37. PSD	Pattern Standard Deviation
38. ROC	Receiver Operating Curve
39. SAP	Standard Automated Perimetry
40. SLT	Selective laser trabeculoplasty
41. SD	Standard Deviation
42. SITA	Swedish Interactive Threshold Algorithm
43. UV	Ultraviolet light
44. UBM	Ultrasound biomicroscopy
45. VFI	Visual Field Index

CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

1.1 ANATOMY OF THE CORNEA

The cornea is the transparent, dome shaped surface that covers the front of the eye. It is the principal ocular refractive surface that provides around two-thirds of the eye's total optical power.[1,2] Corneal transparency is vital for good vision and is the result of many factors including the structural anatomy and physiology of its cellular components. The mean horizontal corneal diameter is between 11.5 to 12.0 mm and exceeds the vertical diameter by approximately 1.0 mm.[3] The cornea is steeper centrally and flatter in the periphery and thus assumes an aspheric shape, the average corneal curvature being about 43.25 Dioptres (D). The cornea is thinnest at the centre (500-600 μm) and thicker towards the periphery (600–800 μm).

HISTOLOGY OF THE CORNEA

The cornea consists of six layers and is mainly composed of collagen. (Figure 1) [4]

a) Epithelium

The corneal epithelium is composed of about 4 to 6 layers of non-keratinized, stratified squamous epithelium resting on a basement membrane.[5] The epithelial layer is fast growing and easily regenerates and is covered by a tear film that provides nourishment.[6] The primary functions of the epithelium are to act as a protective outer barrier and to provide a smooth optical surface that is critical to a good quality of vision.

b) Bowman's membrane

Bowman's layer is a thin but tough layer of irregularly arranged type 1 collagen fibers that protects the stroma. Bowman layer is not a true membrane but an acellular condensate of the

most anterior portion of the stroma. Unlike corneal epithelium, it will not regenerate and can form a scar if breached.[1,7]

c) Stroma

The stroma constitutes around 85 – 90 % of the corneal thickness and therefore is the predominant structural and optical component of the cornea. The stroma is made up of type I and type V collagen fibrils embedded in an extracellular proteoglycan matrix. Proteoglycans are macromolecules composed of a protein core with covalently linked glycosaminoglycan side chains consisting of dermatan sulfate, keratan sulfate and heparan sulfate components.[8] The precise organization of the stromal collagen fibrils is one of the key factors responsible for corneal transparency.[9-11] The collagen fibrils are arranged into bundles called lamellae. The fibrils in each lamella are organised in a parallel manner to each other except in areas where the lamellae diverge.[12]

The microscopic arrangement of the stromal lamellae also influences the corneal biomechanical charectersistics.[13,14] The lamellae vary considerably, with anterior lamellae (0.5-30 μm wide and 0.2-1.2 μm thick) being smaller as compared to posterior lamellae (100-200 μm wide and 1.0-2.5 μm thick). The anterior lamellae originate at the limbus and pass obliquely, often interweaving with a certain proportion terminating directly in the Bowman's membrane. [15-18] The posterior lamellae are more organised and run in an orthogonal direction from limbus to limbus along the superior-inferior or nasal-temporal meridians.[19,20]

The corneo–scleral junction is a transition zone where the lamellae interweave in a circumferential manner to form a ligamentum circulare corneae.[21,22] The increased corneal

thickness and higher tensile strength at the limbus is attributed to this circumferential band of collagen.[23,24]

Keratocytes are specialised fibroblasts and the principal cell type residing in the corneal stroma. They are responsible for synthesising collagen molecules, glycosaminoglycans and play a crucial role in maintaining the corneal structure and function. [25,26]

d) Dua's layer

This is a newly described, acellular layer in the pre-Descemet's cornea consisting of five to eight lamellae (6-15 μm thick) of type 1 collagen bundles that are arranged in transverse, longitudinal and oblique directions.[27]

e) Descemet's membrane

Descemet's membrane is the basement membrane that lies in between the corneal stroma and the endothelium. It is a homogeneous acellular layer secreted by the corneal endothelium and is composed of type IV and type VIII collagen.[28,29] Thickness ranges from around 3 μm at birth to 8-10 μm in adults.[2]

f) Endothelium

The corneal endothelium is a monolayer (4-6 μm thick) of cells arranged in a hexagonal mosaic (Figure 2). [30] Corneal endothelial cells adhere to the Descemet's membrane through hemidesmosomes on their basal surface. These cells are conjoined at their lateral borders with incomplete gap and tight junctions.[31,32] The endothelial cell density declines from 3000 to 4000 cells/mm2 at the second decade to around 2600 cells/mm2 by the eighth decade of life. [33]The main function of this layer is to control corneal hydration and to facilitate nutrition and this is performed by the apical gap junctions.[34]The corneal endothelium does not readily

regenerate and cell loss is compensated for by polymegathism and pleomorphism of the surrounding cells.[35]

Figure 1: Corneal structure [4]

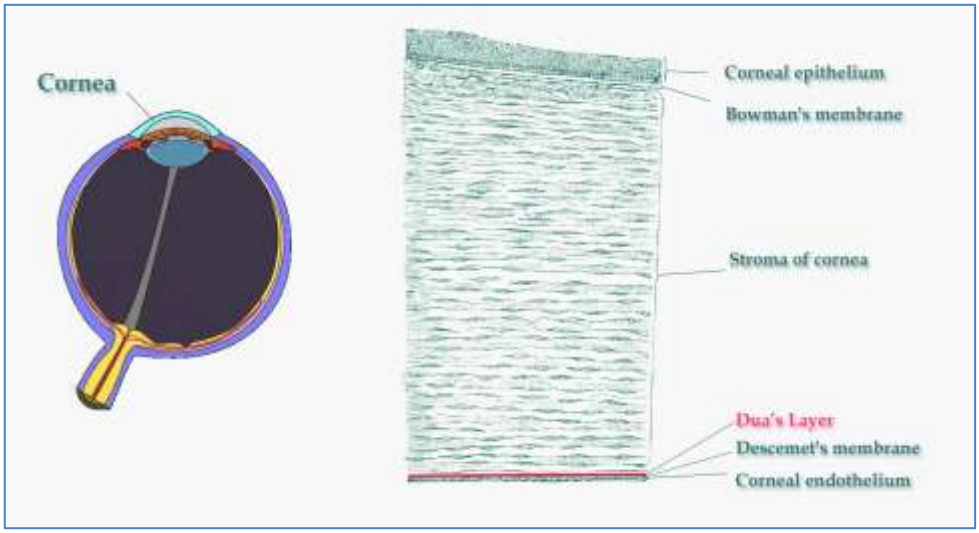
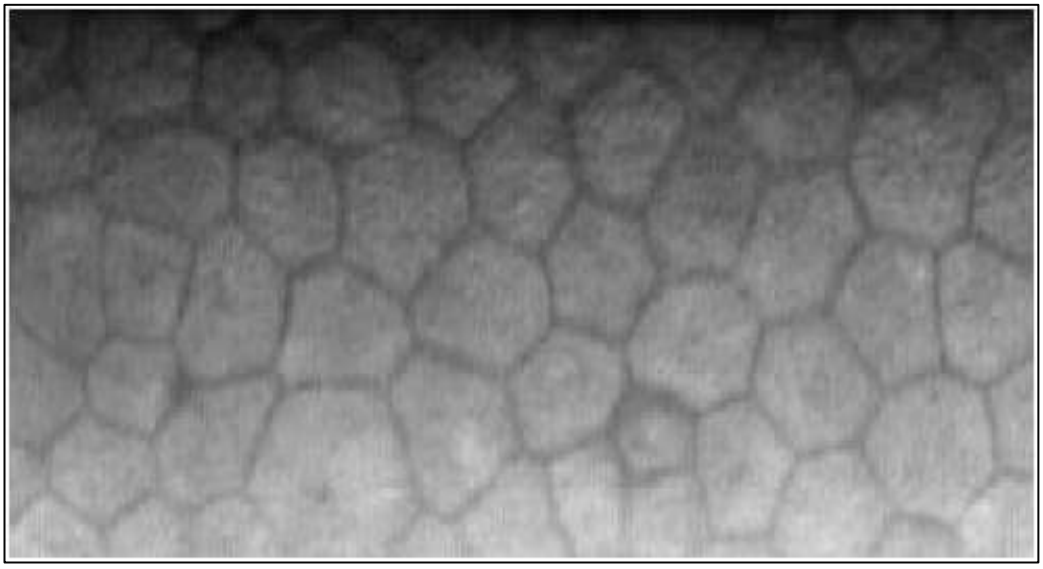


Figure 2 – Corneal endothelium on specular microscopy [30]



1.2 CORNEAL PARAMETERS

The most significant corneal parameters in Ophthalmology practice include thickness, curvature, elevation, transparency and biomechanical properties.

1.2.1 CORNEAL THICKNESS

The central corneal thickness (CCT) is a widely used diagnostic parameter for risk profiling in ocular hypertension and glaucoma and for planning refractive surgery.[36,37]Data on CCT measurements has already been evaluated in several populations. Doughty and Zaman performed a meta-analysis of corneal thickness literature and found that the mean CCT in normal white adults was 535 μm.[38] Several factors can affect CCT in the general population, such as age, gender, environmental and genetic factors, and race. The Ocular Hypertension Treatment Study (OHTS) demonstrated a correlation between greater mean CCT and younger age and female gender. [37] In contrast, other population-based studies found a correlation between higher CCT and male gender and older age. [39,40] These differences may be due to the selection bias in the OHTS study and may reflect sampling errors or racial differences in CCT values in the other population-based studies.

Intraocular pressure (IOP) readings are known to be influenced by CCT. There is evidence that IOP may be underestimated in patients with thinner and overestimated in patients with thicker corneas. [41] In ocular hypertensive patients, CCT is presumed to be a powerful predictor of glaucoma development as eyes with CCT of 555 microns or less showed a greater risk of developing glaucoma than those with a CCT of more than 588 microns.[36] Corneal thickness measurements are critically important in the preoperative assessment of candidates undergoing refractive surgery, lamellar corneal surgery and procedures like corneal collagen crosslinking. An overestimation of CCT would render these procedures unsafe and increase

the risk of developing complications including corneal ectasia. An under-estimation of corneal thickness measurements may unnecessarily exclude eligible patients from undergoing surgery.

1.2.2 CORNEAL CURVATURE

For the normal population, the average radius of curvature of the anterior corneal surface is 7.8 mm (43.25D) and the range is from 6.75 mm (36.0D) to 9.25 mm (50.0D). [42] The anterior corneal surface acts like a convex lens and hence the size of the image formed by it is determined by its curvature. A steeply curved cornea will produce a smaller image, while a flatter cornea will produce a larger image of the same object. The corneal curvature changes gradually, becoming flatter as it approaches the limbus.

Most previous studies have found the anterior corneal radius of curvature decreases with age with either similar decreases of horizontal and vertical meridians [43], more decrease in the horizontal meridian [44] or decrease in the horizontal meridian only. [45] Corneal curvature values are essential for the evaluation of ocular disorders including keratoconus, for contact lens fitting and for planning ocular procedures including cataract and refractive surgery.

1.2.3 CORNEAL ELEVATION

Similar to terrain topography, where surface elevation is studied in reference to sea level, corneal surface elevation is measured from a reference, however, the reference is not fixed. The shape of the normal cornea is believed to be closer to a toric ellipsoid shape than a sphere, and thus using a “best fit toric ellipsoid” as the reference surface is recommended for measuring height data.[46] A careful study of anterior and posterior elevation patterns helps to understand the corneal shape and detect corneal shape irregularities like keratoconus. Normal

ranges and cut-points for elevation values differs between imaging devices. For anterior surface elevations, central readings less than 10-12 µm are considered normal, values greater than 15 µm could be indicative of keratoconus, and those in between fall in the grey zone. Cut-points for the posterior surface elevation are about 2-5 µm higher than the anterior surface elevation values.[47]

1.2.4 CORNEAL BIOMECHANICS

The cornea is endowed with a unique combination of mechanical stiffness and optical transparency that enables it to function both as a protective layer and as the principal refractive component of the eye. The corneal shape and curvature are influenced by its inherent biomechanical structure. The structural composition of the cornea gives it viscoelastic properties, meaning it exhibits elements of both elasticity and viscosity. The term 'Hysteresis' describes the biomechanical response of a viscoelastic material to stress and is a measure of the energy dissipated by the material.[48]A lower hysteresis value characterizes an eye wall that quickly returns to its original position after deformation and a higher value implies a greater corneal viscous damping.[49] The corneal hysteresis (CH) has been known to be influenced by the corneal thickness being higher in thicker corneas possibly due to the presence of increased collagen and other tissue components.[50]

Knowledge of the cornea's biomechanical profile is valuable for the diagnosis of certain ocular disorders, [51 -53] for screening prior to refractive surgery [54] and for predicting treatment outcomes.[55] Corneal biomechanical properties are known to influence intraocular pressure measurements[56,57] and may reflect globe biomechanics and therefore be indicative of the possibility of developing glaucomatous damage. [50,58,59] Clinical studies have demonstrated variations in corneal biomechanical properties across the spectrum of glaucoma with lower

CH values in primary open angle glaucoma (POAG), normal tension glaucoma (NTG) and pseudoexfoliation glaucoma as compared to normal eyes.[60 -62] Researchers have also reported a lower corneal hysteresis in corneal disorders including keratoconus, Fuch's dystrophy and post- refractive surgery.[63-69]

1.3 MEASUREMENT OF CORNEAL PARAMETERS

The precise measurement of corneal parameters including thickness, curvature and biomechanics is vital for both diagnostic assessment and treatment of several ocular conditions.

1.3.1 THICKNESS, CURVATURE AND ELEVATIONS

a) Ultrasound Pachymetry

Ultrasound pachymetry is currently considered the gold standard for corneal thickness measurement (figure 3).[70] Previous studies have reported a high degree of repeatability and reproducibility. [71 -74] Miglior demonstrated highly reproducible CCT measurements with intraclass correlation coefficient (ICC) ranges of the intra- and interobserver evaluations being 0.95-0.97 and 0.89-0.95 respectively.[71] However, being a contact technique, it is associated with certain inherent disadvantages. These include the use of a topical anaesthetic which could alter the corneal thickness and non perpendicular probe placement resulting in off axis measurements.[73,75,76] There are also concerns about the possibility of patient discomfort, epithelial damage and infection with contact methods. Furthermore, changes in tissue hydration could affect the ultrasound speed through the cornea and influence readings.[77]

Currently, various non-contact technologies are available that allow assessment of corneal thickness including scheimpflug imaging**,** optical coherence tomography (OCT), specular microscopy and scanning slit technology. [78,79] Statistically and clinically significant

differences in central corneal thicknesss (CCT) values between different systems have been shown in earlier studies. [80-82] This is possibly due to the variation in measurement techniques used by different devices.

Figure 3: Ultrasound pachymetry

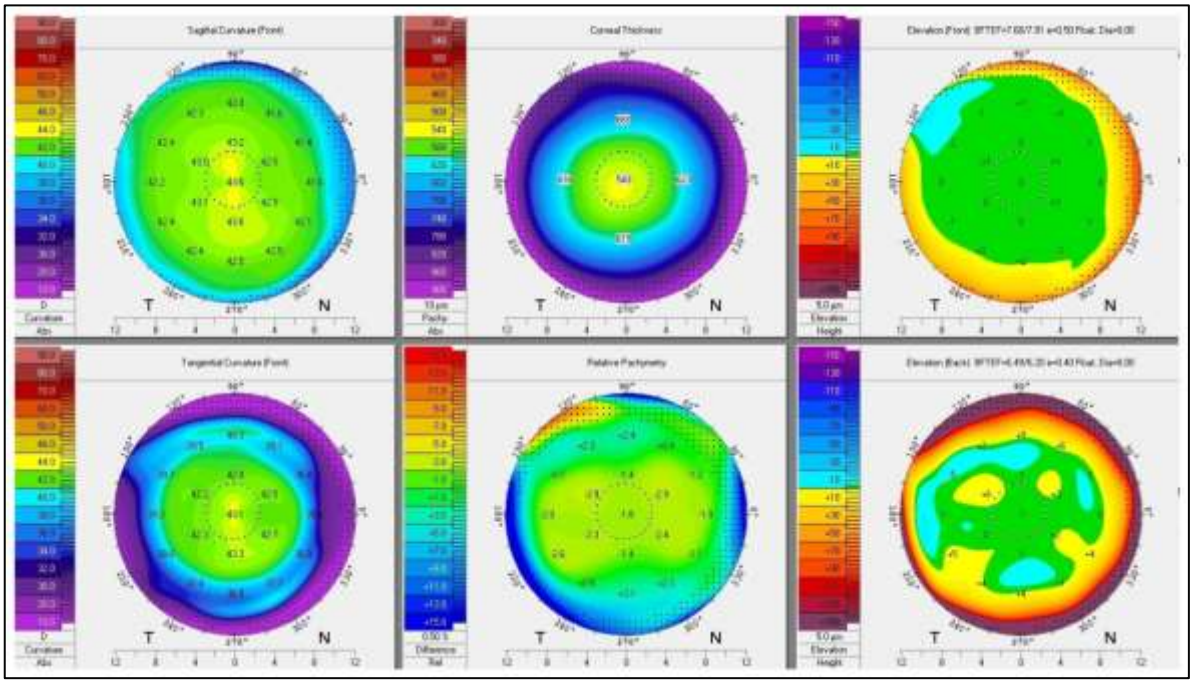
b) Scheimpflug imaging (Pentacam)

The Pentacam (Oculus Inc, Wetzlar, Germany) is a recently developed non-contact imaging device that acquires a three-dimensional scan of the anterior segment (Figure 4).[83] It uses uses a Scheimpflug camera and a monochromatic 475-nm blue light source that rotate together 180° around the optical axis of the eye. The camera takes multiple slit images in less than 2 seconds, capturing 25,000 different elevation points during the scan. A software system is then used to construct a three-dimensional image of the anterior segment. It is a fast and reliable method of evaluating the anterior and posterior corneal curvature, corneal thickness, corneal elevations, anterior chamber depth, angle, and lens density.

Pentacam corneal thickness measurements have been found to be comparable with ultrasound pachymetry, the current gold standard technique for CCT measurements. [84] Recently the Holladay report was introduced in the Pentacam software (Figure 5). This is a comprehensive report comprising the anterior and posterior corneal elevations, curvatures and corneal thickness measurements. Several studies have reported a high degree of repeatability for corneal curvature, thickness and anterior chamber depth measurements in normal and keratoconic corneas.[85-89]

Figure 4: The Oculus Pentacam [83]

Figure 5: Holladay report generated from the Pentacam shows corneal curvature, central corneal thickness, relative pachymetry, anterior and posterior elevations



c) Combined Placido - Ocular Coherence Tomography (Visante OMNI)

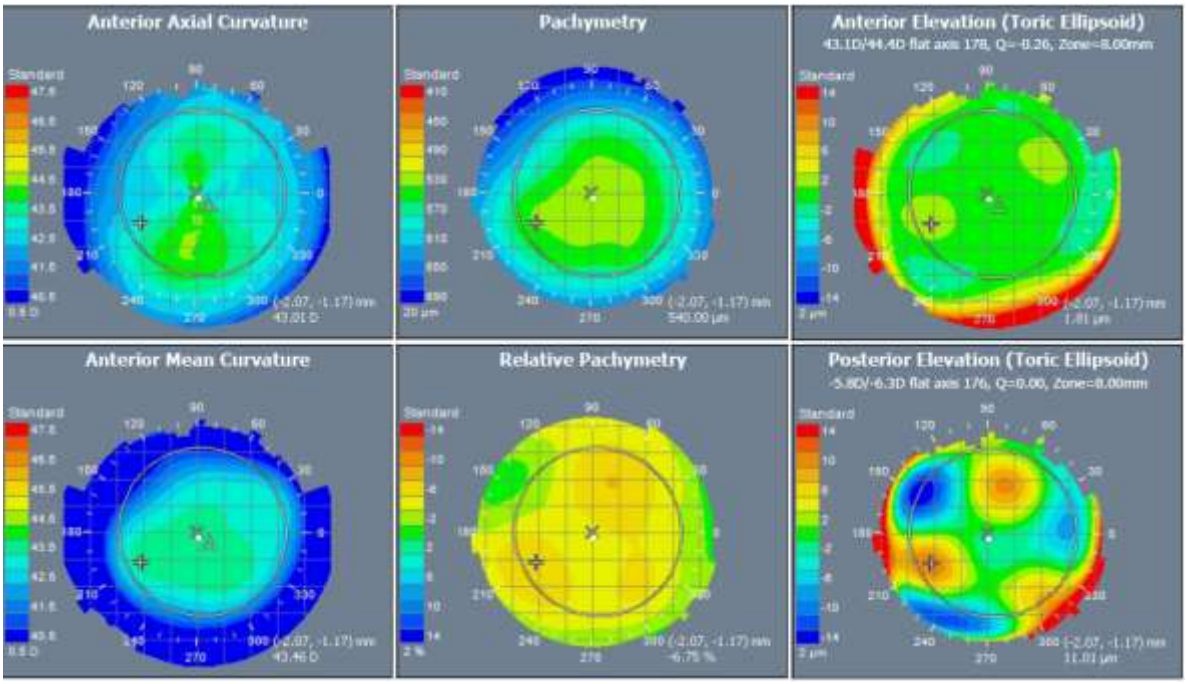
Optical coherence tomography (OCT) is another established noncontact technology that images the anterior segment and provides reliable measurements (figure 6).[90,91] The Visante OMNI (Carl Zeiss Meditec, Jena, Germany) is a novel corneal imaging device. It is a hybrid created by linking the Placido based topographer (Atlas) to an anterior segment optical coherence tomography device (Visante AS-OCT).

The Atlas corneal topographer is based on the Placido principle whereby the anterior corneal surface is illuminated by a series of concentric rings that create a reflected image.[92] Computer analysis of this image generates a detailed corneal curvature color map that is very sensitive in detecting any deviations of the corneal contour. The Visante AS-OCT is a computerized instrument that uses low coherence interferometry to compare the delay of light reflections across tissues against a reference reflection. It provides high-quality cross-sectional corneal images with 17 μm of axial resolution and also provides measurements of corneal thickness, anterior chamber depth and angle. [93] Visante AS-OCT is a time-domain system with a longer wavelength (1310 nm) that penetrates deeply through the sclera and iris; [94] and is able to show all anterior segment structures in a single image.

The Visante OMNI generates posterior corneal elevation data by integrating Atlas corneal topography data with the OCT corneal thickness data. The OMNI also generates a comprehensive Holladay report by integrating the data from the Atlas with the tomography pachymetry data from the OCT (figure 7). A recent study demonstrated that the Visante OMNI generates highly repeatable and reproducible posterior corneal topography measurements. [95]

Figure 6: The Visante OMNI [91]

Figure 7: Holladay report generated from the Visante OMNI shows corneal curvature, central corneal thickness, relative pachymetry, anterior and posterior elevations



1.3.2 BIOMECHANICS

The Ocular response Analyser

The Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Buffalo, NY, USA) is a commercially available medical device that provides in vivo measurements of corneal biomechanical properties (Figure 8).[96] The ORA is an air-puff tonometer that uses bidirectional applanation and measures the central corneal response to indentation by a rapid jet of air.[97] The changes in corneal shape during inward and outward deviation is tracked using an infrared beam and the resulting waveform (Figure 9) [98] allows the assessment of biomechanical parameters that include:

1. **Corneal Hysteresis (CH)**: CH is thought to predominantly reflect the viscoelastic dampening effect of the corneal tissue and has a low correlation with central corneal thickness (CCT).[99-101] CH represents a tissue property that provides more comprehensive information about ocular biomechanics, as measured via the cornea. CH is calculated as the difference between the force-in applanation (P1) and the force-out applanation (P2).

2. **Corneal Resistance Factor (CRF)** : CRF is an indicator of overall corneal resistance that is relatively independent of IOP. CRF is obtained using the formula (P1 − k P2). The constant k was developed through empirical evaluation of the relationship between P1 and P2 and CCT. [97, 99,100]

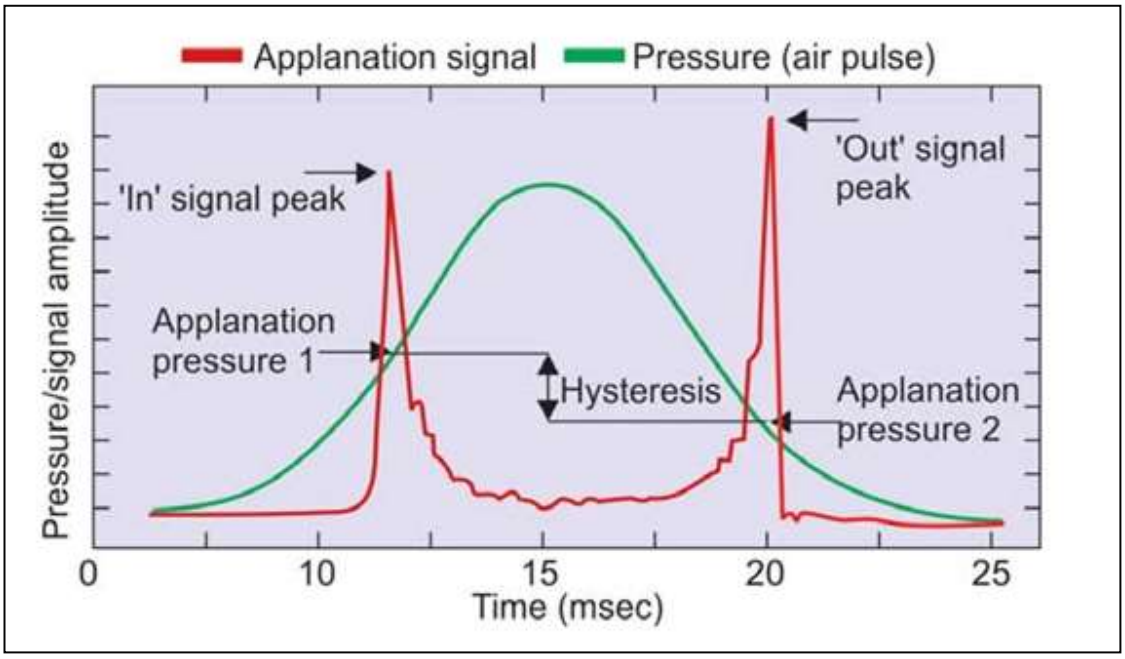
3. **Goldmann-correlated IOP (IOP_G)**: This value is the mean of the biphasic pressure measurements. [97, 99,100]

4. **Corneal-compensated IOP (IOP_{CC})**: This value is a more accurate indicator of true IOP than the IOP_G because it is less affected by corneal properties such as CCT and CH. [97, 99,100]

5. **Waveform signal**: The biomechanical waveform produced by the corneal deformation signal provides a unique description of each eye. Newer versions of the ORA have software available that allows detailed analysis of the deformation signal waveform by providing 37 parameters, each describing a morphological feature of the waveform. The clinical relevance of these waveform parameters has been investigated and the evidence suggests that they are potentially valuable in keratoconus diagnosis. [65,102]

Figure 8: The Ocular Response Analyser [96]

Figure 9: The biomechanical waveform obtained from the Ocular Response Analyser [98]



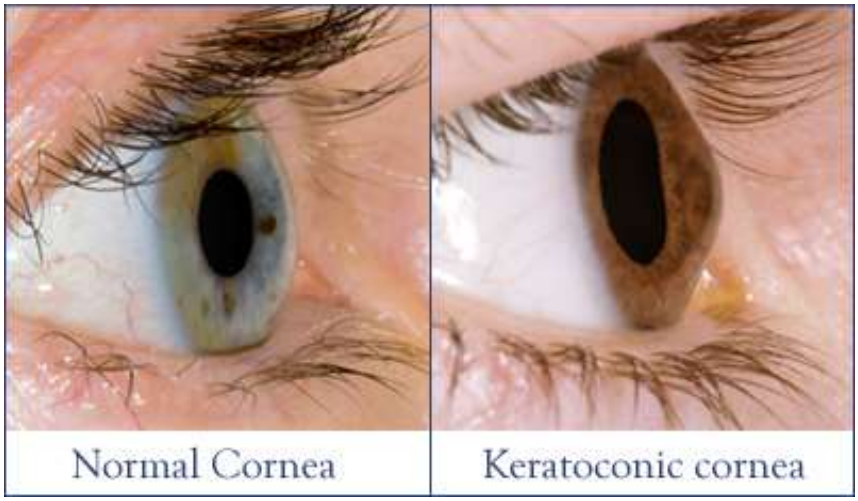
1.4 OCULAR DISEASE RELATED TO CORNEAL THICKNESS AND BIOMECHANICS

1.4.1 Keratoconus

Keratoconus is an ectatic corneal disorder characterized by progressive corneal thinning that results in corneal protrusion, irregular astigmatism and decreased vision (Figure 10).[103] Degeneration of the corneal stromal tissue and the resultant biomechanical alteration causes the cornea to assume a conical shape. The reported incidence of keratoconus varies between 50 and 230 per 100 000 in the general population (approximately 1 per 2000). Classically, keratoconus has its onset at puberty and progresses until the third to fourth decade of life when it usually arrests, however some cases progress beyond the fourth decade.[104]

The progressive alteration of the keratoconic corneal shape may be caused from elastic deformation. Risk factors for keratoconus include constant eye rubbing, floppy eyelid syndrome, ocular or systemic allergy, atopy, eczema as well as family history. [68,105]

Figure 10: Classical conical protrusion seen in keratoconus [103]



Keratoconic corneas have been shown to possess altered collagen fibril orientation and reduced lamellar interweaving particularly around the presumed apex of the cone.[106-108] More recently, it was further shown that the collagen fibrillar mass is unevenly distributed leading to thinning of the central cornea and associated changes in corneal curvature in keratoconus.[109]

Matrix metalloproteases (MMPs) are a group of proteolytic enzymes that are capable of degrading the main components of the corneal extracellular matrix and membranes and causing a reduced stromal collagen content. Due to these actions, MMPs have often been implicated to have a pivotal role in the pathogenesis of keratoconus progression. Research studies have described MMP and tissue inhibitor of matrix metalloproteinase (TIMP) changes in every layer of the keratoconic cornea. Upregulation of MMP-1, MMP-2, MMP-9, MMP-13 and MMP-14 subtypes in KC have been described by some groups whereas others report no change in MMP levels or otherwise, a downregulation of MMP-8 or TIMP-1was noted.[110] Increased activity of other proteinases such as cathepsins might also contribute to the structural deterioration seen in KC.[111]

These factors may result in impaired biomechanics with keratoconic corneas being more elastic and less rigid than normal eyes. [112] Keratoconus has a wide spectrum of disease severity and clinical onset. The early preclinical forms are usually termed ‘‘subclinical’’ or ‘‘forme fruste’’ keratoconus (FFKC). Unfortunately, there is no universally accepted or internationally recognised definition of FFKC eyes. It has already been reported that approximately 50% of clinically normal fellow eyes of patients with a unilateral KC progress to KC within 16 years.[113] Based on these findings, previous published studies have defined FFKC eyes as fellow eyes in a subject with clinically manifest keratoconus in the other eye.[65,

[114] Other studies have used artificial intelligence techniques and computer neural networks incorporated in corneal topography devices to recognize FFKC eyes.[52] Many studies have identified statistically lower CH and CRF values for Keratoconic and FFKC eyes compared with normal eyes.[53,55, 66,67,72,115-117]

a. Diagnosis

Corneal topography is valuable for confirming keratoconus diagnosis along with retinoscopic, biomicroscopic and pachymetric evaluation. The commonly used anterior segment imaging modalities for detecting keratoconus include Placido disk corneal topography and tomography using either scanning slit low contrast interferometry or scheimpflug techniques (Figure 11). [118-123] Placido disk based computer videokeratoscopes have the combined features of a keratometer and a photokeratoscope. Therefore they are sensitive means of detecting subtle topographic changes in early keratoconus and for documenting serial changes in central and peripheral corneal curvature over time. [124-128] Classic Placido-based topographic findings in keratoconus include corneal steepening and protrusion, which usually occur inferior to the visual axis.[122,123]

Several studies that evaluated the reproducibility and repeatability of Scheimpflug imaging have demonstrated reliable corneal measurements in eyes with keratoconus.[120,121]
More recent studies based on the elevation data obtained by scanning-slit topography and Scheimpflug measurements indicate that deformation occurs in both anterior and posterior corneal surfaces in eyes with keratoconus.[129-131] Posterior corneal elevations at the thinnest point are considered to be particularly sensitive in detecting keratoconus. [132] In addition, the Pentacam provides a series of specific anterior surface irregularity topometric indices that

have been developed for the grading and classification of keratoconus.[133-139] These include the following:

1. Index of surface variance (ISV): The unitless standard deviation of individual corneal sagittal radii from the mean curvature and thus an expression of the corneal surface irregularity. It is elevated in all types of corneal surface irregularity (eg, scars, astigmatism, deformities caused by contact lenses, pachymetry). According to the manufacturer's user manual, an ISV greater than 37 is considered abnormal (marked with yellow) and greater than 41is pathological (marked with red).
2. Index of vertical asymmetry (IVA): The measure (expressed in mm) of the mean difference between superior and inferior corneal curvatures. IVA is thus the value of curvature symmetry, with respect to the horizontal meridian as the axis of reflection. An IVA greater than 0.28 is considered abnormal, and greater than 0.32 is considered pathological.
3. Keratoconus index (KI): A unitless index is expressing the ratio between mean radius values in the upper and lower segment (r sagittal superior to r sagittal inferior). A KI value greater than 1.07 is considered abnormal/ pathological.
4. Central keratoconus index (CKI): The ratio between mean radius values in a peripheral ring divided by a central ring: r sag (mean peripheral) to r sag mean center (no units). CKI increases with the severity of central keratoconus. A CKI value greater than 1.03 is considered abnormal/ pathological.
5. Index of height asymmetry (IHA): The mean difference between superior height value minus inferior height value with horizontal meridian as mirror axis (expressed in μm). IHA is calculated by the height data symmetry comparison of the superior and inferior area, and provides the degree of symmetry of height data with respect to the horizontal meridian as the axis of reflection. IHA is similar to the IVA but based on corneal elevation, and is thus more

sensitive. An IHA value greater than 19 is considered abnormal, and greater than 21 is pathological.

6. Index of height decentration (IHD): The value of the decentration of elevation data in the vertical direction (expressed in μm), and is calculated from a Fourier analysis. IHD provides the degree of decentration in the vertical direction, calculated on a ring with radius 3 mm. An IHD value greater than 0.014 is considered abnormal, and greater than 0.016 is pathological.

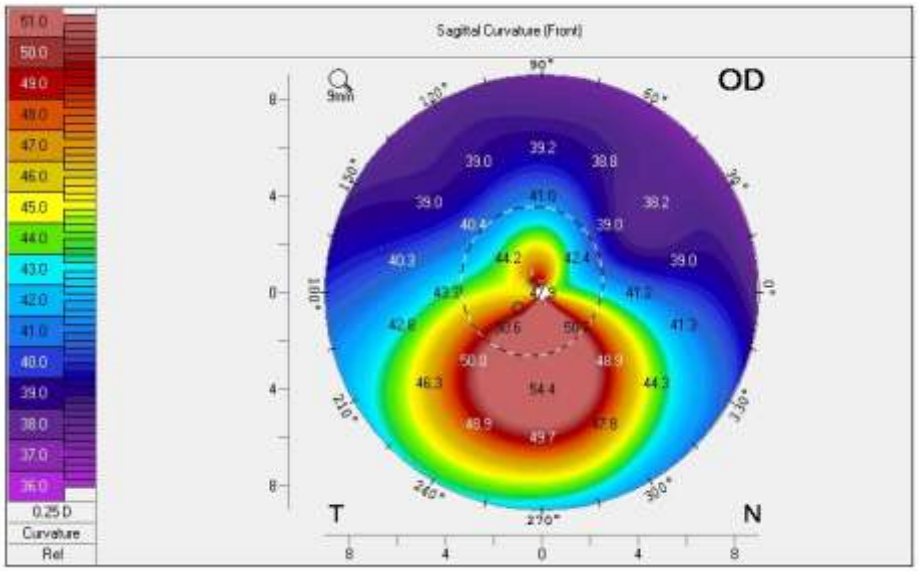
7. Minimum radius of curvature (Rmin): It is a measurement of the smallest radius of sagittal corneal curvature (ie, the maximum steepness of the cone) expressed in mm. Values of Rmin less than 6.71 mm are considered abnormal, considering that the average radius of the anterior corneal surface is 7.87 ± 0.27 mm.

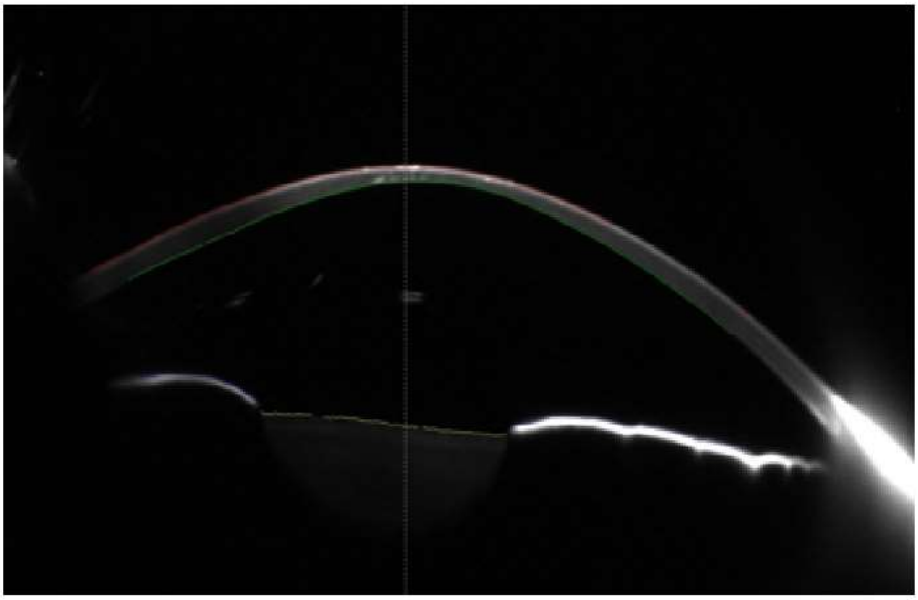
Among these indices, a recent study reported that the index of surface variance (ISV) and the index of height asymmetry (IHA) may be the most sensitive and specific criteria for evaluating early diagnosis and possible progression in keratoconic eyes.[139] Using advanced topographic and tomographic imaging, detection of moderate and advanced keratoconus is not difficult, however FFKC cases could still be missed.

Biomechanical changes may precede topographic changes and may be valuable in detecting these early cases. Analysis of the ORA air pressure curve and infrared signal provides a qualitative analysis of corneal biomechanical properties and seems to be helpful in screening for FFKC.[54] The latest update of the ORA software (version 3.00) provides new waveform parameters and also provides keratoconus-specific parameters. These include the Keratoconus Match index (KMI) or the keratoconus score and the Keratoconus match probability (KMP).[140, 141] KMI compares the similarity of the individual eye's waveform to average

waveform scores of keratoconic eyes in the ORA database. KMI is the outcome of a calculation of 7 waveform scores representing the resemblance of the waveform in the examined eye against the same waveform scores in ectatic eyes in the instrument records. KMP is also calculated based on the same 7 waveform parameters and examines how a certain cornea matches the reference population data. KMP estimates the probability that the scanned eye belongs to one of the following clinically classified populations: Normal, suspect KC, mild KC, moderate KC or severe KC.

Figure 11: Pentacam image showing charecteristic topographic (asymmetric bow tie pattern of inferior steepening) and tomographic (corneal steepening, thinning and apical scarring) features of keratoconus





b. Treatment

Corneal protrusion and thinning in keratoconus results in optical aberrations and distorted vision.[142,143] Mild non-progressive cases can be corrected with spectacles or rigid contact lenses to achieve good functional vision. However, keratoconus tends to progress in 10% to 20% of cases over the second to fifth decades of life. This can lead to contact lens intolerance and the need for corneal transplantation.[144,145] New treatments for keratoconus include intrastromal corneal ring segment implantation,[146-148] conductive keratoplasty [149] and corneal collagen crosslinking (CXL). [150-153] Amongst these, CXL is the only option that aims to arrest keratoconus progression.

Collagen crosslinking

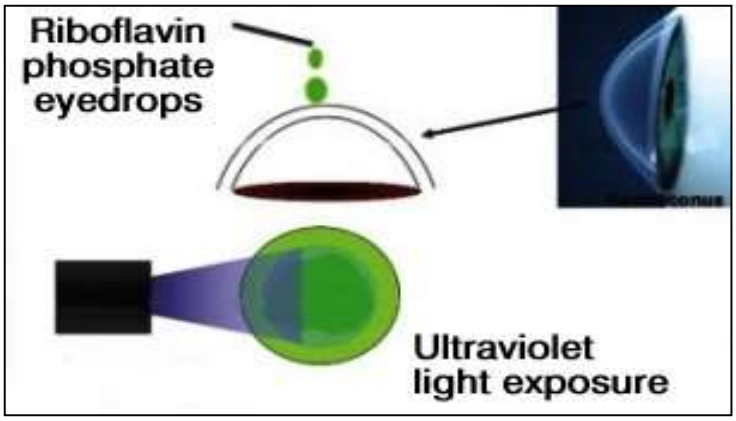
CXL is a day procedure that involves exposing the cornea to the photosensitizer Riboflavin (vitamin B2) and longer wavelength ultraviolet light (370 nm UVA, 3 mW/cm^2). This induces photochemical reactions in the corneal stroma that result in the formation of covalent bonds between the collagen molecules, fibers and microfibrils. In Vitro research studies on porcine, rabbit and human corneas have demonstrated that CXL increases corneal stiffening by more than 300% and increases collagen fiber diameter by 12.2%. [154] Therefore, CXL effectively strengthens the cornea by improving the biomechanical rigidity. [155-157]

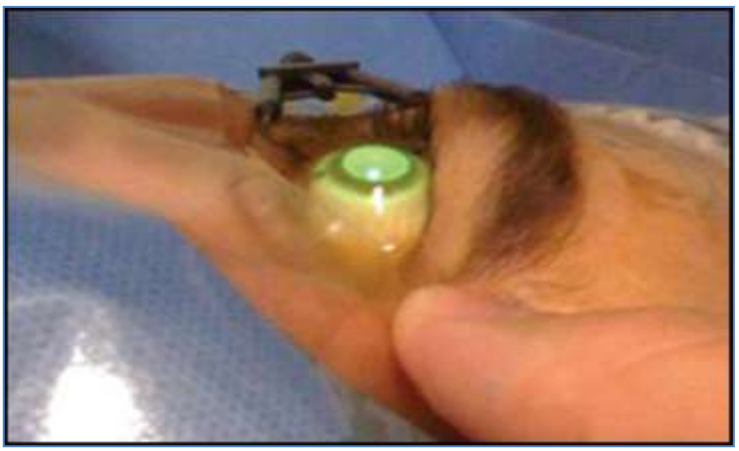
CXL is usually performed according to the traditional or modified Dresden protocol under topical anaesthesia in older patients and rarely under general anaesthesia in very young or uncooperative patients [158]After anaesthesia, a lid speculum is inserted and epithelial tissue is removed to allow penetration of Riboflavin into the corneal stroma (Figure 12).[159] Subsequently, 0.1% riboflavin is applied (2 to 3 drops every 3 minutes) for 30 minutes before irradiation for sufficient saturation of the stroma. The central cornea (8.0 mm diameter) is

then exposed to UVA light with a wavelength of 370 nm and an irradiance of 3 mW/cm2 for 30 minutes. Throughout the UVA exposure, Riboflavin solution is instilled (2 to 3 drops every 3 minutes). Upon completion of treatment, the eye is washed with balanced salt solution and antibiotic eye drops and steroid eye drops are applied. A bandage contact lens is placed in the eye until complete reepithelialisation.

Several clinical studies have demonstrated that CXL is associated with visual improvement and reduction in corneal steepening and is safe and effective in treating progressive keratoconus. [160- 165] Recently trans-epithelial CXL has been described without epithelial debridement and satisfactory results have been reported. [166,167] In addition, accelerated CXL has been developed to shorten the crosslinking procedure time and minimise patient discomfort. This involves delivering an equivalent energy dose while ensuring a proportional biological effect by setting different UV-A powers and exposure times.[168,169]

Figure 12 – Corneal collagen crosslinking [159]



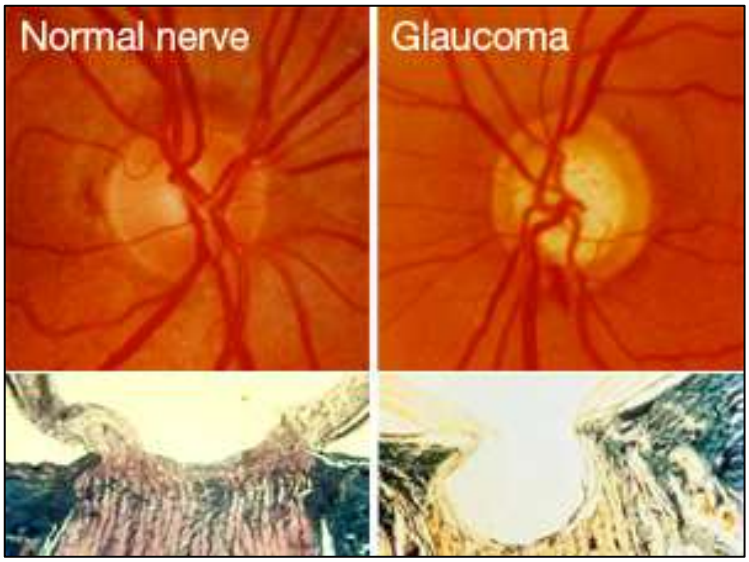


1.4.2 Glaucoma

"Glaucoma" is a term describing a group of ocular disorders with multi-factorial etiology clinically characterized by an IOP-associated optic neuropathy, retinal nerve fiber layer loss and corresponding irreversible visual field loss. [170,171] Glaucoma is the second-leading cause of blindness worldwide after cataracts and affects 1 in 200 people aged 50 and younger, and 1 in 10 people aged 80 and older. The disease is largely asymptomatic with slowly progressive and insidious vision loss that starts peripherally.[172]

Glaucoma could be "primary" or "secondary" depending on the presence or absence of associated ocular pathology contributing to IOP elevation. Primary glaucoma can be broadly classified into two main categories, "open-angle" and "angle- closure" glaucoma according to the manner in which aqueous humour outflow is impaired. Primary open angle glaucoma (POAG) is the most common type of glaucoma prevalent worldwide (figure 13).[173]

Figure 13 – Glaucomatous optic neuropathy [173]



Raised intraocular pressure (above 21 mmHg) is the main and only modifiable risk factor for glaucoma. Other variables associated with visual field (VF) progression in glaucoma include age, gender, race, CCT, optic disc haemorrhage and baseline VF mean deviation.[174] CCT has been reported as a significant predictive risk factor for the development POAG in patients with ocular hypertension (OHT) by the Ocular Hypertension Treatment Study (OHTS) and the European Glaucoma Prevention Study (EGPS). The risk of developing POAG doubled for every 40 μm reduction in CCT from the overall mean CCT of the pooled sample from both studies.[36,175]

Eyes with thinner corneas were demonstrated to be more likely to develop visual field progression in patients with chronic primary angle closure glaucoma (PACG).[36,175,176] Previous studies have also shown that corneal biomechanical parameters CH and CRF are significantly reduced in glaucomatous eyes and that they appear to be predictive risk factors of glaucoma pathogenesis and progression independent of CCT.[50,60]

a. Diagnosis

Diagnosis of glaucoma involves a series of clinical examination procedures which may be supported by imaging modalities. IOP measurement is usually done using the gold standard technique of Goldmann applanation tonometry (GAT). Corneal thickness is measured using either ultrasound pachymetry or non-contact techniques. Subjective examination is performed by ophthalmoscopy, slit-lamp biomicroscopy or stereoscopic optic nerve head photography to assess the color and appearance of the optic nerve head. The anterior chamber angle and trabecular meshwork are subjectively evaluated at the slit lamp via gonioscopy and may be objectively imaged via Ultrasound biomicroscopy (UBM) or Anterior segment – Ocular coherence tomography (AS-OCT).

Various highly sophisticated imaging systems are now available to evaluate the optic nerve head and the retinal nerve fiber layer (figure 14).[177] These include confocal scanning laser ophthalmoscopy (example: Heidelberg Retina Tomograph ; Heidelberg Engineering, Heidelberg, Germany), scanning laser polarimetry (example: GDx Nerve Fiber Analyzer; Laser Diagnostic Technologies Inc., San Diego, California), and ocular coherence tomography (example: time-domain OCT or spectral-domain OCT). The Heidelberg Retina Tomograph (HRT) is the most commonly used confocal scanning laser ophthalmoscopy device. The HRT uses a diode laser beam (wavelength, 670 nm) and captures a series of 32 sequential scans in a total acquisition time of 1.6 seconds. The optical transverse resolution of the HRT is 10 μm and the axial resolution is 300 μm.

Visual field examination is performed via Standard Automated Perimetry most commonly using the using the full threshold SITA 24-2 program of the Humphrey Visual Field (HVF) Analyser (Carl Zeiss Meditech, Dublin, CA, USA). This program determines the severity of glaucoma using summary global indices such as mean deviation (MD) and pattern standard deviation (PSD). [178] Visual field progression may be estimated via the Guided Progression Analysis (GPA) using the Visual Field Index (VFI) (figure 15). The VFI expresses visual function as a percentage of normal age-corrected sensitivity such that the VFI of an eye with a completely normal visual field is 100% and the VFI of a perimetrically blind eye is 0%. [179,180]

Figure 14: The Humphrey Visual Field Analyser and the Heidelberg Retina Tomograph [177]

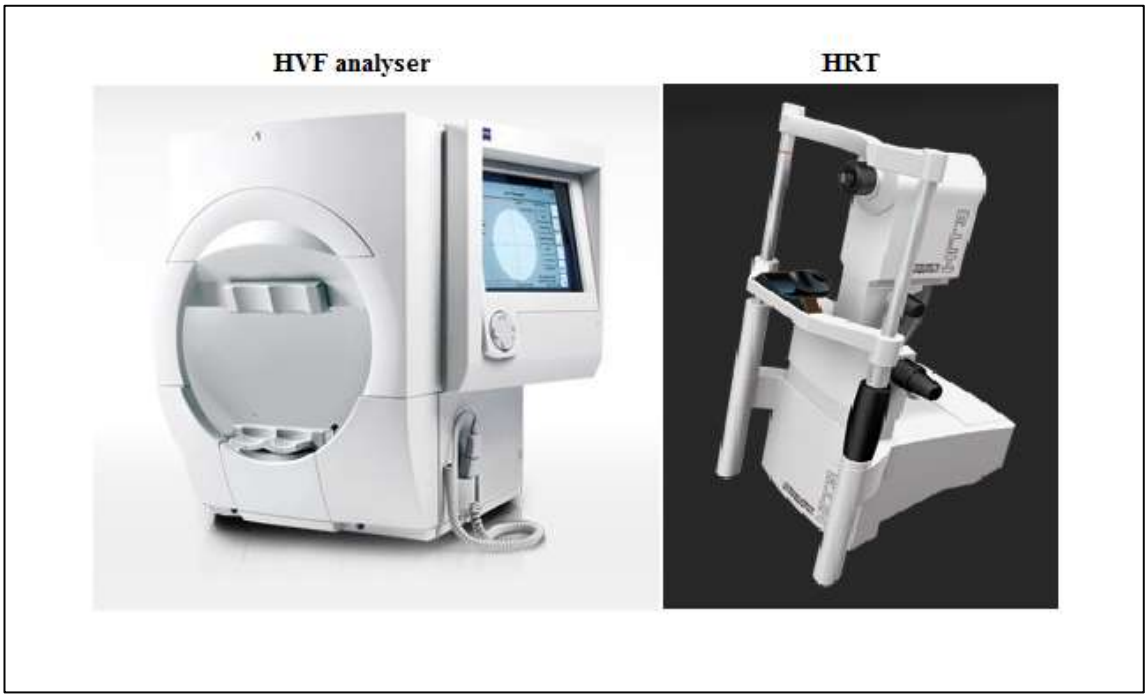
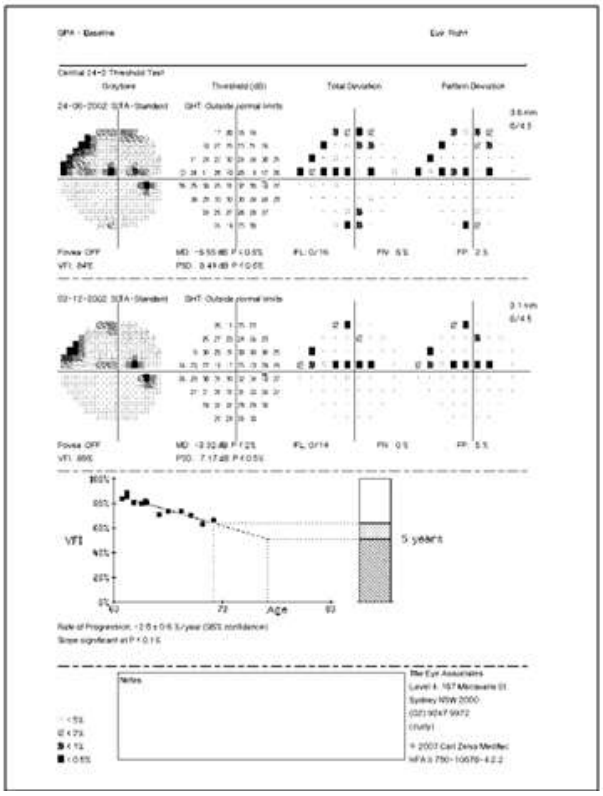


Figure 15: The Guided Progression Analysis (GPA) report



b. Treatment

Topical application of antiglaucoma medications has been the primary mode of glaucoma treatment for the last century. However, long-term local treatment with antiglaucoma medications is often associated with adverse effects, some of which affect the corneal tissue.[181-183]

Prostaglandin analogues (latanoprost 0.005%, travoprost 0.004%, and bimatoprost 0.03%) are the most commonly used ocular hypotensive drugs. Prostaglandins reduce IOP by enhancing aqueous humor outflow through the uveoscleral pathway to the suprachoroidal space and to the episcleral veins. These drugs act primarily through the activation of matrix metalloproteinases (MMPs) that degrade collagen cause a reduction of different collagen types in the eye.The corneal stroma is mainly composed of collagen fibers and prostaglandin analogues seem to affect the corneal stromal structure by decreasing the extracellular matrices.[184,185] The corneal stroma is mainly responsible for corneal thickness and excessive MMP acitivity in the cornea has been implicated as one of the possible causes of corneal thinning in disorders like keratoconus. [110,111]

Several clinical studies have showed that prostaglandin analogues could decrease CCT values. [186-188] Taking into account the importance of CCT in glaucoma evaluation, it is important to determine serial CCT values in these patients to monitor longitudinal changes that might occur owing to medication. Other categories of anti glaucoma medications include beta blockers, alpha agonists and carbonic anhydrase inhibitors. Combined medications offer an alternative for patients who need more than one type of medication. Apart from topical medications, many centres use selective laser trabeculoplasty (SLT) as a first-line option for treatment of glaucoma. For patients unable to tolerate topical medications or for whom

medications alone are inadequate, SLT and trabeculectomy are alternative laser and surgical procedures for glaucoma management.

CHAPTER 2: SUBJECTS AND METHODS

2.1 HUMAN ETHICS

All study protocols were approved by the Macquarie University Human ethics committee (Ethics Ref: 5201001446). All studies were conducted in accordance with institutional guidelines and the tenets of the Declaration of Helsinki.

2.2 RECRUITMENT OF SUBJECTS

All subjects were enrolled after obtaining an informed consent. Normal control subjects were university students and staff with no ocular pathology. Subjects with glaucoma were recuited from a tertiary referral glaucoma practice at Sydney. Keratoconic subjects were recuited from the Department of Ophthalmology and Visual Science, Australian School of Advanced Medicine, Sydney and from a tertiary referral cornea practice at Sydney.

2.3 MEASUREMENT TECHNIQUES

2.3.1 CORNEAL THICKNESS, CURVATURE AND ELEVATIONS

a) Ultrasound Pachymetry

Technique:

After topical anesthetic drops are instilled, the ultrasound probe was placed directly in the center of the subject's eye, creating a 90-degree angle. The ultrasound pachymetry device takes multiple, rapid, and sequential readings during a single applanation of the probe. This gives a mean US pachymetry reading with a standard deviation (SD). The reading was accepted when the SD was less than 5 μm.

b) Scheimpflug imaging (Pentacam)

Technique :

The subject remained seated and positioned in the headrest and alignment was achieved using the table height adjustment, forehead strap, and chin rest. Subjects were instructed to keep both eyes open and look directly at the black fixation target centered in the scanning-slit light for the duration of the scan (2 seconds). The machine was used in automatic release mode to rule out confounding operator-related variables. A subset of normal subjects underwent repeat measurements, once by the same operator and thereafter by another experienced operator to assess intra operator repeatability and inter operator reproducibility. Each measurement was taken approximately 3 to 5 minutes apart.

c) Combined Placido - Ocular Coherence Tomography (Visante OMNI)

Technique:

The subject was initially positioned at the ATLAS topographer that has its own chin and headrest that allows the head to turn at an angle to the unit. Subjects were instructed to blink and then keep both eyes open and look directly at the fixation target and imaging was performed. The participant's information and anterior corneal surface data were automatically transferred to the Visante OCT station by the network link. The subject then sat in front of the Visante OCT device and placed their chin on the chin rest. The subject was directed to fixate at a target and and the optically produced corneal reflex becamevisible as a vertical white line along the center of the cornea. Once the real-time image was optimally aligned, the scan was performed. The intra operator repeatability and inter operator reproducibility was also assessed.

2.3.2 CORNEAL BIOMECHANICS

The Ocular response Analyser

Technique:

During the ORA examination, subjects were seated on a chair in front of the ORA device. They werewell-positioned and relaxed, with their forehead parallel against the head rest without strain. Subjects weree asked to fixate at a target light and the measurement was taken by pressing a button on a personal computer linked to the ORA. A noncontact probe scanned the central corneal area and released an air puff and measured biomechanical values were displayed on the monitor.

2.3.3 GLAUCOMA DIAGNOSIS AND PROGRESSION

a) The Humphrey field analyser (HVF)

Technique:

In clinical practice, the commonest type of perimetry used is the "white-on-white" perimetry in which lights of incremental brightness are presented against a white background. The subject sat with their chin resting in front of an artificial concave dome with a target in the center. A button was given to the patient to be used during the exam and the eye that was not being tested was covered. The subject was instructed to fixate on the target at the center and click the button whenever a light is seen. The computer attached to the device then automatically mapped and calculated the subject's visual field.

Visual field progression was determined by Guided Progression Analysis (GPA) using the Visual Field Index (VFI).[189] VFI is a summary measurement of visual field status expressed as a percent of a normal age-adjusted visual field. Trend based progression analysis was perfomed to calculate the rate of progression or VFI slope using two baseline and subsequent

visual field exams. GPA software differentiates statistically significant progression of visual field loss from random variability and five visual field exams are necessary to generate a rate of visual field progression.

For event-based progression analysis, the probability plot in the GPA report flags points where statistically significant change has occurred. When an individual point has deteriorated from baseline to one follow-up test at $p< 0.05$, an open white triangle appears. If that point is confirmed on a second follow-up test, a half-darkened triangle appears. If that point is confirmed a third time, it is represented by a fully darkened triangle. The progression analysis indicates "possible progression" when three or more points show deterioration in at least two consecutive tests. Similarly, when three or more points show deterioration in at least three consecutive tests, the progression analysis indicates "likely progression."

b) The Heidelberg retina tomograph (HRT)

Technique:

The subject was comfortably seated and the chair height adjusted to permit the forehead to rest on the headrest. The subject was instructed to look into the lens of the camera as it was brought close to the eye. Once the subject's name was entered into the computer and "acquisition" was selected, the camera entered into live mode automatically. With proper fixation, the optic nerve should appear centered in the monitor.

The approximate power of the eye's refraction is dialled into the lens of the camera as the technician views the image on the monitor. Just before taking an image, the subject was instructed to blink and hold their eyes open as they looked at the green fixation light. The

HRT automatically acquired three three-dimensional images with the predetermined acquisition settings and the mean topography image was computed.

2.4 STATISTICAL ANALYSIS

For normal control and glaucoma subjects, the data from one randomly selected eye (Microsoft excel randomisation function) was included for analysis. Keratoconus being a highly asymmetric disease, data from both eyes were included for analysis. Data was presented as the mean ± standard deviation.

The independent samples *t* test was used to compare baseline characteristics between different groups. Change in values over time, treatment effects and differences between devices were evaluated using the paired sample *t* test. The difference in values between more than 2 groups was examined using analysis of variance (ANOVA) with application of Bonnferroni multiple comparison test. The Fishers test was performed to evaluate the difference in percentage between two groups.

The Pearson correlation coefficient was used to measure the strength of the linear relationship between normally distributed variables. When the variables are not normally distributed or the relationship between the variables was not linear, we used the Spearman correlation coefficient. The difference in survival between different groups was compared using the Kaplan Meier analysis. Univariate and multivariate binary logistic regression analysis was performed to determine Odds Ratios of various predictive factors for disease progression.

Bland–Altman analysis was performed to judge the 95% limits of agreement (mean difference ± 1.96 times standard deviation) between different devices.[190] The degree of agreement between two devices is determined by the magnitude of these limits with lower values indicating better agreement and vice versa. The judgement regarding the limit at which two devices could be used interchangeably is a clinical decision. [191] Repeatability and reproducibility of devices were ascertained using the Intraclass correlation coefficients (ICCs). The ICCs range from 0 to 1 and are interpreted as follows: < 0.75 (poor agreement), 0.75 to 0.90 (moderate agreement) and > 0.90 (good agreement).[192] The predictive diagnostic accuracy of variables was assessed using receiver operating curves (ROC) obtained by plotting sensitivity versus 1-specificity for each value. Area under curve (AUC) of ROC curves were calculated and interpreted as follows: 0.90 to 1 = excellent, 0.80 to 0.90 = good, 0.70 to 0.80 = fair, 0.60 to 0.70 = poor, 0.50 to 0.60 = fail.

Statistical analysis was mainly performed using SPSS for Windows version 19 (SPSS Inc., Chicago, IL). A few statistical test results and graphs were also obtained using the Graph Pad Prism 5 (GraphPad Software Inc., California, USA) and MedCalc version 13.1.1 (MedCalc Software Inc., Belgium). A p value less than 0.05 was considered significant for all statistical tests.

CHAPTER 3: THE LONGITUDINAL EFFECT OF TOPICAL ANTIGLAUCOMA MEDICATIONS ON CENTRAL CORNEAL THICKNESS

This chapter is a study that was performed to assess the longitudinal variation in central corneal thickness. Glaucomatous eyes on long-term topical antiglaucoma medications were compared to a group of control glaucoma suspect eyes that were not on any topical treatment. Results have been discussed in detail in comparison to previous studies. This has been published as: Viswanathan D, Goldberg I, Graham SL. Longitudinal effect of topical antiglaucoma medications on central corneal thickness. Clin Experiment Ophthalmol. 2013;41(4)348-54.

3.1 ABSTRACT

Background: To determine the change in central corneal thickness (CCT) over time and whether use of long term topical antiglaucoma medications influences CCT.

Design: Case control study with retrospective and prospective data collection

Participants: 187 eyes of 187 glaucoma patients (mean follow up 6.92 ± 1.67 years) being treated with topical antiglaucoma medications (at least 3 years) with no history of surgery or laser were included and compared with 100 eyes of 100 age matched untreated control subjects (mean follow up 6.58 ± 1.93 years) who were glaucoma suspects with normal intraocular pressure (IOP) not on any treatment.

Methods: Demographic data, CCT and IOP were collected at initial glaucoma diagnosis and at most recent visit and findings were compared between two groups.

Main Outcome Measures: Mean change in CCT in microns (µm)

Results: CCT fell significantly ($p < 0.0001$) in treated eyes but not in control eyes ($p = 0.18$), mean CCT reduction was 12.29 ± 13.65 µm in treated eyes and 1.17 ± 8.75 µm in controls. Amongst treated eyes, CCT reduction was significant ($p < 0.0001$) in those treated with either prostaglandins or a combination of prostaglandin and betablockers, while no significant reduction occurred in eyes treated with only betablockers ($p = 0.15$) when compared with control eyes .

Conclusions: Prostaglandins appear to be associated with a small but significant CCT reduction over time. Serial CCT measurements might be helpful in glaucoma patients, particularly those on prostaglandins.

Key words: Central corneal thickness, glaucoma medications

3.2 INTRODUCTION

Due to its influence on the measurement accuracy of intraocular pressure (IOP) by applanation tonometry, central corneal thickness (CCT) measurement has assumed importance in the management of glaucoma patients.[38,41, 193,194] In addition, the Ocular Hypertension Treatment Study (OHTS) demonstrated that CCT is a powerful predictive factor for the development of primary open-angle glaucoma from ocular hypertension.[195]

Earlier cross-sectional studies suggested that CCT may decrease over time [196-198] ; this was also corroborated by two longitudinal studies, one by Weizer et al on 39 subjects [183] and the other by Brandt et al[199] on OHTS participants who demonstrated a reduction in CCT comparable to other cross- sectional studies. The use of topical antiglaucoma medications could be associated with changes in the corneal structure [181,200] and prostaglandin analogues

may decrease CCT values.[186,187, 201-204] However, in one recent study, prostaglandin analogues were found to increase the CCT value slightly.[205]

As the maximal mean follow up for earlier studies specifically evaluating the effect of topical medications on CCT was less than 48 months, there is a need for investigating both normal and glaucoma treated populations with longer follow-up. We therefore examined whether there are demonstrable CCT changes over time in treated glaucoma subjects compared with non-treated subjects and compared the long term effects of various types of topical antiglaucoma medications on CCT .

3.3 METHODS

A total of 187 eyes of 187 patients (1 eye per patient) with glaucoma being treated with topical antiglaucoma medications were included and were compared with 100 eyes of 100 glaucoma suspects (1 eye per subject) who served as age matched controls. The study was conducted in a tertiary referral glaucoma practice, following the tenets of the Declaration of Helsinki after obtaining approval from the Institutional Review Board (IRB) / Ethics committee.

The inclusion criteria were patients with newly diagnosed glaucoma, either primary open angle glaucoma (POAG) or normal tension glaucoma (NTG) who had not received topical glaucomatous treatment prior to their initial CCT measurement. Patients must have had treatment for at least three years with a particular class of drugs. When more than one class of drugs were used in a patient, they were included as a combination group if each class of drugs was used for at least three years during follow-up. The inclusion criteria for the "control" group were patients who were glaucoma suspects, based on suspicious but not yet

glaucomatous discs with normal visual fields and intraocular pressure (IOP) who did not require treatment. Some subjects had only a family history of glaucoma, but were being checked periodically. Patients with corneal disease including dry eye, past history of ocular trauma, any intraocular surgery, laser treatment, recent contact lens wear and diabetes on drug treatment were excluded. The requirements for at least 3 years of consistent drug use and no laser or surgery substantially reduced the sample size available for this study, but removed confounders that might affect long term CCT.

For the purposes of analysis only, the glaucoma subjects were divided into POAG and NTG subgroups. POAG was defined as a typical glaucomatous optic nerve with focal or diffuse structural damage with or without visual field defect(s) demonstrated in at least 2 consecutive reliable examinations (Glaucoma Hemi-field Test abnormal) and an IOP equal to or greater than 21 mm Hg (Goldmann applanation tonometry). No subjects in this study had a visual field defect without some associated disc change. NTG was defined as for POAG, but with an IOP never recorded as greater than 20mmHg.

At the initial visit, age, gender, race, and family history of glaucoma in first degree relatives were recorded per patient. CCT measurements were performed before IOP measurements after instilling topical anaesthetic eye drops by experienced orthoptists using an ultrasound pachymeter recording a mean of 5 consecutive readings. The pachymeter underwent an upgrade in 2005, therefore CCT measurements taken prior to 2005 were performed using Tomey AL-2000 (Tomey Inc, Nagoya, Japan) and those after 2005 were performed using a newer version, the Tomey SP- 3000 (Tomey Inc, Nagoya, Japan). At the subject's most recent visit, CCT and IOP were again recorded, and their history of topical therapy recorded. CCT measurements at baseline before treatment and after treatment were analysed (see

below).Thus each patient's baseline CCT and IOP data were collected retrospectively and CCT and IOP data at present visit were collected prospectively.

Statistics:

Statistical analysis was performed using SPSS for Windows version 19 (SPSS, Chicago, IL). The independent samples *t* test was used to compare characteristics including age, baseline IOP and baseline CCT between the treated and untreated eyes. The changes in CCT values over time within the different subgroups were evaluated using a paired-sample t test. Fishers test was performed to evaluate the gender difference between the two groups. A p value of $\leq$ 0.05 was considered statistically significant.

3.4 RESULTS

Demographics of the subjects at baseline is shown in Table 1. The mean age of patients in the treated group (n = 187) was 68 ± 10.14 years as compared with 65.77 ± 9.64 years in the untreated group (n = 100). There were no significant differences between the two groups for age at baseline and duration of follow up (independent samples t test, p = 0.08, p = 0.25 respectively) whereas there were significant differences between the groups with respect to gender ratio, baseline values of IOP and CCT as indicated in Table 1. The proportion of females was significantly higher in the untreated control group when compared with the treated group, however a comparison of baseline CCT between males and females in both groups revealed no significant differences (p > 0.05). The treated eyes had a significantly lower CCT (p = 0.01) and significantly higher IOP compared with the untreated control group (p < 0.0001), which was as expected.

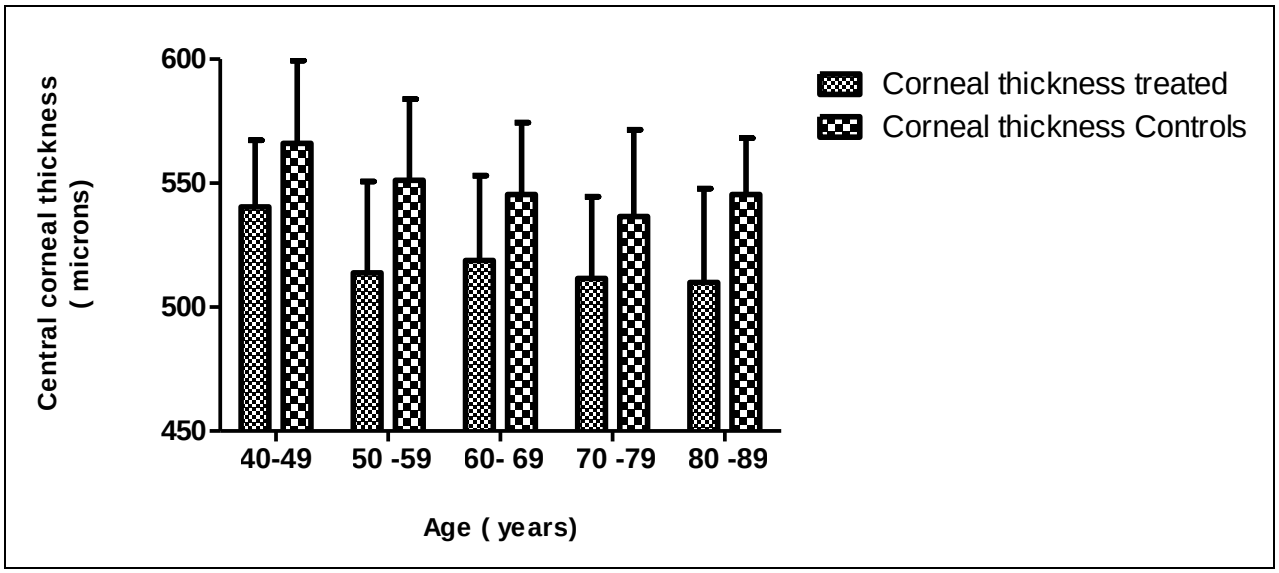
Table 1 – Baseline characteristics of treated and control eyes

Parameter	Treated eyes (n=187)	Control eyes (n=100)	p value
Age (years)	68 ± 10.14	65.77 ± 9.64	0.08
Gender (Male / Female)	103 / 98	35 / 65	0.009
Duration of follow up	6.92 ± 1.67 years	6.58 ± 1.93 years	0.25
CCT (μm)	536.48 ± 35.44	546.79 ± 30.91	0.01
IOP (mmHg)	21.68 ± 4.64	17.33 ± 3	<0.0001

CCT – Central corneal thickness, IOP – Intraocular pressure, μm – Microns, mmHg – Millimeters of mercury

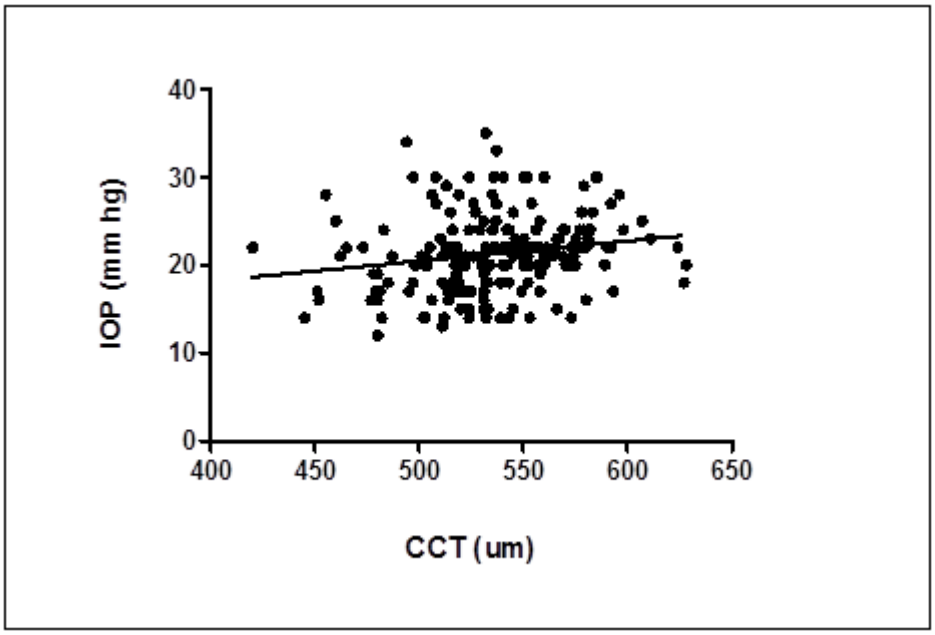
An inverse but non-significant relationship was found between CCT and age in both treated ($r = -0.052$, $p = 0.45$) and untreated eyes ($r = -0.171$, $p = 0.08$) . Linear regression analysis revealed a reduction in CCT per decade by 4.5μm and 5.5 μm in the treated and untreated eyes respectively and this relationship between age and CCT has been shown in Figure 16.

Figure 16: Relationship between age and Central Corneal Thickness



A significant positive correlation ($r = 0.32$, $p < 0.0001$) was observed between baseline CCT and IOP (figure 17), however no significant relationship was found between CCT change and IOP change ($r = 0.07$, $p = 0.35$).

Figure 17: Relationship between IOP and Central Corneal thickness



The distribution of diagnoses of patients according to various medications in the treated group is shown in Table 2. POAG was the commonest form of treated glaucoma followed by NTG. No patient converted from NTG to POAG during the course of the study.

Table 2 – Distribution of diagnosis amongst treated eyes

Medication	POAG N (%)	NTG N (%)	TOTAL N (% *)
Prostaglandins (PG)	56 (69.1)	25 (30.9)	81 (43.3)
Betablockers (BB)	26 (76.5)	8 (23.5)	34 (18.2)
PG + BB	36 (80)	9 (20)	45 (24.1)
PG + BB + CAI	11 (100)	0	11 (5.9)
PG + BB + AA	5 (83.3)	1 (16.7)	6 (3.2)
Miscellaneous	9 (90)	1 (10)	10 (5.3)
TOTAL N (% §)	143 (76.5)	44 (23.5)	187 (100)

PG – Prostaglandins, BB – Beta blockers, CAI – Carbonic anhydrase inhibitors,
AA- Alpha agonists, * Column percent, § Row percent

A comparison of the CCT and IOP values at baseline and follow up for the 2 different types of glaucoma among the treated eyes is shown in Table 3. There was a significant CCT reduction ($p < 0.0001$) in eyes with both NTG and POAG . Although the baseline CCT in eyes with NTG was significantly lower than those with POAG ($p = 0.01$), there was no difference in the amount of CCT change ($p = 0.61$) between the two groups.

Table 3 – Comparison of Central Corneal Thickness and IOP values

Eyes N (%)	Baseline CCT (µm)	CCT at follow up (µm)	Difference (µm)	p value	Baseline IOP (mmHg)	IOP at follow up (mmHg)	Difference (mmHg)	p value
Treated 187 (100%)	536.48 ± 35.44	524.19 ± 34.88	12.29 ± 13.65	<0.0001	21.69 ± 4.61	14.07 ± 1.73	7.56 ± 4.43	<0.0001
POAG 143 (76.5%)	539.56 ± 36.74	526.39 ± 36.19	13.17 ± 13.09	<0.0001	22.73 ± 3.72	14.26 ± 1.62	8.47 ± 3.84	<0.0001
NTG 44 (23.5%)	524.06 ± 30.83	512.02 ± 29.98	12.04 ± 12.70	<0.0001	16.59 ± 2.91	13.54 ± 1.75	3.14 ± 2.53	<0.0001

POAG - Primary open angle glaucoma, NTG - Normal tension glaucoma,
mmHg - Millimeters of mercury, µm - Microns

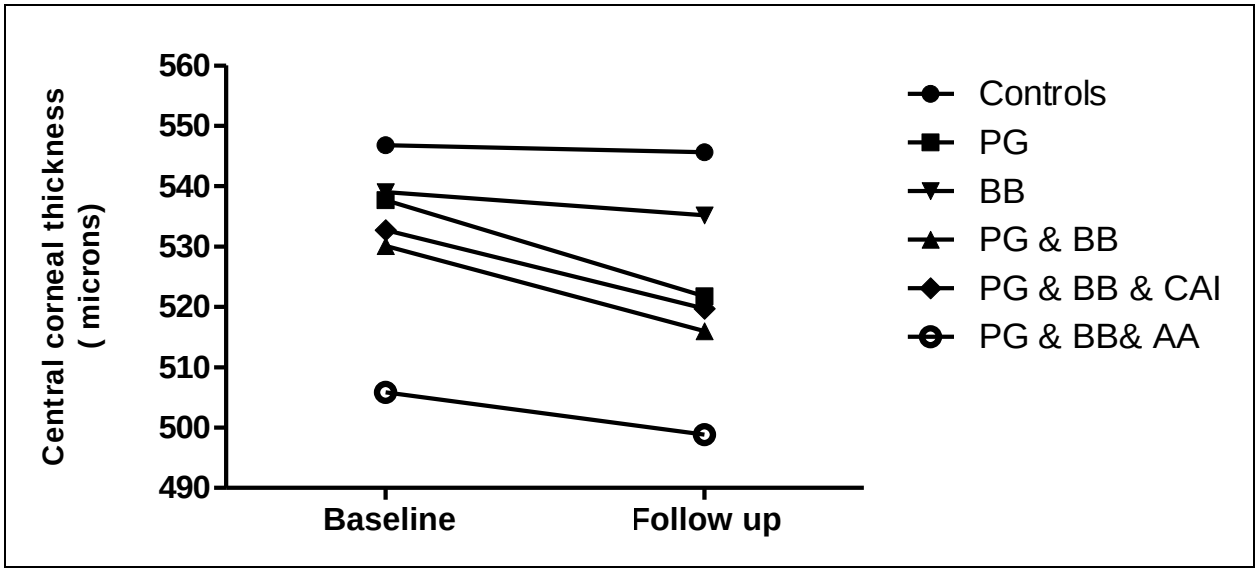
The comparison between CCT at baseline and CCT at follow up for both control eyes and treated eyes classified into different medication groups is shown in Table 4 and Figure 18.

Table 4 – Comparison between Central Corneal Thickness at baseline and at follow up

Eyes	Baseline CCT Microns (μm)	CCT at follow up Microns (μm)	Difference Microns (μm)	p value
Control eyes (n = 100)	546.79 ± 30.91	545.62 ± 31.79	1.17 ± 8.75	0.18
Treated eyes (n = 187)	536.48 ± 35.44	524.19 ± 34.88	12.29 ± 13.65	<0.0001
Prostaglandins (PG)(n = 82)	537.69 ± 37.10	521.79 ± 34.94	15.89 ± 13.00	<0.0001
Beta-blockers (BB) (n = 40)	539.04 ± 28.52	535.17 ± 27.98	3.88 ± 13.00	0.06
PG + BB (n = 45)	530.10 ± 35.50	515.97± 34.97	14.13 ± 13.19	<0.0001
PG + BB + CAI (n = 9)	532.73 ± 25.59	519.73 ± 31.26	13.00 ± 13.54	<0.0001
PG + BB + AA (n = 4)	505.83 ± 34.69	498.83 ± 28.12	7.00 ± 10.60	0.16
Miscellaneous (n = 7)	568.20 ± 33.48	558.60 ± 34.60	9.60 ± 9.55	0.004

PG – Prostaglandins, BB – Beta blockers, CAI – Carbonic anhydrase inhibitors,
AA- Alpha agonists

Figure 18: Comparison between Central Corneal Thickness at baseline and at follow up between controls and different medication groups



PG – Prostaglandins, BB – Beta blockers, CAI – Carbonic anhydrase inhibitors,
AA- Alpha agonists

There was no significant change in the control eyes ($p > 0.05$) whereas a significant reduction in CCT ($p < 0.0001$) was noted in the treated eyes including all medication groups except beta-blockers and the small subgroup who were on a combination of prostaglandins, beta-blockers and alpha agonists . The mean reduction in CCT was 12.29 ± 13.65 µm in the treated eyes and 1.17 ± 8.75 µm in controls . The range of differences in CCT (present CCT minus baseline CCT) was from - 48 microns (thinned) to + 35 microns (thickened). Of 187 eyes, 152 eyes (81.1%) became thinner, 33 eyes (17. 9%) became thicker and 2 eyes (1%) remained exactly the same. Among the treated eyes, 56.1 % eyes thinned more than 10 µm and 4.3% thickened more than 10 µm whereas amongt controls, 14% eyes thinned more than 10 µm and 10% thickened more than 10 µm. There was no significant relationship noted between the amount of observed change in CCT and the duration of follow up in years ($r = - 0.10$, $p = 0.16$) in treated eyes.

3.5 DISCUSSION

Central corneal thickness (CCT) has emerged as an important parameter for glaucoma diagnosis, and management. Our study was designed to investigate whether CCT changes occur over time in eyes with glaucoma treated with topical antiglaucoma medications. This study has shown that CCT decreased by a mean 12.29 ± 13.65 µm (2.26 $\pm$ 2.52 % reduction) in treated eyes over a follow up period of 6.92 ± 1.67 years . Among the treated eyes, the reduction in CCT was most significant in those treated with either prostaglandins or a combination of prostaglandins and betablockers, while there was no significant reduction in eyes treated only with betablockers ($p = 0.16$) when compared with untreated control eyes . The mean reduction in CCT in the eyes treated with prostaglandins was 15.89 ± 13.00 µm amounting to a percentage reduction rate of 2.91 $\pm$ 2.37 % . This is consistent with the

findings of other studies that have shown that prostaglandins are associated with a significant reduction in CCT over time.[186,187, 201-204]

Kim et al [201] recently showed a statistically significant reduction in CCT by 5.4 μm after 24 months of treatment with latanoprost in patients with normal tension glaucoma . Sen et al [187] reported a percentage reduction rate in CCT of 1.9 ± 2.4 % and 2.8 ± 1.8 % during 24 months of treatment with latanoprost and bimatoprost respectively. A recent study also compared the effects of latanoprost, travoprost, and bimatoprost on CCT [202] and observed a statistically significant reduction ($p < 0.001$) in CCT in all groups over 17.19 ± 15.71 months follow up, the reduction of CCT being 14.95 ± 5.04 μm , 15.73 ± 3.25 um and 17.00 ± 6.23 μm in the latanoprost, travoprost, and bimatoprost groups respectively. A reduction of CCT in patients under treatment with topical prostaglandins when compared with topical carbonic anhydrase inhibitors has been reported [206] and Harasymowycz et al [186] reported a 6.9 μm thinning of CCT at the end of a 6-week follow-up after treatment with travoprost.

Brandt et al [199] analysed CCT change (mean 3.8 years apart) in a large cohort of OHTS participants and they observed that CCT decreased by a mean of 1.0 ± 3.4 μm / year among participants originally randomized to observation as compared to 0.5 ± 3.5 μm / year among all participants originally randomized to medication ($p < 0.000$) which is in contrast to our findings . However when they analysed participants treated only with topical prostaglandin analogues, they found had a greater rate of decrease in CCT per year than participants treated only with topical betablockers as found in this study. We detected an overall CCT reduction rate of 2.1 ± 2.4 μm /year among all treated eyes, 2.8 ± 2.5 μm /year among prostaglandin treated eyes, 0.9 ± 2.1 μm /year among betablocker treated eyes and 0.2 ± 1.6 μm /year among untreated control eyes.

The strengths of the study by Brandt et al [199] were the large sample size and the multivariate linear model to study CCT change that took into account all five readings of the first and second CCT measurements, however the limitation was that the observation group also had exposure to topical medications for a variable period of time (just over one year mean duration) between the two CCT measurements and most of these subjects were likely to have been on prostaglandins, whereas the original medication group were predominantly on beta blockers or others. This in fact may explain why the OHTS data suggested less thinning in the original treatment group than the observation group – they had less subjects with prostaglandin therapy – rather than treatment itself being protective to CCT. The prostaglandin changes could easily have occurred within one year of therapy. In addition, the OHTS data included only ocular hypertensive subjects with a mean CCT of around 573 µm, many of whom never developed clinical glaucoma.

The strengths of our study include a large sample, long follow up of nearly 7 years , minimum of 3 years on drug , analysis of treated eyes belonging to both POAG and NTG glaucoma subtypes and an age matched control group with no prior exposure to topical medications. As medications were only used from baseline, the pretreatment CCT values were reliable and free from former antiglaucoma medication effects. We found no time related effect on the amount of CCT change; this is similar to Zhong et al [202] who reported no significant difference in the amount of reduction in CCT between the patients with less than or equal to 6 months treatment and the patients with more than 6 months treatment in the 3 prostaglandin groups. This suggests the CCT thinning caused by prostaglandins mainly occurs within the initial treatment period. Our findings would support this, since crossectional analysis of duration of treatment versus the amount of thinning did not reveal any relationship, suggesting that the change is not progressive atleast after 3 years of treatment .

Interestingly, there were no significant CCT reduction in the group of eyes treated with a combination of prostaglandins, beta-blockers and alpha-agonists. Grueb et al [207] recently described a reversible increase in CCT after topical administration of brimonidine 0.1% which could mean that the CCT reduction caused by prostaglandins might have been compensated by an increase in CCT caused by the alpha-agonists, effectively resulting in no significant change in CCT in that group.

A potential mechanism for prostaglandins to induce thinning could be their effect on matrix metalloproteinases (MMPs). MMPs are a group of enzymes that degrade collagen, a key constituent of basement membrane and extracellular matrix components. [208] The MMP family includes about 20 types of enzymes, which may be found in the anterior segment of the eye, including the corneal epithelium, stroma and endothelium, conjunctiva, lacrimal film, aqueous humor, trabecular meshwork, ciliary muscle cells and lens. [209-212] The IOP lowering effect of prostaglandin analogues is known to be caused by MMP activation in the smooth muscle of the ciliary body. [213] The overactivity of MMP-2 in the cornea induced by prostaglandins may progressively thin the cornea in pathologic conditions such as keratoconus,[214] however other enzymes may also have a role in these processes. [215-217]

Prostaglandins appear to promote collagen gel contraction and affect the corneal stromal structure by decreasing the extracellular matrices and thus might affect the CCT. Liu et al [182] compared the effects of the antiglaucoma drugs latanoprost and timolol on the contraction of corneal fibroblasts cultured in a 3-dimensional collagen gel; they reported that latanoprost stimulated collagen gel contraction mediated by corneal fibroblasts in a concentration and time dependent manner, whereas timolol had no such effect. They postulated this action might affect corneal shape and thickness and thereby influence measurement of IOP. The corneal

stroma is mainly composed of collagen fibres and the reduction in CCT caused by prostaglandins appears to result from their upregulation of local MMPs.

Earlier cross-sectional studies [196-198] demonstrated that thinner corneas are associated with increasing age. Aghaian et al [197] showed a 3µm decrease in CCT per decade of age in a multiracial patient cohort, while the Ocular Hypertension Treatment Study (OHTS) demonstrated a 6.3µm decrease in CCT per decade of age.[195] In our study, linear regression analysis revealed a reduction in CCT per decade of 4.5µm and 5.5µm in the treated and untreated eyes respectively; this agrees with these studies .

In terms of clinical implications, the change is CCT is not likely to be of much clinical significance, although this study was not designed specifically to assess this aspect. The OHTS data also suggested that the changes they noted over time were unlikely to influence clinical decisions. Ehlers et al [193] had demonstrated a 5 mm Hg change in IOP for 70 µm of CCT variation , Doughty and Zaman [71] in their meta-analysis, had reported a change of IOP by 2.5 mm Hg per 50 um of CCT variation. In our study, prostaglandin analogues decreased CCT values by approximately 16 mm over a mean 6.9 years, resulting in falsely lower IOP values by 1.14 and 0.8 mm Hg according to Ehlers and Doughty's IOP correction formulas respectively ; these are not likely to be clinically relevant . However some individuals showed much greater changes up to 48 µm of thinning , this could impact Goldmann applanation tonometry (GAT) measurement accuracy .

The limitations of our study included that fact that the ultrasonic pachymeter was upgraded in 2005 and so some patients underwent CCT measurement using 2 different ultrasonic pachymeters. However, we found no significant difference ($p = 0.19$) in the amount of CCT

change between 2 groups with baseline CCT measured before or after 2005. In addition although the baseline pachymetry measurements were performed by different operators, CCTs at the final visits were measured by the same operator and earlier studies have indicated good intra- and interobserver reproducibility for ultrasonic CCT measurements.[71,72]

Our study had insufficient numbers to examine the effects of the 3 different prostaglandins individually; the majority of patients were on latanoprost. The study may also be limited by the exclusion of subjects undergoing laser and surgery. These groups typically would have been showing either higher IOPs or progressive change, and potentially may have included a larger proportion of patients with thinner corneas, or with greater thinning over time. However we felt there was the potential for both laser and surgery of any kind to impact on the corneal endothelium and its function and therefore chose not to include them. Furthermore, while the "control" group of suspects not on treatment does not represent true normal controls, it does represent an untreated group, none of whom converted to glaucoma during follow up. Ideally we should have followed 100 normal subjects with no risk factors, but we did not have such a sample with baseline data available.

In conclusion, long term treatment with prostaglandin analogues is associated with a small but significant thinning of the central cornea. The potential implication on IOP measurements should be considered in glaucoma practice, but in most cases is not likely to be clinically relevant. Given the known thinning with age, repeat CCT measurements are appropriate in long term glaucoma management.

CHAPTER 4: RELATIONSHIP OF CHANGE IN CENTRAL CORNEAL THICKNESS TO VISUAL FIELD PROGRESSION IN EYES WITH GLAUCOMA

This chapter is a study that analyses whether baseline central corneal thickness or change in corneal thickness functions as a risk factor for visual field progression in eyes with glaucoma. This has been published as: Relationship of change in central corneal thickness to visual field progression in eyes with Glaucoma. Graefes Arch Clin Exp Ophthalmol.2013;251(6):1593-9.

4.1 ABSTRACT

BACKGROUND: To assess the relationship between baseline central corneal thickness (CCT) and/or ongoing CCT change over time with subsequent visual field progression.

METHODS : 163 eyes of 163 patients with medically treated glaucoma were followed up for 6.8 ± 1.8 years. Exclusion - Laser or intraocular surgery. Baseline and follow up CCT, confocal scanning laser tomography and visual fields . CCT and CCT change related to visual field progression using Glaucoma Progress Analysis. Multivariate logistic regression analysis for predictive factors of glaucoma progression.

RESULTS - Thinner baseline CCT was associated with more advanced damage at presentation, with greater mean deviation (MD) ($r = 0.17$, $p = 0.02$) and inversely related to neuroretinal rim area (NRR) ($r = 0.20$, $p = 0.02$). Progressing eyes had significantly thinner ($p = 0.01$) baseline CCT compared to non progressing eyes. The slope of visual field change was significantly greater ($p = 0.05$) for thinner (< 540 µm) as compared to thicker eyes. A small but significant CCT reduction (12.78 ± 13.35 µm , $p < 0.0001$) was noted in all eyes, however there was no significant difference ($p = 0.95$) in the amount of change between progressing

and non progressing eyes. CCT change did not correlate with MD or NRR change. A thinner CCT (Odds ratio = 1.80, p = 0.02), but not CCT change (Odds ratio = 1.07, p = 0.69), was a significant risk factor for glaucoma progression.

CONCLUSIONS - CCT correlates significantly with the amount of glaucomatous damage at presentation. Thinner corneas may be associated with increased risk of visual field progression. CCT reduced slightly over time in eyes with glaucoma; but the magnitude of this change was not related to visual field progression.

KEY WORDS: Central corneal thickness, glaucoma, visual field progression

4.2 INTRODUCTION

The Goldmann applanation tonometer (GAT) remains the gold standard for determining intraocular pressure (IOP).[218] A positive correlation has been demonstrated between central corneal thickness (CCT) and IOP in animal and human studies. This prompted investigators to develop several formulae to try to adjust IOP as measured by GAT for CCT. [38,41,193,219,220]

The Ocular Hypertension Treatment Study (OHTS) and the European Glaucoma Prevention Study (EGPS) reported that CCT was predictive for development of primary open angle glaucoma (POAG) in patients with ocular hypertension such that the risk to develop POAG doubled for every 40 microns decrease in CCT from the overall mean CCT of the pooled sample from both studies.[36,175] Subsequent clinical studies [174, 221-225] on a large number of subjects have shown that subjects with relatively thinner corneas may suffer earlier glaucomatous damage and that CCT is an independent predictive factor for progression of

POAG. [174] In a Bayesian regression model to assess the risk factors for visual field progression, Medeiros et al demonstrated that a thinner CCT was associated with a faster rate of mean deviation (MD) change.[226]

Why CCT is a prognostic risk factor for glaucoma development and progression has not been elucidated precisely. The influence of CCT on glaucoma risk is more than just tonometric artefact [227] as affirmed by a further report from the OHTS investigators which suggested that CCT is a biomarker for structural factors involved in the pathogenesis of POAG.[228] Medeiros et al have questioned this conclusion and have advised caution for the conclusion that CCT is an independent risk factor for glaucoma.[229]

Both cross-sectional [196 -198] and longitudinal studies [183, 199] have suggested that CCT may decrease over time; the relationship of this progressive CCT reduction with visual field progression in glaucoma subjects has been investigated in one study of 39 patients, some of whom had undergone incisional intraocular surgery. [183] We addressed the relationship of central corneal thickness (CCT) to glaucomatous damage and whether CCT or change in CCT over time correlates with subsequent visual field progression in newly diagnosed glaucoma subjects who had only received medical therapy.

4.3 METHODS

We included one hundred and sixty three patients with glaucoma being treated with topical antiglaucoma medications from a tertiary glaucoma practice. Only one eye per subject was used for analysis to avoid intra subject correlations. We adhered to the Declaration of Helsinki. We included patients who had been newly diagnosed with glaucoma, either primary

open angle glaucoma (POAG), or "normal tension glaucoma" (NTG) and who were treatment-naive prior to their initial CCT measurement. Patients with dry eye were excluded on normal clinical criteria (corneal epitheilial defects visualized on fluorescein staining or reduced tear-film breakup time) both at baseline and at follow up examinationas previous publications have reported on reduced CCT in dry eyes.[230] No patients with visually significant cataract were included as this might affect visual field measurements.We also excluded patients with past history of ocular trauma, any intraocular surgery, laser treatment , contact lens wear and diabetics on drug treatment.

POAG was defined as an optic nerve head with typical glaucomatous focal or diffuse structural damage with or without visual field defect(s) demonstrated in at least two consecutive reliable examinations (Glaucoma Hemi-field Test abnormal) [231] and an IOP equal to or greater than 21 mm Hg (Goldmann applanation tonometry). No subjects in this study had a visual field defect without an associated disc change. NTG was defined as for POAG, but with an IOP never recorded as greater than 20mmHg. Reliable visual fields were defined as fixation losses (FLs) <30%, false negatives (FNs) < 20% and false positives (FPs) <20%.

At the initial visit, age, gender, ethnicity and family history of glaucoma in a first degree relative were recorded and baseline CCT measurements were performed before IOP measurements after instilling topical anaesthetic eye drops by experienced orthoptists using an ultrasound pachymeter recording a mean of 5 consecutive readings. The pachymeter was upgraded in 2005, therefore CCT measurements taken prior to 2005 were performed using Tomey AL-2000 (Tomey Inc, Nagoya, Japan) and those after 2005 were performed using a newer version, the Tomey SP- 3000 (Tomey Inc, Nagoya, Japan). Baseline IOPs were single

measurements (Goldmann applanation tonometry) that were performed by the same clinician before commencing topical therapy

Visual field examinations were carried out with static automated perimetry using the full threshold SITA 24-2 program of the Humphrey field analyser (Carl Zeiss Meditech, Dublin, CA, USA). Scanning laser tomography was performed with the Heidelberg retina tomograph (HRT 3- Heidelberg Engineering GmbH, Dossenheim, Germany) at baseline and annually at follow up visits (mean pixel height standard deviation < 40 μm). The patient data including CCT, IOP and visual field data was recorded prospectively but captured for the study analysis retrospectively. The data captured at the final visit for this study was done in real-time, but not prospectively .

Visual field analysis

All 163 patients had at least five visual fields permitting calculation of progression with the Guided Progression Analysis (GPA). GPA uses the Visual Field Index (VFI), a summary measurement of visual field status expressed as a percent of a normal age-adjusted visual field for calculation of glaucoma rate of progression.[179] For a trend based analysis, the slope of the VFI was calculated using the GPA software with the two visual field tests closest in time to the original CCT measurement as the new baseline and then analysing subsequent fields for slope.

For event-based progression analysis the probability plot in the GPA report flags points where statistically significant change has occurred, and when three or more points show deterioration in at least two consecutive tests, the progression analysis indicates "possible progression." In cases where three or more points show deterioration in at least three

consecutive tests, the progression analysis indicates "likely progression." In this study, the baseline examinations were the values closest to the time of measurement of baseline CCT; the software advice of "no", "possible" or "likely" progression was documented. Analysis was performed using both"likely" and "possible" progression as an end point. If classification reverted on subsequent fields improved (back to "no progression"), this was taken as fluctuation and not counted as progression. 'Survival' was the time period from baseline until when glaucomatous eyes first exhibit 'progression'(either 'likely' or 'possible') as determined from serial visual fields using GPA.

For the HRT, change in rim area, retinal nerve fire layer (RNFL) thickness and vertical cup disc ratio (CDR) was recorded between initial and final visits. As there is no accepted algorithm, we did not determine progression with the HRT.[232] Based on baseline CCT, glaucoma subjects were classified into two groups : less than versus 540 microns (Thinner group) or greater (Thicker group). The two groups were compared.

CCT measurement

To validate CCT reproducibility, a group of randomly selected subjects (both treated and untreated) underwent repeat pachymetric measurements. Inter-observer variability was calculated in two ways: firstly, subjects (n = 13) for whom a second, masked operator repeated CCT measurements within a few minutes of the first measurement. The mean difference in CCT was 1.16 μm (95 % CI : 5.38 – 3.05 μm). An intra-class correlation coefficient (ICC) was 0.995 (95 % CI : 0.984 – 0.998), indicating good agreement. Secondly, for a different subset of randomly chosen subjects (n =10), two different masked operators repeated CCT measurements on separate follow-up visits at the same time of day. The ICC was 0.992 (95 % CI : 0.95– 0.99), again indicating good agreement.

To measure intra-observer variability, a subset of subjects (n = 8) underwent repeat CCT measurements by the same operator; the mean difference in CCT was 0.25 μm (95 % CI : 5.56 - 5.06 μm) and ICC was 0.995 (95 % CI : 0.984 – 0.998).

Statistics:

Statistical analysis was performed using SPSS for Windows version 19 (SPSS, Chicago, IL). The independent samples *t* test was used to compare characteristics including age, baseline IOP, and baseline CCT between the two groups. The change in CCT values over time within the two groups was evaluated using a paired-sample t-test. Spearman's correlation coefficient was used to study correlations between CCT values and other parameters. Fishers test was performed to evaluate the difference in percentage of progressive eyes and Kaplan Meier analysis, to compare survival between the two groups . A univariate and multivariate binary logistic regression analysis was also performed and the Odds Ratio of various predictive factors for glaucoma progression, calculated.

4.4 RESULTS

Of an initial 187 eyes, 163 eyes of 163 patients qualified for the study with sufficient reliable visual fields. The two groups were divided by their baseline CCT, either greater than 540 μm (Thicker group) versus 540 μm and below (Thinner group). IOP correlated positively with CCT ($r = 0.20$, $p = 0.01$).

The two groups were comparable with no significant difference in patient age ($p=0.52$). The thicker group had an apparently greater baseline IOP compared with the thinner group, however the difference was not statistically significant ($p = 0.06$). There was a significant reduction in CCT in both groups over the course of the study - Table 5. This change was

significantly greater in the thicker group (p = 0.03). With treatment, there was a significant reduction in IOP (p < 0.0001) in both groups (Table 5). However, the difference in IOP reduction between the groups was not significantly different for both absolute values (p =0.31) and as a percentage (p =0.30).

Table 5: Comparison of age, CCT and IOP between thicker and thinner eyes 163 eyes

	CCT > 540 um (Thicker eyes) (72 / 163 eyes)	**CCT ≤ 540 um (**Thinner eyes) (91 / 163 eyes)
Age (years)	67.97 ± 10.91	69.04 ± 10.56
Mean pre-study CCT (µm)	565.78 ± 19.69	509.51 ± 19.71
Mean post-study CCT (µm)	550.69 ± 22.24	499.07 ±26.17
Mean difference (µm)	15.08 ± 15.26 *	10.43 ± 13.31*
Mean Pre IOP (mmHg)	22.72 ± 3.67	21.29 ± 5.21
Mean post IOP (mmHg)	14.44 ± 1.72	13.73 ± 1.65
Mean difference (mmHg)	8.28 ± 3.48 *	7.56 ± 5.01*

* Statistically significant (p < 0.0001) for mean difference between pre and post values
CCT = central corneal thickness, IOP = intraocular pressure , um = microns
mmHg = Millimeters mercury

Baseline visual field indices and HRT data were compared between the two groups. The thinner group had a significantly worse mean deviation (MD), Pattern standard deviation (PSD) , Visual Field index (VFI) and baseline neuroretinal rim area (NRR) when compared to the thicker eyes. Thinner eyes had a thinner baseline RNFL and a larger vertical cup disc ratio (CDR) compared to thicker eyes, however these were not statistically significant - Table 6. Thinner eyes also had a significantly worse (p < 0.05) MD, PSD and VFI at final follow up as compared with thicker eyes.

Table 6: Comparison of visual field and HRT data between two groups

	Mean pre MD (dB)	Mean pre PSD (dB)	Mean pre VFI (%)	Mean pre NRR (mm^2)	Mean pre RNFL (mm)	Mean pre CDR
CCT > 540 μm Thicker eyes (72 / 163 eyes)	-1.64 ± 2.22	2.71 ± 1.85	96.27 ± 4.89	1.30 ± 0.25	0.21 ± 0.08	0.58 ± 0.13
CCT ≤ 540 μm Thinner eyes (91 /163 eyes)	-3.33 ± 4.55	4.17 ± 3.68	92.04 ± 12.57	1.18 ± 0.29	0.20 ± 0.06	0.63 ± 0.13
p value	0.005	0.003	0.011	0.03	0.41	0.08

CCT = central corneal thickness, MD = Mean deviation, PSD = Pattern standard deviation VFI = Visual Field index, NRR = neuroretinal rim area, RNFL = retinal nerve fire layer, CDR = cup disc ratio, Db = decibels, mm = millimetres, μm = microns

Baseline CCT correlated positively with mean deviation (MD) ($r = 0.17$, $p = 0.02$), neuroretinal rim area ($r = 0.20$, $p = 0.02$) and GPA rate of VFI change ($r = 0.20$, $p = 0.03$) such that thinner corneas were associated with more advanced presentation (Figures 19 and 20) and greater rate of glaucoma progression (Figure 21).

Figure 19 - Correlation between baseline central corneal thickness (CCT) and baseline Mean deviation (MD)

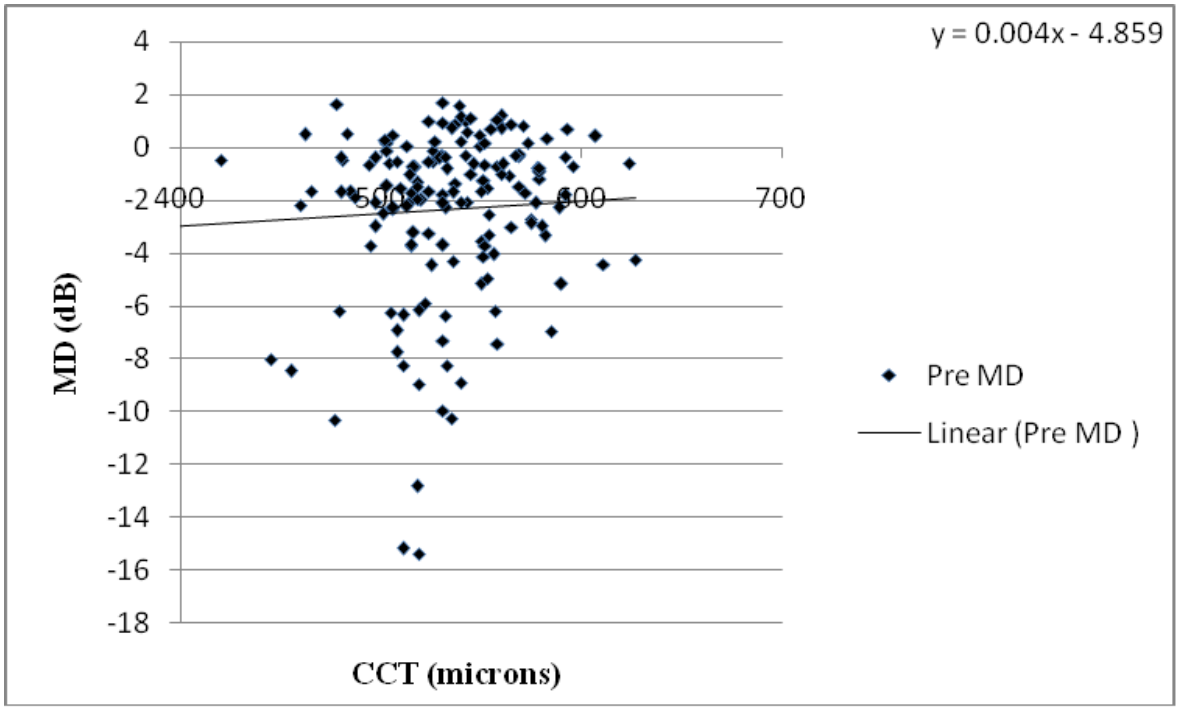


Figure 20: Correlation between baseline central corneal thickness (CCT) and baseline neuroretinal rim area (NRR)

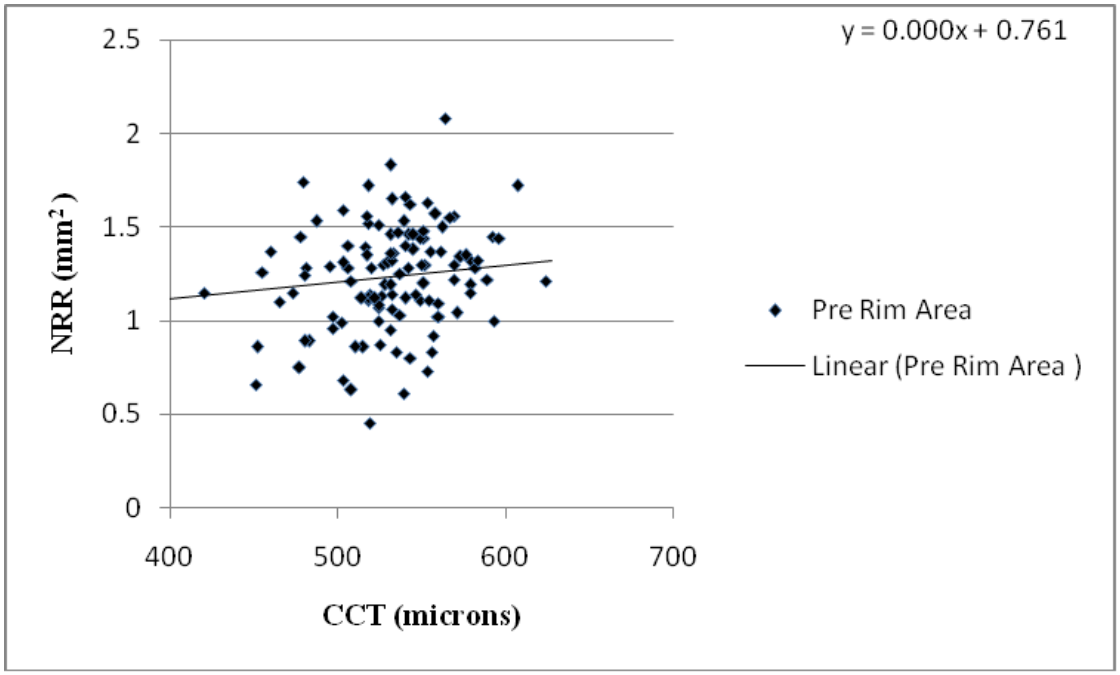
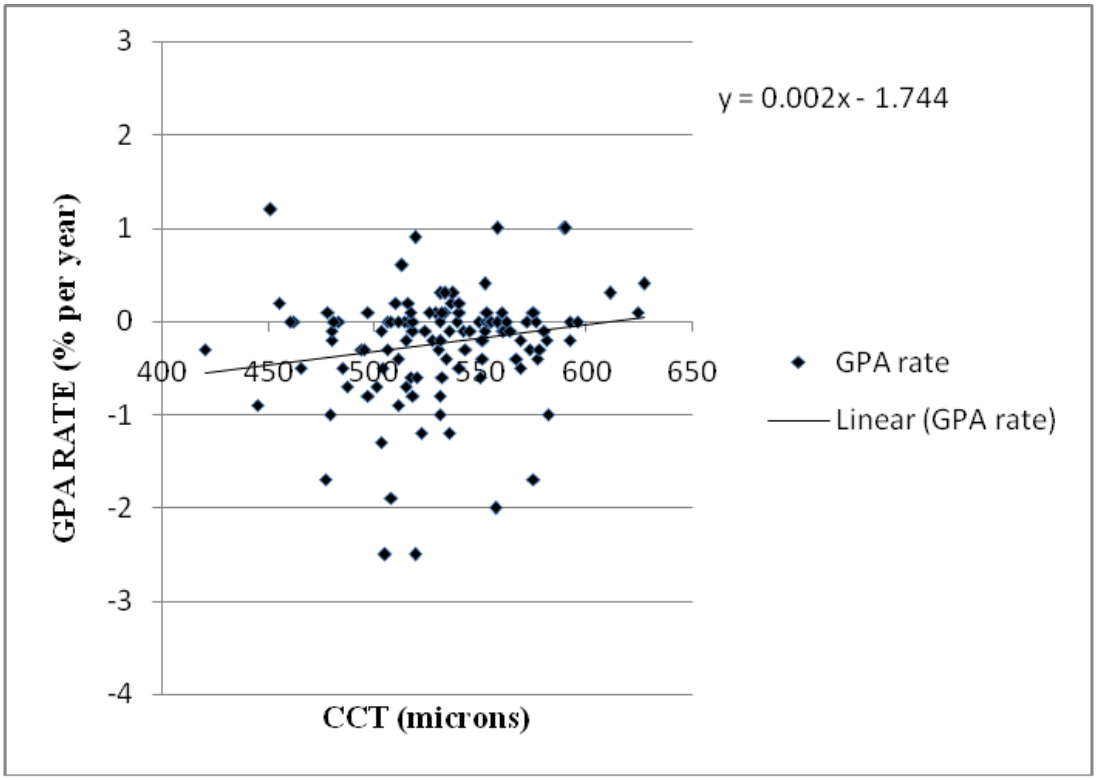


Figure 21: Correlation between baseline central corneal thickness (CCT) and Guided Progression Analysis (GPA) rate of visual field change



Progression of visual field was detected in 26 /163 eyes (16 %) whereas 137/163 eyes (84%) revealed no progression. “Progressing” eyes had significantly thinner (p = 0.01) baseline CCT at 518.85 ± 31.92 μm and significantly worse (p < 0.001) MD at -5.41 ± 5.47dB as compared with “non progressing” eyes with baseline CCT of 536.97 ± 35.82 um and MD of - 1.99 ± 3.06 dB respectively. However, there were no significant differences between the groups for age and the CCT change - Table 7.

Table 7 – Comparison between “progressing” and “non progressing” eyes

	Age (years)	Baseline CCT (μm)	Final CCT (μm)	Difference (μm)
“Progressing eyes” (26 /163)	70 ± 10.4	518.8 ± 31.92	506.25 ± 36.61	12.60 ± 15.68 *
“Non-progressing” eyes (137/163)	68.29 ± 10.76	536.97 ± 35.82	524.55 ± 34.40	12.42 ± 14.09 *
p value	0.44	0.01	0.01	0.95

* Statistically significant (p < 0.001) between pre and post values

CCT = central corneal thickness, μm = microns

The amount of change in CCT did not correlate with the amount of change in MD (r = -0.03, p = 0.7), NRR area (r = -0.05, p = 0.6), and VFI (r = 0.1, p=0.22) respectively. There was a significant reduction in IOP over follow up in both “progressing” (8.17 ± 4.69 mmHg, p < 0.0001) and “non progressing” eyes (7.81 ± 4.37 mmHg , p < 0.0001) , however there were no significant differences between the groups for baseline IOP, treated IOP and the amount of IOP reduction (p = 0.69).

A small but significant reduction of CCT (mean 12.78 ± 13.35 μm, $p < 0.001$) occurred in all eyes. CCT reduction was most significant in those treated with either prostaglandins (mean 15.89 ± 13.00 μm, $p < 0.0001$) or a combination of prostaglandins and betablockers (mean 14.13 ± 13.19 μm, $p < 0.0001$) while there was no significant reduction in eyes treated only with betablockers (mean 3.88 ± 13.00 μm, $p = 0.16$). At follow-up, the mean number of topical medications was significantly higher ($p = 0.03$) for the thinner CCT group (1.54 ± 0.72) as compared to the thicker CCT group (1.32 ± 0.55) reflecting the greater need for topical therapy in these eyes due to their comparitively advanced glaucoma status.

There was no significant difference (Fisher's test, $p = 0.17$) in the use of prostaglandins or a combination of prostaglandins and betablockers amongst "progressing" eyes (69.2%) and "non progressing" eyes (72.1 %). There was a greater proportion of progressing eyes in the thinner group (19/91 eyes = 20.9 %) compared with the thicker group (7/72 eyes = 9.7 %), however, this was not statistically significant (Fishers test , $p = 0.08$).

Multivariate logistic regression analysis incorporating baseline factors including age, gender, CCT, IOP, MD and CCT change demonstrated that a thinner CCT (Odds Ratio = 1.80, 95% CI 1.11 TO 2.95 , $p = 0.02$) but not CCT change (Odds Ratio = 1.07, 95% CI .77 to 1.47, $p = 0.69$) was a significant risk factor for glaucoma progression (Table 8).

Table 8 : Logistic regression analysis of baseline factors for predicting glaucoma progression

Predictive factor	Univariate model		Multivariate model	
	OR (95% CI)	p value	OR (95% CI)	p value
Age per decade older	1.18 (0.80 -1.75)	0.41	1.15 (0.75 -1.76)	0.52
Female gender	1.12 (0.49 - 2.53)	0.78	0.86 (0.80 -1.75)	0.41
Baseline CCT, per 40µm thinner	1.77 (1.11 – 2.80)	0.02	1.80 (1.11 - 2.95)	0.02
CCT change per 10µm thinner	1.00 (0.75- 1.33)	0.99	1.07 (0.77 -1.47)	0.69
Baseline IOP per mm hg higher	0.98 (0.89 – 1.07)	0.69	1.01(0.92 -1.10)	0.85
Baseline MD, per dB greater	1.00 (0.98 – 1.01)	0.99	1.00 (0.98 -1.02)	0.99
Baseline vertical CDR	0.99 (0.98 – 1.01)	0.68	0.99 (0.99 -1.01)	0.88

OR – Odds Ratio, CCT= central corneal thickness, um = microns, IOP = intraocular pressure, mmHg = Millimeters mercury, MD = Mean deviation, Db = decibels, CDR = cup disc ratio, CI = Confidence interval

The GPA rate of visual field change was significantly greater (p = 0.05) for thinner eyes than for thicker eyes, the rates being -0.33 ± 0.65 % per year and -0.11 ± 0.51 % per year respectively. Kaplan Meier analysis demonstrated higher survival in thicker eyes as compared

to thinner eyes ; this was not statistically significant (p = 0.07, log rank test).When Kaplan Meier analysis was performed using both eyes per patient, thicker eyes had a significantly higher (p = 0.001) survival as compared to thinner eyes and the curves diverged further. Although there were relatively more NTG subjects in the thinner cornea group (28.1% in the thinner group versus 11.3% in the thicker group), we did not find any significantly different trends between POAG and NTG for any of the comparisons. This may result from smaller sample sizes in the sub-analyses, but we could not establish any greater change in CCT or greater risk of progression in NTG versus POAG.

4.5 DISCUSSION

CCT is an important parameter in glaucoma management: it impacts on the IOP measurements by applanation tonometry.[38,41,193] and seems to be a predictive factor for the development of POAG. [36,175] We examined the relationships between both baseline CCT and CCT change over time to visual field progression in glaucoma patients .We found that eyes with CCT of 540 microns or less (thinner eyes) had significantly worse perimetric global indices (MD, PSD and VFI) at baseline compared with eyes with CCT thicker than this. HRT data also demonstrated that the eyes with thinner CCT had significantly worse baseline NRR area and baseline CDR compared those with a thicker CCT. Thinner corneas were associated with more advanced damage at presentation. However, there is a wide range of scatter seen in the data such that although statistically significant, one cannot extrapolate that thin corneas are always likely to be worse. This agrees with earlier studies reporting that lower CCT measurements correlated significantly and inversely with the stage of glaucomatous optic neuropathy at presentation.[233,234]

"Progressing" eyes had significantly thinner baseline CCT (518.85 ± 31.92 μm) compared with "non–progressing" eyes (536.97 ± 35.82 μm); there was no significant differences between the two groups for age, amount of CCT change, baseline and final recorded IOP. This correlates well with other studies that have reported that eyes demonstrating visual field progression were associated with a thinner CCT. [221,222,224] In our study, the rate of glaucomatous visual field progression was significantly greater for eyes with a thinner baseline CCT (540 μm or less) compared with eyes with baseline CCT more than 540 μm. There was a greater proportion of "progressing" eyes in the group with thinner baseline CCT and even though Kaplan Meier analysis demonstrated lower visual survival in these eyes, this did not achieve statistical significance. Multivariate analysis did support thinner corneas as associated with increased risk of damage progression.

While several studies [36,174, 175, 221- 225] have consistently reported that thinner CCT is an important predictive factor for glaucoma progression, a few [233 – 235] report that CCT may not be a major determinant of progressive glaucomatous optic nerve damage. In our study, multivariate logistic regression analysis revealed that CCT, but not its change over time (progressive thinning), was a risk factor for glaucoma progression. MD at baseline did not give a higher Odds Ratio, contrary to the notion that more advanced disease is likely to progress more rapidly. This is similar to the findings of an earlier study on risk factors for glaucoma progession.[36,] It also argues against selection bias for eyes with thinner corneas as the explanation for the progression.

Previous cross-sectional [196-198] and longitudinal studies [183, 199] have demonstrated that CCT may decrease over time; with a mean follow up of almost 7 years, we demonstrated a small but significant CCT reduction (mean 12.78 ± 13.35 μm, $p < 0.001$). This amount of change is

not likely to be clinically significant. The relationship of CCT change to visual field progression in glaucoma subjects has been reported in one earlier longitudinal study [183] which was limited by a small sample size and inclusion of normal subjects and glaucoma suspects along with glaucoma patients, some of whom had undergone incisional intraocular surgery. Strengths of our study include a large sample size and exclusion of patients who had undergone laser trabeculoplasty or iridotomy, glaucoma drainage or cataract surgery. This excluded a number of patients who were progressing, as many of these received laser or surgery; we considered such intervention a likely confounder as it might influence CCT. It meant that we recruited patients with mostly mild to moderate disease.

We have in a separate paper analysed the effects of long term medications on CCT and found that prostaglandins or a combination of prostaglandins and betablockers cause a small but significant reduction in CCT compared with betablockers alone.[236] There was however no significant difference in terms of topical prostaglandin use among "progressing" and "non–progressing" eyes .

One potential challenge for our study was upgrading of our ultrasonic pachymeter in 2005; some patients underwent CCT measurement using two different ultrasonic pachymeters. To address this, we compared the amount of CCT change between two groups of eyes, with baseline CCT measured before or after 2005 respectively (the latter group having had both baseline and final CCT measurements with the same pachymeter). We found no significant difference in the amount of CCT change between the two groups (11.26 μm versus 13.26 μm respectively, $p = 0.19$). Although the baseline pachymetry measurements were performed by different operators, all CCTs at the final visit were measured by the same operator; our reproducibility studies demonstrated good inter-observer agreement.

While all eyes showed a small but significant reduction of CCT, there was no difference in the amount of change between "progressing" and "non-progressing" eyes. The amount of change in CCT did not correlate with the change in MD or rim area, in agreement with Weizer et al [183]. Recently Brandt et al [228] reanalysed the OHTS baseline prediction model for the development of POAG after an attempt to adjust IOP for CCT, using various proposed correction formulae. They reported that CCT remained a risk factor for glaucoma progression. CCT appears to be predictive for glaucoma severity at presentation and identifies glaucoma patients at higher risk for visual field progression. Our data supports these findings. However, as suggested,[229] further research is warranted using IOP measurements from CCT independent tonometers before CCT can be validated as an independent risk factor for glaucoma development and progression.

Our study reports that CCT correlates significantly with the stage of glaucomatous damage at diagnosis and that eyes with thinner corneas may have an increased risk for visual field progression. We also report that thinner corneas may progress at a faster rate compared with eyes with thicker corneas and this is a novel finding. While CCT thinned over time in eyes with glaucoma, the amount of change was not related to visual field progression or change in neuroretinal rim area. This observed change in CCT over time is not likely to be clinically significant.

CHAPTER 5: RELATIONSHIP OF STRUCTURAL CHARACTERISTICS TO BIOMECHANICAL PROFILE IN NORMAL, FORME FRUSTE KERATOCONIC, KERATOCONIC AND CROSSLINKED EYES

This chapter describes in detail the influence of corneal structural characteristics on biomechanical behaviour in normal, forme fruste keratoconic, keratoconic and crosslinked keratoconic eyes. Results of this study have been discussed in detail. The paper has been accepted for publication as: Viswanathan D, Kumar NL, Males JJ, Graham SL. Relationship of Structural Characteristics to Biomechanical Profile in Normal, Keratoconic, and Crosslinked Eyes. Cornea. 2015 Jul;34(7):791-6

5.1 ABSTRACT

Purpose: To evaluate the correlation of corneal biomechanical parameters to structural characteristics in normal, forme fruste keratoconic, keratoconic and collagen crosslinked eyes.

Methods: This was a prospective observational study of 50 normal, 10 forme fruste keratoconic, 100 keratoconic and 25 crosslinked eyes. All eyes were imaged using the Scheimpflug camera Pentacam and the Ocular response analyser. The main outcome measures were central corneal thickness (CCT), corneal volume (CV), maximal keratometry (Kmax), corneal hysteresis (CH) and corneal resistance factor (CRF).

Results: Significant differences were noted between all four groups of eyes for CCT, CV, Kmax, CH and CRF values ($p < 0.05$ by ANOVA). CH and CRF correlated negatively (CH: $r = -0.40$, CRF: $r = -0.44$, both $p < 0.0001$) with the Pentacam topographic keratoconus classification. Both CH and CRF correlated positively with CCT and CV for normal,

keratoconic and crosslinked eyes. In contrast, significant negative correlations were observed between CH, CRF and Kmax in keratoconic eyes (CH: r = -0.43, CRF: r = -0.53; both p < 0.0001) whereas no association was noted for normal and crosslinked eyes.

Conclusions: Corneal biomechanical parameters progressively decrease as severity of keratoconus increases. CH and CRF are influenced by corneal structure, with higher values noted in corneas with greater thickness and volume and flatter curvature. Collagen crosslinking appears to normalise the relationship of corneal curvature to biomechanical profile in keratoconic eyes.

5.2 INTRODUCTION

The cornea functions as the principal refractive component of the eye and as a protective layer owing to its unique combination of optical transparency and mechanical stiffness.[1,2] The predominant structural component of the cornea is the stroma that is made up of uniformly distributed collagen fibrils embedded in an extracellular proteoglycan matrix. The distinct microscopic organisation of the cornea endows it with the biomechanical properties of elasticity and viscosity.[12-14]

The Ocular response analyser (ORA; Reichert Ophthalmic Instruments, Buffalo, NY) is the commonest device used to evaluate corneal biomechanical characteristics. The ORA utilizes a rapid air impulse and an advanced electro-optical system to measure changes in corneal shape in response to indentation.[97,99] The corneal hysteresis (CH) and the corneal resistance factor (CRF) are the main biomechanical parameters derived through this deformation process. CH

is a measure of corneal tissue properties that result from viscous damping and CRF appears to indicate the overall resistance of the cornea.[100,106]

Keratoconus (KC) is a degenerative corneal disorder characterised by progressive corneal thinning and conical protrusion resulting in irregular astigmatism and visual impairment [66,105] Altered corneal collagen structure and organization resulting in weakened biomechanics are key factors associated with KC development.[107,108]Keratoconus is usually bilateral but asymmetric and could occur as an early subclinical form known as forme fruste keratoconus (FFKC).[65]

Currently, collagen crosslinking (CXL) using ultraviolet-A (UVA) and Riboflavin is the only treatment available to arrest keratoconus progression. Riboflavin functions as a photosensitizer that is activated by UVA radiation and this photochemical reaction induces the release of oxygen radicals. This leads to the formation of new covalent bonds between collagen fibrils, thereby stiffening the corneal stroma. [154-156] Flattening and regularisation of the conical corneal shape with reduction in myopia and astigmatism is noted as a favorable side effect of CXL in some eyes.[134,164]

Studies have demonstrated that CH and CRF measurements are significantly lower in KC and FFKC eyes.[52,64,69,115,117] Although ex-vivo studies have established an increase in corneal stiffness after CXL, no studies have reported a significant increase in ORA parameters CH and CRF after CXL.[155,156,237] In normal eyes, CH and CRF are known to be influenced by corneal thickness, being higher in thicker corneas probably due to increased collagen.[50] Some studies have reported on corneal structural parameters affecting CH and CRF in normal

eyes, [50,99,106,238, 239] however few have reported on FFKC, keratoconic and crosslinked KC eyes. [52,237]

The Pentacam is a rotating scheimpflug camera (Oculus Inc, Wetzlar, Germany) that is routinely used for evaluating the cornea and diagnosing keratoconus. The Pentacam provides corneal structural parameters including thickness, curvature and volume measurements. The Pentacam also provides a topographic keratoconus classification (TKC) adapted from the Amsler Krumeich grading which is useful in monitoring disease progression.[135,139] The objective of this study is to evaluate the relationship between corneal structural and biomechanical characteristics in normal, FFKC, keratoconic and crosslinked KC eyes.

5.3 METHODS

Study participants

Subjects were recruited prospectively from the Macquarie University Ophthalmology clinic and the Sydney Cornea clinic, Sydney. The study protocol was approved by the Macquarie University human ethics committee and followed the tenets of the Declaration of Helsinki. Fifty normal eyes (50 subjects), 10 FFKC eyes (10 subjects) and 100 Keratoconic eyes (65 subjects) were included. Normal eyes were characterised by the absence of ocular disease, previous ocular surgery or trauma.

Keratoconic eyes (KC) were diagnosed based on the presence of typical corneal signs including steepening, thinning, Fleischer's ring, Vogt striae and apical scarring.[240,241] Keratoconic eyes were also detected by the Pentacam as KC and staged according to disease severity. Eyes with prior corneal hydrops or corneal surgery were excluded. FFKC eyes were defined as fellow eyes in a subject with clinically manifest keratoconus in the other eye.[65]

FFKC eyes were not diagnosed as KC on the Pentacam TKC. Crosslinked eyes were included at 4-6 months after the CXL procedure.

Measurements

Each subject was first measured with the Pentacam and thereafter with the ORA. Contact lens wearers were instructed to avoid wearing lenses for 72 hours prior to measurements.

Pentacam: The Pentacam is a Scheimpflug camera that takes multiple slit images of the anterior segment in less than 2 seconds while rotating 180° around the eye. The device uses a measurement wavelength of 475 nm (blue light-emitting diode) and captures 25,000 different elevation points during the scan. All Measurements were obtained with subjects in a sitting position looking correctly at the fixation target in order to minimise the risk of a displaced apex syndrome. In this study, the auto-measurement mode measuring 25-images-per-scan was chosen. Only scans that the Pentacam's 'Quality specification'(QS) function determined as 'OK' were included for analysis. The corneal parameters measured were: central corneal thickness (CCT in microns), maximal keratometry (Kmax in dioptres), corneal volume (CV in mm^3) and grade of KC according to TKC.

Ocular Response Analyser: The ORA determines biomechanical waveform measurements obtained from inward and outward air puff applanation processes. Subjects were well-positioned with their forehead placed parallel against the head rest and asked to blink gently before taking measurements. All ORA waveforms were reviewed to ensure that they showed adequate amplitude and shape and at least two measurements were performed per subject and averaged for statistical analysis. The ORA parameters CH and CRF were noted and compared between the 3 groups of eyes.

Statistics:

For each normal subject, the data from one randomly selected eye (Microsoft excel randomisation function) were included for analysis. Keratoconus being an asymmetric disorder, data from both eyes was used for analysis. The 4 groups were matched according to age and gender. The measurements obtained from both devices were described as mean ± standard deviation (SD). Differences in values between the 4 groups of eyes was examined using the analysis of variance (ANOVA) test.

Correlations were analysed between TKC, Pentacam and ORA parameters using the Spearman correlation coefficient. Statistical analysis was performed using the Graphpad prism 6 (GraphPad Software Inc., CA, USA). For all analysis, a p value of less than 0.05 was considered statistically significant.

5.4 RESULTS

Significant differences were noted between all four groups of eyes for corneal structural (CCT, CV, Kmax) and biomechanical parameters (CH and CRF) as shown in Table 9.

Table 9: Comparison of corneal structural and biomechanical parameters between groups

Parameter	Control	FFKC	Keratoconic	Crosslinked
No of eyes (subjects)	50 (50)	10 (10)	100 (65)	25 (21)
Mean age ± SD(years)	29.74 ± 6.02	26.30 ± 10.06	27.36 ± 8.40	25.04 ± 7.08
Male gender (%)	35 (70%)	7(70%)	73 (73%)	19 (76%)
Mean central keratometry (D)*	42.69 ± 1.09	42.72 ± 1.53	47.48 ± 4.69	47.52 ± 3.09
Maximal keratometry (D)*	43.53 ± 1.15	44.70 ± 1.79	55.21 ± 7.31	55.66 ± 5.48
Corneal astigmatism (D)*	0.76 ± 0.42	1.40 ± 0.99	3.86 ± 2.12	3.78 ± 1.47
Central corneal thickness (µm)*	542.70 ± 34.77	517.10 ± 34.22	488.19 ± 37.82	489.00 ± 38.86
Thinnest corneal thickness(µm)*	539.58 ± 34.97	509.40± 35.72	450.42 ± 72.39	466.04 ± 35.82
Corneal volume (mm^3)*	59.60 ± 3.98	57.03 ± 3.59	57.09 ± 3.58	58.91 ± 4.08
Anterior chamber depth (mm)*	2.99 ± 0.40	3.21 ± 0.21	3.41 ± 0.32	3.45 ± 0.31
Corneal Hysteresis (mm Hg)*	10.07 ± 1.73	9.17 ± 1.55	8.08 ± 1.77	8.56 ± 1.68
Corneal resistance factor (mm Hg)*	9.82 ± 1.88	8.32 ± 1.48	6.87 ± 2.04	7.47 ± 1.88

* $p < 0.05$ by ANOVA between all groups.

CH correlated negatively ($r = -0.40$, $p < 0.0001$) with the Pentacam topographic keratoconus classification as shown in figure 22. Similarly, a negative correlation was noted between CRF and Pentacam TKC ($r = -0.44$, $p < 0.0001$) as shown in figure 23. Therefore, CH and CRF values decreased as severity of keratoconus increased.

Figure 22: Correlation between CH and TKC

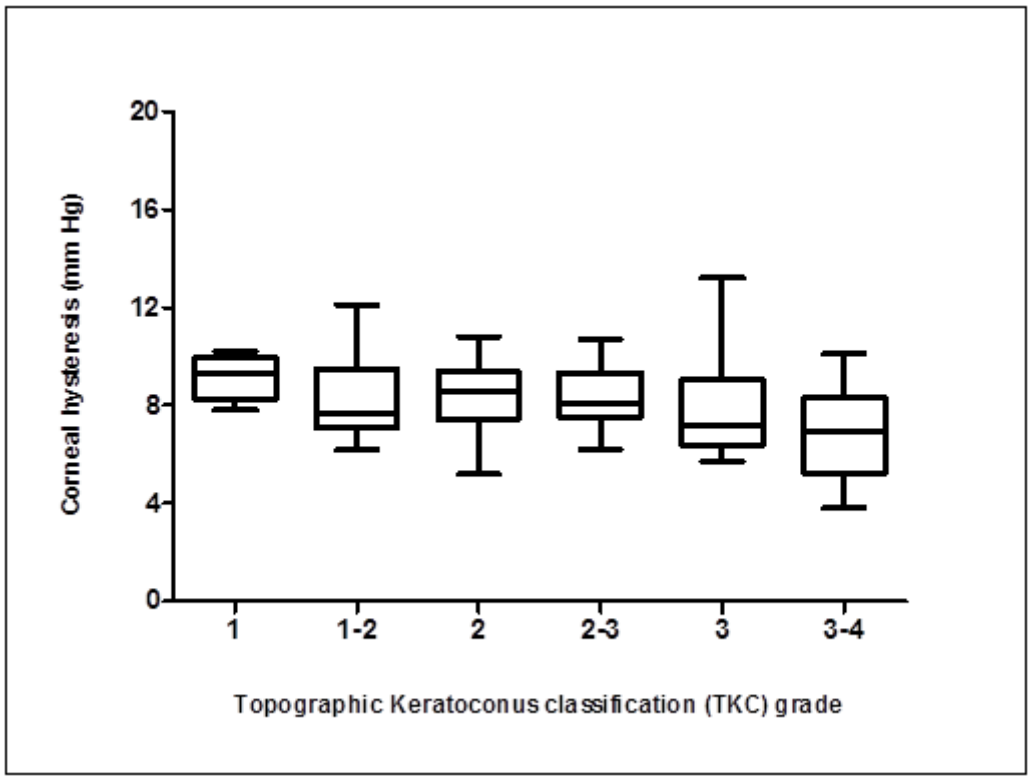
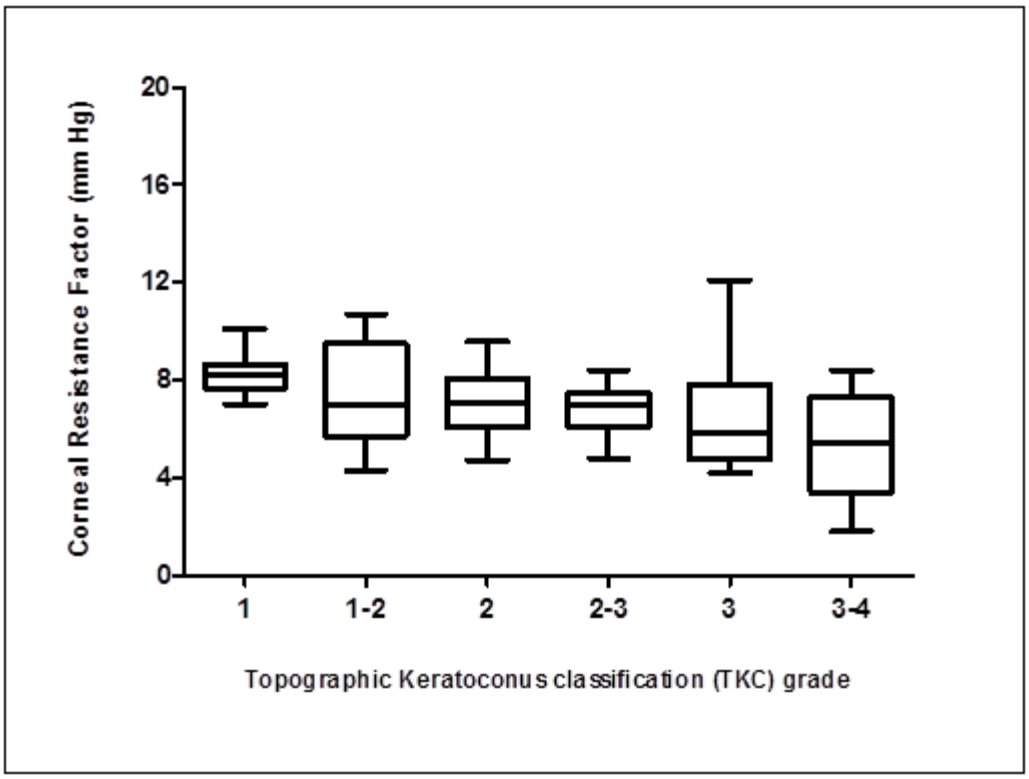
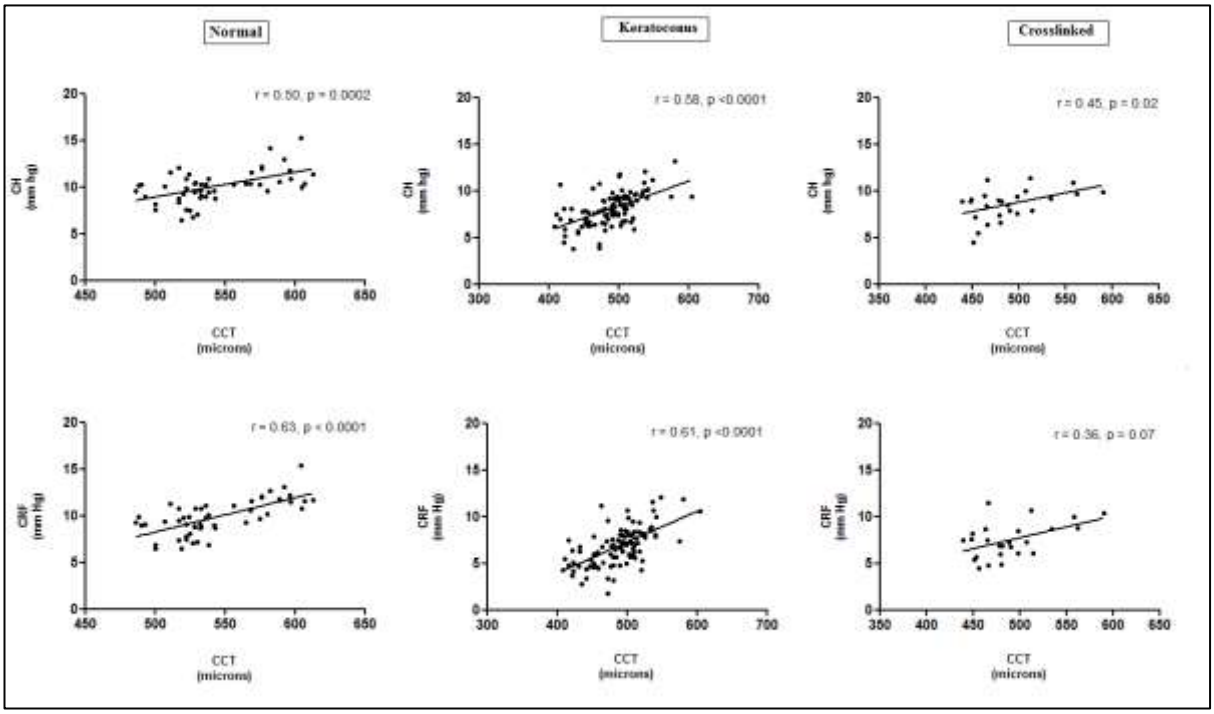


Figure 23: Correlation between CRF and TKC



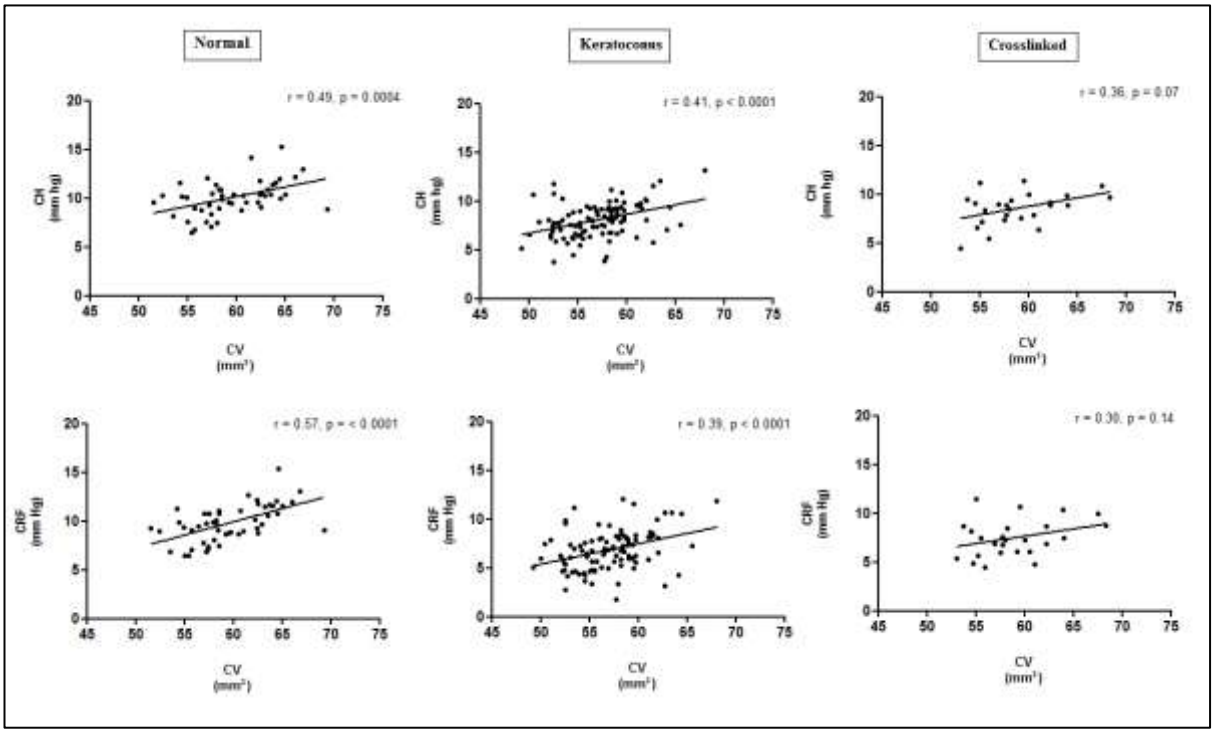
Both CH and CRF correlated positively with CCT in normal, keratoconic and crosslinked eyes as shown in figure 24. Similar positive correlations were noted in FFKC eyes (CH: $r = 0.38$, $p = 0.28$, CRF: $r = 0.47$, $p = 0.18$).

Figure 24: Correlations between CH, CRF and CCT



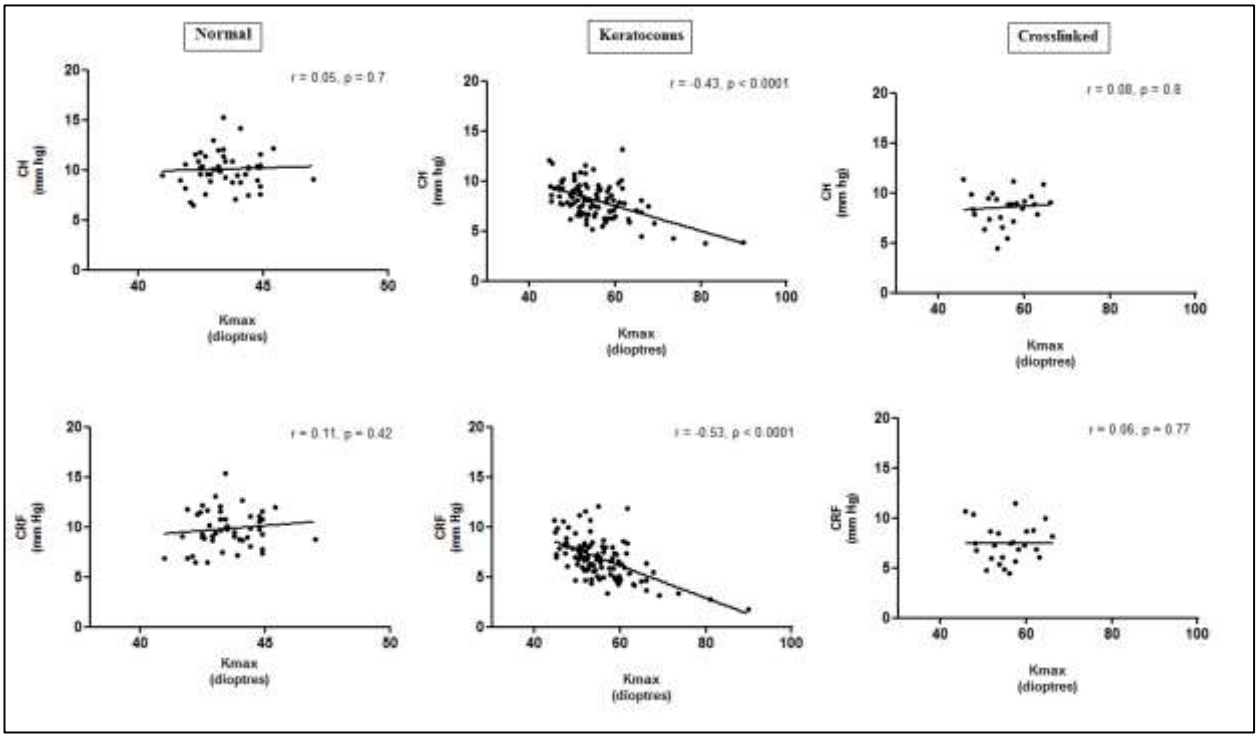
Similarly, positive correlations were noted between CH, CRF and CV in normal, keratoconic and crosslinked eyes as shown in figure 25. Positive correlations were likewise noted in FFKC eyes (CH: $r = 0.22$, $p = 0.54$, CRF: $r = 0.25$, $p = 0.25$).

Figure 25: Correlations between CH, CRF and CV



Therefore, as corneal thickness and volume increase, CH and CRF values are greater. In contrast, significant negative correlations were observed between CH, CRF and Kmax in keratoconic eyes (CH: $r = -0.43$, CRF: $r = -0.53$; both $p < 0.0001$). In FFKC eyes, non significant negative correlations were noted between Kmax and ORA parameters (CH: $r = -0.29$, $p = 0.42$, CRF: $r = -0.24$, $p = 0.51$). Interestingly, no significant associations were noted for normal (CH: $r = 0.05$, CRF: $r = 0.11$; both $p > 0.05$) and crosslinked eyes (CH: $r = 0.08$, CRF: $r = 0.06$; both $p > 0.05$) (figure 26).

Figure 26: Correlations between CH, CRF and Kmax



5.5 DISCUSSION

Corneal thickness, curvature and volume are principal structural attributes of the cornea. In addition, biomechanical parameters CH and CRF provide a more comprehensive characterization of corneal properties. Two major causes for changes in corneal biomechanical profile include pathologies like keratoconus and treatment-related corneal alterations. [242] In this study, we chose to analyse the impact of corneal structural characteristics on the biomechanical profile in normal, forme fruste keratoconic, keratoconic and collagen crosslinked eyes.

Keratoconus is a progressive corneal ectatic disorder characterized by non- inflammatory biomechanical degeneration that results in thinning and conical protrusion. In keratoconic corneas, the intrinsic collagen network is mostly unorganized with decreased fibrillar interweaving. Keratoconic corneas also exhibit increased levels of collagenolysis, loss of keratocytes and reduced collagen cross-links.[243,244] Disease progression is thought to originate with focal degeneration of material properties followed by slippage of collagen fibrils and changes in the stromal extracellular matrix. This results in a cycle of further thinning, increased strain, and redistribution of stress.[109,245]

We noted significant differences between normal, forme fruste keratoconic and keratoconic eyes for CH and CRF values. This is in consistent with earlier studies that demonstrated lower CH and CRF values in FFKC and KC eyes as compared to normal eyes albeit wide overlap.[52,64,69,115,117] In this study, the mean CH values were 10.07 ± 1.73 mm Hg in normal, 9.17 ± 1.55 mm Hg in FFKC and 8.08 ± 1.77 mm Hg in KC eyes. In comparison, Duncan et al reported CH values of 11.0 ± 1.4 mm Hg for normal, 8.8 ± 1.4 mm Hg for FFKC and 7.9 ±1.3

mm Hg for KC eyes.[65] Similarly, Shah et al noted CH values of 10.7 ± 2.0 mm Hg in normal eyes and 9.6 ± 2.2 mm Hg in KC eyes. [115]

Few studies have reported that there is a decrease in CH and CRF values as severity of keratoconus increases.[52,115,246,247] In this study, keratoconus was graded according to the Pentacam TKC which is a precise classification based on the Amsler grading system that incorporates corneal asymmetry indices and corneal thickness progression.[139] Our study corroborated these findings with lower CH and CRF values recorded in higher grades of keratoconus. Although to some extent this may be due to a decrease in corneal thickness and volume, it is mainly because of the altered structure of the stromal proteoglycans leading to lower lamellar adhesion and a lower shear modulus.[248,249]

Significant and positive correlations were observed between CH, CRF and corneal thickness as well as corneal volume in normal, keratoconic and crosslinked eyes. Similar findings were noted in normal and keratoconic eyes by previous studies. [50, 237- 239] The inference from these findings is that thicker corneas contain more collagen fibers and ground substance, resulting in a greater resistance against deformation and a higher damping capacity.

Corneal curvature has also been shown to influence biomechanical characteristics.[237]
A study by Lim et al showed a strong negative correlation between CH and maximum keratometry value in normal eyes such that CH was lowered by 1 mmHg per 6 D of corneal steepening.[250] In the present study, there was a strong negative correlation between CH, CRF and maximal keratometry in keratoconic eyes. However, we observed no associations between CH, CRF and maximal keratometry values in normal and crosslinked eyes. In a

multiple regression model, Gkika et al noted insignificant associations between corneal thickness and curvature values and ORA parameters before and after CXL.[237]

FFKC eyes appear to have associations similar to keratoconic eyes. However, no significance was demonstrated possibly due to limited number of eyes studied. From our findings, it would appear that CXL normalises the relationship of corneal curvature to biomechanical profile in keratoconic eyes. This agrees well with Hersh et al and Raiskup wolf et al who reported an improvement in corneal shape after CXL.[134,164] Previous studies on ORA measurements before and after CXL have showed no significant change in CH and CRF values.[237,251] The ORA provides dynamic measurements that describe the mechanical inertia and viscous properties of the cornea. As CXL does not alter interlamellar cohesion, using a static contact-based technique might enhance detection of the biomechanical effect of CXL on the cornea.[252,253]

To summarise, we demonstrated that CH and CRF are influenced by corneal geometric attributes, being higher in corneas with greater thickness and volume. In keratoconus, negative associations were noted between corneal biomechanical parameters and curvature with lower values noted in advanced cases. However, collagen crosslinking seems to normalise the relationship between corneal curvature and biomechanical values in keratoconic eyes. Further studies with greater number of corneal pathologies including keratoconus suspect eyes are required to determine how corneal structure influences biomechanical profile.

CHAPTER 6: COMPARATIVE ANALYSIS OF CORNEAL MEASUREMENTS OBTAINED FROM A SCHEIMPFLUG CAMERA AND AN INTEGRATED PLACIDO-OPTICAL COHERENCE TOMOGRAPHY DEVICE IN NORMAL AND KERATOCONIC EYES

This study was performed to compare corneal measurements in normal and keratoconic between two commercially available imaging devices that use different technologies. Furthermore, the intra-operator repeatability and inter-operator reproducibility for both devices were assessed. Results of this study have been discussed in detail and the paper has been published as: Viswanathan D, Kumar NL, Males JJ, Graham SL. Comparative analysis of corneal measurements obtained from a Scheimpflug camera and an integrated Placido-optical coherence tomography device in normal and keratoconic eyes. Acta Ophthalmol. 2014 Dec 14. doi: 10.1111/aos.12622

6.1 ABSTRACT

Purpose: To assess the agreement between a Scheimpflug camera (Pentacam) and a combined Placido-optical coherence tomography device (Visante OMNI) in measuring corneal curvature, thickness and elevation values in normal and keratoconic eyes.

Methods: Corneal measurements of 110 normal eyes (one eye per subject) and 70 keratoconic eyes were obtained from both devices and compared. Agreement was determined using the Bland- Altman analysis 95% limits of agreement (LoA).

Results: The Pentacam measured significantly greater keratometry readings in the flattest (K1) and steepest meridians (K2) in normal and keratoconic eyes. The 95% LoA in normal

eyes were -0.32 to 0.59 dioptres (D) (K1) and -0.41 to 0.74 D (K2). In keratoconic eyes, the 95% LoA were -1.35 to 1.92 D (K1) and -1.38 to 1.99 D (K2).

The Pentacam recorded significantly higher central corneal thickness (CCT) values in both groups of eyes. The 95% LoA were -4.31 to 39.89 microns (µm) and -12.92 to 41.35µm in normal and keratoconic eyes respectively. Pentacam anterior and posterior corneal elevations were significantly greater in both groups of eyes. The devices demonstrated excellent repeatability and reproducibility for corneal curvature and thickness but not elevation measurements.

Conclusions : The Pentacam measured significantly greater corneal curvature, thickness and elevation values compared to the Visante OMNI in normal and keratoconic eyes. The devices agree well only for anterior corneal elevations in normal eyes and do not appear to be interchangeable for corneal measurements in clinical practice.

Key words: Corneal curvature, corneal thickness, corneal elevations, keratoconus, Scheimpflug camera, Placido principle

6.2 INTRODUCTION

Refractive surgery is an increasingly popular option for correction of ametropia. Screening patients to ensure their candidature for surgery and to exclude keratoconus or other corneal anomalies is of paramount importance. This involves obtaining precise corneal curvature, thickness and elevation measurements. Furthermore, highly repeatable and reproducible measurements are mandatory to observe time related corneal changes.

Keratoconus is an asymmetric corneal disorder characterized by progressive corneal thinning and protrusion.[109] There is great emphasis on early detection and subsequent management of these cases using newer refractive surgical options.[254,255] Accurate corneal imaging is

therefore essential to diagnosis keratoconus, facilitate patient selection and study the efficacy of these procedures.

Conventional corneal topography systems have been based on the Placido principle that measures the anterior corneal curvature.[256] Clinical studies have demonstrated that examination of the posterior corneal surface can often reveal pathology in eyes that have completely normal anterior curvature.[257] Elevation-based Scheimpflug imaging has the ability to analyse both anterior and posterior corneal surfaces and thus offers an advantage over isolated placido topography.[257, 258]

Ultrasound pachymetry is currently considered the gold standard for corneal thickness measurement. Being a contact technique, it is associated with certain inherent disadvantages.[71,72] The Pentacam (Oculus Inc, Wetzlar, Germany) is a rotating Scheimpflug–based camera that provides a reliable assessment of the anterior and posterior corneal surface and corneal thickness.[259,260] Optical coherence tomography (OCT) is another established technology for imaging the anterior segment and providing noncontact corneal thickness measurements.[90]

The Visante OMNI (Carl Zeiss Meditec, Jena, Germany) is a newer corneal imaging device. This is a hybrid created by linking the Placido based topographer (Atlas) to an optical coherence tomography device (Visante OCT).The OMNI integrates Atlas corneal topography data with the OCT corneal thickness data and provides in addition, posterior corneal elevation data.[95] The purpose of this study is to assess and compare corneal measurements in normal and keratoconic eyes generated by these imaging systems using different technologies.

6.3 METHODS

Study participants

This study was conducted at the Macquarie University Ophthalmology Clinic, Australian School of Advanced Medicine, Sydney. The study protocol was approved by the medical ethics committee and adhered to the principles proposed by the declaration of Helsinki. One hundred and ten normal eyes (110 subjects) and 70 keratoconic eyes (40 subjects) were included after obtaining a written informed consent.

Normal eyes were associated with no ocular disease, previous ocular surgery or trauma, no contact lens use and had refractive errors $\leq$ 1.00 dioptres (D) (spherical equivalent). Keratoconic eyes were diagnosed based on the presence of typical corneal steepening, and one or more of the following signs: corneal thinning, Fleischer's ring, Vogt striae and apical scarring.[109,261] Eyes with previous corneal hydrops or corneal surgery were excluded from the study. Contact lens wearers were instructed to avoid wearing lenses for 72 hours prior to measurements.

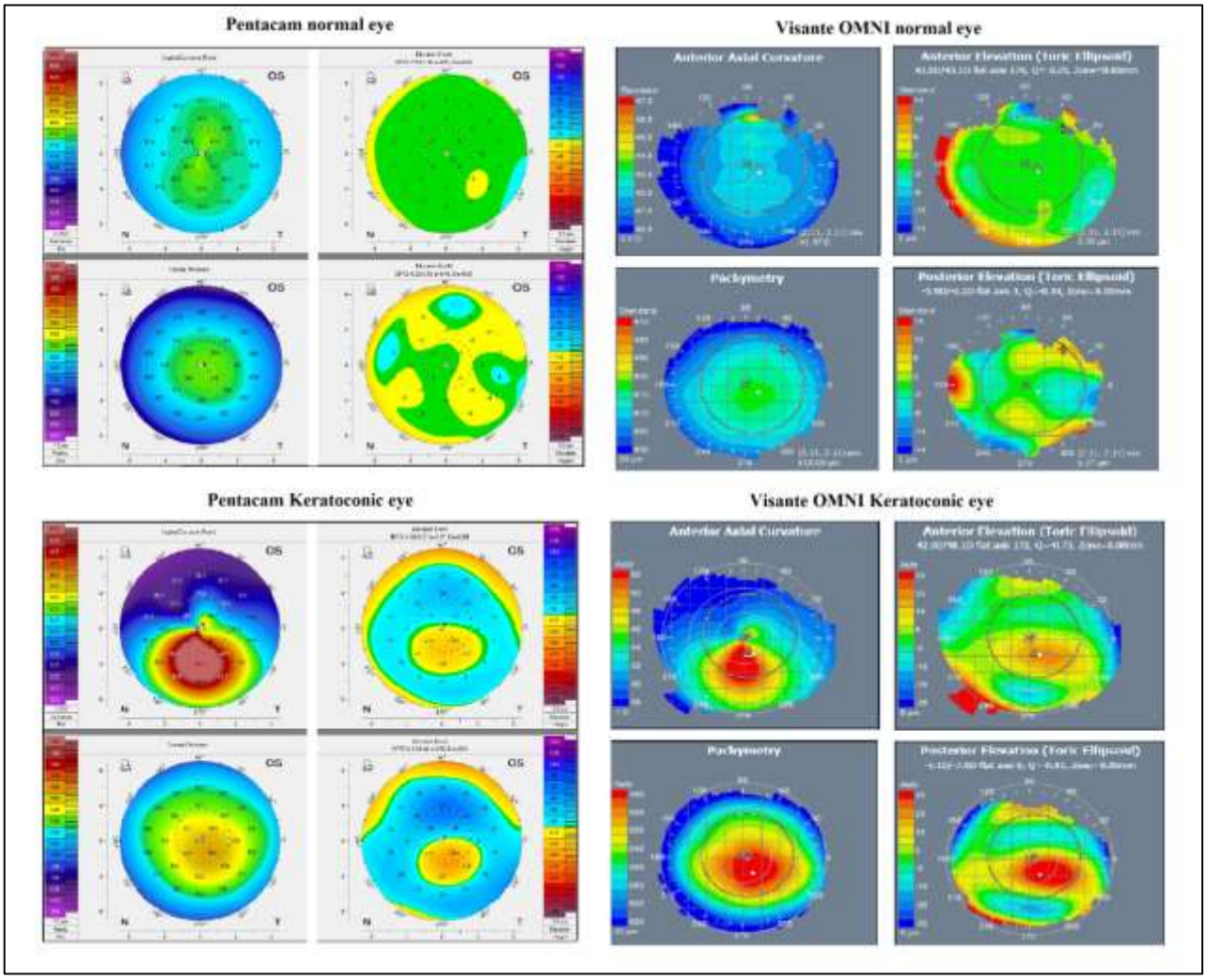
Measurements

Each individual was first measured with the Pentacam and the Visante Omni by a single experienced operator. A subset of 20 normal subjects (20 eyes) underwent repeat measurements, once by the same operator and thereafter by another experienced operator to assess intra operator repeatability and inter operator reproducibility. Each measurement was taken approximately 3 to 5 minutes apart.

The following corneal parameters were measured from both Pentacam and the Visante OMNI (Figure 27) : corneal dioptric powers (Simulated K readings) in the flattest (K1) and

steepest (K2) meridians, central corneal thickness (CCT) and minimum corneal thickness (MCT). Corneal elevations were read in relation to a best fit toric ellipsoid reference (BFTE 8 mm) at the thinnest point of the cornea as this closely resembles the actual corneal surface and therefore is more sensitive to local changes than a reference sphere.[132]

Figure 27: Comparison of corneal parameters between the Pentacam and the Visante OMNI in normal and keratoconic eyes



Pentacam: The Pentacam device uses a measurement wavelength of 475 nm (blue light-emitting diode) and there are 25000 measurement points. A Scheimpflug high resolution camera takes multiple slit images of the anterior segment in less than 2 seconds while rotating 180° around the eye. Measurements were obtained with subjects in a sitting position looking at the fixation target according to the manufacturer's instructions. For this study, the 25-images-per-scan mode and the auto-measurement mode were chosen. Only scans that the Pentacam's 'Quality specification'(QS) function determined as 'OK' were included for analysis.

Visante OMNI: Initially, corneal measurements were performed on each eye of the subject using the Atlas corneal topographer (version 3.0). The subject's information and anterior corneal surface data (7960 data points) are automatically transferred to the Visante OCT via the network link. Then the subject was positioned at the Visante OCT station to measure corneal thickness (global pachymetry: 2048 data points) using the auto corneal vertex alignment. Posterior corneal elevation and curvature data was derived by the system software by integrating the anterior corneal surface data derived from the Atlas with the corneal thickness data from the Visante OCT through precise corneal vertex alignment.[13] The anterior and posterior corneal elevations were obtained by placing the cursor at the thinnest point.

Statistics:

For each normal subject, the data from one randomly selected eye (Microsoft excel randomisation function) was included for analysis. Keratoconus being an asymmetric disorder, data from both eyes was used. Statistical analysis was performed using SPSS software (version 19.0; SPSS Inc, Chicago, Illinois, USA). The measurements obtained from

both devices were described as mean ± standard deviation (SD) and the difference between these values was examined using a paired t test.

Bland–Altman analysis was performed to assess the 95% limits of agreement (mean of the difference ± 1.96 times standard deviation) between both systems.[190] The level of agreement between two devices is determined by the magnitude of these limits with lower values indicating better agreement and vice versa. This judgement regarding the limit at which it is acceptable to use the two devices interchangeably is a clinical decision.[191]

Repeatability and reproducibility were determined using the Intraclass correlation coefficients (ICCs). The ICCs range from 0 to 1 and are interpreted as follows: < 0.75 (poor agreement), 0.75 to 0.90 (moderate agreement) and > 0.90 (good agreement).[192] For all analysis, a p value of less than 0.05 was considered statistically significant.

6.4 RESULTS

One hundred and ten eyes of 110 normal subjects (49 males, 61 females) and 70 eyes of 40 keratoconic subjects (22 males, 18 females) were included. The mean age of normal subjects was 32.49 ± 13.22 years (range 15 – 72 years). The mean age of keratoconic subjects was 33.74 ± 12.35 years (range 17 – 63 years).

Corneal curvature

Compared to Visante OMNI, the Pentacam measured significantly greater simulated keratometry readings in normal and keratoconic eyes (Table 10). In normal eyes, the 95% LoA were from -0.32 to 0.59 dioptres (D) (K1) and from -0.41 to 0.74 D (K2). In keratoconic

eyes, wider 95% LoA were noted from -1.35 to 1.92 D (K1) and from -1.38 to 1.99 D (K2).

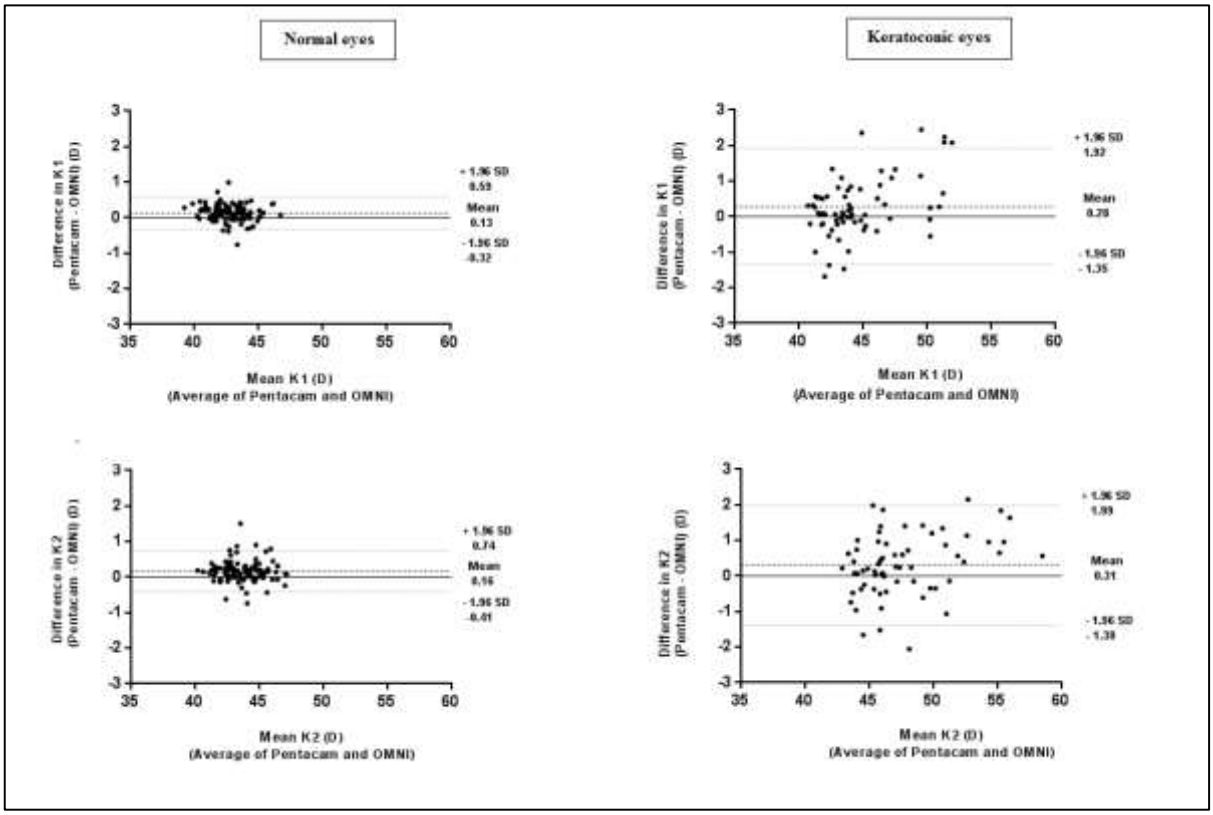
Bland-Altman plots for differences in corneal curvature values are shown in Figure 28.

Table 10: Comparison of corneal curvature values measured by both devices

Parameter	Normal eyes	Keratoconic eyes
Pentacam K1 (D) Visante OMNI K1(D) p value	42.82 ± 1.42 42.69 ± 1.45 < **0.0001**	44.72 ± 3.27 44.44 ± 2.89 **0.007**
Pentacam K2 (D) Visante OMNI K2(D) p value	43.75 ± 1.50 43.59 ± 1.51 < **0.0001**	47.97 ± 4.11 47.66 ± 3.85 **0.005**

K1 = Simulated K reading in the flattest meridian, D = Dioptres
K2 = Simulated K reading in the steepest meridian

Figure 28: Bland-Altman plots and the differences between both devices in corneal curvatures



Corneal thickness

Corneal thickness values obtained from the Visante OMNI were significantly thinner in both groups of eyes (Table 11). In normal eyes, the Visante OMNI recorded lower central corneal thickness (CCT) by 17.79 ± 11.27µm and minimum corneal thickness (MCT) by 25.02 ± 11.96µm. The 95% LoA were from -4.31 to 39.89µm for CCT and from 1.57 to 48.47µm for MCT.

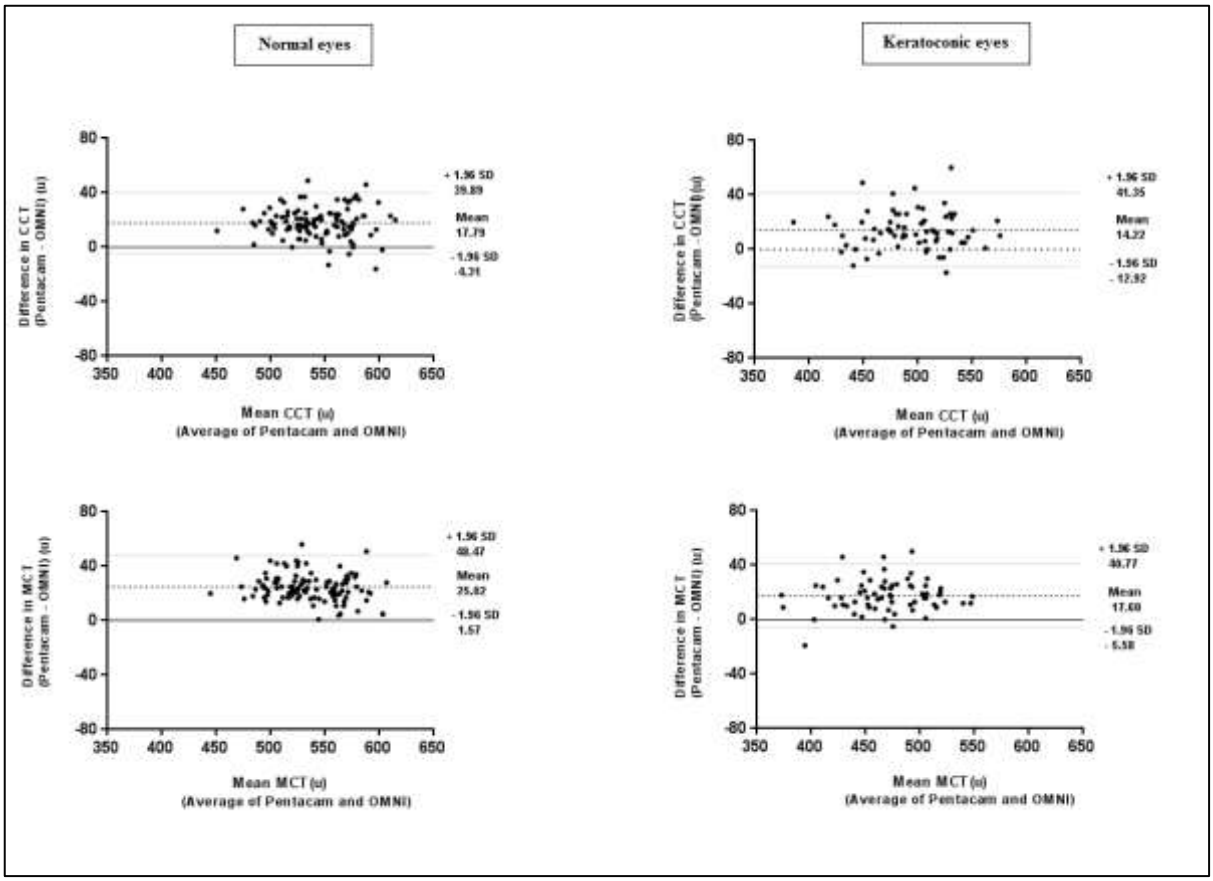
In keratoconic eyes, the Visante OMNI underestimated CCT by 14.22 ± 14.03µm and MCT by 17.60 ± 11.82µm. The 95% LoA were from -12.92 to 41.35µm for CCT and from -5.58 to 40.77µm for MCT. Bland Altman plots for differences in corneal thickness values are shown in figure 29.

Table 11: Comparison of corneal thickness values measured by both devices

Parameter	Normal eyes	Keratoconic eyes
Pentacam CCT (µm) Visante OMNI CCT (µm) p value	552.11 ± 32.61 534.32 ± 32.78 < **0.0001**	501.04 ± 37.61 486.83 ± 37.42 < **0.0001**
Pentacam MCT (µm) Visante OMNI MCT (µm) p value	549.15 ± 32.59 524.13 ± 34.17 < **0.0001**	477.63 ± 40.00 460.03 ± 38.74 < **0.0001**

CCT = Central corneal thickness, µ= Microns, MCT = Minimum corneal thickness

Figure 29: Bland-Altman plots and the differences between both devices in corneal thickness



Corneal elevations

Pentacam anterior and posterior corneal elevations at the thinnest point were significantly higher in normal and keratoconic eyes (Table 12). The 95% LoA were from -2.13 to 4.39µ for anterior elevations and from -6.03 to 12.54 µ for posterior elevations in normal eyes. Similar to corneal curvatures, wider 95% LoA were noted from -17.04 to 25.48µ (anterior elevations) and from -17.25 to 31.34 µ (posterior elevations) in keratoconic eyes.

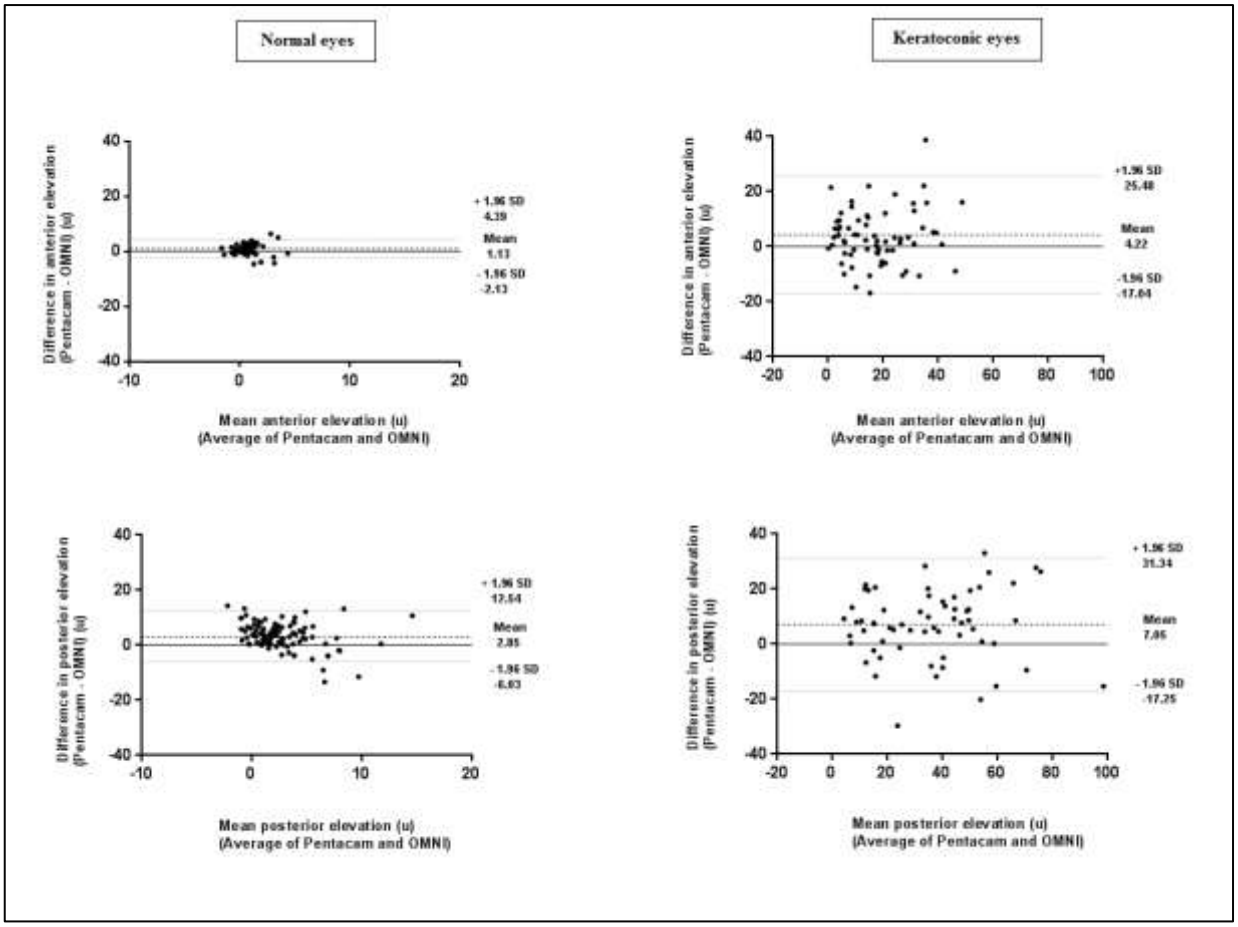
Figure 30 shows the Bland-Altman plots for differences between both devices in corneal elevations. For posterior corneal elevations in normal eyes, the plot shows the pattern in which variation of at least 1 device depends strongly on the magnitude of measurements.

Table 12: Comparison of corneal elevation values measured by both devices

Parameter	Normal eyes	Keratoconic eyes
Pentacam AE (µ) Visante OMNI AE (µ) p value	1.17 ± 1.28 0.04 ± 1.14 **< 0.0001**	20.51 ± 14.70 16.29 ± 12.60 **0.002**
Pentacam PE (µ) Visante OMNI PE (µ) p value	4.24 ± 3.05 0.98 ± 4.16 **< 0.0001**	38.94± 21.77 31.89± 21.24 **< 0.0001**

AE = Anterior elevation at the thinnest point, PE = Posterior elevation at the thinnest point, µ= Microns

Figure 30: Bland-Altman plots and the differences between the 2 devices in corneal elevations



Repeatability and reproducibility

Both Pentacam and Visante OMNI demonstrated excellent intra-operator repeatability and inter-operator reproducibility for corneal curvature and thickness measurements. In contrast, moderate to poor repeatability and reproducibility was observed for elevations, particularly posterior elevation values (Table 13).

Table 13 : Repeatability and reproducibility of both devices

Parameter	Repeatability ICC	Reproducibility ICC
Pentacam K1 (D) Visante OMNI K1(D)	0.995 (0.984 - 0.998) 0.985 (0.952 - 0.995)	0.997 (0.990 - 0.909) 0.995 (0.983- 0.998)
Pentacam K2 (D) Visante OMNI K2(D)	0.993 (0.977 - 0.998) 0.958 (0.874 - 0.987)	0.995 (0.985 - 0.999) 0.994 (0.979- 0.998)
Pentacam CCT (µm) Visante OMNI CCT (µm)	0.984 (0.948 - 0.995) 0.960 (0.878- 0.988)	0.988 (0.961 - 0.996) 0.984 (0.947- 0.995)
Pentacam MCT (µm) Visante OMNI MCT (µm)	0.984 (0.948 - 0.995) 0.968 (0.901- 0.990)	0.994 (0.981- 0.998) 0.990 (0.966 - 0.997)
Pentacam AE (µ) Visante OMNI AE (µm)	0.852 (0.593- 0.952) 0.828 (0.500- 0.942)	0.892 (0.641 - 0.967) 0.854 (0.519 - 0.955)
Pentacam PE (µm) Visante OMNI PE (µm)	0.824 (0.465 - 0.941) 0.741 (0.192 - 0.920)	0.768 (0.393 - 0.923) 0.606 (0.178 - 0.845)

ICC = Intraclass correlation coefficient, K1 = Simulated K reading in the flattest meridian, D = Dioptres, K2 = Simulated K reading in the steepest meridian, CCT = Central corneal thickness, µ= Microns, MCT = Minimum corneal thickness, AE = Anterior elevation at thinnest point, PE = Posterior elevation at thinnest point

6.5 DISCUSSION

This study aimed to compare corneal parameters in normal and keratoconic eyes obtained from two commercially available imaging systems, the routinely used Pentacam and the

recently introduced Visante OMNI. Corneal curvature and thickness measurements are important not only for screening patients for refractive surgery but also for diagnosing corneal disorders and assessing glaucomatous eyes. To the best of our knowledge, this is the first study in the literature to compare these two devices.

The Atlas corneal topographer incorporated in the Visante OMNI works on the Placido principle whereby the anterior corneal surface is illuminated by concentric rings creating a reflected image.[256] A corneal curvature color map is then generated from computer analysis of this image. In comparison, the Pentacam derives keratometry data from cross- sectional Scheimpflug images.

There is a paucity of studies comparing corneal curvature values between the Pentacam and the Atlas topographer. We observed that the Pentacam measured significantly greater keratometry readings in normal and Keratoconic eyes. The 95% LoA were from -0.32 to 0.59 dioptres (D) (K1) and from -0.41 to 0.74 D (K2) in normal eyes. In keratoconic eyes, wider limits were noted from -1.35 to 1.92 D (K1) and from -1.38 to 1.99 D (K2). In contrast, Domenech et al noted wide limits of agreement from to 0.25 to -1.48D (K1) and from 0.38 to -1.54 D (K2) with the Pentacam recording lower keratometry values than the Atlas in normal eyes.[262] This discrepancy is clinically significant as the corneal curvature is one of the principal parameters widely used to detect keratoconus and monitor disease progression.[263,264]

Recently, non contact methods of measuring corneal thickness are preferred over conventional ultrasonic pachymetry. This is due to associated patient discomfort, risk of corneal epithelial defects, potential for transmission of infections and variability of results with this technique.[71,72] The majority of earlier studies comparing the Pentacam to Visante

OCT reported significantly lower CCT values with the Visante OCT in normal eyes.[79,80, 265] Interestingly, O'Donnell et al [266] noted greater CCT measurements with Visante OCT as compared to Pentacam wheras a study by Ponce et al [267] showed no significant discrepancy. Regarding keratoconic eyes, a recent study did not show any significant difference in corneal thickness measurements between both devices.[268] In the present study, the Visante OMNI underestimated CCT by 17.79 ± 11.27 µm and MCT values by 25.02 ± 11.96 µm in normal eyes. Similar findings were noted in keratoconic eyes.

These differences in corneal thickness values may be significant in a clinical setting. An overestimation of CCT may falsely offer reassurance to surgeons that the available corneal thickness is appropriate for refractive surgery. An underestimation may unnecessarily exclude otherwise eligible normal patients from having surgery, or alter their surgical options from LASIK to Surface Ablation or to consider intraocular refractive surgery. Similarly, in keratoconic eyes, an underestimation could potentially prevent patients from undergoing Collagen crosslinking and an overestimation would make the procedure potentially unsafe. Our results indicate that the Pentacam and the Visante OMNI are not interchangeable in clinical practice for corneal thickness values. The reliability of the Pentacam has been reported to be comparable with ultrasound pachymetry which is the gold standard for CCT measurements.[269] The Pentacam also provides more repeatable and reproducible CCT measurements compared to the Visante OMNI and hence would be considered more reliable.

With respect to anterior and posterior corneal elevations at the thinnest point, the Pentacam measured significantly higher values in both normal and keratoconic eyes. Moderate agreement was observed only for anterior corneal elevations in normal eyes. While Bland - Altman analysis demonstrated an inappropriate pattern of agreement for posterior elevations

in normal eyes, wide limits were noted between both devices for anterior and posterior elevations in keratoconic eyes. The 95% LoA for posterior elevations were from -6.03 to 12.54 µm in normal eyes and from -17.25 to 31.34µm in keratoconic eyes. We chose to study posterior corneal elevations at the thinnest point as these values are considered to be particularly sensitive in detecting keratoconus. [132] In our literature search, we did not come across similar studies comparing thinnest point corneal elevations between the Pentacam and the Visante OMNI.

One aspect to be considered is that both devices use different techniques to measure the corneal curvature, thickness and elevation values. While the Pentacam uses a Scheimpflug technique to take cross sectional scans, the OMNI combines two technologies, the Placido technique and the OCT scanning slit technology. The Pentacam measures corneal thickness between the air–tear film interface and the posterior corneal surface and calculates corneal elevations using a software system that constructs a three-dimensional image of the anterior segment from elevation points captured by the rotating camera.[269] The Visante OMNI measures corneal thickness using OCT and then subtracts this data from the anterior corneal surface elevations (measured by ATLAS topographer) to obtain the posterior corneal elevations.[95]

Studies on the individual devices have demonstrated high repeatability and reproducibility for corneal curvature and thickness measurements. [258,270] In this study, both Pentacam and Visante OMNI demonstrated excellent intra-operator repeatability and inter-operator reproducibility for corneal curvature and thickness values. However, lower repeatability and reproducibility was observed for thinnest point elevations, particularly posterior elevation values. Amongst the devices, the Pentacam ICCs were generally noted to be better. This could

be because Visante OMNI measurements require the subject to shift between two devices (Atlas corneal topographer and Visante OCT) thus prolonging scan acquisition time.

Our results compare well with Guilbert et al who compared corneal thickness, curvature and elevation values between a combined Placido-Scheimpflug system and a combined Placido-scanning-slit system and noted poor repeatability only for elevation measurements from both devices.[271] Similarly Núñez et al observed poor repeatability for Pentacam posterior elevation measurements.[272] Recently Srivannaboon et al demonstrated that the Visante Omni provides good repeatable and reproducible posterior corneal elevation values.[95]

However, these studies measured corneal elevations with respect to a best fit sphere (BFS) reference. In this study, we chose a best fit toric ellipsoid (BFTE) reference as this closely resembles the actual corneal surface and therefore is more sensitive to local changes than a reference sphere.[132] The reason for obtaining poorly repeatable elevation measurements has not been elucidated. From our results, it appears that posterior elevations are not reliable for monitoring keratoconus progression.

To summarise, our results indicate that the Pentacam measured significantly greater corneal curvature, thickness and elevation values compared to Visante OMNI in normal and keratoconic eyes. The scatter and variability in measurements is higher for keratoconic eyes than normal eyes probably due to an aberrated corneal surface associated with the condition. The Pentacam and the Visante OMNI agree well only for anterior corneal elevation measurements in normal eyes. Therefore, these two devices are not interchangeable in a clinical setting. One of the strengths of this study is the inclusion of keratoconic eyes in addition to normal eyes. Further studies with larger sample sizes of both normal and abnormal corneas including keratoconus suspect and post refractive surgery are justified.

CHAPTER 7 : SENSITIVITY AND SPECIFICITY OF NOVEL BIOMECHANICAL WAVEFORM PARAMETERS IN DETECTING FORME FRUSTE KERATOCONUS AND KERATOCONUS

This chapter studies the accuracy of newer biomechanical waveform parameters in diagnosing Forme fruste and manifest keratoconus and determines whether these are superior to conventional Ocular response analyser parameters. There are very few studies conducted on this topic and they have been included in the discussion.This study is being submitted for publication to the peer reviewed journal: PLOS one.

7.1 ABSTRACT

Purpose: To evaluate the sensitivity and specificity of Ocular Response Analyser (ORA) indices: Keratoconus Match Index (KMI) and Keratoconus Match Probability (KMP) in distinguishing forme fruste keratoconus (FFKC) and keratoconus (KC) from normal eyes.

Methods: A prospective observational study of 52 normal, 12 FFKC and 110 KC eyes that underwent scheimpflug topography (Oculus Pentacam) and ORA biomechanical assessment. Main outcome measures compared were Pentacam topometric indices and ORA corneal hysteresis (CH), corneal resistance factor (CRF), KMI and KMP. Correlations were analysed between KMI, topographic keratoconus classification (TKC) and topometric indices. Predictive accuracy of KMI was assessed by the Area under curve (AUC) of receiver operating curves (ROC).

Results: Mean KMI was 1.25 ± 0.30 for normal, 0.76 ± 0.59 for FFKC and 0.32 ± 0.53 for KC eyes ($p<0.0001$). Significant negative correlations were noted between KMI and TKC ($r = -0.742$, $p < 0.0001$) and topometric indices. KMI had higher sensitivity and specificity than

CH and CRF in detecting KC (AUC: KMI = 0.929, CH = 0.806, CRF= 0.862) ($p<0.05$) and FFKC (AUC: KMI = 0.771, CH = 0.641, CRF= 0.707 ($p>0.05$). KMP identified 90.1% of normal eyes, 43.2% of FFKC eyes and 22. 09% of KC eyes as normal.

Conclusions: Mean KMI values differ significantly between normal, FFKC and KC eyes. KMI appears to be a reliable indicator of KC diagnosis and severity as compared to CH and CRF values. KMP identifies a significant percent of KC eyes as normal and does not agree well with topographic KC diagnosis.

7.2 INTRODUCTION

Keratoconus (KC) is an ectatic non-inflammatory corneal disorder with an approximate incidence of 1 per 2000 of the general population.[273,274] It is usually bilateral and asymmetric and typically manifests at puberty. Keratoconus is characterised by corneal thinning and conical protrusion causing distorsion of vision.[240, 241, 275] Decrease in collagen fibril diameter and altered collagen fibril alignment resulting in impaired biomechanics have been identified as integral to keratoconus pathogenesis.[106,107]Keratoconus has a wide spectrum of disease severity ranging from early subclinical or forme fruste keratoconus (FFKC) to advanced forms associated with corneal scarring.[276-278]

Currently, the Ocular response analyser (ORA; Reichert Ophthalmic Instruments, Buffalo, NY) is the only commercially available device to assess the corneal biomechanical charectersistics. The ORA is based on a dynamic bidirectional applanation process and provides two principal parameters, the corneal hysteresis (CH) and the corneal resistance factor (CRF).[97] CH reflects corneal viscoelastic properties and CRF indicates the total corneal resistance. [99,100,102,106] CH represents a tissue property that provides more comprehensive

information about ocular biomechanics, as measured via the cornea. Keratoconic eyes are recognised to be more elastic and less rigid than normal eyes and several clinical studies have identified statistically lower CH and CRF values for keratoconic and FFKC eyes as compared to normal eyes. [54,69, 112, 115-117] However, these corneal biomechanical metrics have moderate to poor sensitivity and specificity for discriminating between FFKC, keratoconus and normal corneas.[52, 64, 65]

Recent research studies have demonstrated that ORA waveform parameters are better than CH and CRF values at differentiating keratoconus from normal corneas.[279,280] The latest update of the ORA device provides novel keratoconus-specific waveform parameters that are derived from mathematical characterizations of waveform signals using a custom algorithm. These include the keratoconus match index (KMI) and the keratoconus match probability (KMP).[140,141,281] KMI characterizes the similarity of the individual eye's waveform to average waveform scores of keratoconic eyes in the ORA database. KMP calculates the probability that the examined eye belongs to one of five clinically classified populations: Normal, suspect KC, mild KC, moderate KC or severe KC.

In clinical settings, keratoconus diagnosis is most commonly confirmed using the Oculus Pentacam, a rotating scheimpflug camera (Oculus Inc, Wetzlar, Germany). The Pentacam provides keratoconus-specific topometric indices and a topographic keratoconus classification (TKC) that are useful in monitoring disease progression and treatment efficacy. [134,135,139, 282,283] However, subtle FFKC cases could be missed despite the use of sophisticated topographic and tomographic imaging. Detection of FFKC is critical in the preoperative evaluation of refractive surgery candidates to avoid post-procedure complications like ectasia.[284, 285] A few recent studies report that KMI is a reliable index in

keratoconus detection and staging. [140,141, 281]The principal objective of this study is to evaluate the diagnostic capacity of KMI and KMP in distinguishing FFKC and keratoconus from normal eyes and to correlate these parameters to Pentacam-derived topometric indices.

7.3 METHODS

Study participants

Subjects were recruited prospectively from the Macquarie University Ophthalmology clinic and the Sydney Cornea clinic, Sydney. The study protocol was approved by the Macquarie University human ethics committee and followed the tenets of the Declaration of Helsinki. Fifty two normal eyes (52 subjects), 12 FFKC eyes (12 subjects) and 110 Keratoconic eyes (71 subjects) were included. Normal eyes were charecterised by the absence of ocular disease, previous ocular surgery or trauma.

Keratoconic eyes (KC) were diagnosed based on the presence of typical corneal signs including steepening, thinning, Fleischer's ring, Vogt striae and apical scarring. [240, 241, 275] Keratoconic eyes were also detected by the Pentacam as KC and staged according to disease severity. Eyes with prior corneal hydrops or corneal surgery were excluded. FFKC eyes were defined as fellow eyes in a subject with clinically manifest keratoconus in the other eye.[65] FFKC eyes were not diagnosed as KC on the Pentacam TKC.

Measurements

Each subject was first measured with the Pentacam and thereafter with the ORA. Contact lens wearers were instructed to avoid wearing lenses for 72 hours prior to measurements.

Pentacam: The Pentacam is a Scheimpflug camera that takes multiple slit images of the anterior segment in less than 2 seconds while rotating 180° around the eye. The device uses a measurement wavelength of 475 nm (blue light-emitting diode) and captures 25,000 different elevation points during the scan. All Measurements were obtained with subjects in a sitting position looking at the fixation target according to the manufacturer's instructions. In this study, the auto-measurement mode measuring 25-images-per-scan was chosen. Only scans that the Pentacam's 'Quality specification'(QS) function determined as 'OK' were included for analysis.

The corneal parameters measured were: central corneal thickness (CCT in microns), maximal keratometry (Kmax in dioptres), keratoconus – specific topometric indices and the grade of KC according to TKC. These topometric indices include: index of surface variance (ISV), index of vertical asymmetry (IVA), keratoconus index (KI), central keratoconus index (CKI), index of height asymmetry (IHA), index of height decentration (IHD) and minimum radius of curvature in the 8 mm zone (Rmin).

Ocular Response Analyser : The ORA determines biomechanical waveform measurements obtained from inward and outward air puff applanation processes. Subjects were well-positioned with their forehead placed parallel against the head rest and asked to blink gently before taking measurements. All ORA waveforms were reviewed to ensure that they showed adequate amplitude and shape and at least two measurements were performed per subject and averaged for statistical analysis. The following ORA parameters were noted: CH, CRF, KMI and KMP and compared between the 3 groups of eyes.

Statistics:

For each normal subject, the data from one randomly selected eye (Microsoft excel randomisation function) were included for analysis. Keratoconus being an asymmetric disorder, data from both eyes was used for analysis. The 3 groups were matched according to age and gender. The measurements obtained from both devices were described as mean ± standard deviation (SD). Differences in values between the 3 groups of eyes was examined using analysis of variance (ANOVA) and Bonnferroni multiple comparison test.

Correlations were analysed between KMI, TKC and Pentacam parameters using the Pearson's correlation coefficient (r). The predictive accuracy of KMI was assessed using receiver operating curves (ROC) obtained by plotting sensitivity versus 1-specificity for each value. Area under curve (AUC) of ROC curves were calculated and interpreted as follows: 0.90-1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair, 0.60-0.70 = poor, 0.50-0.60 = fail. Statistical analysis was performed using the Graphpad prism 6 (GraphPad Software Inc., CA, USA) and the Medcalc statistical program version 13.1 (MedCalc Software Inc., Ostend, Belgium). For all analysis, a p value of less than 0.05 was considered statistically significant.

7.4 RESULTS

Pentcam parameters (CCT, Kmax and topometric indices) and ORA parameters (CH, CRF and KMI) were compared in 52 control eyes to corresponding values in 12 FFKC and 110 KC eyes (Table 14). Significant differences were noted between the 3 groups for all measured parameters ($p < 0.0001$ by ANOVA). However, between control and FFKC eyes, Bonnferroni multiple comparison test was significant ($p < 0.05$) only for KMI, not for other parameters. Mean CH was 10.13 ± 1.72 for normal eyes, 9.34 ± 1.57 for FFKC eyes and 8.05 ± 1.79 for

KC eyes (p<0.0001). Mean CRF was 9.86 ± 1.86 for normal eyes, 8.53 ± 1.57 for FFKC eyes and 6.86 ± 2.05 for KC eyes (p<0.0001). Mean KMI was 1.25 ± 0.30 for normal eyes, 0.76 ± 0.59 for FFKC eyes and 0.32 ± 0.53 for KC eyes (p<0.0001).

Table 14: Comparison of Pentacam and ORA parameters between the 4 groups of eyes

Parameter	**Normal**	**FFKC**	**KC**
No of eyes (subjects)	52 (52)	12 (12)	110 (70)
Mean age ± SD (range) (years)	29.56 ± 6.04 (19 -52)	25.55 ± 10.53 (15 -52)	26.74 ± 8.61 (14 -55)
Male gender (%)	37 (71.2%)	8 (72.7%)	78 (74.3%)
CCT (range) (µm)*	543.69 ± 35.56 (486 - 613)	520.73 ± 34.62 (441-558)	487.48 ± 37.93 (408-604)
Kmax (range) (D)*	43.51 ± 1.14 (41 - 47.1)	44.35 ± 2.05 (41.9- 47.4)	55.17 ± 7.38 (44.7- 89.9)
ISV (range)*	14.46 ± 4.85 (7-33)	24.18 ± 6.26 (15 -36)	95.24 ± 47.61 (22 -305)
IVA (range)*	0.11 ± 0.06 (0.03 - 0.33)	0.23 ± 0.10 (0.08 - 0.37)	1.04 ± 0.52 (0.08 - 3.06)
KI (range)*	1.0 ± 0.14 (0.98 - 1.04)	1.12 ± 0.23(1 -1.8)	1.26 ± 0.17 (1.03 - 2.22)
CKI (range)*	0.99 ± 0.14 (0.01 - 1.01)	1.01 ± 0.01 (0.98 -1.02)	1.05 ± 0.06 (0.97 - 1.3)
IHA (range)*	2.44 ±1.69 (0 - 7)	6.61 ± 4.81 (0.8 -16.5)	29.01 ± 24.83 (0.075-122.7)
IHD (range)*	0.01 ± 0.00 (0.00 - 0.02)	0.01 ± 0.01 (0.00- 0.02)	0.09 ± 0.06 (0.01 - 0.39)
Rmin (range)*	7.74 ± 0.22 (7.05 - 8.22)	7.63 ± 0.35 (7.12 -8.25)	6.21 ± 0.75 (3.75 - 7.8)
CH (mm Hg)* (range)	10.13 ± 1.72 (6.5 - 15.4)	9.34 ± 1.57 (7.1-11.8)	8.05 ± 1.79 (3.7-13.1)
CRF (mm Hg)* (range)	9.86 ± 1.86 (6.4 - 15.2)	8.53 ± 1.57 (6.5-10.6)	6.86 ± 2.05 (1.8-12.2)
KMI (range)*	1.25 ± 0.30 (0.70 - 1.86)	0.76 ± 0.59 (6.5-10.6)	0.32 ± 0.53 (-0.89-1.58)

* p < 0.0001 by ANOVA between all groups.

FFKC = forme fruste keratoconus, KC = keratoconus, CCT = Central corneal thickness, Kmax = Maximal keratometry, ISV = index of surface variance, IVA = index of vertical asymmetry, KI = keratoconus index, CKI = central keratoconus index, IHA = index of height asymmetry, IHD = index of height decentration, Rmin = minimum radius of curvature in the 8 mm zone, CH = corneal hysteresis, CRF = Corneal resistance factor, KMI = Keratoconus Match Index

Significant positive correlations were noted between KMI and CCT ($r = 0.586$, $p < 0.0001$) implying that KMI is greater in thicker corneas (Table 15). In contrast, significant negative correlations were demonstrated between KMI and other Pentacam values including Kmax and topometric indices.

Table 15: Correlation coefficients between KMI and Pentacam parameters

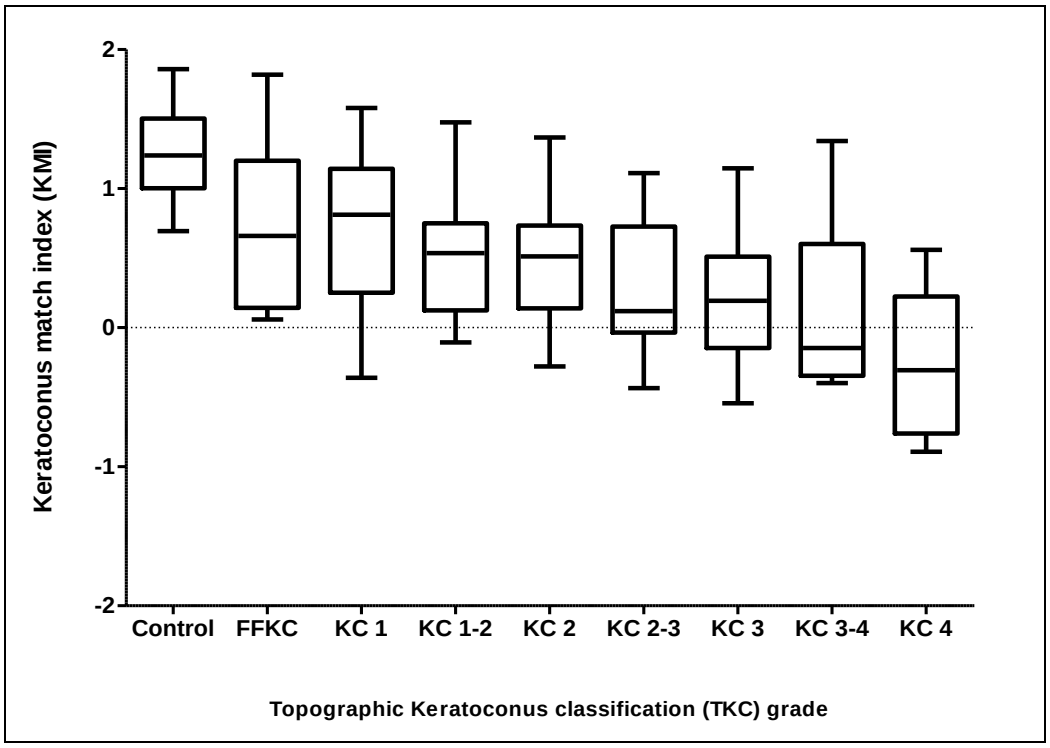
Parameter	Correlation coefficient (r)	P value
CCT	0.586	< 0.0001
Kmax	-0.707	< 0.0001
ISV	-0.734	< 0.0001
IVA	-0.714	< 0.0001
KI	-0.684	< 0.0001
CKI	-0.335	< 0.0001
IHA	-0.468	< 0.0001
IHD	-0.706	< 0.0001
Rmin	0.759	< 0.0001

CCT = Central corneal thickness, Kmax = Maximal keratometry, ISV = index of surface variance, IVA = index of vertical asymmetry, KI = keratoconus index, CKI = central

keratoconus index, IHA = index of height asymmetry, IHD = index of height decentration, Rmin = minimum radius of curvature in the 8 mm zone, KMI = Keratoconus Match Index

KMI also correlated negatively with TKC ($r = -0.742$, $p < 0.0001$). There was a progressive decrease in KMI values from control to FFKC to KC eyes and KMI values reduced further as severity of KC increased (Figure 31).

Figure 31: Correlation between KMI and TKC



The range of KMI values across different stages of Keratoconus according to TKC is shown in Table 16. There were significant differences between the various KC stages ($p < 0.0001$ by ANOVA).

Table 16: Mean KMI values in different KC stages

TKC Keratoconus group	KMI (Mean ± SD)	p value
KC 1	0.68 ± 0.61	< 0.0001
KC 1-2	0.48 ± 0.48	< 0.0001
KC 2	0.43 ± 0.36	< 0.0001
KC 2-3	0.28 ± 0.44	< 0.0001
KC 3	0.15 ± 0.42	< 0.0001
KC 3-4	0.09 ± 0.56	< 0.0001
KC 4	-0.69 ± 0.25	< 0.0001

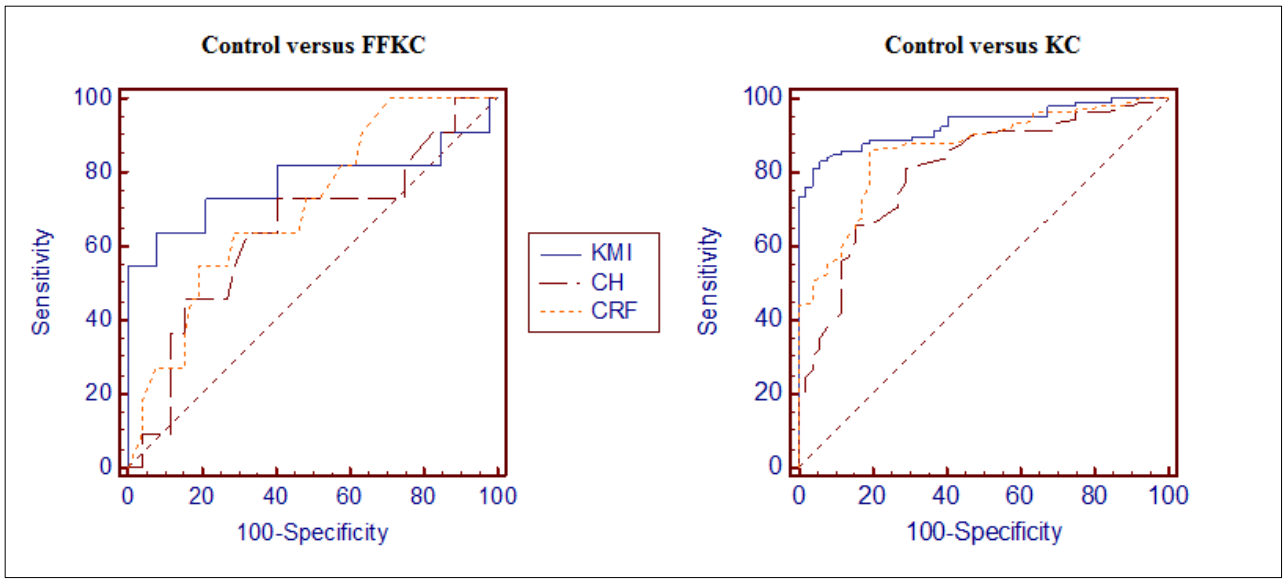
TKC = topographic keratoconus classification, KC = keratoconus, KMI = Keratoconus Match Index

Receiver operating charecteristic (ROC) curve analysis (figure 32) demonstrated that KMI had significantly greater sensitivity and specificity than CH and CRF in discriminating KC from normal eyes (Area under curve: KMI = 0.929, CH = 0.806, CRF= 0.862) ($p<0.05$). Similarly, KMI appears to be more sensitive and specific in differentiating FFKC from normal eyes as compared to CH and CRF (Area under curve: KMI = 0.771, CH = 0.641, CRF= 0.707 ($p>0.05$), however this was not statistically significant.

The optimal cut-off point for KMI to differentiate between normal and KC eyes was ≤ 0.715, with sensitivity of 76.19 % and specificity of 98.08 %. Likewise, the optimal cutoff point for

KMI to distinguish between normal and FFKC was ≤0.816, with sensitivity of 63.64 % and specificity of 92.31 %.

Figure 32: Receiver operating characteristic (ROC) curve analysis of keratoconus match index (KMI), Corneal hysteresis (CH) and Corneal Resistance factor (CRF) in differentiating between normal and KC eyes and between normal and FFKC eyes



Keratoconus match probability (KMP) values in the study groups are presented in Table 17. KMP identified 90.08 % of normal eyes, 43.2% of FFKC eyes and 22.09% of KC eyes as normal. Likewise, 8.44 % of normal eyes, 24.7 % of FFKC eyes and 24.16% of KC eyes were designated as suspect. Interestingly, 1.48 % of normal eyes and 32.1% of FFKC eyes were labelled as keratoconus according to KMP.

Table 17: KMP values in the three study groups

Parameter	Normal (%)	FFKC (%)	KC (%)
KMP Normal	90.08	43.2	22. 09
KMP suspect	8.44	24.7	24.16
KMP mild	1.48	25.7	26.68
KMP moderate	0.00	6	16.91
KMP severe	0.00	0.4	10.17

KMP = Keratoconus Match Probability, FFKC = forme fruste keratoconus, KC = keratoconus

7.5 DISCUSSION

Refractive surgery is commonly performed and screening candidates for keratoconus and FFKC is essential to avoid serious complications. Diagnosis of classic keratoconus is straightforward using clinical examination techniques, corneal topography and tomography. However, detection of early subclinical forms of keratoconus including FFKC can be challenging. Corneal biomechanical instability is one of the key etiological factors for the development of keratoconus and other related corneal ectatic disorders.

The advent of the ORA has made it possible to estimate corneal biomechanical properties using an easy and reliable technique. Clinical studies have demonstrated that ORA CH and CRF measurements are useful in detecting KC and FFKC as an adjunct to conventional corneal imaging, however they are not highly sensitive or specific diagnostic parameters. [52,54,64,65, 69,116, 117] Recent advances in ORA waveform analysis have resulted in the introduction of novel keratoconus-specific parameters including KMI and KMP. [140,141,281] KMI is derived using neural network analysis of ORA waveform data. KMI compares the individual eye's waveform to mean waveform scores of keratoconic eyes in the ORA database.

This study sought to determine the ability of KMI in detecting FFKC and KC and to study its agreement with Pentacam keratoconus staging. Biomechanical parameters could be influenced by age and therefore, the study groups were age matched. [50,286] The Pentacam provides a detailed classification of keratoconus ranging from KC1 to KC4 based on the Amsler Krumeich grading.[134,139] Significant differences were present between normal, FFKC and KC eyes for all measured ORA and Pentacam parameters. However, when control and FFKC eyes were compared, significant differences were noted only for KMI values. Furthermore, KMI values varied consistently with KC severity such that KMI values decreased as keratoconus grade worsened. Our findings agree well with a recent study that reported similar findings in keratoconic eyes. [140] In addition, we looked at FFKC eyes whose KMI values fell in between normal control and keratoconic eyes.

This study also investigated the relationship of KMI to the Pentacam corneal curvature and thickness measurements and to topometric corneal asymmetry indices. These indices are commonly used in keratoconus diagnosis and staging. Significant positive correlations were

noted between KMI and CCT. In comparison, significant negative correlations were noted between KMI and Kmax and topometric indices. This indicates that KMI is lower in thinner and steeper corneas corresponding to higher grades of keratoconus.

Receiver operating curve (ROC) analysis indicated that KMI had greater sensitivity and specificity than CH and CRF values in differentiating KC from normal eyes (Area under curve: KMI = 0.929, CH = 0.806, CRF= 0.862) and these results agree well with an earlier study.[141] The optimal cut-off value of KMI to differentiate between normal and KC eyes was ≤ 0.715 whereas it was ≤ 0.816 to distinguish between normal and FFKC eyes. Mean KMI values of 0.32 ± 0.53 for KC eyes, 0.76 ± 0.59 for FFKC eyes and 1.25 ± 0.30 for normal eyes are consistent with these cut-off values.

KMP estimates the probability that the scanned eye belongs to one of the following categorized populations: Normal, suspect KC, mild KC, moderate KC or severe KC. In this study, 90.08 % of normal eyes, 43.2% of FFKC eyes and 22.09 % of KC eyes were labelled as normal. In contrast, only 78 % of normal eyes and 7.01 % of KC eyes were categorized as normal in a recent study by Labiris et al. [140] Similarly, 8.44 % of normal eyes, 24.7 % of FFKC eyes and 24.16 % of KC eyes were labelled as suspect in this study. In comparison, 22.03 % of normal eyes and 23.68 % of KC eyes were categorized as suspect by Labiris et al. Thus, there appears to be significant discrepancy between different studies regarding KMP classifications.

This study explores the diagnostic ability of KMI and KMP in differentiating FFKC and KC from normal eyes. Our results indicate that KMI has a significant ability to distinguish between normal and keratoconic corneas. KMI values differ significantly between normal and

FFKC eyes, however it does not significantly discriminate between the two groups. Overall, KMI is more sensitive and specific than CH and CRF in differentiating normal from ectatic corneas and could be valuable in screening eyes for refractive surgery. It also appears that KMI is a reliable indicator of keratoconus severity and therefore could potentially be used to monitor keratoconus progression. In the present study, KMP identified a significant percent of KC eyes as normal and does not appear to agree well with topographic KC diagnosis. Studies with greater number of subjects are recommended for validation of these findings and further evaluation of these novel biomechanical parameters.

CHAPTER 8: PROSPECTIVE LONGITUDINAL STUDY OF CORNEAL COLLAGEN CROSSLINKING IN PROGRESSIVE KERATOCONUS

This chapter is a prospective study that analyses the long-term outcome of the corneal collagen crosslinking procedure in progressive keratoconic eyes and compares treated eyes to untreated fellow keratoconic eyes. Results have been discussed in detail in comparison to previous studies and this paper has been published as: Viswanathan D, Males J. Prospective longitudinal study of corneal collagen crosslinking in progressive keratoconus. Clin Experiment Ophthalmol. 2013;41(6):531-6

8.1 ABSTRACT

Background: Collagen crosslinking has been reported to be effective in treating progressive keratoconus and this study aims to evaluate the long term efficacy of this procedure.

Design: Prospective longitudinal interventional study of patients with progressive keratoconus who underwent crosslinking in a tertiary hospital.

Participants: Thirty five patients (fifty one eyes) who underwent crosslinking with a mean follow up of 14.38 $\pm$ 9.36 months (range 6 – 48) were compared with a control group of twenty five fellow eyes that did not undergo crosslinking.

Methods: Crosslinking was performed using 0.1% riboflavin (in 20% dextran T500) and ultraviolet A irradiation (370 nm, 3mW/cm^2,30 minutes) .

Main outcome measures : Maximum keratometry in dioptres (D), logMAR best spectacle corrected visual acuity, cylindrical power, manifest refraction spherical equivalent and central corneal thickness.

Results: Analysis of the treated group demonstrated a significant flattening of maximum keratometry by 0.96 $\pm$ 2.33 D ($p = 0.005$) and a significant improvement in visual acuity by 0.05 $\pm$ 0.13 logMAR ($p = 0.04$). In the control group, maximum keratometry increased significantly by 0.43 $\pm$ 0.85 D ($p = 0.05$) and visual acuity decreased by mean 0.05 $\pm$ 0.14 ($p = 0.2$). No statistical differences were noted regarding cylindrical power, spherical equivalent or corneal thickness in both groups.

Conclusions: Results indicate that collagen crosslinking using riboflavin and ultraviolet-A is effective as a therapeutic option in cases of progressive keratoconus by reducing the corneal curvature and improving the visual acuity in these patients.

Key words : Keratoconus, collagen crosslinking, maximum keratometry

8.2 INTRODUCTION

Keratoconus is a bilateral, asymmetric, progressive ectasia of the cornea characterized by steepening, distortion and thinning of the apical cornea resulting in irregular astigmatism and deterioration of vision. [105,287] The approximate incidence of keratoconus is 1 per 2000 in the general population. [288] Keratoconus begins at puberty and progresses in approximately 20% of patients to such an extent that either lamellar or full-thickness corneal transplantation becomes necessary to preserve vision. [145,288] In many countries, keratoconus remains the most frequent indication for corneal transplantation in patients aged less than 60 years, and

therefore has a significant impact on quality of life over the duration of the affected subject's life. [289- 291]

Keratoconus is characterised by alteration in the normal corneal collagen structure and organization and decreased stromal thickness resulting in corneal biomechanical Weakening. [107,109, 292-294] Biochemical studies of the corneal matrix in keratoconic corneas revealed an increased expression of proteolytic enzymes and decreased concentration of protease inhibitors as compared to normal corneas. [295- 299]

Collagen crosslinking (CXL), a method introduced for the treatment of progressive keratoconus, employs the photosensitizer riboflavin (vitamin B2), which when exposed to longer wavelength ultraviolet light (370 nm UVA), induces chemical reactions in the corneal stroma and ultimately results in the formation of covalent bonds between the collagen molecules, fibers and microfibrils and effectively strengthens the cornea.[154] In Vitro laboratory studies on porcine, rabbit and human corneas have demonstrated that CXL caused a increase in corneal stiffening by more than 300% and an increase in the collagen fiber diameter by 12.2%, thus increasing the corneal biomechanical rigidity. [155 - 157]

Subsequent clinical studies have reported that CXL is associated with visual improvement and reduction in corneal steepening and is safe and effective in treating progressive keratoconus, [150- 153, 160 - 162] however there are few studies with a maximum followup of over 12 months. [163-165] Collagen crosslinking was introduced for the first time in Australia in 2006 as a treatment option for progressive keratoconus and the aim of this study is to study the long term visual and refractive outcome of these cases.

8.3 METHODS

Fifty one eyes of thirty five patients with progressive keratoconus who underwent CXL treatment were included in this long term prospective study. They were compared with a control group of twenty five fellow eyes with keratoconus which did not undergo CXL. The institutional ethics committee approved the study and all patients provided informed consent after receiving a detailed description of the nature of the treatment. The mean age of patients was 24.25 $\pm$ 8.08 years (range 15 – 39), there were 34 males and 17 females. The mean follow up period for treated eyes was 14.38 $\pm$ 9.36 months (range 6 – 48).

For each patient, initially the worse eye was treated , the fellow eye served as a control and in some patients, the fellow eye progressed as well and was treated on compassionate grounds. The mean follow up period for control eyes was 13.88 ± 8.86 months, it was not statistically different (unpaired t test, p = 0.82) from the follow up period for treated eyes. The inclusion criteria for CXL were patients with progressive early to moderate keratoconus (grade I to III according to the Amsler- Krumeich classification) [300] with a corneal thickness of at least 400 microns. The indication for CXL included an increase in maximum keratometry (K) of 1.00 dioptre (D) in 1 year, deterioration in visual acuity (excluding other possible non-cornea–related reasons) and the need for new contact lens fitting more than once in 2 years.

The exclusion criteria for this study were advanced keratoconus with stromal scarring requiring corneal grafting, corneal hydrops, herpetic keratitis, autoimmune and other systemic diseases, pregnancy and breast feeding. The inclusion criteria for control eyes were fellow eyes with keratoconus without apical scarring or having undergone corneal grafting or insertion of intracorneal ring segments.

Contact lens wearers were instructed to discontinue usage of soft lenses for a minimum of 3 days and rigid-gas permeable and hard lenses for a minimum of 2 weeks before the preoperative eye examination. All subjects underwent examination of visual acuity, manifest refraction, corneal topography and corneal pachymetry measurements preoperatively and postoperatively at 1, 6, 12, 18 and 24 months.

Visual acuity measurement included the best spectacle corrected visual acuity (BSCVA) which was obtained using the Early Treatment of Diabetic Retinopathy Study visual acuity test (ETDRS) and analyzed as the logMAR value. Manifest refraction was performed and the manifest refraction spherical equivalent (MRSE) and cylindrical astigmatism were obtained and analysed.

Corneal topography and corneal thickness measurements (pachymetry) were peformed using a non contact rotating Scheimpflug camera (Pentacam, Oculus Inc. Germany) by an experienced orthoptist. Only scans that the Pentacam's 'Quality specification'(QS) function determined as 'OK' were included for analysis. The Pentacam takes 50 pictures of the anterior segment within two seconds and prior clinical studies have demonstrated that the device provides excellent repeatable and reproducible corneal curvature and thickness measurements.[259, 260] For purpose of analysis, the maximal keratometry (Kmax) values were compared between eyes at follow up periods of 6-9 months, 12-18 months and at 24 months and beyond following CXL.

Surgical Technique

Collagen crosslinking was performed using 0.1% riboflavin (in 20% dextran T 500) and ultraviolet A (UVA) irradiation (370 nm,3 mW/cm^2, 30 min) under sterile conditions . The

Innocross – R™ riboflavin isotonic solution **(**Riboflavin 5-phosphate (0.1%) plus 20% Dextran T500 in 2ml syringes) was used for the corneal collagen cross-linking procedure. The UVA machine used was the UV-X™ 1000 (IROC Innocross AG, Zurich , Switzerland). After topical anaesthesia, a lid speculum was inserted and the epithelial tissue was removed in a 9.0 mm diameter area to allow penetration of riboflavin into the corneal stroma.

The photosensitizer 0.1% riboflavin solution was then applied (2 to 3 drops every 3 minutes) to the cornea for 30 minutes before the irradiation to allow sufficient saturation of the stroma. The central cornea (8.0 mm diameter) was then exposed to UVA light with a wavelength of 370 nm and an irradiance of 3 mW/cm2 for 30 minutes. Riboflavin solution was instilled (2 to 3 drops every 3 minutes) during the UVA exposure. After treatment, the eye was washed with 20 mL of a balanced salt solution and antibiotic eye drops (Ofloxacin 0.3%) and steroid eye drops (Dexamethasone 0.1%) were applied and a bandage contact lens was placed in the eye until complete reepithelialisation. Subsequent examinations were performed at 1 week and thereafter at 1, 6,12,18 and 24 months and annually afterwards. At each follow up examination, the refraction, best spectacle corrected visual acuity (BSCVA), corneal topography, central corneal thickness (CCT) were recorded.

Statistical Analysis

To quantify the effect of crosslinking treatment , the changes in Kmax value, astigmatism (based on refraction), spherical equivalent and BCVA were analysed by subtracting each parameter at the respective follow-up examination minus the preoperative value . Statistical evaluation was performed by SPSS software version 19.The paired t test was used to evaluate the differences in the different parameters between the two groups , a p value of ≤ 0.05 was considered to be statistically significant.

8.4 RESULTS

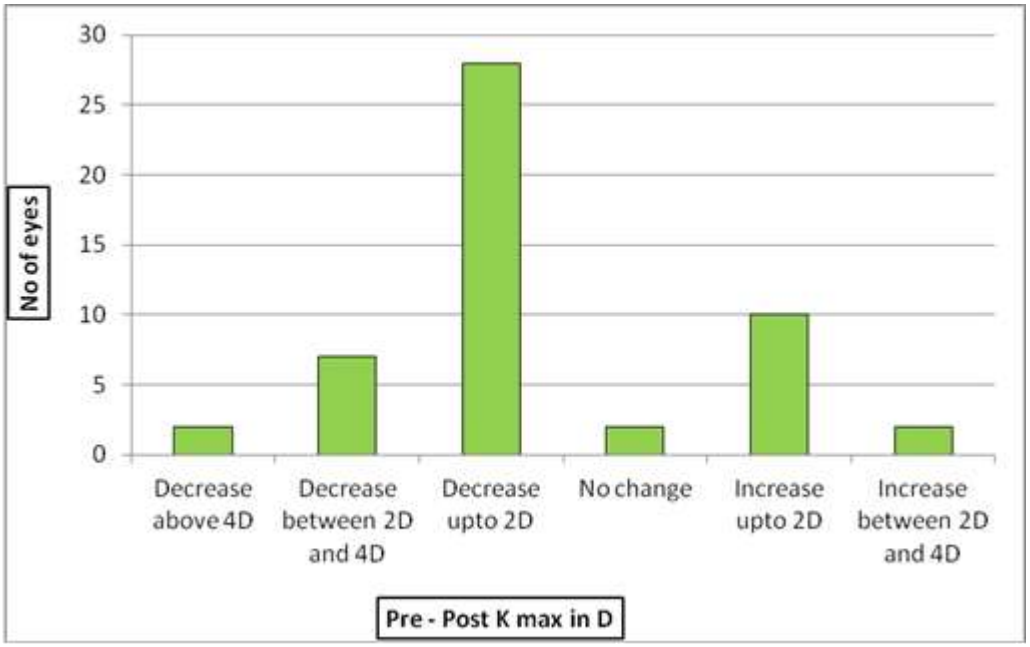
Fifty one eyes of thirty five patients with progressive keratoconus who underwent CXL were compared with a control group of twenty five fellow eyes with keratoconus that did not undergo CXL. At mean follow up of 14.38 $\pm$ 9.36 months, the CXL treated eyes showed a significant reduction ($p = 0.005$) in the maximum keratometry value (Kmax) by 0.96 $\pm$ 2.33 D and a significant improvement ($p = 0.04$) by 0.05 $\pm$ 0.13 in logMAR BSCVA.

There was a mean reduction in spherical equivalent of 0.19 $\pm$ 1.73 dioptres ($p = 0.56$) and a mean reduction in cylindrical power of 0.24 $\pm$ 1.86 dioptres ($p = 0.49$) in the CXL treated group , however these were not statistically significant . The change in CCT was compared and there no significant difference ($p = 0.60$) in the values between pre procedure and final follow up. The above findings are shown in table 18. A comparison of the difference between pre CXL and post CXL Kmax values for all treated eyes has been illustrated in figure 33.

Table 18: Pre and postoperative data for the CXL treated group at mean 14.38 $\pm$ 9.36 months follow up

	BCVA (logMAR)	Spherical Equivalent (Dioptres)	Cylindrical power (Dioptres)	Maximum Keratometry (Dioptres)	CCT (Microns)
Pre CXL	0.21 ± 0.13	-4.56 ± 3.73	-3.99 ± 2.11	49.65 ± 4.91	470.35 ± 39.26
Post CXL	0.16 ± 0.15	-4.36 ± 3.25	-3.75 ± 2.13	48.69 ± 4.56	467.64 ± 43.54
p value	0.04	0.56	0.49	0.005	0.60

Figure 33: Difference between Pre and post CXL Kmax for treated eyes at mean 14.38 ± 9.36 months follow up



Although the treated group had a greater pre CXL Kmax (49.65 ± 4.91 D) and a greater pre CXL spherical equivalent (- 4.56 ± 3.73D) as compared to the control group (48.09 ± 4.81D) and (- 4.32 ± 3.11D) respectively, there were no statistically significant differences (unpaired t test, p = 0.20 and p = 0.88 respectively) between the treated and control groups.

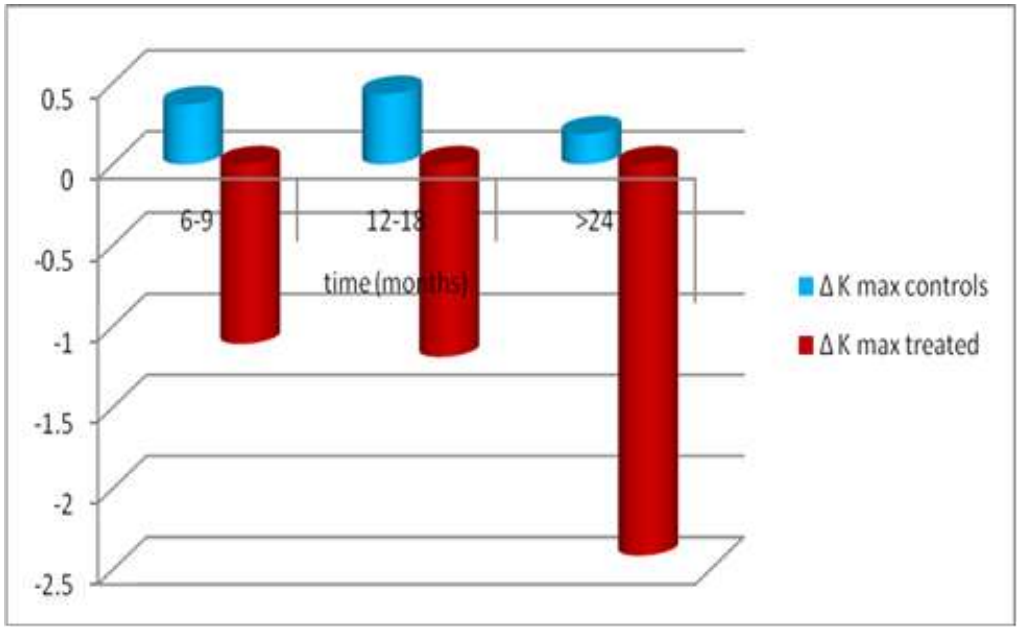
Analysis of the fellow eye control group revealed a significant increase (p = 0.05) in Kmax by 0.46 $\pm$ 1.1 D and a worsening in logMAR BSCVA by 0.05 $\pm$ 0.14 which was not statistically significant (p = 0.2) at mean follow up of 13.88 ± 8.86 months. No statistically significant difference was observed in spherical equivalent, cylindrical power or central corneal thickness (table 19).

Table 19 - Pre and postoperative data for the fellow eye control group at mean 13.88 ± 8.86 months follow up

	BCVA (logMAR)	Spherical equivalent (Dioptres)	Cylindrical power (Dioptres)	Maximum Keratometry (Dioptres)	CCT (Microns)
Baseline	0.14 ± 0.26	-4.32 ± 3.11	-0.07 ± 3.22	48.09 ± 4.81	481.38 ± 43.8
Follow up	0.19 ± 0.19	-4.14 ±3.09	-0.11 ± 3.09	48.69 ± 5.24	479.13 ± 44.93
p value	0.2	0.54	0.84	0.05	0.38

Analysis of the CXL treated group revealed a reduction in K Max by an average of 1.1 $\pm$ 2.08 D (p = 0.009) at 6 - 9 months (n = 51), 1.18 $\pm$ 1.83 D (p= 0.005) at 12 – 18 months (n = 22) and 2.4 $\pm$ 4.41 D (p= 0.14) beyond 24 months (n = 10) . The Kmax increased in the control group by 0.43 $\pm$ 0.85 D (p= 0.17) in 6 -9 months (n = 25), 0.37 $\pm$ 1.05 D (p = 0.17) in 12 – 18 months (n = 8) and 0.19 $\pm$ 1.34 D (p= 0.5) beyond 24 months (n = 5), this comparison (difference in Kmax = Δ Kmax) is shown in figure 34.

Figure 34: Comparison of difference in maximum keratometry (K max) between the CXL treated and fellow control eyes during follow up



Corneal topography demonstrating a reduction in Kmax after CXL by 2 D at 10 months followup is shown in figure 35, there was an associated improvement in logMAR BSCVA from 0.47 to 0.18. In comparison, corneal topography of a fellow control eye demonstrating an increase in Kmax by 2 D at 11 months follow up is shown in figure 36, there was worsening of logMAR BSCVA from 0.12 to 0.78.

Figure 35: Corneal topography showing a reduction in maximum keratometry after CXL on follow up

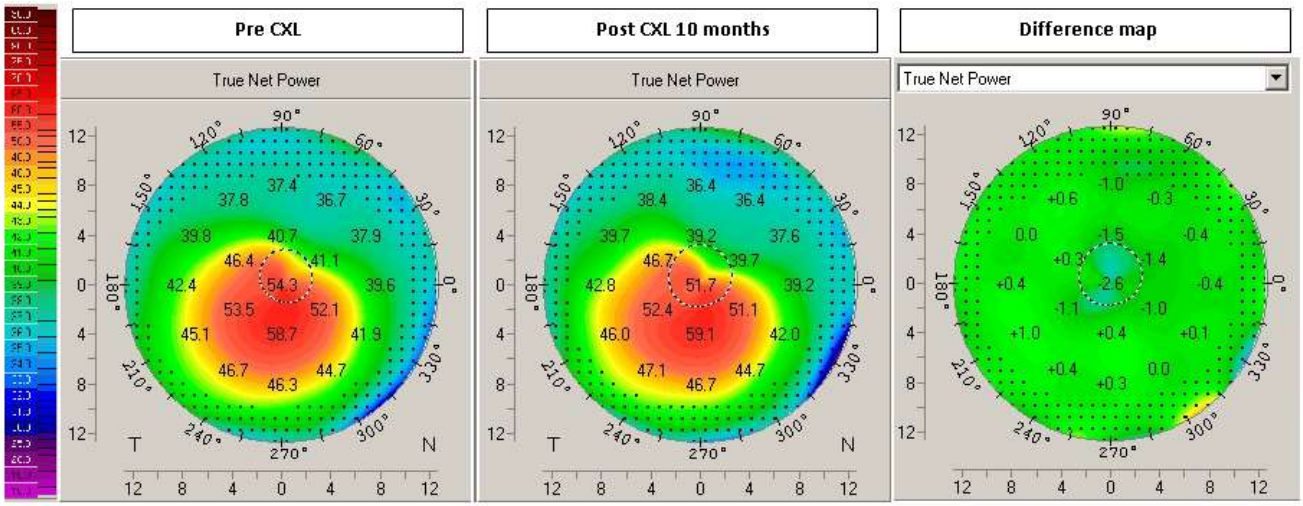
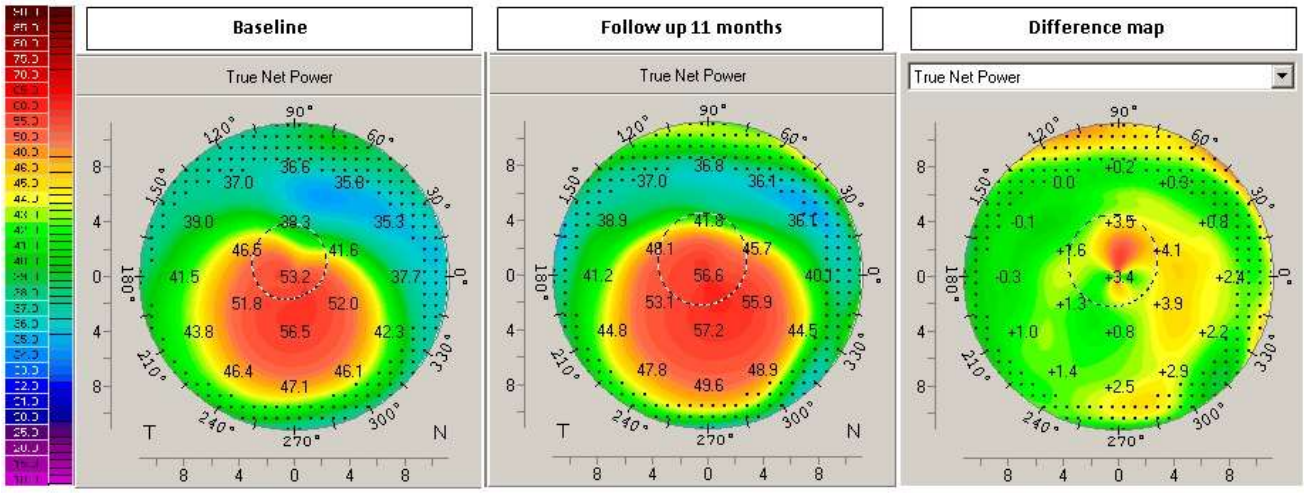


Figure 36: Corneal topography showing an increase in maximum keratometry in a control eye on follow up



8.5 DISCUSSION

Corneal collagen crosslinking is an emerging modality of treatment with encouraging results for the management of progressive keratoconus. CXL often results in visual improvement and reduction in corneal steepening in progressive keratoconus by clinical studies [25-31.] CXL changes the intrinsic biomechanical properties of the cornea, strengthens and stiffens the cornea and arrests the progression of keratoconus . The aim of this study was to report the long term visual and refractive outcome of CXL.

Earlier studies have reported a significant improvement in postoperative BCVA following CXL. [152,153,160 – 164]Among recent studies with 12 months follow up, Hersh et al [301] reported an improvement in postoperative BCVA (mean change -0.13 ± 0.21 logMAR) whereas the French multicenter study [302] reported stabilisation of BCVA in 47.6% eyes and improvement of BCVA in 40 % eyes following CXL. In our study, there was a small improvement in logMAR BSCVA from 0.21 to 0.16 at mean 14 months follow up. In contrast, there was a worsening of logMAR BSCVA in the fellow control group from 0.14 to 0.19 which did not assume statistical significance. Some of the previous studies have reported a significant reduction in the MRSE and cylindrical astigmatism following CXL.[153, 163] In our study, there was a reduction in both MRSE and cylindrical astigmatism in the CXL treated eyes, however this was not statistically significant.

The majority of earlier studies have demonstrated a decrease in Kmax after CXL.[152,153, 160-164] In our study, there was a significant reduction in the K max value in the treated group at mean 14 months follow up and a continued decrease after 12 months and 24 months follow up , this is in good agreement with the other long term studies by Caporossi et al [163] and Raiskup-Wolf

et al. [164] In comparison, there was a significant worsening of Kmax in the fellow eye control group of eyes, this is similar to the findings of the randomised controlled study by Witting-silva et al. [152] This is interesting because two other studies which compared treated eyes with fellow keratoconus eyes as controls did not demonstrate any significant change in K max on follow up in the control eyes.[153,301] The findings of our study emphasize the fact that keratoconus, when left untreated can progress and result in corneal steepening and deterioration of visual acuity . A comparison of corneal thickness measurements revealed no statistically significant change between baseline values and follow up in both the treated eyes and the control eyes, this corroborates the findings of earlier studies.[163,164]

One limitation of our study is that there was no randomisation protocol , the worse eye of each patient underwent CXL and the fellow eye served as control. However, one strength of our study is the long duration of follow up, each patient having had minimum 6 months follow up and at least 40 % patients having more than 1 year of follow up. While a number of conservative and surgical methods of visual rehabilitation exist for keratoconus, none other than CXL address progressive nature of this condition. Results from our study indicate that corneal collagen crosslinking is an effective modality for the treatment of progressive keratoconus by reducing the corneal curvature and improving visual acuity. Further long term follow up of CXL is warranted given the typically young age at treatment.

CHAPTER 9: OUTCOME OF CORNEAL COLLAGEN CROSSLINKING FOR PROGRESSIVE KERATOCONUS IN PAEDIATRIC PATIENTS

This chapter studies in detail the effect of corneal collagen crosslinking procedure in paediatric patients with progressive keratoconus and compares results with previous case reports. This paper has been published as: Viswanathan D, Kumar NL, Males JJ. Outcome of Corneal Collagen Crosslinking for progressive Keratoconus in paediatric patients. BioMed Research International.2014 http://dx.doi.org/10.1155/2014/140461

9.1 ABSTRACT

Purpose : To evaluate the efficacy of corneal collagen crosslinking for progressive keratoconus in paediatric patients.

Methods : This prospective study included 25 eyes of 18 patients (aged 18 years or younger) who underwent collagen crosslinking performed using Riboflavin and ultraviolet-A irradiation (370 nm,3 mW/cm2, 30 min).

Results : The mean patient age was 14.3 ± 2.4 years (range 8 – 17) and mean follow up duration was 20.1 ± 14.25 months (range 6 – 48). Crosslinked eyes demonstrated a significant reduction of keratometry values. The mean baseline simulated keratometry values were 46.34 dioptres (D) in the flattest meridian and 50.06 D in the steepest meridian. At 20 months after crosslinking, the values were 45.67 D (p= 0.03) and 49.34 D (p=0.005) respectively. The best spectacle corrected visual acuity (BSCVA) and topometric astigmatism improved after crosslinking. Mean logarithm of the minimum angle of resolution (logMAR) BSCVA decreased from 0.24 to 0.21 (p = 0.89) and topometric astigmatism reduced from mean 3.50 D to 3.25 D (p = 0.51).

Conclusions: Collagen crosslinking using Riboflavin and ultraviolet-A is an effective treatment option for progressive keratoconus in paediatric patients. Crosslinking stabilises the condition and therefore could potentially reduce the need for corneal grafting in these young patients.

Key words: keratoconus, collagen crosslinking

9.2 INTRODUCTION

Keratoconus is a degenerative corneal disorder charecterised by corneal thinning, conical protrusion, irregular astigmatism and visual impairment . [105] Keratoconic eyes have an altered corneal biomechanical profile and appear to be more elastic and less rigid than normal eyes.[112] Keratoconus usually manifests during adolescence and early adulthood. Young patients are at risk for faster disease progression and corneal grafting often becomes necessary for visual rehabilitation.[303]

Corneal collagen crosslinking (CXL) is a recently introduced treatment for addressing progressive keratoconus. It is a minimally invasive procedure and the only option that halts or slows disease progression. Riboflavin and ultraviolet-A induce cross-linking through photopolymerization of collagen mediated by reactive oxygen species and thus increase corneal biomechanical rigidity and biochemical resistance. [155,156,304]

Several clinical studies have demonstrated that CXL effectively slows Keratoconus progression in adult eyes.[152,161 - 163,165, 305] Recently, CXL has been recommended as an optimal intervention for progressive Keratoconus affecting the paediatric population . [306-309]

Therefore CXL could potentially reduce the need for corneal grafting in these young individuals. This is particularly relevant as paediatric patients have a greater risk of corneal transplant rejection.[309] We observed favourable results after CXL in adult eyes [305] and this study aims to evaluate its efficacy in treating progressive keratoconus affecting paediatric subjects.

9.3 METHODS

Twenty five eyes of 18 patients (5 females, 13 males) with progressive Keratoconus underwent CXL and were enrolled in this prospective study. Only patients who completed a minimum of 6 months follow up after the procedure were included. The institutional ethics committee approved the study and parents provided informed consent prior to treatment.

Inclusion criteria:

Patients aged less than 18 years with progressive early to moderate Keratoconus (grade I to III according to the Amsler- Krumeich classification) with a minimum corneal thickness of at least 400 microns .[158] Indications for treatment included an increase in steep keratometry of 1.00 dioptre (D) or more in 1 year, deterioration in visual acuity and the need for new contact lens fitting more than once in 2 years. Exclusion criteria were advanced Keratoconus with stromal scarring, corneal thickness less than 400 microns, corneal hydrops, severe dry eye, corneal infections, previous ocular surgery and autoimmune diseases.

Tests and evaluation:

Soft contact lenses were discontinued for a minimum of 3 days and rigid-gas permeable and hard lenses for minimum of 2 weeks before preoperative eye examination. Evaluation of

visual acuity, manifest refraction, corneal topography and corneal pachymetry was performed preoperatively and postoperatively in all subjects. The logMAR BSCVA was obtained using the Early Treatment of Diabetic Retinopathy Study chart (ETDRS). Manifest refraction was performed and the manifest refraction spherical equivalent (MRSE) was analysed. Corneal topography and corneal thickness measurements (pachymetry) were peformed using a non contact rotating Scheimpflug camera (Pentacam, Oculus Inc. Germany).

Cross-linking technique:

Corneal collagen crosslinking was performed using 0.1% riboflavin (in 20% dextran T 500) and ultraviolet A (UVA) irradiation (370 nm,3 mW/cm^2, 30 min) under sterile conditions. The UV-X™ 1000 machine (IROC Innocross AG, Zurich , Switzerland) and the Innocross – R™ riboflavin isotonic solution **(**Riboflavin 5-phosphate (0.1%) plus 20% Dextran T500 in 2ml syringes) and were used. The procedure was performed under general anaesthesia in very young patients and under topical anaesthesia in older patients. After anaesthesia, a lid speculum was inserted and the corneal epithelium was soaked with 20% alcohol for 40 seconds. The epithelial tissue was then removed in a 9.0 mm diameter area with a cellulose surgical spear to allow penetration of riboflavin into the corneal stroma. Thereafter, the photosensitizer 0.1% riboflavin was applied (2 to 3 drops every 3 minutes) to the cornea for 30 minutes before irradiation to allow sufficient saturation of the stroma.

Corneal soaking of riboflavin was assessed and then the central 8.0 mm cornea was exposed to UVA light (wavelength of 370 nm and irradiance of 3 mW/cm2) for 30 minutes. Throughout the UVA exposure, Riboflavin solution was instilled (2 to 3 drops every 3 minutes). Upon completion of treatment, the eye was washed with balanced salt solution and antibiotic eye drops (Ofloxacin 0.3%) and steroid eye drops (Dexamethasone 0.1%) were

applied. A bandage contact lens was placed in the eye until complete reepithelialisation. Subsequent follow up examinations were performed at 1 week and thereafter at 1, 6,12,18 and 24 months and annually therafter., The BSCVA, corneal topography, central corneal thickness (CCT) were recorded at each visit.

Statistical Analysis

The changes in simulated keratometry values in the flattest meridian (K1) and the steepest meridian (K2), topometric astigmatism, manifest refraction and BSCVA were analysed to evaluate the effect of crosslinking treatment. This was performed by subtracting each parameter at the respective follow-up examination from the pre-procedure value. Post procedure data was available for all 25 eyes. Statistical evaluation was performed by SPSS software version 19. The paired t test was used to evaluate the differences in the different parameters between pre and post procedure values and a p value of ≤ 0.05 was considered to be statistically significant.

9.4 RESULTS

The mean patient age was 14.3 ± 2.4 years (range 8-17 years), there were 5 females and 13 males. The risk factors for Keratoconus development in the patient population included eye rubbing in 58.8% patients and atopy in 47.10% patients. The outcomes after crosslinking at mean follow up of 20.1 ± 14.25 months (range: 6 - 48 months) are shown in table 20.

Table 20 - Pre and post crosslinking data for treated eyes

Parameter	Pre CXL	Post CXL	p value
BSCVA (logMAR)	0.24 ± 0.19	0.21 ± 0.13	0.89
MRSE (Dioptres)	-5.66 ± 3.47	-4.71 ± 3.11D	0.71
K1 (Dioptres)	46.34 ± 3.13 D	45.67 ± 3.31D	0.03
K2 (Dioptres)	50.06 ± 3.84 D	49.34 ± 3.18 D	0.005
Topometric astigmatism (Dioptres)	3.50 ± 1.36 D	3.25 ± 1.79 D	0.51

BSCVA = best spectacle corrected visual acuity, logMAR = logarithm of the minimum angle of resolution, MRSE = manifest refraction spherical equivalent, K1 = mean simulated keratometry value in the flattest meridian, K2 = mean simulated keratometry value in the steepest meridian

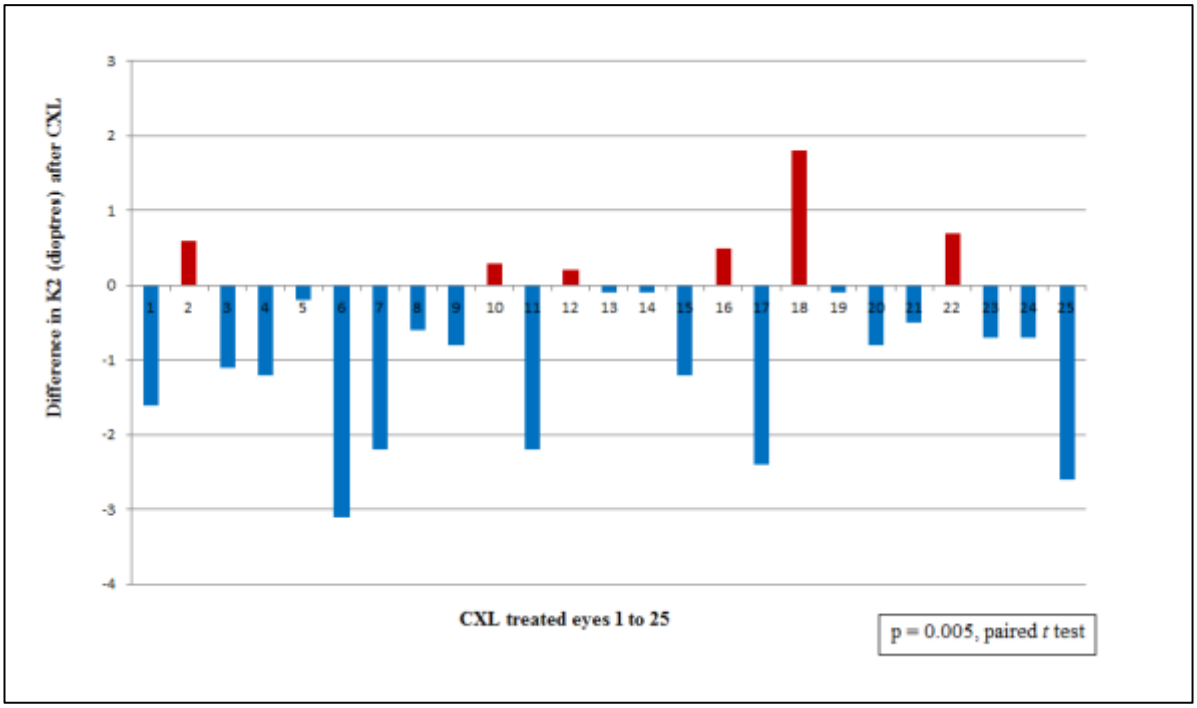
Visual acuity: The mean logMAR BSCVA improved by 0.02 ± 0.19 (p = 0.89) at mean 20 months follow up after CXL.

Manifest refraction: There was a reduction in mean spherical equivalent from -5.66 ± 3.47 D to -4.71 ± 3.11D (p = 0.71) in treated eyes at mean follow up of 20 months.

Corneal topography: There was a significant reduction in keratometry values following crosslinking. The mean simulated keratometry value in the flattest meridian (K1) reduced by 0.66 ± 1.38D (p= 0.03) and the mean simulated keratometry value in the steepest meridian (K2) reduced by 0.72 ± 1.17 D (p=0.009) at 20 months follow up.

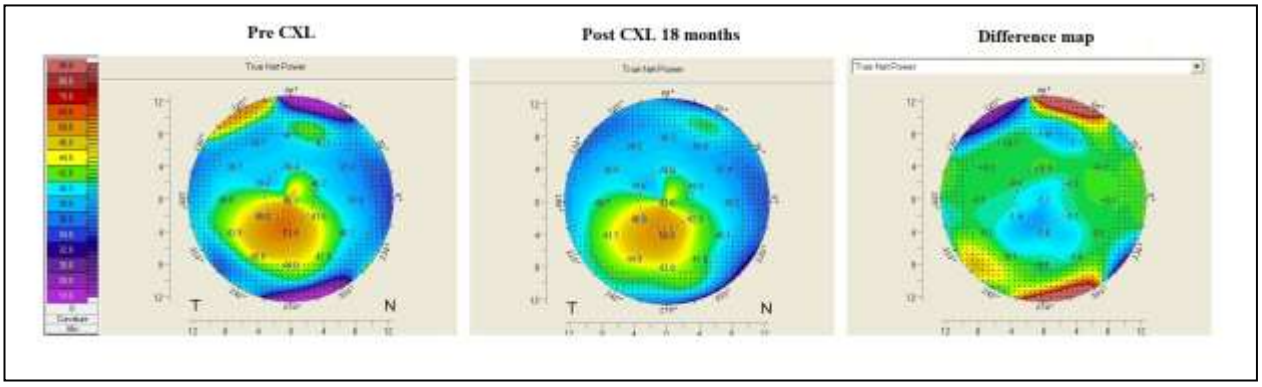
There was a decrease in topometric astigmatism by 0.20 ± 1.44D (p = 0.51) after crosslinking. Figure 37 shows the difference in K2 between pre and post CXL treated eyes at mean 20 months follow up. Corneal curvature was either reduced or remained stable (within 0.5D of pre CXL K2) after CXL in 88 % (22/25) eyes.

Figure 37: Difference in K2 between pre and post CXL treated eyes



Corneal topography of a crosslinked eye is shown in figure 38. At 18 months after CXL, there was a reduction in K2 by 1.8 D in the treated eye. No serious complications like infections or stromal scarring were noted in this series.

Figure 38: Corneal topography showing a reduction in keratometry after CXL



9.5 DISCUSSION

We assessed the topographic, refractive and visual outcome of corneal UV collagen crosslinking in a cohort of paediatric patients with progressive Keratoconus. At 20 months after crosslinking, there was a mean reduction in simulated keratometry values by 0.66D in the flattest meridian and by 0.72D in the steepest meridian. This was associated with an improvement in visual acuity and topometric astigmatism, although these improvements were not statistically significant.

Collagen crosslinking involves a photopolymerization reaction that induces biochemical and microstructural changes within the corneal stroma.[155,156,304] These include the generation of stiffer collagen fibrils and a rearrangement of corneal lamellae within the matrix .[299,310] These structural and biomechanical changes after crosslinking result in a regression of corneal curvature and improved shape thus stabilising Keratoconus and preventing further progression.

We had previously reported favourable results after collagen crosslinking in adult Keratoconic eyes consistent with other published studies.[152,161-163,165,305] Similar to our adult cohort, this study included only patients who had completed a minimum of 6 months follow up after CXL. This is based on previous long term studies that reported an initial worsening of corneal curvature followed by subsequent flattening and stabilisation after CXL.[161,163]

In recent times, the age limit for CXL has lowered considerably.[306-308] In this study, the youngest patient was 8 years old and to the best of our knowledge is the youngest patient reported to undergo crosslinking. Vinciguerra et al evaluated the long term outcome of CXL

for progressive keratoconus in different age groups, including 49 eyes of patients aged below 18 years.[311] Interestingly, their results indicated better functional and morphologic outcomes in young adults (age 18-39 years) as compared to the paediatric age group.

Arora et al conducted a prospective contralateral case control study and included 15 eyes of 15 keratoconic patients that underwent CXL.[309] The criteria for performing CXL was not documented progression, but the advanced keratoconus status in the fellow eye. At 1 year after CXL, significant improvements were noted in logMAR BSCVA and apical keratometry. In comparison, CXL was performed only on progressive paediatric keratoconic eyes in the current study and albeit a longer follow up, significant improvements were noted only for keratometry values and not for BSCVA.

Magli et al recently compared the efficacy of transepithelial CXL (TE-CXL) to conventional epithelium-off CXL in paediatric patients. [312] At 12 months follow-up, they observed that TE-CXL had similar efficacy, but was less painful and had fewer complications than epithelium-off CXL. Similarly, Salman et al performed a prospective case control study on the efficacy and safety of TE-CXL in children and reported satisfactory results. [313] In the current study, crosslinking was performed using the epithelium-off technique and no serious complications were noted.

In their series of paediatric crosslinking, Caporossi et al report worsening in terms of topographic and pachymetic data in 4.6 % of eyes, however the term ‘worsening’ is not defined.[308] We observed worsening of the steep keratometry value (K2) by more than 0.5D in 3 crosslinked eyes. However, an increase in K2 exceeding 1D occurred in only 1 eye which was not associated with a decrease in BSCVA. We presume that this reflects the fast rate of

keratoconus progression in paediatric eyes. Therefore, earlier studies have suggested a closer follow up schedule for children with keratoconus to rapidly identify deterioration.

This study demonstrates that collagen crosslinking can result in a significant reduction in corneal curvature and stabilise progressive keratoconus in patients younger than 18 years. These encouraging results emphasize the need for early treatment in these young patients and long term studies are needed to ascertain whether CXL could prevent or reduce the need for corneal grafting. The optimal timing of intervention however remains debatable with some authors suggesting crosslinking at diagnosis of keratoconus without awaiting disease progression.[314]

CHAPTER 10 A: DISCUSSION AND SUMMARY

Previous research studies in the literature have postulated that the biomechanical properties of ocular tissues including the cornea may be altered as a result of elevated intraocular pressure (IOP). [5,6] In fact high IOP in congenital glaucoma causes expansion of the cornea with ruptures in Descemet's membrane, while juvenile onset glaucoma leads to increasing myopia if untreated. Corneal hysteresis (CH) has a moderate dependence on IOP and the greater the IOP, the lower the CH value. [5,6] Keratoconus (KC) is a progressive corneal disorder and research studies have demonstrated biomechanical weakening and decreasing CH values associated with increasing KC severity. [246,247] However, no studies have determined whether IOP lowering might help halt disease progression in KC by influencing the corneal biomechanics. Corneal collagen cross-linking is a rapidly evolving newer form of treatment for progressive KC that increases the corneal stiffness and stabilises the condition. Based on these principles and findings, the original design of this thesis was to determine if KC progression could be deterred by lowering IOP using glaucoma medications combined with collagen cross-linking.

However, initial literature review raised some potential concerns about the possible effects of prostaglandin drugs on the cornea, and as a result we held off commencing the IOP lowering study until we had more data. Topical application of antiglaucoma medications has always been the primary modality of glaucoma treatment and they broadly belong to four classes of drugs: prostaglandins, beta-blockers, alpha- agonists and carbonic anhydrase inhibitors. However, long-term use of these eye drops is invariably associated with adverse effects, some of which affect the corneal tissue and this could be related to the presence of preservatives. [181-183] We therefore decided to conduct a large glaucoma medication study to determine if there

were any side effects on the cornea from the eye drops used for long term glaucoma management. Findings from our study suggest that prostaglandins do in fact reduce central corneal thickness (CCT) initially but not progressively.

A possible mechanism for prostaglandins to induce corneal thinning could be their effect on matrix metalloproteinases (MMPs). The MMP family includes around 20 types of enzymes that may be present in the anterior segment of the eye, including the cornea, aqueous humor, trabecular meshwork, ciliary muscles and other structures. [23-26] MMPs are a group of enzymes that degrade collagen, a vital constituent of basement membrane and extracellular matrix components in the ocular tissue. [22] Due to these actions, MMPs have often been implicated to have a significant role in the pathogenesis of keratoconus progression. The IOP lowering effect of prostaglandin analogues is recognized to be caused by MMP activation in the ciliary body smooth muscles. [27] MMP overactivity in the cornea induced by prostaglandins could potentially thin the corneal stroma and cause a reduction in corneal thickness. [28]

The proteolytic acitivity of MMPs in the cornea has been implicated as one of the main pathogenic mechanisms of keratoconus. Therefore, KC being a progressive thinning disorder of the cornea, we decided that the use of prostaglandins was not advisable in these cases. Unfortunately alternatives such as beta-blockers and alpha-agonists were also inappropriate in the young atopic population with KC, while carbonic anhydrase inhibitors could affect the corneal endothelial function. We could not therefore justify the use of topical therapy in this population. This led to a redesign of the thesis to concentrate mainly on corneal structural and biomechanical changes in the two diseases (glaucoma and KC) and to examine the results of collagen cross-linking in progressive KC eyes and the entire PhD project therefore evolved.

Based on our initial study findings, performing repeat CCT measurements would be appropriate in long term glaucoma management especially for those on prostaglandin analogues. This is particularly relevant in cases of normal tension glaucoma as they are associated with thin corneas. We noted a big variability in the amount of CCT change with some eyes thinning upto nearly 50 μm.Therefore, the potential implication of CCT change over time on IOP measurements should also be considered , although in most cases is not likely to be clinically significant.

A logical sequence of the glaucoma medications study was to correlate CCT and CCT change to glaucoma risk and progression in a large clinical study. The link between a thin CCT and glaucoma progression has been controversial. Results from our study establish that thinner corneas are indeed related to advanced baseline glaucoma presentation and are associated with a faster rate of visual field progression. However the magnitude of ongoing CCT change is not related to glaucomatous progression and this is a completely novel finding. Therefore, the glaucoma studies performed on a large number of subjects and controls present innovative findings and contribute substantially to glaucoma practice guidelines.

We had hypothesised that there might be potential overlapping mechanisms between glaucoma and KC including a thin cornea and altered biomechanics, although there is no clear established connection between the two diseases. This thesis involved various studies on a large number of KC subjects (nearly 250 eyes). However, despite having thin corneas, we found no signs of glaucoma in any KC subject on routine examination.

To further characterise the pathological features of keratoconus, the relationship between corneal structural and biomechanical charecteristics was studied in detail in a cohort of

normal, forme fruste keratoconic (FFKC), KC and crosslinked KC eyes. We established that CH and CRF are influenced by corneal structure, with higher values seen in corneas with greater thickness and volume. In contrast, as the cornea thins and steepens in progressive KC eyes, there is further biomechanical instability as reflected by lower CH and CRF values. However, in KC eyes after collagen crosslinking, the relationship of corneal curvature to biomechanical profile was similar to normal eyes, possibly due to the induced corneal stiffness after the procedure. No relationship was however noted with intraocular pressure.

Recent advances in corneal biomechanical imaging have led to the development of novel waveform parameters including the keratoconus match index (KMI). We demonstrated that KMI is more sensitive and specific than CH and CRF values in detecting KC and that it correlates well with topographic KC diagnosis. Therefore, results from our studies indicate that corneal biomechanical evaluation is developing as a well established modality to detect and moniter the effects of treatment in KC eyes. Lastly, we evaluated the effects of UV collagen cross-linking in progressive adult and paediatric KC eyes in prospective clinical studies. Our results indicate that cross-linking effectively stabilises KC in adult and paediatric KC eyes accompanied by regression of corneal curvature and improvement in visual acuity noted in some cases.

Despite all current research, some questions remain unanswered, for example: 1. why is a thinner cornea a risk factor for glaucoma? 2. Why does the cornea thin progressively in KC? In order to develop a deeper understanding of the dynamics of these risk factors and disease processes, further detailed longitudinal studies are needed, and alternative investigative approaches such as preoteomics and genetics may help uncover the mechanisms involved.

10 B: CONCLUSIONS AND FUTURE DIRECTIONS

This PhD thesis investigates and discusses in detail the relationship of corneal structure and biomechanical profile to the diagnosis and treatment of two significant ocular disorders : keratoconus and glaucoma.

The conclusions arising from this thesis include:

1. Long term use of topical anti-glaucoma medications exerts an effect on corneal thickness. Prostaglandins appear to be associated with a small but significant CCT reduction over time. Therefore it would be worthwhile performing serial CCT measurements in glaucoma patients, especially those on prostaglandin analogues (chapter 3).

2. Thinner corneas are related to advanced baseline presentation in glaucoma. Thinner CCT could be associated with an increased risk of visual field progression, however the magnitude of ongoing CCT change was not related to visual field progression (chapter 4).

3. We observed no associations between CH, CRF and maximal keratometry values in normal and crosslinked keratoconic eyes. Therefore, it would appear that collagen crosslinking normalises the relationship of corneal curvature to biomechanical profile in keratoconic eyes (chapter 5).

4. Corneal biomechanical parameters progressively decrease as severity of keratoconus increases. CH and CRF are influenced by corneal structure, with significantly higher values noted in corneas with greater thickness and volume and flatter curvature (chapter 5).

5. The Scheimpflug camera (Pentacam) measures significantly greater corneal curvature, thickness and elevation values compared to the combined Placido-optical coherence tomography device (Visante OMNI) in normal and keratoconic eyes. The devices agree well only for anterior corneal elevations in normal eyes and do not appear to be interchangeable for corneal measurements in clinical practice (chapter 6).

6. Both Pentacam and Visante OMNI demonstrate excellent intra-operator repeatability and inter-operator reproducibility for corneal curvature and thickness values but not for corneal elevations, particularly posterior elevation values. Therefore, it appears that posterior elevations are not reliable for monitoring keratoconus progression (chapter 6).

7. ORA waveform parameter KMI appears to be superior to CH and CRF as an indicator of keratoconus diagnosis and severity . KMP identifies a significant percent of keratoconic eyes as normal and does not agree well with topographic KC diagnosis (chapter 7).

8. Corneal collagen crosslinking using Riboflavin and ultraviolet-A is effective as a therapeutic option in cases of progressive keratoconus by reducing the corneal curvature and improving the visual acuity in these patients. Control fellow eyes that did not undergo crosslinking demonstrated progression of keratoconus (chapter 8).

9. Progressive keratoconus in paediatric patients can be effectively managed by collagen crosslinking using Riboflavin and ultraviolet-A. Crosslinking stabilises the condition and therefore, could potentially reduce the need for corneal grafting in these young patients (chapter 9)

Future directions may include:

1. Topical Prostaglandin therapy in glaucoma patients could be associated with corneal thinning that may affect the corneal biomechanical properties. Therefore, measurement of CH and CRF before and after starting these drugs could be worthwhile. This would be particularly relevant in the management rare cases of keratoconus with co-existant glaucoma.

2. CH and CRF could be effective predictors of glaucoma progression, particularly in eyes with thinner corneas and so measuring these biomechanical parameters could be beneficial in glaucoma practice.

3. The air puff based Ocular response analyzer and the Scheimpflug principle based CorVis ST differ in their methodology and therefore may vary in their measurement of corneal biomechanical properties. Therefore, a comparative study of these two devices in normal, FFKC, keratoconic and CXL treated eyes is recommended.

4. Corneal elevation based keratoconus screening programmes incorporated in different imaging devices: the Belin Ambrósio Enhanced Ectasia Display (Scheimpflug camera Pentacam) and the PathFinder Corneal Analysis software program (Atlas topographer) could have dissimilar sensitivity and specificity and therefore would be interesting to compare.

5. Keratoconus suspect eyes and other corneal ectatic disorders including Pellucid marginal degeneration might exhibit interesting biomechanical properties and hence, advanced analysis of ORA waveform parameters are warranted in these cases.

6. Outcomes of the recently introduced accelerated CXL procedure could be different to the conventional CXL procedure in progressive keratoconic eyes and merit a comparative study.

7. Corneal collagen cross-linking might improve the quality of life (QOL) in adult and paediatric keratoconic patients, especially their visual rehabilitation including the dependence on contact lenses and QOL study findings could yield useful data to help manage these cases.

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APPENDIX

Ethics approval

Final Approval- Ethics Application Reference-5201001446 (D)

2/21/11

Ethics Secretariat ethics.secretariat@mq.edu.au
to Dr, Dr, Dr, me

Dear Dr Graham

Re: "Corneal biomechanics in Keratoconus - relationship to intraocular pressure and treatment interventions" (Ethics Ref: 5201001446)

Thank you for your recent correspondence. Your response has addressed the issues raised by the Human Research Ethics Committee and you may now commence your research.

The following personnel are authorised to conduct this research:

Dr Stuart Graham- Chief Investigator/Supervisor
Dr Deepa Viswanathan, Dr John Males & Dr Nikhil Kumar- Co-Investigators

NB. STUDENTS: IT IS YOUR RESPONSIBILITY TO KEEP A COPY OF THIS APPROVAL EMAIL TO SUBMIT WITH YOUR THESIS.

Please note the following standard requirements of approval:

1. The approval of this project is conditional upon your continuing compliance with the National Statement on Ethical Conduct in Human Research (2007).

2. Approval will be for a period of five (5) years subject to the provision of annual reports. Your first progress report is due on 21 February 2012.

If you complete the work earlier than you had planned you must submit a Final Report as soon as the work is completed. If the project has been discontinued or not commenced for any reason, you are also required to submit a Final Report for the project.

Progress reports and Final Reports are available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

3. If the project has run for more than five (5) years you cannot renew approval for the project. You will need to complete and submit a Final Report and submit a new application for the project. (The five year limit on renewal of approvals allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).

4. All amendments to the project must be reviewed and approved by the Committee before implementation. Please complete and submit a Request for Amendment Form available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

5. Please notify the Committee immediately in the event of any adverse effects on participants or of any unforeseen events that affect the continued ethical acceptability of the project.

6. At all times you are responsible for the ethical conduct of your research in accordance with the guidelines established by the University. This information is available at the following websites:

http://www.mq.edu.au/policy/

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/policy

If you will be applying for or have applied for internal or external funding for the above project it is your responsibility to provide the Macquarie University's Research Grants Management Assistant with a copy of this email as soon as possible. Internal and External funding agencies will not be informed that you have final approval for your project and funds will not be released until the Research Grants Management Assistant has received a copy of this email.

If you need to provide a hard copy letter of Final Approval to an external organisation as evidence that you have Final Approval, please do not hesitate to contact the Ethics Secretariat at the address below.

Please retain a copy of this email as this is your official notification of final ethics approval.

Yours sincerely
Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee