TONOMETRY AGREEMENT AND CORNEAL BIOMECHANICAL FEATURES IN NORMAL, GLAUCOMATOUS AND KERATOCONIC EYES

WAN HASLINA BINTI WAN ABDUL HALIM

Doctor of Philosophy

ASTON UNIVERSITY

June 2016

ASTON UNIVERSITY

TONOMETRY AGREEMENT AND CORNEAL BIOMECHANICAL FEATURES IN NORMAL, GLAUCOMATOUS AND KERATOCONIC EYES

Wan Haslina Binti Wan Abdul Halim

Doctor of Philosophy

June 2016

Thesis summary

Intraocular pressure measurement is a routine clinical examination performed in ophthalmic practice. It is vital in clinical monitoring, diagnosis and management of certain eye diseases. There are many types of tonometers currently available to measure the intraocular pressure (IOP). These tonometers employ different technologies compared to the standard Goldman applanation tonometer (GAT). Studies often report inter-tonometry agreement and bias of new tonometers against GAT. However, only a minority have studied the proportionate bias and factors that influence the inter-tonometry bias of a new tonometer. The inter-tonometry agreement is vulnerable to the influence of corneal physical and mechanical properties. The information on reliability and agreement between different tonometers is very important in the management of ocular diseases.

The aim of this thesis was to examine the inter-tonometry agreement between five different tonometers. The influence on IOP of demographic and ocular factors was investigated. This thesis investigates the biomechanical characteristics of the cornea of normal, glaucomatous and keratoconus subjects and the factors that influence biomechanical parameters. The tonometers employed were found to have a good agreement with GAT but the tonometry values were not interchangeable. The bias of each tonometer was influenced differently by central corneal thickness (CCT), specific corneal biomechanical parameters and age. Clinicians should be cautious when examining glaucoma and keratoconus patients with different tonometers, as most demonstrate significant proportionate bias. The corneal biomechanical parameters in subjects with different ocular diagnoses revealed variable significance and was influenced by age, CCT and corneal curvature. Future research to identify unique corneal parameters in different ocular conditions may be of importance especially in screening and diagnosis.

Keywords: Inter-tonometry agreement, tonometry bias, corneal biomechanics, corneal hysteresis

DEDICATION

Bismillahirrahmanirrahim.

I would like to dedicate my work and thesis to my beautiful family.

To my loving parents, thank you for your endless love and prayers. I shall always be indebted to both of you for my happiness and success.

To my best friend, my husband and my better half; Dr Ghazali Yusri AR. Thank you for being my pillar of patience and strength. Every day you teach me how abundant love can be.

To Afif Fathi; my beginning of love and life, Iyad Najmi; my strong and shiny star, Widad Raihanah; my fragrance of love, Ilham Farhanah; my happy inspiration and Imad Wajdi; my champion of tenderness. I am indeed blessed to have you all by my side on this emotional roller-coaster and wondrous PhD journey. Thank you for your patience and all the beautiful years.

All praises be to Allah; The Almighty, The Merciful and The Benevolent.

ACKNOWLEDGEMENT

This doctoral thesis was completed with the help and support of kind people around me.

My utmost appreciation and thanks to my supervisors Dr Shehzad A. Naroo, Professor Sunil Shah and Mr Maged Nessim for their guidance and support during my study. Dr Naroo has been very dedicated, helpful and kind. His guidance has been invaluable for the writing of this thesis. His tireless effort and support to push me for the better is greatly appreciated. I am forever indebted for his patience and support.

I am grateful to Professor Sunil Shah and Mr Maged Nessim for their kind assistance and guidance. Both supervisors have contributed greatly to the study; from the initial phase of my study until the completion of my data collection. Their helpful comments and critiques have been my guidance to better understand my research and also myself. Special thanks to all the resident doctors and supporting staffs of the Ophthalmology Clinic, Birmingham and Midland Eye Centre for their help and support during my data collection.

I would like to acknowledge Dr Chinny Excel, Dr Mark Dunne and Dr Richard Amstrong for their invaluable input on the methodology and statistical aspects of my thesis. I am very appreciative of my supportive colleagues from the Ophthalmology Department, Universiti Kebangsaan Malaysia Medical Centre. Last but not least, I would like to acknowledge the pronounced financial and academic support of the Universiti Kebangsaan Malaysia during my study at Aston University.

TABLE OF CONTENTS

GLOSSARY OF ABBREVIATIONS

A1L : Cord length of the cornea during first cornea applanation

A1T : Time from start to first applanation response

A1V : Speed of the cornea during first applanation response

A2L : Cord length of the cornea during second applanation response

A2T : Time from start to second applanation response

A2V : Speed of the cornea during second applanation response

ANOVA: Analysis of variance

CCT : Central corneal thickness

CCTus : Central corneal thickness by ultrasound method

CH : Corneal hysteresis

CI : Confidence Interval

CR : Coefficient of repeatability

CRF : Corneal resistance factor

CST : Corvis ST

CV : Coefficient of variation

DA : Amplitude of the corneal movement at highest concavity deformation

GaGs : Glycosaminoglycans

GAT : Goldman applanation tonometer

HcR : Radius of corneal curvature at maximum concavity deformation

HcT : Time from start to maximum concavity

HpD : Distance of the most anterior point of the anterior corneal surface during

highest concavity deformation

ICC : Intraclass coefficient correlation

IOP : Intraocular pressure

IOPcc : Intraocular pressure (corneal compensated) by Ocular Response Analyzer

IOPcst : Intraocular pressure by Corvis ST

IOPg : Intraocular pressure (correlated to Goldman applanation tonometer) by Ocular Response Analyzer

IOPgat : Intraocular pressure by Goldman applanation tonometer

IOPicare: Intraocular pressure by iCare tonometer

IOPtono: Intraocular pressure by Tonopen

LASIK : Laser-assisted in situ keratomileusis

LOA : Limit of agreement

mmHg : milimeter of mercury

NTG : Normal tension glaucoma

OHT : Ocular Hypertension

ORA : Ocular Response Analyzer

PGs : Proteoglycans

POAG : Primary open angle glaucoma

PRK : Photorefractive keratectomy

SD : Standard deviation

LIST OF TABLES AND FIGURES

CHAPTER 1: INTRODUCTION

Intraocular pressure (IOP) is the primary risk factor for the development and progression of glaucoma, and is presently the only modifiable risk factor. Guidelines from the National Institute for Clinical Excellence (NICE) CG85 for Glaucoma (NICE Guidelines 2009) and the Royal College of Ophthalmologists recommended that ophthalmic clinicians refer patients to the ophthalmology referral centre when the IOP is higher than 21mmHg, regardless of any glaucomatous change[1, 2]. A recent report suggested a refinement of the NICE guideline to avoid unnecessary referrals that involved repeated applanation tonometry by local accredited optometrists[3]. This has resulted in reduction in the number of referrals to the hospital eye service and is significantly cost-effective[4].

The advent of new tonometers aims towards enhancing the accuracy of IOP measurements. However, the choice of tonometers in each screening centre may be different and can depend on the suitability of patients. The purpose of this study was to highlight the tonometry agreement amongst healthy, glaucomatous and keratoconus patients against the standard applanation tonometer. This study shall report factors that can influence the agreement and provide recommendations to ensure suitable tonometer for IOP measurement. Additionally, corneal biomechanical characteristics of these patients are explored. In vivo corneal biomechanical instruments are used to enable further understanding of the interesting properties of the human cornea in glaucoma and keratoconic eyes. It is hoped that this shall aid in screening and monitoring of disease.

1.1 Intraocular Pressure

The intraocular pressure (IOP) is the pressure exerted by the equilibrium of intraocular fluid production and drainage that causes tension to the ocular wall. In principle, there are two method of measuring an internal pressure, either internal pressure measurement by invasively entering the pressured space (manometry) or relatively by measuring the wall tension aided by mathematical formulae. Even though manometry is the most accurate method for measuring the IOP, it is an invasive procedure and inappropriate for routine clinical practice. Today, all current commercially available tonometers measure relative values of IOP.

1.1.1 Tonometer

In the early years of the development of tonometry, indentation techniques were primarily involved. The technique measured how easily the globe was compressed. In the early 1900s, the common type of indention tonometry, Schiotz tonometry, was first used to measure the IOP. Since then, significant amounts of research and improvements were ongoing to explore on ocular rigidity and IOP [5]. By the mid 1950s, the advent of Goldmann applanation tonometry (GAT) had dominated Schiotz tonometry and it became quickly out of favour[6]. The applanation tonometer had become the preferred method to measure intraocular pressure. The GAT was claimed to be far less affected by ocular rigidity than Schiotz tonometry. The GAT applanates an area of the anterior corneal surface and is based on the principles of the Imbert-Fick formula.

The GAT was initially designed to decrease the effect of ocular rigidity on IOP measurement. The Imbert-Fick principle determines the value of the force that is needed to applanates the ocular wall, which is usually the cornea. The Imbert-Fick principle states that the IOP can be indirectly measured via quantifying the pressure required to flatten a known area of the cornea[7]. The principle assumed that the cornea is dry, perfectly elastic, infinitely thin and spherical, which is in actuality, is not. This ‘law’ was challenged as the model eye features are not true to normal eyes[8]. Thus, Goldmann calibrated his invention against manometric tonometry to evaluate the appropriate area of applanation to enable ‘precise’ tonometry by GAT[6]. It is assumed that the pressure from inside the eyeball that resists the external applanation of tonometry probe was equal and opposite to the attractive capillary forces of the tear film. This is achieved at the applanation area of 3.06mm^2 in eyes with a mean central corneal thickness of approximately 520μm. The calibration was done against a small group of eyes (with

mean central corneal thickness of 520 μm) that may be different to other patient population with different corneal thickness and tear film properties.

It is known that the central corneal thickness (CCT) can influence GAT readings [9-11]. The formula for IOP adjustment was introduced based on the well-known effect of IOP overestimation of thicker corneas and underestimation of thinner ones. However, other studies have shown that individuals also demonstrate wide variations in corneal biomechanical properties [12-14]. The GAT may be less prone to biomechanical influence but it is not entirely independent [15, 16]. It has even been suggested that the biomechanical properties of the eye (e.g. elasticity and rigidity) may be a stronger factor than CCT on applanation tonometry readings and that CCT is just a poor estimation of biomechanical properties [17]. Researchers have proposed specific formulae to calculate the influence of CCT on IOP measurement, but there is no consensus in practice[16, 18]. However, clinicians are aware that the IOP may be over or underestimated because of these ocular and corneal properties [18]. Nonetheless GAT is still regarded as the gold standard for tonometry. All comparisons of the different types of tonometers will be based on GAT in this thesis.

The tonometers that require corneal contact are TonoPen XL® (Tonopen(Bio-Rad, Glendale, California) and iCare® (Icare(Tiolat Oy, Helsinki, Finland). The Tonopen is an electronic hand-held tonometric device that is based on the Mackay-Marg principle. The Tonopen enables tonometry in both supine and sitting position. The Icare is an electronic hand-held tonometer that is based on a rebound principle and is ideal for children as it requires no anaesthesia. The non-contact tonometers are mostly popular for eye care practitioners for screening purposes as they are less operator dependant. In this study, two air-puff tonometers are included; the Corvis® ST (CST)(Oculus Optikgeräte GmbH, Wetzlar, Germany) and Ocular Response Analyzer® (ORA)(Reichert Ophthalmic Instruments, Buffalo, New York). Other than tonometry, the ORA (used since 2006) and CST (launched in September 2011) are non-contact tonometers that are able to measure both IOP and corneal physical parameters that are related to ocular biomechanics [19, 20]. These tonometers are described in section 2.4.

1.1.2 Agreement of Tonometers

It is challenging for all eye care practitioners to use a single tonometry method in their clinical practice as a patient's ocular or physical condition may render them unsuitable for standard applanation tonometry. The current standard of tonometry is GAT, which is

not flawless, but is much preferred due to its low maintenance and historical precedence [18, 21].

In 2012, Cook et al. reported a large meta-analysis of 109 studies on tonometer agreement with GAT [21]. The authors highlighted the sizable variability of agreement [21]. According to that study, amongst the tonometers employed, the non-contact tonometer (including ORA) and the handheld applanation tonometer (Perkins's handheld tonometer) have the closest agreement to GAT. However, the analysis reported that the measurements both within and between studies have a substantial variability. The authors suggested significant inter and intra-observer variability for all 7 tonometers included in the analysis. The repeatability of tonometry measurements of the GATwas also significantly different amongst the studies included in the meta-analysis. This would explain the scale of heterogeneity of the cohorts observed to some extent. However, the author did not report on factors that influenced the inter-method agreement between the tonometers [21].

A recent review noted that the Bland-Altman method is the most popular used in agreement research [22]. The review discussed the lack of standardisation in analysis and reporting in the method comparison studies of medical instruments published in the literature. The authors highlighted that almost 10% of method comparison studies of medically related instruments were analysed inappropriately and may have led to erroneous results [22]. This was supported by two review articles that noted that many agreement studies did not have adequate samples and reported the use of improper statistical measure [21, 23]. Many tonometry agreement studies recruited subjects with variable ocular conditions that could influence agreement [21].

1.2 Corneal Biomechanics

Biomechanics is the science concerned with the internal and external forces acting on the human body and the effects produced by these forces. It has become a subject of high interest since scholars acknowledged that each structure of the human body has its own anatomical, physiological as well as physical properties to enable its function. Many clinical and laboratory studies have shown that the cornea is not a mechanically inert structure [24].

Early evidence mainly from the results of incisional refractive surgery has highlighted the importance of corneal biomechanics in the outcome of the treatment [25-27]. The influence on the cornea of its biomechanical properties has received little attention, due to the lack of feasible and adequate measurement techniques. However, in the past decade, particular attention in corneal biomechanical properties has developed in glaucoma management. This has generated much interest in finding the real corneal biomechanical parameters and their correlation with ocular diseases and tonometry. Increasing interest has also arisen in corneal biomechanics with regard to diagnosis and therapy, e.g. collagen crosslinking (CXL) of keratoconus.

1.2.1 Physical Properties of the Cornea

The foundation of corneal biomechanics is the microanatomy of the cornea. Collagen is the primary structural component and the ground substance of both the cornea and sclera. It has a high tensile strength and provides a resilient, protective coat to the globe. Anisotropy in fibril packing across the cornea has potential implications for the transparency and refractive index of the tissue. Biomechanically, it is possible that the higher packing density of stress-bearing collagen fibrils of the stromal tissue in the pre-pupillary cornea is necessary for maintaining corneal strength, and hence curvature, in a region of reduced tissue thickness [28].

A laboratory study using an X-ray diffraction and femtosecond laser highlighted this difference in the collagen fibrils orientation in the cornea [29]. Collagen fibrils at different depths throughout the entire thickness of the human cornea have different distribution pattern and predominant orientations. Arrangement of the collagen fibrils of orthogonal orientation in the mid and posterior stroma may help to distribute strain in the cornea by allowing it to withstand the pull of the extra ocular muscles, whereas the more isotropic arrangement in the anterior cornea may play an important role in the biomechanics of the cornea by resisting intraocular pressure while at the same time maintaining corneal curvature [30]. Out of the five anatomic layers of the cornea, the epithelium and endothelium contributes least to the biomechanical behaviour of the cornea [31, 32].

With the understanding of the unique corneal microstructure and its contribution to corneal biomechanics, there are many corneal models proposed to demonstrate different corneal properties such as after collagen cross linking treatment [33, 34], photorefractive keratectomy (PRK) and limbal relaxing incisions [35]. Surgical parameters were considered in PRK and laser-assisted in situ keratomileusis (LASIK) to assess

quantitatively the effect of each parameter on the optical outcome, all in the pursuit of successful surgical outcomes.

1.2.2 Biomechanical Properties of the Cornea

Corneal biomechanical properties characterise the response of corneal tissue to an applied force. The cornea is said to be a viscoelastic structure as it is able to return to its initial state after removal of mechanical force.

Elasticity refers to the response and deformation of a material towards an external stress. The stress-strain relationship can be plotted graphically and an elastic material is one that regains its original form in a completely reversible displacement direction along the stress-strain pathway when the imposed stress is removed (Figure 1.1). The measurement of the slope from a representative portion of the graph is termed the modulus of elasticity (Young's modulus). A high modulus indicates a stiffer material.

Ex-vivo studies have shown that the cornea exhibits nonlinear elastic behaviour, such that Young's modulus increases with increasing tissue stress [36, 37]. Moreover, the cornea's elastic modulus varies directionally and regionally, such that a high modulus is exhibited meridionally at the centre and paracentral areas, and circumferentially at the limbus, due to the specific arrangement of collagen fibrils described earlier[38]. Although the normal range of in vivo values of human cornea Young's modulus remains unknown, mathematical modelling has predicted that it varies with IOP, such that a stiffer cornea is manifested at higher levels of IOP[39].

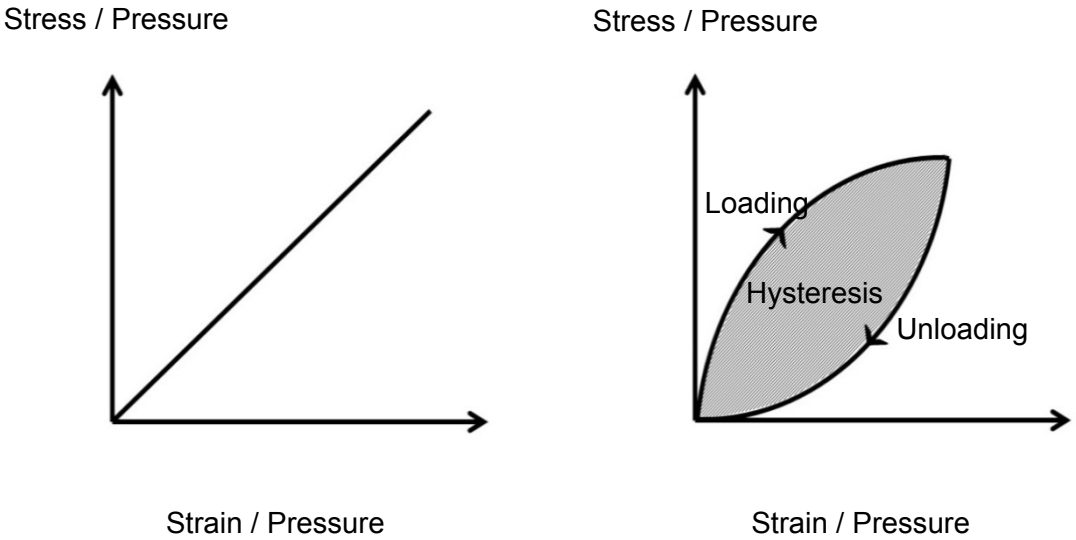


Figure 1.1 The stress/strain curve for an elastic and viscoelastic material

The water content and large molecular components of the cornea will determined its viscosity [40]. Molecules in highly viscous or gel-like substances are strongly connected to each other and are not very flexible. The content of glycosaminoglycans (GaGs) and structure of proteoglycans (PGs) in the ground substance is the basis of viscosity. This ground substance contributes significantly to the mechanical properties of the tissue [41]. It was observed that the viscoelastic behaviour decreases with removal of prostaglandins [42]. Furthermore, the ground substance is important not only as a stress absorber, but also in determining the damping capacity of a tissue [43]. Viscous materials flow when an external stress is applied and, unlike materials with elastic properties, do not regain their original shape when the stress is removed.

As a viscoelastic material, the cornea has elements of both viscosity and elasticity and as a result, energy is dissipated when a stress is applied. Hysteresis refers to the energy lost during the stress-strain cycle. The physical concept of corneal hysteresis is the result of the viscous damping within corneal tissues that is created by the viscosity of GaGs [44] and PGs, as well as by a collagen matrix interaction [45, 46].

1.2.3 In-Vivo Corneal Biomechanical Measurement Devices

The understanding of the importance of the physics of the cornea as a biomaterial and its influence on clinical findings has led to the exploration of other devices in order to investigate the mystery of biomechanics. Until almost a decade ago, only invasive techniques were available to characterise the biomechanical properties of the cornea, which made measurements in a living eye impossible.

Until nearly a decade ago, there were only invasive methods of measuring corneal biomechanical properties which made measurement in a living eye almost impossible. Since the introduction of the Ocular Response Analyser (ORA) in 2005, studies on corneal behaviour in vivo and in many different corneal conditions was made feasible. The development of new in vivo technology to measure the corneal dynamics during deformation using high performing cameras led to the introduction of the Corvis ST in September 2011.

1.2.3.1 CorVis ST

The Corvis© ST (CST) (Oculus Optikgerate GmbH, Germany) is a dynamic Scheimpflug corneal imaging analyser. The CST combines a non-contact tonometer with a high-speed camera to capture a series of horizontal Scheimpflug images, at a rate of 4,300 frames per second, during corneal deformation with an air puff jet. In addition to the deformation response, the CST is also able to measure the IOP and the corneal thickness simultaneously. The corneal image analysis by CST produces nine dynamic corneal parameters that represent different phase of corneal deformation. It has been commercially available since September 2011. Details of its measurement principles and techniques are described more in Section 2.3.

1.2.3.2 Ocular Response Analyser (ORA)

The ORA is a non-contact applanation tonometer that can provide a measure of intraocular pressure (IOPcc) that was claimed to be independent of corneal factors. This device not only provides an assessment of intraocular pressure (IOPcc), but also provides a corrected intraocular pressure (IOPg). Corneal hysteresis measured with the Ocular Response Analyzer, reflects viscous damping in the cornea, which is the capacity of corneal tissue to recover to its shape following the application of external forces.[19]

The ORA releases a precisely metered air pulse that causes the cornea to move inward, successively passing through inward and the outward (as it recovers its shape) applanation phases. On both phases, the intraocular pressure is recorded and the difference in IOP values corresponding to inner and outer applanation phases is corneal hysteresis. Thus, hysteresis reflects resistance to ocular deformation due to the combined effect of the parameters such as corneal thickness, corneal viscoelastic properties and ocular rigidity [47]. Details on the principles and measurement techniques of ORA are provided in section 2.3.

1.2.4 Factors Affecting Corneal Biomechanical Parameters

1.2.4.1 Age

Alterations in the biomechanical properties of the cornea occur with age. Physically, the cross-sectional area of fibrils grows because of the continual deposition of collagen [48] and the accumulation of on-going physiological age-related collagen CXL. Moreover, the gradual degradation of the PGs and GAGs of the viscous ground substance increases corneal stiffness[49]. These changes supported an earlier finding that stiffness of the cornea increase with age[50].

This observation is supported by ex vivo studies on donor corneas. A study that used radial shearing speckle pattern interferometry showed that the stiffness of the human donor cornea increases by a factor of approximately two between the ages of 20 and 100 years [51]. Other laboratory evidence supports the finding that the cornea stiffens with age because of increases in the modulus of elasticity [52], the ocular rigidity coefficient,[53, 54] and cohesive tensile strength [55]. Clinically, a quantitative evaluation by using dynamic image analysis during non-contact tonometry has also showed an increase in corneal viscosity with age[56].

These findings, however, contradict those of clinical studies that have used the ORA to measure biomechanics in vivo. The ORA produces two biomechanical parameters namely corneal hysteresis (CH) and corneal resistance factor (CRF). With increasing age, a reduction in CH [57-65], CRF (45, 68, 73–75) and CCT [66, 67] were noted. Interestingly, some studies using the ORA have also found that CH has no dependence on age[68]. These mixed findings may reflect the different participation of viscous and elastic elements in biomechanics between the cadaveric cornea and live corneal tissue[65, 69]. The cadaveric cornea, a swollen cornea that is deprived of corneal

endothelium pump action, may have larger corneal volume, thereby reducing the viscosity of the ground substance. This inference may be supported by a laboratory study conducted by Daxer et al. [48], which found that the orientation and dimension of collagen fibrils changed with age and thus caused the supposed increase of the corneal strength to be actually reversed. This finding may explain the variation in the biomechanical correlation showed by *ex vivo* studies with the age of the corneal tissue donor.

There was limited reference related with CST. A recent study with the CST on patients younger than 40 year old showed a positive association between age and highest concavity time (HC-time). The authors postulated that the HC-time inversely represents the viscoelasticity profile in patients as a result of physiological cross-linkage of the corneal stoma collagen fibrils in ageing eyes [70].

1.2.4.2 Central Corneal Thickness

The influence of CCT on Goldmann applanation tonometry (GAT) readings was confirmed by previous studies [10, 11, 17]. The adjustment of the GAT IOP was introduced due to the well-known effect of IOP overestimation by thicker corneas and underestimation by thin corneas [14]. It was suggested that biomechanical properties of the eye such as elasticity/rigidity are possibly as important as CCT.

The corneal biomechanical metrics provided by ORA produced an outcome-significant IOP adjustment in at least one quarter of glaucomatous and normal eyes [71]. However, the IOPcc, unlike GAT-IOP, was not significantly correlated with corneal thickness [72, 73]. Furthermore, the differences between GAT-IOP and IOPcc were significantly related to CCT in healthy eyes. On the other hand, the study in glaucoma patients found both pressure measurements provided by the ORA (IOPcc and IOPg) to be positively correlated with CCT [74].

Previous studies have reported that a thinner CCT is an independent risk factor for open-angle glaucoma in patients with ocular hypertension (OHT)[75-79]. A study by Shah et al. [80] found positive relationships between ORA parameters; CH versus CCT, CRF versus CCT and CRF versus CH in normal eyes. In both glaucoma and normal subjects, CCT is positively correlated with CH [81-83]. As the cornea contains more collagen fibres and ground substances, resistance against deformation and damping capacity rises. Thus, the faster the cornea regains its original condition following deformation, the

higher the rigidity of the cornea. The IOP represents a supplementary force that restores the cornea to its original position [84].

Many studies noted that the IOPcc was inversely correlated with CH [65, 68, 85-87]. Kamiya et al. found that eyes with thinner CCT as well as higher IOP values are more predisposed to having lower CH [68]. The CRF increases with rising IOP indicating that resistance against the deformation of the cornea is higher in eyes with higher IOP values. The confounding effects of IOP and CCT on ORA biomechanical parameters was investigated by Galletti et al. leading to new values termed “transformed CH” (CHcorr) and “transformed CRF” (CRFcorr)[88]. These values are not in frequent use now.

1.2.4.3 Corneal Curvature

Corneal curvature significantly affects CH in patients using orthokeratology contact lenses. The CH and CRF correlated negatively with corneal curvature, with longer radii of curvature (flatter corneas) associated with lower CH and CRF values [89]. Chen et al. had successfully induced lower CH and CRF values by the flattening the cornea by using reverse geometrical lenses [90]. A lower CH value (1 mmHg per 6 D) was also measured in the flattened corneas of young myopias [91]. The CRF was also significantly higher in myopic patients using contact lenses compared with those that did not regardless of the duration of usage [92]. Despite these studies, much needs to be learned about the biomechanical patterns in healthy eyes in relation to corneal curvature as an influence on ORA readings.

1.2.4.4 Gender, Axial Length and Myopia

The recent large population-based study of British adults by Foster et al. found a significant positive association between IOP and the axial length of the eye [93]. The study also identified that longer axial length was significantly associated with lower CH and higher IOP, which is in agreement with the findings of a large population-based study among myopic Chinese children [94]. In support of these findings, higher CH was observed among nanopthalmic subjects [95]. It is believed that the sclera as the outermost ocular wall that contributes to axial length; has its own biomechanical properties. This may be related to the differences in the biomechanical properties of the cornea. In the study by Fowler et.al, the IOPg, but not IOPcc, is higher in women than in men [93]. Other studies also showed highly significant associations between both CRF

and CH as well as with age and gender in which mean CRF and CH declined with age and were higher in women than in men [58, 63, 96, 97].

1.2.4.5 Diurnal Differences

Studies have shown that IOP seems to be independent of any variation in hysteresis or CCT [98, 99]. The CCT and CH is generally constant throughout the day [100]. However, highest IOP readings were recorded in the morning and lowest in the afternoon regardless of the change in these corneal properties. Other studies have found no differences in the diurnal variability of corneal biomechanical parameters during daytime or over 24 hours [98, 99, 101, 102].

1.2.4.6 Topical Anaesthesia and Topical Prostaglandin Analogue Therapy

Topical corneal anaesthesia and dry eye does not influence measurements of CH and CRF [103, 104]. A study by Agarwal et al. demonstrated that CH is influenced by IOP [105]. The baseline CH is reported to be a strong predictor and independently associated with the magnitude of IOP reduction with prostaglandin analogue eyedrop. The study also noted that newly treated glaucoma subjects with lower baseline IOP experienced a higher reduction in IOP compared to those in the highest CH quartile [105].

1.2.5 Acquired biomechanical changes of the cornea

Acquired changes in corneal biomechanics can be categorized by corneal modifications that are treatment related, i.e. corneal collagen crosslinking (CXL) and post-refractice surgery; and those resulting from ocular or systemic pathology, i.e. keratoconus, Fuch's endothelial dystrophy, and diabetes mellitus.

1.2.5.1 Laser Refractive Surgery

The corneal biomechanical integrity may reduce after PRK and LASIK [106-110]. A one dioptre correction of myopia with depth of flap excision (ablation depth, ~16 μm) results in a reduction in CH (0.25 mmHg) and CRF (0.37 mmHg) [111]. Additionally, studies have shown that the corneal flap preparation prior to surgery itself can cause a reduction in CH (1 mmHg) and CRF (0.8 mmHg) [107, 112]. Even though studies have shown a reduction of CH and CRF after LASIK, few longitudinal studies showed that no further corneal biomechanical parameter changes occurred after 6-months post treatment [106,

113]. This is in contrast to keratoconus subjects that noted significant changes occurs with disease progression [114].

The use of ORA as a screening tool to determine the mechanical integrity of the cornea has been recognized to help minimize the risk of keratectasia after LASIK. In a patient with normal corneal thickness and topography, a “pre-ectatic” condition of the cornea may be suspected when there is a low (<8 mmHg) CH and a positive difference between CH and CRF (CH − CRF > 0, which means a low P2 value) , which is an important consideration in therapeutic management [115].

1.2.5.2 Primary Corneal Ectasia

Clinically, biomechanical changes of the cornea can manifest as corneal shape changes, shape instability over time and increased sensitivity to any physiological or surgical stimuli. The mechanical properties of the cornea and its constituent materials are essential to relate its geometrical and optical properties with its mechanical behaviour. Keratoconus is a prime example of corneal ectasia which lead to corneal deformation and thinning. It affects dramatically the mechanical behaviour, and several treatments for these diseases attempt at modulating its biomechanical response.

In keratoconus, the CRF and CH values are reduced and a positive difference of CH − CRF was noted. This is especially detected in patients with increasing severity of keratoconus [47, 116-120]. These changes are postulated to be due to the alteration of the ground substance of the keratoconic eye which lead to lower lamellar adhesion and a lower shear modulus [88, 121, 122]. In advanced keratoconus with corneal scarring, CH and CRF values may be higher due to stiffness inflicted by the fibrous scar tissue [88]. There are no observed differences between CH and CRF values in keratoconus, but in comparison to the pellucid marginal degeneration patients, the first peak of the ORA signal was noted to be higher [123]. The significance of this observation is unknown as there is limited information on the corneal biomechanical properties of the latter diagnosis. Zhang et al. have suggested with the use of updated ORA software, new parameters derived from area under the corneal hysteresis waveform can also significantly differentiate keratoconus severity [124].

1.2.5.3 Corneal Cross-linking

Corneal cross-linking therapy (CXL) is a minimally invasive surgical procedure that involves an application of riboflavin solution to the eye for corneal ectasia such as keratoconus. The CXL aims to increase the rigidity of the corneal tissue, thus preventing progression of corneal ectasia. However, an interesting observation by many studies showed that the ORA measurements before and after riboflavin/UVA crosslinking showed no significant differences in CH and CRF up to 1 year after treatment [125-128]. Even though the CH was noted to reduce in the initial several weeks after CXL procedures, the effect subsided with time. This may be due to the effect of oedema and corneal matrix reorganization that occurred immediately after CXL therapy. The absence of corneal biomechanical parameter changes post CXL therapy may be inferred as the limitation of both ORA parameters to display the overall mechanical inertia and viscous properties of the cornea (ground substance, collagen matrix interaction). Other research suggested the use of a static contact method to provide better detection of the effect of CXL on the cornea [129, 130]. Additional calculations were suggested in an updated ORA software version (version 3.0 and above) by assessing the area under the second peak of the ORA signal. It was claimed to be more sensitive to detect changes [125, 131].

1.2.5.4 Corneal Oedema and Corneal Swelling

Corneal oedema is one of the recognised immediate postoperative changes that occurs following cataract surgery and vitrectomy and causes increase of the corneal thickness. Even though corneal thickness increases, corneal hysteresis decreases due to the increased hydration of the cornea [63, 132-134]. The higher water content leads to the dilution of the corneal ground substance and thus, resulted in reduced viscosity. It reflected in reduced corneal damping capacity in these patients. Similar findings were noted as the corneal thickness also increases with corneal swelling in bullous keratopathy or Fuch's corneal dystrophy [19, 135]. However, cataract surgery did not cause any significant permanent change in corneal biomechanics [133, 136, 137]. In post penetrating keratoplasty patients, reduced CH and CRF was noted in patients with thicker corneas due to the altered corneal structure following surgery [138]. Studies looking at normal subjects with induced corneal oedema by contact lens usage , did not show any significant changes with CH [139, 140]. In conclusion, CH does not appear to usefully quantify biomechanical changes induced by corneal swelling compared to CRF.[140]

Studies comparing corneal changes during menstrual cycle showed conflicting results. Goldich et al. [141] found that the CCT and biomechanical parameters significantly varied during the menstrual cycle. The CH and CRF were temporarily decreased at ovulation and this correlates with reduced corneal thickness during this phase. The study suggested that such corneal changes may be important to consider during screening of candidates for laser refractive surgery [141]. However, a study by Seymenoglu et al. [142] suggested no biomechanical changes occurred. Both studies had a small study cohort which affected the power of the research and may explain the different results.

1.2.5.5 Eye Rubbing and Eye Massage

Intensive (20 seconds duration) eye rubbing, i.e. directly over the cornea, leads to a reduction in the viscosity of the PGs and GaGs of the ground substance and, consequently, reduces CH and CRF values [43]. As the ground substance behaves like a thixotropic substance, the pressure and movement forces induced by eye rubbing cause the corneal tissue to be reduced of its viscosity. Eye massage through the upper eyelid also leads to changes in CH and CRF. In this situation, IOPcc decreases, whereas, the CH increases whilst the CRF decreases [143]. These changes are caused by IOP alterations, not by structural modifications, as is the case with eye (cornea) rubbing. After correction for IOP, changes in CH and CRF are no longer observed [143].

1.3 Glaucoma

Glaucoma is one of the leading causes of blindness in the world [144-146]. It is defined as an acquired optic neuropathy which leads to destruction of ganglion cells and fibres and eventually causes irreversible visual field loss. The disturbance of the outflow of aqueous humour, a natural clear nourishing intraocular fluid, resulted in increase of the IOP.

The IOP is recognised as the most important modifiable risk factor in glaucoma treatment. If high IOP left untreated, it can lead to optic nerve damage resulting in progressive, permanent vision loss and then eventually blindness.

1.3.1 Types of glaucoma

In general, glaucoma can be divided into several categories. Namely, primary and secondary glaucoma or open angle and close angle glaucoma. In this region, the most common type is open angle glaucoma. Primary open angle glaucoma (POAG) and normal tension glaucoma (NTG) are the most common glaucoma diagnoses in a glaucoma clinic.

“Open-angle” glaucoma occurs when the drainage of the aqueous out of the eye is not in balance with its production. Clinically, the angle of the anterior chamber of the eye , where the iris meets the cornea is as wide and open as it should be. The obstruction or resistance of the aqueous is caused by the slow clogging of the drainage canals, resulting in increased eye pressure.

Patients with POAG present at an eye clinic with high intraocular pressure and impaired visual function. Patients usually have an ocular finding that is related with optic nerve head damage due to the persistent high pressure. NTG is also called low-tension or normal-pressure glaucoma. In NTG, the optic nerve is damaged even though the eye pressure is within normal values. It is not yet fully understood why some people’s optic nerves are damaged even though they have almost normal pressure levels. Ocular hypertension (OHT) is not a glaucomatous condition but rather an ocular condition that is diagnosed due to recurrent high intraocular pressure without any ocular or visual function anomaly.

Angle-closure glaucoma is a less common type of glaucoma. It can be caused by blocked drainage canals, resulting in a sudden rise in intraocular pressure or a closure or narrowing of the angle between the iris and cornea. It develops rapidly in acute cases and has very aggressive symptoms such as severe headache, loss of vision and severe nausea. In most acute cases the effect can be reversible if treated urgently. For chronic cases, the damage can be progressive and irreversible.

1.3.2 Glaucoma and IOP

Epidemiological studies have demonstrated that even a mild reduction in IOP (up to 1 mmHg) can considerably decrease the risk of worsening of glaucoma [75, 147, 148]. Therefore, an accurate IOP measurement is of paramount importance in the management of glaucoma patients.[149] The evaluation of IOP is used to assess disease control and treatment response, and lowering IOP has resulted in reducing the

rates of disease progression over 5 years [30, 150]. The IOP can be decreased through topical and oral medications, laser procedures and/or other surgical interventions.

1.3.3 Glaucoma and Corneal Biomechanics

While the relationship between glaucoma susceptibility and corneal biomechanical variables (beyond their effects on IOP measurement) has been previously studied, substantial efforts are also being directed towards answering questions about how biomechanical factors in the posterior segment might be related to those in the anterior segment [9].

Many studies that have utilised the ORA have found that the CH and CRF values of the glaucomatous eyes are lower compared to normal and OHT. This lead to an assumption of possible structural relationship between the cornea and the connective tissue of the optic nerve head (ONH) [81, 116, 151, 152]. An association between the biomechanics of the cornea and functional behaviour of the lamina cribrosa was reported [153]. In laboratory-based studies, the surface compliance of the lamina cribrosa has been found to decrease and the cornea to become more rigid with increasing age [75, 154]. Wells et al. found that in glaucoma patients, CH was correlated with the mean cup depth of the ONH and that higher CH values were strongly correlated with the higher deformability of the ONH [155]. Mansouri et al. found a weak association between CH/CRF and structural as well as functional aspects of glaucoma severity [57].

Several studies had looked into the corneal biomechanical properties of OHT, NTG and POAG patients and found that corneal resistance factor (CRF) was significantly less in NTG and maximum in POAG and OHT [12, 151, 156]. Studies have also shown that low corneal hysteresis is associated with glaucoma damage[17, 153, 157]. Previous studies showed that CH and CRF [158] are linked to glaucoma severity [9, 155] and progression[159], and may result in GAT producing lower IOP readings than non-contact tonometers[160].

1.3.4 Ocular Biomechanics and the Risk for Glaucoma

A study suggested that glaucoma risk assessment may be possible based on biomechanical properties [17]. After the correction of corneal thickness and IOP, patients undergoing ocular hypertension treatment (OHT) had higher CH (corrected CH) and CRF values than healthy subjects, although the difference between these values was not statistically significantly [161]. Corrected CH is lower in patients with glaucoma and

normal pressure glaucoma than in healthy subjects, perhaps indicative of a lower tissue-damping capacity in glaucoma [161]. A low CH may be considered an independent indicator of the presence and progression of glaucoma. Even after the reduction of IOP, the CH is lower in glaucomatous eyes than in normal eyes [162, 163].

Conversely, a high CH value might represent a beneficial element in halting the glaucoma progression [17]. Some OHT patients seem to possess a higher corneal-damping capacity, which may be extrapolated to the biomechanics of the ONH [164]. Moreover, there is no significant difference in corrected CH between normal-tension glaucoma and primary open-angle glaucoma. The often cited difference in uncorrected CH between these must thus be ascribed to significant differences in IOP [165]. For example, mean CH is significantly lower in subjects diagnosed with glaucoma compared with glaucoma suspects (ocular hypertensive and normal patients), while CRF is useful for differentiating between subjects with ocular hypertension and glaucoma [75]. When combined, the early evidence suggests that corneal biomechanical factors hold considerable promise in providing IOP-independent predictive variables for glaucoma development or progression. One study had reported that significantly lower CH is seen in subjects with congenital glaucoma when compared with age-matched control subjects,[12] while another study showed that patients with the glaucoma-induced pits of the optic nerve have lower CH than glaucoma patients without these changes [153].

Congdon et al. reported the impact of CCT and CH on various glaucoma damage tests. Both parameters were found to be independently associated with glaucoma damage changes such as a progressive increase of cup-to-disc ratio and visual field defects. The authors concluded that thinner corneas provide lower IOP readings that can affect the decision by practitioners towards applying a wrong target intraocular pressure and withholding adequate IOP-lowering therapy. The study on OHT subjects suggested that CCT as the strongest predictor of conversion from ocular hypertension to primary open-angle glaucoma[17]. Other studies have also observed that the worsening of the visual fields assessment is more likely to occur in eyes with lower CH [17, 165-167]. The biomechanical properties may be more predictive of glaucoma development and progression than IOP level.

1.4 Keratoconus

Keratoconus is an acquired ocular disease and the most common primary corneal ectasia. It is a corneal degenerative disease which is characterised by localised corneal thinning that leads to conical deformation and subsequent distortion of vision. It is a bilateral ocular disease with asymmetrical presentation [168-170]. The most common location of corneal thinning is inferior temporal followed by central and superior [171-173]. The changes often manifest as a change of shape (geometry) or corneal ectasia which affected its mechanical and optical properties. The ectatic changes usually manifest during teenage life during the growth hormone surge [170, 174]. Even though it was thought that the disease stabilizes after the second decade of life, it may progress for the next few decades [174]. Keratoconus can present unilaterally. However the other presumed normal eye may develop the condition later [175]. This acquired corneal ectasia affects both genders. However a higher prevalence is seen in males [175]. Though it may affect any ethnicity, Asians are predisposed to this condition in comparison to Caucasians [176, 177].

Keratoconus can present with variable ocular symptoms and signs which depend on disease severity. The aetiology and pathogenesis of this disease is still poorly understood despite much clinical and laboratory research. However, researchers have proposed genetic, environmental and biochemical factors as possible causes for keratoconus [170, 174].

1.4.1 Classification of Keratoconus

There are many suggested methods for classifying of keratoconus. Several methods have been described in the literature to both evaluate and document progression in keratoconus, but there is no consistent or clear definition of ectasia progression. The Amsler-Krumeich (AK) classification system (Table 1.1) is amongst the oldest and still the most widely used. In the AK system, the severity of keratoconus is graded from stage 1 to 4 using spectacle refraction, central keratometry, presence or absence of scarring, and central corneal thickness [178, 179]. There are other types of classifications that are based on morphology, evolution of clinical signs and index-based assessment (Table 1.2). The AK scale was used in present study.

Table 1.1 Classification based on mean K-readings on the anterior curvature sagittal map, thickness at the thinnest location, and the refractive error of the patient

Stage	Findings
1	Eccentric steepening Myopia, induced astigmatism, or both <5.00 D Mean central K readings <48 D
2	Myopia, induced astigmatism, or both from 5.00 to 8.00 D Mean central K readings <53.00 D Absence of scarring Corneal thickness >400 micron
3	Myopia, induced astigmatism, or both from 8.00 to 10.00 D Mean central K readings >53.00 D Absence of scarring Corneal thickness 300 – 400 micron
4	Refraction not measurable Mean central K readings >55.00 D Central corneal scarring Corneal thickness < 200 micron

Table 1.2 The list of keratoconus classifications based on three different methods.

Keratoconus classification based on morphology. [174]			
Nipple	Diameter of the corneal cone≤5mm, located more commonly infero-nasal quadrant, can be central or paracentral. Refractive error easily correctable with contact lens.		
Oval	Diameter of the corneal cone≥5mm, located more peripheral but commonly in the infero-temporal corneal quadrant. More difficult contact lens correction.		
Keratoglobus	More than 75% of the cornea is ectatic. Very difficult contact lens correction.		
Keratoconus classification with index-based systems.			
Author	Index	Cut of point	Description
Rabinowitz [174]	K value S value	47.2 1.4	Diagnosis is based on central keratometry and inferior-superior asymmetry in keratometric power
Maeda [180]	KPI KCI%	0.23 0%	The KPI value is derived from eight quantitative videokeratography indexes
Smolek/Klyce [181]	KSI	0.25	An artificial intelligent system is employed to detect and assess the severity of keratoconus.

1.4.2 Keratoconus and IOP

The morphological changes associated with keratoconus have been shown to cause errors in applanation tonometer [182]. Underestimates in IOP may occur due to altered corneal parameters such as central corneal thickness and corneal biomechanical changes [183-186]. Mollan et al. suggested Dynamic Corneal Tonometry (DCT) and ORA as suitable tonometric devices for keratoconus due to their relative independence from the central corneal thickness and corneal biomechanics [187]. The DCT is an electronic slit-lamp mounted device which has a probe that applanates the whole corneal surface for tonometry. However, studies reported that DCT gave higher IOP readings than GAT [187, 188]. Even though the DCT measurement in the keratoconic cornea was

found to have no association with corneal thickness and curvature, it may be influenced by other biomechanics properties of the cornea [188, 189].

1.5 Aims of study

Previous studies showed that ORA provide higher IOP measurements compared to GAT [74, 157]. However both tonometers are particularly useful to ascertain a more accurate IOP value especially amongst patients with keratoconus and those presenting after refractive surgery patients. Studies have reported that the most consistent confounding factor of IOP measurements by different tonometers is the variation in corneal biomechanical parameters, namely corneal hysteresis (CH) and corneal resistance factor (CRF) [18, 157, 187, 190]. Although the result may slightly vary in terms of the size of the effect, CCT also affected tonometry agreement [157, 191]. Despite much published literature on tonometry agreement, only a few explored the factors that influence the agreement [157, 192].

This study aim to investigate the agreement of four different tonometers compared to GAT. Additionally the pattern of tonometry bias will be investigated to evaluate any proportionate bias with IOP change in eyes with different diagnoses. Subjects with OHT, NTG and POAG have regular corneal surface that seem ‘similar’ to normal cornea. Keratoconic eyes have abnormal corneal curvature that may ‘distort’ the tonometry measurement. This study hypothesizes that there are differences in tonometry agreement between the different ocular diagnoses. This study will investigate the effect of several demographic variables such (age, gender and ethnicity) on tonometer agreement. The variability of agreement between the instruments employed may be due to corneal physical properties(corneal biomechanics and corneal thickness) . This current study follows the guideline on reporting reliability and agreement studies as suggested in previous literature [193].

The ORA parameters may give further insight into the relationship between corneal biomechanics and IOP measurement in eyes affected by ocular hypertension (OHT), different types of glaucoma, corneal pathologies and normal eyes. The advent of the CST instrument with additional corneal biomechanical parameters may demonstrate further association between these parameters with glaucoma and keratoconus diagnoses. The evaluation of corneal dynamic response parameters by the CST amongst eyes with different clinical diagnosis is still lacking. Thus, this study aims to evaluate the clinical impact of these new and exciting parameters. This study will also

explore the agreement of corneal biomechanical properties by ORA and Corvis ST and investigate factors that influence these parameters.

In summary, this study investigates agreement between GAT, indentation, rebound and non-contact tonometers in different eye diseases and ethnicities. This study relates findings to the corneal biomechanical parameters as determined by ORA and the newer Corvis ST. It will give a better insight into the agreement of IOP measurement between different tonometers and GAT. Additionally, the corneal properties influencing IOP measurements are investigated. The study will explore the choice of tonometry which will be suited to diagnosis and will help improve patients' standard of care.

CHAPTER 2: METHODOLOGY AND INSTRUMENTS

This study aim to examine the agreement between intraocular pressure and the corneal biomechanics in normal, glaucomatous and keratoconic eyes, as described in the section 1.5. This chapter describes the method of comparing measurements of IOP using GAT, Tonopen, iCare, ORA and CST.

2.1 Study Design

This was a prospective cross sectional study. The glaucomatous and keratoconus subjects were recruited from glaucoma and cornea outpatient clinics at the Birmingham and Midland Eye Centre, City Hospital, Birmingham. The healthy subjects were volunteer healthy patients, NHS employees, students and staff of Aston University, Birmingham.

2.1.1 Criteria of selection

The subjects were selected based on the inclusion criteria listed below:

a. Age between 18-85 years old
b. Subjects able to give informed consent
c. Patients with eyes that enable measurement by the instruments

The exclusion criteria employed was:

a. Patients with corneal diseases or eye conditions that prevented valid measurement by the instruments. For example:
    i. central corneal scar
    ii. severe corneal oedema
    iii. severe dry eyes
    iv. ocular surface diseases
b. Patients who had underwent ocular surgery that may affect scleral and corneal rigidity. For example:
    i. post vitrectomy/scleral buckle surgery
    ii. post sclerectomy surgery
    iii. post corneal transplant surgery
    iv. post corneal cross-linking treatment

2.1.2 Study Flow

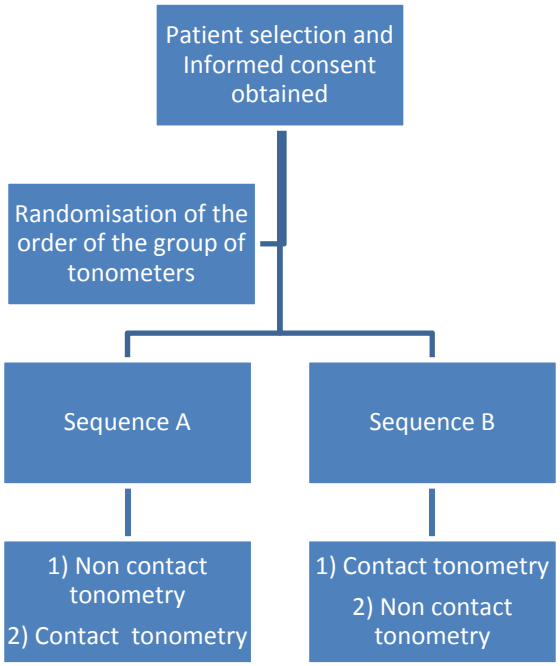
An information leaflet on the research project was provided to the patients prior to thei arrival to the eye clinic. On the examination day, the researcher further explained the purpose of the study and all questions were answered. The participants then signed an informed consent form(see appendix A for information leaflet and informed consent form).The study flow is summarised in Figure 2.1.

Information on the current medications, other medical illness and patient's ethnicity was collected. Subjects were advised to abstain from wearing contact lens at least 24 hours prior to eye examination. All eyes were anaesthetised with the instillation of one drop of Minims Proxymetacaine Hydrochloride topically.

Initially, examination was performed on each eye with Tono-pen, Icare, Corvis ST and ORA, in a randomised order. The randomisation was based on free research sample randomisation software that was downloaded from http://www.randomizer.org. Two repeated measurements were taken with each tonometer.For ORA, the mean IOP of Tonopen and Icare were chosen based on the reliability indicated on the display screen. An IOP measurement with reliability index of 5% or less was recorded as the IOP value.

A pause of approximately 30 seconds was allowed between each measurement taken with the same tonometer and a minimum duration of 5 minutes was allowed between the different tonometer measurements. All the measurements were taken by the researcher (WH) who is an experienced ophthalmologist and trained to operate the non-contact tonometers. The final examination was with GAT by different masked observers. The masked observer was not fixed. However, all observers are ophthalmologists with at least five years of experience in clinical ophthalmology. All subjects were examined by slit lamp examination at the end of each study session to ensure no adverse effect on the cornea.

Figure 2.1 Flowchart of the study



2.1.3 Steps to reduce bias in repeated tonometry

In a normal physiological condition tonometry can be influenced by the body's regular physiological process such as cardiac and respiratory pulses. Thus, for non-contact tonometry the IOP value must be taken multiple times to obtain a mean value of the readings. Most studies of IOP measurement take at least 3 readings for each instrument [79]. For GAT, this is not a problem as the reading is taken at mid-point between the systolic and diastolic IOP. Repeated measurements from a non-contact tonometer are acceptable, as the readings are completed almost within seconds. Thus, the effect of physiological change during the IOP reading is minimised. These repeated IOP measurements for non-contact tonometry in immediate sequence have been shown not to cause reduction of the IOP readings. Studies have investigated the effect of repeated measurements using different sequences between a non-contact tonometer and GAT [194, 195]. Although the intersession result between groups of tonometers was not conclusive, due to the design of the study, it confirmed that there was no significant reduction of IOP measurements between two non-contact tonometers which was taken consecutively in the same session/setting [194, 195].

The order in which the tonometers are used may affect the accuracy and reliability of the GAT more than non-contact tonometer. Studies have reported that higher IOP value by GAT compared to non-contact tonometry when the GAT was used later to

measure the IOP [192, 196, 197]. Therefore, the order of IOP assessment was randomised between non-contact tonometry and GAT in this study (Figure 2.1)

As the non-contact tonometers do not have an aqueous massage effect it follows that IOP assessments made with GAT would always be accurate while assessments made with non-contact tonometry would be accurate only when measured before GAT. This would bias for a lower IOP with the non-contact compared with the GAT and because many non-contact tonometers read up to 3mmHg higher that the GAT (in subjects with IOPs up to 21mmHg), it may lead to the conclusion of a better agreement (between any non-contact and the GAT) than actually exists. However, a recent study showed significantly higher IOP readings compared to GAT and the study employed a randomised sequence for IOP readings [187]. Non-randomisation of the sequence of tonometer examination would introduce a systematic bias into such an experimental design. If the measurements were made too close in time, the between-method differences between GAT and non-contact tonometer may return higher than GAT readings. Thus it would be underestimated and vice-versa for non-contact tonometers that return lower-than-GAT readings. Thus, for this study, a duration of at least 2 minutes gap between each measurement and 5 minutes gap between each tonometry with randomisation of the sequence of tonometer (except GAT), would reduce any ocular massage effect (due to the repeated tonometer applanation on the surface of the cornea) and minimise any bias of IOP measurements.

The inert-observer variability during applanation tonometry was known to affect the accuracy and repeatability of GAT measurement. This study acknowledged that this may affect the outcome in the agreement analysis. In order to reduce this bias, the operators must have at least 5 years of clinical experience in applanation tonometry. This is described in item 2.1.2. Additionally, this study performed repeatability analysis of each tonometer to ensure high reliability and repeatability of tonometry (details in item 2.4.2).

It is well known that following a repeated applanation of the cornea during IOP measurement with Goldmann IOP reading is reduced. The reduction in IOP subsequent to indentation or applanation is probably due to a decreased anterior chamber volume due to increased aqueous outflow or to the negative feedback loop proposed by Stocker et al., which causes a reduction in aqueous production [198]. This postulation was based on a laboratory-based research and was supported by another study[199].

An average of IOP measurements is important for contact tonometers. For example, Goldmann tonometer requires at least a two minutes interval [200] before repeating IOP measurements. This would give the cornea time to regain its proper anatomical and biomechanical properties and reduce bias. In this study, the intra-operator repeatability of all tonometers was assessed by taking two repeated measurements. The repeated measurements were done after two minutes duration. The repeatability performance was analysed and all tonometers showed high to excellent repeatability. The analysis is presented in section 3.2.1. Following that, this study concluded that to reduce repeated tonometry bias, further examination would involve one measurement of GAT (which is principally measured at a balance between a diastolic and systolic pulse pressure), one completed Icare and Tonopen (with standard deviation 5% and less), two CST measurements and one complete ORA examination (consisting of 4 repeated air-puff measurements).

2.2 Study Instruments

In order to reduce bias and ensure valid measurements of the tonometers all instruments was calibrated, checked and cleaned prior to the start of the study and periodically as suggested in the instruments' manuals. This study is aimed to examine the agreement of IOP measurements between these tonometers:

1. Corvis® ST (CST); Oculus Optikgeräte GmbH, Wetzlar, Germany

2. TonoPen XL® (Tonopen); Bio-Rad, Glendale, California

3. iCare® (Icare); Tiolat Oy, Helsinki, Finland

4. Ocular Response Analyzer® (ORA); Reichert Ophthalmic Instruments, Buffalo, New York

5. Goldman applanation tonometer (GAT); Haag-Streit, Bern, Switzerland

2.2.1 Goldmann Applanation Tonometry

Currently, the gold standard for measuring IOP is the Goldmann applanation tonometer (GAT) which is employed in glaucoma clinics worldwide. This technique is operator dependant. It requires a skilled operator as well as contact of the instrument to the patient’s eye. Fluorescein and topical anaesthetic drops must be used. Its measurements are also influenced by the tear film and the physical properties of the cornea (i.e. thickness, rigidity, curvature, and hysteresis)[15]. The corneal

biomechanical influence on applanation tonometry is a subject of high interest. With the knowledge of these limitations, new tonometers have been developed over the past decades in order to improve tonometry methods so that IOP values are less affected.

2.2.2 Tonopen© XL (Reichert)

The Tonopen XL (Tonopen) is an 'electronic' portable applanation tonometer that uses a disposable silicone tip cover, and requires anaesthetic drops. It has a small contact area compared to GAT (2.36mm^2 vs 7.35mm^2, respectively) and is recommended for tonometry on irregular corneal surface [201, 202]. The device utilises micro strain gauge technology and a 1.02 mm transducer tip. Its method is based on Mackay-Marg tonometry principle which involved repeated applanation/indentation of the cornea with its tip. It is battery operated and measures IOP ranging from 5 to 80mmHg. After the cornea is anaesthetised, the operator touches the covered tip of the Tonopen to the centre of the cornea several times. Each corneal indentation will be stored and analysed by the device. The average measurements of several good readings are analysed. Then the digital display on the Tonopen will give the IOP with an estimate of the variability between readings [203]. The mean IOP and the standard deviation of the measurements will be shown on the display screen. Tonometry values with less than 5% standard deviation will be recorded for the study.

The IOP readings by Tonopen are quite consistent and accurate when compared with GAT findings [187, 204]. The Tonopen may be more "user friendly" in the presence of corneal pathology as it applanates a smaller area of the cornea [205]. It has clear advantages in portability, and measuring IOP in different postures. There is evidence that Tonopen measurements are also affected by CCT [192]. In addition, it has a tendency to overestimate IOP, and therefore its measurements need to be correlated with other clinical findings [206]. It is one of the more popular tonometer used by ophthalmologists and optometrists worldwide.

2.2.3 iCare ©

The rebound tonometer iCare® (Icare)(Tiolat Oy, Helsinki, Finland) utilises a small plastic probe which bounces back after touching the corneal surface. It has been commercially available since 2003.The corneal surface contact made by Icare is very minimal and extremely rapid and therefore topical anaesthesia is not required. The deceleration of the probe after the corneal surface contact is measured

electromagnetically and the device produces IOP value on its electronic display panel. With the aid of inbuilt software, it takes the average of 4 out of 6 most probable readings and discards the two outliers. Due to its portability, ease of use, multi-position tonometry, good reliability and freedom from anaesthesia, the Icare is increasingly used in eye clinics worldwide, particularly in those patients who do not tolerate GAT (such as children) [207, 208]. This device may offer a reasonable estimate of IOP in patients with known or suspected glaucoma where IOP cannot otherwise be obtained in clinic [209]. In selected children with glaucoma, home tonometry by Icare rebound tonometry was reliable, easily performed by caregivers, well tolerated and offered valuable IOP information for clinical management [210].

2.2.4 Corvis ST

The Corvis© ST (CST) (Oculus Optikgeräte GmbH, Wetzlar, Germany) is a dynamic Scheimpflug corneal imaging and analyser. The CST combines a non-contact tonometer with a high-speed camera to capture a series of horizontal Scheimpflug images during corneal deformation with an air puff jet.

A high speed Scheimpflug camera records the deformation with full corneal cross-sections, which are then displayed in slow motion on a control panel (Figure 2.2); the camera records 4330 images/s with 8.5 mm horizontal coverage. The image resolution is as much as 640 × 480 pixels [211]. A representative output is shown in Figure 2.3, with several parameters related to the deformation process. During the deformation response, a precisely metered air pulse causes the cornea to applanate the first applanation. The cornea continues to move inward until reaching a point of highest concavity. Since the cornea is viscoelastic, it rebounds from this concavity to another point of applanation (the second applanation) and then to its normal convex curvature.

The CST records throughout the deformation process and therefore gains information concerning the cornea's viscoelastic properties and stiffness, as well as recording standard tonometry and pachymetry data . Table 2.1 lists the corneal biomechanical parameters derived from the CST measurement. Specifically, the CST corneal biomechanical outputs are time from the initiation of the air puff (time0) until the first applanation and second applanation (A-time1 and A-time2), length of the flattened cornea at the first applanation and second applanation (AL1, A-L2), corneal velocity during the first and second applanation moments (AV1, AV2), time from the start until the highest concavity of the cornea is reached; highest concavity time (HcT)), central

curvature radius at the highest concavity; highest concavity curvature (HcR), distance of the two surrounding “knees” at the highest concavity (peak distance) as seen in cross-section (HpD), and maximum deformation amplitude (DA), from start to the highest concavity at the corneal apex [212]. In addition to the deformation response, the CST is also able to measure the IOP and the corneal thickness simultaneously. It was commercially available since September 2011. Figure 2.3 shows the highly detailed dynamics of the cornea during its deformation displayed on the screen both objectively on graphs and subjectively on its dynamic video. This tonometer will be unique to this study as at the present time this protocol of this study is written, there are less than ten research papers in the literature on the Corvis ST.

Table 2.1. The parameters derived from the Corvis ST.

Parameters	Definition
IOP	Non-contact IOP bases on first applanation response
CCT	Central corneal thickness based on optical image analysis
A1T	Time from start to first applanation
A1L	Cord length of the cornea during first cornea applanation
A1V	Speed of the cornea during first cornea applanation
A2T	Time from start to second applanation response
A2L	Cord length of the cornea during second applanation response
A2V	Speed of the cornea during second applanation response
DA	Amplitude of the corneal movement at highest concavity deformation
HcR	Radius of corneal curvature at maximum concavity deformation
HpD	Distance of the most anterior point of the anterior corneal surface during highest concavity deformation
HcT	Time from start to maximum concavity

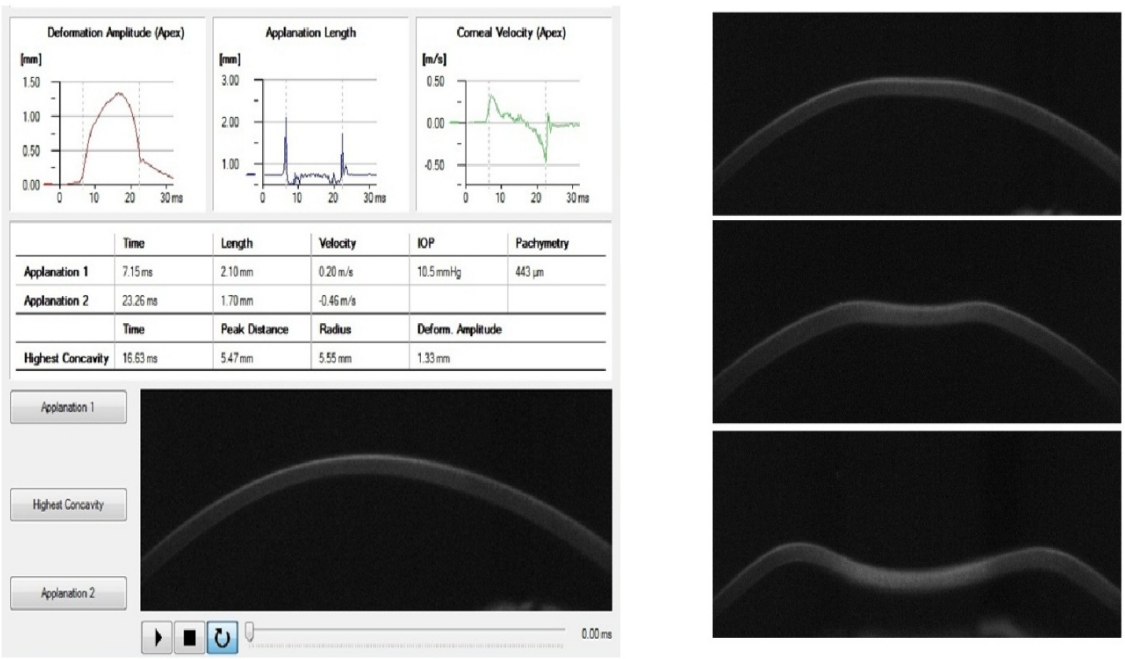


Figure 2.2 The Corvis ST (left) is able to produce detailed images of the cornea during air puff deformation. The instrument is also a validated tonometer and pachymeter.

2.2.4.1 Validity and Reliability of CST

The CST was said to be a valid and reliable alternative for non-contact tonometry but the IOP value is not to be used interchangeably with GAT [211]. However, the study was done with a small and mixed cohort of glaucomatous and normal eyes. In another study of glaucoma suspects and glaucoma patients, corneal deformation amplitude influenced GAT readings more than CCT did [213]. The deformation amplitude was noted as one of the most reliable and accurate indicators of corneal biomechanical properties in these study populations [212, 213].

Huseynova et al. examined the correlation of the biomechanical parameters of ORA and CST among normal subjects in a refractive surgery centre [20]. In the study, the corneal biomechanical parameters of both devices were found to be influenced by the central corneal thickness and IOP. A repeatability and reliability study of the corneal dynamic response parameters of the CST revealed variable repeatability and reproducibility of CST parameters in normal subjects [214]. The authors used a different software version that produced 17 parameters compared to 12 parameters in the present study. The sample size was small (29 subjects of not more than 30 year old and 19 subjects older than 65 years)[214].

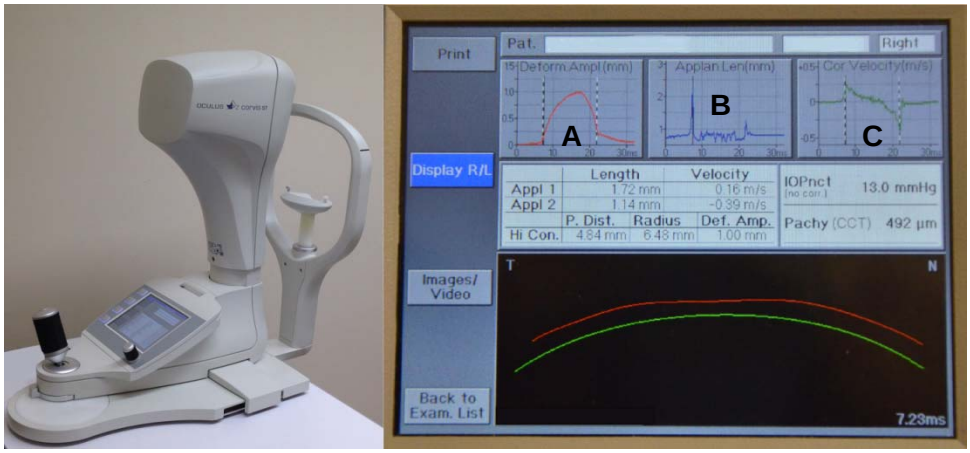


Figure 2.3 Left image: Cornea shape pre-applanation by air puff on the display screen of Corvis ST. Right image: A. First applanation of the cornea surface by air puff, B. Second applanation that resulted in further corneal deflection, C. Corneal deflection at its maximum.

2.2.5 Ocular Response Analyzer (ORA)

The ORA (Reichert Ophthalmic Instruments, Depew, NY, USA) is a non-contact tonometer that uses rapid air pulse to indent the cornea. It has an advance recording system to capture two applanation pressure measurements, one while the cornea moves inward and the other as the cornea moves outward [19].

Two different pressure values were captured as the cornea resists the pressure from the air puff, causing delays in the inward and outward movement of the cornea. The first inward applanation pressure is called “P1,” the second outward applanation pressure is called “P2.” The air pressure increases up to a maximum level Pmax, the air pressure is decreased gradually until the second applanation is detected at pressure P2 [215].

The ORA produced an IOP reading called the Goldmann-correlated IOP (IOPg) which is the average of P1 and P2 (Figure 2.4). The difference between these two pressure values is termed corneal hysteresis (CH = P1 − P2), which is termed as a corneal biomechanical parameter. CH, which is claimed to be the result of the viscous damping within corneal tissues, provides a basis for two additional new parameters; corneal-compensated IOP (IOPcc) and corneal resistance factor (CRF). The IOPcc is an empirical IOP measurement derived from pre and post-LASIK clinical data, which is intended to be less affected by corneal properties than Goldmann applanation tonometry (GAT). CRF appears to be an indicator of the overall “resistance” of the cornea [216], and is expressed by the equation: CRF = k1 × (P1 − 0.7 × P2) + k2. (k1

and k2 are constants). Despite many studies exploring these parameters, the precise meaning of them is not completely understood.

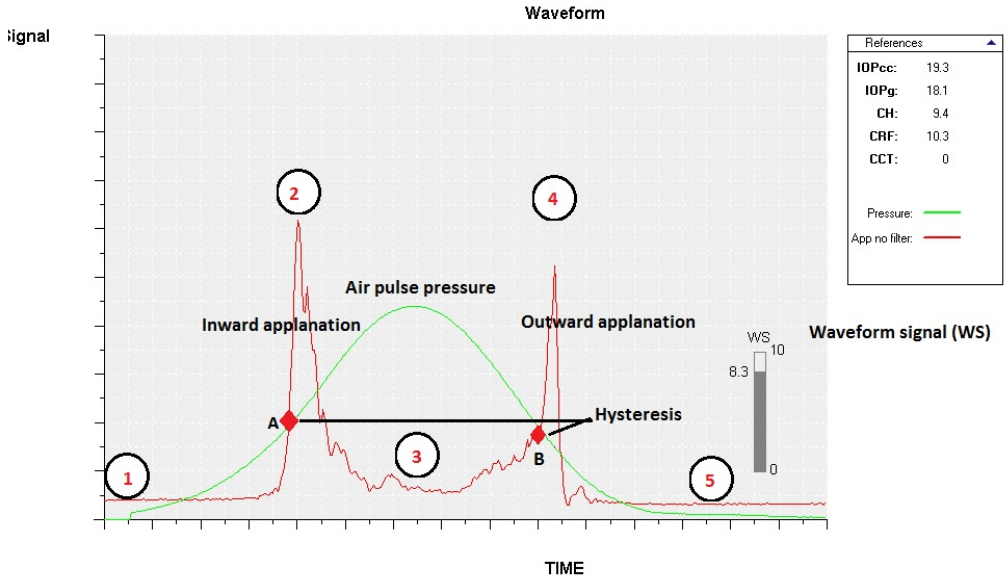


Figure 2.4 Measurement of ocular hysteresis by the Ocular Response Analyzer (ORA). 1, convex cornea; 2, flat cornea (P1); 3, concave cornea; 4, flat cornea (P2) ; 5, convex cornea

CH is a measure of the cornea's viscous damping capacity contrasted to its stiffness, elasticity, or rigidity [46]. There is no evidence for an association between CH and CRF (ORA parameters) and the standard mechanical properties (Young's modulus, rigidity) used to describe elastic materials[156].The CH and CRF are entirely empirical parameters, each of which characterizes the cornea's response to deformation by an air impulse. According to the definitions of CH and CRF, differences in CH and CRF values correlate negatively with the value obtained for P2.

The cornea contains collagen fibres and ground substance, resulting in a high resistance against deformation and a higher damping capacity. The stronger the corneal tension, the faster the cornea regains its original position following deformation. Studies found that higher IOPcc values were associated with lower CH values [65, 68, 85-87]. Therefore, IOP represents an additional force that restores the cornea to its original position (like a slingshot) [84]. In contrast, CRF increases with rising IOP, indicating that resistance against deformation of the cornea is higher in eyes with higher IOP values. The simple ORA measurements of CH and CRF have presumably

characterized the biomechanical state of the cornea, since corneal thickness alone is insufficient to fully achieve this.

2.2.5.1 Measurement Signal

Other than CH and CRF values, the ORA measurement signal has more information about the biomechanical condition of the cornea. Interpretation of results should consider the undulation of the signal as well as the elevation of the first and second peak [217, 218]. The latest version of ORA software (version 3.0) has a keratoconus score in addition as well as waveform parameter.

The tear film has an important effect on ORA measurements. Single measurements should be obtained quickly (within 20s), so as to avoid alterations of the tear film layer resulting from reflections of the infra-red light. A dry cornea leads to overestimated CH values. Sufficiently frequent blinking and adequate fixation are, therefore, essential preconditions. Thus, ORA results in children and patients with nystagmus must be analysed cautiously [219].

Although hysteresis has been considered an important aspect of corneal biomechanical behaviour, there have been conflicting reports concerning hysteresis measurement in some clinical situations [220]. In particular, CH has been shown to decrease during ageing [59], when the cornea tissue is known to stiffen with increasing age [52]. Such reports showed that there is a further need of understanding of the significance of hysteresis as a metric of corneal viscoelasticity and would require development of models, which could help in determining whether the viscous or elastic components are stronger predictors than hysteresis alone for the behaviour of the cornea in various pathological conditions.

2.2.5.2 Reproducibility of ORA measurements

The accuracy, reliability and reproducibility of ORA has been investigated in many studies [131, 221, 222]. The degree of consistency calculated using the intraclass correlation coefficient (ICC) for intra-session repeatability revealed ICCs of 0.731 and 0.881 for CH and CRF, respectively, during a single test series (227).

For reproducibility of measurements between two examinations , an ICC of 0.799 was found, indicating highly consistent measurements during a series of repeated tests [161]. Other studies detected similar values, (0.78 and 0.93) for intra and inter-observer ICCs respectively [221, 222]. A unique feature of the latest software is the 'waveform score,' a parameter intended to facilitate reliability of the measured signal. Signals with waveform scores ≤3.5 should be considered with caution [223].

2.3 Statistics

2.3.1 Justification for analysis of data from one or both eyes.

The choice of analytic approach for this study was made on the basis of research objectives and the inter-ocular correlation of the study variables. For the agreement of IOP measurement between tonometers, the specific eye variables were not of interest, therefore, the most appropriate statistical analysis is at the level of each individual eye [224, 225]. Thus, in this study both eyes have been included in the analysis of inter-method agreement between the tonometers employed.The diseased eyes in this study cohort were mostly asymmetrical. McAlinden et al. reported that in population with asymmetric eye disease, such as in glaucoma and keratoconus, it is acceptable to use data from both eyes [23]. Glynn et al. has demonstrated in their paper that, treating all eyes as the unit of analysis is the best approach for analysis especially with current regression models employed in the analysis of vision research [226]. This study had recruited satisfactory amount of sample to yield a strong power of analysis. The number of sample of each diagnosis is also adequate for a valid regression analysis.

Amstrong had suggested an algorithm in making decision for including one or both eyes in ocular studies[225]. In accordance to the algorithm recommended by Armstrong , strong inter-ocular correlation are manifested by high interclass correlation (ICC) analysis with 95% confidence interval (95%CI). In this study, preliminary analysis of the inter-ocular correlation of all was performed. In summary, the inter-ocular ICC of paired eyes was very weak to moderate (ICC less than 0.75) and the highest ICC for healthy eyes, glaucomatous and keratoconus eyes cohorts in this study were only 0.55 (range of 0.45, 0.64). These indicated that the ICC of paired eyes were weak to moderate[227]. Thus, in this thesis, all eyes that fulfilled the selection criteria were included in this thesis.

2.3.2 Sample size and power calculation

2.3.2.1 Sample size for tonometry agreement study

The total sample size suggested for the agreement study was approximately 185 subjects. In this study, calculation was also done using a formula [228] for sample size calculation for research in assessing agreement of different clinical methods. Based on previous studies on tonometer agreements [187], the standard deviation (S.D.) of the differences (s) was estimated to be 2.0 mmHg. These will produce a sample size of approximately185 eyes.

$$1.96\sqrt{\frac{3s^2}{n}} = confidence\ interval\ of\ limit\ of\ agreement(LOA)$$

When s=2.0 and confidence interval of LOA,

$$1.96\sqrt{\frac{3(2)^2}{n}} = 0.5$$

$$n = \frac{12}{\left(\frac{0.5}{1.96}\right)^2} = 184.39$$

2.3.2.2 Sample size calculation for repeatability study

The repeatability analysis with which we can estimate within-subject standard deviation (Sw) depends on both the number of eyes (n) and the number of observations per subject, m. The width of the 95% confidence interval for the population within-subject standard deviation is$= 1.96 \times \frac{Sw}{\sqrt{2n(m-1)}}$. Therefore, for a Sw of 15% and m of 2, the estimated sample size needed was 85 eyes.

2.4 Statistical analysis

2.4.1 Data distribution

Statistical analyses were performed using SPSS v21.0 (SPSS IBM Inc, Chicago, United States of America). The IOP values were normally distributed for each group of subjects (Shapiro-Wilk test, $p>0.05$). The Shapiro-Wilk (SW) test is chosen as it is a more sensitive and robust test to detect normality for almost all sample size [229]. In large datasets, the test of normality (SW) is very sensitive to small changes in data. Therefore, even though the normality test can be significant for non-normality, the sample distribution may still demonstrate a non-parametric distribution [230]. Hair et al. have described the effects of large sample size on reducing the undesirable effect of non-normality [231].The authors suggested that for sample sizes of more than 200, when non-parametric test is employed eg ANOVA, any unfavourable effect of non-normality may be cancelled out [231].

This study has a total of 389 healthy eyes, 264 glaucomatous eyes and 113 keratoconus eyes. Assumptions of normality were fulfilled for all parameters measured in this study except for HpD, A1L and A2L. However, statistically, non-normality does not affect Type I error rate substantially and parametric tests can be considered robust to non-normality [230, 231]. Where necessary and appropriate, non-parametric test can be used in the analysis of this research data.

In order to reduce statistical test bias, the robust method of analysis is chosen. This is an option available in SPSS software. It is chosen by selecting the bootstrap option before executing the test. Bootstrapping estimates the properties of the sampling distribution from the sample data [232]. By bootstrapping, the analysis is done based on 1000 estimated distributions of samples when possible. In SPSS, it produced confidence intervals of the estimates, either in percentage (95% CI) or a method that is slightly more accurate known as BcA (bias corrected and accelerated confidence interval) [232].

2.4.2 Repeatability Study

Repeatability of measurements is defined as the variation in repeat measurements made on a particular subject under similar conditions i.e. by the same observer and within short duration [233]. In method agreement or comparison studies, the

comparison of the repeatability of each method is important because the repeatability of each method can possibly limit its agreement with others.

For ORA, the manufacturer had suggested that the values from the signal with the best waveform score, is most representative. These measures are automatically chosen by ORA after completing four air-puff measurements. We were unable to do repeat measurement for ORA, due to the fact that the cornea would be subjected to at least eight air-puff measurements in a very short duration. This can expose the cornea to increase chance of air applanation and ocular massage, and reduce the reliability of ORA measurements. This study proceeded with analysis of the ORA based on one best waveform signal.

This study analysed two repeated measurements of GAT, Icare, Tonopen and CST from 85 healthy eyes. For each method, the measurements were done almost consecutively separated by at least 5 minutes between each instruments.

There are many ways to report repeatability of measurement of continuous variables in method comparison studies [193, 233, 234]. One way is by reporting the standard deviation (SD) of the measurement errors, which is similar to an estimate of the within-subject SD (Sw). Other way is to report the SD of the differences between repeated measurements, which is equal to $\sqrt{2} \times within\ subject\ SD$. Another alternative is to report the repeatability coefficient, which is defined by $1.96 \times \sqrt{2} \times within\ subject\ SD$.

Bartlett et al. suggested that the absolute difference between the repeated measurements on a subject must not differ more than the repeatability coefficient 95% of the time [233]. The repeatability coefficient is an estimate. Therefore, it is important to calculate the confidence interval for it to indicate how precisely it has been estimated (CI for CR =$1.96 \times \sqrt{2} \times \sqrt{CI\ of\ within\ subject\ SD}$).

Repeatability (test-retest variability) of the first and second tonometer measurement was quantified as the coefficient of variation (CV), repeatability coefficient (RC) and Intraclass correlation coefficient (ICC) [233]. The definitions of the statistical values above are:

1. Coefficient of variation (CV)

The CV aims to describe the dispersion of the measurement by a method in a way that does not depend on the measurement unit. The higher the CV, the greater the dispersion in the variable, thus the repeatability of measurement is low. CV is defined

as 100 x within-subject standard deviation (Sw) / overall mean and described in percentage (%).

2. Repeatability coefficient (RC)

RC is defined as an estimated average of measurement variability within a group of subjects. Low RC indicates low test-retest variability. The mean difference between two repeated measurements must be normally distributed. The formula for RC is, $2.77 \times within-subject\ standard\ deviation$ (Sw). Sw is derived from a one-way random effect model, which is defined as the square root of the within-subject mean square of error (the unbiased estimator of the component of variance due to random error).

RC is an estimate value, thus, a confidence interval (CI) must be calculated for it to indicate how precisely it has been estimated. For SPSS, the CI of RC is calculated by $\sqrt[2]{CI\ (of\ the\ within\ subject\ variance)} x 1.96 x \sqrt{2}$.

3. Intra-class correlation coefficient (ICC)

In this study, the reliability of measurements by a single observer (intra-operator) was tested. The ICC calculated for this analysis was a two-way mixed type for absolute agreement of the measurements. For clinical measures, ICC was interpreted as follows: less than 0.75 represents poor to moderate reliability; 0.75 to 0.90 represents good reliability; greater than 0.90 represents excellent reliability [227].

This study adopted the assumptions suggested by Bartlett et al. [233]. This study assumed that any bias between methods is constant and the measurement errors variances of methods are equal in the glaucoma and keratoconus cohorts.

2.4.3 Agreement Study

Chapter 3, 4 and 5 of this thesis explore the agreement of IOP measurement by different tonometers within different study groups. The Bland-Altman method of inter-method agreement is employed in this study [228, 234].The GAT is the reference chosen for the inter-method IOP measurement comparison due to its status as being the current accepted “gold” standard of tonometry in ophthalmology clinics worldwide. In accordance with the 4th World Glaucoma Consensus, the Goldmann applanation tonometry is reported to have lowest measurement variability compared to other methods of tonometry [206]. One measurement by GAT, Tonopen and Icare was

sufficient to fulfill the criteria recommended for comparison of tonometers used. For Corvis ST, an average value of 2 measurements is included in this study.

Firstly, a 'two-tailed paired t test' was used to explore mean difference (mean bias) and the standard deviation (SD) of the differences measured the random fluctuations around the mean. With reference to a meta-analysis study and glaucoma consensus, the limit of an acceptable mean difference between tonometers is set at 2 mmHg [21, 235].

Secondly, the limit of agreement (LOA) of the measurements between the tonometers was set at 95% (mean difference ± 1.96 SD), which highlighted how far apart measurements by two (2) methods were more likely to be for 95% of individuals. It was suggested that the ideal range of limit of agreement should not exceed 8mmHg (LOA ± 4.0mmHg), based on the historical inter-observer agreement of GAT [236].

Thirdly, the bias is plotted against the mean value of the measurements of the compared instruments. Horizontal lines that represent the mean difference and the value of LOA are drawn on the plot. A scatter plot of average values against bias was suggested by Bland and Altman and is known as Bland-Altman (BA) plot [228]. The BA plot is used to illustrate the agreement between IOP measurements obtained by the different tonometers against GAT. The difference values in the inter-method agreement plot should be within the limit of agreement line with equal distribution along the mean of the total difference. An example of BA plot with uniform variability can be seen in Figure 3.2a in section 3.2.2.

Further, the BA plot may demonstrate non-uniform variability in the measurement difference between the paired tonometers. An example of the BA plot showing this variability can be seen in Figure 3.2e in section 3.2.2. The distribution of the inter-tonometry bias should be along the line of mean bias in the BA plot and this supports the assumption that the LOA is not dependant on the average tonometry measurement. An inconsistency in the pattern of the distribution and presence of a gradient may indicate a proportional bias. According to Bland and Altman, a log-transformation of the measurements of the tonometers can overcome this problem [234]. If the pattern of inconsistency persists in the transformed plot, a proportionate bias is present. Any significant gradient in the BA plot can be further evaluated by assessing for a correlation between the bias and mean or by performing a linear regression for the difference (bias) model as a function of the average measurement of paired tonometers. Further, a more appropriate estimate of the limit of agreement and

mean bias is calculated according to the changing mean tonometry value. In this study, we performed agreement analysis as suggested by Bland and Altman [228, 234].

2.4.4 Analysis of mean

2.4.4.1 Comparing means

This study compares two similar means of two different groups by performing paired t-tests. For three mean values or more, a one-way ANOVA was performed to explore any significant difference of the variables. For example, comparing means amongst different demographic and categorical characteristics namely ethnicities, gender, ocular diagnoses and age. A Bonferroni correction is made when the categories tested is more than 3. The post-hoc analysis shall be highlighted to demonstrate group with the significant difference, where necessary.

Pearson correlations measure the existence (given by a p-value) and strength (given by the coefficient r between -1 and +1) of a linear relationship between two variables either from the same parameter or from a different one, for example ORA and CST parameters. A significant outcome indicates that a correlation exists with $p<0.05$. An absolute value of r of 0.1 is classified as small/weak, one of 0.3 is classified as medium/moderate and one of 0.5 is classified as large/strong [237].

2.4.4.2 Linear regression analysis

Multivariate regression analysis was alsocarried out to investigate factors affecting tonometry agreement and corneal biomechanical parameters. There are many variables involved in the analysis such as age, IOP and CCT on the corneal biomechanics parameters of each study cohort. Categorical data such as gender and ocular laterality is also involved.

The assumptions of standard regression analysis are outlined below:

1. Linear relationship was established by screening of the scatterplot of the variable against the tonometry bias.
2. There are no outliers.
3. The number of cases should be at least five times the number of cells.

3. All cells for two-way interactions should be greater than one and 80% should be greater than five.
4. The residuals are approximately normally distributed.

Robust regression analysis and the block entry method was chosen to calculate the effect of significant variables/factors on the variability of the inter-method agreement and the variability of each corneal biomechanical parameter [230]. In this study, not all variables investigated conform to the assumptions and follow the non-parametric distribution. However, this study proceeded with the robust method of analysis as described in item 2.4.1.

In the analysis, the CCTus was chosen to represent the measurement for central corneal thickness as the ultrasonic method of pachymetry is considered the gold-standard for pachymetry [238, 239]. In the analysis of the corneal biomechanical parameters, the influence of CCT, IOPcc, age and gender was investigated using the 'enter' method. According to Foster et al. age and gender was a significant influencing factor on the corneal biomechanical parameters in a cohort of British population in Norfolk, United Kingdom [65]. The significant effect of CCT and IOP was discussed in detail in 1.2.4.2 and 1.3.3. The IOPcc was chosen to represent the IOP factor in the regression analysis of the inter-tonometry bias. This is in accordance with previous reports that claimed IOPcc is suitable to represent the corneal-compensated IOP value[20, 157].

In the analysis of factors affecting inter-method bias, this study had chosen linear regression with the enter method. The continuous variables analysed are age, CCTus and all the biomechanical parameters. The dichotomous categorical variable is gender. The effect of CCT and CRF was controlled in the first block. This is in reference to previous studies that showed the significance of these variables as confounding factors that affect the inter-method bias between Icare, ORA and CST with GAT ([157, 202, 211], respectively). The demographic variables (gender and age) and other biomechanical parameters were included in the second block of variables of this analysis.

The effect size of the variables/predictors were calculated and presented as:

1. Adjusted R^2 values represented the variance of the inter-method bias affected by the variables. The significance of this model is assessed by ANOVA with p value <0.05. The F value reported the number of significant predictors and residual predictors.

2. B (the unstandardized coefficient) for each predictor variable shows the predicted increase in the value of the criterion (inter-method measurement bias) for a 1 unit increase in that predictor. The effect of the variable is assessed with p value <0.05.

2. Beta (β) (the standardised coefficient) gives a measure of the contribution of the variable to the model in terms of standard deviation. The B and beta value are reported in tables where applicable.

The robust regression analysis was run by choosing the bootstrapping option in the statistical software, SPSS. The value estimates are stated in the result wherever possible, either by BCa (best corrected accelerated) or percentile method (95%CI). At the end of each model analysis, the histogram, normality plot and the scatterplot were examined to ensure a valid and accurate model. All significant models of the regression analysis for inter-method bias and corneal biomechanical parameters are presented in the result sections.

CHAPTER 3: TONOMETRY AGREEMENT AND CORNEAL BIOMECHANICAL PROPERTIES IN NORMAL EYES

This chapter presents data collected from a cohort of healthy eyes. The first section (section 3.1) describes the demographics of the subjects and the mean value of the variables measured. Section 3.2 reported the inter-tonometry agreement with GAT and repeatability analysis for each tonometers. Influences of demographic variables, CCT and biomechanical parameters on inter-tonometry agreement are also presented and discussed. Section 3.3 then examines corneal biomechanical parameters in healthy eyes by the ORA and CST.

3.1 Demographic

The study recruited three hundred eighty nine (389) normal eyes from a total of 204 healthy volunteers. The mean age of all subjects was 38.1 ± 21.0 years (median; 26 years, max; 86 years, min; 18 years). The subjects consist of 262 female (67.4%) and 127 male (32.6%).

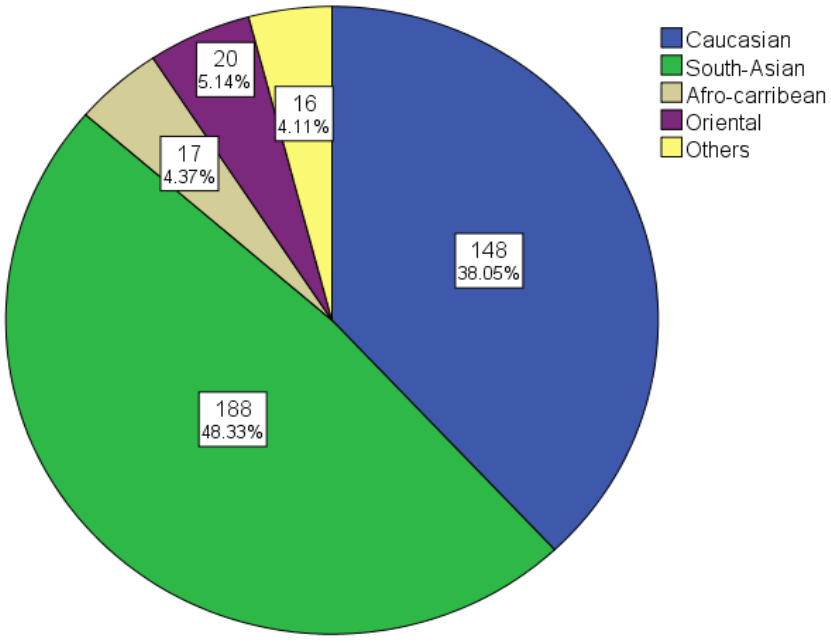


Figure 3.1 The ethnic distribution of healthy subjects.

The mean IOP showed a statistically significant difference amongst the different tonometers. The Tonopen recorded the highest IOP values and the Icare was the

lowest (one-way ANOVA with post-hoc Tukey's test, F=7.82, p<0.00). The mean corneal biomechanical parameters and central corneal thickness for the study population are presented in Table 3.1.

Table 3.1 Mean IOP values, corneal biomechanical parameters and corneal thickness of healthy eyes.

	Mean ± SD	Minimum	Maximum
GAT (mmHg)	14.55 ± 2.63	7.0	21.0
Tonopen (mmHg)	16.58 ± 2.64	9.0	23.0
Icare (mmHg)	13.94 ± 2.90	8.0	22.0
ORA_IOPg (mmHg)	14.38 ± 2.87	7.3	23.4
ORA_IOPcc (mmHg)	15.10 ± 2.86	7.6	23.3
CST_IOP (mmHg)	15.27 ± 1.66	11.0	21.5
CH	10.23 ± 1.55	6.00	14.50
CRF	9.92 ± 1.59	5.70	14.40
A1T (ms)	7.81 ± 0.24	6.97	8.80
A1L (mm)	1.76 ± 0.11	1.29	2.18
A1V (m/s)	0.16 ± 0.02	0.07	0.24
A2T (ms)	22.68 ± 0.38	21.24	23.71
A2L (mm)	1.75 ± 0.27	0.91	2.56
A2V (m/s)	-0.37 ± 0.06	-0.59	-0.23
HcT (ms)	16.47 ± 0.41	14.78	17.79
HpD (mm)	3.41 ± 1.03	1.11	6.01
HcR (mm)	6.78 ± 0.64	4.84	9.38
DA (mm)	1.17 ± 0.10	0.87	1.49
CCTus (µm)	537.47 ± 35.57	436.2	651.00

3.2 Agreement between tonometers in healthy eyes

The information on reliability and agreement of the IOP measurements of other tonometers to GAT is very important to clinicians. The IOP measurements by tonometers available are vulnerable to the influence of corneal physical and mechanical properties. These influencing factors may affect the inter-tonometer measurement bias. This study also explores the demographic and physical factors that may influence the inter-tonometry bias. The instruments employed in this study were detailed in section 2.2.

3.2.1 Repeatability of measurements

The repeatability performance of all tonometers except ORA was analysed in this section. The calculation formula for these and the definition of all statistical measures was discussed in section 2.4.2.

The distribution of the mean difference for A1L, HpD and HcR does not fulfil the assumptions of normality and parametric distribution. Therefore, the RC analysis is not calculated for these parameters. However, the ICC and CV were calculated for these parameters. Table 3.2 listed the RC, ICC and CV of the tonometry values by all the tonometers employed in this study.

Table 3.2 The repeatability of tonometers in healthy eyes.

	Repeatability Coefficient (RC) (95%CI) (mmHg)	Intra-Class Correlation Coefficient (ICC) (95% CI)	Coefficient of Variance (CV) (95%CI)
CST	2.32 (2.07,2.57)	0.89 (0.86, 0.91)	5.64 (5.46,5.82)
Tonopen	2.61(2.33, 2.89)	0.83 (0.78,0.89)	5.52 (5.32, 5.75)
GAT	2.35 (2.09, 2.61	0.87 (0.83,0.90)	5.85 (5.67, 6.03)
Icare	2.5 (2.23, 2.77)	0.92 (0.88,0.95)	6.42 (6.23, 6.61)

From the repeatability analysis presented in Table 3.2, the variability of the tonometers employed in this study was less than 3mmHg, with very good to excellent ICC (Table 3.2). The CV value of less than 10% for these tonometers, indicate low measurement variability and small within method measurement error. The IOP value by Icare has the

highest variability (RC: 6.42%) amongst the tonometers but with an excellent ICC of 0.92. The GAT measurement variability (CV 5.85) and high correlation between repeated measurements (ICC 0.87) was noted to be comparable to Tonopen and CST (CV 5.52, ICC0.83 and CV 5.62, ICC 0.89, respectively).

3.2.2 Inter-tonometry agreement

The agreement of the tonometers was evaluated with intraclass correlation coefficients (ICC), mean difference values, 95% limits of agreement (LOA) and Bland-Altman plots. The GAT was the reference for the inter-method IOP measurement comparison due to its status as being the current "gold standard" of tonometry in ophthalmology clinics worldwide. Mean measurement difference or 'bias' is the difference of mean IOP between the tonometer tested against GAT. The LOA of other tonometers with GAT were calculated.

Table 3.3 Mean difference, expected range of agreement and paired correlation between GAT and all tonometer.

Paired tonometry	Mean Difference ± SD (mmHg)	LOA (95%CI)	ICC	(95%CI)
ICare_GAT	-0.69 ± 2.44	-5.57 to 4.19	0.76	0.69, 0.81
Tonopen _GAT	1.87 ± 2.43	-2.99 to 6.73	0.65	0.23, 0.80
IOPcc_GAT	0.66 ± 2.94	-5.22 to 6.54	0.63	0.54, 0.70
IOPg_GAT	-0.09 ± 2.61	-5.31 to 5.13	0.73	0.66, 0.78
CST_GAT	0.69 ± 2.23	-3.87 to 5.15	0.63	0.53, 0.71

Table 3.3 showed the mean IOP difference of all paired tonometers with GAT. The inter-method mean difference was the highest by Tonopen vs IOP gat with bias of 1.87 ± 2.43mmHg. The mean difference of the Icare, CST and IOPcc was comparable at ± 0.6-0.7mmHg. The IOPg showed the least different to GAT with the mean inter-method difference of -0.09 ± 2.61mmHg. The standard deviations of all inter-tonometry biases were within 3mmHg. Figure 3.2(a-e) illustrates the mean difference and LOA for each paired tonometers. The widest LOA was between Tonopen and GAT (11.76mmHg) and the narrowest was between CST and GAT (9.02 mmHg).

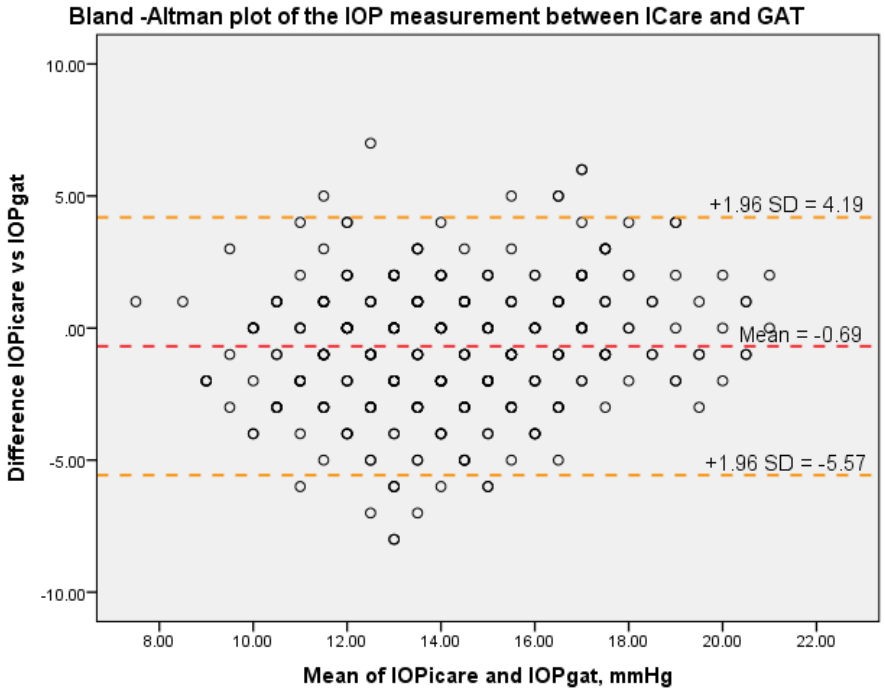


Figure 3.2a The Bland-Altman plots of Icare-GAT tonometers.

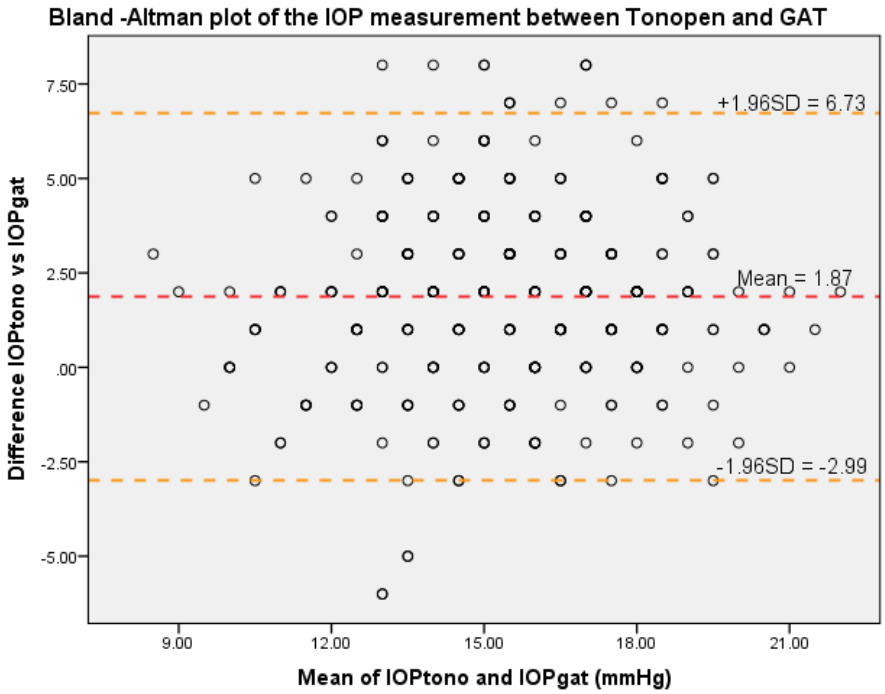


Figure 3.2b The Bland-Altman plots of Tonopen-GAT tonometers.

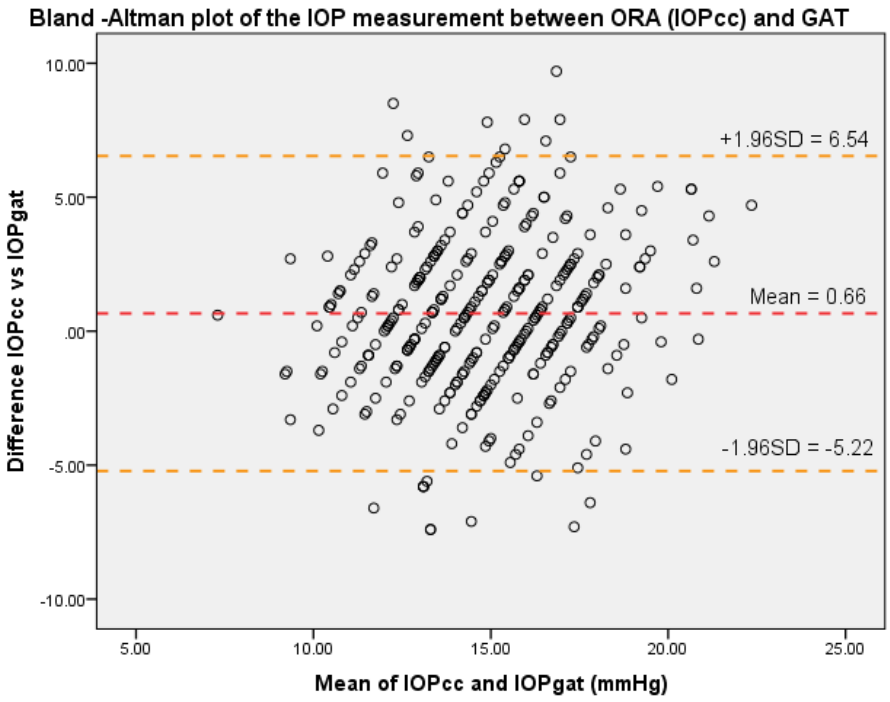


Figure 3.2c The Bland-Altman plots of IOPcc-GAT tonometers.

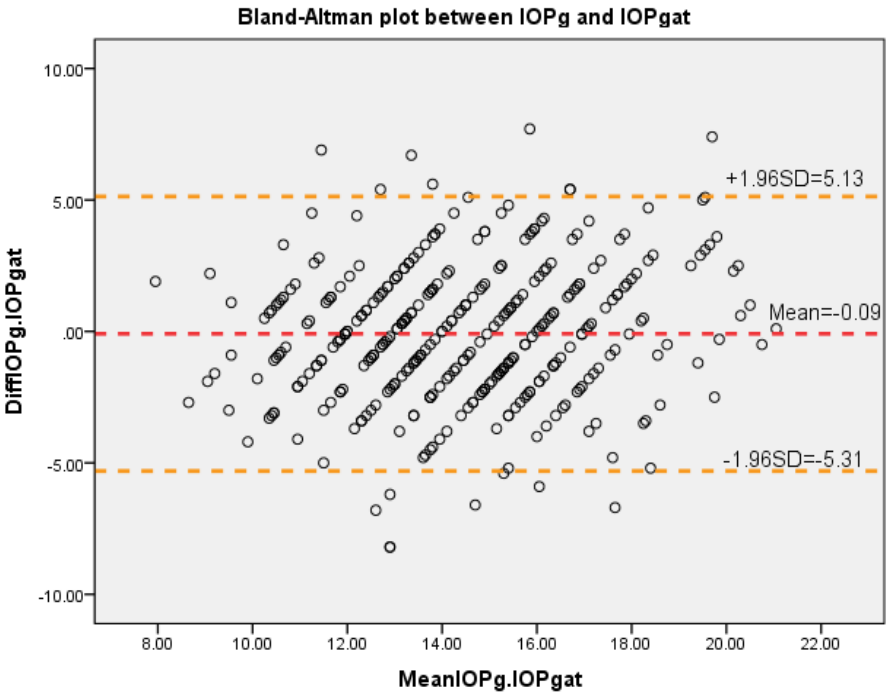


Figure 3.2d The Bland-Altman plots of IOPg-GAT tonometers.

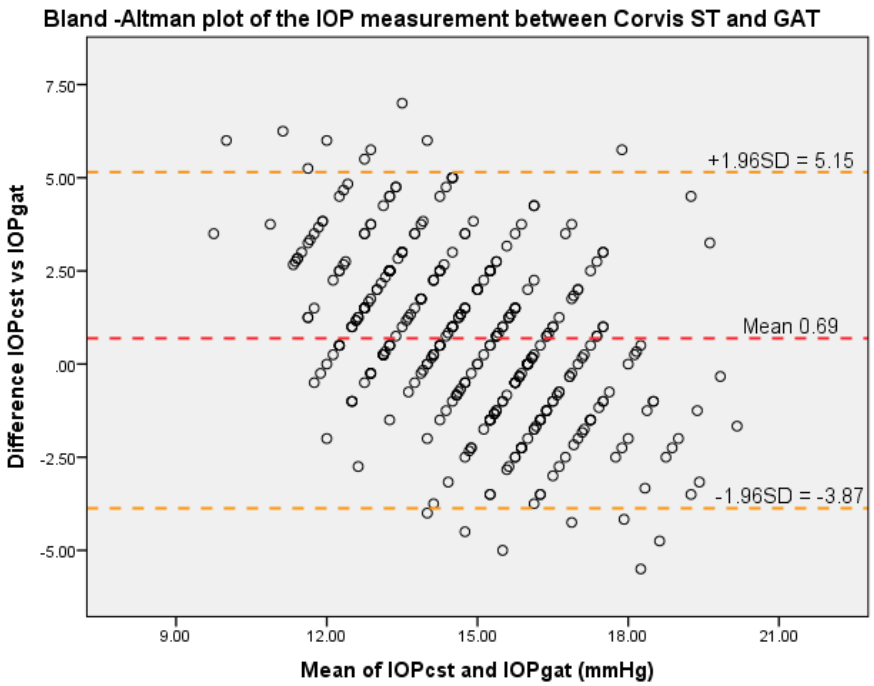


Figure 3.2e The Bland-Altman plots of CST-GAT tonometers.

In Figure 3.2a-e, the average IOP value of paired tonometers against the mean difference is plotted and these are called the Bland-Altman plots. The plots revealed that, the mean difference for Tonopen-GAT, Icare-GAT, IOPg-GAT and IOPcc-GAT were scattered in a constant pattern along the line of average IOP value of the paired tonometers. The bias were also mostly within the line of the estimated limit of agreement.

However, for CST–GAT pair, the mean difference was noted to show a proportional bias against the mean IOP value of the two tonometers (Figure 3.2e). Therefore, a log transformation of the mean difference of the paired method was done and replotted again. The log transformation of the scatter plot is one of the method to confirm existence of significant proportional bias. The log-transformed Bland-Altman plot of IOPct vs GAT in Figure 3. below, showed no difference in the pattern of bias compared to the initial plot in Figure 3.2e. Therefore a linear regression analysis was performed to calculate the LOA estimates at different CST tonometry values.

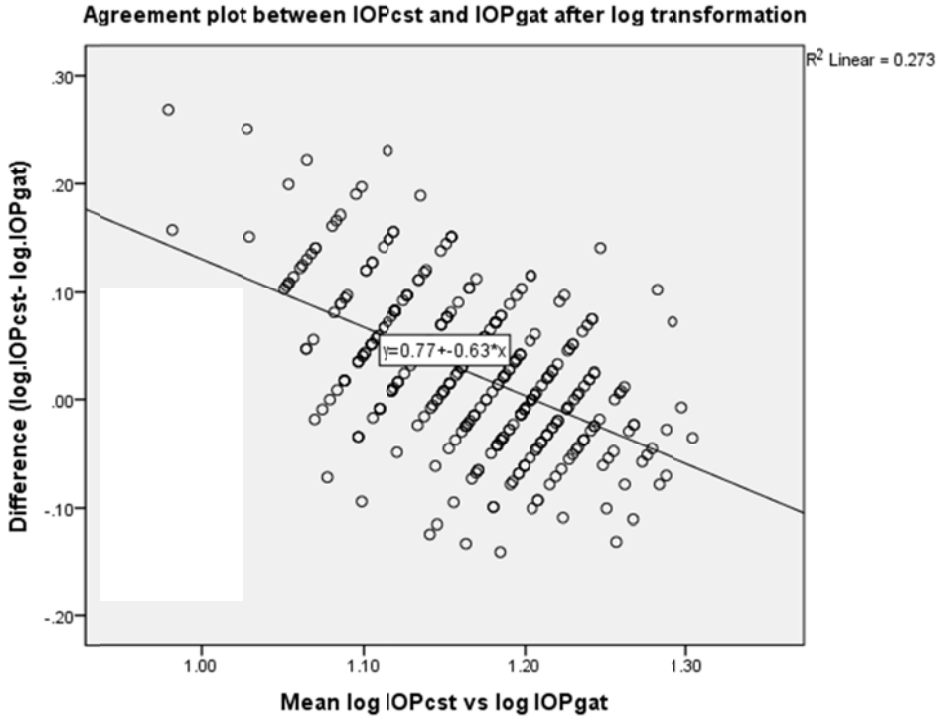


Figure 3.3 The Bland-Altman plot for transformed values (log transformation) of the CST and GAT.

Further linear regression analysis was done on this relationship and revealed that the CST value could significantly predict the mean difference between CST and GAT, p<0.001. The proportionate bias was accounted for by 20% of the increasing CST tonometry value. The regression equation was: mean difference of CST-GAT= $0.2x - 2.32$, $(x = IOPcst)$. Table 3.4 listed the estimated mean difference and limit of agreement between CST and GAT at various CST tonometry values.

Table 3.4 Estimated mean difference and 95% limit of agreement between Corvis ST and GAT tonometer at various CST values.

IOP by CST (mmHg)	Mean difference (CST-GAT), mmHg	Limit of Agreement (LOA) , mmHg
10	-0.32	-4.69 , 4.05
15	0.68	-3.69, 5.05
20	1.68	-2.69, 6.05
25	2.68	-1.69, 7.05

3.2.3 Inter-tonometry Bias

This section further investigates the relationship of the demographic variables and influence of significant factors on the mean difference (bias) between each paired tonometry method. In this analysis, only the CCTus was included to represent the measurement for central corneal thickness.

3.2.3.1 Relationship of inter-tonometry bias with demographic and corneal biomechanical parameters

The bias was compared between gender and ethnic origin with paired t-test (for gender) and ANOVA with Bonferroni correction (for ethnicity). For other continuous variables such as age, CCT and corneal biomechanical parameter value, Pearson's correlation was performed to investigate any significant relationship.

In Table 3.5, paired t-test revealed that female subjects showed significantly more negative Icare and GAT inter-tonometry bias compared to male subjects (-0.78 ±2.40 to -0.48 ±2.26 mmHg, p=0.05). Further, analysis of the bias difference amongst different ethnicity was performed with ANOVA with Bonferroni correction and post-hoc Tukey's analysis (Table 3.6). The highest inter-tonometry bias for CST and IOPcc against GAT was noted to be significant amongst Afro-Caribbean subjects (3.43 ± 2.26 and 1.12 ± 2.06 mmHg, $p<0.05$). However, amongst similar ethnicity, the Icare-GAT bias was noted to be the lowest (-0.07 ± 2.98mmHg).

Table 3.5 Inter-tonometry measurement bias between genders in normal subjects

	Male , N=127	Female, N=262	p†
Tonopen_ GAT (mmHg)	1.84 ±2.42	1.94 ±2.35	0.24
Icare_GAT (mmHg)	-0.48 ±2.26	-0.78 ±2.40	0.05
IOPcc_GAT (mmHg)	1.07 ±3.24	0.43 ±2.64	0.69
IOPg_GAT (mmHg)	0.02 ±2.91	-0.14 ±2.46	0.79
CST_GAT (mmHg)	0.73 ±2.45	0.67 ±2.13	0.58

†paired t-test, all values in mmHg

Table 3.6 Inter-tonometry bias between different ethnicity

	Caucasian (Mean±SD)	South-Asian (Mean±SD)	Afro-carribean (Mean±SD)	Oriental (Mean±SD)	Others (Mean±SD)	p†
Tonopen_GAT (mmHg)	1.44 ± 2.24	2.25 ± 2.43 **	1.86 ± 2.55	1.95 ± 2.39	2.33 ± 1.97	0.64
Icare_GAT (mmHg)	-0.72 ± 2.53	-0.66 ± 2.20	-0.07 ± 2.98	-1.30 ± 2.08	-0.50 ± 2.14	0.00*
IOPcc_GAT (mmHg)	0.48 ±2.89	0.51± 2.62	3.43 ± 2.26 **	0.04 ± 3.66	1.32 ± 3.45	0.04*
IOPg_GAT (mmHg)	-0.18 ± 2.40	-0.28 ± 2.49	2.71 ± 2.66 **	-0.211 ± 3.57	0.42 ± 3.19	0.23
CST_GAT (mmHg)	0.35 ± 2.19	0.91 ± 2.11	1.12 ± 2.06	0.74 ± 2.95	0.50 ± 3.14	0.00*

†ANOVA with Bonferroni correction

*value of significance <0.05

** post-hoc Tukey, p<0.05

Table 3.7 Relationship of the inter-tonometry bias (all unit value in mmHg) with age, CCTus and biomechanical parameters

	Age	CCTus	CRF	CH	A1T	A1L	A1V	A2T	A2L	A2V	HcT	HpD	HcR	DA
IOPicarevsIOPgat	-0.09	0.027	0.21**	0.12*	0.10	-0.02	-0.08	-0.10*	0.03	-0.01	-0.011	-0.03	0.06	-0.07
IOPtono vs IOPgat	-0.32**	-0.01	0.02	0.07	-0.06	-0.08	0.05	0.16**	-0.03	-0.05	-0.01	0.02	-0.11*	-0.03
IOPcc vs IOPgat	0.22**	-0.26**	-0.32**	-0.60**	-0.06	-0.003	-0.06	-0.30**	-0.16**	-0.15**	-0.03	0.06	-0.04	-0.004
IOPg vs IOPgat	0.19**	0.04	0.19**	-0.08	0.17**	0.07	-0.09	-0.38**	-0.04	0.01	-0.03	0.03	0.17**	-0.15**
IOPcst vs IOPgat	0.02	-0.05	-0.003	0.11*	0.11*	-0.03	0.05	-0.09	-0.12*	-0.11*	-0.07	-0.001	-0.06	0.02

Pearson Correlation test, r value

* p< 0.05 level (2-tailed), ** p< 0.01 level (2-tailed)

3.2.3.2 Factors affecting inter-tonometry bias

The inter-method bias was known to be influenced by many interactive factors. Therefore, a multivariate regression analysis was employed to construct a predictive model of the inter-method bias. All regression analysis of the inter-method bias was controlled for the effect of CCTus and CRF. These are known confounding factor from previous inter-tonometry agreement studies described in 2.4.4.1. The assumptions for regression analysis were checked. All assumptions of normality and heteroscedasticity for the residual error plot were met for all analysis.

Multicolinearity of variables can violate the assumptions for a valid regression model. For this cohort, the CH was excluded due to its high correlation with CRF (Pearson's correlation, $r=0.83$, $p<0.01$). Ethnicity was not included in the multivariate analysis due to inadequate sample size in every cell. Simmons et al. suggested that for categorical variable, each cell must have at least 20 sample for a valid regression analysis [240]. Other than Caucasian and South-Asian ethnicity, other ethnic background has less than 20 samples. The ocular laterality effect on all the inter-method bias revealed no significant influence and does not affect the final outcome in all multivariate model, with $p>0.05$.

The effect size of these variables were calculated and presented as Adjusted R^2 value, B (the unstandardized coefficient), Beta also known as β (the standardised coefficient). The value of significance is set at $p<0.05$. The definition of the outcome measures were explained in section 2.4.4.

3.2.3.2.1 Inter-tonometry bias between Tonopen and GAT

A multivariate regression analysis established that age and DA could predict 9.1% of the explained variability of the inter-method IOP bias between Tonopen and GAT, $F=3.10$, $p<0.00$. Older subjects and higher DA could cause reduction of bias between these tonometers. The effect of age on the variability of the inter-method bias is more than DA. (Table 3.8)

Table 3.8 Multivariate regression analysis of factors affecting the inter-tonometry bias between Tonopen and GAT

	B coefficient	Standard Error B	β	P
Age	-0.03	0.01	-0.28	0.00
DA	-5.19	2.29	-0.23	0.02

* value of significance <0.05

3.2.3.2.2 Inter-tonometry bias between Icare and GAT

The adjusted r^2 of the model was 0.044, p<0.00. The model showed that age and CRF explained less than 5% of the inter-method bias variability. The CRF was noted to be the stronger factor that significantly affect the multivariate model with beta coefficients of 0.30 (p<0.00) compared to age (beta coefficients of -0.205, p<0.05). Younger subjects seemed to have higher inter-tonometry bias compared to older subjects. Inversely, subjects with higher CRF could cause an increase in bias. The equation for this regression model is y= -0.02(age) + -0.006(CCTus) + 23.52, (where y= Icare vs GAT bias).

3.2.3.2.3 Inter-tonometry bias between ORA (IOPcc) and GAT

This multivariate regression analysis established that CCTus, IOPcc, CRF, A1T, A2T and age significantly accounted for 52.5% of the inter-method IOP bias variability between IOPcc and GAT (p<0.00). The IOPcc has the strongest unique contribution to the overall bias (β coefficient = 0.70), whilst age has the least effect (β coefficient = 0.09). The inter-method bias between IOPcc and GAT could increase with IOP and age. The corneal biomechanical parameters could negatively affect the bias between these tonometers. The A1T has the highest effect on the bias (β coefficient = - 0.20) and was followed A2V and CRF (β coefficient = -0.12 and 0.12, respectively). CCT has weak but significant negative effect on the overall variability of the bias (β coefficient = -0.09). The equation for this regression model is y= -0.65(IOPcc) -0.21(CRF) -2.29(A1T) + -5.52(A2V) + -0.007(CCT) + 0.013 (age) + 12.18, (where y= IOPcc vs GAT bias).

3.2.3.2.4 Inter-tonometry bias between ORA (IOPg) and GAT

A multivariate regression analysis showed that age, IOPcc, CRF, A1T, A2T and A2V could statistically predict the inter-method bias between IOPg and GAT. These factors accounted for 30.5% of the explained variability of the inter-tonometry bias ($F=22.13$, $p<0.00$). IOPcc has the highest unique contribution on the variability (β coefficient = 0.46), whilst age has the least effect (β coefficient = 0.11). The inter-method bias increased with age, IOPcc and CRF. Amongst the corneal biomechanical parameters, the CRF has the strongest unique contribution compared to A1T, A2T and A2V (β coefficient = -0.23, -0.17 and -0.15, respectively). The equation for this regression model is y= 0.38 (IOPcc) + 0.52(CRF) -2.32(A1T) + -6.12(A2V) + -1.1(A2T) + 0.014 (age) + 30.35, (where y= IOPg vs GAT bias).

3.2.3.2.5 Inter-tonometry bias between CST and GAT

The multivariate regression analysis established that IOPcc, CRF, A1T, A2T and A2V could statistically predict the inter-method IOP bias between Corvis ST and GAT. These factors accounted for 19.9% of the explained variability of the inter-tonometry bias between CST and GAT, $p<0.00$. A1T could cause increment on bias between these tonometers. Inversely, the increment of IOPcc, A2V and CRF could cause a negative effect on the measurement bias. Amongst the variables, the IOPcc has the strongest effect on the inter-method bias between CST and GAT. This is consecutively followed by A2T, A1T, A2V and CRF. Age, gender, CCTus and other corneal biomechanical parameters of CST has no significant contribution to the overall model analysis. The equation for this regression model is y= -0.4(IOPcc) -0.19(CRF) -1.98(A2T) + 2.50(A1T) -5.93(A2V) +34.08, (where y= CST vs GAT bias).

3.2.4 Discussion

This study recruited healthy subjects with multi-ethnic background and age distribution. The proportion of subjects over 40 years old was less than those below 40 years (less than 40 years old, n=250 and 40 year old and more, n =140). In both age groups, Caucasian and South Asian ethnicity are dominant which is consistent with the United Kingdom National Census 2011 for West Midland area [241]. In the census report, residents of Birmingham consisted of more than 80% Caucasian and 8% of South Asian (mainly Indian and Pakistani) ethnic origin.

3.2.4.1 Repeatability of tonometers

This repeatability study observed four tonometers that have different levels of IOP variability. In general, all tonometers showed very good to excellent correlation between repeated measurements with ICC above 0.80. In order to reduce measurement bias, prior to the start of our study, all the instruments underwent calibration as suggested by the manufacturers. This study also followed the measurement technique suggested by the manufacturers. Measurement guideline from previous studies was also noted to reduce measurement error (see section 2.4).

The repeatability coefficient for GAT (CR ±2.35mmHg) in this study was comparable to a previous study, (CR ±2.5mmHg) [194]. The GAT measurement variability (CV 5.85) and high correlation between repeated measurements (ICC 0.87) was noted to be comparable to Tonopen and CST. Unlike other tonometers in this study, the GAT tonometry is supposedly more subjective and operator dependant, thus a higher variability is expected than other automated devices. Previous studies also noted good intra-observer ICC values of 0.81 and 0.79 with a slightly higher variability of 9.0% and 9.7% ([242, 243],respectively), compared to our study (CV of 5.85, variability of 9.2%).

There are two possible explanations for this. First, in this study, the GAT operators were experienced clinicians that are able to operate the tonometer well for accurate tonometry. Secondly, the repeated measurements were taken after at least 2 minutes of the first measurement. Thus, the cornea was able to recover well prior to the second tonometry. In spite that, the differences of the repeatability indices may result from investigator and participant group variability. Nevertheless, this study supported good repeatability of GAT tonometry.

The Icare measured repeated IOP with excellent consistency but it produced the highest variability compared to other tonometers. Previous studies investigating the intra-observer repeatability of Tonopen and Icare showed high repeatability results as for the current study (Tonopen ICC 0.88, CV 5.2 and Icare ICC0.87, CV 5.2) and (Tonopen ICC 0.85 and Icare ICC 0.87) [242, 244]. However, the World Glaucoma Consensus for acceptable precision for tonometry was set as CR of 2.5mmHg or less for GAT intra-observer CV [235]. There is no information regarding the acceptable CV for other tonometers. Other tonometers employed in this study complied with the precision standard set by the consensus. With regard to Tonopen, this study concludes that due to very high repeatability and low variability of CV, despite CR of 2.61mmHg, the Tonopen is still considered precise in its intra-observer repeatability.

The tonometry and pachymetry measurements by CST were highly repeatable (ICC>0.75, CV<10), and this finding was similar to previous studies in healthy eyes [245-247]. This study found the corneal biomechanical parameters by the CST; A1T, A2T, HcR and DA have high repeatability (ICC>0.70, CV <10) which was similar to a previous study [247]. However, previous studies found that only two biomechanical parameters, DA and A1T, showed only fair to good repeatability [212, 246]. The difference in the CST repeatability performance between previous studies and the current study may be related to its software version. The software versions employed in earlier studies were V1.x [247, 248] and V2.x [246]. This study employed an updated CST software version, V6.07r8.

In this study, all five tonometers showed very high intra-observer repeatability as the CV was always below 10% and ICC were greater than 0.80. Thus, due to the very good precision of these tonometers, this study decided to perform single tonometry measurement with the GAT (which is a balance between diastolic and systolic pulse pressure) for further data collection. The Icare and Tonopen produced a mean value with standard deviation the display screen with each completed measurement. Therefore, a completed single measurement with standard deviation of less than 5% is accepted to represent the tonometry value of both instruments. For CST biomechanical parameters, nearly half of these variables showed poor to moderate repeatability. Thus, it is decided an average of two measurements of all CST biomechanical parameters will be regarded as appropriate to represent its value for further analysis.

The intra-observer repeatability of repeated measurements by the ORA was not analysed. In accordance to the recommendation from manufacturer, the IOPg and IOPcc measurements were selected based on the best waveform score (out of four repeated air-puff tonometry) for analysis. This study assumed that the repeatability of the automated tonometry by ORA was based on previous studies that used similar software version (version 3.1) as discussed in section 2.2.5.

3.2.4.2 Inter-tonometry Agreement

The GAT is referred as the “gold standard” for tonometry [235]. Despite recognitions of it to be affected by many factors [16, 18], the GAT is still recognised as clinical standard for IOP measurement by clinicians worldwide. The agreement of tonometry of these instruments with GAT is important for clinicians to know its validity of IOP measurement against clinical standard. This study has embarked on inter-tonometry

agreement analysis between GAT with Tonopen, Icare, ORA and Corvis ST. The outcome measures discussed were mean difference which is interchangeable with 'bias', limit of agreement (LOA) and Bland-Altman plots assessment [234].

3.2.4.2.1 Icare vs GAT

Icare has not been validated with manometry studies in human eyes. However, studies on animal models have shown that Icare Tonolab (rebound tonometry for lab animals) provides very good agreement with manometry studies [249, 250]. In this study, the results of Icare-GAT comparison in healthy eyes showed slight underestimation of the GAT which was in accordance with previous studies [251-253]. The inter-tonometry bias is within limits of ± 2.0 mmHg, which is within the acceptable systematic difference against GAT [21]. However, other studies reported that Icare was noted to overestimate IOP [254-257]. This contrasting result may be due to the differences in the cohort of subjects and sample size recruited. The width of LOA for the Icare-GAT in this study was narrower than other studies [254-257]. Nevertheless, the range is still out of the acceptable LOA limits for it to be considered interchangeable with GAT.

In terms of proportionate bias, this study noted that the mean difference of Icare against GAT was constant across the range of mean IOP. This is in contrast with studies that have shown that IOP by Icare was proportionately related to the increase of mean IOP [251, 252]. These researchers had recruited less than ideal sample size to achieve good statistical power. This indicates that Icare is suitable for screening purposes.

3.2.4.2.2 ORA vs GAT

The inter-tonometry comparison of ORA to GAT in our study revealed mean difference that are within limits of ± 2.0 mmHg, which is the acceptable systematic difference between tonometers [21]. The IOPcc was noted to overestimate GAT, whilst the IOPg was found to be very much closer to GAT measurements (bias of -0.09mmHg), which was similar to a previous study where the bias was nearer to zero [72, 143]. However, the difference of IOP bias by ORA was quite obvious between studies. The current study noted that IOPg by ORA showed the least difference from GAT compared to other tonometer. The inter-tonometry bias was noted to be higher in other studies that

recruited sample with multiple diagnosis[157], healthy subjects[258, 259] and sample of normal twins [260].

For this current study cohort, the IOP by ORA is not interchangeable with GAT. The 95% limit of agreement for IOPg-GAT and IOPcc-GAT in the current study was more than 8mmHg. This is in agreement with previous studies that noted the LOA to be more than the acceptable limit [156, 157]. This study did not found any proportionate bias in the BA plots for both IOPg and IOPcc bias against GAT which is similar with previous studies [72, 260]. However, few studies reported increase of proportionate bias with higher mean IOP [74, 143] . The difference in bias and LOA may be due to the variability of measurement of ORA as a non-contact tonometer. The ORA measurements were completed in less than 10ms, thus are susceptible to the influence of ocular pulse amplitude [157], corneal tear film and CCT [143, 261]. The difference of study cohort and intra-observer bias may also influence the variability.

3.2.4.2.3 Tonopen vs GAT

The tendency for Tonopen to overestimate GAT measurement was consistent with previous studies in normal subjects with mean difference of 1.0, 0.6 and 0.5 mmHg, respectively [187, 262, 263]. A meta-analysis study confirmed that Tonopen overestimated GAT and limit of agreement of Tonopen range were 12mmHg [21]. In contrast, Tonopen was reported to underestimate IOP at high IOP [203, 243]. Our study revealed LOA range of Tonopen-GAT agreement were approximately 10mmHg. This is exceeding the expected standard LOA range of 8mmHg, which was similar to all the studies stated earlier. There was no proportionate bias seen in our study which was similar with a previous study on normal subjects, though the trend was positively affected by CCT [243]. In healthy children, the Tonopen showed higher mean difference compared to GAT and the trend is positive with higher IOP and younger age [264].

3.2.4.2.4 CST vs GAT

The agreement between CST with GAT in this study was noted to be comparable to previous studies of normal eyes (mean difference of 0.69 to 1.5 mmHg, respectively) [265, 266]. In contrast, a similar agreement study on normal and glaucoma subjects revealed that CST underestimates GAT measurement [211]. The limit of agreement of

the CST versus GAT was more than 8mmHg range for the current and all the above studies. The BA plot revealed a proportionate bias where CST overestimated GAT with higher IOP values. CST tonometry of more than 21.6mmHg may increase the bias (mean difference between IOPcs vs GAT) by more than 2 mmHg.

According to a large meta-analysis on tonometry agreement with GAT [21], the most challenging facts concerning the inter-tonometry agreement studies are the heterogeneity of the results in different study population and the use of improper statistics that may lead to different conclusion on the inter-method agreement. This study has clearly outlined the appropriate sample size, method and analysis for a valid method agreement report. The tonometry agreement studies that were compared largely consist of mixed cohorts of glaucoma and healthy eyes. Thus the variability of the inter-tonometry bias amongst the different researchers may be likely due to the heterogeneity of study subjects.

3.2.4.3 Factors Affecting Inter-tonometry Agreement

A major review explored the many within-instrument, physical, physiological, ocular, as well as demographic factors that could influence the measurement of IOP by GAT [18]. To the best of our knowledge, this is the first study that analysed the influence of CST biomechanical variables on the inter-tonometry agreement between Icare, Tonopen, ORA and CST, with GAT. We have chosen the IOPcc that was claimed to represent the corneal-compensated IOP value, to be included in the regression analysis of the inter-tonometry bias [20, 157]. The analysis also included demographic variable i.e.; age and gender. The effect of ethnicity was not analysed by regression as the sample size in each ethnic group did not fulfil the minimum requirement. The effect of multi-colinearity between CH and CRF was taken into consideration. CH was excluded from this part and CRF was chosen for to represent identifiable corneal biomechanical parameters by ORA. The multivariate regression analysis of the inter-method bias was corrected against the confounding effect of CCT, IOPcc, CRF and laterality, using hierarchical method.

3.2.4.3.1 Icare vs GAT

Univariate analysis revealed that inter-method bias between Icare and GAT was significantly affected by corneal biomechanical variables; A2T, CH and CRF. However, the multivariate analysis in this study revealed that the effect CCT, A2T, CH and CRF

were not meaningful when the variables where considered collectively. The CRF and age were found to have small but independent unique contribution that caused changes to the Icare bias. Similar to Tonopen bias, older subjects tend to cause less tonometry bias by Icare. The effect of CCT was confirmed in another study, which found that as the CCT increased, Icare considerably overestimate GAT [244]. Meanwhile, a study noted that only corneal curvature and not CCT, significantly affect the inter-tonometry bias [251]. This study postulated that steeper cornea to decrease the Icare probe velocity, thus causing underestimation of GAT. The study cohort amongst normal and post-keratoplasty subjects may have different distribution of curvature and corneal thickness, thus resulted in the variance of the CCT effect in the regression analysis.

3.2.4.3.2 Tonopen vs GAT

The inter-method agreement between Tonopen and GAT was noted to be affected by age and DA. The effect of these variables was weak but meaningful (r^2=0.09, p<0.00). In this study, age was affecting the bias more than DA. The effect of age was supported previous studies on non-treated subjects attending glaucoma clinics. that showed Tonopen underestimated IOP in older subjects compared to GAT [157]. A large cohort study in South Korea which was a population (Korean) based study revealed no statistically significant association between IOP bias by Tonopen with age [267]. The variable effect of age on the bias may be subjected to the sample size and differences of the study cohort. Our study subjects were healthy subjects with wide age range and satisfactory sample size.

Studies with ORA had established a connection between aging cornea and corneal biomechanics properties, CH and CRF [268]. Increased rigidity in ageing cornea may happen as the result of ultrastructural changes in the collagen fibrils of the corneal stroma [48, 49]. Our study found that DA is the only significant biomechanical parameters affecting the inter-tonometry bias between Tonopen and GAT. No similar studies yet found in the literature. Thus, DA may be a novel measureable parameter that represents an element of corneal biomechanics that is not captured by ORA parameters. Previous studies have established no significant contribution of CCT in the inter-tonometry bias between Tonopen and GAT [65, 192, 202], which was similar with our study. Tonopen indents a very small area of surface and, in comparison with

applanation tonometry by GAT, the IOP measurement was almost static. Thus, the contribution of CCT on the bias may be non-demonstrable.

3.2.4.3.3 CST vs GAT

Since the launch of CST in 2012, studies had reported many findings with regards to its agreement to GAT but only a few studied the factors affecting the inter-method agreement. This study found that the inter-tonometry bias between CST and GAT was affected by IOPcc, CRF, A1T, A2T and A2V. Amongst the factors, IOP contributed the most to the bias, followed by consecutively by A2T, A1T, A2V and CRF. Currently, there is nothing in the literature that investigates the combined effects of demographic profiles, CCT and CRF on the CST tonometry bias in healthy subjects.

This analysis had corrected for the confounding effect of CCT, IOPcc and CRF. Similar to this study, the lack of an effect of CCT, age and axial length on the CST-GAT inter-method bias was also noted in a healthy Chinese cohort [211]. Despite that, CST parameters (A1T, A2T and A2V) had shown significant independent contributions towards the tonometry bias. This may indicate the significance of these parameters as additional element of corneal biomechanical properties other than CRF. In a study on glaucoma subjects, the increment of CST bias against GAT was positively affected by CCT [266]. Previous studies only highlight A1T and DA as the most reliable and reproducible parameters for describing corneal biomechanics [212, 245, 269].

3.2.4.3.4 ORA vs GAT

This study revealed that there was a statistically significant difference of the mean IOPg, IOPcc and Tonopen amongst subjects with different gender, age group and ethnicity. Both IOPcc and IOPg are significantly higher in older subjects. This finding is similar to previous studies which showed a positive influence of CCT [72, 260]. In a large cohort of British people, the IOPcc was higher in males and increased with age[65]. The relationship of age with non-contact tonometry was well known in large population studies. A large tonometry study in a Japanese population [270] with a non-contact tonometer found that the IOP decreases with age, and CCT variation has practically no effect on the age-IOP relationship. This was further supported by a Korean study with a much larger cohort [271]. They found that systolic blood pressure and heart rate were positively associated with IOP pattern.

All the tonometers employed in this study were in good agreement with GAT but a wide range of agreement levels were observed. The LOA of all the tonometers employed in this study was greater than the acceptable limit of approximately 8mmHg (Cook 2012). These indicate that all the tonometers are valid alternatives for GAT tonometry amongst healthy subjects with normal IOP value. However, the IOP values of these tonometers are not interchangeable with GAT. Amongst the paired tonometers, only CST was noted to overestimate GAT when the mean IOP increased. Thus, clinicians should be aware of the proportionate bias by CST when performing tonometry on subjects with high IOP (IOP of more than 22mmHg). The IOP measurement by CST may be more suitable for screening for ocular hypertension than for glaucoma monitoring and management purposes. Age affected the inter-tonometry bias between all paired tonometers with GAT except for CST. The CRF, A1T, A2T, A2V and DA are important biomechanical parameters that influence the inter-tonometry bias.

3.3 Corneal Biomechanical Assessment of Healthy Eyes

In the past decade, particular interest in corneal biomechanical properties has developed in glaucoma management. Apart from geometry, corneal biomechanic parameters of ORA (CH and CRF) were noted as confounding factors for intraocular pressure measurement by applanation tonometry. The in-vivo corneal biomechanical parameters and corneal thickness measured by ORA and Corvis ST provide valuable information and further insight on the biomechanical characteristics of the human corneal tissue.

This study explored the in-vivo corneal biomechanical properties of healthy eyes with ORA and CST. In the initial section (3.2.1), analysis of the repeatability of the corneal biomechanical parameters of CST was performed. The relationships between corneal biomechanical parameters by the Corvis ST (CST) and Ocular Response Analyzer (ORA) were evaluated in section 3.2.2. Further in section 3.2.3, regression analysis was performed to analyse the influence of demographic, central corneal thickness and IOP on the biomechanical parameters. Each corneal biomechanical parameter employed in this analysis was detailed in section 2.3.4 and 2.3.5.

3.3.1 The repeatability of corneal biomechanical parameters and central corneal thickness values by CST.

In this study, intra-observer repeatability of the central corneal thickness and biomechanical parameters of CST were quantified with repeatability coefficients (RC), coefficients of variation (CV) and intraclass correlation coefficients (ICC). The overall mean of each parameters was listed in Table 3.1 in section 3.1.

The ICC (two-way mixed, absolute agreement type) was calculated to measure the intra-session repeatability of the corneal measurements. The magnitude of variability between the measurements was represented by CV (Coefficient of Variation), whilst, the exact amount of variability was represented by CR (Coefficient of Repeatability). The calculation formula for these statistical measures was discussed in section 2.4.2.

Table 3.9 listed the repeatability tests value of the variables. The distribution of mean difference for A1L, HpD and HcR does not fulfil the assumptions of normality and parametric distribution. Thus, the Repeatability Coefficient analysis was not calculated for these parameters. However, the ICC and Coefficient of Variance were calculated for

these parameters. The DA, A1T and A2T are 3 parameters that showed very good to excellent repeatability (ICC more than 0.8) and low variability (CV less than 10%). The HcR also showed good repeatability (ICC 0.71) and low variability (CV 7.7%). A1L and HcT were noted to have low variability but poor repeatability. The repeated measurements of A1V, A2L, A2V and HpD showed poor inter-measurement agreement and higher variability than other parameters.

Table 3.9 The repeatability tests of all tonometry values and biomechanical parameters

	Repeatability Coefficient (95%CI)	ICC (95% CI)	Coefficient of Variance (95%CI)
A1T	0.34 (0.3, 0.38)	0.88 (0.85,0.90)	1.5 (1.47, 1.53)
A1L	-	0.30 (0.15,0.45)	7.3 (7.27, 7.33)
A1V	0.08 (0.07,0.09)	0.29 (0.12,0.43)	18.7 (18.69, 18.71)
A2T	0.57 (0.51, 0.63)	0.87 (0.83,0.89)	0.9 (0.86, 0.94)
A2L	0.88 (0.78, 0.98)	0.28 (0.1,0.43)	35.2 (35.07, 35.33)
A2V	0.13 (0.12, 0.14)	0.68 (0.6,0.74)	24.3 (24.28, 24.32)
HcT	1.16 (1.03, 1.29)	0.48 (0.35,0.58)	2.5 (2.41, 2.59)
HpD	-	0.31 (0.14,0.45)	34.2 (33.92, 34.45)
HcR	-	0.71 (0.64,0.77)	7.7 (7.59, 7.81)
DA	0.12 (0.11, 0.13)	0.9 (0.88,0.92)	3.4 (3.39, 3.41)
CCTcor	17.66(15.73,19.59)	0.98 (0.98,0.98)	1.2 (5.32, 5.75)
CCTus	12.21(10.87, 3.55)	0.92 (0.89, 0.95)	0.83 (0.12, 1.78)

3.3.2 Relationship between ORA and CST biomechanical parameters

The mean value of all the corneal biomechanical variables based on demographic distribution was presented in the early part of this chapter (item 3.1). In this section, Pearson correlation analysis was performed to explore the relationship between the biomechanics parameters of ORA and CST. Pearson correlation measures the existence (given by a p-value) and strength (given by the coefficient r between -1 and +1) of a linear relationship between two variables. A significant outcome indicates that a correlation exists (if $p<0.05$). According to Cohen (1988) an absolute value of r of 0.1 is classified as small/weak, of 0.3 is classified as medium/moderate and of 0.5 is classified as large/strong.

Table 3.10 showed that CH was strongly correlated with CRF ($p<0.01$). The CH and CRF was significantly correlated with all Corvis ST biomechanical parameters except for A1V, HcT, HpD and DA. The correlation of CH with HcR was strong ($r=0.76$, $p<0.01$). However, there was poor correlation between CH with A1T, A1L, A2T, A2L and A2V ($r <0.24$, $p< 0.01$).The CRF showed better correlation with the CST parameters. The Pearson's correlation were small between CRF and A1L, A2T and A2L ($p<0.01$). There was moderate correlation between CRF with A2V and DA, ($r>0.3$, $p<0.01$). A strong correlation was noted between CRF and A1T ($r=0.53$, $p<0.01$) Amongst the CST parameters, DA was strongly correlated with A2V and moderately correlated with A1T, A2T and HcR. The A1T and A2T have moderate correlation with each other ($p<0.05$).

Table 3.10 The relationship of corneal biomechanical variables of ORA and Corvis ST(Pearson's correlation test)

	CH	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
CH	1	0.83**	0.23**	0.14**	0.04	0.12*	0.21**	0.23**	0.04	-0.03	0.76**	-0.09
CRF	0.83**	1	0.51**	0.21**	-0.08	-0.22**	0.25**	0.35**	-0.002	-0.03	0.36**	-0.34**
A1T	0.23**	0.51**	1	0.19**	-0.19**	-0.60**	0.15**	0.40**	-0.08	-0.12*	0.49**	-0.60**
A1L	0.14**	0.21**	0.19**	1	0.29**	-0.11*	0.07	0.12*	0.005	0.02	0.20**	-0.03
A1V	0.04	-0.08	-0.19**	0.29**	1	0.31**	-0.11*	-0.16**	0.002	-0.03	-0.31**	0.31**
A2T	0.12*	-0.22**	-0.60**	-0.11*	0.31**	1	-0.04	-0.31**	-0.01	0.07	-0.42**	0.58**
A2L	0.21**	0.25**	0.15**	0.07	-0.11*	-0.04	1	0.45**	0.09	-0.01	0.34**	-0.23**
A2V	0.23**	0.35**	0.39**	0.12*	-0.16**	-0.31**	0.45**	1	0.15**	-0.12*	0.46**	-0.70**
HcT	0.04	-0.002	-0.08	0.01	0.002	-0.01	0.09	0.15**	1	-0.09	0.07	-0.07
PD	-0.03	-0.03	-0.12*	0.02	-0.03	0.06	-0.01	-0.12*	-0.09	1	-0.03	0.16**
HcR	0.17**	0.36**	0.49**	0.20**	-0.31**	-0.42**	0.34**	0.46**	0.07	-0.03	1	-0.54**
DA	-0.09	-0.34**	-0.60**	-0.03	0.31**	0.58**	-0.23**	-0.70**	-0.07	0.16**	-0.54**	1

** Significance value <0.01 level (2-tailed).

* Significance value < 0.05 level (2-tailed).

3.3.3 Factors affecting the biomechanical parameters by ORA and CST

Multivariate linear regression analysis was carried out to identify factors that affect the corneal biomechanical parameters. Based on previous literature, age, gender, ethnicity, CCTus and IOPcc were chosen as predictors. The biomechanical parameters were analysed as separate criterion for the regression model. Details on the analysis, variables chosen for regression and outcome measures are described in section 2.4.

In Table 3.11, the multivariate analysis of the effect of age, gender, CCT and IOP on corneal biomechanical parameters is presented. Amongst the corneal biomechanics parameters, the regression analysis explained moderate to strong predictability model for A1T, A2T, HcR, DA, CH and CRF. The model described 46% of the variability of CH and A2T, followed by 28% of the explained variability of A1T. The model also described 25% of the variability of DA and CRF. Despite the significance of the regression models of A1L, A1V, A2L and A2V, the model explained less than 12% of these parameters' variability. HcT and HpD regression models showed no significant contribution of the factors analysed.

The CCT and IOPcc made a unique contribution to CH that accounted for 46% of its variability, with IOPcc recording a higher beta coefficient value (β coefficient= -0.53, $p<0.00$) than the CCTus (β coefficient= 0.43, $p<0.00$). The regression model of A2T showed that the total variance (46%) was explained by IOPcc, CCTus and age. The IOPcc has the highest unique contribution (β coefficient= - 0.59, $p<0.00$) on A2T variability, compared to age and CCTus (β coefficient= -0.23 and -0.16, $p<0.00$). The IOPcc was also noted to has the highest and most significant contribution on the variability of A1T (β coefficient= 0.43, $p<0.00$) and DA (β coefficient= -0.53, $p<0.00$). Both DA and A1T also affected by age (β coefficient= 0.11 and -0.12, $p<0.00$) and CCTus (β coefficient= -0.25 and 0.30, $p<0.00$).

Table 3.11 Factors affecting the biomechanical parameters by Corvis ST and ORA

	Standardised coefficient (β) with B coefficients (BCa 95% CI)				Adjusted R^2	p
	Age	Gender	CCTus	IOPcc		
CH	-	-	0.43***	-0.53***	0.46	0.00
CRF	-	-	0.50***	-	0.25	0.00
A1T	-0.12**	-	0.30***	0.43***	0.28	0.00
A1L	-	-	0.26***	0.11*	0.07	0.00
A1V	-	-	-	-0.22**	0.05	0.00
A2T	-0.23***	-	-0.16***	-0.59***	0.46	0.00
A2L	-	-0.13*	0.22***	-	0.07	0.00
A2V	-	-	0.31***	0.17**	0.12	0.00
HcT	-	-	-	-	0.01	0.65
HpD	-	-	-	-	0.01	0.80
HcR	0.16*	-	0.45***	0.28***	0.30	0.00
DA	0.11*	-	-0.25***	-0.43***	0.25	0.00

Age, gender, CCTus and IOPcc, have variable effect on the corneal biomechanics parameters. Amongst the factors, CCTus was noted to affect majority of the CST parameters and both ORA parameters. Higher CCT positively affect A1T, A1L, A2L, A2V, HcR, CH and CRF (β coefficient= 0.30, 0.26, 0.22, 0.31, 0.45, 0.43 and 0.50, $p<0.00$). In contrast, CCT could cause reduction of DA and A2T (β coefficient=-0.25 and -0.16, $p<0.00$). The IOPcc affected A1T, A1L, A1V, A2T, A2v, HcR, DA and CH. The IOPcc has positive unique contribution on A1T, A1L, A2V and HcR (β coefficient= 0.43, 0.11, 0.17 and 0.28, $p<0.01$). This effect was in reverse on A1V, A2T, DA and CH (β coefficient= --0.22, -0.59, 0.43 and 0.53, $p<0.00$). Age could contribute to the reduction of A1T and A2T (β coefficient= -0.12 and -0.23, $p<0.01$), and increment of HcR and DA (β coefficient= 0.16 and 0.11, $p<0.05$). Gender has a negative effect on the A2L (β coefficient= -0.13, $p<0.05$).

3.3.4 Discussion

The importance of corneal biomechanical properties in tonometry, ocular diagnosis and ocular disease management was well known. The ORA, which is the first in-vivo instrument for the measurement of corneal biomechanical parameters was well evaluated by many studies [15, 19, 59, 60, 69, 74, 84, 272-277]. In recent years, Corvis ST (CST) was introduced as a pachy-tonometer. It is able to perform in-vivo Scheimpflug imaging of the corneal deformation under air-pulse pressure [212, 245, 265]. In the current study, the relationship between CST parameters and ORA was analysed. This study analysed the influence of IOP, CCT, gender, age and laterality on the corneal biomechanical parameters by CST and ORA.

This study showed highly significant association between both CRF and CH, which was demonstrated in many studies since the launch of ORA a decade ago [220]. The parameters were derived from the same infra-red wave analysis but empirically calculated using different mathematical algorithms [69]. The results showed that all corneal biomechanical parameters from ORA except HcT and HpD, were significantly correlated with the parameters from CSTc. Both ORA parameters have significant moderate correlation with A1T. Other CST parameters (A1L, A2T, A2L, A2V and HcR) have weak but significant correlation with CH and CRF. However, DA showed an inverse relationship with CRF. Previous studies highlighted A1T and DA as the most reliable and reproducible parameters for describing corneal biomechanics [211, 245, 269]. These CST parameters may be important to represent the viscoelastic properties of the cornea in-vivo.

Similarly, the CRF was also noted to be more representative than CH on corneal viscoelasticity. A study on spectral analysis of the waveform of both ORA and CST found no statistically significant difference between CH and CRF versus DA [278]. The study did not explore direct correlations between corneal biomechanical parameters from both instruments.

The CH was noted to be affected by age, CCT and IOP. However, the CRF increased with thicker CCT and was not influenced by other demographic variables and parameters. In a British population cohort, the mean CH and CRF declined with age and were higher in women than in men [58, 63, 96, 97]. Similar observations were made by other studies that indicated that the ageing cornea could reduce corneal viscoelasticity (92, 96, 124, 125). Despite a few contradictory findings in laboratory studies using donor cornea, the inverse association between age and corneal

viscoelasticity remains strong. However, the CRF was noted to be less influenced compared to CH . In this study, aging positively influenced A1T, A2T, HcR and DA which indicate higher cornea resistance to dynamic external force (air-pulse pressure of the CST).

Similar positive association between age with A2T and DA was noted in a study on younger subjects undergoing refractive surgery [20, 70]. However, a study on a young to middle-aged healthy Brazilian cohort revealed an increase in HcT with age [70]. The author postulated that the HcT inversely represent the viscoelasticity profile in patients as a result of physiological cross-linkage of the corneal stoma collagen fibrils in ageing eyes. The variability on the association of the CST parameters with age maybe resulted from the heterogeneity of the study cohort as well as the CST software version employed. The present study population has a wide age range with multi-ethnic distribution and was examined with a more updated CST software version.

The effect of IOP on the corneal biomechanical properties by ORA was previously analysed in normal and glaucomatous eyes [15, 17, 163]. The ORA's estimate of corneal-compensated IOP (IOPcc) is a mathematically derived tonometry value and claims to be less affected by corneal biomechanical properties and IOP measurement in comparison to other tonometers [157]. Earlier studies have found that IOPcc was not associated with corneal curvature ,central corneal thickness [72, 73, 143]or axial length [72]. Therefore, IOPcc was chosen to represent the IOP for the analysis on factors affecting corneal biomechanics. The present study showed that CH was negatively affected by IOPcc.

Other studies t noted an inverse correlation between IOP and CH [65, 68, 85-87]. Kamiya et al. found that eyes with thinner CCT as well as higher IOP values are more predisposed to having lower CH [68]. By contrast, there is significant positive contribution of IOPcc on CRF in univariate analysis. CRF increases with rising IOP, indicating that resistance against the deformation of the cornea is higher in eyes with higher IOP values. However, upon inclusion of age, gender, IOPcc and CCT in the multivariate analysis, the significant effect of IOPcc disappeared and was shown to be affected by only CCT. The lack of influence of IOPcc on CRF may be due to the strong inter-correlation between IOPcc and CCT. Galletti et al. confirmed the confounding effects of IOP and CCT on ORA biomechanical parameters and suggested new values termed "transformed CH" (CHcorr) and "transformed CRF" (CRFcorr) [88]. The IOP significantly affected seven out of ten CST parameters in the presentstudy. Higher IOP caused significant increment of A1T, A1L, A2V and HcR. The effect of these CST

parameters indicates a 'soft' cornea and showed reduction of the corneal biomechanical properties with increase IOP.

Previous studies have reported that a thinner CCT is an independent risk factor for open-angle glaucoma in patients with ocular hypertension (OHT) [75-79]. A study by Shah et al. [80] found significant and positive relationships between ORA parameters, CH and CCT, CRF and CCT and CRF and CH in normal eyes. In both glaucoma and normal subjects, CCT is positively correlated with CH [81-83]. As the cornea contains more collagen fibres and ground substances, resistance against deformation and damping capacity rises. Moreover, the stronger the corneal tension, the faster the cornea regains its original position following deformation. In addition, IOP represents an additional force that restores the cornea to its original position [84] Central corneal thickness was a significant predictor of A2L, A2V, and also HcR.

3.4 Conclusion

The inter-tonometry method agreement study in this chapter revealed all tonometers have good agreement with GAT. The tonometers are valid alternatives for GAT tonometry amongst healthy subjects with normal IOP values but are not interchangeable with GAT. Clinicians should be aware of the proportionate bias by CST when performing tonometry on subjects with high IOP (more than 22mmHg). Age affected all inter-tonometry mean differences except for CST. The CRF, A1T, A2T, A2V and DA are important biomechanical parameters that influence the inter-tonometry bias. The next chapter looks into the inter-tonometry agreement and corneal biomechanical properties amongst glaucoma subjects.

CHAPTER 4: TONOMETRY AGREEMENT AND CORNEAL BIOMECHANICAL PROPERTIES IN EYES WITH GLAUCOMA AND OCULAR HYPERTENSION

This chapter presents data collected from a cohort of glaucomatous subjects attending the glaucoma clinic at Birmingham and Midland Eye Centre. Details on subject selection, instrumentation and methods of analysis employed in this study are described in Chapter 2. The demographics of study subjects and all study variables are first presented. This chapter is then divided into two main sections (4.2 and 4.3); the first reports the agreement of IOP values of Tonopen, Icare, ORA and CST against GAT in glaucomatous eyes; and the second looks at the relationship of corneal biomechanical parameters of the ocular hypertensive and glaucomatous eyes by the ORA and CST.

4.1 Demographic

A total of 264 eyes were examined from 170 volunteers from a glaucoma clinic. The mean age of all subjects was 66.5 ± 1.2 years (median; 69 years, max; 85 years, min; 24 years). The subjects were 143 male and 121 female. Overall, the study subjects are mainly from the Caucasian background (n= 156, 59.1 %) (Figure 4.1).

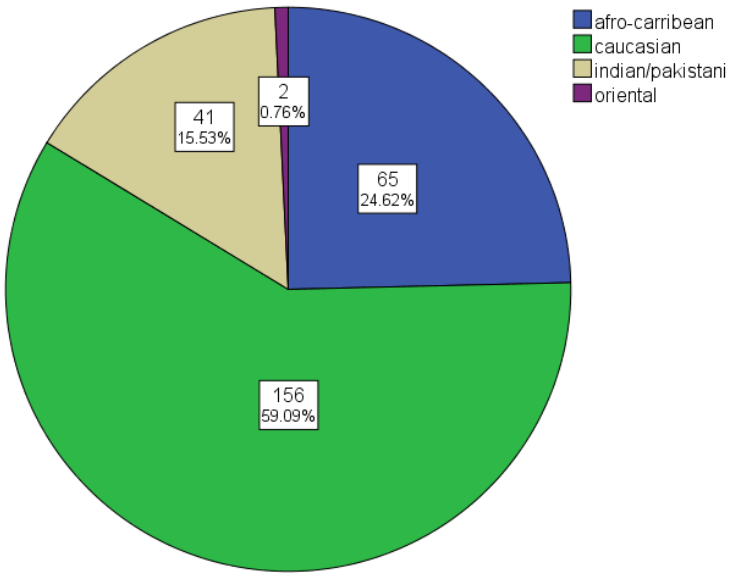


Figure 4.1 The ethnic distribution of glaucomatous subjects

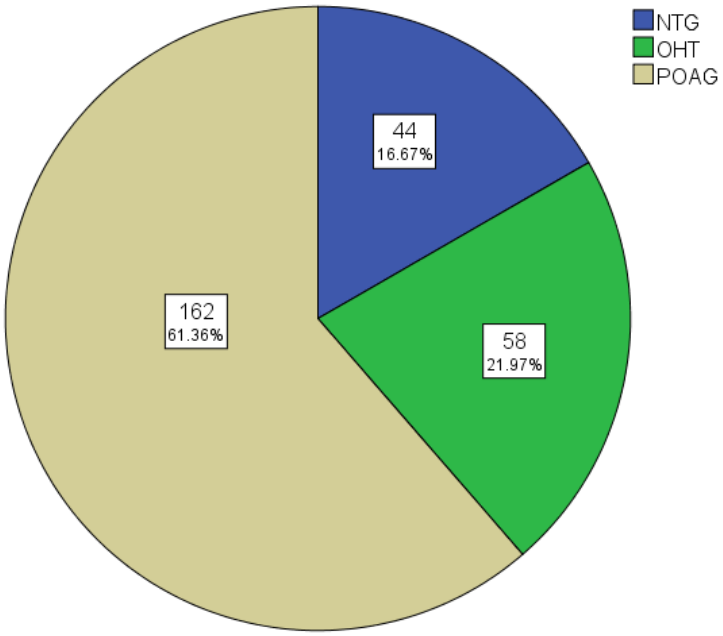


Figure 4.2 Distribution of subjects by glaucoma diagnosis

The IOP values of the glaucomatous eyes analysed in this section are listed in Table 4.1.The IOPcc is the highest amongst the IOP value and the Icare was the lowest. All tonometers measured highest IOP value in OHT eyes, followed by POAG and NTG (One-way ANOVA with post-hoc Bonferroni analysis, $p<0.001$).

Table 4.1 Mean IOP values, corneal biomechanical parameters and corneal thickness of glaucomatous eyes (all mean values are in mmHg)

	All n=264	OHT n=58	NTG n=44	POAG n=162	$p^{\dagger}$
GAT	16.22 ± 4.56	20.26 ± 4.48	12.30 ± 2.98	14.67 ± 3.09	<0.001
Tonopen	16.18 ± 3.74	19.26 ± 4.29	12.91± 3.08	15.09 ± 3.00	<0.001
Icare	15.71± 5.05	19.87 ± 5.18	11.45 ± 3.41	14.02 ± 3.88	<0.001
IOPg	17.64 ± 5.64	21.92 ± 5.71	13.68 ± 4.49	15.61± 4.37	<0.001
IOPcc	18.23 ± 5.13	20.73 ± 5.22	15.06 ± 4.04	17.25 ± 4.79	<0.001
CST	16.26 ± 3.37	18.99 ± 3.68	13.07 ± 2.05	15.09 ± 2.93	<0.001

In table 4.2, mean central corneal thickness and corneal biomechanical parameters distribution for the study population are presented. The CCT, CH, CRF and all of the CST corneal parameters (except A1L and HcT) showed significant differences amongst the different glaucoma diagnoses ($p<0.01$).

Table 4.2 Mean IOP values, corneal biomechanical parameters and corneal thickness of glaucomatous eyes

	OHT n=58 (mean±SD)	NTG n=44 (mean±SD)	POAG n=162 (mean±SD)	p value
CH	11.01 ± 2.89	8.96 ± 1.82	8.20 ± 1.73	<0.001
CRF	11.80 ± 3.01	8.26 ± 1.25	8.65 ± 1.79	<0.001
A1T	8.22 ± 0.51	7.52 ± 0.31	7.77 ± 0.33	<0.001
A1L	1.73 ± 0.15	1.76 ± 0.26	1.76 ± 0.19	0.74
A1V	0.13 ± 0.03	0.15 ± 0.03	0.14 ± 0.03	<0.001
A2T	21.93 ± 0.36	22.52 ± 0.73	22.21 ± 0.48	<0.001
A2L	1.82 ± 0.30	1.55 ± 0.30	1.76 ± 0.35	<0.001
A2V	-0.32 ± 0.07	-0.40 ± 0.10	-0.37 ± 0.08	<0.001
HcT	16.40 ± 0.39	16.64 ± 0.73	16.48 ± 0.51	0.16
HpD	3.16 ± 1.10	3.79 ± 1.21	3.75 ± 1.21	0.02
HcR	7.77 ± 1.07	3.70 ± 1.33	6.89 ± 0.69	<0.001
DA	1.03 ± 0.09	1.49 ± 1.34	1.14 ± 0.12	0.01
CCTus	562.37 ± 40.19	513.93 ± 27.29	521.65 ± 36.33	<0.001

*One-way ANOVA with Bonferroni post-hoc

4.2 Agreement between tonometers in glaucomatous eyes

This section explores the demographic and physical factors that may influence the agreement between the various tonometers and GAT amongst eyes with POAG, NTG and OHT. The details of these instruments were explained in chapter 2 (section 2.2).

The inter-tonometry agreement of all tonometers against GAT is listed according to glaucoma diagnosis in table 4.3, 4.4 and 4.5. All tonometer's mean biases are within the acceptable ± 2.0 mmHg range across all the different glaucoma diagnosis except for IOPcc by ORA. In POAG subjects, the mean IOPcc biases against GAT are slightly higher than the recommended range (2.53 mmHg).

The IOPcc and IOPg (ORA tonometry values) recorded low percentage of acceptable bias (50% and less) compared to other tonometers. Across the different diagnosis, the OHT eyes showed lowest percentage of bias within ±2.0 mmHg compared to other NTG and POAG subjects (except for inter-tonometry comparison of CST and GAT). Further, OHT subjects have higher standard deviation of mean difference in all paired tonometry, with an average value of more than 3.5 mmHg.

Table 4.3 Mean difference, expected range of agreement and paired correlations between GAT and all tonometers amongst the OHT subjects

	Mean Difference ± SD (mmHg)	LOA (95%CI)	ICC(95%CI)	% within 2.0 mmHg
Icare_GAT	-0.23 ± 3.68	-7.44, 6.98	0.83 (0.72,0.93)	57.7
Tonopen _GAT	-0.81 ± 3.44	-7.55, 5.93	0.82 (0.68, 0.89)	57.8
IOPcc_GAT	0.28± 4.35	-8.25, 8.76	0.68 (0.42, 0.82)	35.4
IOPg_GAT	1.27 ± 3.92	-6.41,8.83	0.76 (0.58, 0.87)	47.9
CST_GAT	-1.21 ± 3.65	-8.36, 5.94	0.73 (0.53,0.85)	53.8

Table 4.4 Mean difference, expected range of agreement and paired correlations between GAT and all tonometers amongst the NTG subjects

	Mean Difference ± SD (mmHg)	LOA (95%CI)	ICC(95%CI)	% within 2.0 mmHg
Icare_GAT	-0.85 ± 2.03	-4.84, 3.14	0.86 (0.70, 0.90)	79.5
Tonopen _GAT	0.59± 1.96	-3.25,4.43	0.87 (0.76, 0.93)	88.7
IOPcc_GAT	2.07 ± 2.84	-3.5, 7.64	0.75 (0.30, 0.89)	50
IOPg_GAT	0.65 ± 2.43	-4.11,5.41	0.87 (0.74, 0.94)	46.9
CST_GAT	1.10 ± 1.73	-1.70, 3.90	0.83 (0.54, 0.92)	63.8

Table 4.5 Mean difference, expected range of agreement and paired correlation between GAT and all tonometer amongst the POAG subjects

	Mean Difference ± SD (mmHg)	LOA (95%CI)	ICC(95%CI)	% within 2.0 mmHg
Icare_GAT	0.68 ± 2.52	-4.22,5.58	0.78 (0.69, 0.85)	64.6
Tonopen _GAT	0.34 ± 2.03	-3.65, 4.33	0.82 (0.75, 0.87)	70.2
IOPcc_GAT	2.53± 3.49	-4.31, 9.37	0.57 (0.15, 0.76)	39.6
IOPg_GAT	0.94 ± 3.14	-5.21, 7.09	0.69 (0.53, 0.78)	54.2
CST_GAT	0.40 ± 2.00	-3.52, 4.32	0.78 (0.69, 0.84)	74.3

The mean difference (bias) in each comparison is plotted against the average of tonometry values and presented as Bland-Altman plots in section 4.2.1 for further evaluation of the inter-tonometry agreement.

4.2.1 Inter-tonometry agreement

Figures 4.3-4.7a-c are the BA plots of inter-tonometry comparison, in the different subject groups (OHT, NTG and POAG).

4.2.1.1 Agreement between Icare and GAT

From Figure 4.3(a)-(b), the average of IOP Icare_GAT in the NTG and OHT groups are evenly distributed along the line of the mean difference of the paired tonometers. The biases are almost all within the estimated limit of agreement. The wide limit of agreement in the OHT group was mentioned earlier in section 4.2. However, the distribution of the average IOP value in the POAG group plot, was noted to show an upward / positive pattern (Figure 4.3c).

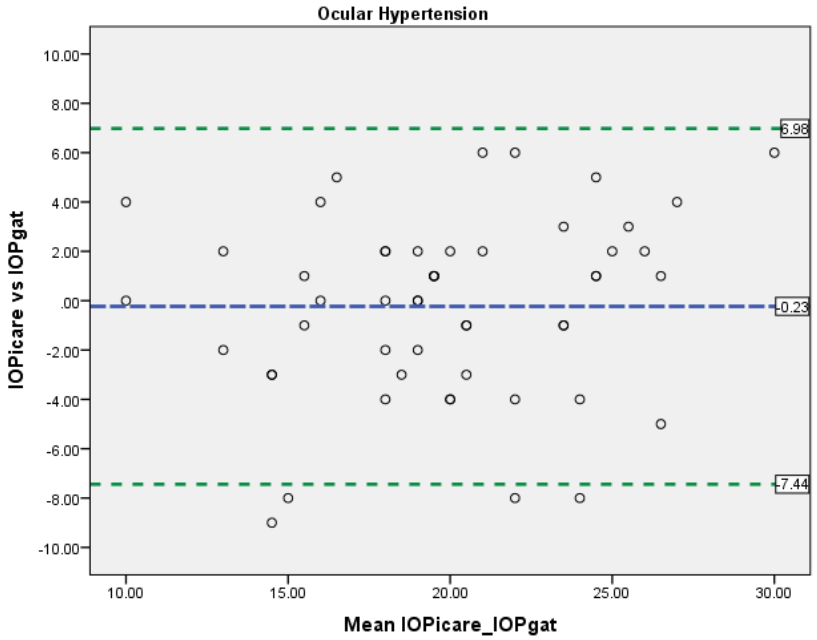


Figure 4.3(a) Bland-Altman plot of IOP by Icare vs GAT in OHT

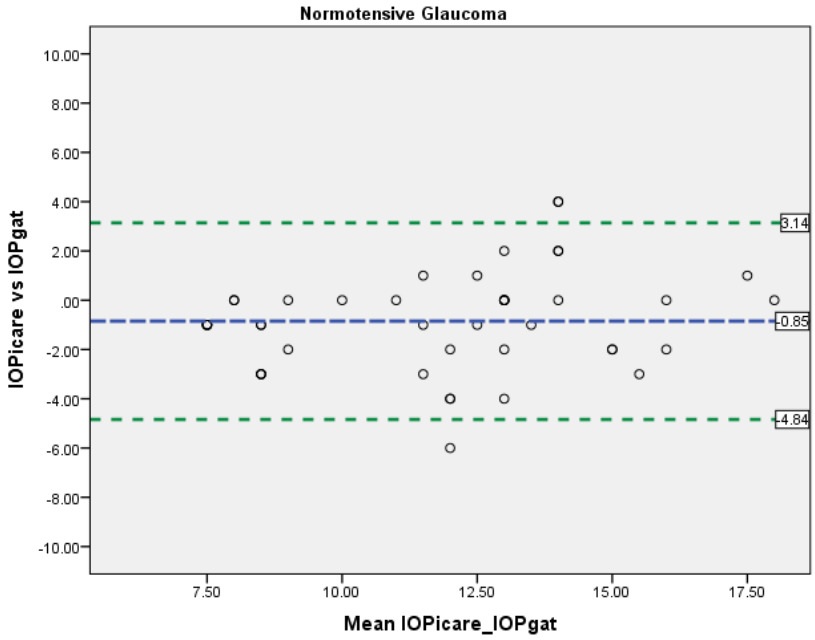


Figure 4.3(b) Bland-Altman plot of IOP by Icare vs GAT in NTG

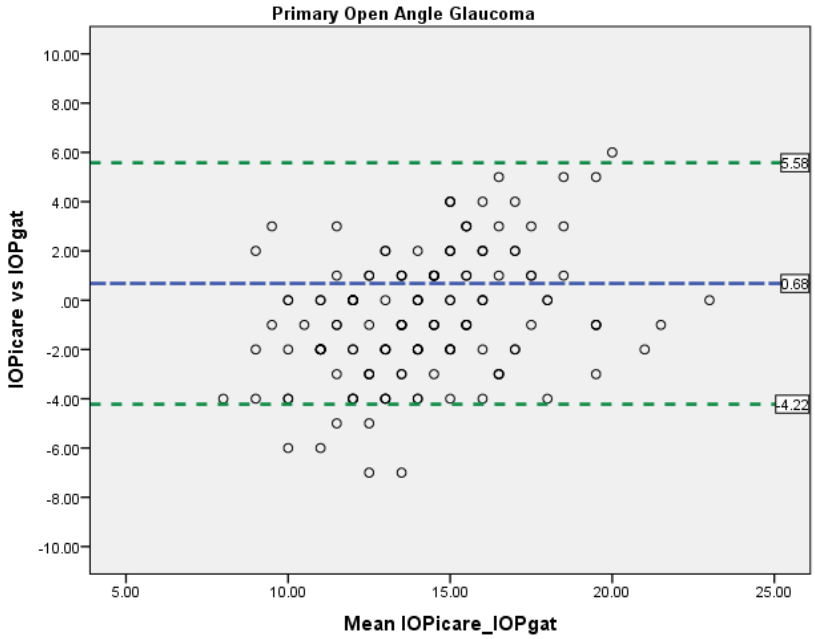


Figure 4.3(c) Bland-Altman plot of IOP by Icare vs GAT in POAG

4.2.1.2 Agreement between Tonopen and GAT

According to Figure 4.4(a)-(c), the average value of Tonopen-GAT in all the glaucoma subgroups, are scattered in a well distributed pattern along the line of mean IOP difference of the paired tonometers. The biases are also mostly within the line of the

estimated limit of agreement. This indicates that the bias is fairly constant across the range of all average IOP value plotted. The LOA in the OHT subjects is apparently wider than other group, as mentioned before.

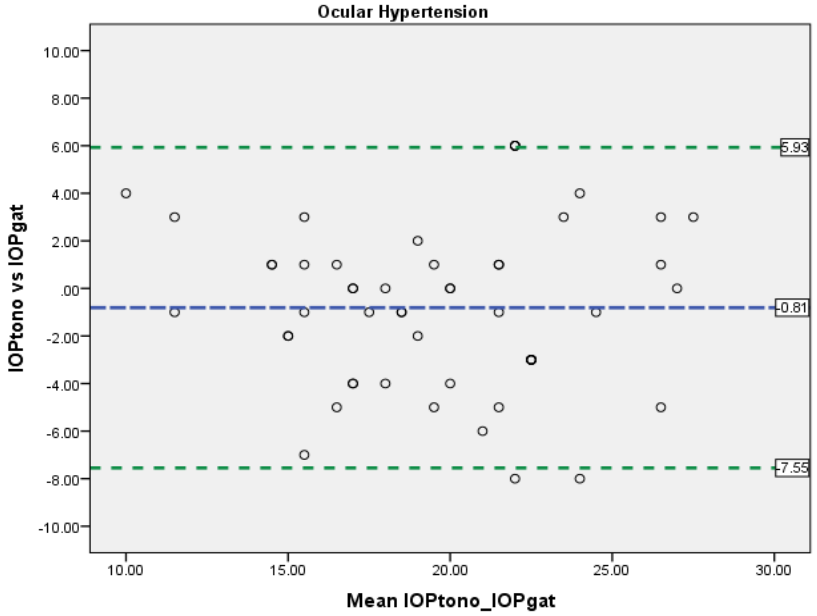


Figure 4.4(a) Bland-Altman plot of IOP by Tonopen vs GAT in OHT

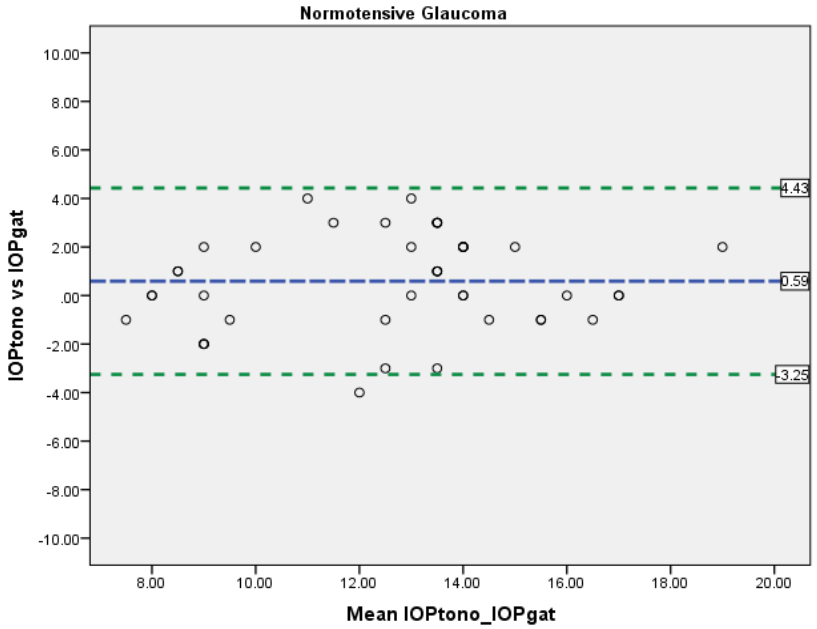


Figure 4.4(b) Bland-Altman plot of IOP by Tonopen vs GAT in NTG

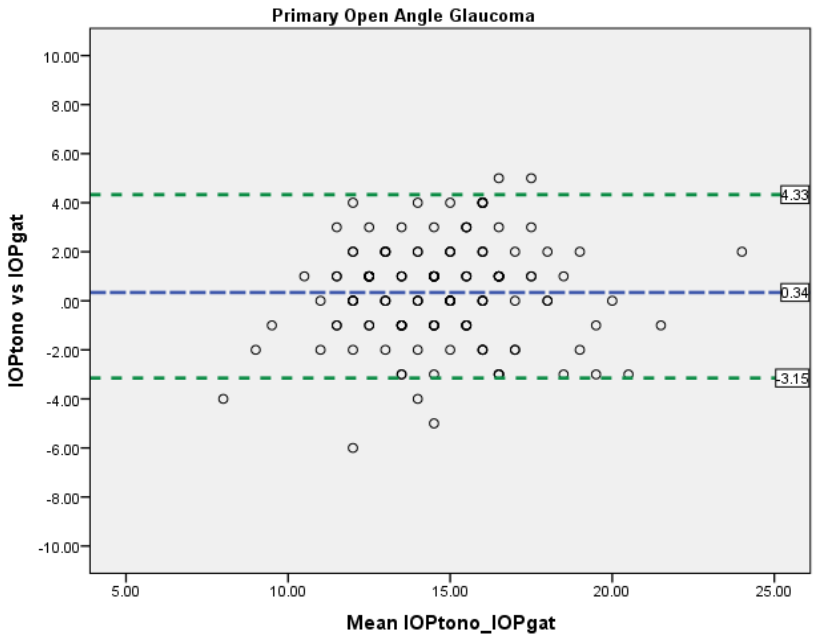


Figure 4.4(c) Bland-Altman plot of IOP by Tonopen vs GAT in POAG

4.2.1.3 Agreement between IOPg and GAT

In Figure 4.5(a)-(c), the mean difference of IOPg and GAT value along the line of the mean of bias are in non-uniform distribution. This may indicate that bias changes with average IOP value. Further analysis of this relationship is presented in section 4.2.2.

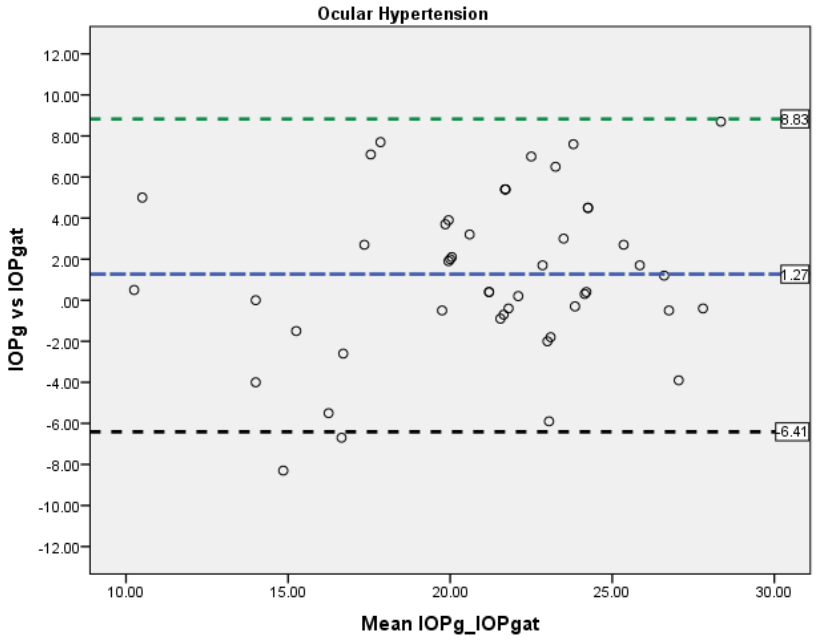


Figure 4.6(a) Bland-Altman plot of IOP by IOPg vs GAT in OHT

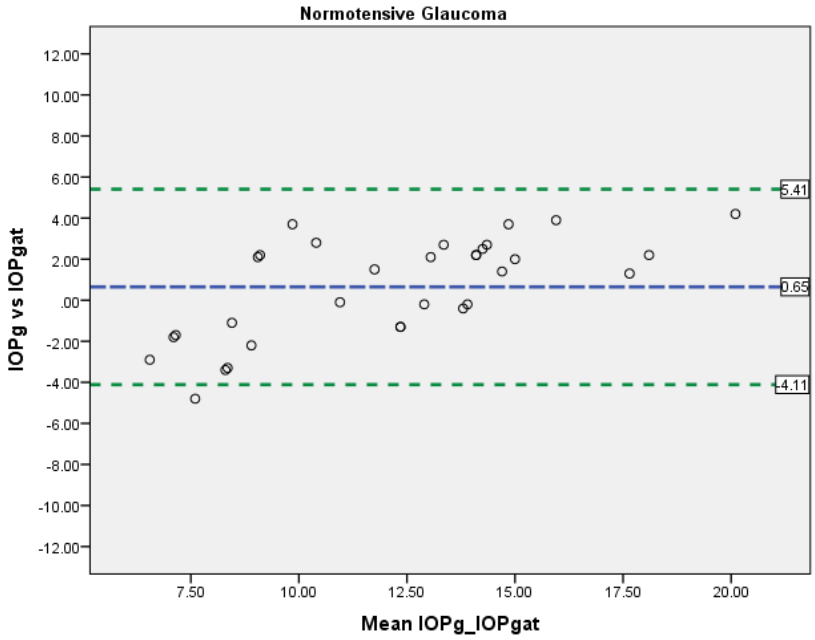


Figure 4.5(b) Bland-Altman plot of IOP by IOPg vs GAT in NTG

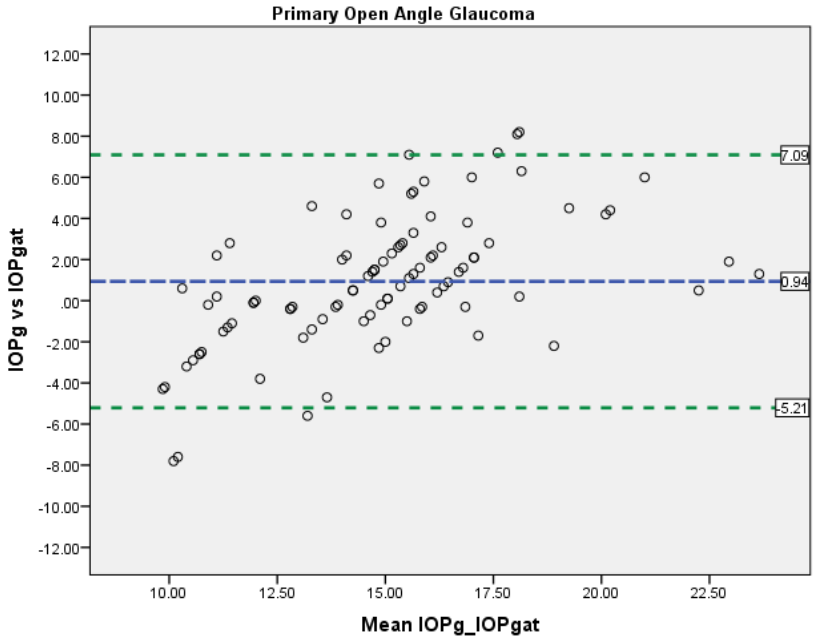


Figure 4.5(c) Bland-Altman plot of IOP by IOPg vs GAT in POAG

4.2.1.4 Agreement between IOPcc and GAT

The BA plots in Figure 4.6(b)-(c) of the inter-tonometry bias between IOPcc and GAT are scattered in a positive pattern against the average IOP value for NTG and POAG subjects.The BA plot in Figure 4.6(b) revealed, again, that the LOA of the inter-tonometry bias in the OHT group is the widest. Further analysis of the relationship between bias and average IOP value of IOPcc and GAT is presented in section 4.2.2.

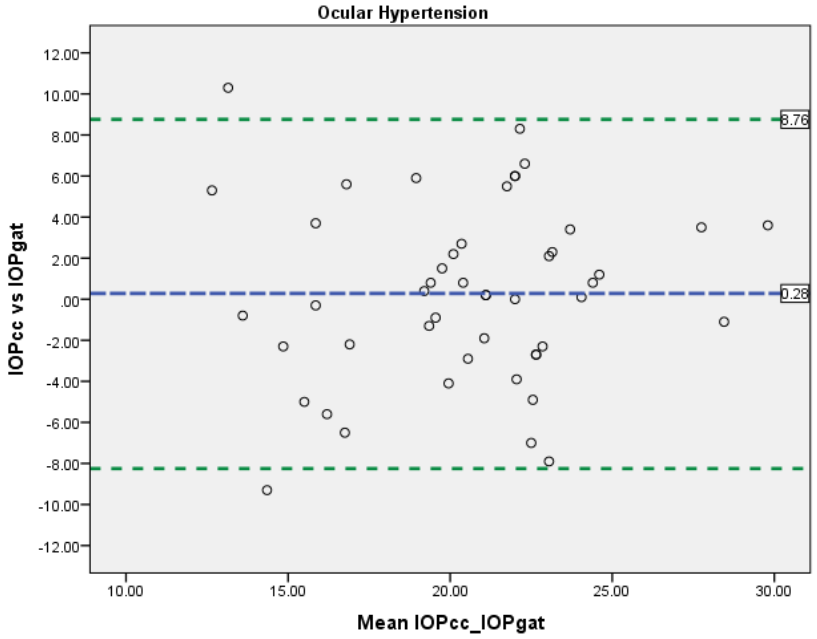


Figure 4.6(a) Bland-Altman plots of IOP by IOPcc against GAT in OHT

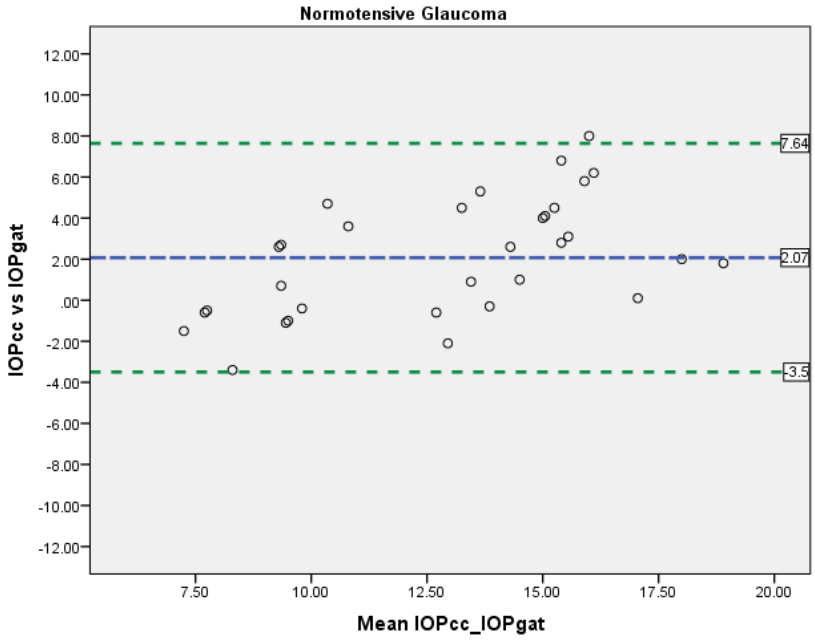


Figure 4.6(b) Bland-Altman plots of IOP by IOPcc against GAT in NTG

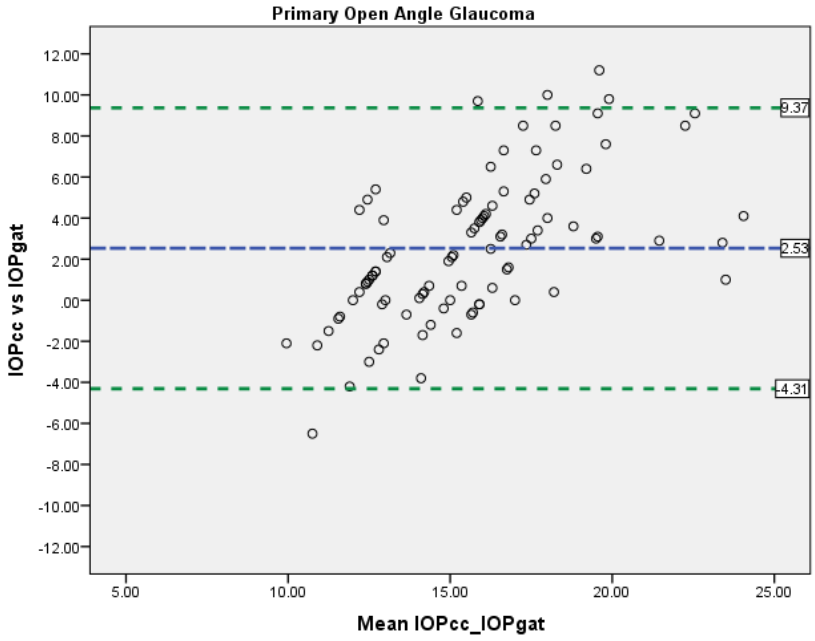


Figure 4.6(c) Bland-Altman plots of IOP by IOPcc against GAT in POAG

4.2.1.5 Agreement between CST and GAT

The BA plots for the CST versus GAT are presented in Figure 4.7(a)-(c). In Figure 4.7(b), the OHT group showed almost a well-distributed bias along the mean bias value across the different average IOP range. However, for NTG and POAG groups, the plots showed a negative pattern of distribution of the inter-tonometry bias against the average IOP value of CST and GAT(Figure 4.7(b) and 4.7(c),respectively). These relationships are further analysed in item 4.2.2.

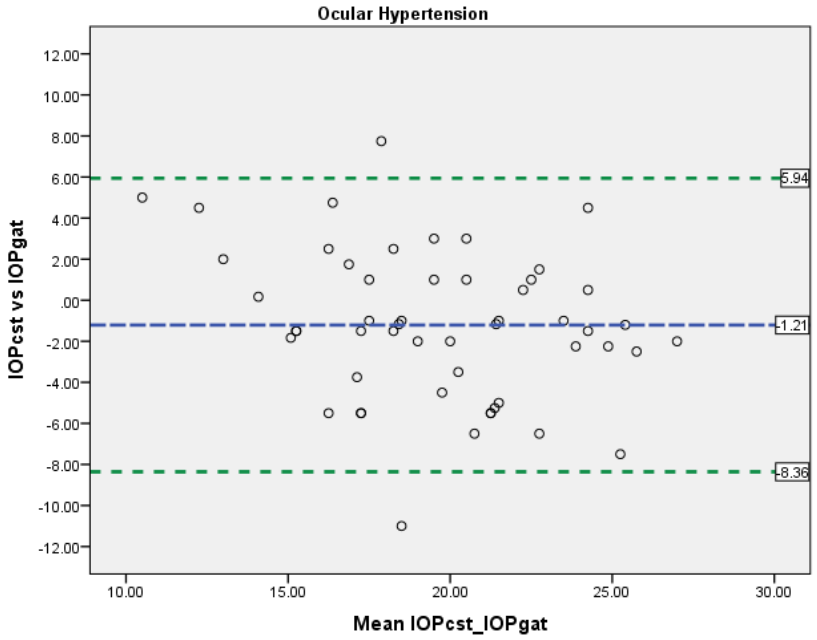


Figure 4.7(a) Bland-Altman plots of IOP by IOPcc against GAT in OHT

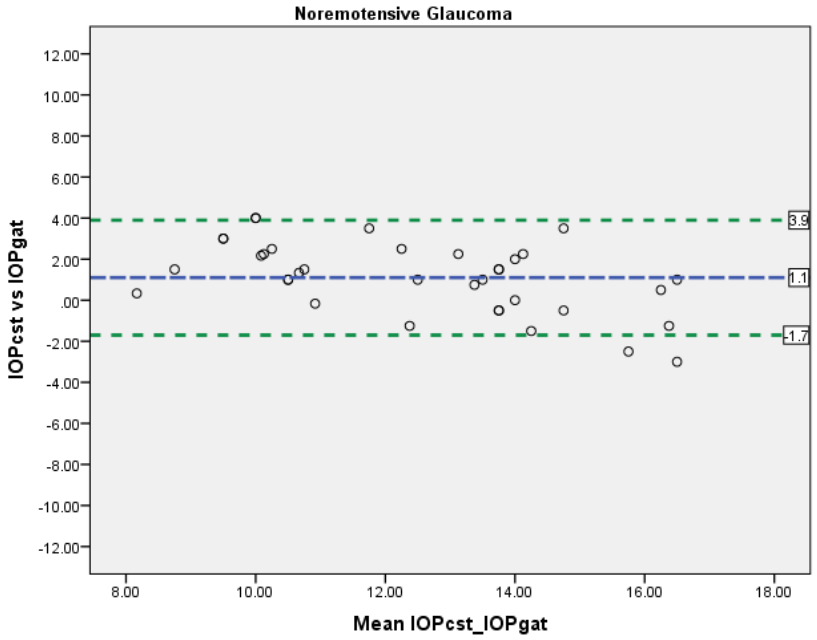


Figure 4.7(b) Bland-Altman plots of IOP by IOPcc against GAT in NTG

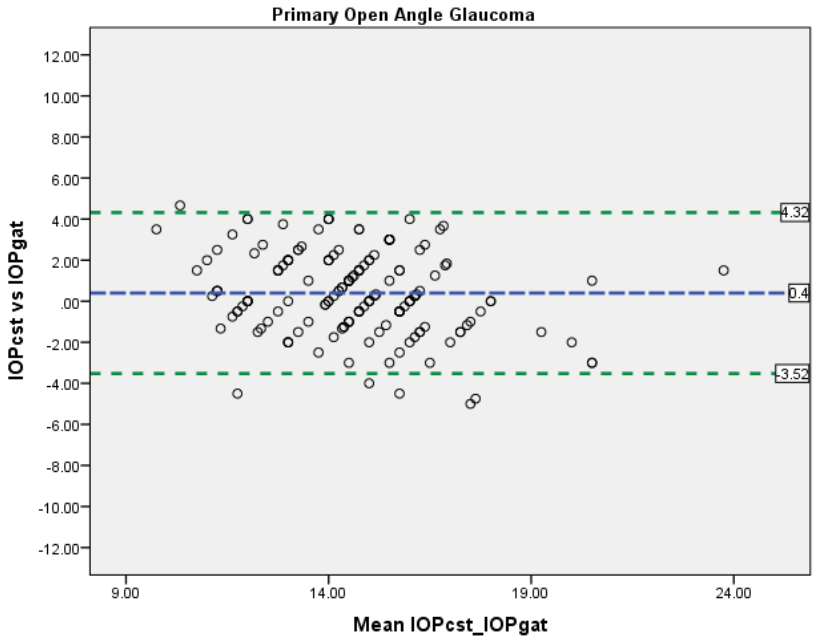


Figure 4.7(c) Bland-Altman plots of IOP by IOPcc against GAT in POAG

4.2.2 Proportionate bias of the inter-tonometry agreement

In the majority of the BA plots in section 4.2.1, inconsistent bias values are noted with changing average IOP value between the paired tonometers. Tables 4.6, 4.7 and 4.8 listed the regression analysis performed on the average IOP value against the bias for each tonometer pair, according to the glaucoma diagnosis. Linear regression models of each inter-tonometry bias at different average IOP values of the paired tonometers are presented in Table 4.9.

Table 4.6 The effect of mean IOP on the mean difference (bias) between tonometers in OHT subjects: a linear regression analysis

	Adjusted R^2	B (Bca 95%CI)	Standard Error B	β	p
Icare_GAT	0.01	0.14 (-0.13, 0.39)	0.14	0.16	0.25
Tonopen _GAT	-0.02	-0.02 (-0.23, 0.24)	0.12	-0.02	0.88
IOPcc_GAT	-0.02	0.09 (-0.32, 0.48)	0.17	0.08	0.61
IOPg_GAT	0.02	0.19 (-0.01, 0.51)	0.16	0.21	0.16
CST_GAT	0.06	-0.22 (-0.49, -0.02)	0.12	-0.27	0.05*

Table 4.7 The effect of mean IOP on the mean difference (bias) between tonometers in NTG subjects: a linear regression analysis

	Adjusted R^2	B (Bca 95%CI)	Standard Error B	β	p
Icare_GAT	0.02	0.15 (-0.13, 0.34)	0.08	0.21	0.17
Tonopen _GAT	0.003	0.10 (-0.06, 0.23)	0.08	0.14	0.37
IOPcc_GAT	0.26	0.46 (0.22, 0.74)	0.13	0.53	<0.001
IOPg_GAT	0.44	0.47 (0.29, 0.65)	0.09	0.67	<0.001
CST_GAT	0.26	-0.39 (-0.61, -0.16)	0.12	-0.53	<0.001

Table 4.8 The effect of mean IOP on the mean difference (bias) between tonometers in POAG subjects: a linear regression analysis

	Adjusted R^2	B (Bca 95%CI)	Standard Error B	β	p
Icare_GAT	0.12	0.32 (0.18, 0.49)	0.08	0.35	<0.001
Tonopen _GAT	0.002	0.06 (-0.01, 0.22)	0.08	0.08	0.37
IOPcc_GAT	0.36	0.70 (0.49, 0.98)	0.12	0.61	<0.001
IOPg_GAT	0.31	0.61 (0.40, 0.87)	0.12	0.56	<0.001
CST_GAT	0.06	-0.24 (-0.40, -0.08)	0.08	-0.25	<0.001

Table 4.9 Regression model of measurement biases as a function of average measurement of paired tonometer

	OHT	NTG	POAG
Icare vs GAT	-	-	$y = 0.32x - 5.17$
Tonopen vs GAT	-	-	-
IOPcc vs GAT	-	$y = 0.46x - 3.84$	$y = 0.7x - 8.45$
IOPg vs GAT	-	$y = 0.47x - 5.06$	$y = 0.61x - 8.1$
CST vs GAT	$y = -0.27x + 4.11$	$y = -0.39x + 5.97$	$y = -0.24x + 3.84$

y = tonometry bias (mmHg), x = average IOP of paired tonometer (mmHg)

There is no proportionate bias between Tonopen and GAT in any glaucoma subgroup. The bias between Icare and GAT is only statistically significant for positively proportionate changes in the POAG subgroup (p<0.00). Positive contribution of the average IOP value between ORA (IOPcc, IOPg) and GAT on the inter-tonometer bias, is also statistically significant in both the NTG and POAG groups. However, for CST and GAT inter-tonometry bias, a contrasting effect was noted. A statistically significant negative contribution of the average IOP value on the measurement bias is noted in all glaucoma subgroups.

The inter-tonometry biases of the paired tonometers are further investigated in the next section (4.2.3) to understand the influence of demographic and ocular factors (IOP, CCT and corneal biomechanical parameters) that may affect the variance.

4.2.3 Inter-tonometry bias

In this section, the influence of the demographic variables, age, CCT, IOPcc and biomechanical factors on the inter-tonometry biases are analysed. The biases between gender and ethnic origins are analysed with paired t-test (for gender) and ANOVA with Bonferroni correction (for ethnicity). Paired t-test revealed no statistically significant influence on the inter-tonometry bias between female and male subjects amongst all glaucoma groups ($p>0.05$). Similarly, there was no significant association of ethnicity with inter-tonometry bias across all glaucoma diagnoses ($p>0.05$).

4.2.3.1 Factors affecting the inter-tonometry measurement bias

Multivariate regression analysis is employed to construct a predictive model of the inter-method bias. The demographic variables (gender and age) and biomechanical parameters (CRF, A1T, A2T, A2L, A2V, HcR and DA) were included in the analysis. In order to reduce bias of analysis, each linear regression is analysed by robust regression method as described item 2.4.4.1. All B coefficient values are accompanied by the bias corrected accelerated 95% confidence interval (BCa 95%CI) values. The multivariate model of each inter-tonometry bias was corrected for the effect of IOP, CRF and CCT.

Analysis of correlation between corneal biomechanical parameters showed that only CH was strongly correlated with CRF. The Pearson's r value are 0.74 in OHT, 0.79 in NTG and 0.72 in POAG subjects ($p<0.01$). As multicolinearity of variables can violate the assumptions for a valid regression model, the CH was excluded due to its high correlation with CRF. Ethnicity is not included in the multivariate analysis due to inadequate sample size in every cell. Simmons et al. suggested that for categorical variable, each cell has at least 20 samples [240]. Other than Caucasian, other ethnic background has less than 20 samples in each glaucoma cohort. Thus, the ethnicity is not included in the multivariate analysis. The ocular laterality effect on all the inter-method bias revealed no significant influence and does not affect the final outcome in all multivariate model, with $p>0.05$.

The effect size of these variables were calculated and presented as:

1. Adjusted R^2 value represented the variance of the inter-method bias affected by the variables. The F value reported the number of significant predictors and residual predictors. The value of significance is set at $p<0.05$.

2. B (the unstandardized coefficient) for each predictor variable shows the predicted increase in the value of the criterion (inter-method measurement bias) for one unit increase in that predictor.

3. Beta (β) (the standardised coefficient) gives a measure of the contribution of the variable to the model in terms of standard deviation.

4.2.3.1.1 Inter-tonometry bias between Tonopen and GAT

A multivariate regression analysis in OHT subjects established no significant contribution of all the variables studied on the predicted bias between Tonopen and GAT. The regression analysis yields adjusted R^2 of 0.04 and p value of 0.25. Similar finding is noted in POAG subjects where there is no statistically significant model for the contribution of the variables tested on the inter-tonometry bias (R^2= 0.04, Adjusted R^2 =0.27, p=0.32). Table 4.10 listed the result of multivariate regression analysis for NTG.

Table 4.10 Factors affecting the inter-tonometry bias between Tonopen and GAT in NTG subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	P
IOPcc	0.50 (0.19, 0.82)	0.06	1.08	0.00
A1T	-4.82 (-3.75, -6.01)	0.70	-0.85	0.00
A1V	-29.13 (-21.05,-37.34)	7.61	-0.40	0.00
DA	0.51 (0.19, 0.69)	0.17	0.32	0.01

Note: R^2= 0.76, Adjusted R^2= 0.72 (p=0.00)

4.2.3.1.2 Inter-tonometry bias between Icare and GAT

Table 4.11-4.13 presented the linear regression model of predictors of inter-tonometry bias in each glaucoma subgroups. The A1V has the strongest effect of the bias followed by HcR and age.

Table 4.11 Factors affecting the inter-tonometry bias between Icare and GAT in OHT subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	P
A1V	-61.08 (-92.42,-29.75)	15.47	-0.51	0.00
HcR	1.02 (0.09,1.95)	0.46	0.28	0.03
Age	-0.07 (-0.14,-0.01)	0.03	-0.27	0.03

Note: R^2= 0.52 , Adjusted R^2= 0.44 (p=0.00)

Table 4.12 Factors affecting the inter-tonometry bias between Icare and GAT in NTG subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	P
IOPcc	0.26 (0.09, 0.44)	0.09	0.55	0.01
Age	-0.11(-0.17,-0.05)	0.03	-0.59	0.00
A2L	2.08 (0.31, 3.85)	0.86	0.31	0.02
A1T	-1.99 (-3.95,-0.03)	0.95	-0.34	0.05

Note: R^2= 0.65 , Adjusted R^2= 0.56 (p=0.00)

Table 4.13 Factors affecting the inter-tonometry bias between Icare and GAT in POAG subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	P
IOPcc	0.18 (0.06,0.29)	0.06	0.31	0.00
A2L	1.58 (0.13,3.03)	0.73	0.24	0.03

Note: R^2= 0.16 , Adjusted R^2= 0.12 (p=0.01)

4.2.3.1.3 Inter-tonometry bias between ORA (IOPcc) and GAT

A multivariate regression analysis has established linear model of factors that influence inter-tonometry bias in OHT, NTG and POAG subjects. All linear models are presented in Table 4.14, 4.15 and 4.16.

Table 4.14 Factors affecting the inter-tonometry bias between ORA (IOPcc) and GAT in OHT subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	p*
IOPcc	0.65 (0.47,0.82,	0.04	0.70	0.00
CCTus	-0.01(-0.06,0.04)	0.004	-0.09	0.04

Note: R^2= 0.45 , Adjusted R^2= 0.42 (p=0.00)

Table 4.15 Factors affecting the inter-tonometry bias between ORA (IOPcc) and GAT in NTG subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	p
IOPcc	0.39 (0.21, 0.58)	0.09	0.59	0.00
Age	-0.11 (-0.18,-0.03)	0.04	-0.41	0.01

Note: R^2= 0.68 , Adjusted R^2= 0.63 (p=0.00)

Table 4.16 Factors affecting the inter-tonometry bias between ORA (IOPcc) and GAT in POAG subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	P
IOPcc	0.85 (0.75, 0.95)	0.05	1.08	0.00
CRF	-0.31(-0.50, -0.13)	0.09	-0.18	0.00
CCT	-0.01 (-0.2, -0.01)	0.00	-0.17	0.00
A2T	1.30 (0.48,2.12)	0.41	0.19	0.00
HcR	0.95 (0.48,1.41)	0.23	0.20	0.00
A1T	-1.64 (-3.01, -0.27)	0.69	-0.14	0.02
A1L	-3.27 (-5.03,-1.51)	0.88	-3.71	0.00
A1V	21.97 (7.11, 36.84)	7.46	0.17	0.00

Note: R^2= 0.86 , Adjusted R^2= 0.84 (p=0.004)

4.2.3.1.4 Inter-tonometry bias between ORA (IOPg) and GAT

A multivariate regression analysis has established linear model of factors that influence inter-tonometry bias in OHT, NTG and POAG subjects. All linear models are presented in Table 4.17.

Table 4.17 Factors affecting the inter-tonometry bias between ORA (IOPg) and GAT in glaucoma subjects

Group	Significant Factor	B Coefficient (Bca 95% CI)	Standard Error B	β	p
OHT[1]	IOPcc	0.38 (0.12,0.62)	0.05	0.46	0.00
NTG[2]	IOPcc	0.33 (0.17, 0.49)	0.08	0.57	0.00
	A1L	2.67 (0.10, 5.23)	1.25	0.28	0.04
POAG[3]	IOPcc	0.36 (0.23, 0.50)	0.07	0.51	0.00

[1] R^2= 0.21 , Adjusted R^2= 0.15 (p=0.02)
[2] R^2= 0.63 , Adjusted R^2= 0.57 (p=0.00)
[3] R^2= 0.27, Adjusted R^2= 0.24 (p=0.00)

4.2.3.1.5 Inter-tonometry bias between CST and GAT

A multivariate regression analysis has established linear model of factors that influence inter-tonometry bias in NTG subjects. The linear models are presented in Table 4.18. There is no statistically significant model for the contribution of the variables tested on the inter-tonometry bias in OHT subjects (R^2= 0.17, Adjusted R^2 =0.09, p=0.11) and POAG subjects (R^2= 0.01, Adjusted R^2= -0.03, p=0.90).

Table 4.18 Factors affecting the inter-tonometry bias between CST and GAT in NTG subjects: a multivariate regression analysis

	B Coefficient (Bca 95% CI)	Standard Error B	β	p
IOPcc	-0.29 (-0.40, -0.16)	0.06	-0.68	0.00
Age	-0.13 (-0.18, -0.08)	0.02	-0.81	0.00

Note: R^2= 0.61, Adjusted R^2= 0.55 (p=0.00)

4.2.4 Discussion

In this chapter the inter-tonometry agreement analysis and corneal biomechanical characteristics of subjects from a glaucoma outpatient clinic are presented. This cohort has subjects that are diagnosed to have underlying Primary Open Angle Glaucoma (POAG), Ocular Hypertension (OHT) and Normal Tension Glaucoma (NTG). The ethnic distribution of the glaucoma subjects in this cohort is in accordance to the Birmingham's ethnic proportion[241]. The OHT subjects are noted to have higher mean

tonometry value than the NTG and POAG. The inter-tonometry difference and agreement of the tonometry pair amongst the different glaucoma diagnoses are discussed further in the item below.

This is the first study that extensively analyses the inter-tonometry agreement between four tonometers (Icare, Tonopen, ORA and CST) against GAT, amongst different glaucoma diagnoses. The inter-tonometry bias of each tonometry pair is further explored by analysing the influence of demographic as well as CCT, IOP and corneal biomechanical parameters of ORA and CST. A similar study was conducted by Sullivan-Mee et al. on glaucoma subjects with the Pascal Dynamic Tonometer, ORA and GAT [157]. In the study, the CH and CRF were included in the multivariate regression analysis. The authors noted a persistent influence of CCT, CH and CRF in the almost all pair of tonometry comparison. However, in this study, the strong correlation between CH and CRF is considered to violate the assumption for a valid regression analysis. Thus, CH was excluded and CRF was chosen for to represent corneal biomechanical parameters by ORA. The multivariate regression analysis of the inter-method bias was corrected against the confounding effect of CCT, IOPcc and CRF, using hierarchical regression method.

4.2.4.1 Icare vs GAT

In glaucomatous eyes, previous tonometry agreement studies were done on pooled subjects from glaucoma clinic [279-282]. Only two studies were found to report the specific finding in POAG and NTG. Brusini et al. had studied the inter-tonometry agreement of Icare and GAT in POAG subjects [191]. Whilst in NTG, a study looked into the inter-tonometry bias amongst NTG [253]. To date, no specific study was found in the literature on tonometry agreement of Icare and GAT on OHT subjects or compare between the three glaucoma diagnoses.

In this study cohort, the bias between Icare and GAT for OHT and NTG subjects showed negative inter-tonometry bias which indicates underestimation of IOP by GAT. In POAG, Icare tonometry overestimates GAT by 0.68mmHg. However, a statistical analysis with One-way ANOVA revealed a non-significant difference. In NTG, we noted that Icare underestimates GAT by -0.85mmHg. A comparative study of NTG (97 eyes) and normal (89 eyes) in South Korea, noted Icare significantly underestimates GAT by - 0.23mmHg [253]. Interestingly, tonometry bias in POAG subjects by Brusini et al. revealed an underestimation of 1mmHg of IOP GAT value by Icare [191].

The LOA for the Icare-GAT in OHT subjects (approximately ± 7mmHg) is apparently wider compared to the NTG and POAG subjects (approximately ± 4mmHg and ± 5mmHg). Wider LOA indicate lesser agreement between the tonometry values of the paired tonometer. The difference is apparent on the Bland-Altman plots presented. The plots revealed that inter-tonometry agreement (within ± 2.0 mmHg) in OHT subjects (57.7%) are much less than NTG (79.5%) and POAG (64.6%). Interestingly, the BA plots revealed despite the poor inter-tonometry agreement in OHT, there is no significant proportionate bias with increasing average IOP value of the paired tonometer. Similar uniformity of bias was noted in NTG patients. In POAG, there is significantly positive pattern of inter-tonometry bias with increment of average IOP value.

According to a systemic review by Cook et al.[21], the difference in tonometry between Icare and GAT in this glaucoma cohort is within acceptable limit and showed very good agreement (mean difference within ± 2.0 mmHg). However, the range of IOP agreement in POAG and especially in OHT is outside the acceptable range. Thus, the IOP value between Icare and GAT may be considered interchangeable only in NTG subjects, in which the IOP range is significantly lower than the other glaucoma groups.

The difference in the Icare and GAT inter-tonometry bias between the different glaucoma diagnoses was further investigated. In the initial univariate model, CCT was significantly correlated with the inter-tonometry bias by Icare in OHT and POAG. A previous study reported that bias between Icare and GAT was significantly influenced by CCT in both glaucomatous and normal eyes [282]. However, the multiple regression analysis in our study revealed that CCT was not significantly associated with the bias when corneal biomechanical parameters by ORA and CST were considered at the same time. In this study, multivariate regression analysis was corrected for the effect of CCT, IOP and CRF. The bias in OHT is noted to be moderately and significantly influenced by age, A1V and HcR. The A1V parameter by Corvis ST is noted to have the strongest negative influence on the variability of bias, followed by HcR. Older subjects have significant negative influence on the inter-tonometry bias between Icare and GAT. In NTG, the variability of bias was moderately affected by intraocular pressure, age, A2L and A1T. Increasing intraocular pressure and A2L value in NTG eyes significantly related to positive bias change. Older subject and increase in A1T cause a negative change in the inter-tonometry bias. In POAG, the inter-tonometry bias was also positively affected by A2L and IOP. Even though the influence of these factors is significant, the relationship is weak. Shin et al. found that after adjusting for

age, CCT, spherical equivalent, keratometry, and axial length, CH and CRF remained significantly associated with Icare in the NTG and normal subjects.[253]

4.2.4.2 Tonopen vs GAT

In this study, the Tonopen overestimates GAT in POAG and NTG subjects by 0.59 mmHg and 0.34mmHg, respectively. The tendency for Tonopen to overestimate GAT measurement was noted in previous studies on normal subjects [187, 262, 263]. A meta-analysis study also supported that Tonopen overestimated GAT with wide range of agreement [21]. Similar finding was noted in a study on POAG subjects, where the mean difference of IOP between Icare and GAT is 0.5mmHg [202]. In OHT group, the average IOP value is significantly higher than other glaucoma diagnosis. However, interestingly, the Tonopen is noted to underestimate GAT by 0.81 mmHg in this group. The negative difference may be explained by the tendency of Tonopen to underestimate IOP GAT at high IOP [14]. Our study revealed LOA range of Tonopen-GAT agreement in OHT subjects is approximately 13mmHg. The range of inter-tonometry agreement in NTG and POAG is very similar at approximate range of ± 4mmHg. This inter-tonometry agreement between Tonopen and GAT is very good especially in NTG and POAG. The IOP value of both tonometer may be likely interchangeable in similar subjects. There is no proportionate bias seen in our study which was similar with our normal cohort and a previous study on glaucomatous subjects [243]. This indicates that the inter-tonometry bias between Tonopen and GAT is constant and uniform up to ±10 mmHg and not influenced by the change in the average IOP value.

There is no significant influence of the age, CCT, IOP and corneal biomechanical parameters on the variability of bias between Tonopen and GAT in both POAG and OHT subjects. Previous studies have similar result that showed no significant contribution of CCT in the inter-tonometry bias between Tonopen and GAT in mix glaucoma subjects [192, 251]. Tonopen indents a very small area of surface and, in applanation tonometry by GAT; the IOP measurement was almost static. Thus, the contribution of CCT on the bias may be non-demonstrable. The inter-tonometry bias in NTG is affected moderate to strongly, by DA, A1V, A1T and IOP, consecutively. Similar to our NTG cohort, DA is a significant biomechanical parameter affecting the inter-tonometry bias between Tonopen and GAT in normal subjects. Despite no significant inter-tonometry bias pattern noted on the BA plot of NTG subjects, increment of 1mmHg of ocular pressure from the mean value may increase the bias by 0.5mmHg.

4.2.4.3 ORA vs GAT

The IOPcc was noted to have significantly higher against GAT in both NTG (2.07mmHg) and POAG subjects (2.53 mmHg) than OHT. In OHT, the bias is very minimal at 0.28mmHg. However, the IOPg was noted to be overestimating GAT in all glaucoma groups and especially in OHT subjects but the difference is not significant. Previous studies on healthy eyes noted positive bias between both IOPg and IOPcc, against GAT [72, 143]. The difference of IOP bias by ORA was quite obvious between studies. This bias was noted to be higher in other studies that recruited sample with a mix of glaucoma diagnosis [157].The current study noted that IOPg by ORA have the least bias to GAT compared to other tonometry. The inter-tonometry comparison of ORA to GAT in our study revealed mean difference of IOPg in all glaucoma group are within limits of ± 2mmHg, which is the acceptable systematic difference between tonometers which indicate good inter-tonometry agreement[21]. Unexpectedly, the IOPcc was noted to be outside the range of good agreement in NTG and POAG subject.

Overall, the IOP by ORA is not interchangeable with GAT. The limit of agreement for IOPg-GAT and IOPcc-GAT in the current study was more than 8mmHg with in all glaucoma subgroup especially for bias of IOPcc in OHT subjects. Previous studies also noted the LOA in glaucoma subjects is more than the acceptable limit [157, 283]. This study did not found any proportionate bias in the BA plots for both IOPg and IOPcc bias against GAT in OHT. However, significant proportionate bias change is noted in with increasing average IOP in both NTG and POAG group. We found no similar study in glaucoma subgroup. However, few studies in normal subjects reported higher mean IOP causes proportionate increase in the inter-method difference of ORA IOP with GAT [74]. The difference in bias and LOA may be due to the variability of measurement of ORA as a non-contact tonometer. The tonometry was done in less than 10ms, thus it may be susceptible to the influence of ocular pulse amplitude [157], corneal tear film and CCT [261, 284].

In the multivariate analysis, the inter-tonometry bias was noted to be significantly affected by IOP in all glaucoma subgroup. Older subjects seemed to cause further underestimation of the inter-tonometry bias in NTG subjects. In a large cohort of British population study, the IOPcc was noted higher in male and increased with age. The CCT is noted to have significant but minimal influence on the variability of the inter-tonometry bias in both OHT and POAG. This finding is similar with previous studies which showed positive influence of CCT [72, 260]. Interestingly, CRF, A2T, HcR, A1T,

A1L and A1V have significant effect on the changes in the mean difference between IOPcc against GAT. Amongst all, A1L is noted to have the strongest effect on the variability of IOPcc bias. The variability of IOPg bias against GAT was weakly contributed by IOP in all glaucoma groups. In NTG, A1L is noted to significantly influence the change of bias with a moderately positive effect on the inter-tonometry bias. To the best of our knowledge no similar study is available in the literature that includes CST parameters in the analysis of inter-tonometry agreement.

4.2.4.4 CST vs GAT

The agreement between CST with GAT in NTG and POAG subjects was noted to be comparable to previous studies of normal eyes [265, 266](mean difference of 0.65 and 1.1 mmHg, respectively). Both NTG and POAG subjects showed very good agreement range with LOA of less than ±4mmHg. In contrast, a similar agreement study on normal and glaucoma subjects revealed that CST underestimates GAT measurement [211]. In OHT, CST is noted to underestimate GAT. The limit of agreement of the CST against GAT in this group is more than 14mmHg. The BA plot revealed significant proportionate bias in all glaucoma diagnosis. The CST underestimated GAT with higher average IOP values.

Multivariate analysis on the inter-tonometry bias between CST and GAT revealed that its variability was affected by age and IOPcc, only in NTG subject. In POAG, the univariate analysis of the bias was noted to be significantly affected by age, A1T, A1L, HcT and DA. This may indicate the significance of these parameters to represent additional element of corneal biomechanical properties other than CRF. However the effect was not sustained when the factors are analysed together. Multivariate analysis showed that the bias in OHT and POAG is not significantly influence by all the variables. At the time this was written, no similar article study the combination effect of demographic profiles, IOP, CCT and CRF on the CST tonometry bias in different glaucoma subjects. In a study on OHT and glaucoma subjects, the increment of CST bias against GAT was positively affected by CCT [266]. The insignificant effect of CCT, age and axial length on the CST-GAT inter-method bias was also noted in a healthy cohort [211] but no similar study done glaucoma subjects yet.

4.3 Corneal Biomechanical Assessment of Glaucomatous and OHT Eyes

This study explored the in-vivo corneal biomechanical properties of glaucomatous and OHT eyes with ORA and CST. In the initial section (4.3.1), the relationships between corneal biomechanical parameters by the Corvis ST (CST) and Ocular Response Analyzer (ORA) were evaluated in section. Further in section 4.3.2, regression analysis was performed to analyse the influence of demographic, central corneal thickness and IOP on the biomechanical parameters.

4.3.1 Relationship between ORA and CST biomechanical parameters

The mean value of all the corneal biomechanical variables based on demographic distribution was presented in the early part of this chapter (item 4.1). In this section, Pearson correlation analyses were performed to explore the relationship between the biomechanics parameters of ORA and CST in glaucoma subjects.

The correlation between all corneal biomechanical parameters is listed according to the diagnosis in Table 4.19, 4.20 and 4.21. The correlation of age, CCT and IOPcc is also listed according to glaucoma diagnosis in Table 4.22, 4.23 and 4.24.

Table 4.19 The relationship between corneal biomechanical variables of ORA and Corvis ST in OHT subjects

	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
CH	0.74** (0.56,0.86)	0.44** (0.18,0.66)	0.16 (-0.21,0.48)	-0.27 (-0.52,0.08)	-0.02 (-0.32,0.26)	0.08 (-0.24,0.40)	0.36* (0.10, 0.61)	0.04 (-0.26,0.33)	-0.07 (-0.37,0.23)	0.42** (0.15,0.64)	-0.27 (-0.51,-0.03)
CRF		0.64** (0.44, 0.79)	0.33* (0.02,0.58)	-0.29 (-0.47,-0.09)	-0.22 (-0.47,0.03)	0.06 (-0.23,0.32)	0.54* (0.27, 0.75)	0.01 (-0.31,0.35)	-0.06 (-0.40,0.25)	0.43** (0.08,0.68)	-0.49** (-0.66,-0.31)
A1T			0.28 (0.05,0.49)	0.62** (-0.76,-0.47)	-0.62** (-0.74,-0.50)	-0.02 (-0.29,0.27)	0.55** (0.39,0.70)	0.02 (-030,0.37)	-0.26 (-0.52,0.03)	0.45** (0.18,0.65)	-0.61 (-0.77,-0.41)
A1L				-0.03 (-0.31,0.23)	-0.26 (-0.54,0.04)	0.30 (-0.11,0.63)	0.46** (0.18,0.70)	0.14 (-0.17,0.45)	-0.17 (0.53,0.19)	0.61** (0.43,0.75)	-0.35 (-0.57,-0.14)
A1V					0.50** (0.18,0.67)	-0.12 (-0.40,0.23)	-0.47** (-0.65,-0.27)	-0.02 (-0.37,0.30)	0.33* (0.05,0.54)	-0.20 (-0.52,0.13)	0.26 (-0.06,0.58)
A2T						-0.06 (-0.37,0.29)	-0.36* (-0.61,-0.04)	-0.22 (-0.50,0.08)	0.32* (0.04,0.57)	-0.28 (-0.53,0.01)	0.57** (0.27,0.78)
A2L							0.36 (0.06,0.58)	-0.01 (-0.35,0.29)	-0.06 (-0.34,0.28)	0.16 (-0.16,0.41)	-0.20 (-0.49,0.16)
A2V								-0.05 (-0.43,0.32)	-0.22 (-0.49,0.05)	0.49** (0.69,0.21)	-0.63** (-0.82,-0.35)
HcT									-0.19 (-0.47,0.12)	0.21 (-0.11,0.54)	0.07 (-0.29,0.44)
HpD										-0.21 (0.52,0.11)	0.12 (-0.15,0.41)
HcR											-0.47** (-0.68,-0.23)

Pearson's correlation with bootstrapping (Bca 95% CI) *p value <0.05, **p value<0.0

Table 4.20 The relationship between corneal biomechanical variables of ORA and Corvis ST in NTG subjects

	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
CH	0.79** (0.60,0.89)	-0.16 (-0.50,0.36)	-0.05 (-0.2,0.25)	0.40* (-0.26,0.67)	0.04 (-0.23,0.45)	0.02 (-0.38,0.48)	0.21 (-0.07,0.47)	0.14 (-0.26,0.48)	-0.08 (-0.47,0.40)	0.06 (-0.47,0.34)	-0.20 (-0.44,0.45)
CRF		0.12(-0.26,0.63)	0.03 (-0.35,0.34)	0.37* (-0.06,0.64)	-0.15 (-0.41,0.11)	-0.05 (-0.42,0.35)	0.35 (0.08,0.53)	0.33 (-0.04,0.65)	-0.01 (-0.42,0.49)	0.20 (-0.20,0.48)	-0.19 (-0.36,-0.08)
A1T			0.30 (0.05,0.60)	-0.13 (-0.41,0.23)	-0.41* (-0.59,-0.33)	-0.07 (-0.32,0.19)	0.23 (-0.06,0.55)	0.27 (-0.05,0.61)	-0.13 (-0.37,0.15)	0.22 (0.03,0.63)	-0.01 (-0.58,0.04)
A1L				0.20 (-0.29,0.75)	-0.11 (-0.63,0.22)	0.05 (-0.40,0.44)	0.22 (-0.21,0.55)	0.27 (-0.04,0.48)	0.19 (-0.16,0.50)	0.07 (-0.23,0.54)	0.13 (-0.56,0.30)
A1V					0.02 (-0.18,0.23)	-0.22 (-0.57,0.32)	-0.25 (-0.53,0.05)	-0.17 (-0.45,0.12)	-0.31 (-0.66,0.38)	-0.12 (-0.36,0.43)	-0.08 (-0.36,0.43)
A2T						0.19 (-0.18,0.45)	-0.48** (-0.76,0.03)	-0.29 (-0.52,0.02)	-0.15 (-0.48,0.19)	-0.19 (-0.36,-0.08)	0.13 (-0.12,0.33)
A2L							0.29 (-0.09,0.60)	0.04 (-0.36,0.37)	0.14 (-0.24,0.52)	0.12 (-0.13,0.37)	-0.06 (-0.30,0.01)
A2V								0.63** (0.37,0.79)	0.32 (-0.06,0.59)	0.22 (-0.21,0.73)	0.07 (-0.78,0.21)
HcT									0.26 (-0.06,0.49)	-0.10 (-0.56,0.62)	0.37* (-0.67,0.62)
HpD										-0.08 (-0.53,0.63)	0.32 (-0.40,0.53)
HcR											-0.83** (-0.94,-0.48)

Pearson's correlation with bootstrapping (Bca 95% CI) *p value <0.05, **p value<0.0

Table 4.21 The relationship between corneal biomechanical variables of ORA and Corvis ST in POAG subjects

	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
CH	0.72** (0.59,0.82)	-0.09 (-0.31,0.15)	0.04 (-0.14,0.22)	0.30** (0.14,0.46)	0.36** (0.19,0.51)	0.36** (0.19,0.51)	0.27* (-0.15,0.22)	0.36** (0.15,0.53)	0.13 (-0.11,0.34)	0.05 (-0.22,0.31)	0.29** (0.10,0.44)
CRF		0.36** (0.15,0.54)	-0.04 (-0.26,0.19)	-0.07 (-0.30,0.16)	-0.02 (-0.26,0.20)	0.26* (0.06,0.44)	0.45** (0.26,0.61)	0.19 (-0.00,0.36)	0.12 (-0.08,0.32)	0.21 (-0.03,0.43)	-0.14 (-0.30,0.01)
A1T			0.08 (-0.11,0.27)	-0.31** (-0.51,-0.08)	-0.36** (-0.58,-0.12)	0.08 (-0.10,0.26)	0.51** (0.34,0.65)	-0.26* (-0.44,-0.09)	-0.07 (-0.30,0.17)	0.21 (-0.02,0.42)	-0.49** (-0.64,-0.33)
A1L				0.54** (0.09,0.36)	-0.01 (0.23,0.20)	0.06 (-0.21,0.33)	-0.01 (-0.25,0.26)	-0.05 (-0.18,0.10)	-0.17 (-0.37,0.04)	0.21 (-0.01,0.46)	0.50 (-0.15,0.23)
A1V					0.46** (0.24,0.64)	0.08 (-0.18,0.30)	-0.35** (-0.56,-0.08)	0.19 (0.02,0.35)	-0.05 (-0.27,0.15)	0.02 (-0.21,0.26)	0.42** (0.17,0.59)
A2T						0.21 (-0.05,0.43)	-0.51** (-0.67,-0.34)	0.21 (0.02,0.40)	0.11 (-0.11,0.33)	-0.14 (-0.36,0.07)	0.65** (0.47,0.79)
A2L							0.09 (-0.11,0.30)	0.18 (-0.05,0.42)	-0.05 (-0.26,0.17)	0.28* (0.05,0.47)	0.06 (-0.17,0.28)
A2V								0.06 (-0.14,0.25)	0.03 (-0.18,0.23)	0.35** (0.12,0.57)	-0.68** (-0.76,-0.60)
HcT									-0.05 (-0.25,0.14)	0.07 (-0.24,0.38)	0.17 (-0.10,0.42)
HpD										-0.10 (-0.31,0.11)	0.22* (-0.00,0.42)
HcR											0.41** (-0.60,-0.16)

Pearson's correlation with bootstrapping (Bca 95% CI) *p value <0.05, **p value<0.0

Table 4.22 The relationship between age, CCT and IOPcc with the corneal biomechanical variables in OHT subjects ¥

	CH	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
Age	-0.38* (-0.62,-0.12)	-0.34* (-0.53,-0.16)	-0.36* (-0.54,-0.16)	-0.16 (-0.46,0.15)	-0.04 (-0.32,0.31)	0.09 (-0.18,0.33)	0.04 (-0.23,0.30)	-0.32* (-0.49,-0.12)	0.28 (0.01,0.56)	-0.09 (-0.39,0.20)	-0.16 (-0.40,0.05)	0.45** (0.24,0.65)
CCT	0.38* (-0.04,0.70)	0.26 (-0.14,0.66)	0.15 (-0.23,0.50)	-0.10 (-0.41,0.25)	-0.23 (-0.46,0.00)	0.14 (-0.17,0.40)	0.27 (-0.05,0.58)	0.11 (-0.20,0.43)	0.09 (-0.21,0.38)	-0.04 (-0.34,0.27)	0.28 (-0.06,0.57)	-0.07 (-0.36,0.25)
IOPcc	-0.21 (-0.54,0.18)	-0.10 (-0.36,0.20)	0.38 (0.07,0.62)	0.12 (-0.20,0.42)	-0.24 (-0.58,0.15)	-0.57** (-0.78,-0.26)	0.04 (-0.23,0.34)	0.18 (-0.15,0.49)	0.04 (-0.24,0.34)	-0.11 (-0.42,0.21)	0.21 (-0.15,0.58)	-0.38* (-0.59,-0.14)

Table 4.23 The relationship between age, CCT and IOPcc with the corneal biomechanical variables in NTG subjects ¥

	CH	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
Age	0.40* (0.07,0.66)	0.28 (-0.11,0.57)	-0.25 (-0.43,-0.09)	-0.46* (-0.71,-0.14)	0.22 (-0.32,0.59)	0.09 (-0.16,0.63)	0.13 (-0.32,0.53)	-0.15 (-0.43,0.12)	-0.29 (-0.59,0.05)	-0.26 (-0.64,0.20)	0.12 (-0.49,0.47)	-0.32 (-0.63,0.59)
CCTus	0.06 (-0.45,0.56)	0.04 (-0.43,0.63)	0.10 (-0.21,0.45)	0.34 (0.04,0.58)	-0.23 (-0.61,0.30)	-0.07 (-0.34,0.17)	-0.03 (-0.35,0.27)	0.33 (0.14,0.51)	0.33 (-0.02,0.64)	0.27 (-0.17,0.65)	-0.15 (-0.68,0.61)	0.39* (-0.47,0.68)
IOPcc	0.07 (-0.43,0.45)	0.33 (-0.11,0.69)	0.53** (0.31,0.80)	0.24 (-0.20,0.58)	0.15 (-0.26,0.47)	-0.47** (-0.63,-0.36)	-0.18 (-0.56,0.21)	0.32 (-0.01,0.61)	0.31 (-0.17,0.69)	-0.29 (-0.61,0.06)	0.38* (0.00,0.64)	-0.33 (-0.47,-0.38)

¥Note: Pearson's correlation with bootstrapping (Bca 95% CI) ,where * p value <0.05, ** p value<0.0

Table 4.24 The relationship between age, CCT and IOPcc with the corneal biomechanical variables in POAG subjects

	CH	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	PD	R	DA
Age	-0.26* (-0.42,-0.07)	-0.14 (-0.32,0.04)	0.14 (-0.09,0.37)	-0.18 (-0.40,0.11)	-0.17 (-0.37,0.04)	-0.19 (-0.42,0.06)	-0.05 (-0.34,0.27)	0.06 (-0.16,0.27)	-0.06 (-0.28,0.15)	0.12 (-0.11,0.35)	0.21 (0.00,0.39)	-0.01 (-0.21,0.21)
CCT	0.18 (-0.80,0.38)	0.43** (-0.80,0.38)	0.26* (0.00,0.49)	-0.06 (-0.28,0.18)	-0.28 (-0.47,-0.04)	-0.33** (-0.54,-0.10)	0.21 (-0.02,0.43)	0.42** (0.24,0.59)	0.20 (-0.04,0.40)	-0.05 (-0.28,0.17)	0.23* (-0.12,0.52)	-0.33** (-0.56,-0.06)
IOPcc	-0.46** (-0.62,-0.28)	0.10 (-0.15,0.33)	0.55** (0.37,0.69)	-0.09 (-0.29,0.12)	-0.42** (-0.58,-0.22)	-0.62** (-0.73,-0.50)	0.20 (-0.33,0.11)	0.46** (0.25,0.63)	-0.31** (-0.47,-0.14)	-0.03 (-0.24,0.20)	0.16 (-0.07,0.38)	-0.56 (-0.70,-40)

Note: Pearson’s correlation with bootstrapping (Bca 95% CI) ,where * p value <0.05, ** p value<0.0

4.3.2 Factors affecting the biomechanical parameters by ORA and CST

Linear regression analysis was carried out to identify factors that affect the corneal biomechanical parameters. Based upon the previous literature, age, gender, CCTus and IOPcc were chosen as predictors. The IOPcc was chosen to represent IOP in the regression analysis as it was claimed to represent the corneal-compensated IOP value [20, 157, 187].

Multivariate regression analysis was executed to evaluate the effect of age, gender, CCT and IOP on the biomechanical parameters in this study. Enter (block entry) method was chosen with robust regression to detect the significant factors for the best predictive model of each biomechanical parameters employed in this study. Table 4.25 summarises the results for OHT subjects, whilst Table 4.26 and 4.27 summarised the result for NTG and POAG subjects respectively.

According to Table 4.25, in OHT subjects, almost 25% of the variability of CH and CRF was contributed by age and gender. Male subjects contributed to increment of the CH and CRF, whilst the increment of age causes a negative effect on the values ($p<0.01$). The effect of age, gender and IOP was significantly large in A1T with a total 46% predictive value ($p<0.01$).The influence of age is more than IOP and gender on the A1T parameter (Beta coefficient values of 0.49, 0.39 and 0.44, respectively, $p<0.01$). The DA is noted to be strongly and positively predicted by age more than IOP (Beta values of 0.57 and -0.40, respectively, $p<0.01$). Both age and IOP (and contributed 39% to the variability of DA ($p<0.01$). The CCT is noted to significantly affect the A1V and A2L. However, the overall regression model for both parameters are not statistically significant ($p>0.05$).

Table 4.25 Factors affecting the biomechanical parameters by Corvis ST and ORA in OHT subjects: a multivariate regression analysis

	Standardised coefficient (β) with B coefficients (BCa 95% CI)				Adjusted R^2	p
	IOPcc	CCTus	Age	Gender		
CH	-0.15 -0.09(-0.26,0.08)	0.29 0.02(-0.00,0.04)	**-0.15*** -0.07(-0.14,-0.02)	**0.28*** 1.54(0.19,3.03)	0.25	**0.00****
CRF	-0.12 -0.08(-0.27,0.06)	0.16 0.01(-0.01,0.03)	**-0.33*** -0.08(-0.14,-0.02)	**0.46*** 2.77(1.16,4.57)	0.24	**0.00****
A1T	0.39* 0.04(0.02,0.07)	0.11 0.00(-0.01,0.05)	**-0.49*** -0.02(-0.03,-0.01)	**0.44*** 0.44(0.21,0.69)	0.46	**0.00****
A1L	0.09 0.03(-0.06,0.13)	-0.21 -0.01(-0.02,0.01)	-0.30 -0.03(-0.07,0.00)	0.15 0.05(-0.05,0.15)	0.04	0.24
A1V	-0.26 -0.02(-0.04,0.01)	**-0.32*** 0.00(-0.01,0.01)	-0.05 0.00(-0.02,0.02)	-0.20 -0.01(-0.03,0.01)	0.11	0.07
A2T	**-0.56**** -0.42(-0.06,0.02)	0.11 0.01(-0.02,0.03)	0.020 0.001(0.00,0.02)	0.01 0.01(-0.20,0.19)	0.29	**0.00****
A2L	0.11 0.01(-0.01,0.03)	0.25 0.00(-0.01,0.01)	0.07 0.00(-0.01,0.01)	-0.13 -0.08(-0.26,0.12)	0.02	0.54
A2V	0.20 0.00(-0.01,0.01)	-0.01 0.00(-0.01,0.01)	-0.41 -0.02(-0.04,-0.01)	0.14 0.02(-0.02,0.06)	0.12	0.07
HcT	0.05 0.00(-0.02,0.03)	0.13 0.01(-0.02,0.04)	0.29 0.01(0.00,0.02)	-0.09 -0.07(-0.33,0.18)	-0.01	0.46
HpD	-0.10 -0.02(-0.11,0.07)	0.04 0.01(-0.07,0.11)	0.02 0.00(-0.03,0.03)	-0.04 -0.08(-0.84,0.58)	-0.09	0.96
HcR	0.28 0.06(0.00,0.13)	0.29 0.01(0.00,0.02)	-0.15 -0.01(-0.03,0.01)	0.04 0.10(-0.56,0.77)	0.08	0.13
DA	**-0.40**** -0.01(-0.01,-0.0)	0.02 0.00(-0.01,0.01)	**0.57**** 0.04(0.02,0.05)	-0.17 -0.03(-0.07,0.11)	0.39	**0.00****

** level of significance, p< 0.01

*level of significance, p< 0.05

Table 4.26 Factors affecting the biomechanical parameters by Corvis ST and ORA in NTG subjects: a multivariate regression analysis

	Standardised coefficient (β) with B coefficients (BCa 95% CI)				Adjusted R^2	p
	IOPcc	CCTus	Age	Gender		
CH	0.17 0.07(0.07,0.22)	0.21 0.01(-0.10,0.04)	0.35 0.06(-0.01,0.12)	0.29 1.01(-0.31,2.32)	0.18	0.06
CRF	**0.44*** 0.13(0.01,0.23)	0.24 0.01(0.00,0.04)	0.30 0.03(-0.00,0.07)	0.31 0.77(-0.07,1.57)	0.28	**0.01***
A1T	**0.53**** 0.04(0.02,0.07)	0.15 0.00(0.00,0.01)	-0.03 -0.01(-0.08,0.13)	-0.11 -0.07(-0.36,0.14)	0.22	**0.04***
A1L	0.20 0.01(-0.01,0.04)	0.26 0.02(-0.01,0.06)	-0.28 -0.06(-0.16,0.05)	-0.09 -0.05(-0.26,0.16)	0.18	0.06
A1V	0.20 0.01(-0.03,0.05)	-0.13 0.00(0.00,0.01)	0.11 0.00(-0.01,0.00)	0.27 0.01(-0.01,0.04)	0.03	0.32
A2T	0.51 -0.09(-0.19,-0.03)	0.17 -0.01(-0.02,0.00)	-0.11 -0.01(0.00,0.02)	0.23 0.06(-0.60,0.74)	0.13	0.11
A2L	-0.17 -0.10(-0.04,0.02)	-0.03 0.00(-0.01,0.00)	0.21 0.01(-0.01,0.02)	-0.29 -0.17(-0.39,0.06)	-0.04	0.57
A2V	**0.38*** 0.01(0.00,0.02)	**0.41*** 0.00(0.00,0.00)	0.23 0.02(-0.03,0.05)	-0.36 -0.07(-0.16,0.02)	0.25	**0.02***
HcT	0.33 0.06(-0.01,0.15)	0.34 0.01(0.00,0.03)	-0.04 0.00(-0.03,0.03)	-0.14 -0.20(-0.71,0.29)	0.14	0.10
HpD	-0.35 -0.10(-0.19,0.04)	0.12 0.01(-0.01,0.03)	-0.22 -0.02(-0.07,0.04)	-0.20 -0.47(-1.64,0.61)	0.12	0.12
HcR	0.41 0.13(0.00,0.28)	-0.03 0.00(-0.02,0.03)	0.31 0.04(-0.02,0.10)	-0.26 -0.67(-1.38,-0.10)	0.12	0.12
DA	**-0.37*** -0.12(-0.28,-0.01)	0.23 0.01(0.00,0.04)	-0.32 -0.04(-0.10,0.00)	-0.01 -0.03(-0.68,0.37)	0.21	**0.04***

** level of significance, p< 0.01

*level of significance, p< 0.05

In NTG subjects (Table 4.26), approximately 21-24% of the variability of CRF, A1T and DA parameters is significantly predicted by IOP. The effect of IOP on CRF and A1T are positive, whilst on DA, IOP has a negative influence ($p<0.05$). The A2V in this group is influenced by both IOP and CCT and predicted 25% of the A2V value ($p<0.05$).

Table 4.27 Factors affecting the biomechanical parameters by Corvis ST and ORA in POAG subjects: a multivariate regression analysis

	Standardised coefficient (β) with B coefficients (BCa 95% CI)				Adjusted R^2	p
	IOPcc	CCTus	Age	Gender		
CH	**-0.55**** -0.22(-0.30,0.15)	**0.28**** 0.01(0.01,0.02)	-0.14 -0.02(-0.03,0.01)	**0.23*** 0.80(0.14,1.43)	0.36	**0.00****
CRF	-0.03 -0.10(-0.11,0.08)	**0.38**** 0.02(0.01,0.03)	-0.13 -0.02(-0.04,0.01)	0.18 0.62(-0.07,1.42)	0.18	**0.00****
A1T	**0.52**** 0.04(0.03,0.05)	0.10 0.00(-0.01,0.02)	-0.01 0.00(-0.04,0.04)	**0.17*** 0.11(0.01,0.22)	0.32	**0.00****
A1L	-0.14 -0.01(-0.02,0.00)	0.08 0.01(0.00,0.002)	-0.10 -0.01(-0.04,0.02)	0.12 0.05(-0.03,0.14)	0.01	0.29
A1V	**-0.28*** -0.02(-0.03,0.00)	-0.15 0.00(0.00,0.00)	-0.14 0.00(-0.01,0.00)	-0.02 -0.01(-0.12,0.10)	0.11	**0.01****
A2T	**-0.62**** -0.07(-0.09,-0.05)	-0.05 -0.01(-0.03,0.02)	0.01 0.00(-0.05,0.06)	-0.10 -0.10(-0.28,0.10)	0.38	**0.00****
A2L	-0.18 -0.02(-0.04,)	0.28 0.00(0.00,0.01)	0.10 0.00(-0.01,0.01)	-0.03 -0.02(-0.18,0.16)	0.05	0.07
A2V	**0.31**** 0.01(0.00,0.10)	**0.29**** 0.01(0.00,0.01)	0.03 0.00(-0.01,0.01)	-0.02 -0.01(-0.04,0.03)	0.17	**0.00****
HcT	**-0.35**** -0.06(-0.08,-0.03)	**0.33**** 0.01(0.00,0.10)	0.01 0.01(-0.10,0.10)	**0.23*** 0.34(0.05,0.63)	0.24	**0.00****
HpD	-0.10 -0.03(-0.09,0.04)	0.09 0.00(0.00,0.01)	0.13 0.01(-0.01,0.03)	0.03 0.06(-0.47,0.61)	-0.01	0.59
HcR	-0.06 -0.01(-0.04,0.03)	**0.30*** 0.01(0.00,0.01)	0.18 0.01(0.00,0.02)	0.19 0.29(0.07,0.60)	0.15	**0.01****
DA	-0.26 -0.03(-0.09,-0.01)	0.04 0.01(-0.01,0.04)	0.10 0.04(-0.01,0.12)	-0.10 -0.12(-0.41,0.05)	0.04	0.11

** level of significance, p< 0.01

*level of significance, p< 0.05

In POAG subjects, the CH was mildly influenced by IOP, CCT and gender. The CRF was only affected by CCT. For CST parameters, A2V and HcT are both weakly affected by the IOP and CCT. The HcR is only affected by CCT. The influence of gender on A1T, HcT and CH are generally minimal.

4.3.3 Discussion

Amongst all glaucoma diagnoses, the CRF, CH, A1T, A2L, HcR and CCT are noted to be highest in OHT. High CCT in OHT subjects was previously reported in Ocular Hypertension Treatment Trial (OHTT) [285]. Analysis of correlation showed that CH was strongly correlated with CRF ($p<0.01$) in all glaucoma subgroup. This study showed highly significant association between both CRF and CH, which was demonstrated in many studies since the launch of ORA since a decade ago [184, 220]. Both parameters was derived from the same infra-red wave analysis but CRF was calculated using different mathematical calculation and algorithm[69].

The corneal biomechanical parameters from ORA were significantly correlated with different parameters from CST according to glaucoma diagnosis. In POAG and OHT, both ORA parameters (CH and CRF) have significant moderate correlation with A1T and A2V. In NTG subjects, CH and CRF are only significantly correlated with A1V only. The similarities of relationship between ORA and CST parameters (A1T and A2V) in OHT and POAG may be indicative of the influence of IOP on the corneal biomechanics of both subjects. This is a novel finding and may be investigated to further characterise the parameters that may identify the different glaucoma diagnosis. A study on spectral analysis of the waveform analysis of ORA and CST noted statistically significant difference of both instruments in normal eyes [278].

Other CST parameters; A1V, A1L, A2T, A2L, HcT and DA have weak but significant correlation with CH in POAG subjects. DA showed significant unique relationship with CH in POAG and CRF in OHT. Studies in healthy and glaucoma subjects highlighted A1T and DA as the most reliable and reproducible parameters for describing corneal biomechanics [212, 245, 269]. The study did not explore direct correlation between corneal biomechanical parameters from both instruments. To the best of our knowledge, there is no available study that looked into the characteristics of CST corneal biomechanical parameters in POAG, OHT and NTG. These CST parameters may be important to represent the viscoelastic properties of the cornea in-vivo for glaucoma eyes.

In the multivariate analysis, this study analysed the collective contribution of age, gender, CCT and IOP on the corneal biomechanical parameters by ORA and CST. In OHT subjects, age has significant negative contribution on CH, CRF, A1T and DA. Female subjects are noted to positively influence CH, CRF and A1T. The contribution of age and gender to the variability of these parameters are moderate to strong. In NTG, no significant influence of age and gender noted on the ORA and CST parameters. The CCT only affected the A2V parameter whilst the IOP have significant influence on the variability of CRF, A1T, A2V and DA. However, in POAG, IOPcc is noted to positively contribute to CRF, A1T, A2V and DA.

In POAG, the CH was influenced by age and gender where being female and older increases the CH value. Female subjects tend to have higher CRF, A1T, A2T and HcT, how the effect was very small. Subjects with high central corneal thickness significantly influence CRF value. Subjects with higher IOP are noted to have higher A1T and A2V. However, the IOP influence was reversed in HcT, A2T and DA. The effect on these parameters may indicate rapid deformation and deflection of the cornea to air-puff pressure. Thus, can be a demonstration of reduced rigidity of the cornea in POAG subjects. The CCT is noted to significantly contribute to positive value in CH, CRF, A2V, HcT and HcR. The CH, A1T, A1V, A2T, A2V, HcT are influenced by IOP. The contribution of these factors on the corneal biomechanical parameters is noted to be weak but significant.

In NTG, the IOP influenced the CRF, A1T, A2V and DA. Only A2V is affected by CCT in this study group. The significant contribution of these factors, although significant, is weak (R^2=0.22-0.28). Previous studies found that IOP more than CCT and age contribute to the variability of CST corneal biomechanical parameters factors [20, 266, 286]. The CH and CRF by ORA are known to be affected by IOP, age and CCT in normal subjects [59, 65].

The CH was noted to be affected by age, CCT and IOP in subjects with corneal deformity and glaucoma [81-83]. However, the CRF increases with thicker CCT and not influenced by other demographic variables and parameters. In a study of a British population, the mean CH and CRF declined with age and were higher in women than in men [65], which is in accordance to the findings in POAG and OHT subjects in this study. For this glaucoma cohort, the significant effect of age was noted on CH, CRF, A1T and DA in OHT eyes. Similar observations were made by other studies that indicated ageing cornea could reduce corneal viscoelasticity (92, 96, 124, 125). Despite a few contradictory findings in laboratory studies using donor cornea, the

inverse association between age and corneal viscoelasticity are present in many studies [220]. The effect of IOP on the corneal biomechanical properties by ORA was previously analysed in normal and glaucomatous eyes [15, 17, 163]. In both glaucoma and normal subjects, CCT is positively correlated with CH [81-83]. As the cornea contains more collagen fibres and ground substances, resistance against deformation and damping capacity rises. Moreover, the stronger the corneal tension, the faster the cornea regains its original position following deformation. The IOP may serves as an additional force that restores the cornea to its original position [84].

4.4 Conclusion

Overall, all the tonometers employed in this study have variable range of agreement with GAT. All tonometers showed good agreement with GAT in all glaucoma subgroups except for IOPcc. The Tonopen are not affected by change of the average IOP values. The LOA of all tonometers was greater than the acceptable range, except for Tonopen in NTG and POAG groups. Thus the IOP values of these tonometers are non-exchangeable to GAT tonometric values. Clinicians should be aware of the proportionate bias by CST when performing tonometry on subjects with different glaucoma diagnosis as the effect of age, IOP, CCT and corneal biomechanical parameters are also variable. Age affected the inter-tonometry bias between IOPcc, Icare and CST with GAT in variable contribution across the glaucoma diagnosis. The CRF, A1T and DA are important biomechanical parameters that influence the inter-tonometry bias. Subjects of each glaucoma diagnosis showed variable relationship between ORA and CST corneal biomechanical parameters.

CHAPTER 5: TONOMETRY AGREEMENT AND CORNEAL BIOMECHANICS EVALUATION IN KERATOCONIC EYES

This chapter presents data from a cohort of keratoconus subjects. The subjects were recruited from the out-patients clinic at Birmingham and Midland Eye Centre. Details on subject selection, instrumentation and methods of analysis employed in this study are described in Chapter 2. The initial section, 5.1, describes the demographics of the study subjects. The inter-tonometry agreement study is presented in section 5.2. In section 5.3, results of the corneal biomechanical study by the ORA and CST are reported.

5.1 Demographics

This study recruited 113 eyes from a total of 69 subjects diagnosed with keratoconus. The mean age of all subjects was 29.3 ± 9.8 years (median; 29.0 years, max; 58 years, min; 18 years). The subjects consist of 15 female (27.8%) and 54 male (72.2%). The keratoconic eyes were classified according to the Amsler-Krumeich grading described in section 1.5.2.

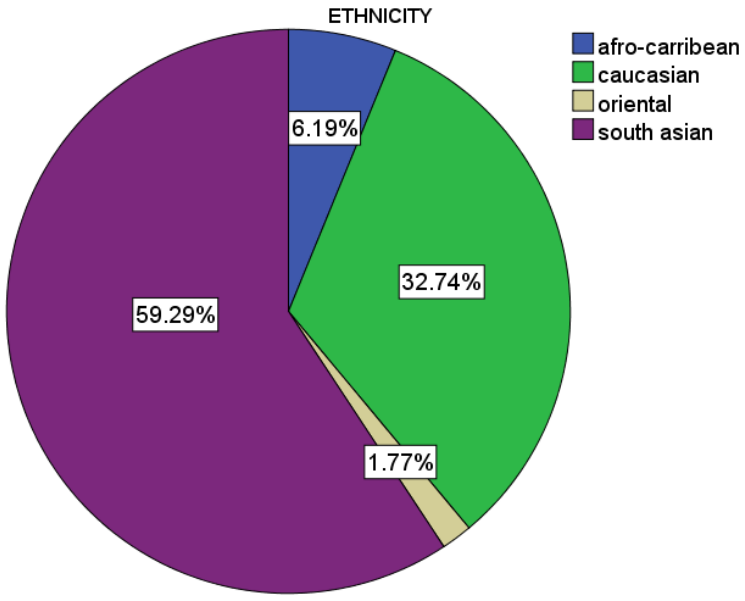


Figure 5.1 The ethnic distribution of the keratoconus subjects

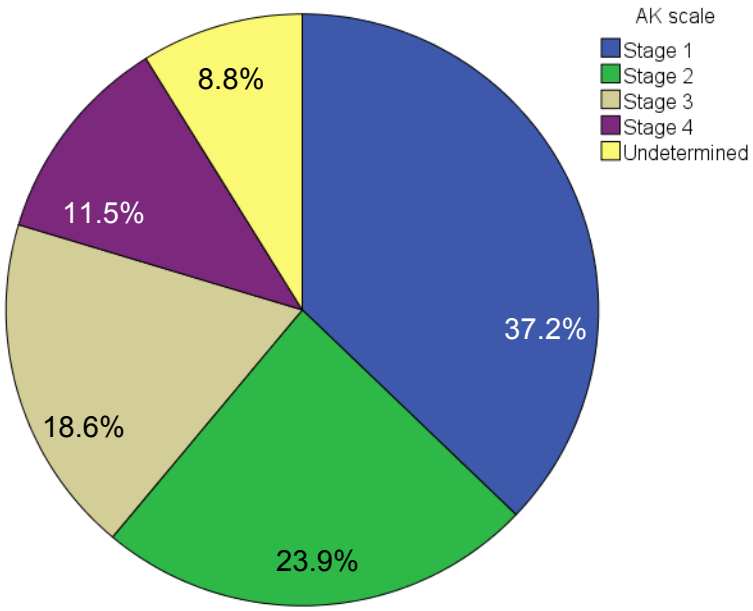


Figure 5.2 The distribution of keratoconic eyes according to the Amsler-Krumeich classification

Table 5.1 Mean IOP values, corneal biomechanical parameters and corneal thickness of keratoconic eyes

	Mean ± SD	Minimum	Maximum
GAT (mmHg)	11.75 ± 3.00	6.00	16.00
Tonopen (mmHg)	11.96 ± 2.79	5.00	18.00
Icare (mmHg)	9.37 ± 3.50	4.00	17.00
ORA_IOPg (mmHg)	9.84 ± 3.72	2.90	19.70
ORA_IOPcc (mmHg)	12.62 ± 2.93	6.00	20.80
CST_IOP (mmHg)	12.19 ± 1.96	6.70	16.50
CH	8.69 ± 2.18	4.40	15.10
CRF	7.35 ± 2.49	2.70	13.80
A1T (ms)	7.38 ± 0.28	6.61	7.98
A1L (mm)	1.64 ± 0.26	0.87	2.12
A1V (m/s)	0.16 ± 0.03	0.10	0.15
A2T (ms)	22.82 ± 0.47	21.38	23.87
A2L (mm)	1.56 ± 0.42	0.91	2.41
A2V (m/s)	-0.48 ± 0.09	-0.69	-0.31
HcT (ms)	16.38 ± 0.65	13.52	17.68
HpD (mm)	3.56 ± 1.25	2.22	6.02
HcR (mm)	5.14 ± 1.35	2.51	10.78
DA (mm)	1.32 ± 0.18	1.03	1.75
CCTus (μm)	451.65 ± 65.25	246.00	544.00

5.2 Agreement between tonometers in keratoconus eyes

This section examines the agreement of IOP measurements between these tonometers; the Corvis® ST, Tonopen Avia®, Icare® and Ocular Response Analyzer® with Goldman applanation tonometer (GAT). Further, the influence of demographic, central corneal thickness and cornea biomechanical factors on the inter-tonometry agreement is analysed.

5.2.1 Inter-tonometry agreement

The agreement of the tonometers was evaluated with intraclass correlation coefficients (ICC), mean difference (bias), 95% limits of agreement (LOA) and Bland-Altman plots (Bland & Altman, 1986).

Table 5.2 showed the mean IOP difference of all paired tonometers with GAT in keratoconic eyes. The inter-method bias was high by Icare and IOPcc with the mean difference values of more than 2.0 mmHg. The mean difference of the Icare, CST and IOPcc was comparable at less than 0.5 mmHg. The Tonopen showed the least different to GAT measurement. The widest LOA was by Icare_GAT tonometer pair (LOA range of 13.50 mmHg) and the narrowest was by IOPcc_GAT pair (LOA range of 10.01 mmHg).

Table 5.2 The inter-tonometry bias, limit of agreement and paired correlation of tonometers against GAT

Paired tonometry	Mean Difference ± SD (mmHg)	LOA (95%CI)	ICC	(95%CI)
Icare vs GAT	-2.25 ± 3.43	4.50 to -9.00	0.46	0.11, 0.66
Tonopen vs GAT	0.07 ± 2.55	5.09 to -4.95	0.72	0.59, 0.81
IOPcc vs GAT	0.47 ± 3.38	7.13 to -6.19	0.58	0.34, 0.73
IOPg vs GAT	-2.31 ± 3.38	4.35 to -8.97	0.55	0.15, 0.75
CST_IOP vs GAT	-0.11 ± 2.80	5.41 to -5.52	0.59	0.39, 0.72

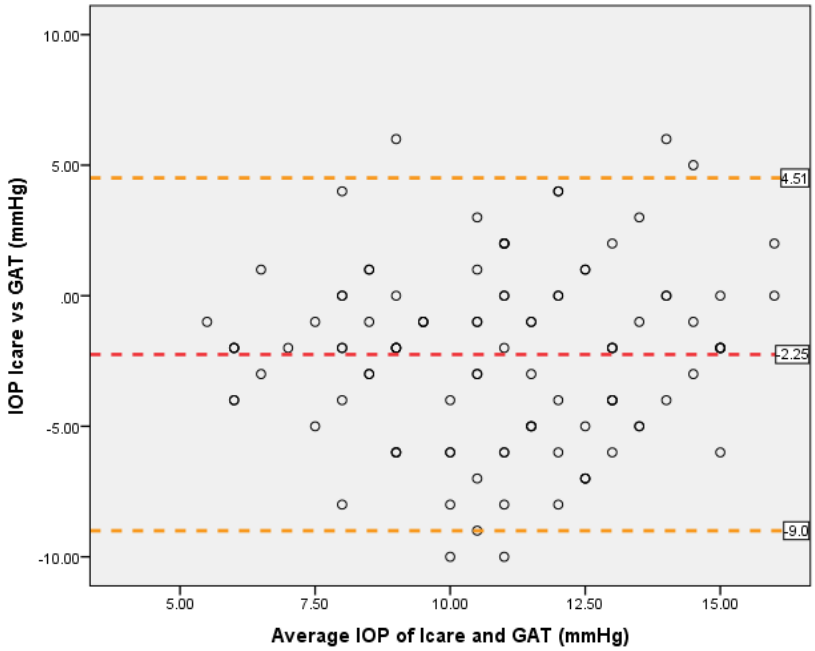


Figure 5.3 The tonometry agreement plots between Icare and GAT

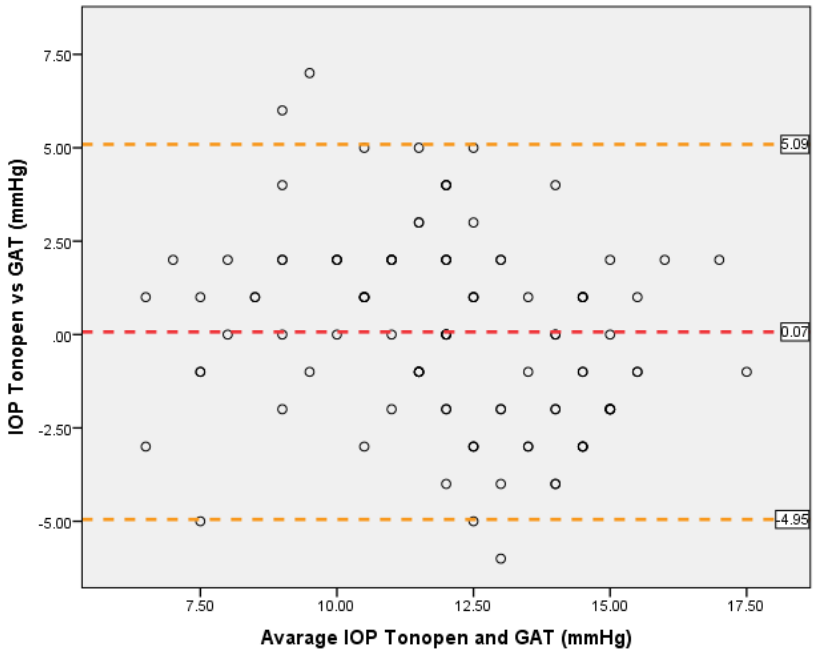


Figure 5.4 The tonometry agreement plots between Tonopen and GAT

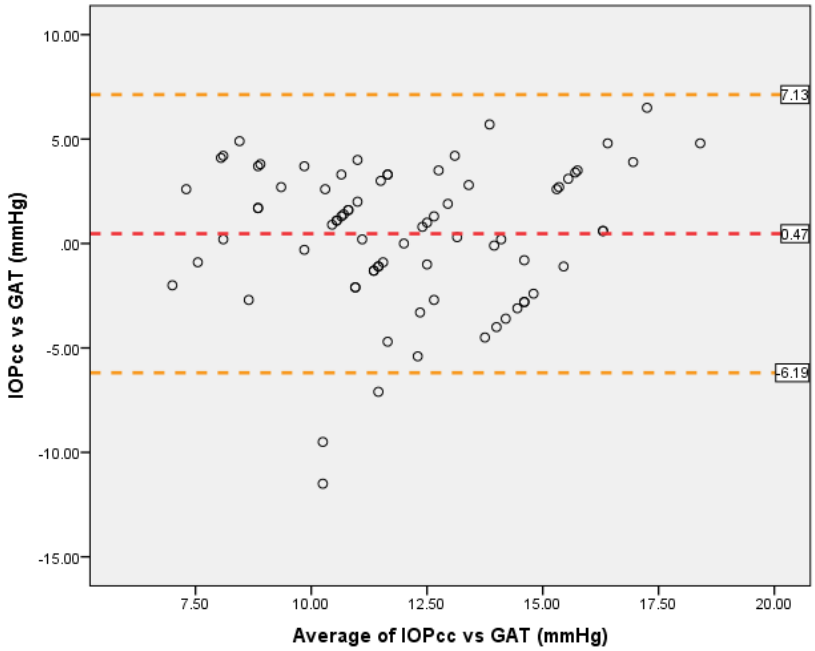


Figure 5.5 The tonometry agreement plots between IOPcc and GAT

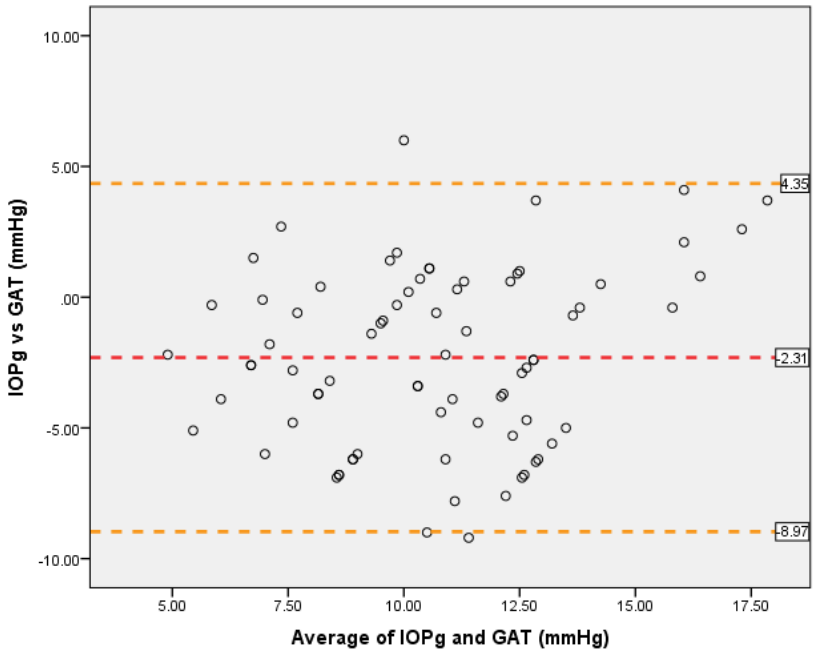


Figure 5.6 The tonometry agreement plots between IOPg and GAT

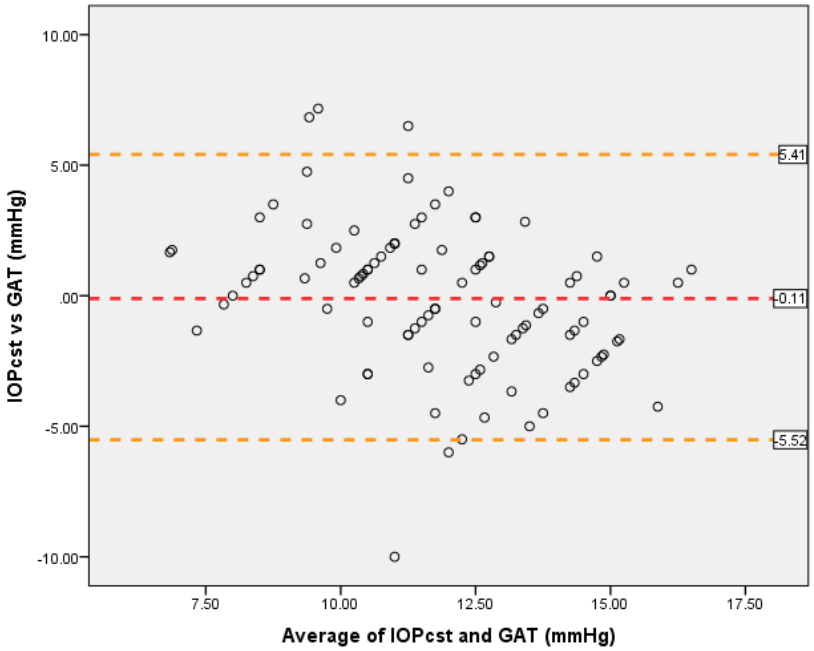


Figure 5.7 The tonometry agreement plots between IOPcst and GAT

In Figure 5.3-5.7, the Bland-Altman plots revealed that, the mean difference for paired tonometers Icare-GAT, IOPg-GAT and IOPcc-GAT were scattered in a constant pattern along the line of average IOP value. The biases were also mostly within the line of the estimated limit of agreement except for IOPcc, with a few outliers.

5.2.2 Proportionate bias of the inter-tonomery agreement

The inter-tonometry biases for CST-GAT and Tonopen-GAT pairs are noted to show a proportional bias against the mean IOP value of the two paired tonometers.(Figure 5.5 and 5.8) Further linear regression analysis was done on this relationship to predict the inter-tonometry bias based on the average IOP value. The inter-tonometry bias of Icare-GAT is found to be weak but significantly reduced by 0.24 mmHg with each 1mmHg increment of the average IOP (adjusted R^2=0.05, F=5.56, with p=0.02). Similarly, the inter-tonometry bias between CST and GAT showed a negative proportionate bias of 0.43mmHg with each 1 mmHg average IOP increment (adjusted R^2=0.11, F= 12.20 with p<0.01). Table 5.3 and 5.4 listed the estimated mean difference and limit of agreement of both tonometer pairs at various IOP average values.

Table 5.3 Estimated mean difference and 95% limit of agreement at various average Icare and GAT value

Average Icare and GAT	Icare vs GAT	Limit of Agreement (LOA)
5	1.74	-4.92 , 8.40
10	0.54	-6.12, 7.20
15	-0.66	-7.32, 6.00
20	-1.86	-8.52, 4.80
25	-3.06	-9.72, 3.60

¥values in mmHg

Table 5.4 Estimated mean difference and 95% limit of agreement at various average IOPcst and GAT value

Average IOPcst and GAT	IOPcst vs GAT	Limit of Agreement (LOA)
5	2.78	-2.74, 8.30
10	0.63	-4.89, 6.15
15	-1.52	-7.04, 4.00
20	-3.67	-9.19, 1.85
25	-5.82	-11.34,-0.30

¥values in mmHg

5.2.3 Inter-tonometry bias

This section further investigates the relationship of the demographic variables and influence of physical and corneal biomechanical variables on the inter-tonometry bias. For continuous variables such as age, CCT and corneal biomechanical parameter value, a Pearson's correlation was initially performed to investigate any significant correlation.

A one-way ANOVA with post-hoc Bonferroni test was also executed to evaluate the difference of inter-tonometry bias amongst different keratoconus severity (Amsler-Krumeich scale). Amongst all inter-tonometry bias, only Icare-GAT paired tonometer

showed significant increase in mean negative bias with increasing keratoconus severity,($F=3.70$, $p<0.05$). Whereas, the inter-tonometry biases for others are comparable amongst different keratoconus grades.

Table 5.5 listed the correlation between all continuous variables. The CCT was noted to have significant moderate and positive correlation with all inter-tonometry biases ($p<0.05$). The HcR also has significant positive correlation with all biases except for Tonopen-GAT bias ($p>0.05$). Age has significant correlation with Tonopen-GAT inter-tonometry bias only ($p<0.01$). The inter-tonometry bias of Icare-GAT showed significantly positive and moderate correlation to A2L and HcR ($p<0.05$). The IOPcc-GAT bias is significantly related to CCT, CH, A2V, HcT and HcR with weak to moderate correlation ($p<0.05$). Other than CCT, the IOPg-GAT bias is moderately and significantly correlated to A1T, A2T, A2L, A2V and HcR ($p<0.05$). For CST-GAT bias, it is positively correlated with CCT, A1T, A1L, A2V, HcT and HcR with weak to moderate relationship ($p<0.05$).

Table 5.5 Relationship of the inter-tonometry bias (value in mmHg) with age, CCTus and biomechanical parameters in keratoconic eyes

	AGE	CH	CRF	CCT	Mean K	A1T	A1L	A1V	A2T	A2L	A2V	HcT	HpD	HcR	DA
Icare-GAT	-0.05	0.18	0.21	0.41**	-0.41**	0.17	0.19	0.03	-0.03	0.33*	0.20	0.22	0.06	0.37**	-0.11
Tonopen-GAT	-0.31*	0.22	0.24	0.34*	-0.26*	0.18	0.28*	0.14	0.08	0.11	0.13	0.28*	0.07	0.23	-0.02
IOPcc-GAT	0.03	-0.13	0.02	.33*	-0.33*	0.11	0.17	0.06	-0.16	0.21	0.12	0.41**	0.24	0.27*	0.00
IOPg-GAT	-0.05	0.36**	0.55**	0.33*	-0.24	0.22	0.14	0.03	-0.21	0.24	0.26	0.28*	0.18	0.27*	-0.08
CST-GAT	0.07	.24	0.18	0.39**	-0.31*	0.18	0.26	0.07	-0.02	0.16	0.16	0.30*	0.31*	0.23	-0.01

Pearson's correlation (BCa 95% CI)
*p value <0.05, **p value<0.0

5.2.3.1 Factors affecting inter-tonometry bias

The inter-method bias can be influenced by many interactive factors. Therefore, multivariate regression analysis was employed to construct a predictive model of the inter-method bias. The robust regression analysis was chosen and further details on the method and the variables included were described in section 2.4.4.

The demographic variables (gender and age), biomechanical parameters (CRF, A1T, A1L, A1V, A2T, A2L, A2V, HpD, HcR and DA) and corneal curvature (mean K) were included in the analysis. In the analysis, the CH was excluded due to its high correlation with CRF (Pearson's correlation, r=0.77 (BCa 95% CI= 0.62, 0.89) with p<0.01). This is to reduce multi-colinearity of both variables that may violate the assumptions for a valid regression model. Ethnicity was not included in the multivariate analysis due to very small sample size in Oriental and Afro-carribean.

5.2.3.1.1 Inter-tonometry bias between Tonopen and GAT

This study established no significant contribution of all the variables studied on the predicted bias between Tonopen and GAT.

5.2.3.1.2 Inter-tonometry bias between Icare and GAT

Similar to the Tonopen and GAT paired tonometers, this analysis established no significant contribution of all the variables studied on the predicted bias between Icare and GAT.

5.2.3.1.3 Inter-tonometry bias between ORA (IOPcc) and GAT

This multivariate regression analysis established that mean K significantly accounted for 19 % of the IOPcc-GAT inter-tonometry bias (p<0.05). The mean K has unique contribution to the overall bias (β coefficient = -0.67).

5.2.3.1.4 Inter-tonometry bias between ORA (IOPg) and GAT

Linear regression analysis showed that CRF, HcR and DA could statistically predict the IOPg and GAT inter-method bias in keratoconic eyes. These factors accounted for 38% of the explained variability of the inter-tonometry bias ($F=3.80$, $p<0.01$). DA is the highest unique contribution on the variability (β coefficient = 0.57), followed by CRF (β coefficient = 0.57) and HcR (β coefficient = 0.53) with $p<0.05$. The equation for this regression model is y= 16.86(DA) + 0.76(CRF) + 1.33(HcR) + 16.21, (where y= IOPg vs GAT bias).

5.2.3.1.5 Inter-tonometry bias between CST and GAT

The inter-method IOP bias between Corvis ST and GAT was established to be significantly predicted by mean K and HpD. These factors accounted for 27% of the explained variability of the inter-tonometry bias between CST and GAT, $F=2.48$, $p<0.01$. The corneal curvature causes a negative effect on the measurement bias (β coefficient = -0.05, $p=0.012$) whilst the HpD positively contributes to the bias. The model for predicting the CST-GAT bias is y=-0.25 (Mean K) + 1.02(HpD) -48.74, (where y=CST-GAT).

5.2.4 Discussion

This chapter studied keratoconus subjects recruited from an outpatient clinic. This study adopted the Amsler-Krumeich (AK) scale to grade the severity of keratoconus. Information of subjects' refraction status, clinical findings and corneal curvature was extracted from the medical record for the purpose of classification for keratoconus severity.

In this cohort, majority of the subjects have AK scale grade 1. The study recruitment clinic is a referral centre for keratoconus treatment in the region. Thus, the majority of the advance keratoconus either had undergone keratoplasty surgery and the progressive conditions were often already treated with corneal cross-linking therapy. There are 10 eyes that could not be graded due to unavailability of the K Mean since the videokeratography instrument was faulty during their appointment. These 10 eyes have not been included.

Amongst all tonometers studied against the GAT, the Icare and IOPg showed high inter-tonometry bias. Both underestimated GAT by more than 2mmHg. There is no information on the agreement of Icare and GAT in keratoconic eyes. The IOPg was noted to underestimate IOP by GAT in keratoconus [187, 287]. The highest acceptable bias for tonometers is ±3 mmHg [21]. Thus, both Icare and IOPg are still within acceptable limit of agreement with GAT. The Tonopen, IOPcc and CST showed very good agreement with inter-tonometry bias of less than 0.5mmHg. However, in this cohort, the LOA for all tonometers exceeded the range for acceptable limit of agreement. A similar study found that the Tonopen overestimated IOP measurement by more than 3mmHg and was noted to be less dependent on CCT than GAT. [187] The study also found that the IOPcc has the least bias against GAT and is one of the most acceptable modalities for tonometry in healthy and keratoconic eyes. [187]

Assessing the Bland-Altman plots for proportionate bias shows both Icare and CST were noted to show a negative bias pattern with increasing average IOP. This indicated that with the increment of IOP, Icare and CST underestimate the GAT. No information on proportionate bias of Icare and CST against GAT was found for keratoconus subjects specifically. However, proportionate bias of Icare was noted in earlier study in normal and glaucoma subjects [281]. The authors found instead of a negative pattern, Icare further overestimates the GAT with increasing average IOP. [281] Thus, clinicians should be more caution on examining keratoconus subjects with CST and Icare. The tonometers can be an added alternative to tonometry but is not interchangeable with GAT.

The Tonopen-GAT bias is noted to be independent of all factors analysed in this study but shows no significant difference with keratoconus severity. Previous study in keratoconus subjects noted that Tonopen is independent of CCT but weakly correlated with corneal curvature [187]. The authors also showed that Icare may underestimate IOP in healthy steep cornea and overestimate IOP in normal flat corneas. This is supported by Salvetat et al. that found an inverse influence of corneal curvature and IOP on the Icare-GAT agreement in normal eyes [251]. Even though the Icare-GAT bias was noted to be significantly different according to keratoconus severity, the bias is noted to be independent of all the corneal curvature, corneal thickness and corneal biomechanical parameters. Low number of subjects in grade 2, 3 and 4 of the AK scale may contribute to the insignificant result for other tonometry pair in this study. This study found that corneal curvature has weak but significant contribution towards the variability of IOPcc-GAT and CST-GAT paired tonometers. In addition to that, HpD

also contributes to the variability of CST-GAT bias. The CRF, HcR and DA significantly affected IOPg-GAT bias only. The effect of these significant variables is weak to moderate.

These biases can be due to other factors such as location of applanation or indentation. The keratoconic corneal surface has thinned area that can be located either centrally or off-centre. This may affect tonometry especially for Tonopen, Icare and GAT as the tonometers may measure on ectatic thinned cornea or thicker corneal area. This may lead to heterogeneous readings that may affect the significance of the regression analysis. Additionally, the tonometers paired with the GAT may have unique measurement principles. Tonometry in keratoconus can be different than in normal or glaucoma subjects. The ectatic cornea may be the causative factor in itself and may not be represented by all the corneal biomechanical parameters tested in this study. Further investigation on the characteristics of the ectatic cornea in keratoconus is reported in the next section.

5.3 Corneal Biomechanical Assessment of Keratoconic Eyes

The corneal biomechanical parameters between different keratoconus classification and gender are evaluated in this section with ANOVA (with Bonferroni post-hoc test) and t-test. The relationships between continuous variables (corneal biomechanical parameters by CST and ORA, CCT, IOPcc and age) were evaluated with Pearson's correlation. Further, multivariate regression analysis was performed to measure the effect of age, central corneal thickness, CRF and IOP on each biomechanical parameter. Details of the type of analysis used were explained in item 2.4.

5.3.1 Relationship between ORA and CST biomechanical parameters

A one-way ANOVA (robust method) with post-hoc Bonferroni test was executed to evaluate the mean value of all corneal biomechanical parameters according to keratoconus severity (Amsler-Krumeich scale). Statistically significant difference was noted in all parameters except CH, CRF, A1L, A2T and HpD. From the post-hoc analysis, statistically significant increase of A1V and DA are noted with increasing keratoconus severity ($p<0.00$). Meanwhile, a statistically significant reducing trend is noted in A1T, A2L, A2V, HcT and HcR ($p<0.00$) with increasing keratoconus severity. The CCT also showed a significant reducing pattern with increasing severity ($p<0.01$).

In table 5.6, the CCT is seen to have a significantly weak to moderate correlation with CST parameters except A1V, A2L and HpD. The CH was strongly correlated with CRF ($p<0.01$). The CH and CRF was significantly correlated with all Corvis ST biomechanical parameters except for A1V, HcT, HpD and DA. The correlation of CH with HcR was strong ($r=0.76$, $p<0.01$). However, there was poor correlation between CH with A1T, A1L, A2T, A2L and A2V ($r <0.24$, $p< 0.01$).The CRF is more correlated with the CST parameters compared to CH. A moderate correlation was noted between CRF and A1T ($r=0.53$, $p<0.01$). However, the correlation of CRF with A2V, DA, A1L, A2T and A2L ($p<0.01$) are weak. Amongst the CST parameters, DA was moderately correlated with A2V and has weak correlation with A1T, A2T and HcR. The A1T and A2T have weak correlation with each other ($p<0.05$). Age has no correlation with both ORA and CST parameters in keratoconus subjects.

Table 5.6 The relationship of CCT and corneal biomechanical variables of ORA and Corvis ST in keratoconic eyes

	CCT	CH	CRF	A1T	A1L	A1V	A2T	A2L	A2V	HcT	HpD	HcR	DA
Age	0.18 (-0.11,0.44)	0.002 (-0.19,0.20)	-0.06 (-0.26,0.15)	0.13 (-0.14,0.38)	0.002 (-0.22,0.22)	0.01 (-0.29,0.31)	-0.18 (-0.44,0.20)	0.03 (-0.23,0.28)	-0.02 (-0.22,0.19)	-0.15 (-0.54,0.06)	0.20 (-0.01,0.39)	0.06 (-0.17,0.27)	0.09 (-0.13,0.30)
CCT		0.04 (-0.18,0.31)	0.20 (-0.03,0.43)	**0.58** (0.40,0.73)**	**0.32** (0.08,0.54)**	-0.16 (-0.33,0.04)	**-0.30* (-0.55,-0.01)**	0.20 (-0.40,0.44)	**0.54** (0.34,0.72)**	**0.26* (0.03,0.44)**	0.11 (-0.13,0.31)	**0.55** (0.32,0.80)**	**-0.43** (-0.63,-0.23)**
CH			**0.77** (0.62,0.89)**	-0.07 (0.31,0.22)	0.001 (-0.20,0.22)	0.17 (-0.11,0.41)	-0.09 (-0.35,0.16)	-0.13 (-0.34,0.14)	0.00 (-0.38,0.38)	-0.14 (-0.31,0.12)	0.00 (-0.28,0.25)	-0.14 (-0.34,0.14)	0.01 (-0.32,0.29)
CRF				0.17 (-0.05,0.39)	0.04 (-0.17,0.31)	0.16 (-0.12,0.41)	-0.23 (-0.49,0.02)	-0.07 (-0.31,0.20)	0.09 (-0.18,0.39)	-0.04 (-0.21,0.22)	0.02 (-0.25,0.30)	-0.08 (-0.35,0.27)	-0.05 (-0.35,0.17)
A1T					**0.30* (0.07,0.52)**	**-0.25* (-0.44,-0.05)**	**-0.41** (-0.65,0.11)**	0.08 (-0.14,0.31)	**0.47** (0.24,0.65)**	0.08 (-0.27,0.26)	0.06 (-0.22,0.31)	**0.37** (0.09,0.66)**	**-0.49** (-0.66,-0.29)**
A1L						**0.30* (0.07,0.52)**	-0.23 (-0.47,0.04)	-0.09 (-0.35,0.20)	0.24 (-0.10,0.49)	0.12 (-0.15,0.28)	0.28* (0.07,0.47)	0.20 (-0.06,0.48)	-0.22 (-0.45,0.04)
A1V							**0.27* (0.02,0.48)**	-0.15 (-0.36,0.10)	**-0.44** (-0.61,-0.23)**	0.10 (-0.16,0.41)	0.09 (-0.14,0.30)	**-0.29* (-0.50,-0.11)**	**0.52** (0.33,0.66)**
A2T								0.01 (-0.19,0.20)	**-0.41** (-0.59,-0.19)**	-0.18 (-0.43,0.31)	-0.18 (-0.43,0.12)	-0.06 (-0.41,0.21)	**0.49** (0.23,0.71)**
A2L									**0.32** (0.09,0.52)**	**0.37** (0.17,0.52)**	0.09 (-0.17,0.33)	**0.47** (0.26,0.62)**	-0.17 (-0.39,0.04)
A2V										0.11 (-0.08,0.30)	-0.01 (-0.28,0.28)	**0.57** (0.31,0.81)**	-0.83 (-0.90,-0.72)
HcT											0.07 (-0.23,0.24)	**0.27* (-0.03,0.51)**	0.09 (-0.29,0.31)
HpD												0.15 (-0.16,0.39)	-0.05 (-0.29,0.18)
HcR													**-0.56** (-0.74,-0.42)**

Pearson's correlation with bootstrapping (Bca 95% CI)

*p value <0.05, **p value<0.0

5.3.2 Factors affecting the biomechanical parameters by ORA and CST

The corneal biomechanical parameters were known to be influenced by many factors. Previous studies found that CCTus and IOPcc contribute to the variability of CST corneal biomechanical parameters factors [20]. CH and CRF by ORA are known to be affected by IOP and CCT [59, 65], as well as age and gender [65]. Thus, multivariate regression analysis was executed to evaluate the effect of age, gender, CCT, corneal curvature and intraocular pressure effect on the biomechanical parameters by ORA and Corvis ST. Enter analysis method was chosen for the best predictive model of each biomechanical parameters employed in this study.

Table 5.7 Factors affecting the biomechanical parameters by Corvis ST and ORA

	Standardised coefficient (β)					Adjusted R^2	p
	Age	Gender	CCTus	IOPcc	K		
CH	-0.11	-0.41**	0.19	-0.55**	-0.01	0.41	0.00
CRF	-0.11	0.46**	0.17	-0.12	-0.06	0.22	0.00
A1T	-0.01	-0.03	0.39 **	0.48**	0.01	0.48	0.00
A1L	-0.15	-0.13	0.24	0.02	-0.01	0.02	0.31
A1V	0.03	0.09	0.20	0.02	0.03	0.03	0.24
A2T	0.01	0.00	0.06	-0.63**	0.05	0.33	0.00
A2L	0.05	0.11	-0.05	-0.01	-0.48**	0.12	0.03
A2V	0.00	0.13	0.09	0.03	-0.72**	0.59	0.00
HcT	-0.05	0.12	-0.02	0.19	-0.32**	0.13	0.02
HpD	0.13	0.05	0.03	-0.10	-0.07	-0.06	0.93
HcR	0.08	-0.02	0.13	-0.01	-0.54**	0.38	0.00
DA	0.09	0.05	0.12	-0.25*	4.58**	0.41	0.00

** level of significance, p< 0.0 *level of significance, p< 0.05

Notes:

Adjusted R^2= the variance of the parameter affected by the predictor variables

In Table 5.7, the multivariate analyses of the effect of age, gender, CCT, IOP and mean K value on corneal biomechanical parameters are listed. Amongst the corneal biomechanics parameters, the regression analysis showed a moderate to strong predictability model for A1T, A2T, A2V, HcR, DA, CH and CRF.

Both corneal biomechanical parameters by ORA were influenced by age whilst CH was additionally affected by IOPcc. Age has no significant influence on all corneal biomechanical parameters in keratoconus subjects ($p>0.05$). The CST corneal biomechanical parameters are significantly affected by IOPcc (A1T, A2T, CH and DA) and mean K (A1V, A2L, A2V, HcT, HcR and DA), whilst CCT significantly contributed to the variability of A1T only.

5.3.3 Discussion

In recent years a rapid influx of information has developed with the advent of in vivo and ex vivo instruments to evaluate biomechanical properties of the cornea. Significant scientific interest has been focused on two commercially available air-puff tonometer that also able to measure biomechanical properties of the cornea in vivo; ORA and CST. These tono-pachymeters have been employed in studies to look at the accuracy of IOP measurements, the diagnosis of keratoconus and screening patients at risk for acquired ectasia after laser refractive surgery.

This study recruited subjects with variable keratoconus severity. However, no significant difference was noted in the ORA parameters amongst different keratoconus severities. Although, previous studies noted a lower CH and CRF in keratoconic compared to healthy eyes [19, 60, 118, 120, 288, 289], the sensitivity and specificity of these parameters to diagnose keratoconus are poor [88, 290, 291].

In this cohort female subjects were noted to have lower CH and higher CRF than males. In addition to gender influence this study found that CH was negatively influenced by IOPcc.The influence of gender was noted in a recent large population-based study of healthy British adults in Norfolk, United Kingdom [93]. Many studies that have also reported the IOPcc was inversely correlated with CH.[65, 68, 85-87] The result may be related to the state of the ectatic thinner cornea. In support of this eyes with thinner CCT as well as higher IOP values are more predisposed to having lower CH.[68] By contrast, CRF increases with rising IOP indicating that resistance against the deformation of the cornea is higher in eyes with higher IOP values.

The majority of the CST parameters showed a significant change with more advance keratoconus disease. A1V and DA are two parameters that are significantly higher with worsening of the keratoconus grade. Meanwhile, the A1T, A2L, HcT, HcR and CCT noted to be significantly reduced with more advance keratoconus grade. These findings are supported by a study that compared CST parameters of keratoconus subjects with normal subjects [292]. The keratoconic eyes have greater DA with faster corneal applanation velocity. The author postulates that due to less effective collagen fibres, the corneal mechanical strength is reduced, leading to less resistance to air pulse or indentation. Therefore, the thin ectatic cornea was applanated easily and bounced back in less time with a shorter radius change. The low A1T, A2L and HcT represent reduced time and length for the corneal applanation and indent. Low HcR value indicates a much less radius change during the air-pulse indentation.

5.4 Conclusion

In keratoconus subjects, the Tonopen, IOPcc by ORA and CST are in good agreement with GAT and can be a valid alternative for GAT. However, the wide LOA of all paired tonometers indicates that the IOP values of these tonometers are not interchangeable with GAT. The clinicians should be aware of the proportionate bias by CST and Icare against GAT when performing tonometry on keratoconus subjects with high IOP. The corneal curvature, DA, HcR and CRF are important biomechanical parameters that influence the inter-tonometry bias.

CHAPTER 6: DISCUSSION
TONOMETRY AGREEMENT AND CORNEAL BIOMECHANICS IN CLINICAL PRACTICE

The tonometer is an important screening tool for the detection of glaucoma. Tonometers are not only used by ophthalmic practitioners in eye clinics, but have expanded to optical shops for screening, and even home use for self-monitoring of the IOP. There are many techniques to measure IOP but all tonometers have features that may have a substantial and widely variable influence on IOP measurement [160]. The advent of new tonometry methods has enabled clinicians to address the limitation of previous tonometers and is welcomed by clinicians. However, it is very challenging for scientists to design a practical tonometer that can measure 'true' IOP.

Glaucoma experts recommend the use of a single type of tonometer in monitoring patients. However, in practice, this is not always achievable especially when assessing referrals, screening and making diagnosis of a wide array of corneal and ocular conditions. Furthermore, the patient may be seen at different clinics with different instruments. This study aimed to be more practical in the approach of filling the gap between the 'on paper' recommendations of tonometry with actual ophthalmic practice. Many researchers have addressed factors that influence the IOP measurement of a tonometer. However, limited information is available that addresses factors that affect tonometry agreement. This study embarked on finding out the differences between tonometers with standard tonometry (GAT), and factors that influence the agreement between tonometers.The inclusion of a new in-vivo corneal biomechanical assessment instrument, the CST, in this study has added another novel aspect to this work. The relationship between the parameters of two commercially available corneal biomechanical instruments is studied. This analysis is then extended to investigate the factors that may influence these parameters - information that is also currently lacking in the literature.

This study reports variable inter-tonometry bias, limit of agreement, proportionate and agreement-influencing factors in different ocular diagnosis. Amongst the tonometers tested, the Tonopen and CST have proven to have good agreement with GAT in all study subjects. In healthy subjects, all tonometers showed good agreement with GAT. However, the IOPcc was more susceptible to overestimate IOP in glaucoma subjects

compared others. In keratoconus, the Tonopen, IOPcc and CST are more agreeable to GAT compared to Icare and IOPg.

Even though the bias was high in the healthy eyes, the Tonopen is independent of the average IOP values. It has not shown any proportionate tonometry bias except in the keratoconus group. This may be due to the fact that both the Tonopen and GAT, share the same tonometric principle. The Tonopen is an electronic applanation tonometer that also adapts the Imbert-Fick principle [293]. It was made to be independent of the tear film effect. The small area of applanation needed plus the advantage of having the readings monitored for error may have made it less susceptible to bias [201, 202], and this may help with the agreement with GAT. The Tonopen and GAT measure the IOP by central cornea applanation. In keratoconic eyes, the location of the cone and the variability of the cornea thickness and slopes may have affected the IOP measurement by both Tonopen and GAT. This may have caused further underestimation of the GAT readings with increment of average IOP values. However, the bias is still within good agreeable limits and this supports the suggestion for Tonopen use in ectatic or irregular corneas [201]. Age is a dominant factor that affects the inter-tonometry agreement of normal and glaucoma subjects. As the aging cornea becomes more rigid it caused Tonopen to further underestimates the bias.

The CST shows a very comparable mean IOP value but slightly overestimates compared to GAT in healthy and POAG subjects. The bias increases with average IOP in normal subjects. In contrast, the CST underestimates when compared to GAT in OHT subjects. This indicates that the CST's tonometric performance is variable in different subjects. The OHT subjects have highest CCT and the corneal biomechanical parameters that indicate a high resistance feature. The air-puff pressure of the CST may have been automatically adjusted during measurement of OHT subjects and the resistant cornea caused underestimation of its reading.

Table 6.1 Summary of the inter-tonometry agreement study in all study subjects

		Icare-GAT	Tonopen-GAT	IOPcc-GAT	IOPg-GAT	CST-GAT
Normal	≤ 2 mmHg bias	Y	Y	Y	Y	Y
	±GAT	↓	↑	↑	↓	↑
	Proportionate bias	=	=	=	=	Y
	Factors	age, CCT	age, DA	IOPcc, CRF,A1T, A2V,CCT,age	IOPcc, CRF,A1T,A2 T,A2V, age	IOPcc, CRF, A1T, A2T , A2V
OHT	≤ 2 mmHg bias	Y	Y	Y	Y	Y
	±GAT	↓	↓	↑	↑	↓
	Proportionate bias	=	=	=	=	↓
	Factors	A1V, HcR, Age	None	IOP,CCT	IOP	IOP,CCT
NTG	≤ 2 mmHg bias	Y	Y	N	Y	Y
	±GAT	↓	↑	↑	↑	↑
	Proportionate bias	=	=	↑	↑	↓
	Factors	Age	Age, A2V	None	IOP, A1L	IOP, age
POAG	≤ 2 mmHg bias	Y	Y	N	Y	Y
	±GAT	↑	↑	↑	↑	↑
	Proportionate bias	↑	=	↑	↑	↓
	Factors	IOP, A2L	None	IOPcc, CRF, CCT, A2T,HcR	IOP	IOP, age
Kerato-conus	≤ 2 mmHg bias	N	Y	Y	N	Y
	±GAT	↓	↑	↑	↓	↓
	Proportionate bias	=	↓	=	=	=
	Factors	None	None	K	DA,CRF,HcR	K, HpD

Note:

↑ :overestimate

↓ : underestimate

Y : yes

N : No

= : constant bias

The ORA produces two IOP measure: the IOPcc and IOPg. The IOPcc has persistently shown an overestimation compared to GAT readings in all study groups. The bias is in accordance to the acceptable level in the normal and keratoconus sub-groups. Proportionate bias is seen in all except the keratoconus group. There is a weak influence of corneal curvature on the agreement between IOPcc and GAT but a similar effect is not seen in IOPg. IOPg overestimates compared to GAT with increasing average IOP values in the glaucoma group but an inverse finding is noted in the normal and keratoconus group. Corneal biomechanical parameters by both ORA (CRF) and CST (CRF, DA) influenced IOPg agreement with GAT readings. As the IOPcc has good agreement with GAT values, no proportionate bias and minimal effect of corneal curvature; it is proposed that IOPcc is more superior for tonometry in keratoconus subjects [187, 251].

The CST is a fairly new tono-pachymeter. At the moment, there are only a few studies available in the literature that looks into tonometry agreement with GAT in glaucoma and keratoconus sub-groups. This study reports reliable tonometry by CST in all study subjects. In normal subjects, increases in average IOP value can increase the bias. However, in keratoconus and NTG it can cause an underestimation compared with GAT values. Age can cause CST to further underestimate the GAT in NTG. In healthy and keratoconic eyes, increased CRF and CST's parameters (cornea applanation parameters and during peak corneal deformation) which indicate increase in corneal resistance; can cause similar effect. Therefore, clinicians should be aware of the bias when doing CST tonometry in elderly subjects or in patients with corneal changes such as patients that have undergone cross-linking therapy, or laser vision correction, or corneal graft or with underlying corneal scars. This would require further analysis with these types of patients to establish the effect.

This study reported a significant role of corneal applanation parameters of CST (A2T and A1T) as well as corneal deflection parameters (HcR and DA) in the agreement of tonometers amongst different glaucoma sub-groups and various keratoconus severity grades. This can be deduced as an indicator of functional corneal biomechanical parameters in these subjects. Similarly to the findings of this study, the deformation amplitude was recommended as a potential diagnostic parameter, in other studies, and deserves further research and clinical attention [294, 295]. A study comparing normal and keratoconic eyes revealed significant difference in the repeatability of several parameters and found deformation amplitude to be highly reliable [295]. Tian et al. suggested that DA to be considered as the most viable diagnostic parameter and

deserve clinical attention in healthy, glaucomatous and keratoconus subjects [294, 296].

Table 6.2 List of factors affecting corneal biomechanical parameters in all study cohorts

	Normal	OHT	NTG	POAG	Keratoconus
CH	IOP,CCT	gender, age	None	IOP, CCT, gender	IOP, gender
CRF	CCT	gender, age	IOP	CCT	gender
A1T	IOP,CCT, Age	gender, age	IOP	IOP, gender	IOP, CCT
A1L	CCT, IOP	None	None	None	None
A1V	IOP	CCT	None	IOP	None
A2T	IOP, age, CCT	IOP	None	IOP	IOP
A2L	CCT, gender	None	None	None	K
A2V	CCT, IOP	None	CCT, IOP	IOP,CCT	K
HcT	None	None	None	IOP,CCT, gender	K
HpD	None	None	None	None	None
HcR	CCT, IOP, age	None	None	CCT	K
DA	IOP, CCT, age	age, IOP	IOP	None	K, IOP

This study has reported good reliability of CST parameters in normal subjects that was in agreement with other studies [214, 294, 295, 297]. No information is yet available on the reliability of CST parameters in glaucoma subjects. In this study, the relationship between CRF and CH with CST parameters is significantly variable in different corneal diagnoses.

The CST dynamics during applanation and at maximum corneal concavity are significant additional parameters that represent other properties of corneal biomechanics. In normal and POAG subjects, the CRF is influenced by CCT whilst CH is affected more by IOP than CCT, this is in agreement with previous studies [81-83, 157]. The CST parameters are also affected by both IOP and CCT, though corneal curvature is associated with more reduced parameter value. Age affected CST parameters more in normal than any other subjects. This study has not compared the

corneal biomechanical parameters of diseased groups against an age matched healthy cohort. It is hoped further works can be done to further analyse the differences and shed more light on the characteristics of corneal biomechanical parameters in glaucoma diagnoses and keratoconus patients.

This study has successfully achieved its aims in investigating two main aspects of clinically relevant information to assist clinicians in daily practice. It has effectively assessed the intra-operator inter-tonometry agreement of the four tonometers against GAT and factors that is associated with it. This research has also extensively reported on the factors affecting tonometry agreement bias and corneal biomechanics. The study cohorts reports the findings in 3 main glaucoma diagnoses, similar studies are not currently available in the literature. Each cohort was represented by a good sample size to achieve a good power of analysis. It has abided by the proposed guidelines for agreement study, which was noted to be significantly lacking in the current literature.

This study acknowledges several limitations. The ophthalmologists performing the GAT were always experienced ophthalmologists, with at least 5 years of clinical experience, but the same ophthalmologist did not perform all GAT measurements. In fact the ophthalmologists changed every six to twelve months. This may have introduced unexpected operator bias for the tonometer. Additionally, another limitation of our study is that the inter-tonometry analysis between different pair of the 5 tonometers was not done in this study. We only performed analysis against the standard tonometer GAT.

This study did not include tear film evaluation and corneal curvature in the assessment of factors affecting the agreement for all study subjects. The tear film is known to affect the IOP measurement by GAT [75, 298]. Corneal curvature has also been reported to influence tonometry [18, 39]. These factors could be confounders and should be considered in future studies. Ethnicity was described in the demographics of subjects but it was not included as one of the variables for analysis of factors affecting tonometry agreement and corneal biomechanics. The distribution of each ethnicity listed is very small for some groups and uneven rendering regression analysis would be in-valid.

Across the study subjects, it was noted that the GAT values for POAG and normal eyes were almost similar, inspite the difference in the standard deviations which represented by variability and sample size. The POAG and NTG patients were all diagnosed and treated with antiglaucoma. This observation may highlight the effect of glaucoma medications on IOP which can influence the corneal biomechanical parameters.

Additionally, the corneal biomechanical properties may be altered due to the chronic use of prostaglandin analogue. A most recent report by Meda et al. noted this significant effect in POAG eyes[299]. The assessment of the corneal biomechanics of the glaucomatous eyes in this study may need further evaluation as to include the effect of different type of antiglaucoma and treatment duration. Prospective study looking at newly diagnosed glaucoma eyes may shed more information on the effect on the corneal biomechanics and effect on tonometry agreement.

There are additional analyses that can be achieved with the current study data. Further analysis will involve cross inter-tonometry agreement between all tonometers involved. This information will aid clinicians in assessing reliability of instruments, especially in subjects where a certain type of tonometry is not feasible. A comparative analysis of corneal biomechanical parameters between each glaucoma and keratoconus diagnosis against healthy subjects will definitely shed more information on the new CST parameters. It will enable identification of possible parameters that can characterise each ocular diagnoses. This will lead to a more focused and probable diagnostic parameters that will aid in the screening of glaucoma and keratoconus patients. Additional recruitment of moderate to severe keratoconus subjects will enable more analysis and information on certain characteristics of keratoconus progression. Future research can be done on other corneal ectasia subjects such as post refractive ectasia subjects.

In conclusion, this body of work suggests that if GAT was not used then Tonopen and CST would be recommended as reliable monitoring devices in glaucoma and keratoconus subjects due to their excellent repeatability, independence to IOP change and corneal properties. Clinicians should be cautious of CST use in keratoconus patients due to its potential to underestimate IOP. Tonopen is suitable for glaucoma screening purposes due its excellent repeatability and constant measurement with changing IOP. Age is a potential confounding effect that affects tonometry agreement with GAT. Thus, measurements in elderly should be done with caution due to it’s the tendency to underestimate GAT with high IOP. Deformation amplitude is a potential corneal biomechanical parameter in glaucoma and keratoconus subjects. It is more affected by CCT than IOP and corneal curvature. More analysis and studies should highlight its potential as a diagnostic parameter in keratoconus and glaucoma screening.

REFERENCES

1. Excellence, N.I.f.H.a.C., *Glaucoma: diagnosis and management of chronic open angle glaucoma and ocular hypertension.*, in *NICE clinical guideline 85*. 2009, National Institute for Health and Clinical Excellence: London.

2. Ophthalmologists, T.C.o.O.a.T.R.C.o., *Guidance on the referral of glaucoma suspects by community optometrists*. 2010, The College of Optometrists and The Royal College of Ophthalmologists: London.

3. Group, B.C.C. *Avoiding unnecessary referral for glaucoma: use of a repeat measurement scheme*. Quality and Productivity: Proven Case Study [Webpage] 2011 April 2016; Available from: https://www.nice.org.uk/localPractice/collection.

4. Parkins, D.J. and D.F. Edgar, *Comparison of the effectiveness of two enhanced glaucoma referral schemes.* Ophthalmic Physiol Opt, 2011. **31**(4): p. 343-52.

5. Friedenwald, J.S., *Clinical significance of ocular rigidity in relation to the tonometric measurement.* Trans Am Acad Ophthalmol Otolaryngol, 1949. **53**: p. 262-4.

6. Goldmann, H. *Applanation tonometry*. in *Transact. 2ng Glaucoma Conference*. 1957. New York: J Macy Jr Foundation.

7. Yanoff M, D.J., *Ophthalmology*. 2009, Mosby/Elsevier: New York. p. 1118.

8. Koster, W., *Beiträge zur Tonometrie und Manometrie des Auges.* Graefe's Arch Ophthalmol, 1895: p. 113-158.

9. Congdon, N.G., et al., *Central corneal thickness and corneal hysteresis associated with glaucoma damage.* Am J Ophthalmol, 2006. **141**(5): p. 868-75.

10. Francis, B.A., et al., *Effects of corneal thickness, corneal curvature, and intraocular pressure level on Goldmann applanation tonometry and dynamic contour tonometry.* Ophthalmology, 2007. **114**(1): p. 20-6.

11. Ko, Y.C., C.J.l. Liu, and W.M. Hsu, *Varying Effects of Corneal Thickness on Intraocular Pressure Measurements with Different Tonometers.* Eye, 2004. **19**(3): p. 327-332.

12. Broman, A.T., et al., *Influence of corneal structure, corneal responsiveness, and other ocular parameters on tonometric measurement of intraocular pressure.* J Glaucoma, 2007. **16**(7): p. 581-8.

13. Singh, R.P., et al., *Central corneal thickness, tonometry, and ocular dimensions in glaucoma and ocular hypertension.* J Glaucoma, 2001. **10**(3): p. 206-10.

14. Iester, M., et al., *Incorporating corneal pachymetry into the management of glaucoma.* Journal of cataract and refractive surgery, 2009. **35**(9): p. 1623-8.

15. Liu, J. and C.J. Roberts, *Influence of corneal biomechanical properties on intraocular pressure measurement: quantitative analysis.* J Cataract Refract Surg, 2005. **31**(1): p. 146-55.

16. Doughty, M.J. and M.L. Zaman, *Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach.* Surv Ophthalmol, 2000. **44**(5): p. 367-408.

17. Congdon, N.G., et al., *Central corneal thickness and corneal hysteresis associated with glaucoma damage.* American journal of ophthalmology, 2006. **141**(5): p. 868-75.

18. Chihara, E., *Assessment of true intraocular pressure: the gap between theory and practical data.* Surv Ophthalmol, 2008. **53**(3): p. 203-18.

19. Luce, D.A., *Determining in vivo biomechanical properties of the cornea with an ocular response analyzer.* Journal of cataract and refractive surgery, 2005. **31**(1): p. 156-62.

20. Huseynova, T., et al., *Corneal biomechanics as a function of intraocular pressure and pachymetry by dynamic infrared signal and Scheimpflug imaging analysis in normal eyes.* Am J Ophthalmol, 2014. **157**(4): p. 885-93.

21. Cook, J.A., et al., *Systematic review of the agreement of tonometers with Goldmann applanation tonometry.* Ophthalmology, 2012. **119**(8): p. 1552-7.

22. R Zaki, A.B., R Ismail, NA Ismail, *Statistical Methods Used to Test for Agreement of Medical Instruments Measuring Continuous Variables in Method Comparison Studies: A Systematic Review.* PLoS ONE, 2012. **7**(5: e37908).

23. McAlinden, C., J. Khadka, and K. Pesudovs, *Statistical methods for conducting agreement (comparison of clinical tests) and precision (repeatability or reproducibility) studies in optometry and ophthalmology.* Ophthalmic Physiol Opt, 2011. **31**(4): p. 330-8.

24. Roberts, C., *The cornea is not a piece of plastic.* Journal of refractive surgery, 2000. **16**(4): p. 407-13.

25. Roy, P., et al., *Computational models of the effects of hydration on corneal biomechanics and the results of radial keratotomy.* Journal of biomechanical engineering, 1996. **118**(2): p. 255-8.

26. Rabinowitz, Y.S., *Ectasia after laser in situ keratomileusis.* Current opinion in ophthalmology, 2006. **17**(5): p. 421-6.

27. Simon, G. and Q. Ren, *Biomechanical behavior of the cornea and its response to radial keratotomy.* Journal of refractive and corneal surgery, 1994. **10**(3): p. 343-51; discussion 351-6.

28. Boote, C., et al., *Collagen fibrils appear more closely packed in the prepupillary cornea: optical and biomechanical implications.* Investigative ophthalmology & visual science, 2003. **44**(7): p. 2941-8.

29. Abahussin, M., et al., *3D collagen orientation study of the human cornea using X-ray diffraction and femtosecond laser technology.* Investigative ophthalmology & visual science, 2009. **50**(11): p. 5159-64.

30. Aoyama, A., et al., *Target intraocular pressure for stability of visual field loss progression in normal-tension glaucoma.* Jpn J Ophthalmol, 2010. **54**(2): p. 117-23.

31. Elsheikh, A., D. Alhasso, and P. Rama, *Assessment of the epithelium's contribution to corneal biomechanics.* Experimental eye research, 2008. **86**(2): p. 445-51.

32. Elsheikh, A., et al., *Numerical study of the effect of corneal layered structure on ocular biomechanics.* Current eye research, 2009. **34**(1): p. 26-35.

33. Ahearne, M., et al., *Non-destructive mechanical characterisation of UVA/riboflavin crosslinked collagen hydrogels.* The British journal of ophthalmology, 2008. **92**(2): p. 268-71.

34. Albanese, A., et al., *Keratoconus, cross-link-induction, comparison between fitting exponential function and a fitting equation obtained by a mathematical model.* Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2009. **63**(9): p. 693-6.

35. Alastrue, V., et al., *Biomechanical modeling of refractive corneal surgery.* Journal of biomechanical engineering, 2006. **128**(1): p. 150-60.

36. Hjortdal, J.O., *Regional elastic performance of the human cornea.* J Biomech, 1996. **29**(7): p. 931-42.

37. Hoeltzel, D.A., et al., *Strip extensiometry for comparison of the mechanical response of bovine, rabbit, and human corneas.* J Biomech Eng, 1992. **114**(2): p. 202-15.

38. Hjortdal, J.O., *On the biomechanical properties of the cornea with particular reference to refractive surgery.* Acta Ophthalmol Scand Suppl, 1998(225): p. 1-23.

39. Orssengo, G.J. and D.C. Pye, *Determination of the true intraocular pressure and modulus of elasticity of the human cornea in vivo.* Bull Math Biol, 1999. **61**(3): p. 551-72.

40. Kesmarky, G., et al., *Plasma viscosity: a forgotten variable.* Clin Hemorheol Microcirc, 2008. **39**(1-4): p. 243-6.

41. Minns, R.J., P.D. Soden, and D.S. Jackson, *The role of the fibrous components and ground substance in the mechanical properties of biological tissues: a preliminary investigation.* J Biomech, 1973. **6**(2): p. 153-65.

42. Tanaka, E., et al., *The proteoglycan contents of the temporomandibular joint disc influence its dynamic viscoelastic properties.* J Biomed Mater Res A, 2003. **65**(3): p. 386-92.

43. Liu, W.C., et al., *Effects of eye rubbing and breath holding on corneal biomechanical properties and intraocular pressure.* Cornea, 2011. **30**(8): p. 855-60.

44. Kiroglu, Y., et al., *Giant arachnoid granulation in a patient with benign intracranial hypertension (2009: 1b).* Eur Radiol, 2009. **19**(4): p. 1046.

45. Nishimura, M., et al., *Role of chondroitin sulfate-hyaluronan interactions in the viscoelastic properties of extracellular matrices and fluids.* Biochim Biophys Acta, 1998. **1380**(1): p. 1-9.

46. Dupps, W.J., Jr. and S.E. Wilson, *Biomechanics and wound healing in the cornea.* Experimental eye research, 2006. **83**(4): p. 709-20.

47. Ogbuehi, K.C. and T.M. Almubrad, *Accuracy and reliability of the Keeler Pulsair EasyEye non-contact tonometer.* Optom Vis Sci, 2008. **85**(1): p. 61-6.

48. Daxer, A., et al., *Collagen fibrils in the human corneal stroma: structure and aging.* Invest Ophthalmol Vis Sci, 1998. **39**(3): p. 644-8.

49. Malik, N.S., et al., *Ageing of the human corneal stroma: structural and biochemical changes.* Biochim Biophys Acta, 1992. **1138**(3): p. 222-8.

50. Friedenwald, J.S. and R.D. Stiehler, *The Mechanism of Formation of the Aqueous.* Trans Am Ophthalmol Soc, 1937. **35**: p. 184-200.

51. Knox Cartwright, N.E., J.R. Tyrer, and J. Marshall, *Age-related differences in the elasticity of the human cornea.* Invest Ophthalmol Vis Sci, 2011. **52**(7): p. 4324-9.

52. Elsheikh, A., et al., *Characterization of age-related variation in corneal biomechanical properties.* J R Soc Interface, 2010. **7**(51): p. 1475-85.

53. Ytteborg, J., *Influence of bulbar compression on rigidity coefficient of human eyes, in vivo and encleated.* Acta Ophthalmol (Copenh), 1960. **38**: p. 562-77.

54. Pallikaris, I.G., et al., *Ocular rigidity in living human eyes.* Invest Ophthalmol Vis Sci, 2005. **46**(2): p. 409-14.

55. Randleman, J.B., et al., *Depth-dependent cohesive tensile strength in human donor corneas: implications for refractive surgery.* J Refract Surg, 2008. **24**(1): p. S85-9.

56. Ishii, K., et al., *Elastic hysteresis in human eyes is an age-dependent value.* Clinical & Experimental Ophthalmology, 2013. **41**(1): p. 6-11.

57. Mansouri, K., et al., *Association between corneal biomechanical properties and glaucoma severity.* American journal of ophthalmology, 2012. **153**(3): p. 419-427 e1.

58. Shen, M., et al., *Biomechanical properties of the cornea in high myopia.* Vision research, 2008. **48**(21): p. 2167-71.

59. Kotecha, A., et al., *Corneal thickness- and age-related biomechanical properties of the cornea measured with the ocular response analyzer.* Investigative ophthalmology & visual science, 2006. **47**(12): p. 5337-47.

60. Ortiz, D., et al., *Corneal biomechanical properties in normal, post-laser in situ keratomileusis, and keratoconic eyes.* Journal of cataract and refractive surgery, 2007. **33**(8): p. 1371-5.

61. Kida, T., J.H. Liu, and R.N. Weinreb, *Effects of aging on corneal biomechanical properties and their impact on 24-hour measurement of intraocular pressure.* American journal of ophthalmology, 2008. **146**(4): p. 567-572.

62. Kamiya, K., K. Shimizu, and F. Ohmoto, *The changes in corneal biomechanical parameters after phototherapeutic keratectomy in eyes with granular corneal dystrophy.* Eye, 2009. **23**(9): p. 1790-5.

63. Alio, J.L., et al., *Factors influencing corneal biomechanical changes after microincision cataract surgery and standard coaxial phacoemulsification.* Journal of cataract and refractive surgery, 2010. **36**(6): p. 890-7.

64. Hager, A., et al., *Effect of central corneal thickness and corneal hysteresis on tonometry as measured by dynamic contour tonometry, ocular response analyzer, and Goldmann tonometry in glaucomatous eyes.* Journal of glaucoma, 2008. **17**(5): p. 361-5.

65. Foster, P.J., et al., *Intraocular pressure and corneal biomechanics in an adult British population: the EPIC-Norfolk eye study.* Investigative ophthalmology & visual science, 2011. **52**(11): p. 8179-85.

66. Lee, E.S., et al., *Central corneal thickness of Korean patients with glaucoma.* Ophthalmology, 2007. **114**(5): p. 927-30.

67. Wong, T.T., et al., *The relationship of intraocular pressure with age, systolic blood pressure, and central corneal thickness in an asian population.* Invest Ophthalmol Vis Sci, 2009. **50**(9): p. 4097-102.

68. Kamiya, K., et al., *Factors affecting corneal hysteresis in normal eyes.* Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie, 2008. **246**(10): p. 1491-4.

69. Kotecha, A., *What biomechanical properties of the cornea are relevant for the clinician?* Survey of ophthalmology, 2007. **52 Suppl 2**: p. S109-14.

70. Valbon, B.F., et al., *Effects of age on corneal deformation by non-contact tonometry integrated with an ultra-high-speed (UHS) Scheimpflug camera.* Arq Bras Oftalmol, 2013. **76**(4): p. 229-32.

71. Fowler, C.W. and T.N. Dave, *Review of past and present techniques of measuring corneal topography.* Ophthalmic Physiol Opt, 1994. **14**(1): p. 49-58.

72. Medeiros, F.A. and R.N. Weinreb, *Evaluation of the influence of corneal biomechanical properties on intraocular pressure measurements using the ocular response analyzer.* J Glaucoma, 2006. **15**(5): p. 364-70.

73. Wasielica-Poslednik, J., et al., *Reproducibility of ocular response analyzer measurements and their correlation with central corneal thickness.* Graefes Arch Clin Exp Ophthalmol, 2010. **248**(11): p. 1617-22.

74. Martinez-de-la-Casa, J.M., et al., *Ocular response analyzer versus Goldmann applanation tonometry for intraocular pressure measurements.* Invest Ophthalmol Vis Sci, 2006. **47**(10): p. 4410-4.

75. Gordon, M.O., et al., *The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma.* Arch Ophthalmol, 2002. **120**(6): p. 714-20; discussion 829-30.

76. Miglior, S., et al., *Predictive Factors for Open-Angle Glaucoma among Patients with Ocular Hypertension in the European Glaucoma Prevention Study.* Ophthalmology, 2007. **114**(1): p. 3-9.

77. Leske, M.C., et al., *Predictors of long-term progression in the early manifest glaucoma trial.* Ophthalmology, 2007. **114**(11): p. 1965-72.

78. Kass, M.A., et al., *The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma.* Arch Ophthalmol, 2002. **120**(6): p. 701-13; discussion 829-30.

79. Brandt, J.D., et al., *Central corneal thickness in the Ocular Hypertension Treatment Study (OHTS).* Ophthalmology, 2001. **108**(10): p. 1779-88.

80. Shah, S., et al., *The use of the Reichert ocular response analyser to establish the relationship between ocular hysteresis, corneal resistance factor and central corneal thickness in normal eyes.* Contact Lens and Anterior Eye, 2006. **29**(5): p. 257-262.

81. Detry-Morel, M., J. Jamart, and S. Pourjavan, *Evaluation of corneal biomechanical properties with the Reichert Ocular Response Analyzer.* Eur J Ophthalmol, 2011. **21**(2): p. 138-48.

82. Fontes, B.M., et al., *Corneal biomechanical metrics in eyes with refraction of -19.00 to +9.00 D in healthy Brazilian patients.* J Refract Surg, 2008. **24**(9): p. 941-5.

83. Kamiya, K., et al., *Factors affecting corneal hysteresis in normal eyes.* Graefes Arch Clin Exp Ophthalmol, 2008. **246**(10): p. 1491-4.

84. McMonnies, C.W., *Assessing corneal hysteresis using the Ocular Response Analyzer.* Optom Vis Sci, 2012. **89**(3): p. E343-9.

85. Oncel, B., et al., *Diurnal variation of corneal biomechanics and intraocular pressure in normal subjects.* European journal of ophthalmology, 2009. **19**(5): p. 798-803.

86. Alhamad, T.A. and K.M. Meek, *Comparison of factors that influence the measurement of corneal hysteresis in vivo and in vitro.* Acta Ophthalmol, 2011. **89**(5): p. e443-50.

87. Hirneiss, C., et al., *Corneal biomechanics measured with the ocular response analyser in patients with unilateral open-angle glaucoma.* Acta ophthalmologica, 2011. **89**(2): p. e189-92.

88. Galletti, J.G., T. Pfortner, and F.F. Bonthoux, *Improved keratoconus detection by ocular response analyzer testing after consideration of corneal thickness as a confounding factor.* J Refract Surg, 2012. **28**(3): p. 202-8.

89. Lim, L., et al., *Cornea biomechanical characteristics and their correlates with refractive error in Singaporean children.* Investigative ophthalmology & visual science, 2008. **49**(9): p. 3852-7.

90. Chen, D., A.K. Lam, and P. Cho, *A pilot study on the corneal biomechanical changes in short-term orthokeratology.* Ophthalmic Physiol Opt, 2009. **29**(4): p. 464-71.

91. Lim, L., et al., *Corneal biomechanics, thickness and optic disc morphology in children with optic disc tilt.* The British journal of ophthalmology, 2008. **92**(11): p. 1461-6.

92. Cankaya, A.B., et al., *The effect of contact lens usage on corneal biomechanical parameters in myopic patients.* Cornea, 2012. **31**(7): p. 764-9.

93. Foster, P.J., et al., *Intraocular pressure and corneal biomechanics in an adult British population: the EPIC-Norfolk eye study.* Invest Ophthalmol Vis Sci, 2011. **52**(11): p. 8179-85.

94. Song, Y., et al., *Corneal hysteresis and axial length among Chinese secondary school children: the Xichang Pediatric Refractive Error Study (X-PRES) report no. 4.* Am J Ophthalmol, 2008. **145**(5): p. 819-26.

95. Altan, C., et al., *Corneal biomechanical properties and intraocular pressure measurement in patients with nanophthalmos.* The British journal of ophthalmology, 2012. **96**(6): p. 806-10.

96. Altan, C., et al., *Biomechanical properties of axially myopic cornea.* Eur J Ophthalmol, 2012. **22 Suppl 7**: p. S24-8.

97. Huang, Y., et al., *Corneal biomechanics, refractive error, and axial length in Chinese primary school children.* Investigative ophthalmology & visual science, 2011. **52**(7): p. 4923-8.

98. Gonzalez-Meijome, J.M., et al., *Intraoffice variability of corneal biomechanical parameters and intraocular pressure (IOP).* Optometry and vision science : official publication of the American Academy of Optometry, 2008. **85**(6): p. 457-62.

99. Kida, T., J.H. Liu, and R.N. Weinreb, *Effect of 24-hour corneal biomechanical changes on intraocular pressure measurement.* Investigative ophthalmology & visual science, 2006. **47**(10): p. 4422-6.

100. Laiquzzaman, M., et al., *Diurnal variation of ocular hysteresis in normal subjects: relevance in clinical context.* Clin Experiment Ophthalmol, 2006. **34**(2): p. 114-8.

101. Kida, T., J.H. Liu, and R.N. Weinreb, *Effects of aging on corneal biomechanical properties and their impact on 24-hour measurement of intraocular pressure.* Am J Ophthalmol, 2008. **146**(4): p. 567-572.

102. Shen, M., et al., *Diurnal variation of ocular hysteresis, corneal thickness, and intraocular pressure.* Optom Vis Sci, 2008. **85**(12): p. 1185-92.

103. Ehongo, A., V. De Maertelaer, and S. Pourjavan, *Effect of topical corneal anaesthesia on ocular response analyzer parameters: pilot study.* Int Ophthalmol, 2009. **29**(5): p. 325-8.

104. Firat, P.G. and S. Doganay, *Corneal hysteresis in patients with dry eye.* Eye (Lond), 2011. **25**(12): p. 1570-4.

105. Agarwal, D.R., et al., *The relationship between corneal hysteresis and the magnitude of intraocular pressure reduction with topical prostaglandin therapy.* Br J Ophthalmol, 2012. **96**(2): p. 254-7.

106. Pepose, J.S., et al., *Changes in corneal biomechanics and intraocular pressure following LASIK using static, dynamic, and noncontact tonometry.* Am J Ophthalmol, 2007. **143**(1): p. 39-47.

107. Chen, M.C., et al., *Corneal biomechanical measurements before and after laser in situ keratomileusis.* J Cataract Refract Surg, 2008. **34**(11): p. 1886-91.

108. de Medeiros, F.W., et al., *Differences in the early biomechanical effects of hyperopic and myopic laser in situ keratomileusis.* J Cataract Refract Surg, 2010. **36**(6): p. 947-53.

109. Shah, S., et al., *The use of the Ocular Response Analyser to determine corneal hysteresis in eyes before and after excimer laser refractive surgery.* Contact lens & anterior eye : the journal of the British Contact Lens Association, 2009. **32**(3): p. 123-8.

110. Shah, S. and M. Laiquzzaman, *Comparison of corneal biomechanics in pre and post-refractive surgery and keratoconic eyes by Ocular Response Analyser.* Cont Lens Anterior Eye, 2009. **32**(3): p. 129-32; quiz 151.

111. Hamilton, D.R., et al., *Differences in the corneal biomechanical effects of surface ablation compared with laser in situ keratomileusis using a microkeratome or femtosecond laser.* Journal of cataract and refractive surgery, 2008. **34**(12): p. 2049-56.

112. Gatinel, D., et al., *Corneal hysteresis, resistance factor, topography, and pachymetry after corneal lamellar flap.* J Refract Surg, 2007. **23**(1): p. 76-84.

113. Ortiz, D., et al., *Corneal biomechanical properties in normal, post-laser in situ keratomileusis, and keratoconic eyes.* J Cataract Refract Surg, 2007. **33**(8): p. 1371-5.

114. Kamiya, K., K. Shimizu, and F. Ohmoto, *Comparison of the changes in corneal biomechanical properties after photorefractive keratectomy and laser in situ keratomileusis.* Cornea, 2009. **28**(7): p. 765-9.

115. Kerautret, J., et al., *Biomechanical characteristics of the ectatic cornea.* J Cataract Refract Surg, 2008. **34**(3): p. 510-3.

116. Sporl, E., et al., *[Biomechanical condition of the cornea as a new indicator for pathological and structural changes].* Ophthalmologe, 2009. **106**(6): p. 512-20.

117. Mikielewicz, M., et al., *Air-pulse corneal applanation signal curve parameters for the characterisation of keratoconus.* Br J Ophthalmol, 2011. **95**(6): p. 793-8.

118. Fontes, B.M., et al., *Corneal biomechanical metrics and anterior segment parameters in mild keratoconus.* Ophthalmology, 2010. **117**(4): p. 673-9.

119. Saad, A., et al., *Biomechanical properties of keratoconus suspect eyes.* Invest Ophthalmol Vis Sci, 2010. **51**(6): p. 2912-6.

120. Fontes, B.M., et al., *Ocular response analyzer measurements in keratoconus with normal central corneal thickness compared with matched normal control eyes.* Journal of refractive surgery, 2011. **27**(3): p. 209-15.

121. Akhtar, S., et al., *Ultrastructural analysis of collagen fibrils and proteoglycans in keratoconus.* Acta Ophthalmol, 2008. **86**(7): p. 764-72.

122. Gefen, A., et al., *Biomechanical analysis of the keratoconic cornea.* J Mech Behav Biomed Mater, 2009. **2**(3): p. 224-36.

123. Mahmoud, A., et al., *Comparison of biomechanical and topographic parameters in normal and pathologic corneas.* Invest Ophthalmol Vis Sci, 2007. **48**: p. 1843.

124. Zhang, L., et al., *Second-generation corneal deformation signal waveform analysis in normal, forme fruste keratoconic, and manifest keratoconic corneas after statistical correction for potentially confounding factors.* J Cataract Refract Surg, 2015. **41**(10): p. 2196-204.

125. Goldich, Y., et al., *Can we measure corneal biomechanical changes after collagen cross-linking in eyes with keratoconus?--a pilot study.* Cornea, 2009. **28**(5): p. 498-502.

126. Gkika, M., et al., *Evaluation of corneal hysteresis and corneal resistance factor after corneal cross-linking for keratoconus.* Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie, 2012. **250**(4): p. 565-73.

127. Greenstein, S.A., K.L. Fry, and P.S. Hersh, *In vivo biomechanical changes after corneal collagen cross-linking for keratoconus and corneal ectasia: 1-year analysis of a randomized, controlled, clinical trial.* Cornea, 2012. **31**(1): p. 21-5.

128. Sedaghat, M., M. Naderi, and M. Zarei-Ghanavati, *Biomechanical parameters of the cornea after collagen crosslinking measured by waveform analysis.* Journal of cataract and refractive surgery, 2010. **36**(10): p. 1728-31.

129. Kurita, Y., et al., *Contact-based stiffness sensing of human eye.* IEEE Trans Biomed Eng, 2008. **55**(2 Pt 1): p. 739-45.

130. Wollensak, G., et al., *Interlamellar cohesion after corneal crosslinking using riboflavin and ultraviolet A light.* Br J Ophthalmol, 2011. **95**(6): p. 876-80.

131. Spoerl, E., et al., *Detection of biomechanical changes after corneal cross-linking using Ocular Response Analyzer software.* J Refract Surg, 2011. **27**(6): p. 452-7.

132. Hager, A., et al., *Changes in corneal hysteresis after clear corneal cataract surgery.* Am J Ophthalmol, 2007. **144**(3): p. 341-6.

133. Kucumen, R.B., et al., *Corneal biomechanical properties and intraocular pressure changes after phacoemulsification and intraocular lens implantation.* J Cataract Refract Surg, 2008. **34**(12): p. 2096-8.

134. de Freitas Valbon, B., et al., *Central corneal thickness and biomechanical changes after clear corneal phacoemulsification.* Journal of refractive surgery, 2012. **28**(3): p. 215-9.

135. del Buey, M.A., et al., *Biomechanical properties of the cornea in Fuchs' corneal dystrophy.* Invest Ophthalmol Vis Sci, 2009. **50**(7): p. 3199-202.

136. Kamiya, K., et al., *Evaluation of corneal biomechanical parameters after simultaneous phacoemulsification with intraocular lens implantation and limbal relaxing incisions.* Journal of cataract and refractive surgery, 2011. **37**(2): p. 265-70.

137. Kamiya, K., et al., *Time course of corneal biomechanical parameters after phacoemulsification with intraocular lens implantation.* Cornea, 2010. **29**(11): p. 1256-60.

138. Laiquzzaman, M., K. Tambe, and S. Shah, *Comparison of biomechanical parameters in penetrating keratoplasty and normal eyes using the Ocular Response Analyser.* Clin Experiment Ophthalmol, 2010. **38**(8): p. 758-63.

139. Lu, F., et al., *Central corneal thickness and corneal hysteresis during corneal swelling induced by contact lens wear with eye closure.* American journal of ophthalmology, 2007. **143**(4): p. 616-22.

140. Lau, W. and D. Pye, *Changes in corneal biomechanics and applanation tonometry with induced corneal swelling.* Invest Ophthalmol Vis Sci, 2011. **52**(6): p. 3207-14.

141. Goldich, Y., et al., *Variations in corneal biomechanical parameters and central corneal thickness during the menstrual cycle.* J Cataract Refract Surg, 2011. **37**(8): p. 1507-11.

142. Seymenoglu, G., et al., *Corneal biomechanical properties during the menstrual cycle.* Current eye research, 2011. **36**(5): p. 399-403.

143. Lam, A.K. and D. Chen, *Effect of ocular massage on intraocular pressure and corneal biomechanics.* Eye, 2007. **21**(9): p. 1245-6.

144. Pizzarello, L., et al., *VISION 2020: The Right to Sight: a global initiative to eliminate avoidable blindness.* Arch Ophthalmol, 2004. **122**(4): p. 615-20.

145. Congdon, N., et al., *Causes and prevalence of visual impairment among adults in the United States.* Arch Ophthalmol, 2004. **122**(4): p. 477-85.

146. Friedman, D.S., et al., *Prevalence of open-angle glaucoma among adults in the United States.* Arch Ophthalmol, 2004. **122**(4): p. 532-8.

147. Leske, M.C., et al., *Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial.* Arch Ophthalmol, 2003. **121**(1): p. 48-56.

148. Chauhan, B.C., et al., *Canadian Glaucoma Study: 3. Impact of risk factors and intraocular pressure reduction on the rates of visual field change.* Arch Ophthalmol, 2010. **128**(10): p. 1249-55.

149. Robert, Y.C., *What do we measure with various techniques when assessing IOP?* Surv Ophthalmol, 2007. **52 Suppl 2**: p. S105-8.

150. *The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. Collaborative Normal-Tension Glaucoma Study Group.* Am J Ophthalmol, 1998. **126**(4): p. 498-505.

151. Abitbol, O., et al., *Corneal hysteresis measured with the Ocular Response Analyzer in normal and glaucomatous eyes.* Acta Ophthalmol, 2010. **88**(1): p. 116-9.

152. Mansouri, K., et al., *Association between corneal biomechanical properties and glaucoma severity.* Am J Ophthalmol, 2012. **153**(3): p. 419-427 e1.

153. Bochmann, F., G.S. Ang, and A. Azuara-Blanco, *Lower corneal hysteresis in glaucoma patients with acquired pit of the optic nerve (APON).* Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie, 2008. **246**(5): p. 735-8.

154. Albon, J., et al., *Age related compliance of the lamina cribrosa in human eyes.* Br J Ophthalmol, 2000. **84**(3): p. 318-23.

155. Wells, A.P., et al., *Corneal hysteresis but not corneal thickness correlates with optic nerve surface compliance in glaucoma patients.* Invest Ophthalmol Vis Sci, 2008. **49**(8): p. 3262-8.

156. Lau, W. and D. Pye, *A clinical description of Ocular Response Analyzer measurements.* Investigative ophthalmology & visual science, 2011. **52**(6): p. 2911-6.

157. Sullivan-Mee, M., et al., *Factors Influencing Intermethod Agreement Between Goldmann Applanation, Pascal Dynamic Contour, and Ocular Response Analyzer Tonometry.* J Glaucoma, 2012. **22**(6): p. 487-95.

158. Hager, A., et al., *[The influence of corneal hysteresis and corneal resistance factor on the measurement of intraocular pressure].* Ophthalmologe, 2007. **104**(6): p. 484-9.

159. De Moraes, C.V., et al., *Lower corneal hysteresis is associated with more rapid glaucomatous visual field progression.* Journal of glaucoma, 2012. **21**(4): p. 209-13.

160. De Moraes, C.G.V., et al., *Modalities of Tonometry and their Accuracy with Respect to Corneal Thickness and Irregularities.* Journal of Optometry, 2008. **1**(2): p. 43-49.

161. Sporl, E., et al., *[Biomechanical condition of the cornea as a new indicator for pathological and structural changes].* Der Ophthalmologe : Zeitschrift der Deutschen Ophthalmologischen Gesellschaft, 2009. **106**(6): p. 512-20.

162. Iordanidou, V., et al., *Modifications in corneal biomechanics and intraocular pressure after deep sclerectomy.* Journal of glaucoma, 2010. **19**(4): p. 252-6.

163. Sun, L., et al., *Recovery of corneal hysteresis after reduction of intraocular pressure in chronic primary angle-closure glaucoma.* American journal of ophthalmology, 2009. **147**(6): p. 1061-6, 1066 e1-2.

164. Shah, S., et al., *Ocular response analyser to assess hysteresis and corneal resistance factor in low tension, open angle glaucoma and ocular hypertension.* Clin Experiment Ophthalmol, 2008. **36**(6): p. 508-13.

165. Ang, G.S., et al., *Corneal biomechanical properties in primary open angle glaucoma and normal tension glaucoma.* Journal of glaucoma, 2008. **17**(4): p. 259-62.

166. Mangouritsas, G., et al., *Association between corneal hysteresis and central corneal thickness in glaucomatous and non-glaucomatous eyes.* Acta Ophthalmol, 2009. **87**(8): p. 901-5.

167. Anand, A., et al., *Corneal hysteresis and visual field asymmetry in open angle glaucoma.* Invest Ophthalmol Vis Sci, 2010. **51**(12): p. 6514-8.

168. Zadnik, K., et al., *Biomicroscopic signs and disease severity in keratoconus. Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study Group.* Cornea, 1996. **15**(2): p. 139-46.

169. Zadnik, K., et al., *Between-eye asymmetry in keratoconus.* Cornea, 2002. **21**(7): p. 671-9.

170. Kennedy, R.H., W.M. Bourne, and J.A. Dyer, *A 48-year clinical and epidemiologic study of keratoconus.* Am J Ophthalmol, 1986. **101**(3): p. 267-73.

171. Weed, K.H., C.N. McGhee, and C.J. MacEwen, *Atypical unilateral superior keratoconus in young males.* Cont Lens Anterior Eye, 2005. **28**(4): p. 177-9.

172. Auffarth, G.U., L. Wang, and H.E. Volcker, *Keratoconus evaluation using the Orbscan Topography System.* J Cataract Refract Surg, 2000. **26**(2): p. 222-8.

173. Prisant, O., J.M. Legeais, and G. Renard, *Superior keratoconus.* Cornea, 1997. **16**(6): p. 693-4.

174. Rabinowitz, Y.S., *Keratoconus.* Surv Ophthalmol, 1998. **42**(4): p. 297-319.

175. Li, X., et al., *Longitudinal study of the normal eyes in unilateral keratoconus patients.* Ophthalmology, 2004. **111**(3): p. 440-6.

176. Georgiou, T., et al., *Influence of ethnic origin on the incidence of keratoconus and associated atopic disease in Asians and white patients.* Eye (Lond), 2004. **18**(4): p. 379-83.

177. Pearson, A.R., et al., *Does ethnic origin influence the incidence or severity of keratoconus?* Eye (Lond), 2000. **14 (Pt 4)**: p. 625-8.

178. Krumeich, J.H. and G.M. Kezirian, *Circular keratotomy to reduce astigmatism and improve vision in stage I and II keratoconus.* J Refract Surg, 2009. **25**(4): p. 357-65.

179. Amsler, M., *Keratocone classique et keratocone fruste, arguments unitaires.* Ophthalmologica, 1946. **111**(2-3): p. 96-101.

180. Maeda, N., et al., *Automated keratoconus screening with corneal topography analysis.* Invest Ophthalmol Vis Sci, 1994. **35**(6): p. 2749-57.

181. Smolek, M.K. and S.D. Klyce, *Current keratoconus detection methods compared with a neural network approach.* Invest Ophthalmol Vis Sci, 1997. **38**(11): p. 2290-9.

182. Bohm, A., et al., *[Measuring intraocular pressure in keratoconus. Effect of the changed biomechanics].* Ophthalmologe, 1997. **94**(11): p. 771-4.

183. Brooks, A.M., I.F. Robertson, and A.M. Mahoney, *Ocular rigidity and intraocular pressure in keratoconus.* Aust J Ophthalmol, 1984. **12**(4): p. 317-24.

184. Moshirfar, M., et al., *Corneal biomechanics in iatrogenic ectasia and keratoconus: A review of the literature.* Oman J Ophthalmol, 2013. **6**(1): p. 12-7.

185. Stabuc Silih, M. and M. Hawlina, *Influence of corneal thickness on comparative intraocular pressure measurements with Goldmann and non-contact tonometers in keratoconus.* Klin Monbl Augenheilkd, 2003. **220**(12): p. 843-7.

186. Read, S.A. and M.J. Collins, *Intraocular pressure in keratoconus.* Acta Ophthalmol, 2011. **89**(4): p. 358-64.

187. Mollan, S.P., et al., *Accuracy of Goldmann, ocular response analyser, Pascal and TonoPen XL tonometry in keratoconic and normal eyes.* Br J Ophthalmol, 2008. **92**(12): p. 1661-5.

188. Barreto, J., Jr., et al., *Dynamic contour tonometry and goldman applanation tonometry in eyes with keratoconus.* Clinics, 2006. **61**(6): p. 511-4.

189. Meyenberg, A., et al., *Dynamic contour tonometry in keratoconus and postkeratoplasty eyes.* Cornea, 2008. **27**(3): p. 305-10.

190. Salvetat, M.L., et al., *Corneal Deformation Parameters Provided by the Corvis-ST Pachy-Tonometer in Healthy Subjects and Glaucoma Patients.* J Glaucoma, 2015. **24**(8): p. 568-74.

191. Brusini, P., et al., *Comparison of ICare tonometer with Goldmann applanation tonometer in glaucoma patients.* J Glaucoma, 2006. **15**(3): p. 213-7.

192. Tonnu, P.A., et al., *The influence of central corneal thickness and age on intraocular pressure measured by pneumotonometry, non-contact tonometry, the Tono-Pen XL, and Goldmann applanation tonometry.* Br J Ophthalmol, 2005. **89**(7): p. 851-4.

193. Kottner, J., et al., *Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed.* J Clin Epidemiol, 2011. **64**(1): p. 96-106.

194. AlMubrad, T.M. and K.C. Ogbuehi, *The effect of repeated applanation on subsequent IOP measurements.* Clin Exp Optom, 2008. **91**(6): p. 524-9.

195. Sorensen, P.N., *The noncontact tonometer. Clinical evaluation on normal and diseased eyes.* Acta Ophthalmol (Copenh), 1975. **53**(4): p. 513-21.

196. Popovich, K.S. and M.B. Shields, *A comparison of intraocular pressure measurements with the XPERT noncontact tonometer and Goldmann applanation tonometry.* J Glaucoma, 1997. **6**(1): p. 44-6.

197. Jorge, J., et al., *Clinical performance of non-contact tonometry by Reichert AT550 in glaucomatous patients.* Ophthalmic Physiol Opt, 2003. **23**(6): p. 503-6.

198. Stocker, F.W., *On changes in intraocular pressure of the other eye while tonography is done on one eye.* Trans Am Ophthalmol Soc, 1956. **54**: p. 63-9; discussion, 69-71.

199. Moses, R.A. and C.H. Liu, *Repeated applanation tonometry.* Am J Ophthalmol, 1968. **66**(1): p. 89-91.

200. Recep, O.F., et al., *Accurate time interval in repeated tonometry.* Acta Ophthalmol Scand, 1998. **76**(5): p. 603-5.

201. Azuara-Blanco, A., et al., *Tono-Pen determination of intraocular pressure in patients with band keratopathy or glued cornea.* Br J Ophthalmol, 1998. **82**(6): p. 634-6.

202. Salvetat, M.L., et al., *Comparisons between Pascal dynamic contour tonometry, the TonoPen, and Goldmann applanation tonometry in patients with glaucoma.* Acta Ophthalmol Scand, 2007. **85**(3): p. 272-9.

203. Iester, M., et al., *New Tonopen XL: comparison with the Goldmann tonometer.* Eye (Lond), 2001. **15**(Pt 1): p. 52-8.

204. Minckler, D.S., et al., *Clinical evaluation of the Oculab Tono-Pen.* Am J Ophthalmol, 1987. **104**(2): p. 168-73.

205. Hines, M.W., B.F. Jost, and K.L. Fogelman, *Oculab Tono-Pen, Goldmann applanation tonometry, and pneumatic tonometry for intraocular pressure assessment in gas-filled eyes.* Am J Ophthalmol, 1988. **106**(2): p. 174-9.

206. Li, J., et al., *Clinical comparison of the Proview eye pressure monitor with the Goldmann applanation tonometer and the Tonopen.* Arch Ophthalmol, 2004. **122**(8): p. 1117-21.

207. Li, Y., et al., *Comparison of the Icare Tonometer and the Hand-held Goldmann Applanation Tonometer in Pediatric Aphakia.* J Glaucoma, 2012. **22**(7): p. 550-4.

208. Lambert, S.R., et al., *Rebound tonometry in children: a report by the American Academy of Ophthalmology.* Ophthalmology, 2013. **120**(4): p. e21-7.

209. Flemmons, M.S., et al., *Icare rebound tonometry in children with known and suspected glaucoma.* J AAPOS, 2011. **15**(2): p. 153-7.

210. Flemmons, M.S., et al., *Home tonometry for management of pediatric glaucoma.* Am J Ophthalmol, 2011. **152**(3): p. 470-478 e2.

211. Hong, J., et al., *A new tonometer--the Corvis ST tonometer: clinical comparison with noncontact and Goldmann applanation tonometers.* Invest Ophthalmol Vis Sci, 2013. **54**(1): p. 659-65.

212. Hon, Y. and A.K. Lam, *Corneal deformation measurement using Scheimpflug noncontact tonometry.* Optom Vis Sci, 2013. **90**(1): p. e1-8.

213. Leung, C.K., C. Ye, and R.N. Weinreb, *An ultra-high-speed Scheimpflug camera for evaluation of corneal deformation response and its impact on IOP measurement.* Invest Ophthalmol Vis Sci, 2013. **54**(4): p. 2885-92.

214. Bak-Nielsen, S., et al., *Repeatability, reproducibility, and age dependency of dynamic Scheimpflug-based pneumotonometer and its correlation with a dynamic bidirectional pneumotonometry device.* Cornea, 2015. **34**(1): p. 71-7.

215. Elsheikh, A., et al., *Assessment of the ocular response analyzer as a tool for intraocular pressure measurement.* J Biomech Eng, 2009. **131**(8): p. 081010.

216. Reinstein, D.Z., M. Gobbe, and T.J. Archer, *Ocular biomechanics: measurement parameters and terminology.* Journal of refractive surgery, 2011. **27**(6): p. 396-7.

217. Spoerl, E., et al., *Detection of biomechanical changes after corneal cross-linking using Ocular Response Analyzer software.* Journal of refractive surgery, 2011. **27**(6): p. 452-7.

218. Touboul, D., et al., *Early biomechanical keratoconus pattern measured with an ocular response analyzer: curve analysis.* Journal of cataract and refractive surgery, 2011. **37**(12): p. 2144-50.

219. Kirwan, C., M. O'Keefe, and B. Lanigan, *Corneal hysteresis and intraocular pressure measurement in children using the reichert ocular response analyzer.* Am J Ophthalmol, 2006. **142**(6): p. 990-2.

220. Terai, N., et al., *Identification of biomechanical properties of the cornea: the ocular response analyzer.* Current eye research, 2012. **37**(7): p. 553-62.

221. Kynigopoulos, M., et al., *Repeatability of intraocular pressure and corneal biomechanical properties measurements by the ocular response analyser.* Klinische Monatsblatter fur Augenheilkunde, 2008. **225**(5): p. 357-60.

222. Moreno-Montanes, J., et al., *Reproducibility and clinical relevance of the ocular response analyzer in nonoperated eyes: corneal biomechanical and tonometric implications.* Investigative ophthalmology & visual science, 2008. **49**(3): p. 968-74.

223. Lam, A.K., D. Chen, and J. Tse, *The Usefulness of Waveform Score from the Ocular Response Analyzer.* Optom Vis Sci, 2010. **87**(3): p. 195-9.

224. Karakosta, A., et al., *Choice of analytic approach for eye-specific outcomes: one eye or two?* Am J Ophthalmol, 2012. **153**(3): p. 571-579.e1.

225. Armstrong, R.A., *Statistical guidelines for the analysis of data obtained from one or both eyes.* Ophthalmic Physiol Opt, 2013. **33**(1): p. 7-14.

226. Glynn, R.J. and B. Rosner, *Regression methods when the eye is the unit of analysis.* Ophthalmic Epidemiol, 2012. **19**(3): p. 159-65.

227. Portney, G.L. and M. Krohn, *Tonography and projection perimetry. Relationship according to receiver operating characteristic curves.* Arch Ophthalmol, 1977. **95**(8): p. 1353-6.

228. Bland, J.M. and D.G. Altman, *Statistical methods for assessing agreement between two methods of clinical measurement.* Lancet, 1986. **1**(8476): p. 307-10.

229. Ahad NA, T.S., Othman AR, Yaacob CR, *Sensitivity of Normality Tests to Non-normal Data.* Sains Malaysiana, 2011. **40**(6): p. 637-641.

230. P., F.A., *Disocovering statistics using IBM SPSS statistics: And sex and drugs and rock 'n' roll*. 4th ed. 2013, London: Sage.

231. Hair J. F., B.W.C., Babin B. J., Anderson R. E., *Multivariate Data Analysis*. 7th ed. 2010, New Jersey: Pearson.

232. Efron B., T.R., *An introduction to the bootstrap*. 1993, New York: Chaapman & Hall.

233. Bartlett, J.W. and C. Frost, *Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables.* Ultrasound in Obstetrics and Gynecology, 2008. **31**(4): p. 466-475.

234. Bland, J.M. and D.G. Altman, *Measuring agreement in method comparison studies.* Stat Methods Med Res, 1999. **8**(2): p. 135-60.

235. Weinreb R.N. , B.J.D., Garway-Heath D., Medeiros F.A. , *4th Consensus Meeting: Intraocular Pressure*, in *Consensus Meeting*. 2007, World Glaucoma Association: Florida.

236. Parker, V.A., J. Herrtage, and N.J. Sarkies, *Clinical comparison of the Keeler Pulsair 3000 with Goldmann applanation tonometry.* Br J Ophthalmol, 2001. **85**(11): p. 1303-4.

237. Cohen, J., *The statistical power of abnormal-social psychological research: a review.* J Abnorm Soc Psychol, 1962. **65**: p. 145-53.

238. Giasson, C. and D. Forthomme, *Comparison of central corneal thickness measurements between optical and ultrasound pachometers.* Optom Vis Sci, 1992. **69**(3): p. 236-41.

239. Miglior, S., et al., *Intraobserver and interobserver reproducibility in the evaluation of ultrasonic pachymetry measurements of central corneal thickness.* Br J Ophthalmol, 2004. **88**(2): p. 174-7.

240. Simmons, J.P., L.D. Nelson, and U. Simonsohn, *False-Positive Psychology: Undisclosed Flexibility in Data Collection and Analysis Allows Presenting Anything as Significant.* Psychological Science, 2011. **22**(11): p. 1359-66.

241. *Population Census 2011 : Birmingham*. 2011.

242. Schweier, C., et al., *Repeatability of intraocular pressure measurements with Icare PRO rebound, Tono-Pen AVIA, and Goldmann tonometers in sitting and reclining positions.* BMC Ophthalmol, 2013. **13**: p. 44.

243. Tonnu, P.A., et al., *A comparison of four methods of tonometry: method agreement and interobserver variability.* Br J Ophthalmol, 2005. **89**(7): p. 847-50.

244. Nakakura, S., et al., *Intradevice and Interdevice Agreement Between a Rebound Tonometer, Icare PRO, and the Tonopen XL and Kowa Hand-held Applanation Tonometer When Used in the Sitting and Supine Position.* J Glaucoma, 2015. **24**(7): p. 515-21.

245. Nemeth, G., et al., *Repeatability of ocular biomechanical data measurements with a Scheimpflug-based noncontact device on normal corneas.* J Refract Surg, 2013. **29**(8): p. 558-63.

246. Ali, N.Q., D.V. Patel, and C.N. McGhee, *Biomechanical responses of healthy and keratoconic corneas measured using a noncontact scheimpflug-based tonometer.* Invest Ophthalmol Vis Sci, 2014. **55**(6): p. 3651-9.

247. Chen, X., et al., *Reliability of corneal dynamic scheimpflug analyser measurements in virgin and post-PRK eyes.* PLoS One, 2014. **9**(10): p. e109577.

248. Nemeth, G., et al., *Analysis of age-dependence of the anterior and posterior cornea with scheimpflug imaging.* J Refract Surg, 2013. **29**(5): p. 326-31.

249. Saeki, T., et al., *The efficacy of TonoLab in detecting physiological and pharmacological changes of mouse intraocular pressure--comparison with TonoPen and microneedle manometery.* Curr Eye Res, 2008. **33**(3): p. 247-52.

250. Pease, M.E., J.C. Hammond, and H.A. Quigley, *Manometric calibration and comparison of TonoLab and TonoPen tonometers in rats with experimental glaucoma and in normal mice.* J Glaucoma, 2006. **15**(6): p. 512-9.

251. Salvetat, M.L., et al., *Comparison of iCare tonometer and Goldmann applanation tonometry in normal corneas and in eyes with automated lamellar and penetrating keratoplasty.* Eye (Lond), 2011. **25**(5): p. 642-50.

252. Martinez-de-la-Casa, J.M., et al., *Performance of the rebound, noncontact and Goldmann applanation tonometers in routine clinical practice.* Acta Ophthalmol, 2011. **89**(7): p. 676-80.

253. Shin, J., et al., *The Effect of Corneal Biomechanical Properties on Rebound Tonometer in Patients With Normal-Tension Glaucoma.* American Journal of Ophthalmology, 2015. **159**(1): p. 144-154.

254. Nakamura, M., et al., *Agreement of rebound tonometer in measuring intraocular pressure with three types of applanation tonometers.* Am J Ophthalmol, 2006. **142**(2): p. 332-4.

255. Davies, L.N., et al., *Clinical evaluation of rebound tonometer.* Acta Ophthalmol Scand, 2006. **84**(2): p. 206-9.

256. Fernandes, P., et al., *Comparison of the ICare rebound tonometer with the Goldmann tonometer in a normal population.* Ophthalmic Physiol Opt, 2005. **25**(5): p. 436-40.

257. Jorge, J., et al., *Comparison of the IOPen and iCare rebound tonometers with the Goldmann tonometer in a normal population.* Ophthalmic Physiol Opt, 2010. **30**(1): p. 108-12.

258. Hagishima, M., et al., *Effect of corneal astigmatism on intraocular pressure measurement using ocular response analyzer and Goldmann applanation tonometer.* Graefes Arch Clin Exp Ophthalmol, 2010. **248**(2): p. 257-62.

259. Morita, T., et al., *Intraocular pressure measured by dynamic contour tonometer and ocular response analyzer in normal tension glaucoma.* Graefes Arch Clin Exp Ophthalmol, 2010. **248**(1): p. 73-7.

260. Carbonaro, F., et al., *Comparison of three methods of intraocular pressure measurement and their relation to central corneal thickness.* Eye (Lond), 2010. **24**(7): p. 1165-70.

261. Xu, G., D.S. Lam, and C.K. Leung, *Influence of ocular pulse amplitude on ocular response analyzer measurements.* J Glaucoma, 2011. **20**(6): p. 344-9.

262. Bandyopadhyay, M., et al., *Comparison of Goldmann applanation tonometry with the Tonopen for measuring intraocular pressure in a population-based glaucoma survey in rural West Bengal.* Ophthalmic Epidemiol, 2002. **9**(3): p. 215-24.

263. Bafa, M., et al., *Clinical comparison of the measurement of the IOP with the ocular blood flow tonometer, the Tonopen XL and the Goldmann applanation tonometer.* Acta Ophthalmol Scand, 2001. **79**(1): p. 15-8.

264. Bradfield, Y.S., et al., *Comparison of Tono-Pen and Goldmann applanation tonometers for measurement of intraocular pressure in healthy children.* J aapos, 2012. **16**(3): p. 242-8.

265. Smedowski, A., et al., *Comparison of three intraocular pressure measurement methods including biomechanical properties of the cornea.* Invest Ophthalmol Vis Sci, 2014. **55**(2): p. 666-73.

266. Reznicek, L., et al., *Evaluation of a novel Scheimpflug-based non-contact tonometer in healthy subjects and patients with ocular hypertension and glaucoma.* Br J Ophthalmol, 2013. **97**(11): p. 1410-4.

267. Kim, N.R., et al., *Comparison of goldmann applanation tonometer, noncontact tonometer, and TonoPen XL for intraocular pressure measurement in different types of glaucomatous, ocular hypertensive, and normal eyes.* Curr Eye Res, 2011. **36**(4): p. 295-300.

268. Kotecha, A., et al., *Intraocular pressure measurement precision with the Goldmann applanation, dynamic contour, and ocular response analyzer tonometers.* Ophthalmology, 2010. **117**(4): p. 730-7.

269. Ambrosio, R., Jr., et al., *Evaluation of corneal shape and biomechanics before LASIK.* International ophthalmology clinics, 2011. **51**(2): p. 11-38.

270. Nomura, H., et al., *The relationship between age and intraocular pressure in a Japanese population: the influence of central corneal thickness.* Curr Eye Res, 2002. **24**(2): p. 81-5.

271. Baek, S.U., C. Kee, and W. Suh, *Longitudinal analysis of age-related changes in intraocular pressure in South Korea.* Eye (Lond), 2015. **29**(5): p. 625-9.

272. Franco, S. and M. Lira, *Biomechanical properties of the cornea measured by the Ocular Response Analyzer and their association with intraocular pressure and the central corneal curvature.* Clinical and Experimental Optometry, 2009. **92**(6): p. 469-475.

273. Pepose, J.S., et al., *Changes in corneal biomechanics and intraocular pressure following LASIK using static, dynamic, and noncontact tonometry.* American journal of ophthalmology, 2007. **143**(1): p. 39-47.

274. Kerautret, J., et al., *Biomechanical characteristics of the ectatic cornea.* Journal of cataract and refractive surgery, 2008. **34**(3): p. 510-3.

275. Hurmeric, V., et al., *The relationship between corneal biomechanical properties and confocal microscopy findings in normal and keratoconic eyes.* Cornea, 2010. **29**(6): p. 641-9.

276. Narayanaswamy, A., et al., *Comparison of ocular response analyzer parameters in chinese subjects with primary angle-closure and primary open-angle glaucoma.* Archives of ophthalmology, 2011. **129**(4): p. 429-34.

277. Fontes, B.M., et al., *Corneal biomechanical metrics in eyes with refraction of -19.00 to +9.00 D in healthy Brazilian patients.* Journal of refractive surgery, 2008. **24**(9): p. 941-5.

278. Tejwani, S., et al., *Biomechanics of the cornea evaluated by spectral analysis of waveforms from ocular response analyzer and Corvis-ST.* PLoS One, 2014. **9**(8): p. e97591.

279. Sinha, G., et al., *IOP agreement between I-Care TA01 rebound tonometer and the Goldmann applanation tonometer in eyes with and without glaucoma.* Int Ophthalmol, 2015. **35**(1): p. 89-93.

280. Sahin, A., L. Niyaz, and N. Yildirim, *Comparison of the rebound tonometer with the Goldmann applanation tonometer in glaucoma patients.* Clin Experiment Ophthalmol, 2007. **35**(4): p. 335-9.

281. Marini, M., et al., *Comparing applanation tonometry and rebound tonometry in glaucomatous and ocular hypertensive eyes.* Eur J Ophthalmol, 2011. **21**(3): p. 258-63.

282. Rao, A., et al., *Relationship of central corneal thickness and intraocular pressure by iCare rebound tonometer.* J Glaucoma, 2014. **23**(6): p. 380-4.

283. Lau, W. and D.C. Pye, *Associations between diurnal changes in Goldmann tonometry, corneal geometry, and ocular response analyzer parameters.* Cornea, 2012. **31**(6): p. 639-44.

284. Lam, A., et al., *Comparison of IOP measurements between ORA and GAT in normal Chinese.* Optom Vis Sci, 2007. **84**(9): p. 909-14.

285. Kymes, S.M., et al., *Management of ocular hypertension: a cost-effectiveness approach from the Ocular Hypertension Treatment Study.* Am J Ophthalmol, 2006. **141**(6): p. 997-1008.

286. Salvetat, M.L., et al., *Corneal Deformation Parameters Provided by the Corvis-ST Pachy-Tonometer in Healthy Subjects and Glaucoma Patients.* J Glaucoma, 2014. **24**(8): p. 568-74.

287. Goldich, Y., et al., *Goldmann applanation tonometry versus ocular response analyzer for intraocular pressure measurements in keratoconic eyes.* Cornea, 2010. **29**(9): p. 1011-5.

288. Shah, S., et al., *Assessment of the biomechanical properties of the cornea with the ocular response analyzer in normal and keratoconic eyes.* Invest Ophthalmol Vis Sci, 2007. **48**(7): p. 3026-31.

289. Kirwan, C., M. O'Keefe, and B. Lanigan, *Corneal hysteresis and intraocular pressure measurement in children using the reichert ocular response analyzer.* American journal of ophthalmology, 2006. **142**(6): p. 990-2.

290. Wolffsohn, J.S., et al., *Changes of corneal biomechanics with keratoconus.* Cornea, 2012. **31**(8): p. 849-54.

291. Fontes, B.M., et al., *Ability of corneal biomechanical metrics and anterior segment data in the differentiation of keratoconus and healthy corneas.* Arquivos brasileiros de oftalmologia, 2010. **73**(4): p. 333-7.

292. Tian, L., et al., *Corneal biomechanical assessment using corneal visualization scheimpflug technology in keratoconic and normal eyes.* J Ophthalmol, 2014. **2014**: p. 147516.

293. Stepanik, J., *THE MACKAY - MARG TONOMETER.* Acta Ophthalmologica, 1970. **48**(6): p. 1140-1144.

294. Tian, L., et al., *Assessment of ocular biomechanics using dynamic ultra high-speed Scheimpflug imaging in keratoconic and normal eyes.* J Refract Surg, 2014. **30**(11): p. 785-91.

295. Ye, C., et al., *Variability of Corneal Deformation Response in Normal and Keratoconic Eyes.* Optom Vis Sci, 2015. **92**(7): p. e149-53.

296. Tian, L., et al., *Corneal biomechanical characteristics measured by the CorVis Scheimpflug technology in eyes with primary open-angle glaucoma and normal eyes.* Acta Ophthalmol, 2016. **94**(5): p. e317-24.

297. Yu, A.Y., et al., *Correlation between corneal biomechanical properties, applanation tonometry and direct intracameral tonometry.* The British journal of ophthalmology, 2012. **96**(5): p. 640-4.

298. Whitacre, M.M. and R. Stein, *Sources of error with use of Goldmann-type tonometers.* Surv Ophthalmol, 1993. **38**(1): p. 1-30.

299. Meda, R., et al., *The impact of chronic use of prostaglandin analogues on the biomechanical properties of the cornea in patients with primary open-angle glaucoma.* Br J Ophthalmol, 2017. **101**(2): p. 120-125.

APPENDIX

Patient Information Sheet

Assessing the agreement between different tonometers

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

What is the purpose of the study?

The measurement of the pressure inside the eye is very important in the management of a variety of eye conditions including glaucoma and corneal diseases.

While the eye pressure in the eye clinics is commonly measured using Goldmann tonometer, more technologically advanced instruments are now available for this purpose. In our study, we will investigate how these different instruments agree with each other. We will be measuring the eye pressure in patients affected by a variety eye conditions and also individuals not affected by any ocular disease.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

You will be given an option of either to complete the examination for the study during your appointment day or on a separate appointment. Each person will have measurements from the different instruments in a different order which is randomised.

Your eyes' pressure and the properties of the front window of your eyes (cornea) will be measured with devices which do not touch the surface of your eyes (non contact). The non contact instruments are Ocular Response Analyzer® (ORA) by Reichert

Technologies and Corvis ST® by Oculus Optigereate GmbH. During the examinations you will feel a puff of air going into your eyes each time we test the eye. This will not cause any pain or discomfort.

Then, we will administer one set of eye drops to numb the surface of your eyes before assessing the pressures using the instruments that will make contact with the surface of your eyes. The contact instruments are Goldmann applanation tonometer (GAT), Tono-pen and ICare. These instruments will only touch your eyes slightly and you might feel some pressure on your eyes but no pain or discomfort.

As part of your normal examination, we usually take the eye pressure measurement using the Goldmann applanation tonometry, and will do so even if you decide not to take part in the study.

The measurement duration will differ from one machine to another but would not exceed 5 minutes each. The entire process of informed consent and measurements will take no more than 40 minutes, thus it will not significantly affect your waiting time if you choose to be measured during your routine outpatient appointment.

What do I have to do?

You will not have any specific things to do while having your intraocular pressure measured.
You do not have to make any extra visits unless you choose to return on a special visit just for the tests.

What is being tested?

We will assess the pressure inside your eyes and other eye measurements using different techniques and different instruments. We will compare the measurements to those of other patients.

What are the potential side effects of the procedure?

Some of the techniques of examination do not require any eye contact and are not known to cause any side effect. Some tonometers included in our study, will involve a gentle touch on the front window (cornea) of your eyes. You should not feel any discomfort because your eyes will be anaesthetised. However, this can very rarely irritate the surface of the eye. Nevertheless, an ophthalmologist will check your eyes before you leave the clinic.

In the very rare event that you suffer any discomfort that does not settle the same day following your visit to the clinic and having the measurements, you need to be reviewed at the Eye Hospital A&E. If you cannot get back to the Eye Hospital, then you should visit your own doctor who can help.

What are the other possible disadvantages and risks of taking part?

Your appointment may take a little longer than usual, but all other treatment and follow-up arrangements are unchanged.

What are the possible benefits of taking part?

You may have no direct benefit from this study but hopefully the results will give us a better idea about the new devices to measure the eye pressure accurately.

What happens when the research study stops?

Your direct involvement in this study only lasts for the time taken to measure the pressure inside your eyes. The measurements will be kept for duration of the study until the research is completed. The data will then be destroyed.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed and documented.

Harm: In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against Sandwell & West Birmingham Hospitals NHS Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Will my taking part in the study be kept confidential?

Yes. All information and measurements are kept so you are not able to be identified, so your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

For further information about the study or should you have any concerns about your involvement please contact :

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any point. However, we would need to use the information collected up to your withdrawal.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. This information will be gathered by one of the clinical members of staff either directly from you at the time you enrol in the study or from your clinical notes at a later date. This information will be anonymised, and only clinical members of staff involved directly with your clinical care will have access to any identifiable data.

Our procedures for handling, processing, storage and destruction of your data are compliant with the *Data Protection Act 1998*. You have the right to view the data we have on record about you and to correct any errors.

With your permission we would like to inform your GP that you have participated in this study.

What will happen to my personal information?

The data will be stored in a secure environment on the Birmingham & Midland Eye Centre Site. Only members of Mr Nessim's research team will have access to the data. All data will be anonymised and only clinical members or staff involved directly with your clinical care will have access to any identifiable data. The data will be stored for 2 years and then be destroyed

What will happen to the results of the research study?

It is intended that the results of the research will be presented at scientific meetings, and published in relevant clinical and academic journals. We also feed these results back to participants through patient support groups and information in clinic. You will not be identified in any report or publication.

Who is organising and funding the research?

The Sandwell & West Birmingham Hospitals Trust is organising this study. No funds are required and no profit will be made. You will not receive any payment for participating in the study.

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the Birmingham East, North and Solihull Research Ethics Committee.

And finally …

You will be given a copy of the information sheet and a signed consent form. Thank you for taking the time to read this sheet and considering involvement in this research study

Study Number:
Patient Identification Number

CONSENT FORM: Assessing the agreement between tonometers

Chief Investigator: Mr Maged Nessim

Please initial box

1. I confirm that I have read and understand the information sheet dated 20.09.13 (version 1.7) for the above study. I have had the opportunity to consider the nformation provided, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree with my GP being informed of my participation in the study. ☐
5. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of person taking consent	Date	Signature

When completed, 1 copy for the patient, 1 for researcher site file, 1(original) to be kept in the medical notes.