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AN ANALYSIS OF TEAR BREAK UP TIME, TEAR MENISCUS HEIGHT AND AREA, THE GRADE OF KERATOCONUS AND DRY EYE SYMPTOMS IN KERATOCONIC AND NON-KERATOCONIC INDIVIDUALS

by

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ABSTRACT

Keratoconus, being one of the most common corneal disorders has various structural and functional implications. A keratoconic cornea may exhibit a variety of structural changes occurring within each individual layer, affecting the collagen structure and therefore the transparency of the cornea. Research has revealed the presence of symptoms being experienced by these patients, symptoms affecting vision as well as the comfort of the ocular system. These symptoms may be likened to those being experienced by patients who suffer from dry eye syndrome. Dry eye symptoms often occur as a result of changes within the structural and functional components of the tear system and therefore may occur in cases of keratoconus.

One of the structural alterations which has been demonstrated in previous research may be changes occurring within the tear meniscus. The tear meniscus has been shown to be a valid and reliable indicator of tear volume and has therefore been used as a diagnostic factor for dry eye. The presence of dry eye symptoms in keratoconic individuals has been documented in previous research, however, the tear meniscus dimensions have not been extensively investigated. Could it be possible that these symptoms may be related to changes within the structure of the tears, namely tear meniscus dimensions specifically?

The aim of this study was to determine whether significant differences in symptoms and tear meniscus dimensions exist between keratoconic subjects and those of control subjects. If so, could a correlation be established between the symptoms being experienced and the alterations in the tear meniscus structure? This study involves the Ocular Surface Disease Index (OSDI) questionnaire, which is a subjective measure of dry eye symptoms, giving an indication of the severity of symptoms being experienced. Two diverse types of instrumentation are utilized in order to measure the dimensions of the tear meniscus. These instruments being the Keratograph 4 and the iVue Optical Coherence Tomographer (OCT). Through the use of the Keratograph 4, non-invasive tear break up time (NTBUT) measurements and corneal topography readings were additionally measured.

The study comprised of 50 subjects, being separated into 25 keratoconic and 25 control subjects which could be used for comparison. The results of the study could be summarized as follows:

- Keratoconic subjects are shown to experience symptoms of a greater severity compared to control subjects as given by the OSDI scores.
- NTBUT measurements are not shown to be significantly different between keratoconic versus control subjects and these measurements do not seem to be correlated with the symptoms being described by the OSDI scores in the keratoconic group, however, a correlation was shown to be present in the control subject group.
- The symptoms being experienced in cases of keratoconus do not appear to suggest a link in terms of the severity of keratoconus as measured by corneal topography.
- No significant link is shown to be present between the subjective complaints of ocular symptoms and the height of the tear meniscus as measured using either instrument.
- The tear meniscus height (TMH) as measured using the Keratograph 4 as well as the OCT colour scan have not been shown to be statistically significantly different between the two different subject groups.
- The tear meniscus height as measured using the OCT where the photograph was measured displays a statistically significant difference between the two subject groups.
- The tear meniscus area (TMA) as measured using the OCT is not shown to be statistically significantly different between the two subject groups and does not show a significant correlation when compared to the symptoms being expressed by the OSDI scores.
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“Support is essential to the realization of every successful outcome” – Jeffrey Benjamin
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CHAPTER 1

INTRODUCTION
The cornea is an aspheric, transparent structure responsible for the majority of the refracting power of the eye as a result of its precise structure (Meek and Knupp, 2015). The specific layered arrangement of the cornea provides efficient transparency thereby aiding in vision and clarity (Meek and Knupp, 2015). Any disruption of this detailed structure may lead to alterations in vision as well as ocular comfort as is the case in keratoconus. Keratoconus is a non-inflammatory corneal ectasia where the cornea develops a characteristic conical shape due to the thinning taking place within the layers of the cornea (Goebels et al., 2015). Keratoconus may result in drastic effects to both functional vision as well as comfort of the ocular system. The symptomatic complaints given by these patients may be likened to those experienced by patients suffering from dry eye disease (Fink et al., 2005).

Dry eye disease often occurs due to alterations within either the structure or quality of the tear film (Ichihashi et al., 2015). Most of the tear film is contained within the tear menisci of the upper and lower lids, in contact with the globe (Chan et al., 2015). Different dimensions of the tear meniscus can be measured such as the TMH and TMA. In most cases of dry eye disease, where subjects complain of severe dry eye symptoms, these patients are shown to have a lower TMH when compared to control patients (Tung et al., 2014; Nguyen et al., 2012). Due to the similarity in symptoms being experienced by subjects with dry eye disease and those affected by keratoconus, could the same tear meniscus characteristics be obtained in cases of keratoconus due to the various changes occurring within the tear film? Could these dry eye symptoms be linked to any other objective tear film test results?

The aim of this study was to investigate whether a significant difference could be found in terms of the TMH and TMA between a keratoconic group versus a group of control subjects. These tear meniscus dimensions being obtained using two different instruments. The two specified methods include the Oculus Keratograph 4 and the iVue OCT. Another aim was to determine whether a correlation could be found between the symptoms being experienced and the tear meniscus dimensions measured as well as whether these symptoms and signs depend on the severity of keratoconus measured.

A total of 50 subjects were selected to participate in this study, being composed of 25 keratoconic and 25 control subjects. The experimental group, being the keratoconic subjects, were not confined to a single ethnic group and consisted of 15 females and 10 males ranging between the ages of 19 and 56 (mean = 25.08, standard deviation = 10.63). The keratoconic subjects consisted of patients attending the contact lens clinic at the University of Johannesburg. The control group was made up of students studying optometry at the University of Johannesburg, with an age range of 18 to 23 (mean = 19.28, standard deviation
The severity and type of symptoms being experienced were determined using the OSDI questionnaire where each subject is required to rate 12 pertinent questions according to the type, severity and effect of the symptoms experienced. The grade of keratoconus for the experimental group was determined using corneal topography obtained through the use of the Keratograph 4. A grading of keratoconus is given by the Keratograph 4 ranging from grade 1-5 where grade 5 indicates that the severity of keratoconus is at such a level that a reading could not be obtained. NTBUT measurements were obtained using the Keratograph 4 in order to determine whether a rapid break up time may be associated with the symptoms and severity of keratoconus. TMH measurements were obtained using both types of instrumentation mentioned above. Using the Keratograph 4, only one photographic image of the lower lid margin is obtained. Using the OCT, two different types of images can be measured once obtained, a photographic image of the lower lid as well as a colour scan or coloured image of the same region. TMA measurements were obtained using the OCT, specifically the colour scan.

The data collected could then be analyzed using the Statistical Package for the Social Sciences (SPSS) software package and Medcalc software as this was the software appropriately suited to the data set. The necessary statistical tests were performed including Kolmogorov-Smirnov, Shapiro-Wilk, Mann-Whitney U and various other methods of testing. Medcalc statistical software was used furthermore in order to calculate Spearman’s rank correlation, correlation scatter plots as well as to generate Bland-Altman plots.

Chapter 2 contains the literature pertaining to the important components within the study being the tear film, dry eye disease, keratoconus and the instrumentation used within this study. Chapter 3 gives a brief description of the methods used to analyze the data sets obtained. The experimental procedures and methods are explained in detail in chapter 4. Chapter 5 details the results of the study through the use of scatter plots, box and whisker plots, Bland-Altman plots and various tables signifying the results of the statistical analysis. Chapter 6 details the discussion, conclusions reached throughout the duration of the study as well as limitations and ways to improve further research. Chapter 7 includes a list of references used throughout this thesis. The appendices are included in chapter 8.
CHAPTER 2

LITERATURE REVIEW
2.1 THE TEAR FILM

2.1.1 INTRODUCTION TO THE TEAR FILM

The tear film, otherwise known as the precorneal tear layer can be described as a thin coating of fluid covering the most anterior surface of the eye (Craig et al., 2010; Montés-Micó et al., 2010; Zhou and Beuerman, 2012). A particular relationship exists between the functional components of the anterior ocular system; these functional components being the meibomian and lacrimal glands, eyelids, corneal and conjunctival epithelium as well as the tear film (Holland et al., 2013). The functional components mentioned above consist of an intertwined connection concerning the anatomy of each structure, its composition, physiology as well as the function each structure performs (Holland et al., 2013). The tear film performs a multitude of utilities including cleansing, immunologic, refractive, antimicrobial and lubricating functions (Murube, 2009).

The tear film has been defined as a trilaminar structure, being composed of three distinct strata namely, the lipid, aqueous and mucin layers which play an essential role in the health and stability of the ocular system (Craig et al., 2010; Zhou and Beuerman, 2012; Besharse and Dana, 2010). The detailed composition of the tear film maintains a smooth ocular surface necessary for the refraction of light, as well as the preservation of a healthy ocular surface by providing lubrication and a vital source of nutrients (McClellan, 1997; Paulsen and Berry, 2006; Holland et al., 2013; Korb, 2002; Zhou and Beuerman, 2012). The frontal region of the eye is subjected to an assortment of hazardous external factors; the tear film forms a specialized coating which serves to protect the exposed surface against potential pathogens, irritants, allergens or pollutants (Holland et al., 2013). The tear film therefore forms an integral component of an effective and healthy ocular system. A critical homeostatic balance exists amongst the structures forming the anterior surface, disruption or disturbance of this balance may lead to tear dysfunction resulting in severe symptoms of discomfort which may affect the quality of life of the patient involved.

2.1.2 STRUCTURE AND FUNCTION OF THE TEAR FILM

The unique trilaminar structure of the tear film can be defined as a thin, transparent layer coating the front surface of the eye, each layer has a specific biochemical composition necessary to perform a host of functions. The outermost component of the tear film, the lipid layer, can be defined as a heterogeneous mixture, which forms the smooth initial interface between the ocular surface and the external environment (Holland et al., 2013; Craig et al.,
This superficial lipid coating is principally derived from the meibomian glands which are tubule-acinar holocrine glands located within the tarsal plate of both the upper and lower lids, distributing this oily secretion across the anterior surface of the eye (Craig et al., 2010; Rolando and Zierhut, 2001; Holland et al., 2013; Stahl et al., 2012). Furthermore, the glands of Moll and Zeiss are responsible for the secretion of additional lipid components, which are released onto the eyelid margin (Korb, 2002; Stahl et al., 2012). The entire content of each meibomian gland is released due to the pressure created during the blink reflex, which results in the secretion of meibum on to the surface of the eye (Korb, 2002; Rolando and Zierhut, 2001). The meibomian glands receive innervation from both parasympathetic and sympathetic nerve fibres and this secretion is thought to be under partial hormonal control (Korb, 2002).

The thickness of the lipid layer has been estimated to range between ten and 600 nanometers, with variability amongst individuals (Craig et al., 2010). There are two distinct phases formed by the lipid layer, a polar coating which is a thin, inner region that spreads itself over the aqueous layer and is composed of free fatty acids, phospholipids as well as cerebrosides (Craig et al., 2010; Johnson and Murphy, 2004; Davidson and Kuonen, 2004). The non-polar region is located superficially, forming the outer phase of the lipid layer; it contains lipids such as wax esters, triglycerides, sterol esters and hydrocarbons (Craig et al., 2010; Stahl et al., 2012). These two layers form an integrated mixture, with each component contributing to the specific homeostatic balance which exists within the structure of the tear film.

There are many important functions performed by the lipid layer, it acts as a hydrophobic barrier in order to prevent excess evaporation of tears, unnecessary evaporation may lead to instability of the tear film which in turn would alter the effectiveness of the visual system (Johnson and Murphy, 2004; Craig et al., 2010; Korb, 2002). This specialized layer is also responsible for enhancing the spreading of the tear film over the ocular surface, preventing the tears from spilling over the margin of the eyelids and serves to prevent sebaceous lipids from entering and altering the composition of the tear film (Johnson and Murphy, 2004; Craig et al., 2010; Stahl et al., 2012; Korb, 2002; Foulks, 2007).

The middle region of this three layered structure is known as the aqueous layer and makes up the thickest portion of the tear film (Stahl et al., 2012; Craig et al., 2010). This watery layer is predominantly secreted by the main lacrimal gland which is a compound tubuloalveolar gland, with additional secretions from the accessory glands of Krause and Wolfring (Besharse and Dana, 2010; Rolando and Zierhut, 2001; Korb, 2002). The volume of
aqueous is constantly being replenished by these specified glands, in order to maintain 
homeostasis (Holland et al., 2013). The aqueous phase is composed of a variety of elements 
such as electrolytes, proteins, immunoglobulins, metabolites and vitamins (Johnson and 
Murphy, 2004; Korb, 2002). The osmolality of the tears is maintained by the concentration of 
electrolytes present, thereby maintaining the veracity of the epithelium (Korb, 2002). Some 
of these electrolytes include potassium, sodium, chloride and magnesium, any increase in the 
concentration of these components would result in damage to the surface of the ocular system 
due to hyperosmolarity (Craig et al., 2010). The aqueous layer also contains important 
antimicrobial components, namely lysozyme, lactoferrin and lipocalin (Besharse and Dana, 
2010; Stahl et al., 2012).

Lysozyme is one of the most important bacteriolytic enzymes present within the tears, 
it is a long-chain, glycolytic enzyme which is considered to be the first defence mechanism 
against potential ocular pathogens (Davidson and Kuonen, 2004). The antimicrobial activity 
of lysozyme has been shown to kill gram-positive bacteria via catalytic hydrolysis of the 
peptidoglycans present within the bacterial cell wall and constitutes approximately 20-40 
percent of the entire protein content found in basal as well as reflex tears (Davidson and 
Kuonen, 2004; McDermott, 2013). Due to the destructive effects on the cell wall, an 
unwavering osmotic environment is unable to be maintained and therefore lysis of the 
organism takes place (McDermott, 2013).

Lactoferrin exhibits an extensive antimicrobial spectrum; being effective against both 
gram-positive and gram-negative bacteria as well as parasites, fungi, yeasts and viruses (Zhou 
and Beuerman, 2012). The volume of lactoferrin present within the tears is similar to that of 
lysozyme, representing approximately 21 percent of the total protein content within the tears 
and is also found to be present in other biological fluids such as nasal discharge and saliva 
(McDermott, 2013; Korb, 2002). The destructive nature of lactoferrin results in the cell 
membrane of the invading organism being disrupted thereby depriving the organism of 
essential nutrients (Dartt et al., 2011A). Lactoferrin has the ability to bind iron which in turn 
inhibits the invading organism from replicating and thereby halts further activity of the 
specific pathogen (Korb, 2002).

Lipocalin is a specific tear constituent secreted by the main lacrimal gland, accounting 
for approximately 25 percent of the total protein content of the tears (McDermott, 2013). 
Lipocalin functions similarly to lactoferrin in that it halts the ability of the organism to bind 
iron molecules which results in a bacteriostatic effect (Dartt et al., 2011A; McDermott, 
2013). In addition to these activities, lipocalin has the capability of binding other constituents
which may have an effect on the immune system such as Immunoglobulin A, Immunoglobulin G, copper and endogenous lipid molecules (Davidson and Kuonen, 2004; Korb, 2002).

Secretory Immunoglobulin A (sIgA) is another important defence system present within the constituents of the tears; it functions to protect the eye against infection, infestation, attachment and colonization of pathogenic organisms (Dartt et al., 2011A; Davidson and Kuonen, 2004). This major immunoglobulin is primarily produced by the plasma cells within the lacrimal glands of the ocular system with additional amounts being produced by specialized conjunctival tissue (Dartt et al., 2011A). sIgA, after being produced by the plasma cells; is bound to a secretory component at the surface of the plasma cell which aids in stabilizing sIgA (McDermott, 2013). The complex being formed by sIgA and the secretory component is then endocytosed and transported across the cell to be secreted into the tear film (McClellan, 1997; McDermott, 2013). sIgA has various mechanisms of action, it has the ability to cause aggregation of molecules causing these molecules to be confined within the tear film, it may also cause neutralization of the molecule, thereby inhibiting the molecule from binding to a specific host cell (Dartt et al., 2011A).

The third and innermost component of the tear film is the mucous layer that can be defined as a glycocalyx gel consisting of a heterogenous group of glycoproteins which function to prevent adherence and interaction of foreign microbes (Holland et al., 2013). This mucin layer is composed of urea, salts, glucose, enzymes and is viscoelastic in nature therefore allowing the aqueous component to spread out uniformly, compensating for any imperfections or gaps within the film (Davidson and Kuonen, 2004; Rolando and Zierhut, 2001). Mucin is secreted into the tear film through conjunctival goblet cells as well as the lacrimal glands (Rolando and Zierhut, 2001; Holland et al., 2013). This specialized glycocalyx gel functions to provide viscosity as well as lowering the surface tension to allow for unvarying lubrication of the hydrophobic ocular surface, this is achieved through the firm attachment of the mucin layer to the corneal epithelium (Holland et al., 2013; Korb, 2002). The mucous layer is also responsible for forming a greasy coating over unwanted foreign bodies in order to protect the anterior ocular structures from damage due to abrasion and the force of blinking (Korb, 2002; Craig et al., 2010).

Ocular mucins play a major role in stabilization of the tear film; instability may result from epithelial cell loss, a reduction in aqueous production or damage to the glycocalyx which causes a build-up of mucous on the anterior surface of the ocular system (Davidson and Kuonen, 2004). These mucins may be broken up into two broad categories namely;
secretory or membrane-bound mucins (Davidson and Kuonen, 2004). Secretory mucins can be seen in the form of secretory granules which are stored and only released when exposed to an appropriate stimulus whereas membrane-bound mucins form a supportive structure onto which the mucous layer is anchored (Besharse and Dana, 2010).

2.1.3 THE TEAR MENISCI

The tear menisci may be defined as the region of tears found to be present at the margin of both the upper and lower lids and is responsible for the majority of the volume of tears present within the ocular system (Benitez-del-Castillo and Lemp, 2013). The tear meniscus contains approximately 75-90% of the entire volume of tears and is therefore a vital component when trying to determine the volume of the tear film (Santodomingo-Rubido and Wolffsohn, 2006). Upon blinking, tears are redistributed, originating from the tear menisci and spreading across the anterior surface of the eye (Herranz and Corrales Herran, 2012). Various dimensions of the tear film may be investigated including the TMH, TMA, depth as well as its curvature. The volume of tears contained within the tear menisci provide an accurate indication as to whether the tear film lacks in quantity which may result in symptoms of dry eye. Structural failure of the tear film may result in symptoms such as foreign body sensation, burning, reflex tearing as well as visual distortion (Wang et al., 2006). TMH has been used as a valuable indicator when attempting to determine whether a subject may suffer from dry eye disease (Srinivasan et al., 2007; Dogru et al., 2006).

There are various methods available when attempting to determine the height of the tear meniscus, some of which include a slitlamp graticule, optical coherence tomography, a Keratograph, a meniscometer, a Tearscope as well as various image capture techniques (Srinivasan et al., 2007; Santodomingo-Rubido and Wolffsohn, 2006; Oguz et al., 2000; Arriola-Villabos et al., 2015; Ng et al., 2014). Both superior and inferior TMH may be measured, however, in most cases, measurements of the superior tear meniscus may be complicated due to the curvature of the top lid (Johnson and Murphy, 2005; Johnson and Murphy, 2006). In cases where both superior and inferior tear menisci are measured, it has been found that the difference between these two menisci is minimal (Wang et al., 2006; Chen et al., 2009). Other studies, however, find a significant difference in the TMH of the upper and lower lids (Shen et al., 2009). The inferior tear meniscus is more commonly measured due to the ease of observation and measurement (Johnson and Murphy, 2005; Johnson and Murphy, 2006). In general, the inferior TMH in normal individuals tends to be approximately 130 µm according to Santodomingo-Rubido and Wolffsohn (2006). The study
by Bitton et al., (2007) presented mean TMH measurements of between 240 and 250 µm. The study by Nguyen et al. (2012) where the lower TMH is measured using the OCT, the mean values obtained ranged between 140 and 250 µm. The study by Uchida et al., (2007) utilized the Tearscope interference device in order to determine the inferior TMH, this study found a mean TMH in normal subjects of 220 µm.

The quantity of the tear film is often an indication of the presence of dry eye disease, a reduction in the quantity of the tear film may often result in symptoms of dry eye disease (Srinivasan et al., 2007; Pine et al., 2015). The study by Nguyen et al. (2012), however, has shown the presence of a negative correlation between lower TMA measurements and symptom scores described by the Dry Eye Questionnaire (DEQ). Lower TMH measurements have been shown to be present in cases of dry eye (Ibrahim et al., 2010). In the study by Arriola-Villalobos et al. (2015), it was determined that lower TMH may be utilized as a diagnostic criteria for dry eye, aqueous deficient dry eye specifically. In a study performed by Miller et al. (2004), the TMH measurements of contact lens and non-contact lens wearers were compared and it was found that contact lens wear does not seem to play a significant role in the height of the tear meniscus. The study by Uchida et al., (2007) found the inferior TMH to be significantly lower in dry eye subjects compared to the normal population. The same findings were established when measured using the OCT, in the study performed by Yuan et al., (2010). In the study performed by Stahl et al., (2006) the inferior TMH was shown to be higher in the experimental group versus the control group, with the experimental group consisting of subjects with nasolacrimal duct obstruction.

As can be observed in various items of research, the measurement of tear meniscus dimensions serves as a valuable diagnostic tool when diagnosing various types of dry eye disease (Santodomingo-Rubido and Wolffsohn, 2006; Yuan et al., 2010; Johnson and Murphy, 2005; Fukuda et al., 2013). These dimensions serve as a valued technique when assessing the tear function and structure in clinical examinations.

2.1.4 TEAR SECRETION

Tear secretion is a very complex and intricate process involving specific structures within the ocular system. An intricate neural loop exists between the central nervous system, the lacrimal functional unit and autonomic, sensory as well as motor nerves (Maskin and Thomas, 2007). The lacrimal glands secrete tears after receiving stimulatory information from the ocular surface via this neural loop (Bartlett and Jaanus, 2008). The ocular surface, more specifically the cornea, is richly innervated with nerve fibres. Extraneous stimuli such
as dust or irritants as well as emotional stimuli may result in stimulation of the nerve fibres within the cornea (Maskin and Thomas, 2007). These nerve fibres found within the cornea, then synapse with efferent nerve fibres in the brainstem, sending commands to the lacrimal and meibomian glands thereby resulting in production of tears (Maskin and Thomas, 2007; Bartlett and Jaanus, 2008). At the same time, a stimulatory message is sent to the eyelid muscles in order to stimulate the blink reflex (Maskin and Thomas, 2007). Alterations in tear production, which may result in hypo or hypersecretion of tears may lead to ocular surface inflammation causing dry eye disease. Hyposcretion can occur as a result of various medications which may induce symptoms such as tear deficiency; some of these drugs may include diuretics, antihistamines, antidepressants as well as antipsychotic agents (Korb, 2002). Conversely, hypersecretion is a rare ailment which may lead to tear instability where a patient experiences dry eye symptoms, however, the eyes are constantly lubricated (Korb, 2002). It has been postulated that a significant relationship exists between the production and drainage of the tears (Paulsen et al., 2003).

2.1.5 ELIMINATION OF THE TEARS

Tears are eliminated from the eye by a process known as tear drainage, there are numerous mechanisms in which tear fluid may be circulated towards the lacrimal puncta in order for drainage to take place (Dartt et al., 2011A; Korb, 2002). These mechanisms include the force of gravity causing the tear fluid to be moved in a downward direction, capillary attraction which forces tears to migrate towards the puncta as well as movement of the tear film produced by the act of blinking (Korb, 2002). The lacrimal puncta may be defined as small, circular openings located on the inner surface of the lid, the diameter of these apertures may be altered during the process of tear drainage (Lee and Higginbotham, 1999). A large volume of tears can be lost through the course of evaporation with the remainder being eliminated via this process of drainage (Dartt et al., 2011A). The lacrimal system consists of the punctum, canaliculi, the lacrimal sac and the lacrimal duct (Yanoff et al., 2009). There is a specific sequence in which tears may be drained, initially passing through the puncta, into the canaliculi, flowing into the lacrimal sac and finally flowing through the lacrimal ducts (Dartt et al., 2011A; Korb, 2002; Yanoff et al., 2009). The puncta lead to tiny structures known as canaliculi, these miniature canals are lined with stratified squamous epithelium and may be broken up into two separate parts which can be seen to span a vertical path before turning medially to form horizontal canals (Bye et al., 2013). These two regions then converge to form one common canal, leading towards the nasolacrimal sac (Bye et al., 2013). From the
nasolacrimal sac, tears move through the nasolacrimal ducts which are situated within the osseous canal, following a lateral path and exiting into the nose (Bye et al., 2013).

Tears are propelled into the puncta by way of the muscle action taking place within the orbicularis fibres which are in contact with the punctum (Korb, 2002). The contraction of this muscle draws the puncta in a nasal direction, resulting in compression, this compression leads to a build-up of fluid which shortens the canaliculi thereby pumping the tears into the lacrimal sac (Korb, 2002; Önerci, 2013). From the lacrimal sac, drainage of tears occurs through the nasolacrimal ducts, where the tears pass through these ducts leading into the inferior meatus of the nose (Paulsen et al., 2003; Craig et al., 2010). The nasolacrimal ducts are composed of a double layer of specialized epithelial cells with the superficial portion being columnar epithelium while the underlying layer is composed of basal cells (Paulsen et al., 2003). The contraction of the orbicularis muscle resulting in the alternation of negative and positive pressure within the lacrimal sac has been termed the lacrimal pump, allowing the contents of the sac to be released under the inferior turbinate within the nose (Davson, 2012). The presence of a specific valve within the lacrimal system namely the valve of Hasner prevents retrograde flow of tears within the lacrimal system (Nerad, 2012).

2.1.6 TYPES OF TEARS

Tears may be divided into three broad categories namely, basal, reflex and psychic tears; these secretions are determined by interactions between the endocrine glands and the nervous system (Johnson and Murphy, 2004). The accessory glands of Krause and Wolfring are responsible for the secretion of basal tears which are redistributed by the process of blinking and provide a thin film of fluid over the corneal surface to aid in maintaining homeostasis (Hamrah and Pavan-Langston, 2008; Murube, 2009). Reflex tears on the other hand, are produced by the actions of the main lacrimal glands (Hamrah and Pavan-Langston, 2008). Located within the superior fornices are the accessory glands of Krause and Wolfring, however, the neural stimulation to these specific glands is unknown (Holland and Mannis, 2006). The lacrimal gland is located superotemporal relative to the globe, where the contents are drained into particular ducts positioned within the superior temporal fornix (Holland and Mannis, 2006). Secretion of tears from these specialized sebaceous glands occurs in response to parasympathetic stimulation via the facial nerve (Holland and Mannis, 2006). The average volume of basal tears secreted by these accessory glands varies from five to nine microliters, depending on the patient (Hamrah and Pavan-Langston, 2008). This basal secretion has a flow rate of approximately 0.5 to 2.2 microlitres per minute (Hamrah and Pavan-Langston,
Reflex tears may be seen to decrease with increasing age, however, basal tears are said to be maintained throughout life, not being subjected to the effects of aging (Hamrah and Pavan-Langston, 2008). Reflex tears can be seen as tears which overflow the ocular surface in response to nervous stimulation and may be further subdivided into peripheral sensory and central sensory subtypes (Vingerhoets and Cornelius, 2012). Reflex tearing occurs in response to irritants reaching the ocular surface; the stimulus occurring in response to these irritants is then transmitted via the trigeminal nerve to the parasympathetic branch of the autonomic nuclei which connect to the facial nerve thereby stimulating the lacrimal gland (Holland and Mannis, 2006).

The volume of tears contained within the ocular system may be affected by countless changes which may take place in the outer surrounding environment, some of which include light, wind, temperature as well as humidity (Cavallotti and Cerulli, 2008). The cornea contains a rich supply of nerve endings which have the ability to detect subtle changes occurring within the external environment and in turn, responds to these changes accordingly (Schrage et al., 2011). The ocular system responds in the following manner, the blink reflex occurs in order to counteract the change taking place, there is dilation of conjunctival vessels and in addition to this, there is reflex tearing which causes an increase in tear volume (Schrage et al., 2011).

2.1.7 INNERVATION

Tear secretion is said to be under partial autonomic neural control, the lacrimal gland receives innervation from both the parasympathetic and the sympathetic nervous systems and sensory nerves are also found to be present (Tomlinson and Khanal, 2005; Dartt et al., 2011A). The majority of the cells present in the lacrimal system receive innervation, however, there are certain populations of cells which do not receive direct innervation (Dartt et al., 2011A). These cells present within the lacrimal system still retain the ability to respond to neural stimulation due to the presence of gap junctions which allow for the chemical and electrical connection between cells (Dartt et al., 2011A). There are specific neurotransmitters contained within both the parasympathetic and sympathetic nerves situated within the lacrimal gland. The parasympathetic nerves contain acetylcholine and vasoactive intestinal peptide while the sympathetic nerves contain norepinephrine and neuropeptide Y (Dartt et al., 2011A). Acetylcholine results in stimulation of the lacrimal gland resulting in secretion of water, electrolytes as well as proteins while norepinephrine stimulates secretion of proteins via the lacrimal gland (Dartt et al., 2011A). The stimulation responsible for signaling the
lacrimal gland to secrete the necessary components originates from stimulation of the afferent sensory nerves residing within the cornea (Dartt et al., 2011A). By way of a neural reflex arc, a stimulus at the surface of the eye will result in the activation of sympathetic and parasympathetic nerves, causing the release of neurotransmitters (Dartt et al., 2011A). The process of signal transduction occurs once the neurotransmitters have interacted with specified receptors on the cell surface causing a flow of intracellular events (Dartt et al., 2011A).

2.1.8 TEAR FILM DYSFUNCTION

Dysfunction of the tear film results from any deficiencies in the tear film composition, systemic diseases affecting the ocular surface, inefficient distribution of the tear film as well as any medications affecting the ocular surface (Rolando and Zierhut, 2001). Deficiencies may be present in any of the three layers of the tear film; aqueous deficiency being one of the subtypes of dry eye (Rolando and Zierhut, 2001). Changes in the composition of the aqueous layer will result in injury to the ocular surface, factors such as reduction of growth factors, stimulation of pro-inflammatory cytokines and amplified electrolyte concentration (Rolando and Zierhut, 2001). Certain cases of tear film dysfunction may exhibit tear volumes which are normal, however, the composition of the tears may be thick and foamy, lipid deficiencies may occur due to alterations in the functioning of the meibomian glands such as obstruction (Rolando and Zierhut, 2001). Disturbances in the mucin layer may occur due to vitamin A deficiency, reduction in vitamin A levels can affect the function of the goblet cells leading to loss of glycoproteins (Rolando and Zierhut, 2001; Berman, 1991). Dry eye syndromes considered to be mucin deficient may also include conditions such as Stevens-Johnson syndrome and cicatricial pemphigoid, where the composition of mucin is said to be altered due to the disease process.

There are various systemic diseases which may alter the tear film, causing a destructive effect on the ocular surface. Most of these conditions are those which have an association with connective tissues such as rheumatoid arthritis, scleroderma, Sjögren’s syndrome and many others (Rolando and Zierhut, 2001). In patients presenting with systemic diseases such as these, an assessment of the tear film should be done and in most cases, some form of tear dysfunction will be found.

Due to the importance of an effective and healthy tear film, various methods have been developed in order to assess the numerous components of this tear layer. The tests used to assess the tear film may be broken up into different categories depending on the variables
being tested, these variables include tear production, tear distribution, tear volume and tear evaporation (Tomlinson and Khanal, 2005). These various techniques can assess one of two variables, either tear quality or tear quantity, both of which are essential to the function of the tear layer (Hamrah and Pavan-Langston, 2008).

2.1.9 TEAR FILM TESTS

The Schirmer I test is one of the most common methods utilized in clinical practice, to determine the secretion of tears (Hamrah and Pavan-Langston, 2008). The secretion of both basal and reflex tearing is assessed using the Schirmer techniques in which filter paper strips are placed over the lower palpebral conjunctiva where the strip would be in contact with tear fluid (Hamrah and Pavan-Langston, 2008; McGinnigle et al., 2012). The strip is held in a stable position by indenting the bottom end of the strip over the lower lid region. After approximately five minutes, the strip is removed and the region of wetting which can easily be observed, is measured in order to determine whether these values fall within the norm for the general population (Hamrah and Pavan-Langston, 2008). While the Schirmer I test may be useful, a lack of standardization and resultant reflex tearing due to its invasive nature may result in the Schirmer I test not giving an accurate indication of the volume of tear film present (Khanal et al., 2008). A variation of this method is known as Schirmer 2, where an anaesthetic drop is instilled prior to the use of specialized filter paper. This topical anaesthetic is thought to inhibit secretion from the main lacrimal gland and therefore, only the basal tear secretion is measured with this technique (Newman et al., 2008).

Another technique used to measure tear quantity, in which the mechanism is very similar to that of the Schirmer test, is known as the phenol red thread test. This method utilizes a cotton thread with a specialized phenol coating which is placed into the lower conjunctival fornix in contact with the ocular surface (Goyal, 2013). The specialized phenol coating is susceptible to changes in pH conditions, exhibiting a colour change when saturated with fluid, more specifically the tears of the ocular surface (Goyal, 2013). After being in contact with the surface of the eye for approximately 15 seconds, the length of altered colour along the span of the thread can be measured in millimetres (Goyal, 2013). The length along which a colour change is observed is generally between nine and 20 millimetres, indicating a healthy ocular tear film (Goyal, 2013).

Tear break up time (TBUT) is a method which can be utilized to assess the quality of the tear film; these tests can either be invasive or non-invasive. The more invasive form of this test involves instillation of sodium fluorescein dye which may induce reflex tearing
thereby disrupting the ocular surface and altering the tear film. While viewing the ocular surface using a slit lamp biomicroscope and a cobalt blue filter, the stability of the tear film may be assessed by determining the time at which the tear film exhibits its first dry or hypofluorescent spot (Cohen et al., 2006; Doughty, 2014; Ramos et al., 2014). The appearance of dry spots indicates the instability of the tear film, the values given by TBUT should range between 15 and 30 seconds with any value less than ten seconds indicating some form of tear deficiency (Cohen et al., 2006; Sweeney et al., 2013). NTBUT involves the projection of keratometer mires or a grid-like pattern onto the precorneal tear film, this grid-like pattern is observed for distortion or abnormalities which would indicate that the tear layer is starting to destabilize or dry out (Ramos et al., 2014; Sweeney et al., 2013; McGinnigle et al., 2012). The pattern is observed until the regular configuration starts to distort, representing the break up time of the tear film which is also measured in seconds (Chaudhuri and Vanathi, 2012).

Fluorophotometry is a method which can be utilized to investigate the drainage of tear fluid (Tomlinson and Khanal, 2005). This technique is based on the specified time in which the rate of disappearance of a dye marker is measured (Tomlinson and Khanal, 2005). A specified dye, sodium fluorescein, is instilled into the eye and the rate of disappearance is monitored over a period of approximately 30 minutes (Tomlinson and Khanal, 2005). The rate of elimination of the fluorescein dye occurs rapidly over the first five minutes, this elimination of fluorescein dye may be due to the reflex tearing which occurs as a result of the instillation of a foreign substance (Tomlinson and Khanal, 2005). For the remainder of the 30 minute period, the correlation between the rates of elimination of fluorescein dye can be seen to correspond with the rate of tear turnover using a mathematical relationship (Tomlinson and Khanal, 2005). In this way, tear turnover rates may be used to investigate dry eye or a differential diagnosis thereof (Tomlinson and Khanal, 2005).

Tear film interferometry is a valuable and non-invasive method which can be used to determine the stability of the ocular tear layer (Benitez-del-Castillo and Lemp, 2013). Interferometry uses the concept of light that is reflected off the tear film, more specifically the lipid interface as well as the aqueous-mucin interface of the tear film. These reflected images may be evaluated in order to determine whether instability is present (Benitez-del-Castillo and Lemp, 2013; Nichols et al., 2002). Interference occurs between the light reflected off of these two surfaces, this may result in constructive interference or destructive interference, resulting in different colour fringes (King-Smith et al., 1999). The images given by interferometry are in the form of interference patterns. Depending on the thickness of the
lipid layer, the colours of these interference patterns may vary (Benitez-del-Castillo and Lemp, 2013). Due to the specific functions of the lipid layer which serve to halt excess evaporation, a correlation may be determined between the thickness of the lipid coating and the rate of evaporation of the tear film (Benitez-del-Castillo and Lemp, 2013; Craig and Tomlinson, 1997).

Tear osmolarity tests may be used in order to determine the specific particle concentration within the tear film, thereby assessing the quality of the tears. Tear osmolarity is a laboratory test and may be used to evaluate the balance between tear evaporation and tear secretion (Wallace, 2004). The measurement of tear osmolarity is crucial when assessing the tear film as hyperosmolarity may result in ocular surface damage.

The various tear tests mentioned above can be made use of in clinical practice, some may not be as widely utilized as others. These tests will allow the examiner to form a diagnosis regarding the tear structure and function. The critical homeostatic balance present within the composition of the tear film is of great importance with any disruption leading to tear dysfunction. The various methods of tear testing may be used in order to diagnose dry eye disease which is a debilitating condition found to be prevalent in today’s society.
2.2 DRY EYE

2.2.1 INTRODUCTION TO DRY EYE

The tear film forms the initial refracting surface of the eye, allowing the eye to function optimally, providing vision along with ocular comfort (Ridder et al., 2011; Gumus et al., 2011). Dry eye disease may be classified as a complex, multifactorial disorder affecting both the tear film as well as the ocular surface (Barabino et al., 2012; Ridder et al., 2011). For many years the definition of dry eye disease has been a controversial topic, however, the following definition was proposed by the Dry Eye Workshop in 2007 by the chairman, Michael Lemp: ‘A multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface, accompanied by increased osmolarity of the tear film and inflammation of the ocular surface’ (Lemp et al., 2007). Dry eye disease, which may otherwise be referred to as keratoconjunctivitis sicca (KCS) or dysfunctional tear syndrome, may result in a variety of ocular symptoms including irritation, scratchiness, grittiness, burning and ocular fatigue (Johnson, 2009; Benitez-del-Castillo and Lemp, 2013). The lacrimal functional unit is implicated in dry eye disease; this lacrimal functional unit is made up of three important components namely; the main and accessory lacrimal glands, the surface of the ocular system as well as its connecting innervation (Stern et al., 2004).

The ocular surface generally consists of a standard quantity of tears as well as anti-inflammatory components which aid in preserving and protecting the ocular system when exposed to external factors which may have an effect on the tear film (Stern et al., 2004). The eye has natural protection mechanisms against stresses such as wind, changes in humidity, bacteria or pathogens (Stern et al., 2004). Structures such as the conjunctival epithelial cells as well as goblet cells act in protecting the ocular surface through the secretion of mucin. Alterations in this mucin concentration or volume of tears may result in ocular surface damage (Stern et al., 2004).

The prevalence of dry eye disease has been shown to differ depending on various external factors such as temperature, humidity, gender, visual tasks being performed, contact lens wear as well as the effects of aging (Brewitt and Sistani, 2001; Benitez-del-Castillo and Lemp, 2013). The prevalence of dry eye disease may range from five to 30 percent due to different population characteristics (Benitez-del-Castillo and Lemp, 2013). Studies have shown that dry eye disease may have a relationship with gender showing an increased prevalence in females. Dry eye disease is an ocular disorder which is generally associated
with individuals over 40 years of age (Onwubiko et al., 2013; Dartt et al., 2011B). The link between age and dry eye disease has been thought to be due to the inevitable changes which take place during the natural process of aging, with changes occurring in terms of tear production, evaporation rate and tear dynamics as the aging process progresses (Onwubiko et al., 2013). There are various factors associated with KCS; factors which may result in a greater incidence for dry eye disease, some of which include medications, connective tissue diseases, gender, age, androgen insufficiency, occupation and environmental factors as well as postmenopausal oestrogen therapy (Benitez-del-Castillo and Lemp, 2013; Dartt et al., 2011B; Lee et al., 2002).

2.2.2 SYMPTOMS AND SIGNS

The understanding of KCS may be a challenge as some patients may present with symptoms, however, no signs may be seen and vice versa. One of the most important diagnostic criteria used in dry eye disease is the presence of distressing symptoms (Holland and Mannis, 2006). Patient history is essential when attempting to manage this condition, of particular importance is the duration of the symptoms, any factors which may exacerbate these symptoms as well as any relieving factors (Holland and Mannis, 2006). Patients often complain of symptoms such as scratchiness, grittiness, irritation, a foreign body sensation, sensitivity to light, itching and burning (Nichols, 2006; Holland and Mannis, 2006). These symptoms arise from activation of the sensory nerves within the cornea and the conjunctiva (Levin and Albert, 2010). The human body is innervated with sensory nerves throughout; however, the cornea is one of the most densely innervated regions found within the body and is therefore susceptible to transient changes in the external environment (Baudouin, 2001). Innervation of the cornea is necessary for a host of important functions within the ocular system including; sensations, the process of wound healing as well as protection (Lanza et al., 2013). These sensory nerves originate predominately from the trigeminal ganglion with some of the innervation originating from the sympathetic nerve fibres of the superior cervical ganglion (Yew et al., 1988). Alterations in corneal nerve innervation may disrupt the process of tear production resulting in changes to tear volume and ultimately leading to dry eye disease (Lanza et al., 2013).

There are numerous signs involved in dry eye disease, patients with this disorder may present with the characteristic signs such as corneal surface staining, rapid TBUT indicating tear instability and reduced tear secretion (Asbell and Lemp, 2011). The presence of these
signs does not conclusively prove that the patient is suffering from dry eye disease; several diagnostic tests may have to be performed in order to confirm this diagnosis.

2.2.3 CLASSIFICATION OF DRY EYE

Dry eye disease may be classified into one of two broad categories namely aqueous deficient dry eye, resulting from changes in tear volume and evaporative dry eye caused by excessive tear loss (Barabino et al., 2012; Redmond and While, 2008). Aqueous deficient dry eye is characterised by a reduction in the secretion of tears as well as an abnormality in the functional efficiency of the lacrimal gland unit, leading to a lesser volume of tear fluid (Barabino et al., 2012).

There are two subtypes of aqueous deficient dry eye, being Sjögren’s syndrome dry eye and non-Sjögren’s syndrome dry eye (Barabino et al., 2012). Sjögren’s syndrome can be defined as a systemic disorder in which the immune system initiates an attack targeting certain regions within the body, some of which include the skin, lungs and gastrointestinal tract (Nguyen and Peck, 2009). Primary Sjögren’s syndrome results in specific gland destruction, principally the salivary and lacrimal glands. Due to this mechanism, patients often present with KCS and xerostomia denoting dryness of the mouth (Nguyen and Peck, 2009). Sjögren’s syndrome is diagnosed by the presence of the characteristic symptoms, the presence of auto-antibodies as well as tissue infiltration via lymphocytes (Asbell and Lemp, 2011). Sjögren’s syndrome frequently presents in association with additional conditions such as rheumatoid arthritis, scleroderma or Systemic Lupus Erythematosus, which is then termed secondary Sjögren’s syndrome (Barabino et al., 2012; Nguyen and Peck, 2009). Non-Sjögren’s syndrome dry eye may occur as a result of dysfunction within the lacrimal system itself which does not take place due to autoimmunity factors, disruption within the neural loop or obstruction of the glands (Barabino et al., 2012; Asbell and Lemp, 2011). There are numerous features which may cause exacerbation of this disorder, such as age-related issues, an unstable tear film, altered tear osmolarity and reduced tear volume (Barabino et al., 2012).

Evaporative dry eye occurs due to an increase in the evaporation rate of the tear film rather than a change in tear secretion dynamics, this may imply abnormalities within the lipid layer of the tears as this layer is responsible for limiting the quantity of evaporation taking place (Barabino et al., 2012; Albietz, 2001). Extrinsic factors have also been shown to have an effect on evaporative dry eye, some of these factors being humidity, blinking changes, medications or contact lens wear (Ridder et al., 2011; Albietz, 2001; McGinnigle et al., 2012). There may be an increase in the exposed surface of the eye allowing excessive
evaporation to take place; this may include conditions such as exophthalmos, lid deformities or proptosis (Benitez-del-Castillo and Lemp, 2013). The predominant factor in this subtype of dry eye has been shown to be meibomian gland dysfunction (Benitez-del-Castillo and Lemp, 2013; Albietz, 2001). The lipid layer of the tear film serves to limit excess evaporation. In cases where this layer is not functioning efficiently the volume of tear evaporation cannot be controlled. Meibomian gland dysfunction can be defined as a chronic form of blepharitis in which the resultant inflammation causes changes within the tear film structure causing complaints of dry eye symptoms (Copeland and Afshari, 2013). The mechanism responsible for this disturbance is thought to be hyperkeratinisation which may lead to an increase in the viscosity of meibum produced via the meibomian glands (Benitez-del-Castillo and Lemp, 2013). Alterations in the physical characteristics of the meibomian secretion may result in a cloudy, dense exudation which has the ability of plugging the gland orifices (Asbell and Lemp, 2011). This may result in what is referred to as metaplasia, the characteristic appearance of the gland orifice due to the thickened meibum secretion which lies within the gland and cannot be expelled (Asbell and Lemp, 2011). A condition such as this, if left without treatment, may result in meibomian gland dropout, or complete degeneration of the meibomian glands (Asbell and Lemp, 2011). An increase in tear evaporation will result in tear hyperosmolarity which is one of the pathophysiological causes of KCS. There are various skin disorders which may be associated with evaporative dry eye; conditions such as atopic dermatitis and psoriasis, these skin abnormalities may aggravate the tear dysfunction symptoms even further (Asbell and Lemp, 2011).

2.2.4 PATHOPHYSIOLOGY

It has been postulated that there are two important hallmarks in dry eye disease, tear instability and tear hyperosmolarity (Benitez-del-Castillo and Lemp, 2013). A stable tear film is necessary in order to maintain the health and functional aspects of the eye, the tears are reformed and redistributed with every blink in order to maintain this homeostatic mechanism (McGinnigle et al., 2012). Blink rate is important and generally falls within the range of 17 times per minute (McGinnigle et al., 2012). The blink rate of patients with dry eye disease has been shown to be significantly reduced; this finding may account for the fact that the tear film is relatively unstable, resulting in the layer of tears breaking up causing discontinuity (McGinnigle et al., 2012; Benitez-del-Castillo and Lemp, 2013). The instability of the tear film is thought to be the dominating factor underlying many forms of dry eye disease.

Tear osmolarity has been shown to be the principle pathogenic factor involved in dry
eye disease contributing to ocular surface damage and a cascade of inflammatory events (Benitez-del-Castillo and Lemp, 2013; Albietz, 2001; McGinnigle et al., 2012).

Hyperosmolarity occurs due to either a reduction in tear secretion or an excessive volume of tear evaporation (Benitez-del-Castillo and Lemp, 2013). Tear osmolarity has been said to be the single best marker in the determination of the presence and severity of dry eye disease (McGinnigle et al., 2012; Lemp et al., 2011). The osmolarity of the tear film may range between 285 to 295 milliosmoles per litre with slight deviations amongst the literature (Benitez-del-Castillo and Lemp, 2013; Boyd, 2011). There are various alterations which take place at the ocular surface due to the increase in osmolarity; there is a significant increase in inflammatory tear cytokines which results in a torrent of inflammatory actions (Lemp et al., 2011). The proliferation of these cytokines causes an increase in the apoptotic cell death of the surface cells leading to alterations in the expression of mucin (Lemp et al., 2011; Benitez-del-Castillo and Lemp, 2013). The alterations occurring at the surface of the cells cause a resultant loss of lubrication leading to injury of the anterior surface of the eye (Benitez-del-Castillo and Lemp, 2013). Osmolarity tests have the ability to differentiate between normal and dry eye subjects with great accuracy; however, these tests may be complicated to perform (Tomlinson et al., 2006).

2.2.5 DRY EYE DIAGNOSIS

The diagnosis of dry eye is complicated as there is no gold standard in terms of diagnostic criteria with the causes of dry eye being numerous. Dry eye disease is a difficult condition to diagnose, a range of objective tests as well as subjective complaints of symptoms may be used (Khanal et al., 2008). Some of the more common objective tests include the Schirmer test, the phenol red thread test, TBUT, fluorophotometry, tear film interference patterns as well as tear osmolarity tests. The varieties of tests that can be performed on the tear film are plentiful; these are some of the more frequent tests encountered during clinical practice. The clinical procedure for performing these tests has been previously discussed; however, the diagnostic limits must be confirmed. When using the Schirmer test, if the region of the strip saturated with tear fluid is five millimetres or less, this is diagnostic of dry eye disease (Pleyer and Foster, 2006). Using the phenol red thread test, a wetting value of less than ten millimetres is considered an abnormal result and may be indicative of dry eye syndrome (Alves et al., 2004). TBUT, whether the invasive or non-invasive form of this test, is indicative of tear film instability when the measured break up time is less than approximately ten seconds (Holland and Mannis, 2006). The stability of the tear film may be
assessed using the method known as interferometry as discussed earlier, the variation in interference patterns may allow the examiner to determine the constancy of the tear film. These interference patterns may vary from a greyish colour indicative of a tear film which may show signs of instability to a blue-white colour indicating satisfactory stability of the tear film. Interference patterns displaying variable coloured fringes may indicate a highly unstable tear film which may result in dry eye syndrome (King-Smith et al., 1999).

Dry eye disease often results in damage to the ocular surface; the extent of damage may be assessed by means of ocular surface staining. There are different stains which may be used in order to evaluate injury to the anterior surface of the eye; some of which include fluorescein, Rose bengal and Lissamine green stains (Bron, 2001; McGinnigle et al., 2012). Fluorescein is the standard stain used in many diagnostic procedures, this specific dye will fluoresce a bright green colour in order to reveal damaged regions of the cornea as well as the conjunctiva (Bron, 2001). Fluorescein has the unique ability to stain devitalized cells but is unable to stain mucous (Albietz, 2001). The staining of mucous occurs with the stain known as Rose bengal, which also possesses the ability to stain dead and degenerated cells (Bron, 2001). Research has suggested that the capability of Rose bengal in staining certain cells is due to the fact that there is a lack of adequate mucin in dry eye disease thereby allowing cells which would usually be protected by this mucin layer, to be exposed to the dye (McGinnigle et al., 2012; Bron, 2001). Lissamine green has the ability to stain damaged regions in a similar manner to Rose bengal, the only difference being that this dye stains a green colour and therefore compromised cells may be identified with ease (Albietz, 2001). Lissamine green is thought to be less toxic compared to Rose bengal and is better tolerated by patients (Bron, 2001; Albietz, 2001).

There is a collection of other objective tear tests which can be performed in order to determine the presence of dry eye disease, these may not be performed routinely but are available if needed by an eye care practitioner. Some of these methods include evaporimetry, interferometry and abberometry (McGinnigle et al., 2012). Evaporimetry may be defined as a quantitative tear test, being used to determine the evaporation rate of the tear film; this is measured under specified conditions of controlled humidity and the rate of evaporation will inevitably be raised in dry eye disease subjects (Herranz and Corrales Herran, 2012).

Wavefront abberometry involves the use of colour coded maps in order to assess the presence of any disturbances within the tear film structure, these disruptions may be due to the instability of the tears which occurs during dry eye disease (Benitez-del-Castillo and Lemp, 2013; McGinnigle et al., 2012). Using these and various other testing methods, an
examiner is able to confirm the diagnosis of dry eye syndrome as conclusively as possible. Thereafter, treatment options may be investigated in order to relieve the patient of these symptoms of discomfort.

2.2.6 DRY EYE TREATMENTS

The treatments available for dry eye disease are often palliative instead of curative; these treatment methods are predominantly effective at relieving patient symptoms in order to increase patient comfort. Dry eye disease management is aimed at reducing ocular surface inflammation which is the main contributor towards the damage occurring at the surface of the eye during this disease process (Barabino et al., 2012). Tear replacement is often the most suitable method for treating dry eye disease, tear volume is unquestionably altered in this disease and therefore tear substitutes play a role in the management strategy (Barabino et al., 2012). Replacement of tears may also result in a reduction in tear osmolarity, thereby attempting to maintain homeostasis while providing a protective layer over the ocular surface (Holland et al., 2013; Barabino et al., 2012). Many commercially available artificial tear products contain components which act to increase the viscosity of the tear film, provide adequate lubrication as well as adhering to the surface of the eye for a maximum amount of time, thereby relieving symptoms of irritation (Holland et al., 2013). One of the preservatives commonly found in certain artificial tear brands which has been shown to result in severe ocular toxicity is benzalkonium chloride (Holland et al., 2013). Benzalkonium chloride has been shown to have detrimental effects on the tear film by disrupting the viability of cellular membranes, leading to consequences such as apoptosis or necrosis (Holland et al., 2013). It is preferable for patients to use preservative-free treatments in order to eliminate the risk of the toxicity effects (Holland et al., 2013; Albietz, 2001).

Cyclosporine A is a naturally occurring immunosuppressive agent which has been widely utilized in order to control rejection of newly transplanted organs as well as to treat various autoimmune diseases (Barabino et al., 2012; Scholar, 2001). Topical Cyclosporine A used for ophthalmic purposes is known as Restasis™, it is used to treat the underlying inflammation involved in dry eye disease (Barabino et al., 2012). Restasis™ is an ophthalmic solution which has been shown to increase the rate of tear production and is responsible for the relief of chronic symptoms by reducing the inflammatory response thereby preventing further damage (Dartt et al., 2011B; Yorio et al., 2011; Dunphy et al., 2015). Restasis™ contains a small percentage of Cyclosporine A which is accountable for the inhibition of T-cell activation resulting in the disruption of lacrimal gland function which
ultimately leads to an alteration in tear film secretion causing dry eye disorders (Dunphy et al., 2015). The inhibition of these T-cells allows for minimal disruption of lacrimal gland function therefore causing increased tear production and alleviation of dry eye symptoms (Barabino et al., 2012; Dunphy et al., 2015). Cyclosporine A has also been shown to be effective in reducing the rate of apoptosis of the conjunctival epithelial cells (Barabino et al., 2012).

Corticosteroid therapy is an effective immunosuppressive treatment used to reduce inflammation in dry eye disease (Holland et al., 2013; Barabino et al., 2012). Some of the mechanisms whereby this inflammation is reduced include; a reduction in cell adhesion molecules, inhibition of inflammatory cytokine production, synthesis of matrix metalloproteinases as well as lymphocyte apoptosis (Barabino et al., 2012; Dartt et al., 2011B). Studies have shown that therapy with topical corticosteroids, preferably non-preserved, may result in relief of dry eye symptoms within two weeks of use (Barabino et al., 2012). Coupled with the advantages, the disadvantages of corticosteroid therapy include side effects such as ocular hypertension, cataracts, glaucoma and infection (Barabino et al., 2012; Dartt et al., 2011B).

Tetracyclines are anti-biotic agents which may be used as a treatment option for dry eye disease due to their numerous anti-inflammatory properties (Dartt et al., 2011B; Barabino et al., 2012). Tetracyclines function by disrupting protein synthesis, this disruption occurs at the level of the ribosomes and is effective against both gram-positive and gram-negative bacteria (Barabino et al., 2012). These agents are effective in the management of dry eye disease due to the various anti-inflammatory properties which can be exerted including; B cell activation, collagenase activity and inhibition of matrix metalloproteinases (Barabino et al., 2012; Holland et al., 2013). Two of the most commonly used tetracyclines include; doxycycline and minocycline (Dartt et al., 2011B; Barabino et al., 2012).

Essential fatty acids are important for maintaining the homeostatic functions of the ocular surface, more specifically omega 3 and 6 polyunsaturated fatty acids (Barabino et al., 2012; Holland et al., 2013). On consumption, these fatty acids produce anti-inflammatory effects which function towards controlling the inflammatory responses during dry eye disease (Barabino et al., 2012). Not only have these products been effective in the treatment of dry eye, but research has shown the beneficial effects of omega 3 and 6 in the management of chronic conditions such as rheumatoid arthritis (Holland et al., 2013).

Cases of very severe dry eye disease may be treated through the use of contact lenses, this is not a standard treatment used for this condition; however, patients experiencing
corneal epithelial disease may need a more permanent treatment method. Therapeutic scleral lenses create a defensive barrier over the front surface of the eye providing protection against any further trauma (Holland et al., 2013; Albietz, 2001). Scleral lenses are specifically designed to provide a scleral-bearing fit, allowing for a fluid-filled reservoir which is in constant contact with the front surface of the eye (Holland et al., 2013). The ocular surface is therefore permanently lubricated, protected against inflammatory mediators within the tears as well as trauma from external agents (Holland et al., 2013).

Autologous serum has been developed as a new treatment for severe dry eye, serum is the fluid component of blood and therefore contains important components which can be used in order to treat various conditions (Poon et al., 2001). Serum enhances epithelial growth and health due to the presence of specific substances, some of which include albumin, growth factors and immunoglobulin (Poon et al., 2001; Geerling et al., 2004). Patients who do not respond to conventional treatments for dry eye disease with persistent symptoms may be treated with autologous serum eye drops which have been shown to reduce signs and symptoms significantly (Noble et al., 2004; Geerling et al., 2004; Ogawa et al., 2003). Autologous serum has also been shown to be beneficial in relieving symptoms and signs in conditions such as chronic graft-versus-host disease, cases of persistent epithelial defects as well as Sjögren’s syndrome (Ogawa et al., 2003; Poon et al., 2001; Tsubota et al., 1999). The preparation of serum eye drops differs which could subsequently affect the concentration of serum given, however, the complications reported with the use of serum eye drops are minimal (Rauz and Saw, 2010).

Amniotic membrane consists of a foetal component derived from the mammalian placenta (Rauz and Saw, 2010). The amniotic membrane consists of specific layers, a basement membrane along with a stromal matrix made up of three collagenous layers (Rauz and Saw, 2010). This amniotic membrane has been shown to have anti-inflammatory, anti-angiogenic and anti-scarring properties (Rauz and Saw, 2010). Amniotic membrane has recently been used to treat conditions such as severe dry eye, persistent corneal epithelial defects, chemical and thermal burns as well as reconstruction of conjunctival defects (Rauz and Saw, 2010).

One of the most invasive techniques for managing dry eye which cannot be treated by any alternative means includes transplantation of the submandibular gland (Geerling et al., 1999). When treating dry eye disease, the tear substitutes used to replenish the deficiency in the tears must mimic the physiology of the tears as accurately as possible. Submandibular saliva differs in terms of both enzymatic and electrolyte content, however, the use of this
saliva in treating dry eye disease has shown to be beneficial (Geerling et al., 1999). Transplantation of salivary glands (either sublingual, parotid or submandibular) in order to treat dry eye disease has been shown to decrease signs and symptoms of dry eye disease as the tear volume is being substituted continuously (Guthoff and Katowitz, 2007).
2.3 KERATOCONUS

2.3.1 INTRODUCTION TO KERATOCONUS

Keratoconus can be defined as a progressive, non-inflammatory corneal ectasia resulting in thinning and distortion of the anterior corneal surface (Abolbashari et al., 2013; Saijyothi et al., 2012). Keratoconus is a progressive disorder which may be bilateral but asymmetric in most cases (Abolbashari et al., 2013). The extreme thinning which occurs within the cornea is a consequence of changes taking place within the corneal stroma (Joseph et al., 2011). Keratoconic patients present with irregular astigmatism as well as large amounts of myopia (Joseph et al., 2011; Assiri et al., 2005; Sarac et al., 2011). As keratoconus evolves, the cornea becomes oedematous due to a build up of fluid, scarring also develops which may cause detrimental effects to the vision of the patient (Traboulsi, 2011). The pathogenesis of keratoconus is considered to be linked to oxidative stress within the corneal surface itself (Saijyothi et al., 2012). Keratoconus is generally initiated at puberty with gradual and advanced changes occurring over time (Abolbashari et al., 2013). Keratoconus has higher prevalence in Asian, Middle Eastern and Mediterranean populations (Mohd-Ali et al., 2012), with studies showing this condition to be present in one out of 2000 individuals (Abolbashari et al., 2013; Saijyothi et al., 2012; Traboulsi, 2011). Certain characteristic changes occur in keratoconic corneas including DNA damage to mitochondrial cells, altered concentration of antioxidant enzymes and cytotoxic compound accumulation (Saijyothi et al., 2012).

2.3.2 POSSIBLE CAUSES

The underlying cause of keratoconus is yet to be determined, it has been said that there may be a multifactorial aetiology linked to this condition (Mohd-Ali et al., 2012). Research has shown that there is speculation of a genetic link, environmental effects as well as an association with certain systemic diseases (Traboulsi, 2011; Mohd-Ali et al., 2012). The modes of inheritance have been thought to be sporadic, autosomal recessive as well as autosomal dominant (Burdon and Vincent, 2013). Studies have shown a positive familial trait in patients suffering with keratoconus, relatives of these individuals in most cases have been shown to present with undiagnosed keratoconus (Traboulsi, 2011). Even in the presence of this link, the familial history of keratoconus is not correlated with the clinical severity of this disease (Traboulsi, 2011). Despite the uncertainty regarding the genetic link in keratoconus, evidence has shown an association between keratoconus and Trisomy 21 suggesting a genetic
predisposition with respect to chromosome 21. Other than chromosome 21, defects have also been identified on chromosomes 13 and 17, however, the exact mechanism of these deficits has not yet been recognised (Chaudhuri and Vanathi, 2012).

Environmental factors which have been shown to have an influence on the development of keratoconus include frequent and persistent eye rubbing, atopy of the eyes as well as contact lens wear (Traboulsi, 2011; Mohd-Ali et al., 2012). Previous research has shown an existing relationship between allergy symptoms and constant eye rubbing, the itching sensation experienced in conditions of ocular allergy result in a persistent need to relieve this feeling resulting in relentless fisting of the eyes (Villarreal and Kaiserman, 2011). Tenacious rubbing of the eyes has been shown to be a significant predictor in the development of unilateral keratoconus specifically; mechanical trauma to the ocular surface may result in changes to the internal tissues leading to the progression of keratoconus (Traboulsi, 2011). The mechanical pressure exerted by rubbing may result in microscopic injuries to the epithelial cells; leading to the release of cytokine molecules which in turn cause the differentiation of myofibroblast cells (Traboulsi, 2011). The force of rubbing ultimately leads to changes within the biomechanical structure of the cornea leading to the progressive thinning characteristic of keratoconus (Traboulsi, 2011). Rubbing of the eyelids may also induce inflammatory effects leading to chemosis as well as hyperaemia of the front surface of the eye (Villarreal and Kaiserman, 2011). Keratoconic patients are urged to avoid tenacious eye rubbing as this constant stimulus may hasten the progression of keratoconus (Villarreal and Kaiserman, 2011).

2.3.3 ASSOCIATED FACTORS

Atopy may be defined as a tendency or genetic predisposition of an individual to hypersensitivity reactions leading to increased levels of IgE antibodies in the bloodstream thereby resulting in allergic reactions to specific stimuli (Smolin et al., 2005; Traboulsi, 2011). Some of the most frequently encountered atopic conditions include atopic dermatitis, allergic rhinitis, asthma as well as hay fever (Smolin et al., 2005). The hypersensitivity reaction occurring is a type 1 reaction in which exposure to an allergen will elicit an immediate defensive immune response leading to production of immunoglobulin E (Braun and Anderson, 2007; DeFranco et al., 2007). Evidence has shown the existence of a varied relationship that exists between atopic conditions and keratoconus (Traboulsi, 2011). In patients with keratoconus, research has shown that the incidence of atopy which may be linked to the etiology of keratoconus is approximately 35 percent (Rahi et al., 1977).
The literature suggests that keratoconus may be linked to a lengthy history of contact lens use where continuous contact lens wear may decrease the overall corneal thickness leading to an increase in the curvature of the cornea therefore changing the irregularity of the corneal surface (Traboulsi, 2011). The theory involving contact lens wear as a risk factor is still debatable with some research suggesting that the development of keratoconus may not be linked to the use of contact lenses (Selser, 1994). Warpage of the cornea may occur due to years of continuous rigid contact lens wear, however, this does not necessarily mean that contact lens wear is considered a risk factor (Selser, 1994; Friedman and Kaiser, 2007). It has been hypothesized that contact lens wear may result in damage to the epithelial cells of the cornea therefore aiding in the progression of keratoconus and is therefore considered a risk factor (Wang and Swartz, 2010).

Another common risk factor for keratoconus is the presence of other specific systemic conditions (Chaudhuri and Vanathi, 2012). The most common systemic conditions associated with keratoconus have been found to be Ehlers-Danlos syndrome, Marfan’s syndrome, Down’s syndrome, Crouzon’s syndrome, osteogenesis imperfecta as well as various connective tissue disorders (Chaudhuri and Vanathi, 2012; Hom and Bruce, 2006). The incidence of Down’s syndrome, a chromosomal anomaly associated with keratoconus, has been shown to range between eight and 15 percent (Bennett and Hom, 2004; Levin and Albert, 2010; Kenney and Brown, 2003). Various corneal dystrophies have also been found to have an association with keratoconus, dystrophies including lattice dystrophy, Fuch’s endothelial dystrophy and granular dystrophy (Burdon and Vincent, 2013). The exact association has not yet been determined, however, there may be a genetic link behind the occurrence of these dystrophies or there may be similarities amongst the protein pathways involved (Burdon and Vincent, 2013).

2.3.4 DIAGNOSTIC SIGNS

There are numerous signs which can be seen in patients with keratoconus. When examining a keratoconic patient, one of the first signs observed is a scissoring reflex seen with retinoscopy where two separate streaks of light are seen to move away from and toward each other making retinoscopy problematic to perform (Barbara, 2011). The observation of this scissor reflex is indicative of the presence of irregular astigmatism which is characteristic of keratoconus and may be produced by the coma aberration (Barbara, 2011; Rosenfield et al., 2009). Another distinguishing reflex signaling the presence of keratoconus is known as the oil drop reflex or Charleux sign and occurs as a result of internal reflection of light when
performing ophthalmoscopy (Barbara, 2011; Ng et al., 2014).

The slitlamp is a valuable diagnostic tool which can be used when dealing with patients who have keratoconus. The signs seen with slitlamp are numerous and may vary depending on the severity of the condition. One of the most renowned slitlamp signs seen in cases of this progressive disorder is a brown coloured deposit which forms within the cornea known as Fleischer’s ring (Wang and Swartz, 2010). This deposition is present in the epithelial layer of the cornea and is composed of an iron oxide known as hemosiderin which forms completely or partially around the border of the protruding cone (Wang and Swartz, 2010; Yanoff et al., 2009). The exact mechanism behind the formation of this deposit is not entirely known, however, it is thought to occur due to the instability of the tear film in keratoconic individuals (Wang and Swartz, 2010). Throughout the progression of this disorder, Fleischer’s ring may become more densely pigmented but may narrow, a cobalt blue filter may be used in order to increase the visibility of this deposition (Barbara, 2011).

Due to the build-up of pressure within the corneal tissue, another common presentation which may be observed when performing a slitlamp examination is the appearance of Vogt’s striae (Barbara, 2011). These striae may be seen as discrete, vertical lines, usually parallel to the steep axis seen at the level of Descemet’s membrane and located within the stromal layer of the cornea (Sinjab, 2011; Barbara, 2011). Vogt’s striae have been thought to be a sign of stress taking place at the level of the collagen fibrils within the corneal tissue. When applying pressure to the corneal surface, these striations seem to disappear temporarily, however, appear to return when the applied pressure is released (Barbara, 2011). These stress lines may also be seen to transiently disappear when wearing a rigid gas permeable contact lens, most likely due to the pressure the lens exerts over the cornea.

Another definitive sign which can often be seen in cases of keratoconus is one which occurs within the stromal layer of the cornea, resulting from an influx of fluid into the structure of the cornea (Bilgin et al., 2013). This complication is known as corneal hydrops, the process whereby aqueous moves into the corneal tissue is thought to be due to small ruptures which occur in Descemet’s membrane, providing a passage for fluid to enter (Bilgin et al., 2013). In some cases, excess and vigorous eye rubbing commonly seen in keratoconus may be the traumatic cause of these breaks within Descemet’s membrane (Bilgin et al., 2013). Other risk factors seen to be associated with corneal hydrops include Down’s syndrome, eccentric cones as well as keratoconic patients who have associated ocular allergies such as vernal conjunctivitis (Bilgin et al., 2013; Thota et al., 2006). In cases of corneal hydrops, patients may present with characteristic ocular symptoms such as abrupt
pain, photophobia and severe variations in vision (Thota et al., 2006). In some cases, corneal hydrops is a self-limiting condition which may settle over a period of time, however, a residual scar usually remains. Along with residual scarring, these individuals may develop sporadic complications such as corneal neovascularization, perforation and infection (Thota et al., 2006). In severe cases, incessant oedema may result in central scarring of the cornea which may be rectified by means of a corneal transplant (Thota et al., 2006).

One of the characteristic external signs indicating the presence of keratoconus is known as Munson’s sign (Sinjab, 2011). This sign may be seen as a V-shaped contour of the lower lid due to the protrusion of the cornea which can be seen when the patient is asked to look down towards the ground (Sinjab, 2011; Smolin et al., 2005). Munson’s sign has been shown to be more distinct in severe cases of keratoconus and may not be distinguishable in very moderate cases (Sinjab, 2011). Rizzuti’s sign may be seen as a beam of light close to the nasal limbus which can be produced through the use of lateral reflection of the cornea from the temporal side (Smolin et al., 2005). This sign can be seen in more severe stages of keratoconus due to the projection of the cornea causing paracentral thinning (Smolin et al., 2005; Chinmaya, 2011).

One of the frequent diagnostic signs evident in keratoconic patients is the irregularity and distortion of keratometric mires (Roy et al., 2007). These mires are often seen as steep and oval shaped with the possibility of obtaining keratometry readings being mostly unmanageable (Saxena, 2011). When attempting to obtain keratometry readings, measurements cannot be obtained as the readings pass the limits of the measurement scale (Sharma, 2006). In various cases of keratoconus, instrumentation such as the Oculus Keratograph 4 may be utilized in order to obtain keratometric readings as well as a grading of severity.

2.3.5 GRADING OF KERATOCONUS

There are various methods which can be utilized to grade keratoconus. The severity of keratoconus may be graded according to the cone apex, broken up into three broad categories being mild, moderate and advanced (Nejabat et al., 2012). Keratoconus may also be classified according to the variations in the shape of the corneal cone, the cone shapes may be categorized as either nipple, oval or globus in shape (Nejabat et al., 2012; Hom and Bruce, 2006). A nipple cone is considered to be smaller compared to the other cones and is located centrally, whereas an oval cone has been shown to be located inferiorly and show a greater prevalence for breaks in Bowman’s as well as Descemet’s membrane (Nejabat et al., 2012;
Hom and Bruce, 2006). The globus cone is relatively large and encompasses almost the entire corneal surface (Nejabat et al., 2012; Hom and Bruce, 2006). In general terms, the cone seen in keratoconus may be located centrally or paracentrally, a central cone may have an increased detrimental effect on vision as it may be positioned within the centre of the visual axis (Nejabat et al., 2012).

Some studies suggest utilizing keratometry readings as a means for grading the severity of keratoconus. Assiri et al., (2005) suggested that keratoconus could be classified as either early, moderate or advanced depending on the average keratometry readings obtained. The study by Zadnik et al., (1998) categorized keratometry readings according to the flattest reading which was generally shown to be steeper with more severe keratoconus, using these keratometry readings, keratoconus was graded from mild to severe. Other studies advocate the use of the first definite apical clearance lens (FDACL) technique in order to determine the severity and progression of keratoconus (Edrington et al., 1999). This technique advocates the use of rigid gas permeable lenses fitted in order to determine the flattest lens that vaults the cone apex and using this, a classification is created where keratoconic patients are graded according to the curvature of the corneal apex (Edrington et al., 1999).

Corneal higher order aberrations have also been utilized when attempting to grade keratoconus according to severity. It has been determined that keratoconic corneas exhibit aberrations which are significantly greater than those revealed in normal corneas (Alió and Shabayek, 2006). By utilizing the root mean square (RMS) value given, keratoconus may be graded according to the various components present (spherical component, cylindrical component, spherical equivalent and average keratometry values). By using these components as well as the axial length of the eye, a classification system was developed by Alió and Shabayek, (2006). Alió and Shabayek, (2006) classified keratoconus into four different grades according to the mean central keratometry readings, the RMS value obtained, the presence of scarring as well as the measurement of corneal thickness.

The KISA index has been applied for several studies when grading keratoconus. The KISA index is an algorithm which combines the amount of central corneal steepening, the diopteric asymmetry, the AST index (degree of regular corneal astigmatism) and the SRAX index (degree of irregular corneal astigmatism) in order to calculate a value for the grade (Alió and Shabayek, 2006; Bennet and Weissman, 2015; Wang and Swartz, 2008). The KISA index has been shown to have significant clinical correlation and is used as an effective and reliable method for determining the severity of keratoconus (Azar and Koch, 2002).

Another grading scheme used for keratoconus is known as the Massachusetts Eye and
Ear Infirmary (MEEI) grading scheme which combines the case history given by the patient, the clinical signs seen throughout the examination as well as the corneal topography readings in order to generate a score (Smolin et al., 2005). This score ranges between 0-9 with 0 indicating that the patient has a normal cornea, 1-3 indicating that this patient may be a keratoconus suspect, 4-5 indicating early keratoconus and 6-9 indicating advanced keratoconus (Smolin et al., 2005).

One other valuable method of grading keratoconus is the grading given by the Oculus Keratograph 4. When obtaining a topographical map of the surface characteristics of the cornea using the Keratograph, a grading of keratoconic severity is also given. This grading ranges from grade 1 to grade 5 with intervals in between according to the severity of keratoconus present. Personal communication with Oculus, Wetzlar revealed that this grade is calculated utilizing four different parameters namely the Index of Surface Variance (ISV), Keratoconus Index (KI), RMin (minimum value of the curvature of the cornea) as well as eccentricity. The eccentricity refers to the measures of eccentricity in the 30 degree meridian measured in the four principal meridians namely nasal, temporal, superior and inferior. Utilizing these four parameters, a classification is determined known as the Topographical Keratoconus Classification, this being the classification given by the Oculus Keratograph 4. The exact details of these given parameters cannot be discussed as this is proprietary information.

2.3.6 PATHOPHYSIOLOGY

Corneal nerves play an essential role in the healing process of the epithelial surface as well as cell degeneration, modifications in the corneal structure may affect these crucial functions. Alterations in the corneal nerve structure are seen in keratoconic corneas, however, the mechanism whereby this affects the advancement of keratoconus is unknown (Ozgurhan et al., 2013). When corneas of keratoconic patients are observed using biomicroscopy, the nerves are shown to be more prominent which is a characteristic sign in the early diagnosis of keratoconus (Ozgurhan et al., 2013; Mannion et al., 2005). The corneal nerves have shown to have amplified fragility along with reduced sensitivity due to the sub-basal changes taking place within the corneal tissue (Ozgurhan et al., 2013; Mannion et al., 2005). The corneal nerve plexus has been shown to be altered in keratoconic corneas, there is a decrease in the nerve density compared to healthy corneas (Ozgurhan et al., 2013). Research has shown that the corneal nerves may exhibit a keratocyte nuclei-based casing around the borders of these nerves, this occurs at the level of Bowman’s membrane (Mannion et al., 2005). Keratoconus
has been shown to be a rapidly advancing disorder with the initial changes occurring at the epithelial level, progressing towards changes at the level of Bowman’s membrane and following on, to affect the anterior stroma of the cornea (Mannion et al., 2005). Due to these significant changes taking place within the corneal tissue, the tissue does not receive a constant supply of nutrients necessary for its normal function, therefore deterioration takes place and the cornea can be seen to stretch and distort resulting in the cone structure which can easily be observed (Mannion et al., 2005). This distending cornea is often found to be directly within the area of the visual axis, resulting in adverse effects on vision.

The pathophysiology and mechanisms behind the development of keratoconus are not entirely understood, however, oxidative stress is thought to be one of the contributing factors in the pathogenesis of this corneal ectasia (Saijyothi et al., 2012). Due to alterations within the corneal structure occurring in keratoconus, the cornea displays underlying faults in its ability to process reactive oxygen types (Kenney and Brown, 2003). The cornea is continuously exposed to ultraviolet radiation which is responsible for producing reactive oxygen species (Wojcik et al., 2013). The reactive oxygen species result in demolition and demise of cells, affecting the viability of the corneal tissue bringing about oxidative harm to molecules such as lipids, DNA as well as proteins (Wojcik et al., 2013).

Antioxidants present within the cornea are accountable for the perseverance of the corneal tissue, allowing the cornea to perform its characteristic and essential functions (Cheung et al., 2013). Ocular surface defence as well as the processes involved in corneal wound healing are performed by small molecule antioxidants (Saijyothi et al., 2012). The tear film is said to contain five important antioxidants, being tyrosine, glutathione, ascorbic acid, cysteine and uric acid (Saijyothi et al., 2012). When analysing the tears of keratoconic patients, researchers have shown a substantial reduction in glutathione levels with uric acid and tyrosine levels being elevated (Saijyothi et al., 2012). When exposed to ultraviolet light, the cornea becomes increasingly vulnerable to the effects of oxidative stress, particularly when an imbalance exists between the antioxidant concentration and the reactive oxygen species (Wojcik et al., 2013).

Minute functional cells within the corneal tissue known as keratocytes are responsible for maintaining the structure and function of the collagen constituents contained in the cornea as well as the extracellular matrix components (Ozgurhan et al., 2013). When a corneal injury occurs, these specialized keratocytes work along with endothelial and epithelial cells in order to preserve the structure of the cornea (Nishida, 1998). Collagen and proteoglycans, which are extracellular matrix proteins may be generated by the stromal keratocytes when exposed
to a destructive force (Nishida, 1998). Within the human body as well as within ocular structures, cell death is inevitable, cells within the body undergo a process known as apoptosis (Kenny and Brown, 2003; Levin and Albert, 2010). Apoptosis can be described as a function of certain cells whereby the cell self-terminates due to a pre-programmed response (Kenney and Brown, 2003).

Within the corneas of keratoconic patients, the apoptosis of corneal keratocytes has shown to be amplified (Levin and Albert, 2010; Kim et al., 1999). It has been hypothesized that the rate of keratocyte apoptosis is increased due to the susceptibility or weakness of the cornea as a result of keratoconus (Kim et al., 1999). This automated cell death is initiated by the release of specific cytokines, some of these cytokines include tumour necrosis factor-alpha and interleukin-1 (Cheung et al., 2013). Studies have shown that chronic rubbing of the eyes may contribute to the pathogenesis of apoptosis as mechanical trauma further exacerbates this process (Levin and Albert, 2010; Kenney and Brown, 2003). Along with apoptosis, there are several other morphological changes which can take place within the cornea, altering the specific characteristics of the cornea. Some of these morphological alterations include DNA disintegration, decline in the concentration of cells, growth of apoptotic cell groups and the condensation of chromatin molecules (Cheung et al., 2013).

Other notions thought to be related to keratoconus are namely degradation of corneal tissue or lack of maintenance thereof. While the alternative theory is slippage between the collagen fibrils within the cornea (Meek et al., 2005).

2.3.7 TREATMENT METHODS

The damage and destruction occurring within the corneal tissue is inevitable in the pathogenesis of keratoconus. Treatment methods are aimed at preventing further progression of this condition and aiding the patient in obtaining effective and improved vision in order to improve quality of life. In mild cases of keratoconus or in the early stages of this condition, spectacle lenses or the use of soft contact lenses may be indicated (Mandell, 1997). As the severity of this condition increases, these methods of treatment are no longer effective in improving visual acuity (Mandell, 1997). Due to the progression of the cone along with alterations of the corneal tissue and escalation of astigmatism, spectacle wear may no longer provide sufficient visual acuity for these patients. Soft contact lenses have also been a means of treatment for keratoconic patients and these lenses may be highly successful during the early stages of keratoconus. As the condition develops, however, the soft contact lens moulds to the shape of the cone and cannot correct for the imperfections in order to create a smooth
refracting surface for clear and crisp vision.

Following significant progression and amplified severity, rigid gas permeable contact lenses may be an effective method to consider, the use of rigid lenses allows for the creation of a smooth, optical surface compensating for the defects within the surface of the cornea (Mandell, 1997). The development of an even, newly formed exterior corrects for the existing imperfections within the front surface of the keratoconic cornea, providing the patient with clearer, less distorted vision (Mandell, 1997). Cavities may be observed between the distorted cornea and the contact lens, the tear film spreads itself out in order to fill these crevices thereby neutralizing the presence of optical aberrations (Bennett and Hom, 2004). The neutralization of optical aberrations is achieved through the refractive index which, in terms of the cornea and the tear film, is almost identical (Bennett and Hom, 2004). There are numerous fitting techniques which may be employed when dealing with a keratoconic cornea. There has been widespread debate regarding whether touching the apex of the cone may induce severe corneal scarring following long term use, some suggest the presence of slight apical clearance may be preferable (Leung, 1999).

One of the most common fitting philosophies actively being used in clinical practice may be defined as the three point touch technique which has otherwise become known as the divided support method (Hom and Bruce, 2006; Nejabat et al., 2012; Leung, 1999). Using this system, the desired contact lens is fitted with a light area of touch where the contact lens comes into contact with the apex of the corneal cone (Hom and Bruce, 2006). In addition to this region of central bearing, two supplementary points of touch, approximately 180 degrees from the apex may be observed within the midperipheral portions of the contact lens (Hom and Bruce, 2006; Stein et al., 2012). Fitting the lens in such a way allows for the distribution of weight across the entire surface of the cornea, thereby avoiding excessive strain on one specific portion of the corneal surface (Bennett and Hom, 2004; Nejabat et al., 2012; Leung, 1999). When evaluating this fit with fluorescein dye, the observed pattern has a bull’s eye appearance. During the early developmental stages of this fitting technique, the idea was to halt the progression of keratoconus by creating an area of touch thereby stabilizing the cone.

In the technique known as apical bearing, lenses with a large total diameter are used in conjunction with a base curve that is flat thereby resulting in bearing, specifically on the apex of the keratoconic cone (Bennett and Hom, 2004; Leung, 1999). This type of fit is one of the easiest to achieve as contact with the protruding cone may be inevitable in keratoconic corneas. The concern associated with this fitting technique is that the region of bearing may result in severe corneal scarring to the affected portion of the cornea (Romero-Jiménez et al.,
The visual acuity obtained with a fit such as this has been shown to be superior to the apical clearance method of fitting (Romero-Jiménez et al., 2010; Leung, 1999).

The third fitting technique, apical clearance, requires clearance (otherwise known as vaulting) off the apex of the cone, the lens obtains its support from the paracentral cornea rather than the apex (Romero-Jiménez et al., 2010; Leung, 1999). Clinicians advocated this method of fitting as there is no contact with the peak of the cone, however, the quality of vision obtained through lenses that were fitted this way was not sufficient (Romero-Jiménez et al., 2010).

An additional lens which can be used in cases of keratoconus is known as a hybrid lens. This type of contact lens is made up of a soft hydrogel outer skirt forming the periphery of the lens, with a rigid gas permeable portion located centrally (Hom and Bruce, 2006; Bennett and Hom, 2004). The use of this combination of two different types of lenses allows for increased comfort as well as effective centration of the lens (Hom and Bruce, 2006). Occasionally, these lenses may be fitted too tightly, restricting the movement of the lens over the cornea resulting in hypoxia which may cause corneal oedema as well as neovascularization (Hom and Bruce, 2006; Bennett and Hom, 2004).

Due to problems encountered with both soft as well as rigid contact lenses, a lens design was developed in order to provide the optics of a rigid lens while maintaining superior comfort provided by means of a soft lens. This lens design is known as a piggyback system, providing better centration, additional protection of the cornea as well as optimal vision (Bennett and Hom, 2004). This specialized fitting design utilizes both a rigid gas permeable lens as well as a traditional hydrogel soft lens being communally fitted onto the same cornea (Bennett and Hom, 2004). The hydrogel lens is fitted initially, forming the interface between the cornea and the rigid lens, providing adequate movement, centration and comfort (Bennett and Hom, 2004). A rigid gas permeable lens is then fitted over the surface of the soft lens, correcting for the irregularities and distortions within the surface of the cornea (Bennett and Hom, 2004). In most cases, the refractive power is placed into the soft lens with the hard lens providing the newly formed anterior surface of the ocular system (Bennett and Hom, 2004).

In severe cases of keratoconus, when the use of contact lenses may not provide significant benefit to the patient, corneal surgery may be indicated. One of the most common procedures used to stabilize keratoconic corneas is known as corneal cross-linking, this method of treatment is widely utilized and aims at improving the stability and biomechanical structure of the affected cornea (Romero-Jiménez et al., 2010). Cross-linking is performed in order to halt the progression of keratoconus, preventing further corneal damage and thereby
preserving visual acuity (Legare et al., 2013). The specific substance utilized during this procedure is known as riboflavin, this substance is a hydrophilic photosensitizer which becomes excitable when exposed to any form of ultraviolet radiation (Raiskup and Spoerl, 2014; Legare et al., 2013; Caporossi et al., 2010).

Cross-linking is generally performed under topical anaesthesia as a significant amount of corneal epithelium is removed (O’Brart, 2014). The cross-linking procedure is performed by initially removing a central region of corneal tissue approximately six to nine millimetres in diameter, this diameter may vary across various studies (Romero-Jiménez et al., 2010; O’Brart, 2014). Removal of corneal tissue allows for effective absorption of riboflavin into the stroma of the cornea (O’Brart, 2014; Legare et al., 2013). Once this region of tissue is removed, a solution of 0.1% riboflavin is applied to this region of the cornea followed by exposure to ultraviolet radiation, specifically ultraviolet-A (Romero-Jiménez et al., 2010; O’Brart, 2014). This specific concentration of riboflavin is utilized due to its absorption coefficient, a solution of 0.1% would result in the stroma absorbing approximately 90% of the riboflavin thereby protecting the surrounding structures from ultraviolet damage (Raiskup and Spoerl, 2013).

The mechanism behind the use of ultraviolet radiation at approximately 365-370 nm entails the activation of riboflavin molecules which generates reactive oxygen species (Romero-Jiménez et al., 2010; Raiskup and Spoerl, 2013). The wavelength chosen is sufficient to absorb the maximum amount of riboflavin within the corneal stroma (Raiskup and Spoerl, 2013). The riboflavin solution is applied to the cornea at intervals of three to five minutes for at least a total of twenty minutes, this may vary between various studies (O’Brart, 2014). The formation of covalent bonds between collagen fibrils is induced by the presence of reactive oxygen species, increasing the stability of the corneal stroma through the creation of these additional bonds (Romero-Jiménez et al., 2010; Raiskup and Spoerl, 2013). Photochemical reactions occur, resulting in additional covalent bonds being created between amino acids along each chain of molecules, cross-linkages are formed in this way (Raiskup and Spoerl, 2013; Legare et al., 2013). Additional covalent bonds being formed between neighbouring collagen fibrils increase the stability of the corneal stroma, creating a resistance to further deformation of the cornea due to the process of keratoconus (Legare et al., 2013; Caporossi et al., 2010). The cross-linking procedure has been limited to corneas with a thickness of at least 400nm, in corneas thinner than 400nm, adverse reactions may occur in the corneal endothelium (Romero-Jiménez et al., 2010; O’Brart, 2014).

Following the completion of the cross-linking procedure, the patient experiences
severe ocular pain for the first 48 hours and vision remains blurry for at least two weeks following the procedure (O’Brart, 2014). After approximately three weeks of recovery, the patient may resume contact lens wear as the epithelium should be healed sufficiently (O’Brart, 2014). Within six months, keratocyte re-population occurs and corneal nerve sensitivity is said to return to normal after approximately twelve months, where nerve fibres have been regenerated and the sub-epithelial plexus has been restored (O’Brart, 2014).

As with many other surgical techniques, the procedure of corneal cross-linking has its adverse effects, some of these complications include infectious keratitis, sterile infiltrates as well as the progression of the ectasia (O’Brart, 2014). Debridement of the corneal epithelium exposes the corneal surface to potentially harmful pathogens which may enter and infect the corneal tissue causing complications (O’Brart, 2014). The presence of sterile infiltrates may occur post-operatively, this may resolve within a few days or the use of topical corticosteroids may be indicated for treatment (O’Brart, 2014). In rare cases of keratoconus, the cross-linking procedure may not stabilize the ectasia resulting in further progression and destruction of the cornea, however, this is not a common finding amongst patients who have undergone this cross-linking procedure (O’Brart, 2014).

In specific cases of keratoconus where the damage and degradation of the cornea is so severe that the use of contact lenses may result in no observable benefit to the patient and the nature of destruction may not be rectified using cross-linking, a penetrating keratoplasty may be performed. Research has suggested that the percentage of keratoconic patients requiring a keratoplasty procedure is between ten and 22% of the population affected by this corneal ectasia (Bennett and Hom, 2004). The keratoplasty technique involves removing the complete thickness of the affected cornea and replacing this with a donor cornea in order to create a new refractive surface (Romero-Jiménez et al., 2010).

2.3.8 DRY EYE AND SYMPTOMS IN KERATOCONUS

The symptoms being described by keratoconic patients may be likened to those experienced by dry eye patients. It is unclear, however, whether these symptoms are of the same origin, whether keratoconic patients do indeed suffer from dry eye disease or whether these symptoms just appear to be similar. There is little research detailing the presence of dry eye in keratoconic subjects. The study by Karamichos et al., (2015) where tear profiles of keratoconic and dry eye patients were investigated, found that the tear metabolome between keratoconic and dry eye patients is similar.

The symptoms described by keratoconic individuals vary substantially with most
symptoms being related to the inaccurate visual acuity experienced in this condition. However, patients may also complain of symptoms such as light sensitivity and discomfort (Wang and Swartz, 2010). The study by Dogru et al., (2003) where various clinical features of keratoconus were investigated, demonstrated the presence of symptoms such as eye fatigue, irritation and foreign body sensation. Symptoms may also vary due to the stage of progression of the disease, with early keratoconus showing a lack of symptoms (Saxena, 2011; Bühren et al., 2007). In the early stages of keratoconus, patients generally notice a blurring of vision as well as distortion (Levin and Albert, 2010). During the more advanced stages of keratoconus, patients may begin to complain of symptoms such as headaches, eyestrain, photophobia, glare as well as night blindness (Levin and Albert, 2010).

The symptoms of discomfort experienced by keratoconic patients may be due to the presence of associated conditions such as atopic dermatitis, keratoconic subjects have often been found to suffer from various other atopic conditions which may play a role (Rudikoff et al., 2014). One of the prominent symptoms described by keratoconic patients is itching which in most cases, is due to the presence of an atopic history (Bennett and Hom, 2004). Approximately 50% of keratoconic patients have raised serum levels of Immunoglobulin E due to the presence of associated conditions, this in turn results in severe itching and therefore vigorous rubbing of the eyes (Bennett and Hom, 2004).

The study by Kymes et al., (2004) discusses the effect that keratoconus may have on the quality of life of these patients due to the symptoms of ocular discomfort as well as the effect on visual function. This study shows that keratoconic individuals experience a loss of function in their general surroundings that is disproportionate to the clinical measures seen by clinicians (Kymes et al., 2004). Keratoconic patients tend to have increased anxiety and reactivity to various stressful situations (Kymes et al., 2008).

Studies where OSDI questionnaires were utilized in order to determine the symptoms being described by keratoconic subjects are scarce. One study performed by Carracedo et al., (2015) made use of this specific questionnaire when comparing the symptoms of keratoconics versus healthy subjects and found the mean OSDI score to be significantly higher in the keratoconic group, signifying the severity in symptoms being experienced.

2.3.9 OTHER CLINICAL FINDINGS IN KERATOCONUS

The study by Dogru et al., (2003) where various clinical parameters were measured in keratoconic versus control subjects showed significant differences between the two study groups. NTBUT measurements were shown to be significantly reduced in the keratoconic
group with a mean value of 6.8 seconds as opposed to 14.5 seconds for the control group (Dogru et al., 2003). NTBUT measurements by Dogru et al.’s, (2003) study seemed to be significantly lower as the grade of keratoconus increased suggesting a decrease in tear film stability as keratoconus progresses. The study by Mohd-Ali et al., (2011) also showed a significant difference between the NTBUT measurements of keratoconic versus normal subjects with those of keratoconics being significantly lower.

Other clinical measures such as TMH have also been measured in keratoconic subjects in order to determine whether a significant difference exists. The study by Sarac et al., (2011) found no significant difference between the height and the area of the tear meniscus when comparing keratoconic subjects with a group of control subjects. In the study by Carracedo et al., (2015) it was found that keratoconic subjects have lower tear volumes compared to control subjects. This study by Carracedo et al., (2015) found no significant difference in TBUT measurements between the two groups, however, using other findings such as the decreased mucin production in keratoconic tears, concluded that keratoconic subjects suffer from greater tear film instability compared to healthy individuals.

The presence of uncharacteristic clinical results in keratoconus has been published, however, there still remains a lack of specific symptomology relating to clinical findings that has not been extensively investigated.
2.4 INSTRUMENTATION

2.4.1 THE OCULAR SURFACE DISEASE INDEX

The OSDI is a comprehensive method used to evaluate the severity of subjective dry eye symptoms being experienced in ocular disease. This questionnaire was first developed in 1997 and comprises of 12 questions which are broken up into three separate sub-scales, with each sub-scale being composed of specific types of questions. Questions numbered one through to five are related to the specific types of symptoms being experienced, questions six through to nine determine whether these symptoms cause interference with daily tasks while questions 10 through 12 assess the association of any environmental factors which may result in exacerbation of these symptoms (Alió and Azar, 2008).

When completing the OSDI questionnaire, there are five options to choose from ranging from symptoms which occur all of the time given by a value of four to those that occur none of the time given by a value of zero with intervals in between zero and four. Subtotals for the three separate subscales are calculated and a total for the entire questionnaire is recorded. Using the total for the three subscales along with the number of questions answered out of 12 (which must be specified), these values can be put into a formula in order to determine the severity of symptoms being experienced, given as a percentage. Depending on the percentage obtained, the severity may be determined with a larger percentage indicating symptoms of greater severity. The overall percentage given by the OSDI questionnaire is calculated using the specified calculation shown below:

\[
OSDI = \frac{(\text{SUM OF SCORES}) \times 25}{\text{NUMBER OF QUESTIONS ANSWERED}}
\]  

(1)

A sample of the OSDI questionnaire may be found in section 8.1 Appendix A.

2.4.2 THE KERATOGRAPH

2.4.2.1 CORNEAL TOPOGRAPHY

When measuring specific characteristics of the anterior ocular surface, the Keratograph 4 has been shown to be a reliable source of information (Bruce and Bohl, 1992). This apparatus may be utilized for a variety of different functions. The Oculus Keratograph 4 in particular, utilizes software which may be made use of in order to obtain keratometry as well as corneal topography readings. This non-invasive computerized system consists of a
placido ring arrangement of high resolution which allows for optimal illumination without affecting the comfort of the patient (Agarwal et al., 2010). Corneal topography represents a three-dimensional imaging technique in which a map may be obtained pertaining to the surface curvature of the cornea. There are various maps which may be obtained through the use of the Oculus Keratograph 4, some of which include axial maps, height maps, refractive maps and irregularity maps (Agarwal, 2006). The axial maps provide information as to whether the cornea would be classified as normal, astigmatic or irregular whereas the height maps give an indication of the corneal height at various points on the surface of the cornea (Agarwal, 2006). The refractive power of the cornea is provided by the refractive map while the irregularity maps indicate the regularity or lack thereof at the surface of the cornea (Agarwal, 2006). Corneal topography may be utilized for various reasons, some of which include monitoring the corneal surface prior to and following ocular surgery, as well as specific contact lens fitting for specified conditions such as keratoconus (Melki and Azar, 2006).

Corneal topography maps are essential when diagnosing and monitoring the progression or severity of a corneal condition such as keratoconus. The use of corneal topography allows the examiner to view the geometry of the cornea allowing earlier diagnosis of this corneal ectasia thereby permitting prompt intervention (Holladay, 2009). When evaluating a keratoconic cornea, it is important to take note of the irregularity or lack thereof as well as magnitude and range of the corneal curvature (Sinjab, 2012). An instantaneous map may be obtained whereby the radius of curvature of the cornea is measured allowing the observation of minor irregularities within this surface (Holladay, 2009). These instantaneous maps may also be utilized in order to determine the apex of the cornea in keratoconic patients, the position of the apex as well as the corneal thickness at the center of the apex can be observed (Holladay, 2009).

The Keratograph 4 may also be utilized in order to determine the severity of keratoconus according to a grading scale. Once topography measurements have been taken, using the multitude of information given, a grading of keratoconic severity may be given. The scale ranges from grade 1 through to 5 with intermediate intervals, 1 representing keratoconus in its mildest form and 5 indicating the severity of the effects at the surface of the cornea. The exact mechanism of how this grading is calculated could not be confirmed as this is proprietary information, a breakdown of this system has, however, been given in section 2.3.5.
2.4.2.2 NON-INVASIVE TEAR BREAK UP TIME

NTBUT has been shown to be one of the most valuable tests when trying to diagnose dry eye, it has been shown to be highly specific (Jiang et al., 2014). The Keratograph was developed as one of the first commercial devices with the software allowing an automated testing technique in order to obtain NTBUT measurements (Jiang et al., 2014). The patient is seated behind the Keratograph, with the patient’s side of the instrument consisting of the forehead rest, chin rest and illuminating system. The illuminating system consists of red LED lights, at a wavelength of 653 nm, projecting a placido disc pattern onto the corneal surface. Once the patient is correctly aligned, the NTBUT setting is selected. Once the eye can be viewed centrally, the software prompts the examiner to ask the patient to blink twice. The second blink initiates the recording of measurements, with video recording taking place until significant distortion of the tear film occurs or the measurement is disrupted due to blinking (Bennett and Weissman, 2015; Yokoi and Komuro, 2004).

The Keratograph 4 software records two different values, the first being the time taken for the first distortion in the tear film to appear, the second being the mean of the TBUT (Fuller et al., 2013; Cox et al., 2015). These values are represented on a colour-coded map displaying the zones in which tear break up occurs. This colour-coded map is subdivided into 24 bands, with each band representing 15 degrees. The colours of each block range from red to yellow to green as the break up time increases.

2.4.2.3 TEAR MENISCUS HEIGHT

TMH measurements can also be obtained using the Keratograph 4. The tear meniscus function may be selected, allowing the examiner to view the lower lid margin with the tear meniscus situated at the border of the lid margin (Arriola-Villalobos et al., 2015). No illuminating lights are projected onto the ocular surface during this measurement, therefore not affecting the tear film dynamics. Photographs may be obtained of the lower lid margin. Using these photographs, specific measurements may be obtained. The Keratograph 4 is enabled with its own measuring function allowing the examiner to place a curser from one point to the next, specifying the length being measured. Measurements obtained using the Keratograph 4 are given in millimeters which can then be converted to microns (µm) (Arriola-Villalobos et al., 2015).
2.5 OPTICAL COHERENCE TOMOGRAPHY

OCT has become widely utilized in clinical practice, this imaging technique is non-invasive and provides cross-sectional and comprehensive images of internal ocular structures. This instrument has become a valuable tool in the diagnosis and monitoring of various ocular pathologies. The foremost principle behind OCT is the use of interferometry in order to measure the reflectance of light scattered off the particular structure which is affected by contributing factors such as the refractive index. OCT makes use of infrared beams which form a multitude of axial maps across the ocular surface, of which the resolution is dependent on the coherence length of the light (Steinert and Huang, 2008). Coherence may be defined as the correlation or lack thereof between two separate beams of light which may originate from the same source of origin (Brezinski, 2006). The correlation that exists between the two beams of light may either be complete or unfinished, and the result of this will influence whether the beams are said to be completely or partially coherent (Brezinski, 2006). The uses of OCT are numerous, it is extensively utilized in various health care facilities worldwide, and of particular importance is its utilization in ocular health care.

OCT imaging may be applied when investigating aspects of both the anterior and posterior ocular structures. High resolution, cross-sectional images of posterior ocular structures may be obtained using posterior segment OCT, some which include the macula, retinal nerve fiber layer as well as the optic disc (Garg, 2014). Using this technique, various pathologies may be detected such as retinal detachments, age related macular degeneration, retinal hemorrhages and various retinal degenerations (Garg, 2014).

Anterior segment OCT may be used in determining corneal thickness, monitoring the progression of corneal ectasias, determination of the anterior chamber angle as well as visualization of various anterior chamber structures. The use of OCT technology in examining the ocular tear film has been more commonly utilized in recent years. Tear film thickness may be measured, using a vertically orientated cross section over the central region of the cornea as well as the upper and lower eyelids (Wang et al., 2006). By making use of anterior segment OCT, the tear meniscus may be imaged and various measurements may be obtained including tear meniscus height, area as well as curvature (Arriola-Villalobos et al., 2015).

Various studies have advocated the use of the OCT as a reliable indicator of the tear meniscus dimensions (Del Águila-Carrasco et al., 2015; Johnson and Murphy, 2005; Nguyen et al., 2012). The study by Del Águila-Carrasco et al., (2015) took 34 subjects wearing
different types of daily disposable contact lenses and measured the TMH using an OCT. The study investigated whether different soft contact lens materials influenced TMH differently (Del Águila-Carrasco et al., 2015). The study by Nguyen et al., (2012) investigated the correlation between lower TMH using the OCT and various objective tear film tests. Fourier-Domain OCT (FD-OCT) was utilized during this specified study and it was determined that this method is a precise technique when attempting to determine the height of tear meniscus. A study by Johnson and Murphy (2005) compares the various methods of TMH measurement, with OCT being a reliable source of information when taking such measurements.

In cases where measurements were taken using the OCT, ImageJ was utilized in order to perform measurements on the scans taken. ImageJ is an image processing program developed by the National Institute of Health. This program can be calibrated according to custom settings when measuring specific aspects of certain images. The ImageJ program allows the user to process, analyze, measure, edit, save and print various images of varying quality. ImageJ is calibrated in such a way that measurements are given in pixels prior to the scans being measured. This program must be calibrated in such a way that the measurements are given in millimeters and can then be converted to microns. When measuring images of known distances, it could be determined that 1mm was the equivalent of 248 pixels. The ImageJ software must be calibrated in order to measure specified scans, the scale settings can be selected and using these settings, the ratio of pixels to mm can be specified and the exact variables required for example length and area can be chosen. ImageJ is a valuable tool in studies such as this.

Utilizing the instrumentation and tools discussed within this chapter, measurements of keratoconic severity, NTBUT, TMH, TMA and severity of symptoms could be determined for both subject groups,
CHAPTER 3

OVERVIEW OF METHODS OF ANALYSIS
3.1 INTRODUCTION

The data values obtained throughout this study were analyzed using both SPSS and Medcalc software programs which were the most appropriate programs for the types of data presented.

3.2 SPSS SOFTWARE

SPSS has been in use for many years and proves to be a simple and easily understandable program used by health care practitioners amongst others, in order to perform statistical analyses. The features of the software amongst many others include calculation of the following:

- Descriptive statistics: means, medians, standard deviations, interquartile ranges, ranges, minimum and maximum data values
- Tests for normality namely the Kolmogorov-Smirnov and Shapiro-Wilk tests
- Non-parametric testing methods such as the Mann-Whitney U test

3.2.1 KOLMOGOROV-SMIRNOV AND SHAPIRO-WILK TESTS

The Kolmogorov-Smirnov goodness of fit test was utilized in order to determine whether a specific distribution of data measurements conform to a theoretical distribution model with the null hypothesis stating that the population of data measurements is normally distributed (Sheskin, 2003). The Kolmogorov-Smirnov test requires that a cumulative probability distribution be constructed between the two different sample groups being tested with a null hypothesis which states the following:

\[ H_0: F(X) = F_0(X) \]  

When accepting the null hypothesis, the two different sample groups are shown to be normally distributed and by rejecting the null hypothesis, the sample groups are shown not to be normally distributed. One limitation of the Kolmogorov-Smirnov test is its sensitivity to large values within the sample. The Shapiro-Wilk test is based on the correlation between data values and is said to be a better indication of normality compared to the Kolmogorov-Smirnov test. The Shapiro-Wilk test follows the same assumption with a null hypothesis stating that the two distributions are shown to be normally distributed. If the \( \rho \) value is shown to be less than the critical level chosen, this null hypothesis can be rejected.
3.2.2 MANN-WHITNEY U TEST

The Mann-Whitney U test is an alternative to the t-test as the data given is non-parametric. The Mann-Whitney U test is utilized in order to compare the medians of two different distributions (Pallant, 2005). The Mann-Whitney U test converts the data to ranks, ordered from the smallest to the largest numbers across two different groups to be tested and compared (Pallant, 2005). This test is based on the principle that if a significant difference is found to be present between the two data sets, it can be determined that these data sets originate from different populations and therefore the null hypothesis which states that the data values originate from the same sample group can be rejected (Black, 2009). This test provides a U statistic and is compared to a significance level of 0.05, if the significance level is less than or equal to the value of 0.05 then it can be concluded that the differences between the two groups are statistically significant.

3.2.3 BOX AND WHISKER PLOTS

Box and whisker plots may be considered as explanatory illustrations which show the general distribution of a dataset. Various components of the specific dataset may be seen using such plots including the median, maximum and minimum values, interquartile range and any outliers present. An example of such a plot can be seen below in Figure 3.2.3.1.

![Box and Whisker Plot](image)

**Figure 3.2.3.1** Figure representing an example of a box and whisker plot, used to visualize a distribution of data measurements between two different subject groups.
The above figure represents a box and whisker plot, these types of plots were used extensively throughout this study and therefore an accurate understanding of the different components is necessary when interpreting the results given. The above plot represents the distribution of data values for two different subject groups as indicated on the x-axis, namely the keratoconic (group 1) and the control group (group 2). As the name suggests, each subject group consists of a box (A) with whiskers projecting above and below the box (B).

The entire box represents the interquartile range of data values with the top border of the box indicating the upper quartile while the bottom border indicates the lower quartile. The thick black line running through the centre of the box (A) is indicative of the median or otherwise known as the middle quartile. The end of each whisker consists of a short horizontal line, with the top whisker indicating the maximum value (excluding outliers) and the bottom whisker indicating the minimum value (excluding outliers).

Outliers can be seen as values which tend to fall outside of the range of the box and whisker plot, not included within the minimum and maximum values (C). Each outlier is represented as a case number pertaining to the subject that the measurements were taken on (C). The 50 eyes of each subject within the two groups were numbered starting from 1 to 100, therefore labelled as the case number indicated by each outlier as can be seen in Figure 3.2.3.1 (C).

3.3 MEDCALC SOFTWARE

3.3.1 BLAND-ALTMAN PLOTS

Medcalc is another software program being used for statistical analysis. Medcalc was utilized in this study in order to generate Bland-Altman plots and Spearman’s rank correlation with scatter plots. Bland-Altman plots are generated in order to determine the agreement between two specified components (Bland and Altman, 1986; Hofman et al., 2015). The Bland-Altman difference plots are generated by calculating the difference between two different components and plotting these differences against their corresponding means (Bland and Altman, 1986; Burkhardt and Weiss, 2008).
Figure 3.3.1.1 Figure representing an example of a Bland-Altman plot, used to compare two different sets of data measurements.

Figure 3.3.1.1 illustrates an example of a Bland-Altman plot, generally used to determine the difference between two sets of measurements. The x-axis represents the means of the two different data sets or groups of measurements \( \frac{X+Y}{2} \). The y-axis represents the differences between the two different data measurements. The solid horizontal line passing through the plot (A) represents the mean difference between the two variables, this represents the estimated bias between the two sets of data measurements. The mean difference is surrounded by a confidence interval with the standard deviations representing the random fluctuations occurring around this mean difference (Bland and Altman, 1986; Burkhardt and Weiss, 2008). The dotted lines located superior and inferior indicate the limits of agreement (B) (upper and lower limits). Any value falling outside of these limits is said to be an outlying value, such as the one data value being observed below the lower limit of agreement (C). The limits of agreement are shown to be within 1.96 standard deviations of the mean difference. Values that cluster around a value of zero display a mean difference that is almost non-existent and therefore the two variables may be considered interchangeable.

3.3.2 SPEARMAN’S RANK CORRELATION

Medcalc was utilized to determine whether a correlation exists between the variables present within this study. This correlation requires that data be arranged according to rank as
the data readings increase in value (Bland, 2000; Nagle and Spencer, 2000). The statistic given is therefore not dependent on the original distribution of data measurements. Rank correlation is performed in order to determine the correlation coefficient \( r \). By utilizing the specified significance level \( \rho \), it can also be decided to accept or reject the null hypothesis between two sets of measurements which states that a significant relationship cannot be found between the two variables being tested. A positive value for \( r \) indicates the presence of correlation between two variables (as one variable increases so too does the other in a proportionate nature). A negative value indicates negative correlation (as one variable increases the other is shown to decrease). The correlation coefficient can be any value up to and including a value of 1, with the distance from 1 indicating the strength or weakness of the correlation (Bland, 2000; Nagle and Spencer, 2000). The critical value for Spearman’s rank correlation is chosen as a \( \rho \) value of 0.05 (95% confidence level). In cases where the \( \rho \) value falls below 0.05, the null hypothesis can be rejected therefore indicating that the correlation is statistically significant. Scatter plots displaying a graphical representation of the data set could be generated in order to have a visual impression of the presence or absence of any correlation, an example of which can be observed in Figure 3.3.2.1.

![Figure 3.3.2.1](image)

**Figure 3.3.2.1** Figure representing an example of a scatter plot generated by plotting the data of variable X against the data of variable Y. As can be seen, there is a lack of correlation shown to be present between the two variables as the data points are distributed in a random pattern.

Figure 3.3.2.1 represents an example of a scatter plot calculated in order to view the data points between two variables being tested. Variable X and Y are compared in order to view a visual representation of the correlation shown to be present. In cases where a
correlation is found to exist, the data points will be clustered together in an organized manner, generally forming a straight diagonal line, showing the proportional increase of one variable compared to the other. Lack of correlation can be seen in Figure 3.3.2.1 as the data points are distributed in a random nature with no characteristic pattern being observed.

These statistical methods were employed in order to analyze the data obtained throughout this study, the results of which are presented in chapter 5.
CHAPTER 4

EXPERIMENTAL METHODS
4.1 INTRODUCTION

The experimental procedures in this study were performed on both eyes of 50 subjects. All experimental procedures were performed according to the principles outlined by the declaration of Helsinki. Ethical clearance was obtained by the University of Johannesburg prior to the collection of data measurements. The subjects who took part in this study comprised of two different groups, the experimental group consisting of keratoconic subjects and the control group which comprised of normal healthy individuals, each group being composed of 25 subjects. The experimental group consisted of keratoconic patients attending the contact lens clinic at the University of Johannesburg for contact lens fittings. These keratoconic subjects were not confined to a single ethnic group and consisted of 15 females and 10 males ranging between the ages of 19 and 56 (mean age = 25.08, standard deviation = 10.63). Most of the subjects were referred from other institutions or government clinics in order to be fitted with contact lenses to aid in visual function.

Routine preliminary tests were performed on each of these keratoconic subjects, including a comprehensive case history, motilities, pupils, saccadics and visual acuity. Retinoscopy and subjective refractions were attempted on each patient, however, in most cases this proved to be futile and hence no binocular tests were performed. Binocular tests were not included as the subjects could not fixate accurately on a target due to the decreased visual acuity. The presence of keratoconus was confirmed using various diagnostic procedures, including retinoscopy, corneal topography, keratometry and slitlamp biomicroscopy. Retinoscopy reveals the presence of a scissoring reflex where two beams of light can be seen to move in opposite directions as a result of the astigmatism present in keratoconus. The corneal topography system, namely the Oculus Keratograph 4 was utilized in order to view the surface characteristics of the cornea in the experimental (keratoconic) subject group.

The Keratograph 4 gives a grading of keratoconus ranging between 1 and 5, indicating the severity of keratoconus. This grading is calculated by combining the various components given by the corneal topography maps (the exact calculation could not be obtained as this is proprietary information), however, the parameters used to determine the grading are given in section 2.3.5. In extreme cases of keratoconus, corneal topography readings could not be obtained. Keratometry was attempted on each of the keratoconic subjects, however, due to the corneal distortion seen in keratoconus, the keratometric mires were severely distorted and therefore these measurements were not accurately obtained for
each subject. Slitlamp procedures were performed in order to observe diagnostic signs characteristic of keratoconus, some of these signs include Vogt’s striae, fleischer’s ring, thinning of the cone, scarring and endothelial folds as discussed in section 2.3.4.

Once the preliminary tests had been performed and the presence of keratoconus could be confirmed, the subjects were invited to partake in the study. The study details and procedures were explained to the keratoconic subjects and informed, written consent was obtained prior to data collection.

The control group (mean age = 19.28, standard deviation = 1.14) of 25 subjects was composed of optometry students attending the University of Johannesburg. The first year students were approached as their schedule allowed for availability in order to partake in this research study. Corneal topography measurements were performed at the University of Johannesburg on each of the control subjects to confirm the absence of keratoconus. Ophthalmoscopy and slitlamp biomicroscopy procedures were performed on each of the control subjects to ensure the absence of any ocular pathology which would result in exclusion from the study. Following the above mentioned procedures, the subjects were invited to partake in the study. Subjects were excluded from participation in this study based on the following set of criteria: the presence of any ocular pathology or recent ocular surgeries which may impair the effective functioning of the lacrimal system thereby affecting the structure of the tear meniscus. Another exclusion factor included subjects wearing contact lenses or recent contact lens wear as the contact lens wear may have an effect on the dimensions of the tear meniscus. Subjects taking any medications which could have an influence on the function of the tear film were also excluded from the study. Once it was determined whether the subject fulfilled the specified criteria and was willing to partake in the study, the study details were explained and written consent was obtained prior to data collection.

Both the experimental (keratoconic) and control groups were encouraged to ask questions if any study procedures were unclear. Following written consent, measurements could be collected on each of the 50 subjects.

4.2 OSDI QUESTIONNAIRE

Each subject was presented with an OSDI questionnaire to complete. This questionnaire was thoroughly explained to each individual in order to ensure the accurate understanding of the questions being asked. Each subject was required to complete the OSDI questionnaire as accurately as possible and subjects were encouraged to ask questions if
clarification was needed when answering the specific questions. Following the completion of these questionnaires, the OSDI scores could be manually calculated at a later stage using the equation (1) specified in chapter 2 (section 2.4.1).

Following the completion of the OSDI questionnaires, measurements could be obtained using both sets of instrumentation namely the Oculus Keratograph 4 and the iVue OCT. In an attempt to ensure that constant temperature conditions were maintained throughout, the air conditioning was set at a standard setting of 22 degrees Celsius for all measurements. This was done in order to rule out changes in the tear dimensions due to the temperature of the surrounding atmosphere.

4.3 THE KERATOGRAPH 4

The Keratograph 4 was utilized for three separate types of measurements namely, corneal topography readings, NTBUT as well as TMH measurements, all of which were obtained on both subject groups. In the case of each measurement obtained using the Keratograph 4, the patient was asked to keep their head as still as possible, to blink and thereafter, to hold their eyes open during the acquisition of the measurements. For NTBUT, three measures were taken for each eye, while in the case of TMH, five measurements were taken for each eye. In between each measurement, the machine was refocused prior to obtaining the next measurement.

4.3.1 CORNEAL TOPOGRAPHY

Corneal topography measurements were obtained for both the keratoconic and the control group of subjects as mentioned in section 4.1. The presence and grade, therefore the severity of keratoconus, could be determined using the corneal topography measurements, while in the control group of subjects, the absence of keratoconus could be confirmed. The subjects were seated comfortably, chin resting on the chin rest and the forehead resting comfortably against the forehead rest. The subject was then asked to look straight ahead at the fixation target present in the centre of the placido ring structure on the patient’s side of the instrument. The fixation target consists of an orange ring with a black centre, the patient was asked to look directly into the centre of the orange ring. The red placido ring structure of lights will fluoresce at the commencement of the measurement. From the examiners side, the corneal topography setting was selected and adjustments were made using the joystick situated in front of the examiner.

The Keratograph 4 presents signaling to the examiner (using direction-giving arrows)
indicating the directions in which the joystick should be moved in order for the measurement to be taken. Once the mires on the examiner's screen are clear and aligned, an automatic measurement was obtained. Corneal topography measurements were taken on both eyes of the 50 subjects. In cases where measurements failed (in the keratoconic group specifically), these measurements were repeated and in cases where the second attempt was not successful, the absence of results was recorded. The results were represented on a topography map showing the surface characteristics of the cornea. This colour coded topography map shows areas of elevation and depression along the corneal surface, providing large amounts of information regarding the corneal characteristics of these subjects. Despite the amount of information given by the Keratograph 4 when obtaining a topography map, the examiner was able to select the specific map needed for this study which specified the grade of keratoconus as given by the corneal topography system of the Keratograph 4. Keratoconus is graded from grade 1 to grade 5 as discussed in section 2.3.5. For easy reference purposes, the grade of keratoconus is divided into grade case numbers in order to represent the intervals between different grades in an easily understandable manner. These grade case numbers were not given by the Keratograph 4 but rather calculated manually in order to represent the grade as one numerical value. The grade case numbers are represented as follows:

- Grade 1 indicated by case number 1
- Grade 1-2 indicated by case number 2
- Grade 2 indicated by case number 3
- Grade 2-3 indicated by case number 4
- Grade 3 indicated by case number 5
- Grade 3-4 indicated by case number 6
- Grade 4 indicated by case number 7
- Grade 4-5 indicated by case number 8
- Grade 5 indicated by case number 9 (a measurement could not be obtained)

### 4.3.2 Non-Invasive Tear Break Up Time

NTBUT was obtained using the Keratograph 4 and these measurements were performed on both eyes of all subjects. With the subject correctly aligned as with corneal topography, the subject was directed to look straight ahead at the center of the placido disc rings where the orange circle with the black centre can be observed as seen with corneal topography. The patient was instructed to keep their head as still as possible while obtaining
the measurements. The NTBUT setting was selected, where the red placido ring structure lights up for the commencement of the measurement. Once the NTBUT setting was selected, the subject was instructed to blink twice and thereafter, to hold their eyes open for a long as possible to obtain accurate measurements. The second blink activating the initiation of the measurement while the measurement ceases once tears over the front surface of the cornea have evaporated entirely or once the subject has blinked again, disrupting the measurement. In cases where measurements were disrupted, new measurements were attempted, however, in some keratoconic cases, TBUT’s could not be measured. NTBUT using the Keratograph 4 functions similarly to the corneal topography measurements in that the measurements are automatic. Once the tear film starts to break up, the Keratograph 4 records the measurements. Three measurements were taken on both eyes of each subject and after each measurement, the joystick was used to refocus and a new measurement was taken. The three measurements were taken at approximately 10 second intervals giving the examiner time to refocus the instrument.

Two measurements are given by the Keratograph 4 system as specified in section 2.4.2.2, the first measurement was of particular importance in this study, being the first time the tear film starts to break up. The second measurement, being the mean of the break up time was of no significance throughout thus study. Thereafter, a mean for the NTBUT measurements, as given by the first value could be calculated from the three values obtained.

4.3.3 TEAR MENISCUS HEIGHT

Utilizing the Keratograph 4, TMH measurements could be measured. With the subject seated comfortably behind the instrument, the tear meniscus setting was applied. The Keratograph 4 allows the examiner to view the front surface of the eye with a black and white photographic image appearing on the screen. The examiner is able to adjust the position of the camera in order to view the lower lid margin. The patient was instructed to keep their head still, resting against the chin and forehead rests, as well as to focus on the target displayed. Due to the positioning of the patient in order to image the lower lid margin, only half of the orange circle (target) as seen with corneal topography can be seen and the patient was therefore asked to focus on the top of the orange circle as a fixation point. Once the position of the camera had been adjusted and the examiner could view the lower lid margin clearly by adjusting the focus, photographs were obtained with the tear meniscus situated above the lower lid margin. These photographs were obtained on both eyes of each subject, in both the keratoconic and the control group of subjects (an example of such a photograph may
be viewed in Figure 4.3.3.1). Five photographs were obtained from each eye in order to calculate an average, giving a better indication of the TMH.

Following the acquisition of these photographs, each image could be magnified to twice its original size in order to accurately view the height of the tear meniscus. Measurements were taken from the margin of the lower lid to the top of the tear meniscus. A reflection of light could be observed at the top of the tear meniscus, this reflection being the light reflected off the top surface of the meniscus. Using the tools and software available on the Keratograph 4, individual photographs could be opened and could be measured. Measurements entailed selecting the measurement setting on the Keratograph 4 which allows the examiner to move a cursor from one region of the photograph towards another region and the length of the chord is determined. In an attempt to ensure consistency, each measurement was taken to the top of this reflection as specified (an example of this may be seen in figure 4.3.3.2). Each photograph could be saved with the measurements on them for further viewing and reference, if necessary. The Keratograph 4 was calibrated such that the measurements of TMH obtained are given in millimeters (mm) which were then converted to microns (µm). Each measurement was obtained by the same individual in order to ensure the consistency of the measurements.

Figure 4.3.3.1 Figure representing a photograph of the lower lid margin obtained using the Keratograph 4 which was utilized in order to determine the height of the lower tear meniscus in the 50 subjects included within this study.
Figure 4.3.3.2 Figure representing a magnified view of the lower lid margin as imaged using the Keratograph 4. Using the Keratograph 4 software, a measurement line may be plotted and given in millimeters (mm), as indicted by the yellow measurement indicator, shaped like an “I” (0.22 mm). The quality of this image could not be improved any further.

4.3.4 VERNIER MEASUREMENTS

The accuracy of the measurement system using the Keratograph 4 software is not entirely known. In order to determine the accuracy of the Keratograph 4 (as well as ImageJ) a vernier was used as a tool for comparison. The vernier was set at two different intervals, being 500 microns and 250 microns. The accuracy of the gap for each of the two intervals was established using two thickness gauges of 500 microns and 250 microns. The thickness gauge was placed between the two arms of the vernier and the vernier was locked in place at the specified thickness (250 and 500 µm). Once set at these specified intervals, photographs were taken using the Keratograph 4 imaging system as well as a canon 60D camera making use of a 100mm macro-lens. An example of the image obtained with the canon camera can be seen in Figure 4.3.4.1. The images of the two vernier settings (of known thickness as confirmed by the thickness gauge) were measured using the Keratograph 4. The measuring tool was selected and the cursor was moved from the start of the gap to the end of the gap within the vernier, in order to determine the distance as accurately as possible. This procedure was done for both thicknesses (as specified above) and can be observed in Figure 4.3.4.2. Five separate measurements were done on each of the two thickness settings. Following the completion of a measurement, the examiner would exit the image and reselect the image again in order to maintain consistency when measuring the thickness of the gap. A mean was calculated for the five measurements taken on each of the two thicknesses.
Using the vernier images obtained with the canon camera, the width of the gap was measured using the ImageJ software. These images were saved onto a memory card and imported to a laptop where the ImageJ software could be utilized. The ImageJ software, as discussed in section 2.5, may be utilized to measure various parameters of photographic images. Applying the ImageJ software, the thickness of each of the two images (500 and 250 microns) could be measured as seen in Figure 4.3.4.3 and compared with those measured using the Keratograph 4. In this way, the accuracy of each of the two scan measurement systems (Keratograph 4 and ImageJ) could be compared to a gap of known thickness.

![Figure 4.3.4.1](image) Figure representing the vernier caliper set at a known interval of 500 µm. An image such as this one could be measured using both the Keratograph 4 measuring system and ImageJ in order to determine the accuracy of these measurement techniques.
**Figure 4.3.4.2** Figure representing the vernier caliper set at a known gap of 500 µm being measured using the Keratograph 4 software. Using the Keratograph 4, a measurement line may be plotted and given in millimeters (mm), as indicated by the yellow measurement indicator, shaped like an “I” (0.46 mm). The quality of this image could not be improved any further.

**Figure 4.3.4.3** Figure representing the vernier caliper set at a gap of 500 µm being measured using the ImageJ software program. The measurement indicator may be seen as the vertical yellow line indicating the thickness being measured with this software. Looking at the measurement block given within this figure, the interval being measured was shown to be 500 µm.
4.4 OPTICAL COHERENCE TOMOGRAPHY

The OCT was also utilized to obtain TMH and TMA measurements. The subject was seated comfortably behind the instrument, chin on the chin rest and forehead against the forehead rest. The subject was instructed to look straight ahead at a target within the OCT (the target can be seen as a green cross within the cornea anterior module (CAM) lens when measurements are being taken). Once the personal particulars of the subject had been entered into the computer, the eye being measured was selected and the anterior segment settings were activated. Before these measurements were obtained, the CAM lens was attached to the front portion of the instrument in order to obtain anterior segment scans. With the subject looking straight ahead, the instrument is adjusted in order to view the central portion of the lower lid margin. Due to the fact that the lower lid margin is focused on, the patient is instructed to view the top portion of the green cross. With the subject correctly aligned and scan quality within acceptable limits, the scans can be obtained by pressing the button on the joystick. Both eyes of each subject were scanned, with five scans being obtained on each eye in order to calculate an average. Once a scan had been obtained, the instrument was pulled back and refocused in order to take the next scan, ensuring that scans were obtained as accurately as possible. Once the scan had been taken, two separate images can be viewed. One of the images being a black and white photograph of the specific portion of the lower lid margin (as seen in Figure 4.4.1 (B)). The second image being a colour scan indicating the angle formed at the junction between the lower lid margin and the cornea of the same area (as seen in Figure 4.4.1 (A)).

The OCT scans obtained on each eye of the 50 subjects were exported from the OCT system and saved onto a memory stick in order for measurements to be performed. The OCT software was not utilized when measuring these scans as the units given by the OCT were not specified and could not be confirmed. When measuring the two different types of scans given by the OCT, ImageJ software was applied. ImageJ software, as discussed in section 2.4.3, was calibrated such that measurements were given in millimeters and converted into microns to determine both TMH and TMA. When measuring the TMH, each scan was manually imported into the ImageJ program, and the length setting was applied. Measuring the OCT photograph entailed moving the cursor from the border of the lower lid margin to the top of the light reflection seen at the uppermost border of the tear meniscus (as with the Keratograph 4 images) as can be seen in Figure 4.4.2. When measuring the OCT colour scan, the tear meniscus could be seen as the curved line joining the lower lid margin and the
anterior surface of the cornea, the cursor was placed at the starting point and end point of this line creating a straight line thereby determining the distance (as can be seen in Figure 4.4.3). Even though the tear meniscus can be seen as a curved line in the colour scan, in this study, we chose to use a straight horizontal line joining the two regions as calculating lengths of curved lines required further complicated calculations.

When measuring the area of the tear meniscus, only the colour scans were utilized as the entire tear meniscus structure can be viewed using the colour scans. When looking at the colour scans, a triangular shaped region can be observed, this area being the area between the lower lid margin, the cornea and the superior border of the tear meniscus. When selecting the area settings on the ImageJ program, each image is magnified to three times its original size and a triangular shaped region can be plotted at the borders of these three structures specified above. As with the TMH, the curved lines were ignored and straight lines were plotted when creating the triangular region to be measured (as seen in figure 4.4.4). Once this triangular region had been plotted, the ImageJ program automatically calculates the area of the specific region in millimeters as calibrated and converted to microns (µm). Utilizing the five photographs and five colour scans obtained, a mean could be determined for both the photograph and colour scan in terms of TMH as well as a mean for the TMA of each eye for all subjects.

Following the completion of all measurements, statistical analysis was performed using the SPSS and Medcalc software programs.
Figure 4.4.1 Figure representing a scan obtained using the OCT where both the colour scan (A) and the photograph (B) may be observed. TMH was measured using both the colour scan and the photograph whereas only the colour scan (A) could be used to measure TMA.

Figure 4.4.2 Figure representing the magnified view of an OCT photograph where the height of the tear meniscus was measured using ImageJ software. The region being plotted by the yellow vertical line represents the height of the tear meniscus being measured as 157 µm.
Figure 4.4.3 Figure representing the colour scan obtained using the OCT where the height of the tear meniscus was determined using ImageJ software. The yellow horizontal line seen in the colour scan represents the height being measured which can be seen to be 222 µm.

Figure 4.4.4 Figure representing a magnified view of a colour scan obtained using the OCT where a triangular region was plotted in order to determine the area of the tear meniscus as measured using ImageJ.
CHAPTER 5

RESULTS
5.1 NORMALITY OF DATA

Normality tests are performed on the data sets in order to determine whether they are normally distributed. The assumption of normality is met in cases where a distribution of scores is found to be symmetrical and the data points have been shown to be proportional (Pallant, 2005). Once tests for normality have been performed, it can be decided whether to utilize parametric or non-parametric testing methods to assess the data sets. In cases where the data is normally distributed, parametric testing methods may be used which are found to be more robust. In cases where the data is not found to be normally distributed, non-parametric testing methods are utilized, in which fewer assumptions can be made regarding the probability of the distributions. The Kolmogorov-Smirnov and the Shapiro-Wilk tests for normality were selected when assessing these data. Using the results obtained from these normality tests, it is possible to validate the use of either the parametric or non-parametric testing methods.

Table 5.1 Representation of the two different tests performed in order to determine the normality of data. Both the Kolmogorov-Smirnov and the Shapiro-Wilk tests were utilized for both subject groups, being the keratoconic and the control groups. For both tests, the test statistic and the significance level are represented. OSDI being the OSDI percentage obtained, NTBUT representing the non-invasive TBUT measurements, TMH-OC representing measurements obtained using the OCT where the colour scan was measured. TMH-OP indicating that the OCT was used as a method of measurement, however, the photograph was measured. TMH-K represents measurements of the TMH using the Keratograph 4. TMA-OC represents tear meniscus area measured using the OCT, where the colour scan was measured. Measurements obtained by the keratoconic group are indicated by the letter K at the end of each component given and measurements obtained by the control group are indicated by a C at the end of each component. These test statistics are compared to a significance level of 0.05.
Tests of Normality

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</tbody>
</table>

From the values represented in Table 5.1, it can be seen that in most cases the data sets are not normally distributed. The null hypothesis states that the distribution of results is shown to be normally distributed and is drawn from the same distribution. The null hypothesis is rejected if $\rho$ is found to be less than 0.05. Using the Kolmogorov-Smirnov test, the data sets which were found to be normally distributed included the following (and are indicated by an *): OSDI-K, TMH-OCK, TMH-OPK, and TMH-KC. When these data were assessed using the Shapiro-Wilk test, it can be seen that only OSDI-K and TMH-OPK were found to be normally distributed. In the case of the remaining variables, the null hypothesis could be rejected thereby indicating that the data distributions are not normal. For this reason, mostly non-parametric tests were performed on the data sets. Non-parametric testing methods utilize ranking or an ordered arrangement of data measurements rather than the physical nature of the numbers themselves. The statistical analysis performed on these data include the use of the Mann-Whitney U test in order to determine whether the data sets are statistically significantly different. When examining the correlation between numerous variables, Spearman’s rank correlation is chosen as the method of choice due to the lack of normality within the data set.
5.2 SYMPTOMS (OSDI SCORE)

The OSDI scores of each subject were calculated using the equation (1) specified in chapter 2. An overall score was assigned to each subject based on the number of questions answered and the sum of scores calculated for each of the sub-sections in the OSDI questionnaire. Due to the fact that symptoms are subjective complaints and generally cannot be reported separately in terms of each individual eye, one OSDI score is obtained for each of the 50 subjects within the study.

Table 5.2.1 Descriptive statistics represented for the OSDI scores obtained from each of the 50 subjects. These descriptive statistics include the mean, median, standard deviation, range and interquartile range for both subject groups. OSDI scores are represented as a percentage depending on the severity of the symptoms being experienced with higher values indicating symptoms of greater severity.

<table>
<thead>
<tr>
<th>Descriptive Statistics – OSDI</th>
<th>Keratoconic</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>58.468</td>
<td>9.442</td>
</tr>
<tr>
<td>Median</td>
<td>54.9</td>
<td>6.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>20.617</td>
<td>11.98</td>
</tr>
<tr>
<td>Range</td>
<td>83.3</td>
<td>54.2</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>28.4</td>
<td>9.35</td>
</tr>
</tbody>
</table>

As observed in Table 5.2.1, the descriptive statistics for the OSDI scores display a large difference when comparing the values obtained from the keratoconic group with those obtained from the control group. The mean OSDI score of the keratoconic group may be observed to be almost six times that of the control group. A large difference may also be observed between the medians of the two subject groups, with the median of the keratoconic group being almost nine times the median of the control group. As illustrated in Table 5.2.1, it may be assumed that keratoconic individuals experience symptoms of greater severity compared to the control group of subjects. As can be seen in Table 5.1, when looking at the normality of the data using both the Kolmogorov-Smirnov and the Shapiro-Wilks tests, it can be observed that the OSDI scores for the keratoconic group are shown to be normally distributed. The control group, however, have been shown not to be normally distributed in terms of the OSDI scores.
Table 5.2.2 Mann-Whitney U test results displayed in order to determine whether the difference between the two groups is statistically significant for the symptoms being described by the OSDI scores. Represented are the $\zeta$ statistic and the significance level.

<table>
<thead>
<tr>
<th>Test statistics</th>
<th>Mann Whitney U</th>
<th>$\zeta$</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSDI score</td>
<td>50</td>
<td>-8.288</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The null hypothesis for the Mann-Whitney U test states that the median of one distribution is equal to the median of the second distribution. As can be observed from the values given in Table 5.2.2, the significance level is less than 0.05 therefore indicating that the null hypothesis can be rejected. By rejecting the null hypothesis, it can be determined that a statistically significant difference can be observed in terms of the symptoms described by the OSDI scores for the keratoconic and control groups, with the keratoconic group displaying greater severity.

Figure 5.2.1 Box and whisker plot representing the OSDI scores obtained for each of the two subject groups, namely the keratoconic and control groups as indicated on the x-axis. OSDI scores as indicated on the y-axis are represented as a percentage depending on the severity of the symptoms being experienced.
Figure 5.2.1 displays the distribution of OSDI scores for both the keratoconic and the control group of subjects, allowing for a graphical representation of the numerical data. The box for each subject group indicates the interquartile range (50%), with the thick black line situated approximately in the centre of the box indicating the median of the OSDI scores for each of the two groups (shown to be higher for the keratoconic group). The two whiskers projecting from each end of the box reach an end point which represents the maximum and minimum OSDI scores obtained for the group. The maximum for the keratoconic group has been shown to be 100% while the maximum for the control group is 54.2% with a minimum of 0. The OSDI scores for the keratoconic group are seen to be grouped around the interquartile range of data distributions with no outliers being observed. The distribution of data for the control group, however, displays a cluster of outliers. Six outliers can be observed, falling outside of the specified range, as indicated by their case number discussed in chapter 3. The severity of symptoms being experienced by keratoconic individuals can be observed when assessing Figure 5.2.1 with symptoms generally ranging from 40 to 70 percent which is more than double that of the control group, where most of the OSDI scores are found to be below 20 percent. As observed in Table 5.2.2, the difference in symptoms between the two groups is shown to be statistically significantly different.

Figure 5.2.2 Bland-Altman plot representing the data measurements in terms of the OSDI scores obtained for each of the two subject groups. The mean difference as well as the limits of agreement are represented. The means of the two subject groups (OSDI score) are represented on the x-axis and differences represented on the y-axis. Data measurements are represented as a percentage.
Bland-Altman plots may be used to determine the agreement between two different types of instrumentation or two different sets of circumstances. Figure 5.2.2 depicts the agreement between the symptoms as expressed by the OSDI scores for each of the two subject groups. The mean difference, which can be seen as the centre line passing through the plot in Figure 5.2.2, is a value of 49%. This mean difference is larger than zero, therefore indicating the difference in symptomology between the keratoconic and the control group. Most of the data points fall within the 95% limits of agreement on either side of the mean difference. One outlying value is shown to be present below the lower limit of agreement, or below -4.9 being the bottom limit. This plot illustrates the difference in symptomology between the two subject groups, where keratoconics suffer from symptoms of greater severity.

5.3 THE KERATOGRAPH 4

5.3.1 GRADE OF KERATOCONUS

As discussed in chapter 2, the Keratograph 4 was utilized for various pertinent findings including: corneal topography measurements, NTBUT and TMH measurements. The corneal topography measurements were obtained for both the keratoconic and the control subjects, however, only the grade of keratoconus has been utilized in the results to follow. The grade of keratoconus given by the Keratograph 4 is graded between grade 1 and grade 5, with intervals found to range between these values as can be seen below.

Table 5.3.1.1 Represented are the frequencies for the grade of keratoconus given by the Keratograph 4. Represented are the different grades as given by the Keratograph 4. For simplicity, each of these grades were given a grade case number for reference. The frequency of each grade as well as the total percentage for each isolated grade is represented, this applies to 25 keratoconic patients and therefore 50 eyes.
As can be seen in Table 5.3.1.1, the 25 keratoconic patients displayed a variation in severity of keratoconus according to the Keratograph 4 measurements (refer to section 4.3.1). The majority of the keratoconic subject group were graded as grade 5, where a measurement could not be obtained due to the severity of the keratoconus. Grade 5 makes up 38% of the keratoconic subject group indicating the severity of the keratoconus being experienced, grade 4 along with grade 4-5 did not feature in this subject group. The mildest form of keratoconus, being grade 1 only represents 2% of the subject group. Using the grading’s obtained with the Keratograph 4, it could be determined whether the severity of keratoconus could possibly be linked to various other objective measurements.

**5.3.2 GRADE OF KERATOCONUS VERSUS SYMPTOMS (OSDI SCORES)**

When comparing the grade of keratoconus with the symptoms being experienced by the keratoconic subjects, only one grade can be used for each of the 25 subjects. The reason for this being that the OSDI score can only be expressed as one value expressing the severity of symptoms. As a result of this, the grade for each subject was taken as the grade of keratoconus in the worse eye. This grade was therefore compared with the OSDI score calculated for each individual as well as the other clinical parameters to follow.

**Table 5.3.2.1** Representation of the grade of keratoconus as given by the Keratograph 4 versus the mean OSDI score indicating the severity of symptoms corresponding to each of the grades given. Symptoms are represented as a percentage once the OSDI scores had been calculated.
As can be seen from the values above, the grade of keratoconus showing the greatest severity in symptoms is a grade 2, which has a mean OSDI score of 76.11%. The lowest mean OSDI score is given by grade 2-3, with grade 2-3 displaying a score of 45.47%, with similar values being obtained by subjects with grade 1 keratoconus. No value is given for grades 4 and 4-5 as these grades did not feature when corneal topography measurements were obtained for the keratoconic group of subjects. Grade 5 which indicates that a reading of keratoconic grade could not be obtained, this grade obtained 54.78% showing the severity of keratoconus within this subject group.
Figure 5.3.2.1 Box and whisker plot illustrating the distribution of OSDI scores obtained by the keratoconic group versus the grade of keratoconus as given by the Keratograph 4. OSDI scores are given as a percentage with greater severity shown by a higher percentage while the grades of keratoconus are displayed on the x-axis, with the absence of grade 4 and 4-5 as these grades were not obtained for this specific subject group.

A graphical representation of the symptoms being experienced versus the grades of keratoconus can be observed in the figure above. The thick black line shown to be present in each of the boxes is indicative of the median OSDI score obtained for each specific grade. The value for each median score is given in the box to the right of each black line. There was only one subject with a grade of 1 and therefore only one solid black line may be observed with the box being absent. The largest interquartile range can be observed for grade 5, where corneal topography measurements could not be accurately obtained due to the severity of keratoconus present. One outlier may be observed as case number 29, seen outside of the box for grade 3. This box and whisker plot displays the variation in terms of severity of keratoconus for this specific subject group.

Table 5.3.2.2 Spearman’s rank correlation coefficient calculated for the grade of keratoconus versus the OSDI scores determined from the keratoconic group of subjects. The correlation coefficient, significance level as well as the 95% confidence interval for the correlation coefficient is represented.
Table 5.3.2.2 shows Spearman’s rank correlation calculated for the keratoconic group where the OSDI scores were compared to the grade of keratoconus obtained. From the values obtained using this correlation test (represented in Table 5.3.2.2) it can be seen that there is a weak (Cohen, 1988) negative correlation present between the two variables. The null hypothesis states that the two variables being tested have no correlation. When looking at the significance level, this value is much larger than 0.05 and therefore the null hypothesis cannot be rejected. The small negative correlation shown to be present between the two variables is not statistically significant. These two variables do not exhibit a relationship.

Figure 5.3.2.2 Scatter plot demonstrating the data obtained for the OSDI scores versus the grade of keratoconus obtained by the keratoconic subject group. OSDI scores are represented as a percentage while the grade case number is represented according to the scaling system ranging from 2 to 9 in this specific plot, depending on the severity of keratoconus.

The scatter plot in Figure 5.3.2.2 displays the lack of correlation between the OSDI scores and the grade of keratoconus as given by the Keratograph 4. The correlation coefficient and significance level given in Table 5.3.2.2, show that the weak correlation is not
a statistically significant finding. When observing the scatter plot above, due to the
distribution of data points being scattered in a random nature, no specific pattern of
correlation can be observed.

5.3.3 GRADE OF KERATOCONUS VERSUS NTBUT-K

Table 5.3.3.1 Represented are the various grades of keratoconus as given by the Keratograph 4 along
with each grade case number and the mean NTBUT measurements obtained utilizing the same
instrument. The grades for those subjects where a measurement could be obtained for NTBUT are
represented and the TBUT is given in seconds.

<table>
<thead>
<tr>
<th>Grade of keratoconus (GRADE-K)</th>
<th>Grade case number</th>
<th>Mean NTBUT-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1-2</td>
<td>2</td>
<td>4.77</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>8.18</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>6</td>
<td>3.40</td>
</tr>
<tr>
<td>Grade 5</td>
<td>9</td>
<td>3.42</td>
</tr>
</tbody>
</table>

The grades of keratoconus displayed in Table 5.3.3.1 are given for those subjects
where NTBUT measurements could be obtained due to the fact that in some subjects, these
measurements were unsuccessful. The NTBUT measurements are shown to be similar for
grades 1-2 and 2 with a large change observed in grade 3 where the NTBUT almost doubles.
In cases where corneal topography measurements could not be obtained (grade 5), the mean
NTBUT is shown to be much shorter with a value of 3.42 seconds. The fastest TBUT is given
by subjects with a grade of 3-4, where the mean TBUT is shown to be only 3.40 seconds
indicating the instability of the tear film in these subjects.
Figure 5.3.3.1 Box and whisker plot indicating the grade of keratoconus as given by the Keratograph 4 versus the NTBUT obtained by the keratoconic group for each grade. Grade 4 and 4-5 are not included within this illustration as these grades did not feature in this subject group.

When observing Figure 5.3.3.1, the median NTBUT measurements can be seen as a thick black line passing through the box for each grade, and as indicated by the value labelled to the right of each. Both grades 1 and 2-3 were only obtained by one eye of two different keratoconic subjects and therefore, a single NTBUT value is indicated for each of the two, with the boxes being absent. The grade displaying the greatest amount of variation in terms of NTBUT measurements is grade 3, as indicated by the whiskers showing great variation towards the maximum value (top whisker). One outlying value may be observed by case number 14, which can be seen to fall above the maximum value of grade 2.

Table 5.3.3.2 Spearman’s rank correlation represented for the NTBUT and the grade of keratoconus obtained for the keratoconic group of subjects, both of which were obtained using the Keratograph 4. Being represented is the correlation rank coefficient, significance level and the 95% confidence interval for the correlation coefficient.
Spearman’s rank correlation coefficient is represented for the NTBUT versus the grade of keratoconus in the keratoconic group of subjects. The significance level observed is greater than 0.05 and therefore the null hypothesis can be accepted stating that the correlation is not shown to be statistically significant.

![Figure 5.3.3.2](image)

**Figure 5.3.3.2** Scatter plot representing the data in terms of the grade of keratoconus versus the NTBUT in the keratoconic group of subjects. The NTBUT is represented in seconds while the grade of keratoconus is graded on a scale of 2 to 9 according to the grade case number in this particular group.

The scatter plot in Figure 5.3.3.2 is a representation of the grade of keratoconus obtained using the Keratograph 4 where the grade case number can be observed versus the NTBUT, also measured through the use of the Keratograph 4. As can be seen in Table 5.3.3.1, only five of the grade case numbers are represented, being 2, 3, 5, 6 and 9. This may be observed in Figure 5.3.3.2 where points have been plotted for only these grades whereas the remaining grades did not feature in this subject group. When observing the values in

<table>
<thead>
<tr>
<th>Spearman's Rank Correlation – GRADE-K vs NTBUT-K</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
<td>-0.175</td>
</tr>
<tr>
<td>Significance level</td>
<td>0.5171</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
<td>-0.617–0.351</td>
</tr>
</tbody>
</table>
Table 5.3.3.2, along with the scatter plot in Figure 5.3.3.2, it can be seen that no significant correlation seems to exist between these two variables. The grade of keratoconus does not have an effect on the time taken for the tears to break up.

### 5.3.4 GRADE OF KERATOCONUS VERSUS TMH-KK

Table 5.3.4.1 Descriptive statistics representing the TMH obtained using the Keratograph 4 and how these heights relate to the severity of keratoconus as seen with the grading results given by the Keratograph 4. The mean of the TMH is represented for each grading of keratoconus and each grade case number is represented for simplicity purposes. The means are represented in microns (µm). These results are represented for the keratoconic group of subjects only.

<table>
<thead>
<tr>
<th>Grade of keratoconus (GRADE-K)</th>
<th>Grade case number</th>
<th>Mean TMH-KK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>256.00</td>
</tr>
<tr>
<td>Grade 1-2</td>
<td>2</td>
<td>298.00</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
<td>248.80</td>
</tr>
<tr>
<td>Grade 2-3</td>
<td>4</td>
<td>275.33</td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>238.40</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>6</td>
<td>306.00</td>
</tr>
<tr>
<td>Grade 5</td>
<td>9</td>
<td>216.40</td>
</tr>
</tbody>
</table>

Observing the data contained in Table 5.3.4.1, the grades of keratoconus as given by the Keratograph 4 are compared with the height of the tear meniscus using the same instrument. Grade 4 and 4-5 are not included within this table as these grades did not feature for the keratoconic subject group. As can be seen in the table above, the height of the tear meniscus (means) can be seen to vary between the different grades of keratoconus with no specific trend being seen. Grade 3-4 displays the largest mean for TMH while the smallest mean is obtained for grade 5 where the severity of keratoconus is at its highest. The means for the different grades are shown to be similar, falling within the range of between 200 and 300 µm, except for grade 3-4, where the mean is shown to be above 300 µm.
Figure 5.3.4.1 Box and whisker plot demonstrating the grades of keratoconus obtained using the Keratograph 4 versus the height of the tear meniscus using the same instrument. The TMH measurements are given in microns (µm). Grades 4 and 4-5 are omitted as these grades were not featured for this subject group.

As can be seen from the graphical representation given above, the median TMH values can be seen to differ for the various grades with no specific trend being observed. Grade 3 has shown to exhibit variation as the box spans a larger distance with the maximum value as indicated by the top whisker being larger compared to the remaining grades. Grade 1-2 displays little variation as the box has been shown to be the smallest compared to the remaining grades. No outlying values can be seen within this plot and therefore the TMH values obtained using the Keratograph 4 fall within a specific range.

Table 5.3.4.2 Spearman’s rank correlation coefficient calculated for the grade of keratoconus as given by the Keratograph 4 versus the TMH, obtained using the same instrument. The coefficient of rank correlation, significance level and 95% confidence interval for the correlation coefficient is represented.
Spearman’s rank correlation represented for the keratoconic group of subjects comparing the severity of keratoconus as measured using the Keratograph 4 versus the height of the tear meniscus measured using the same instrument. From the correlation coefficient obtained, it can be seen that a weak (Cohen, 1988) negative correlation is shown to exist between these two variables. When looking at the significance level given in Table 5.3.4.2, this value is larger than 0.05 and therefore it can be concluded that this finding is not shown to be statistically significant.

Figure 5.3.4.2 Scatter plot representing the grade of keratoconus versus the TMH, both of which were obtained using the Keratograph 4 in order to view whether a correlation is shown to exist between the two variables. The grade of keratoconus is unitless and the TMH measurements are given in microns (µm).

Figure 5.3.4.2 illustrates a graphical representation of the data set for the keratoconic group of subjects. Seen in Table 5.3.4.2, it was determined that a weak, negative of correlation is shown to exist between the grade of keratoconus as given by the Keratograph 4 versus the height of the tear meniscus measured using the same instrument. When observing the scatter plot seen above, this weak correlation cannot be visually observed as the data
points are shown to be distributed in a random nature, no specific trend is observed within the data measurements. This data set has not shown statistical significance.

5.4 OPTICAL COHERENCE TOMOGRAPHY

5.4.1 GRADE OF KERATOCONUS VERSUS TMH-OPK

Table 5.4.1.1 Representation of the TMH obtained using the OCT where the photograph was measured in the keratoconic group of subjects. These measurements were compared to the grade of keratoconus obtained using the Keratograph 4, with the grade case number also being represented. The TMH measurements are expressed in microns (µm).

<table>
<thead>
<tr>
<th>Grade of keratoconus (GRADE-K)</th>
<th>Grade case number</th>
<th>Mean TMH-OPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>148.40</td>
</tr>
<tr>
<td>Grade 1-2</td>
<td>2</td>
<td>138.10</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
<td>199.92</td>
</tr>
<tr>
<td>Grade 2-3</td>
<td>4</td>
<td>216.47</td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>211.36</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>6</td>
<td>223.00</td>
</tr>
<tr>
<td>Grade 5</td>
<td>9</td>
<td>209.20</td>
</tr>
</tbody>
</table>

Table 5.4.1.1 represents the mean TMH obtained using the OCT, where the photograph was measured and the severity of keratoconus was compared. It was thought that a pattern may be observed between the severity of keratoconus as given by the Keratograph 4 and the height of the tear meniscus measured. However, when observing the data given, the smallest TMH mean is given for grade 1-2 while the largest TMH mean is given for grade 3-4. The TMH means seem to vary between the different grades of keratoconus and this variation does not seem to follow a characteristic pattern.
Figure 5.4.1.1 Box and whisker plot illustrating the grade of keratoconus as given by the Keratograph 4 versus the TMH obtained using the OCT where the photograph was measured. The TMH measurements are given in microns (µm) and all grades are given except for grades 4 and 4-5 which are omitted.

The above figure is a graphical representation of the data given in Table 5.4.1.1, where the height of the tear meniscus is compared to the severity of keratoconus. In grades 1 and 2-3, only one eye for two different subjects obtained this grade and therefore only one value is represented for each. For the remaining grades, the median TMH values are represented by the thick black lines and indicated by the numerical value given to the right of each. Grade 5 displays the largest amount of variation as this box and whisker plot spans the largest distance with the maximum value (top whisker) shown to be larger compared to the remaining grades. The height of the tear meniscus obtained using the OCT photograph seems to vary with the change in grade of keratoconus but this variation does not seem to follow a characteristic pattern.

Table 5.4.1.2 Representation of Spearman’s rank correlation calculated for the grade of keratoconus obtained using the Keratograph 4 versus the TMH measurement given by the OCT, where the photograph was measured. The coefficient of correlation, significance level and 95% confidence interval for the correlation coefficient is represented.
The correlation coefficient obtained from Spearman’s rank correlation demonstrates a strong correlation as indicated using the intervals given by Cohen (1988). Looking at the significance level obtained, this value is smaller than the critical value of 0.05 therefore indicating that the strong correlation shown to be present is statistically significant.

Figure 5.4.1.2 Scatter plot representing the data for the grade of keratoconus as determined by the Keratograph 4 versus the height of the tear meniscus using the OCT where the photograph was measured for the keratoconic group. There are no units for the grade of keratoconus and the TMH measurements are given in microns (µm).

The scatter plot seen in Figure 5.4.1.2, demonstrates the strong positive correlation shown to be present between the grades of keratoconus obtained using the Keratograph 4 versus the height of the tear meniscus, when measuring the OCT photograph. The correlation shown to exist is statistically significant, as indicated by the significance level given in Table 5.4.1.2, therefore indicating that a significant relationship exists between the severity of keratoconus and the height of the tear meniscus (TMH-OPK). When examining the visual
interpretation of the data (as seen in Figure 5.4.1.2), the increase in TMH corresponding to an increase in keratoconic grading and therefore severity can be observed.

5.4.2 GRADE OF KERATOCONUS VERSUS TMH-OCK

Table 5.4.2.1 Representation of the TMH obtained using the OCT where the colour scan was measured in the keratoconic group of subjects. These measurements were compared to the grade of keratoconus obtained using the Keratograph 4, with the grade case number also being represented. The TMH measurements are expressed in microns (µm).

<table>
<thead>
<tr>
<th>Grade of keratoconus (GRADE-K)</th>
<th>Grade case number</th>
<th>Mean TMH-OCK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>180.20</td>
</tr>
<tr>
<td>Grade 1-2</td>
<td>2</td>
<td>243.70</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
<td>201.02</td>
</tr>
<tr>
<td>Grade 2-3</td>
<td>4</td>
<td>276.47</td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>291.72</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>6</td>
<td>230.40</td>
</tr>
<tr>
<td>Grade 5</td>
<td>9</td>
<td>302.92</td>
</tr>
</tbody>
</table>

As can be seen from the values represented above, when measurements are obtained from the colour scan, the largest TMH mean is given by grade 5 while the smallest TMH mean is given by grade 1. When observing the largest and smallest TMH mean values, these seem to display a relationship with the severity of keratoconus, greater severity showing a higher TMH, a characteristic trend seems to be present. However, the TMH means for the grades between grade 1 and 5 seem to vary with no specific trend being observed but all seem to fall within the range of between 200 µm and 300 µm. It seems that the grade of keratoconus does not have an effect on the height of the tear meniscus as measured by the OCT where the colour scan is measured.
Figure 5.4.2.1 Box and whisker plot indicating the grade of keratoconus as given by the Keratograph 4 versus the TMH values obtained using the OCT where the colour scan was measured. The TMH values are given in microns (µm) with grades 4 and 4.5 being omitted.

As can be seen in the figure above, the box and whisker plots seem to display less variation compared to those given for the TMH values where the photograph was measured (seen in Figure 5.4.1.1). The median TMH values, given for each of the separate grades seem to fall within a similar range other than grades 1 and 5. Grades 1 and 2-3 are indicated by one value with the absence of a box as only one eye for two different keratoconic subjects obtained these two grades of severity. An outlying value as indicated by case number 23 may be observed to fall outside of the range of grade 3. Grade 5 displays the largest maximum value (top whisker) while grade 2 displays the smallest minimum value as indicated by the bottom whisker.

Table 5.4.2.2 Representation of Spearman’s rank correlation calculated for the grade of keratoconus as given by the Keratograph 4 versus the TMH obtained using the OCT where the colour scan was measured. The correlation coefficient, significance level and the 95% confidence interval for the correlation coefficient is represented.
Spearman’s rank correlation coefficient as seen in Table 5.4.2.2 demonstrates a weak negative correlation between the grade of keratoconus and the height of the tear meniscus obtained using the OCT (colour scan). Due to the magnitude of the correlation coefficient, it may be classified as weak according to the intervals specified by Cohen (1988). When observing the significance level, it is a value that is larger than the critical value of 0.05 and therefore the weak, negative correlation shown to be present is not statistically significant. These two variables do not exhibit a relationship.

![Scatter plot](image.png)

**Figure 5.4.2.2** Scatter plot representing the data for the grade of keratoconus as given by the Keratograph 4 versus the height of the tear meniscus given by the OCT where the colour scan was measured for the keratoconic group of subjects. The grade of keratoconus is unitless while the TMH measurements are given in microns (µm).

The scatter plot above is a graphical representation of the numerical data given for the keratoconic group of subjects. The findings of Spearman’s rank correlation found a weak negative correlation was shown to be present, however, this finding is not statistically
significant. When looking at the scatter plot in Figure 5.4.2.2, the data points are shown to be scattered randomly with no distinct pattern being observed, confirming that a visual representation of correlation cannot be observed.

5.4.3 GRADE OF KERATOCONUS VERSUS TMA-OCK

Table 5.4.3.1 Descriptive statistics representing the area of the tear meniscus obtained using the OCT where the colour scan was measured versus the grading of keratoconus as given by the Keratograph 4. The mean TMA measurements are expressed in microns\(^2\) (\(\mu m^2\)). The grade case numbers are also included.

<table>
<thead>
<tr>
<th>Grade of keratoconus (GRADE-K)</th>
<th>Grade case number</th>
<th>Mean TMA-OCK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>4000</td>
</tr>
<tr>
<td>Grade 1-2</td>
<td>2</td>
<td>9200</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
<td>8040</td>
</tr>
<tr>
<td>Grade 2-3</td>
<td>4</td>
<td>19467</td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>23680</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>6</td>
<td>14000</td>
</tr>
<tr>
<td>Grade 5</td>
<td>9</td>
<td>32280</td>
</tr>
</tbody>
</table>

The results demonstrated in Table 5.4.3.1 represent the comparison between the area of the tear meniscus being measured by the OCT versus the severity of keratoconus obtained using the Keratograph 4. When observing the results obtained from this analysis, it can be seen that no trend can be observed between the TMA and the severity of keratoconus. Grade 5 represents the largest mean TMA while grade 1 represents the smallest mean TMA. The largest and smallest values seem to display a characteristic pattern, however, the grading’s in between seem to increase and decrease in a variable manner. No specific trend can be observed when looking at Table 5.4.3.1. Grade 3 displays a large value of 23680 \(\mu m^2\) while grade 3-4 drops to a value of 14000 \(\mu m^2\). The grade of keratoconus does not seem to play a role in the area of the tear meniscus being measured.
Figure 5.4.3.1 Box and whisker plot illustrating the grade of keratoconus given by the Keratograph 4 versus the TMA obtained using the OCT where the colour scan was measured. All grades are given except for grades 4 and 4-5, the TMA measurements are given in microns$^2$ ($\mu$m$^2$).

As can be observed in the box and whisker plot above, the grade illustrating the largest median is grade 5 while the grade displaying the smallest median is grade 1. The grades shown to be present between grades 1 and 5 show variable results which do not seem to increase or decrease in a characteristic manner with grade 2 showing a small median value of 4400 $\mu$m$^2$ while grade 2-3 displays a value of 19467 $\mu$m$^2$. Two outlying values can be observed, indicated by case numbers 23 and 31, number 23 falling outside of the range of grade 2 while number 31 falls outside of the range of grade 3. The area of the tear meniscus does not seem to show a specific trend when compared to the severity of keratoconus.

Table 5.4.3.2 Representation of Spearman’s rank correlation calculated for the grade of keratoconus as given by the Keratograph 4 versus the TMA obtained using the OCT. The correlation coefficient, significance level and 95% confidence interval for the correlation coefficient is represented.
The correlation coefficient seen in Table 5.4.3.2, calculated using Spearman’s rank correlation displays a weak positive correlation (Cohen, 1988). This correlation, however, has a significance level which is larger than the critical value of 0.05 and therefore the null hypothesis cannot be rejected. No statistically significant relationship can be observed between the grade of keratoconus given by the Keratograph 4 versus the TMA, obtained using the OCT.

**Figure 5.4.3.2** Scatter plot representing the data for the grade of keratoconus versus the area of the tear meniscus obtained using the OCT. The grading of keratoconus does not have a unit and the TMA is given in microns$^2$ ($\mu$m$^2$).

The scatter plot given in Figure 5.4.3.2 represents the data of each of the two variables being tested. However, this correlation was not found to be statistically significant. The distribution of data measurements do not seem to create a characteristic pattern and therefore there is no visual indication of correlation being observed.
5.5 NON-INVASIVE TEAR BREAK UP TIME

The Keratograph 4 was utilized to obtain NTBUT for both the keratoconic and the control subject groups. The Keratograph 4 displays two different readings when measuring the NTBUT as discussed in section 4.3.2. The first measurement was utilized throughout this study, this being the first time a dry spot appears on the corneal surface. As can be seen in Table 5.1, the NTBUT for both the keratoconic and the control group have been shown not to be normally distributed using both tests for normality.

Table 5.5.1 The descriptive statistics in terms of the NTBUT obtained for both subject groups. The descriptive statistics include the mean, median, standard deviation, range and interquartile range. The NTBUT is represented as a time measurement in seconds for each of the two groups.

<table>
<thead>
<tr>
<th>Descriptive Statistics- NTBUT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Keratoconic</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>4.94</td>
</tr>
<tr>
<td>4.7</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Keratoconic</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>3.3</td>
</tr>
<tr>
<td>3.8</td>
</tr>
<tr>
<td>Standard deviation</td>
</tr>
<tr>
<td>Keratoconic</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>4.71</td>
</tr>
<tr>
<td>3.216</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Keratoconic</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>Interquartile range</td>
</tr>
<tr>
<td>Keratoconic</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

As can be seen in Table 5.5.1, the mean NTBUT values for the keratoconic and the control groups do not seem to be largely different with a difference of 0.24 seconds. The median value is shown to be larger in the control group while the range is much larger for the keratoconic group of subjects. This may suggest that the presence of keratoconus does not necessarily have an effect on the NTBUT.

Table 5.5.2 Representation of the non-parametric test statistic represented for the NTBUT when comparing the keratoconic and control groups. The test statistic along with the significance level is represented.

<table>
<thead>
<tr>
<th>Test statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann Whitney U</td>
</tr>
<tr>
<td>NTBUT</td>
</tr>
<tr>
<td>547</td>
</tr>
<tr>
<td>-0.479</td>
</tr>
<tr>
<td>0.63</td>
</tr>
</tbody>
</table>

When observing the values obtained in Table 5.5.2, it can be seen that the NTBUT measurements between keratoconic and control subjects do not display a statistically significant difference. The significance level is larger than 0.05 thereby indicating that the
null hypothesis cannot be rejected and therefore no difference is shown to be present. Therefore, the presence of keratoconus does not seem to have a significant effect on the break up time of the tear film.

**Figure 5.5.1** Box and whisker plot representing the NTBUT, measured in seconds obtained for each of the two subject groups. The median, maximum and minimum values are represented for each of the two subject groups. Measurements are given in seconds.

Figure 5.5.1 provides a graphical representation of the descriptive statistics related to the NTBUT for both subject groups. The thick black line seen to be passing through each of the two blocks represents the median value for each of the two subject groups in terms of NTBUT. Both maximum and minimum values are represented for each, as indicated, however, the maximum value for the keratoconic group is an outlying value of 20 seconds represented by case number 8. The keratoconic group also displays an additional outlying value represented by case number 14. The median value for the control group is shown to be slightly larger, being 3.80 seconds with the same minimum value of 1 second. Two outlying values may also be observed for the control group, represented by case numbers 68 and 70. When looking at the results obtained from the hypothesis testing, it can be seen that the data values given for the NTBUT measurements obtained by both subject groups do not display a statistically significant difference.
Figure 5.5.2 Bland-Altman plot representing the data in terms of the NTBUT measurements obtained using the Keratograph 4 for both subject groups. The mean difference, as well as the limits of agreement are represented. The means of the two subject groups (NTBUT) are represented on the x-axis while the differences are represented on the y-axis. Measurements are given in seconds.

The Bland-Altman plot (seen in Figure 5.5.2) demonstrates the differences in TBUT measurements between the keratoconic and control subject groups. The mean difference is shown to be close to zero, being a value of -0.4 seconds. Data points are shown to fall within the 95% limits of agreement on either side of the mean difference other than one outlying point shown to be present above the upper limit of agreement.

5.5.1 NTBUT VERSUS SYMPTOMS (OSDI SCORE)

Table 5.5.1.1 Spearman’s rank correlation coefficient calculated in order to determine the correlation between the NTBUT and the OSDI scores for the keratoconic group of subjects. Represented are the correlation coefficient, the significance level as well as the 95% confidence interval for the correlation coefficient.

<table>
<thead>
<tr>
<th>Spearman’s Rank Correlation – NTBUT-K vs OSDI-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>

As observed in Table 5.5.1.1 from Spearman’s rank correlation, when looking at the correlation coefficient for these two variables, a weak negative correlation is shown to be
present. Observing the significance level, it can be seen that the null hypothesis cannot be rejected as this value is much larger than the critical value of 0.05. Due to this, the small negative correlation seen is shown to be statistically insignificant between the OSDI scores and the NTBUT measurements. No relationship can be displayed between these two variables as determined by Spearman’s rank correlation.

![Scatter plot](image)

**Figure 5.5.1.1** Scatter plot representing the NTBUT versus the OSDI scores, both obtained by the keratoconic group. The OSDI scores are represented as a percentage while the NTBUT are represented as a time measurement in seconds.

The scatter plot which can be observed in Figure 5.5.1.1, is a graphical representation of the data set for the keratoconic group. There is no relationship that can be observed between the data measurements of the two variables. These measurements are randomly distributed, neither variable displaying any dependence on the other.

**Table 5.5.1.2** Spearman’s rank correlation for the NTBUT versus the symptoms expressed by the OSDI scores in the control group of subjects. Represented are the correlation coefficient, the significance level as well as the 95% confidence interval for the correlation coefficient.

<table>
<thead>
<tr>
<th>Spearman's Rank Correlation – NTBUT-C vs OSDI-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>
From the values obtained in Table 5.5.1.2, it can be seen that a medium positive correlation may be seen to exist between these two variables when looking at the correlation coefficient obtained (Pallant, 2005). The significance level for these two variables in terms of the control group of subjects shows that the null hypothesis can be rejected in this case. Therefore, the distribution of data measurements is not due to random sampling and a significant correlation seems to exist between the symptoms being experienced and the times taken for the tear film to break up. The significance level shows that although the correlation is of a medium nature, it is still a significant finding. Dependence can be seen between these two variables, as one variable increases, so too does the other. These results differ from those obtained from the keratoconic group where no correlation was shown to be present between the symptoms being expressed by the OSDI scores versus the NTBUT obtained using the Keratograph 4.

Figure 5.5.1.2 A scatter plot representing the NTBUT and the symptoms described by the OSDI scores obtained by the control group of subjects. The OSDI score being represented as a percentage with the NTBUT being represented as a time measured in seconds.

The scatter plot observed in Figure 5.5.1.2 represents the OSDI scores versus the NTBUT for the control group of subjects. From the values given in Table 5.5.1.2, it can be seen that a medium correlation seems to exist between the symptoms being expressed by the OSDI scores and the NTBUT for the control group of subjects. When looking at the scatter plot in Figure 5.5.1.2, a perfect linear correlation cannot be seen as the correlation coefficient does deviate from 1, however, does seem to show a visual representation of correlation present.
5.6 TEAR MENISCUS HEIGHT MEASUREMENT (KERATOGRAPH 4)

TMH measurements can be obtained using the photography system of the Keratograph 4 where photographs can be examined of the lower lid margin as discussed in chapter 4. From the normality test performed (see Table 5.1), it can be seen that the TMH using the Keratograph 4 is shown to be normally distributed in the control group. The keratoconic group, however, is not normally distributed.

Table 5.6.1 Descriptive statistics represented for the keratoconic and the control group of subjects. These descriptive statistics relate to the TMH measurements that were obtained using the Keratograph 4. Represented are the mean, median, standard deviation, range and interquartile range for each group. Values are represented in microns (µm).

<table>
<thead>
<tr>
<th>Descriptive Statistics - TMH-KERATOGRAPH</th>
<th>Keratoconic</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>268.48</td>
<td>247.12</td>
</tr>
<tr>
<td>Median</td>
<td>238</td>
<td>240</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>95.287</td>
<td>42.82</td>
</tr>
<tr>
<td>Range</td>
<td>456</td>
<td>228</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>101</td>
<td>59</td>
</tr>
</tbody>
</table>

From the descriptive statistics shown in Table 5.6.1, a difference can be observed between the means of the keratoconic versus the control subjects in terms of the TMH values obtained with the Keratograph 4. The medians can be seen to be separated by a value of only 2µm. As observed, the range and interquartile ranges for the keratoconic group are almost double that of the control group, the keratoconic group, however, was not shown to be normally distributed. Non-parametric testing methods were further utilized in order to determine whether the differences between the two subject groups are shown to be statistically significantly different.

Table 5.6.2 Non-parametric test statistics performed for the TMH obtained with the Keratograph 4 for both subject groups. The Mann-Whitney U test results are represented. The test statistic as well as the significance level is given.

<table>
<thead>
<tr>
<th>Test statistics</th>
<th>Mann Whitney U</th>
<th>$\zeta$</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMH-K</td>
<td>1207</td>
<td>-0.296</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Table 5.6.2 represents the non-parametric test statistics for the TMH obtained with the Keratograph 4. From the results observed, it can be seen that the null hypothesis cannot be rejected as the significance level is larger than 0.05. Therefore the distribution of measurements for the keratoconic and the control groups in terms of TMH using the Keratograph 4 are not statistically significantly different. There is no compelling evidence to show that the distribution of data measurements are different for these two groups when using this specific type of instrumentation.

The box and whisker plot observed above is a graphical representation of the values given in Table 5.6.1. The TMH values obtained using the Keratograph 4 are compared for each of the two subject groups. Looking at the median values seen in Figure 5.6.1, these values due not differ significantly for the two groups, with a difference of 2 µm. The maximum values for each of the two groups, as indicated by the numerical number given at the top of each box, are shown to fall outside of the interquartile range for both subject groups. Two outliers are displayed for the keratoconic group as indicated by case numbers 1 and 2. One outlier being displayed for the control group as indicated by case number 91.

**Figure 5.6.1** Box and whisker plot representing the TMH values obtained using the Keratograph 4 for both subject groups. TMH values are represented in microns (µm). Medians along with maximum and minimum values are represented.
Figure 5.6.2 Bland-Altman plot representing the data measurements obtained for the TMH measured using the Keratograph 4 between the two different subject groups. The mean difference as well as the limits of agreement are represented. The means of the TMH values for the two different groups are represented on the x-axis while the differences are represented on the y-axis. Measurements are given in microns (µm).

Figure 5.6.2 demonstrates the Bland-Altman plot used to depict the relationship between the TMH measurements obtained using the Keratograph 4 for the two different subject groups. The mean difference is shown to be a value of 21.4 µm. All data points seem to be clustered around the mean difference with two outlying values being seen above the upper limit of agreement (238.7 µm).

5.6.1 TMH-K VERSUS SYMPTOMS (OSDI SCORE)

Table 5.6.1.1 Spearman’s rank correlation coefficient represented for the keratoconic group of subjects. This correlation coefficient represents the correlation between the TMH obtained using the Keratograph 4 versus the OSDI scores. The correlation coefficient, significance level as well as the 95% confidence interval for the correlation coefficient is represented.

<table>
<thead>
<tr>
<th>Spearman's Rank Correlation – TMH-KK vs OSDI-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>
From the values obtained using Spearman’s rank correlation for the keratoconic group of subjects, it can be seen that a weak positive correlation appears to be present between the symptoms being described by the OSDI scores and the TMH obtained using the Keratograph 4. When looking at the significance level obtained, this correlation is not statistically significant. No relationship seems to be present between the symptoms being described and the height of the tear meniscus in keratoconus as measured using the Keratograph 4.

![Scatter plot](image)

**Figure 5.6.1.1** Scatter plot demonstrating the data between the TMH using the Keratograph 4 and the OSDI scores in the keratoconic group of subjects. The OSDI scores are represented as a percentage while the TMH are represented in microns (µm).

The scatter plot in Figure 5.6.1.1 demonstrates a visual representation of the lack of correlation between symptoms being described by the OSDI scores and the height of the tear meniscus in keratoconic subjects as measured using the Keratograph 4. No relationship seems to be evident when looking at the distribution of points within the scatter plot, the points appear to be distributed in a random nature with no distinct pattern being observed. The height of the tear meniscus does not appear to play a significant role in the presence of symptoms being experienced.

**Table 5.6.1.2** Spearman’s rank correlation calculated for the height of the tear meniscus obtained using the Keratograph 4 versus the symptoms expressed by the OSDI scores in the control group of subjects. Represented are the correlation coefficient, the significance level as well as the 95% confidence interval for the correlation coefficient.
Spearman’s correlation data are demonstrated in Table 5.6.1.2 for the control group of subjects. The symptoms as described by the OSDI scores are compared with the TMH measurements obtained using the Keratograph 4 for the control group. As can be seen from the significance level obtained, this value is much larger than 0.05 therefore indicating that the null hypothesis cannot be rejected. Due to this calculation, it can be concluded that the weak negative correlation which seems to be present when looking at the correlation coefficient is not statistically significant.

Figure 5.6.1.2 Scatter plot representing the data for the control group of subjects. The TMH obtained using the Keratograph 4 is compared to the symptoms being described by the OSDI scores. The OSDI scores are expressed as a percentage while the TMH are expressed in microns (µm).

The scatter plot represented in Figure 5.6.1.2 demonstrates the lack of correlation between the OSDI scores and the TMH-K. In the control group of subjects, as with the keratoconic group, there is no specific relationship between the subjective symptoms described by the OSDI scores and the height of the tear meniscus when measured using the Keratograph 4. The data points are randomly distributed with no link being observed between
the two different variables. We therefore cannot make any assumptions relating symptoms and the height of the tear meniscus.

5.7 OPTICAL COHERENCE TOMOGRAPHY

Optical coherence tomography (OCT) is another method which can be utilized in order to measure tear meniscus dimensions. Both the height and the area of the tear meniscus could be measured using the OCT. The height being measured on both the OCT photograph and colour scan, as discussed in chapter 4. The area of the meniscus being measured on the colour scan only.

5.7.1 TMH-OP

Table 5.7.1.1 Descriptive statistics representing the data for the TMH using the OCT, with OP indicating that the photograph was measured. The descriptive statistics are given for both the keratoconic and control subjects. Represented are the mean, median, standard deviation, range and interquartile range for both groups. Values are represented in microns (µm).

<table>
<thead>
<tr>
<th></th>
<th>Keratoconic</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>213.72</td>
<td>168.4</td>
</tr>
<tr>
<td>Median</td>
<td>221.7</td>
<td>159.85</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>49.844</td>
<td>44.528</td>
</tr>
<tr>
<td>Range</td>
<td>210</td>
<td>231</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>65</td>
<td>45</td>
</tr>
</tbody>
</table>

The descriptive statistics are represented for both subject groups in terms of the TMH where the photograph was measured. When observing the values shown in Table 5.7.1.1, it can be seen that the mean TMH for the keratoconic group seems to be much larger than that of the control group, differing by approximately 45 microns. The medians display an even larger difference, differing by approximately 62 microns. When observing the results for the Kolmogorov-Smirnov test, which can be seen in Table 5.1, the normality results may be observed. These results indicate that the keratoconic group of subjects are shown to be normally distributed, whereas the control group are not normally distributed. As a consequence of these findings, non-parametric tests were further performed on the OCT data set.
Table 5.7.1.2 Non-parametric test statistics calculated for the keratoconic versus the control subject groups. The Mann-Whitney U test results are represented, including the test statistic and the significance level.

<table>
<thead>
<tr>
<th>Test statistics</th>
<th>Mann Whitney U</th>
<th>( \chi )</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMH-OP</td>
<td>561</td>
<td>-4.541</td>
<td>0.00</td>
</tr>
</tbody>
</table>

When examining the values obtained in Table 5.7.1.2, the Mann-Whitney U test, with a test statistic of -4.541 and a significance level of 0.00, the null hypothesis can be rejected. The null hypothesis states that the medians of the two different groups are shown to be equal. By rejecting the null hypothesis, it can be determined that the median TMH values between the keratoconic and the control groups are found to be statistically significantly different. Therefore, keratoconus may have an effect on the height of the tear meniscus when measured using the OCT where the photograph was examined. When comparing these results with the photographs obtained with the alternate instrumentation (Keratograph 4), it could be seen that no significant difference seems to exist between the two subject groups.

Figure 5.7.1.1 Box and whisker plot representing the TMH measurements obtained using the OCT with OP indicating that the photograph was measured in order to obtain these measurements. The heights are represented for both the keratoconic and the control subject groups. Measurements are given in microns (µm).
The box and whisker plot displayed in Figure 5.7.1.1, being a graphical representation of the data represented in Table 5.7.1.1, shows the differences in descriptive statistics between the two subject groups. From this figure, the large differences between the medians can be observed, with that of the keratoconic group being much larger than the median of the control group. The minimum values between the two groups are shown to be quite similar being separated by a difference of 6 µm while the maximum values show a variation of 14 µm. The interquartile range is also shown to be much larger in the keratoconic group versus the control group of subjects. Three outliers are shown to be present for the control group being represented by case numbers 65, 91 and 92. No outliers can be seen for the keratoconic subject group.

Figure 5.7.1.2 Bland-Altman plot representing the data measurements in terms of the TMH obtained using the OCT where the photograph was measured for each of the two subject groups. The mean difference as well as the limits of agreement are represented. The means of the TMH are represented on the x-axis while the differences are represented on the y-axis for the two subject groups. Measurements are given in microns (µm).

Figure 5.7.1.2 demonstrates both means and differences obtained by both subject groups (keratoconic and control) in terms of the TMH obtained using the OCT where the photograph was measured. The mean difference, as illustrated by the centre line is a value of 51 µm, this value is more than double that of the mean difference demonstrated when measurements were taken using the Keratograph 4 (Figure 5.6.2). As can be seen by the results obtained in table 5.7.1.2, the distribution of data values between the keratoconic and
the control group in terms of TMH obtained using the OCT (photograph) are statistically significantly different.

5.7.2 TMH-OP VERSUS SYMPTOMS (OSDI SCORE)

Table 5.7.2.1 Spearman’s rank correlation calculated for the OSDI scores versus the TMH obtained with the OCT. OPK indicating that the OCT was used, the photograph was measured and these measurements were done for the keratoconic group of subjects. Represented are the correlation coefficient, the significance level and the 95% confidence interval for the correlation coefficient.

<table>
<thead>
<tr>
<th>Spearman's Rank Correlation - TMH-OPK vs OSDI-K</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
<td>0.0920</td>
</tr>
<tr>
<td>Significance level</td>
<td>0.6617</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
<td>-0.315–0.470</td>
</tr>
</tbody>
</table>

The correlation coefficient shown in Table 5.7.2.1 displays a weak positive correlation. When looking at the significance level obtained, it is much larger than 0.05 and therefore the null hypothesis cannot be rejected and this finding is not shown to be statistically significant. From this, it can be concluded that no significant correlation seems to exist between the symptoms experienced and the height of the tear meniscus photograph obtained with the OCT in keratoconic subjects.
Figure 5.7.2.1 Scatter plot displaying the data for the OSDI scores versus the TMH photographs obtained with the OCT. These data measurements pertain to the keratoconic group. Symptoms are given as a percentage while the TMH are measured in microns (µm).

The scatter plot in Figure 5.7.2.1 give a visual confirmation of the conclusions determined in Table 5.7.2.1, that there is no significant correlation between the symptoms experienced in keratoconus and the height of the tear meniscus using the photograph obtained with the OCT. The data values are shown to be distributed in a random nature with no specific pattern being observed. Therefore the symptoms of keratoconus do not display a dependence on the height of tears present in keratoconic subjects.

Table 5.7.2.2 Spearman’s rank correlation coefficient for the symptoms being expressed by the OSDI scores and the height of the tear meniscus using the photograph obtained using the OCT in the control group of subjects. Represented are the correlation coefficient, the significance level and the 95% confidence interval for the correlation coefficient in the control group.

<table>
<thead>
<tr>
<th>Spearman’s Rank Correlation - TMH-OPC vs OSDI-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>

Spearman’s correlation calculated for the control group of subjects, from the statistics calculated in the table above, it can be seen that the null hypothesis cannot be rejected as with
the keratoconic group of subjects. There is therefore no statistical correlation observed between the symptoms of dry eye and the height of the tear meniscus (TMH-OP) in the control group of subjects.

![Graph](image)

**Figure 5.7.2.2** Scatter plot representing the data points for the OSDI scores versus the height of the tear meniscus using the photograph obtained with the OCT in the control group of subjects. OSDI symptoms are represented as a percentage while the TMH is represented in microns (µm).

The scatter plot in Figure 5.7.2.2, being a graphical representation of the data distribution between the two subject groups, confirms a lack of correlation between symptoms of dry eye and the height of the tear meniscus using the photograph in the control group of subjects (when the OCT is used to obtain measurements). The data points are distributed in a random nature with no specific trend being observed between these two variables.

### 5.7.3 TMH-OC

**Table 5.7.3.1** Descriptive statistics for the TMH obtained with the OCT, with OC indicating that the colour scan was measured in this case. These statistics are displayed for both the keratoconic and the control subject groups. Represented are the mean, median, standard deviation, range and interquartile range for both groups. These values are given in microns (µm).
The descriptive statistics in Table 5.7.3.1 are also characteristic of the TMH measurements obtained using the OCT, in this case, however, the colour scans were measured in order to determine the heights. The difference between the means do not seem to be as large as the difference observed in Table 5.7.1.1, where the OCT photograph was measured. The interquartile range, however, shows large variation between the two groups with that of the keratoconic group being almost double that of the control group. The medians display a difference of approximately 29 µm.

Table 5.7.3.2 Non-parametric tests performed for the keratoconic versus the control subject groups, in terms of TMH measured with the OCT where the colour scan was measured. The Mann-Whitney U test was performed and represented are the test statistic and the significance level.

<table>
<thead>
<tr>
<th>Test statistics</th>
<th>Mann Whitney U</th>
<th>z</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMH-OC</td>
<td>1043.5</td>
<td>-1.112</td>
<td>0.266</td>
</tr>
</tbody>
</table>

When observing the results in Table 5.7.3.2, it can be seen that the significance level is larger than 0.05 and therefore the null hypothesis can be accepted. From this, it can be concluded that there is no statistically significant difference between the TMH measurements of the two subject groups where the colour scans were measured using the OCT.
Figure 5.7.3.1 Box and whisker plot representing the TMH measurements obtained using the OCT with OC indicating that the colour scan was measured in order to obtain these measurements. The heights are represented for both the keratoconic and the control subject groups. Measurements are given in microns (µm).

When observing the distribution of values in Figure 5.7.3.1, the median for the keratoconic and the control subject groups are shown to be separated by an amount of 29.2 µm. The maximum value for the keratoconic group is only slightly smaller than that for the control group. The minimum value for the keratoconic group is also smaller than that for the control group, being separated by a difference of 39 µm. Outliers can be seen for both the keratoconic and the control groups. Four outliers can be observed for the control subjects, each being numbered according to their case numbers being 81, 82, 91, and 98. The keratoconic group, however, only displays one outlier represented by case number 23.
Figure 5.7.3.2 Bland-Altman plot representing the data measurements for the TMH obtained using the OCT where the colour scan was measured for each of the two subject groups. The mean difference as well as the limits of agreement are represented. The means of the TMH are represented on the x-axis while the differences are represented on the y-axis for the two subject groups. Measurements are given in microns (µm).

Figure 5.7.3.2 represents the Bland-Altman plot used to determine the agreement between TMH values obtained using the OCT where the colour scan was measured for both subject groups. The mean difference is shown to be a value of 22.1 µm which is similar to the mean difference obtained when the Keratograph 4 was used as the instrument of choice. This value is closer to zero compared to that of Figure 5.7.1.2 where the photograph was measured. All data points seem to fall within the 95% limits of agreement on either side of the mean difference, other than one outlying value which can be seen to fall below the lower limit of agreement (-218.2 µm).

5.7.4 TMH-OC VERSUS SYMPTOMS (OSDI SCORE)

Table 5.7.4.1 The correlation coefficient calculated using Spearman’s rank correlation for the symptoms described by the OSDI scores versus the TMH obtained using the OCT where the colour scan was measured. Both measurements were done for the keratoconic group of subjects.

<table>
<thead>
<tr>
<th>Spearman's Rank Correlation – TMH-OCK vs OSDI-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>
When observing the correlation coefficients along with the significance levels, it can be seen that a small negative correlation seems to exist between the symptoms experienced versus the TMH measured (colour scan) in the keratoconic group of subjects. This small correlation, however, is not shown to be statistically significant. The height of the tear meniscus (TMH-OC) does not seem to play a significant role in relation to symptomology of the keratoconic subjects.

Figure 5.7.4.1 Scatter plot representing the symptoms described by the OSDI scores versus the TMH obtained with the OCT where the colour scan was measured in the keratoconic group of subjects. The OSDI scores are represented as a percentage while the TMH is represented in microns (µm).

As can be seen in Figure 5.7.4.1, a lack of correlation exists between the symptoms of dry eye being described by the keratoconic group of subjects and the height of the tear meniscus measured by the OCT, in which the colour scan was measured when observing the scatter plot. Figure 5.7.4.1 depicts a graphical representation of the data where no characteristic pattern can be observed.

Table 5.7.4.2 Spearman’s rank correlation calculated for the control group of subjects. Correlations were calculated for the symptoms as described by the OSDI scores versus the TMH obtained with the OCT where the colour scan was measured. Represented is the correlation coefficient, the significance level and the 95% confidence interval for the correlation coefficient.
As with the keratoconic group of subjects, it can be seen that there is a lack of statistically significant correlation between the symptoms of dry eye and the height of the tear meniscus measured with the OCT, where the colour scan was measured. The weak negative correlation seen when looking at the correlation coefficient is not statistically significant.

As can be seen in Figure 5.7.4.2, a visual representation of the lack of significant correlation can be seen between these two variables namely, the OSDI scores and TMH measured with the OCT (colour scan) in the control group of subjects. No dependent relationship can be found between symptoms versus the height of the tears in either of the subject groups.

5.8 BLAND-ALTMAN PLOTS COMPARING INSTRUMENTS

5.8.1 TMH-K VERSUS TMH-OP
Figure 5.8.1.1 A Bland-Altman plot representing the data measurements when comparing the TMH obtained using the Keratograph 4 to those obtained with the OCT where the photograph was measured in the keratoconic group of subjects. Measurements are given in microns (µm).

In the case above, the TMH obtained using the Keratograph 4 and the OCT (photograph) were compared. The x-axis representing the means of the two different experimental methods while the y-axis represents the differences between the data measurements of the two methods. The central line of the plot represents the mean difference which is a value of 49.1 µm. The central line is shown to be quite a distance from zero with zero representing no variation between the two variables being tested. The two dotted lines seen on the top and bottom regions of the plot are indicative of the 95% limits of agreement. The limits of agreement in Figure 5.8.1.1 span approximately 400 µm, with the majority of the data measurements falling within these limits on either side of the mean difference with one outlying value shown to be present above the top limit. Most of the data measurements are shown to fall between approximately 150 µm and -100 µm when looking at the plot which indicates the variation between the measurements, this excludes the outlying value.
Figure 5.8.1.2 A Bland-Altman plot representing the mean differences between the TMH obtained with the Keratograph 4 versus those obtained with the OCT where the photograph was measured in the control group of patients. All data measurements are represented in microns (µm).

Figure 5.8.1.2 demonstrates the mean differences calculated when comparing the two different types of instrumentation used to measure TMH in the control group of patients. As can be seen in Figure 5.8.1.2, the mean difference which is the central line demonstrated on the plot is a value of 78.7 µm. The mean difference in this case deviating from zero indicating the variation which can be observed between the two measuring techniques. The majority of the data measurements are shown to fall within the limits of agreement except for one outlier shown to be present below the bottom limit of agreement (-19.4 µm). The limits of agreement span approximately 200 µm.
5.8.2 TMH-K VERSUS TMH-OC

Figure 5.8.2.1 A Bland-Altman plot representing the differences in testing methods between the Keratograph 4 and the OCT colour scan when measuring the TMH in the keratoconic group of subjects. The means and differences are represented for both testing methods and measurements are given in microns (µm).

The Bland-Altman plot displayed in Figure 5.8.2.1 represents the means and differences between the Keratograph 4 values and the OCT values where the colour scan was measured. When comparing the mean difference in Figure 5.8.2.1 with that in Figure 5.8.1.1 where the OCT photograph was measured, a large difference can be observed between the two OCT scans. The mean difference is closer to zero, being a value of 18.3 µm which is small in comparison to the values obtained for the TMH. Two outlying values can be observed above the upper limit of agreement while the other data measurements are shown to be within the limits of agreement. Due to the distribution of the data measurements which are seen to fall between the limits of agreement as well as the value of the mean difference being small, this shows the improved interchangeability of these two types of instrumentation when measuring TMH.
Figure 5.8.2.2 Bland-Altman plot representing the data measurements for the Keratograph 4 versus the OCT where the colour scan was measured in the control group of subjects. The mean difference as well as the limits of agreement are represented. The means of the two methods represented on the x-axis and differences represented on the y-axis. Data measurements are represented in microns (µm).

The Bland-Altman plot displayed in Figure 5.8.2.2 demonstrates the mean difference between the TMH measurements. The 95% limits of agreement are represented by the dotted lines, plotted on either side of the mean difference, these limits of agreement span approximately 290 µm. The majority of the data values can be seen to fall within these limits of agreement with two outlying values shown to be present below the bottom limit of agreement (-126.4 µm). Most of the data points are shown to be clustered around the mean difference, being a value of only 19 µm. Due to the distribution of data measurements, the interchangeability between these two types of instrumentation can be observed.
5.8.3 TMH-OP VERSUS TMH-OC

Figure 5.8.3.1 Bland-Altman plot representing the means and differences between the two different OCT scans, namely the photograph and the colour scans obtained for the keratoconic group of subjects. Represented are the mean differences as well as the limits of agreement. All data measurements are given in microns (µm).

Bland-Altman plot representing the means and differences calculated between the TMH measurements using the photograph versus the height measurements obtained using the OCT where the colour scan was measured. These measurements are expressed for the keratoconic group of subjects in order to compare the measurements taken off two different scans using the same instrumentation. As can be seen in Figure 5.8.3.1, the data measurements seem to be centered within the limits of agreement. No outlying values are shown to be present, the data measurements fall between these specified limits. The limits of agreement in this case are shown to be 150.4 and -212 µm, the data measurements therefore fall between these specific values covering a range of approximately 362 µm. Due to the variation in the data measurements, it can be seen that these two methods may not necessarily be used interchangeably.
Figure 5.8.3.2 Bland-Altman plot demonstrating the two alternative types of scans used to measure the TMH with the OCT. The photograph is compared with the colour scan in the control group of subjects. The mean difference as well as the limits of agreement are represented. All data measurements are given in microns (µm).

The Bland-Altman plot in Figure 5.8.3.2 is a representation of the TMH obtained using the OCT, where the OCT photograph is compared with the colour scan in the control group of subjects. The limits of agreement for the control group being different to those obtained for the keratoconic group, the upper limit being 81.5 and the lower limit being -200.8 µm. The majority of the data values seem to fall between these limits of agreement which span approximately 280 µm with only one outlying value shown to be present below the lower limit. The remainder of the data values are found to be within these limits, however, deviating largely from zero. These methods cannot be used interchangeably as each yield variably different results.

5.9 TEAR MENISCUS AREA

Table 5.9.1 Descriptive statistics representing the data for the TMA measured using the OCT. The descriptive statistics are given for both the keratoconic and the control group of subjects. Represented are the mean, median, standard deviation, range and interquartile range for both subject groups. Values are represented in microns$^2$ (µm$^2$).
The descriptive statistics in terms of TMA are represented in Table 5.9.1, the statistics for both subject groups are represented where the colour scan was measured in order to determine the area of the meniscus. Observing the values obtained in Table 5.9.1, a large difference may be observed between the means of the TMA of the two different subject groups. The mean of the keratoconic group is shown to be larger than that of the control group with the interquartile range of the control group being just over half that of the keratoconic group. The medians are separated by 2800 µm², with the median of the keratoconic group being larger compared to the control group. When looking at the results of the Kolmogorov-Smirnov test in Table 5.1, it can be seen that the TMA for both subject groups is shown not to be normally distributed and therefore non-parametric tests will be performed for this data set.

Table 5.9.2 Non-parametric test statistic namely, the Mann Whitney U test performed for the data measurements of TMA obtained using the OCT in both subject groups. The results for Mann Whitney U, the $\chi$ statistic as well as the significance level is represented.

<table>
<thead>
<tr>
<th>Test statistics</th>
<th>Mann Whitney U</th>
<th>$\chi$</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA</td>
<td>1003.500</td>
<td>-1.397</td>
<td>0.163</td>
</tr>
</tbody>
</table>

Observing the values obtained in Table 5.9.2, it can be seen that the significance level is greater than 0.05 and therefore the null hypothesis cannot be rejected. Without rejecting the null hypothesis, it can therefore be said that the results for the TMA obtained using the OCT are not shown to be statistically significantly different. There is therefore no statistically significant difference between the TMA of the keratoconic group versus the control group of
subjects. It can be assumed that keratoconus does not play a contributing role to the size of the tear meniscus.

**Figure 5.9.1** Box and whisker plot representing the TMA measurements obtained using the OCT. Measurements are represented for both the keratoconic and the control group of subjects. Measurements are given in microns$^2$ ($\mu$m$^2$).

Figure 5.9.1 displays the differences in TMA between the two subject groups. When looking at the box and whisker plot, there does not seem to be a large difference between the median values seen in the centre of each box. When looking at Table 5.9.1, however, the difference seems to be large but it has been shown that this difference is not statistically significant (as seen in Table 5.9.2). The interquartile range can be seen to be larger in the keratoconic group versus the control group. Both subject groups display outlying values with each of these values being represented by the case measurement number. Four outlying values can be observed in the keratoconic group as indicated by case numbers 9, 23, 49 and 50, whereas the control group display six separate outlying values as indicated by case numbers 81, 82, 91, 92, 97 and 98.
Figure 5.9.2 Bland-Altman plot representing the data measurements obtained in terms of the TMA using the OCT where the colour scan was measured for each of the two different subject groups. The mean difference as well as the limits of agreement are represented. Measurements are given in microns$^2$ ($\mu m^2$).

Figure 5.9.2 represents the Bland-Altman plot used to determine the agreement between the TMA measurements obtained for each of the two subject groups using the OCT. The mean difference is shown to be a value of 3655 $\mu m^2$ which is small in comparison to the TMA measurements obtained. As determined using the Mann-Whitney U test in Table 5.9.2, it was determined that the TMA values are not shown to be statistically significantly different between the two subject groups. Most of the data points are distributed between the limits of agreement, except for two outlying values. One of these outliers is shown to be below the lower limit of agreement (34433.6 $\mu m^2$), while the other two outlying points are shown to be above the upper limit of agreement (41743.6 $\mu m^2$).

5.9.1 TMA VERSUS SYMPTOMS (OSDI SCORE)

Table 5.9.1.1 Spearman’s rank correlation calculated for the symptoms described by the OSDI scores versus the TMA obtained using the OCT, measured in the keratoconic group of subjects. The correlation coefficient, significance level and 95% confidence interval for the correlation coefficient is represented.
Looking at Spearman’s rank correlation results calculated for the OSDI scores versus the TMA in the keratoconic group of subjects, it can be seen that the significance level is greater than 0.05. Therefore the null hypothesis cannot be rejected indicating that no statistically significant correlation exists between the symptoms being experienced and the area of the tear meniscus in the keratoconic group of subjects.

### Table 5.9.1.2

<table>
<thead>
<tr>
<th>Spearman’s Rank Correlation – TMA-OCK vs OSDI-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>

The scatter plot in Figure 5.9.1.1 is a graphical representation of the data expressed in Table 5.9.1.1. This scatter plot gives a visual confirmation of the results obtained in Table 5.9.1.1, the finding that there is a lack of correlation between symptoms and the area of the tear meniscus in the keratoconic group of subjects. The data measurements are shown to be randomly distributed, with no specific pattern being observed.

**Figure 5.9.1.1** Scatter plot representing the data obtained for the dry eye symptoms described by the OSDI score versus the TMA obtained using the OCT in the keratoconic group of subjects. OSDI scores being represented as a percentage while TMA are represented in microns² (µm²).

**Table 5.9.1.2** Spearman’s rank correlation comparing the symptoms described by the OSDI scores versus the TMA obtained with the OCT. These correlations are represented for the control group of subjects. The coefficient of correlation, the significance level as well as the 95% confidence interval for the correlation coefficient is represented.
Spearman’s rank correlation results comparing symptoms as described by the OSDI scores with TMA in the control group of subject’s yields similar results to those of the keratoconic group. The weak negative correlation seen when looking at the correlation coefficient is not statistically significant when comparing dry eye symptoms being experienced and the area of the tear meniscus when measured using the OCT.

<table>
<thead>
<tr>
<th>Spearman's Rank Correlation – TMA-OCC vs OSDI-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of rank correlation (rho)</td>
</tr>
<tr>
<td>Significance level</td>
</tr>
<tr>
<td>95% confidence interval for rho</td>
</tr>
</tbody>
</table>

**Figure 5.9.1.2** Scatter plot representing the relationship between the dry eye symptoms as described by the OSDI scores versus the TMA measurements obtained using the OCT in the control group of subjects. OSDI scores are represented as a percentage whereas the TMA are represented in microns² (µm²).

The scatter plot in Figure 5.9.1.2 displays a visual representation of the lack of correlation between dry eye symptoms and the TMA in the control group, similar to those results observed in the keratoconic group of subjects. The symptoms of dry eye, therefore do not depend on the size of the tear meniscus, these variables are not dependent on one another.
5.10 VERNIER MEASUREMENTS

In order to provide a control for determining the accuracy of both the measurement methods, a vernier was used. The vernier, set at two specified gaps (widths) was measured using both measurement techniques. The Keratograph 4 and ImageJ were utilized as discussed in section 4.3.4. The measurements represented below are those obtained for the vernier set at 500 µm.

Table 5.10.1 Representation of the data measurements obtained using the vernier as a control measurement. Measurements were taken of the vernier set at two different thicknesses with only the 500µm gap represented below. The image number is listed along with the measurements obtained using the Keratograph 4 and those measured using ImageJ. All measurements of both measurement techniques are expressed in microns (µm).

<table>
<thead>
<tr>
<th>IMAGE NUMBER</th>
<th>KERATOGRAPH</th>
<th>IMAGEJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>510</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>476</td>
</tr>
<tr>
<td>3</td>
<td>490</td>
<td>500</td>
</tr>
<tr>
<td>4</td>
<td>460</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>490</td>
<td>492</td>
</tr>
<tr>
<td>MEAN</td>
<td>490</td>
<td>493.6</td>
</tr>
</tbody>
</table>

Table 5.10.1 represents the vernier measurements taken as a control in order to determine the accuracy of the two measurement techniques namely the Keratograph 4 and ImageJ. A mean was calculated for each of the two methods and can be observed in Table 5.10.1, with the mean measured by ImageJ being closer to the specified distance versus the measurements taken using the Keratograph 4. The difference between the two measurement techniques is 3.6 µm, however, this may result in a significant difference when determining the accuracy of two different measurement methods with ImageJ measuring a value which is closer to 500 µm compared to the Keratograph 4.

Table 5.10.2 Descriptive statistics represented for the vernier measurements taken for a specified distance of 500 µm. Represented are the mean, median, standard deviation, variance, maximum and minimum values in microns (µm). The normality results are also included.
The descriptive statistics represented in Table 5.10.2 pertain to those photographs taken using the vernier. The procedure followed when obtaining these measurements is detailed in section 4.3.4. The medians, observed in Table 5.10.2 are shown to be separated by a value of 10 µm with the median of ImageJ shown to be higher, while the standard deviations are shown to vary by an amount of 8.28 µm with that of the Keratograph 4 shown to be higher. The Kolmogorov-Smirnov test for the normality of data was also performed on each of the two data sets, yielding a finding of normally distributed results. Due to this finding, a parametric testing procedure, namely the paired samples t-test, could be performed on these data.

**Table 5.10.3** Table representing the paired samples t-test conducted for the vernier measurements obtained using both the Keratograph 4 and ImageJ. Represented are the mean difference, the t statistic as well as the significance level (two-tailed probability).

<table>
<thead>
<tr>
<th>Test statistics – Paired samples t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>VERNIER</td>
</tr>
</tbody>
</table>

The paired samples t-test was used in order to determine whether a statistically significant difference is shown to be present between the vernier measurements taken using two different measurement techniques (Keratograph 4 and ImageJ). The null hypothesis for this specified test states that the mean of sample group one is equal to the mean of sample group two. From the results obtained, the significance level is greater than 0.05 and therefore the null hypothesis cannot be rejected. Accepting the null hypothesis concludes that the
means of the two measurement techniques are shown not to be statistically significantly different.

![Bland-Altman plot](image)

**Figure 5.10.1** Bland-Altman plot representing the two different measurement techniques (Keratograph 4 and ImageJ) utilizing vernier images of a known thickness of 500 µm. All measurements are represented in microns (µm).

The Bland-Altman plot, seen in Figure 5.10.1 shows the interchangeability of the two measurement techniques as shown using the vernier of a known thickness. The mean difference between the two techniques is represented as the central horizontal line being a value of 3.6 µm. According to the results of the paired samples t-test, as can be observed in Table 5.10.3, there is no statistically significant difference between these two techniques which agrees with the Bland-Altman plot in Figure 5.10.1.

### 5.11 SUMMARY OF RESULTS

The results obtained in this study detail the severity of symptoms being experienced by the keratoconic group of subjects. These symptoms are shown to be statistically significantly different between the two subject groups, signaling the effect that keratoconus may have on the ocular comfort of the subjects affected. These symptoms, although shown to be greater in severity in the keratoconic subject group, do not seem to increase with an increase in the grade of keratoconus. The symptoms being described by the keratoconic subjects do not show any correlation with other clinical parameters measured within this study namely NTBUT, TMH-K, TMH-OP, TMH-OC and TMA. The control group, however, exhibit a significant correlation when comparing the symptoms being described and the
measures of NTBUT.

The grade of keratoconus does not show correlation with most of the clinical measures within this study namely, NTBUT, TMH-K, TMH-OC and TMA. The only parameter shown to be correlated with the grade of keratoconus is the height of the tear meniscus where the photograph was measured in the experimental group (TMH-OPK).

The TMH measured using the Keratograph 4 displays no statistically significant difference between the two subject groups. The same finding was obtained for the height of the tear meniscus measured using the OCT where the colour scan was measured for the two subject groups. The photographs obtained using the OCT, however, displayed a statistically significant difference between the two subject groups. The TMA measurements between the two subject groups, showed that a statistically significant difference could not be found between the two subject groups.

The vernier data displayed a lack of significant difference between these measurement techniques, therefore yielding the conclusion that these techniques may be used interchangeably when measuring various scans such as those obtained throughout this study.
CHAPTER 6

DISCUSSION AND CONCLUSIONS
6.1 NORMALITY

The results presented include the data obtained from the 50 subjects partaking in the study, 25 of which represent keratoconic subjects while the remaining 25 represent the control group. Both the Kolmogorov-Smirnov and Shapiro-Wilk tests were performed in order to determine the presence of normality for both subject groups. The results of these tests, presented in Table 5.1, indicate the absence of normality (mostly) within the data sets for both subject groups. Using the Kolmogorov-Smirnov test, it was determined that only four of the 12 variables are shown to be normally distributed while the Shapiro-Wilk test found that only two of the variables were shown to be normally distributed and therefore non-parametric testing methods were selected when analyzing these data. The normality of data for keratoconic studies has been documented in various items of research. The study by Piñero et al. (2010) where the effect of intracorneal ring segments was examined on refractive, keratometric as well as aberrometric data, found that the keratoconic data was mostly not normal. The study by Jinabhai et al. (2012) advocated the use of non-parametric testing methods as the contrast sensitivity data for a keratoconic subject group proved to be mostly not normal in its distribution. The study by Sarac et al. (2011), however, studied the differences in tear meniscus dimensions between keratoconic and normal subjects; this data was found to be normally distributed and advocated the use of parametric testing methods. In terms of the tear meniscus dimensions measured in the study by Tung et al. (2014) the same findings were obtained, the data for the keratoconic group was shown to be normally distributed.

6.2 SYMPTOMS (OSDI SCORE)

The presence of dry eye symptoms in keratoconus has been documented in the literature as discussed in chapter 2. Keratoconus has been defined as a progressive condition which results in increased ocular discomfort as this condition progresses, leading to augmented changes to functional vision as well as comfort (Kymes et al., 2004). Keratoconic patients suffer from symptoms which range from decreased visual acuity and distorted vision (Mandell, 1997) to symptoms of discomfort such as itching or sensitivity to light (Naderan et al., 2015). The symptoms experienced by keratoconic subjects are similar to those suffered by dry eye patients namely itching, burning, redness and dryness (Fink et al., 2005). Investigation into the severity of these symptoms was performed using the OSDI questionnaire, which has been psychometrically tested and was found to be an effective and
dependable measure when trying to determine the presence and effects of dry eye symptoms (Li et al., 2012A). A study conducted by Schiffman et al. (2000), performed in order to determine the reliability and validity of the OSDI, concluded that the OSDI questionnaire is a valuable and reliable tool when measuring the severity of dry eye disease.

The severity of dry eye symptoms experienced by the keratoconic group in this study, as determined by the OSDI score, is clearly evident when observing the results obtained. As shown in Table 5.2.1, the symptom severity experienced by the keratoconic group is almost nine times that of the control group. When comparing the two subject groups (keratoconic versus control) in terms of the OSDI scores (Table 5.2.2), a statistically significant difference exists. The OSDI questionnaire is infrequently used when dealing with keratoconic subjects and previous studies using the OSDI questionnaire in general are scarce. Our findings fall in line with those obtained by Carracedo et al., (2015), where the OSDI questionnaire was utilized in order to determine the severity of symptoms in keratoconus and it was found that keratoconics suffer from more severe symptoms compared to control subjects. Studies in which questionnaires were given to keratoconic subjects include the study by Gothwal et al. (2013), where the Impact of Visual Impairment (IVI) questionnaire was given to 160 keratoconic subjects living within an Indian community in order to determine the influence of disease severity on quality of life. The overall conclusion of this specified study (Gothwal et al., 2013) showed that the vision-related quality of life scores were similar in cases of mild versus severe keratoconus, demonstrating that the severity of keratoconus may not necessarily have a relationship with vision-related quality of life.

It has been postulated that as the severity of keratoconus increases, the severity of the dry eye symptoms may increase proportionately as with dry eye disease. In a study by Dogru et al. (2003), changes occurring at the surface of the tears were investigated in keratoconic patients. It was determined that the specific changes occurring at the interface between the cornea and the lids, being the tear film, along with the changes occurring at the surface of the cornea due to the pathogenesis of keratoconus may play a significant role when looking at the instability of the tears (Dogru et al., 2003). Changes occurring at the level of the corneal nerves are said to play an important role, where a loss in trophic effects of the corneal nerves are said to be associated with the pathogenesis of keratoconus (Dogru et al., 2003). Due to changes such as the loss of goblet cells and squamous metaplasia, this may result in alterations to the tear film resulting in instability, altered surfacing and a poor wetting effect (Dogru et al., 2003).

Research has speculated as to whether inflammation may play a role in the
pathogenesis of keratoconus. In a study performed by Lema and Durán (2015), it was found that degradation of tissue structure in a condition such as keratoconus may be linked to the expression of inflammatory mediators, some of which include cell adhesion molecules, proinflammatory cytokines and matrix metalloproteinases (Lema and Durán, 2005). This study by Lema and Durán (2015) also revealed a relationship between the severity of keratoconus and increased levels of inflammatory markers. The inflammatory events taking place at the ocular surface due to the presence of this corneal ectasia can be seen within the composition of the tears, revealed by the presence of degradation products (Lema and Durán, 2005). In most cases, the presence of inflammatory markers has been shown to be exacerbated by association with various atopic conditions.

In a study performed by Sarac et al. (2011), it was determined that approximately 81.5% of keratoconic patients suffer from symptoms of dry eye. Of the sample group, approximately 70% were shown to suffer from tear deficient dry eye, which occurred due to the instability of the tear film, with instability increasing as the severity of keratoconus progressed (Sarac et al., 2011). When reviewing the literature available on keratoconus, Rabinowitz (1998) found keratoconus to be a variable condition where symptoms may depend on the phase and progression of the disease. Symptoms do not generally appear during the early stages of the condition but may develop as the severity of the condition increases (Rabinowitz, 1998). In a study performed by Johnson (2009), in which the association between dry eye symptoms and signs was evaluated, it was stated that the duration of any specific ocular disease may have an effect on the severity of the symptoms being experienced. Due to the progression of keratoconus, it has been thought that the severity of dry eye symptoms would be more evident as the severity of the keratoconus increases.

In results obtained from this study, as can be seen in Table 5.3.2.2, no significant correlation could be found between the symptoms described by the OSDI score and the grade of keratoconus obtained through the use of corneal topography readings. It seems that symptoms do not increase as the grade of keratoconus increases. In various items of research where keratoconus has been discussed, it has been stated that keratoconus is a difficult condition, due to the fact that symptoms and signs of keratoconus may not always be observed in conjunction with one another (Rabinowitz, 1998). From the results obtained in this study, the severity of dry eye symptoms in the keratoconic group of subjects is clearly evident with a lack of correlation in terms of severity being displayed. The lack of correlation between symptoms and the grade of keratoconus has also been found by Gothwal et al.,
(2013). A factor which may have an effect on the description of symptoms being experienced could be the understanding of the OSDI questionnaire. In cases where the questions presented within the OSDI questionnaire are not fully understood, the result may be inaccurate. Subjects were encouraged to ask questions if clarification was needed, however, there may still have been some misunderstanding resulting in variable OSDI scores.

In most cases, the presence of symptoms in keratoconic patients may be due to the associated conditions which often accompany this corneal ectasia. The correlation between keratoconus and various other conditions has been documented for many years and it has been found that a positive correlation exists between keratoconus and a number of disorders including collagen vascular diseases, Marfan’s syndrome, Down’s syndrome, eye rubbing and atopy amongst others (Bawazeer et al., 2000). Atopy is one of the main causes of itching, discomfort and irritation in patients who have keratoconus, often found with associated eye rubbing (Harrison et al., 1989; Lema and Durán, 2005).

In a study performed by Harrison et al. (1989), 67 keratoconic subjects were tested and it was determined that 56.7% of these subjects presented with some form of atopic disease including asthma, hayfever and eczema. An incidence of 35% for atopic tendencies was found in the study performed by Rahi et al. (1977), where a group of 182 keratoconic subjects were tested for the presence of atopic disease, with hayfever being the most common condition present. The study by Rahi et al. (1977) also displayed an escalation of the serum IgE levels within the atopic group of subjects, these levels are generally found to be greater in keratoconic subjects. Harrison et al. (1989), also concluded that a positive family history of keratoconus was shown to be present in an atopic subject group with familial incidence shown to be present in 86.4% of subjects included in the specified study.

The majority of keratoconic subjects suffer from associated conditions such as atopy, as discussed previously. Generally, the presence of associated conditions causes a lot of the symptoms being described by keratoconic subjects as described by Rudikoff et al., (2014). The specific symptoms being described by keratoconic subjects may not be the exact symptoms being described by dry eye subjects, however, these symptoms appear to be similar. The symptoms seem to be comparable but may not be of the same origin, the exact origin of keratoconic symptoms is not entirely known. The study performed by Karamichos et al., (2015) concluded that the tear metabolome of dry eye and keratoconic subjects are shown to be the same and this could be a possible reason as to why these symptoms seem to be identical.
6.3 GRADING OF KERATOCONUS

Uncertainty exists when trying to determine the severity of keratoconus. Due to the unpredictability of this corneal ectasia, drastic clinical signs of keratoconus may not be correlated with the best corrected vision being obtained. Based on both clinical signs and topographic patterns, an estimate of keratoconic severity may be determined. Topography has been widely utilized and shown to be a valuable tool in diagnosing conditions such as keratoconus (Karimian et al., 2008). Various instruments may be utilized in order to determine the topographical features of the keratoconic cornea. In a study performed by Vázquez et al. (2014), Pentacam Scheimpflug Tomography was utilized in order to determine the topographical findings in keratoconic patients versus normal patients. In the specified study, patients were divided into three experimental groups based on the topographical findings, with group one being comprised of patients with inconspicuous topographical findings while groups two and three (55 patients combined), indicated abnormalities within the topographical maps of the better and worse eyes, respectively. A grading system known as the Keratoconus Severity Score (KSS) was used to determine the severity of keratoconus present. This grading scheme ranges from 0 to 5 with 0 indicating the presence of normal topography while a grade 5 demonstrates the occurrence of severe keratoconus (Vázquez et al., 2014). According to the results obtained from Vázquez et al.’s study, 20% of patients in groups two and three combined obtained a KSS of 5. The majority of the keratoconic patients within Vázquez et al.’s (2014) study obtained a KSS of 3 (60%) indicating that the keratoconus was of a moderate form.

The topographical findings acquired during our study demonstrate the severity of keratoconus using the grading scale obtained with the Keratograph 4. As discussed in chapter 4, the severity based on the topographical pattern ranges between 1 and 5 with intervals between each grade. As demonstrated in Table 5.3.1.1, the frequency of each grade is shown with grade 5 demonstrating the greatest prevalence amongst the keratoconic group of subjects. Grade 5 made up 19 of the 50 keratoconic eyes (25 subjects), with this grade indicating that the keratoconus had progressed to a level where a measurement could not be obtained using the Keratograph 4. A total of nine eyes obtained a grading of 3 while grade 4-5 did not feature within this subject group. Only one eye obtained a grading of 1 indicating mild keratoconus. The keratoconic grading results differ from those obtained by Vázquez et al. (2014) where the majority of the study population obtained a KSS of 3 indicating moderate keratoconus. Although these grading schemes are not identical, each give an
equivocal indication of the severity of the topographical changes present, indicating the severity of keratoconus experienced.

A study by Goebels et al. (2015) was done in order to investigate indices for grading the various stages of keratoconus. Using the Pentacam, two indices could be obtained namely, the Keratoconus Index (KI) and the Topographic Keratoconus Classification (TKC). The Topographic Modeling System as well as the Ocular Response Analyzer were also used in order to determine the grade of keratoconic severity (Goebels et al., 2015). The results of this study suggested that the various indices used for classifying keratoconus cannot be used interchangeably (Goebels et al., 2015). One topographical method should be chosen and utilized in order to determine keratoconic severity. In this study, the chosen method being the grade of keratoconus given by the Keratograph 4. When observing the data given in Tables 5.3.4.1, 5.4.1.1 and 5.4.2.1, the grade of keratoconus versus the mean of the TMH is given using each of the two different types of instrumentation, namely the Keratograph 4 (Table 5.3.4.1) and the OCT (Tables 5.4.1.1 and 5.4.1.2) for the keratoconic group of subjects. Using the Keratograph 4, no specific trend can be observed when looking at the TMH means versus the grade of keratoconus; the means do not increase or decrease depending on the grade of keratoconus. When examining the data obtained using the OCT, it can be seen that; in the case of the OCT photograph, the largest mean TMH is obtained by grade 3-4 while grade 1-2 displays the smallest mean TMH. When looking at the colour scan, grade 5 displays the largest TMH mean while grade 2 displays the smallest mean TMH. Due to the similarity in symptoms between keratoconic and dry eye subjects, it has been postulated that the same findings would be obtained for keratoconic subjects in terms of TMH; a decrease in TMH values as can be observed with dry eye subjects. However, it is also possible that the TMH would increase as the severity of keratoconus increases due to an increase in symptoms and therefore elevated reflex tearing, however, no trend or pattern can be observed when comparing these means to the grade of keratoconus. There is a scarcity in terms of research comparing the severity of keratoconus and the height of the tear meniscus in conditions such as keratoconus.

The one and only parameter shown to be correlated with the grade of keratoconus is the height of the tear meniscus as measured using the OCT photograph (TMH-OPK). This study illustrates a lack of correlation with the remaining clinical variables when compared with the grade of keratoconus. In our study, it was determined that no significant correlation exists between the grade of keratoconus (therefore the severity measured) and an objective
measure of tear quality such as NTBUT. Figure 5.3.3.2 demonstrates the lack of correlation between the NTBUT and the grade of keratoconus obtained.

6.4 NON-INVASIVE TEAR BREAK UP TIME

Break up time of the tear film is thought to be an accurate indication of tear film stability with a shorter break up time indicating an increasingly unstable tear film (Yanoff et al., 2009; Shaarawy et al., 2009). Conditions such as dry eye often present with lower TBUT (Probst and Tsai, 2012), resulting in this test being sensitive to changes within the tear film. However, variable TBUT results may also be obtained in subjects who do not suffer from instability of the tear film (Himebaugh, 2007). Alterations in TBUT in dry eye patients has been extensively documented, however, investigation into the TBUT changes occurring in keratoconic subjects has not been extensively investigated. In keratoconic subjects, it has been assumed that the TBUT would occur more rapidly due to the various changes taking place at the surface of the tears. As can be seen in Table 5.5.1, the mean NTBUT for the two subject groups do not differ significantly. A difference of 0.24 seconds can be observed between the means of the two subject groups, with the standard deviation, however, being much larger for the keratoconic group due to the variation in measurement times. The test statistics in Table 5.5.2 confirm the finding that the NTBUT is not significantly different between keratoconic and control subjects suggesting that the presence of keratoconus does not play a vital role in the break up of the tears.

It is thought that the break up of the tear film may result in an increase in symptoms being described, however, results shown in Table 5.5.1.1 suggest a lack of correlation between the OSDI scores and NTBUT values in the keratoconic group of subjects. Subjective complaints regarding symptoms being experienced may not be significantly correlated with an objective test such as NTBUT. This lack of correlation has been extensively documented in various items of research (Begley et al., 2003; Johnson, 2009; Pult et al., 2011). The study performed by Johnson (2009) confirms the lack of correlation that can be observed in cases of dry eye where symptoms being described by subjects show no correlation with objective test results. The study performed by Begley et al., (2003) where patient reported symptoms were compared with the results of clinical examinations for dry eye, showed the same results. In the study performed by Lemp et al., (2011), tear osmolarity was investigated as a diagnostic criteria for dry eye disease. Although tear osmolarity is shown to correlate well with the severity of dry eye disease, no significant correlation is shown to exist in terms of the symptoms being experienced by dry eye subjects. The study by Pult et al., (2011),
however, seems to suggest a moderate correlation between the dry eye symptoms as described by OSDI scores and objective tests including NTBUT and TMH measurements.

When Spearman’s rank correlation is calculated for the control group of subjects, comparing the OSDI scores versus the NTBUT, the correlation coefficient is a value of 0.452, a moderate positive correlation (Pallant, 2005). When observing the significance level ($\rho$ value), this allows for the rejection of the null hypothesis. By rejecting the null hypothesis which states that $r \neq 1$, it can be determined that the data comparing these two variables (OSDI scores and NTBUT measurements) is shown to be correlated. A correlation would have been expected when investigating the keratoconic group of subjects as the severity of symptoms is far greater. The lack of correlation in the keratoconic group may be due to the unpredictability and variability within the keratoconic group of subjects.

6.5 TEAR MENISCUS HEIGHT

TMH measurements have been utilized as a valuable tool in the diagnosis of dry eye syndrome (Mainstone et al., 1996). It has been postulated that lower TMH measurements may be correlated with various other objective measures of dry eye such as TBUT and the Schirmer test where a significant positive correlation has been observed between lower TMH measurements and both Schirmer and TBUT results (Tung et al., 2014). The results in Table 5.6.1 present the descriptive statistics representing the TMH obtained using the Keratograph 4 for both the keratoconic and the control group of subjects. As can be seen in this table, a small difference can be observed between the means of the two subject groups, with the difference between them being a value of 21.36$\mu$m. When these means are compared, the mean of the keratoconic group is displayed as the larger value of the two groups. The medians are only separated by a value of 2$\mu$m, with the median of the control group being slightly larger than that of the keratoconic group. Both the range and interquartile ranges, however, demonstrate a large difference between the two subject groups, with the interquartile range of the keratoconic group being almost twice that of the control group. The distribution of data measurements between the two groups can be observed diagrammatically when looking at the box and whisker plot in Figure 5.6.1 where the two subject groups are being compared. As can be seen in this figure, the distributions appear similar with the maximum and minimum values indicating the most variation.

When looking at the test statistic represented in Table 5.6.2, the results of the Mann Whitney U test show that the null hypothesis cannot be rejected. Due to this, it can be seen that the TMH measurements obtained using the Keratograph 4 are not shown to be
statistically significantly different between the two subject groups. A change in the height of the tear meniscus would have been expected within the keratoconic group due to the significant physiological changes taking place within the cornea and the tear film of these keratoconic subjects. In order to determine whether a significant link could be observed between the symptoms described by the OSDI scores and the height of the tear meniscus, Spearman’s rank correlation was calculated. As can be seen in Table 5.6.1.1, Spearman’s rank correlation confirms the lack of correlation between these two variables in the keratoconic group of subjects. The null hypothesis which states that the two variables are not shown to be correlated cannot be rejected and therefore, a change in symptoms with the fluctuation in TMH cannot be concluded. The scatter plot in Figure 5.6.1.1 gives a visual representation of the lack of correlation, the data are shown to be distributed in a random nature with no specific pattern being observed between these two variables. The same result was obtained when comparing these two variables in the control group of subjects, no significant correlation was shown to be present as shown in Table 5.6.1.2 and Figure 5.6.1.2.

The study performed by Tung et al., (2014) where the height and area of the tear meniscus was measured in dry eye and control subjects confirmed a lack of correlation between the symptoms described by the OSDI scores and the tear meniscus parameters being measured. The study by Pult et al., (2011), however, found the presence of a moderate relationship between symptoms described by the OSDI scores and the height of the tear meniscus being measured using a slitlamp biomicroscope.

The OCT has been widely utilized as an imaging technique, employing low-coherence interferometry in order to obtain high resolution, two-dimensional images of ocular tissues (Johnson and Murphy, 2005). The OCT was used in order to obtain TMH measurements where two diverse images were compared. Table 5.7.1.1 represents the descriptive statistics obtained from each of the two subject groups where the OCT photograph was measured in order to determine the height of the tear meniscus. When observing these descriptive statistics, a large difference may be observed between the means of the keratoconic versus the control group, this difference being approximately 45µm. The difference displayed in the medians is shown to be even larger, being separated by an amount of approximately 62µm.

The Mann-Whitney U test was employed in order to determine whether a significant difference is shown to be present between the keratoconic and control subject groups in terms of the tear meniscus height in the photographs attained using the OCT. The test statistics and significance level obtained in Table 5.7.1.2 indicate that the null hypothesis can be rejected, this infers that the TMH values obtained using the OCT where the photograph was measured
are shown to be statistically significantly different between the two subject groups. The null hypothesis states that the medians of the two groups are identical, by rejecting this hypothesis, it may be concluded that a significant difference exists. The variation between the keratoconic and control groups may be easily observed when looking at Figure 5.7.1.1, where the distribution of data between the two groups can be seen. From the test statistics calculated, it can be concluded that keratoconus may play a role in the height of the tear meniscus as measured by the OCT, using the photograph specifically. This finding was not present when the TMH of the two subject groups was measured using the Keratograph 4, where no statistically significant difference was present.

The difference between the two different subject groups has been displayed using the Mann-Whitney U test, this finding contradicts what was originally postulated, the fact that keratoconic patients may have a smaller TMH compared to the control group. This assumption falls in line with previous findings indicating that patients with dry eye syndrome tend to have reduced TMH (Li et al., 2012B; Veinekar, 2012). The same finding was thought to be present in keratoconus due to the characteristic symptomology which is similar to that of dry eye syndrome. In the study performed by Ibrahim et al. (2010), where Visante OCT was utilized in order to investigate the TMH in a sample of patients diagnosed with dry eye, it was found that both the upper and lower tear menisci were shown to be significantly lower in dry eye patients when compared to a group of control patients. In a separate study performed by Tung et al. (2014) tear meniscus parameters are shown to be significantly lower in various dry eye conditions such as aqueous tear deficiency and Sjögren syndrome when measured using the OCT. Another study performed by Qiu et al. (2012), utilizes FD-OCT in order to determine the TMH of the lower lid in subjects with various tear film dysfunctions. Throughout this study by Qiu et al., (2012) it was also determined that the lower tear meniscus is shown to be decreased in subjects with any type of tear dysfunction.

The descriptive statistics in Table 5.7.3.1 are presented where the tear meniscus measurements were obtained using the colour scan produced by the OCT, where both the keratoconic and control groups are compared. Observing these descriptive statistics, the difference between the means of the two groups does not seem to be as apparent as that observed in Table 5.7.1.1 where the photograph was measured. The means of the two groups are separated by a value of 17.32µm, which is minor compared to the difference observed in Table 5.7.3.1. The difference between the medians has also been shown to be small, a value of approximately 29.2µm while the interquartile range displays the largest amount of variation, where the two subject groups are separated by a value of 51µm, the keratoconic
group being more than double that of the control group. The same test statistic was calculated in order to determine the statistical significance, the Mann-Whitney U test. The results of this test indicate that the null hypothesis could not be rejected in this case and therefore no statistically significant difference was found to be present between the two subject groups when measuring the TMH of the colour scan. When observing the distribution of values in Figure 5.7.3.1, the boxes displayed by each of the two subject groups in the box and whisker plot seem to fall within a similar range with little variation being seen between the two groups.

The difference in results for each of the two types of OCT scans, namely photograph and colour scan may be due to the difference in clarity of the two images. The borders of the tear meniscus may be easier to discriminate using the colour scan as the definite change in colour can be observed. The photograph (as with the Keratograph 4 images), being black and white, present difficulty when attempting to determine the border of the tear meniscus and this may be a reason for the variable results between the two scans. In the colour scans, there is no light reflection seen at the top of the tear meniscus as with the photograph. This light reflection may cause interference and confusion when trying to determine the defined border of the tear meniscus.

Spearman’s rank correlation was calculated in an attempt to determine whether correlation exists between the symptoms being described by the OSDI scores and TMH obtained using the OCT (photograph). The results of this correlation test can be observed in Table 5.7.2.1. From these results it can be determined that no significant correlation seems to exist between the dry eye symptoms and the height of the tear meniscus measured using the OCT where the photograph was measured for the keratoconic group of subjects. The same can be said for the control group of patients as seen in Table 5.7.2.2, where no correlation is shown to be present. The scatter plots shown in Figures 5.7.2.1 and 5.7.2.2 confirm the lack of correlation as the data are shown to be distributed in a random nature with no specific pattern being observed when the OCT photograph is measured. The study performed by Nguyen et al. (2012) looks at the correlation between the lower TMH (measured using the OCT) and clinical aspects, one of which would be the symptoms described by the Dry Eye Questionnaire (DEQ). This specific study by Nguyen et al. (2012) found symptoms to be negatively correlated with the height of the lower tear meniscus. The study by Nguyen et al. (2012), utilizes the DEQ rather than the OSDI questionnaire, although the OSDI has been shown to be a valuable tool when evaluating dry eye disease, it does not seem to correlate well with the objective clinical measures of dry eye disease (Nguyen et al., 2012).
Bland-Altman plots can be generated in an attempt to compare two specific variables, where the results may give an indication of whether two different testing techniques may be used interchangeably (Hofman et al., 2015). These graphs are constructed in order to generate a plot where the differences between instrumentation are plotted against their corresponding means. A mean difference is calculated which acts as a reference point for comparison between the two diverse types of instrumentation. Bland-Altman plots have been constructed in order to compare the TMH obtained using the Keratograph 4 with that of the OCT (both photograph and colour scan). When observing these plots, it can be seen that in most cases, the two different instruments cannot be used interchangeably.

Figure 5.8.1.1 represents the Bland-Altman plot comparing the values obtained using the Keratograph 4 with those obtained using the OCT (photograph) in the keratoconic group. Both image scans obtained are easily comparable as both represent the lower lid margin where the tear meniscus can be seen and measured accordingly. The results of this test, however, show that these two methods of measuring TMH cannot be used interchangeably when measuring the height of the meniscus in the keratoconic group of subjects. This plot was repeated for the control group of subjects, yielding the same result, a lack of interchangeability between the Keratograph 4 and the OCT (photograph). A study performed by Arriola-Villalobos et al., (2015), compares the TMH obtained using the Keratograph 5M with those obtained using the Spectralis OCT which is a FD-OCT. The Bland-Altman plots generated in this study show the presence of poor agreement between the two measuring techniques. Repeatability and reproducibility seemed to be demonstrated using the FD-OCT, however, this was not the case using the Keratograph 5M. Our results are similar in that no correlation is present between the two different measurement devices in both subject groups.

Slightly different results were obtained when observing the colour scan given by the OCT compared with the Keratograph 4 in the keratoconic subject group. As seen in Figure 5.8.2.1, an improved interchangeability is shown to be present between the two measurement techniques. In the case of the control group an improved interchangeability is also observed when measurements were obtained using the colour scan of the OCT versus the Keratograph 4, with the mean difference being smaller compared to the OCT photograph. Comparisons were made using a single type of instrumentation, namely the OCT, where the photograph and colour scan measurements could be equated. Looking at the Bland-Altman plots in Figures 5.8.3.1 and 5.8.3.2, a lack of interchangeability was shown to be present between the OCT photograph and the OCT colour scan in both subject groups.
6.6 TEAR MENISCUS AREA

When measuring the dimensions of the tear meniscus, it was decided that in addition to the TMH, measurements of TMA would be included, these measurements would be obtained using the OCT. Only the colour scan provides a lateral view of where the cornea contacts the lower lid margin and therefore the triangular area of the tear meniscus can be observed. Table 5.9.1 represents the descriptive statistics associated with TMA for both the keratoconic and the control subject groups represented in $\mu m^2$. A difference may be observed between the means of the two subject groups with the mean of the keratoconic group being larger by 3502.67 $\mu m^2$. The range between the two groups is separated by a value of 15400 $\mu m^2$ while the interquartile range of the keratoconic groups is just less than double that of the control group. When observing the results obtained in Table 5.9.2, it can be seen that the null hypothesis cannot be rejected and therefore no statistically significant difference may be observed between the TMA of each of the two subject groups. Keratoconus does not seem to play a role in altering the area of the tear meniscus. When observing the box and whisker plot in Figure 5.9.1, the data for the two subject groups appear to fall within the same range with no large variation being seen. Each group is shown to have several outliers being represented by the measurement case number, falling above the maximum value of the interquartile range.

Other studies, where the area of the tear meniscus has been measured, include the study by Zhou et al. (2009) where the TMH, depth, as well as area were measured using FD-OCT, in dry eye as well as control patients. This was done in order to determine the reproducibility of FD-OCT. The results of this study suggested that obtaining measurements regarding the TMA are complicated by the curvature of the air-meniscus interface, however, FD-OCT has proven to be a highly reproducible measure in terms of tear meniscus parameters (Zhou et al., 2009). In a similar study performed by Qiu et al. (2012) tear meniscus parameters were obtained for subject groups with various forms of dry eye syndrome. Anterior segment OCT using the FD-OCT technique was completed on each of the subject groups in order to obtain the TMH, depth as well as area. The results of this study concluded that all three of the tear meniscus parameters were found to be significantly lower in the dry eye subject groups compared to the control groups (Qiu et al., 2012).

When comparing the area of the tear meniscus obtained using the OCT and the symptomology being described by the OSDI scores, in this study the results can be seen in Table 5.9.1.1 and Table 5.9.1.2 for the keratoconic and the control groups respectively. For both subject groups, the null hypothesis cannot be rejected and therefore no significant
correlation is shown to exist between the symptoms being experienced and the TMA being measured. The scatter plots in Figures 5.9.1.1 and 5.9.1.2 display this lack of correlation with the data being distributed in a random nature with no trend being observed within the data set.

6.7 VERNIER MEASUREMENTS

In order to determine the accuracy of measurements being taken between the two measurement techniques, namely the Keratograph 4 software and ImageJ, measurements were taken of a vernier as discussed in chapter 4. As can be seen in Table 5.10.1, the difference between the two measurement techniques is separated by a value of 3.6 µm, with the mean of ImageJ being slightly larger. The medians were shown to be separated by an amount of 10 µm, with that of ImageJ being larger than that of the Keratograph 4. Using the Kolmogorov-Smirnov test, both the Keratograph 4 and ImageJ results are shown to be normally distributed and therefore the paired samples t-test could be used to determine statistical significance.

From the results of the paired samples t-test, it can be seen that the null hypothesis can be accepted therefore showing that these two measurement techniques provide similar results. Therefore, the Keratograph 4 measurement tool and ImageJ may be used interchangeably when measuring scans such as those obtained throughout this study. The vernier images provide support for the accuracy between the two measurements techniques when taking subjective measurements of the TMH and TMA of the various scans obtained.

6.8 LIMITATIONS OF THE STUDY

Throughout the procedures employed during this study, great care was taken in order to ensure that consistent and accurate measurements were obtained for the specified subject groups. This study, however, did have its limitations which could not be overcome. The first limitation encountered was the characteristics of both subject groups. The 25 keratoconic subjects partaking in this study consisted of patients attending the contact lens clinic at the University of Johannesburg. Although no intentional discriminations were made regarding race, gender or age, the following distribution was obtained, the subject group was made up of Africans predominantly (76%) who were mostly female (64%). The keratoconic group consisted of subjects with ages ranging between 19 and 56. The control group, however, consisted of students studying optometry at the University of Johannesburg and therefore ages ranged between 18 and 23 with the group consisting of 52% Africans who were
predominantly females (84%). Due to the differences within the distribution of subjects, this may not be an accurate indication of the differences between keratoconic and control parameters.

Age plays a vital role in the structure and function of the tear film, this may have had an impact as the control group was younger compared to the keratoconic group. Various studies have demonstrated the effects of aging on the tear film, a significant correlation has been shown to be present between abnormalities within meibomian glands and aging individuals (Ding and Sullivan, 2012). Due to the scarcity and availability of young keratoconic subjects attending the clinic, the subject group could not be limited according to age which would have been preferable.

The unpredictability of keratoconus may be a limitation of the study. Due to the severity of this ectasia in a portion of the keratoconic subject group, some of the data measurements could not be obtained i.e. the grading of keratoconus given by corneal topography. Due to the severity of dry eye symptoms being experienced by some of the keratoconic subjects, acquisition of measurements could be difficult as the subject may feel severe discomfort when holding their eyes open in order to take the measurement. In cases where five measurements could not be obtained due to patient discomfort, an average of the remaining measurements was calculated. The OSDI is also a subjective indication of the symptoms being experienced and therefore may not be the most accurate indication of symptom severity.

A further limitation was the manner in which scans were measured, these scans were measured by the same examiner and is therefore a subjective measurement. There may be a significant amount of error associated with the examiner taking the measurements. When measuring each of the individual scans, human error may play a role in the measurements obtained. Due to the tools available, this was the only manner in which scans could be measured but there may be human error associated with this subjective method of measurement.

6.9 SUGGESTIONS FOR FURTHER RESEARCH

Perhaps future studies could include subjects falling within a similar age range as age may play a vital role in the quality and quantity of the tear film. Race may also be a contributing factor and therefore future studies should ensure the distribution of race to be as uniform as possible. Many of the procedures performed within this study were entirely subjective such as the subjective measurements where the start and end points of the tear
meniscus were observed and measured. Due to the possible human error involved with this method, perhaps future studies could incorporate a standardized scaling system where automatic and controlled measurements could be obtained using specific instrumentation. Future studies may incorporate measurements of tear osmolarity in order to determine whether keratoconus and changes in osmolarity may be related. Comparison of corneal topography versus the regions of the front surface of the eye that result in rapid drying may also be compared in order to determine whether certain regions being affected by keratoconus may have an adverse effect on the tear film.

Other grading scales could be used in order to determine the severity of keratoconus. Grading scales which are internationally recognized would probably give a different indication of keratoconic severity, some of these indices include the KISA index and the Massachusetts Eye and Ear Infirmary (MEEI) grading scheme. A possible solution could be to create a grading system specifically designed for South Africa, owing to the prevalence of keratoconus amongst the South African community.

6.10 CONCLUSION

The results of this study confirm the difference in symptomology between keratoconic and control subjects. The severity of keratoconic symptoms has been conclusively shown, however, the reasons for the similarity in symptoms between keratoconic and dry eye subjects remains unknown. Previous research has documented the lack of correlation between symptoms and signs of dry eye, the results of this study support this finding. The clinical parameters used throughout this study, being the NTBUT measurements as well as the TMH measurements; do not exhibit a relationship when compared with the symptoms expressed by the OSDI scores for either of the two subject groups. The findings of this study, although detailed in previous research confirm this lack of correlation and therefore does not shed any new light on the condition of keratoconus and why these findings may be so.

The differences in TMH measurements between the two subject groups could only be shown when measured using the OCT where the photograph was measured (TMH-OP). Due to the differences in clarity between the two measurement techniques utilized throughout this study, it can be assumed that the OCT photographs provide better clarity and therefore a better indication of the difference in height measurements between the two subject groups. TMH-OPK was also the only variable shown to be correlated with the grade of keratoconus and therefore may indicate its usefulness.
When examining the data obtained throughout this study, the main findings can be summarized as follows:

- The results show that the dry eye symptoms being experienced in keratoconus are of a greater severity compared to symptoms being experienced by the control group of subjects. These symptoms, however, do not show correlation with the grade of keratoconus.

- The severity of keratoconus being experienced (grade) cannot be correlated with any of the clinical measures throughout the study namely NTBUT-K, TMH-KK, TMH-OCK, TMA-K. The only parameter shown to be correlated with the grade of keratoconus is the TMH measured using the OCT where the photograph is measured (TMH-OPK), significant correlation is displayed between these two variables.

- No statistically significant difference was shown to be present between the NTBUT, the TMH-K or TMA values obtained for the two subject groups. The only variable, other than OSDI score shown to be statistically significantly different between the two subject groups was the TMH measurements obtained using the OCT where the photograph was measured (TMH-OP).

- While both the Keratograph 4 and the OCT provide an accurate representation of the tear meniscus dimensions, these different types of instrumentation cannot be used interchangeably when trying to obtain measurements such as these.

- The vernier data displays the accuracy of the two scan measurement techniques utilized throughout this study.


CHAPTER 8
APPENDICES
## 8.1 APPENDIX A: OSDI QUESTIONAIRRE

### Ocular Surface Disease Index® (OSDI®)$^2$

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

<table>
<thead>
<tr>
<th>Question</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>Half of the time</th>
<th>Some of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Eyes that are sensitive to light?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2. Eyes that feel gritty?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3. Painful or sore eyes?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4. Blurred vision?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5. Poor vision?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Subtotal score for answers 1 to 5**

<table>
<thead>
<tr>
<th>Question</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>Half of the time</th>
<th>Some of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Reading?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>7. Driving at night?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>8. Working with a computer or bank machine (ATM)?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>9. Watching TV?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Subtotal score for answers 6 to 9**

<table>
<thead>
<tr>
<th>Question</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>Half of the time</th>
<th>Some of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Windy conditions?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>11. Places or areas with low humidity (very dry)?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>12. Areas that are air conditioned?</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Subtotal score for answers 10 to 12**

Add subtotals A, B, and C to obtain D

\[ D = \text{sum of scores for all questions answered} \]

**Total number of questions answered**

(Do not include questions answered N/A)

Please turn over the questionnaire to calculate the patient’s final OSDI® score.
Evaluating the OSDI® Score

The OSDI® is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI® is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.

Assessing Your Patient’s Dry Eye Disease

Use your answers D and E from side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below. Find where your patient’s score would fall. Match the corresponding shade of red to the key below to determine whether your patient’s score indicates normal, mild, moderate, or severe dry eye disease.

1. Data on file, Allergan, Inc.
APPENDIX A:
Information for participants

1. You are invited to take part in the following research project:

An analysis of tear break up time, tear meniscus height and area, the grade of keratoconus and dry eye symptoms in keratoconic and non-keratoconic individuals

MASTERS RESEARCHER: DEANNE NICHOLAS
SUPERVISOR: PROFESSOR WDH GILLAN, DEPARTMENT OF OPTOMETRY

2. Participation in the study is on a voluntary basis and you are free to withdraw from the study at any point, though you are requested not to do so lightly because such action might negatively influence the study.

3. You are encouraged to ask questions, at any stage of the project, should you feel unclear about any aspect of the study.

4. Your participation will aid in further understanding of certain human tear function dynamics, though you might not personally experience any direct advantage during the project.

5. Procedures being utilized throughout this study are of a non-invasive nature. No medications or invasive techniques will be used; there is no risk of harm or injury to subjects partaking in this study.

6. The information obtained from you as a subject will be treated as confidential and will not be made available to others without your written consent but may be used in scientific papers or presentations. Your confidentiality will be protected at all times.

7. You are welcome to request any results pertaining to your involvement in this study should you be interested.

I, ________________________________________________ hereby voluntarily consent to participate in the research project: **An analysis of tear break up time, tear meniscus height and area, the grade of keratoconus and dry eye symptoms in keratoconic and non-keratoconic individuals**

Subject Identification Number: ________________________________________________

Signature of subject: _______________________________ Date: _____________________

Signature of researcher: _______________________________ Date: _____________________

Signature of supervisor: _______________________________ Date: _____________________

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APPENDIX B:
Possible benefits and risks of the research project

An analysis of tear break up time, tear meniscus height and area, the grade of keratoconus and dry eye symptoms in keratoconic and non-keratoconic individuals

Researcher: Deanne Nicholas, BOptom (UJ)
Supervisor: Professor WDH Gillan, DPhil (RAU)

Summary of project methods and routine:

1. You will be required to complete a brief confidential questionnaire that mainly requests biographical details and other pertinent information relevant to the purposes of the study.

2. Routine optometric test procedures are performed on all subjects prior to data collection. These techniques include tests for visual function as well as ocular health. Control group subjects are screened for any ocular pathology or predisposing ocular conditions which may interfere with the results of the study. Keratoconic patients undergo preliminary tests in order to confirm the diagnosis of keratoconus.

3. Once chosen as a selected subject, measurements of tear meniscus heights are taken on all subjects using both the Oculus Keratograph and the iVue OCT. Additional measurements of non-invasive tear break up time as well as the degree of keratoconic severity is also collected.

No known risks are associated with this project. All testing procedures that are used in the data collection have been used commonly in optometric practice worldwide and are of a non-invasive nature. The procedures involved in this study will be performed by a qualified optometrist under the supervision of a professor in the Department of Optometry. The benefits acquired during this study will enrich the scientific knowledge of the tear meniscus within the disease process of keratoconus. You will thus be making an important contribution to the current body of knowledge by participating in this study.
APPENDIX C:

Consent form

I have read and understood the information provided by the researcher relating to the objectives of the study and my participation therein. If I did not understand, I was given time to ask questions and gain clarity of issues pertaining to the study and to what will be required of me. I understand that participation in this study is completely voluntary and that I may withdraw at any time without penalty.

I am consciously aware that the results of this study are of a scientific nature and thus may be published in scientific papers and/or included in a Masters dissertation. I agree to this, provided that my confidentiality is properly protected. Should I be interested in viewing any of the publications, the researchers agreed to make the relevant material available to me for inspection.

I hereby fully consent to participate in this research project:

______________________________  ______________________
Name of study participant     Signature

______________________________
Name of researcher     Signature

______________________________
Name of supervisor     Signature

______________________________
Place     Date
APPENDIX D:

Subject Questionnaire

Subject name: _________________________________ Date: __________________
Identification number: _______________________________________________________
Sex: ___________________________________________________________________
Race:   _________________________________________________________________
Date of birth: __________________________________ Age:  __________________
Occupation:    ___________________________________________________________
General health: Good/Fair/Poor _____________________________________________
Do you have any systemic conditions, such as diabetes, hypertension, hyperlipedemia,
thyroid disease?
________________________________________________________________
Do you take any medication regularly? If so, what medication/s and for which condition/s?
_______________________________________________________________________
Do you wear spectacles and/or contact lenses? _________________________________
If so, for what purpose? __________________________________________________
Have you ever had ocular surgery? __________________________________________
If so, include details. ______________________________________________________
Do you have any history of ocular diseases? ___________________________________
If so, include details. ______________________________________________________
Do you have any history of epilepsy or neural disorders, severe head trauma or sever
medically diagnosed migraine attacks?
_______________________________________________________________________
If so, include details. ______________________________________________________
Do you have any visual symptoms, such as double vision, flashes of light or fluctuating
vision?
_______________________________________________________________________
If so, how often (daily, weekly, less often)? _________________________________
Are the visual symptoms accompanied by headaches? ___________________________
Any other comments that you feel might be relevant?
_______________________________________________________________________
_______________________________________________________________________

THANK YOU. YOUR PARTICIPATION IS GREATLY APPRECIATED.
8.3 APPENDIX C: PUBLICATIONS

The following publications were derived from the preliminary work done in preparation for this thesis,

PUBLICATION TITLES

8.3.1 Meibomian Gland Imaging: a review

8.3.2 An investigation of the relationship between tear meniscus height and the subjective severity of ocular symptoms in keratoconus
Meibomian gland imaging: A review

The meibomian glands of the upper and lower eyelids play a valuable role in secreting the lipid layer of the tear film. Disturbances in meibomian gland function may result in altered secretion and variations in tear composition which may lead to meibomian gland dysfunction and evaporative dry eye, leading to ocular discomfort. To diagnose and monitor the structural and functional changes occurring within the glands of the eyelids, various imaging techniques are available. Some of the methods used to evaluate the tears and therefore the meibum within the tears include evaporimetry, interferometry, tear osmolarity and meibometry. With these techniques, changes in the lipid layer of the tear film can be quantified and alterations in meibomian gland function assessed. Meibography is an additional method that can be used; it has the unique feature of allowing the assessment of meibomian gland morphology during ocular surface disease processes. The aim of this review is to create an improved understanding of the meibomian glands and the ways that they may be investigated in order to expand on the treatment methods available.

Introduction

Meibomian glands may be classified as holocrine glands embedded within the tarsal plate of both the superior and inferior lid margins. Individual meibomian glands consist of a central duct that passes through the length of the gland. Extending from this central duct are glandular structures termed acini. The acini are minute grape-like structures which contain modified sebaceous cells known as meibocytes. Meibocytes are responsible for the synthesis of a lipid secretion known as meibum, which passes through numerous ductules into the central canal. From the central canal, the meibum is expelled onto the eyelid margin via a compressive force, and forms the superficial layer of the tear film.

The layer of meibum within tears forms an integral part of the tear film and has various important functions. The meibum secretion is responsible for stabilising the tear film and providing a hydrophobic barrier, thereby preventing excessive evaporation of tears from the ocular surface. Meibum also provides lubrication, thus preventing ocular irritation and in turn affording a smooth optical surface. Antimicrobial properties are also present within the meibomian secretion, providing protection against external antigens that may come into contact with the ocular surface.

Meibomian gland function is thought to be regulated by neuronal, hormonal and vascular factors. Abnormalities within these regulating mechanisms, or disturbances in the functioning of the meibomian glands, may lead to meibomian gland disease which can take on various forms. There may be an absence or complete deficiency of the meibomian glands that may occur as a congenital condition. In some cases, the meibomian glands may be replaced by an extra row of eyelashes, a condition known as dystichiasis which may be inherited or acquired. The remaining glands may be enlarged or distorted. Lastly, the most common form of meibomian gland disease is known as meibomian gland dysfunction (MGD), where changes may occur in the ducts, orifices, acini and secretory activities of the glands.

The various structural changes which take place during meibomian gland disease can be assessed using numerous imaging techniques. One of these imaging methods, termed meibography, is a technique that allows assessment of the morphology of individual meibomian glands. Using the images obtained, a qualitative analysis can be done based on the structural changes taking place, permitting the diagnosis of dry eye disease. Some of these structural changes include loss of glandular tissue (gland dropout), dilation of the ducts within the glands, and stasis of the meibum.

Meibomian gland dysfunction

MGD or posterior blepharitis can be defined as an abnormality occurring in the functions of the meibomian glands owing to either obstruction or changes in the secretory activities of the glands. MGD can be classified as primary or secondary to systemic diseases; it can be focal or diffuse and...
may lead to symptoms of ocular irritation, alteration of the tear film or inflammation of the meibomian gland orifices. Numerous structural changes can take place in MGD that will result in the functions of the glands being altered.

Some of the structural changes occurring within the lids may include thickening, rounding or distortion of the lid margin owing to obliteration of the meibomian gland orifices. The mucocutaneous junction of the lid may become irregular, occasionally it may be displaced posteriorly or it may become elevated. The orifices of the meibomian glands may change in number owing to the disease process, they may duplicate or reduce in number, and become pouted or narrowed. The meibomian glands may be expressed (by applying digital pressure to the lids) and the secretions be assessed; the secretions may be classified as clear, cloudy, granular or inspissated (toothpaste consistency). These changes can be seen in meibomian gland disease where the meibomian glands do not function efficiently or where a disturbance exists within the functional mechanisms of these glands.

Obstructive MGD is the most common form of MGD and may result in hyposecretion or the absence of lipid-rich meibum, which may lead to excessive and rapid evaporation of the tear film. Increased evaporation of the tear film may result in the inability to tolerate contact lens wear, an unstable tear film or blepharitis. Other forms of meibomian gland dysfunction include meibomitis, meibomian neoplasia and meibomian seborrhoea. The seborrheic type of MGD results from hypersecretion of meibum, which induces an inflammatory reaction resulting in ocular irritation. It has not yet been established whether the seborrheic form results from true hypersecretion of meibum or the accumulation of static meibum that is expressed from the ductules. The seborrheic form of MGD demonstrates normal tear evaporation rates as opposed to the obstructive form of MGD. Meibomitis can be defined as an inflammation of the meibomian gland orifices along with solidification of the meibum within the glands.

The prevalence of MGD has been found to be approximately 39% – 50% in the general population worldwide and is one of the primary causes of evaporative dry eye. Dermatological diseases such as acne rosacea and psoriasis may be found in association with MGD. Owing to the prevalence of meibomian gland dysfunction, it has become increasingly important to accurately examine and assess the meibomian glands of dry-eye patients. There are various techniques available for assessment of the tears and the meibum it contains, namely evaporationometry, interferometry, tear osmolarity and meibometry.

**Meibography**

Meibography is a specialised imaging technique used to visualise the morphology of the meibomian glands. Wise et al. stated that this technique utilises ultraviolet light to transilluminate the glands of an everted eyelid. The meibomian gland structure can be viewed by placing the tip of a transilluminating light probe against the everted eyelid which is thus illuminated from behind. Both red and white light sources can be used to image the glands which can then be viewed through a slitlamp biomicroscope from which assessments can be made. The images are captured by an infrared photography system. Meibography provides an **in vivo** method of assessing the morphology of the glands, allowing clinicians to observe any loss of glandular tissue as well as the meibum within the glands. This imaging method, which has been termed contact meibography, was considered very useful in the clinical environment; however, it was found to be time consuming and expensive, and caused patient discomfort owing to its invasive nature. In 2008, non-contact meibography was introduced, a method in which an infrared light source is used, which does not come into contact with the eyelid of the patient and therefore results in less discomfort. An infrared charge-coupled device is attached to a slitlamp biomicroscope without the need for a probe. Conventional meibography allows only the observation of a small central region of the lid margin whereas non-contact meibography encompasses a larger, wider region of the upper and lower lids for extensive examination. The light and dark contrast is opposite to that of contact meibography, in that the glands appear light instead of dark when using the non-contact method. The upper and lower lids can be assessed to determine the degree of a transilluminating light probe against the everted eyelid which is thus illuminated from behind. Both red and white light sources can be used to image the glands which can then be viewed through a slitlamp biomicroscope from which assessments can be made. The images are captured by an infrared photography system. Meibography provides an **in vivo** method of assessing the morphology of the glands, allowing clinicians to observe any loss of glandular tissue as well as the meibum within the glands. This imaging method, which has been termed contact meibography, was considered very useful in the clinical environment; however, it was found to be time consuming and expensive, and caused patient discomfort owing to its invasive nature. In 2008, non-contact meibography was introduced, a method in which an infrared light source is used, which does not come into contact with the eyelid of the patient and therefore results in less discomfort. An infrared charge-coupled device is attached to a slitlamp biomicroscope without the need for a probe. Conventional meibography allows only the observation of a small central region of the lid margin whereas non-contact meibography encompasses a larger, wider region of the upper and lower lids for extensive examination. The light and dark contrast is opposite to that of contact meibography, in that the glands appear light instead of dark when using the non-contact method. The upper and lower lids can be assessed to determine the degree
of meibomian gland dropout, which refers to the loss or destruction of glandular tissue.\textsuperscript{2,3} As demonstrated in Figure 1a, the destruction of glandular tissue can be seen by the distortion or non-linearity within the glands. Normal meibomian gland structure can be seen in Figure 1b, where the glands have a regular and linear appearance.

In 2012, a mobile meibography system in the shape of a pen was introduced; the system uses an infrared LED attached to a handheld camera system.\textsuperscript{3} This is a convenient and simple method of assessing the glands as it is portable and eliminates the need for a slitlamp biomicroscope.\textsuperscript{3}

The Oculus Keratograph 4 has become an important tool that can be used for contact lens assessment, corneal topography, measuring tear meniscus height, pupillometry as well as many other procedures.\textsuperscript{15} The Keratograph 4 may also be used to image the meibomian glands of the upper and lower lids.\textsuperscript{15} Using this multi-functional apparatus, both still and video images of the meibomian glands can be captured by means of an infrared illumination system that can be manually adjusted.\textsuperscript{2,15} These meibography methods enable an examiner to accurately assess the structural and functional changes that occur within the meibomian glands as these changes could presage MGD. To determine the extent of damage to the glands, the images obtained during meibography are compared with a series of gradings, ranging from mild to severe loss or damage. These grading scales enable the examiner to qualitatively assess the changes occurring during the disease process.

**Grading scales**

The extent of meibomian gland dropout has been shown to be directly correlated with ocular surface conditions.\textsuperscript{3,4,4} There are various factors to be considered when assessing the meibomian glands. These include the percentage of partial glands, the presence of chalazia and the number of glands present within the lid margin.\textsuperscript{7} In the presence of an ocular surface disease, the glands become enlarged and tortuous whilst the ducts appear to be dilated, eventually leading to complete destruction of the gland.\textsuperscript{5} The changes occurring within the structures of the meibomian glands can be qualitatively assessed using a grading scale.\textsuperscript{3,2,17} Three main grading methods have been identified: the meiboscore method, the meiboscale and the meibograde method.\textsuperscript{3,12}

The meiboscore method assesses the areas of partially or completely destroyed meibomian gland structure compared with the normal regions of the eyelid margin.\textsuperscript{3} The meibograph images are assessed and assigned a value proportional to the area of destruction, ranging from 0 to 3, with 3 indicating that the involved area encompasses more than 66% of the lid margin.\textsuperscript{3} A score of 2 implies that the area of the lid margin involved encompasses between 33% and 66%. Less than 33% of the area is involved if a score of 1 is obtained, and 0 implies that the lid has no partial or missing glands.\textsuperscript{3} The numerical values for the upper and lower lids are summed together\textsuperscript{3} to obtain a score ranging from 0 to 6. The meiboscale is a five-grade pictorial scale used to grade meibography images, as opposed to previous scales where four different grades were used.\textsuperscript{12} This subjective method, developed by Pult,\textsuperscript{12} measures the area of meibomian gland loss\textsuperscript{3} from degree 0 to degree 4. Degree 0 implies that there is no meibomian gland loss; degree 1 is indicative of 25% or less. Degree 2 indicates 26% – 50%, 51% – 75% is indicated by degree 3, and degree 4 indicates loss of over 75% of the meibomian glands within the lid.\textsuperscript{12}

The meibograde method provides a more comprehensive grading scale.\textsuperscript{3} It is based on three important structural changes that can occur within the meibomian glands: gland dropout, gland shortening and gland distortion.\textsuperscript{3,16} Each of these three categories is assigned a meiboscore ranging from 0 to 3 as mentioned above; thereafter the scores from the three individual categories are summed to obtain a total score ranging from 0 to 9 for each eyelid.\textsuperscript{2} These grading scales are extensively used and can be useful in the evaluation of MGD and evaporative dry eye.\textsuperscript{12}

Studies have shown that meibomian gland dropout is found to be increased in patients wearing contact lenses and in patients of greater age.\textsuperscript{2,5} Dropout is also more commonly observed in glands of the lower lid as opposed to glands of the upper lid,\textsuperscript{2,3} which may be related to the different structural features of the upper and lower tarsus.\textsuperscript{3} The upper tarsus has been shown to be anatomically larger than the lower tarsus.\textsuperscript{3}

**Other methods of imaging**

Laser confocal microscopy is a relatively new technology that can be used as a diagnostic tool in evaluating various factors involved in ocular surface diseases.\textsuperscript{2,3,17} One of the disadvantages of this imaging technique is that it is invasive and the use of topical anaesthetic is necessary.\textsuperscript{17} Once the patient has been anaesthetised, the eyelid is everted and a Tomo-Cap is used to applanate the palpebral conjunctiva, thereby obtaining digital images of the meibomian gland structures.\textsuperscript{2,17} This method has the advantage of being able to resolve microscopic structures within the eyelid margin.\textsuperscript{17} Using this innovative technology, Matsumoto et al.\textsuperscript{17} performed a study in which two new parameters could be described relating to the meibomian glands, namely glandular acinar unit diameter and acinar unit density.\textsuperscript{3,17} These parameters can now be studied extensively and quantitative measures obtained. Another parameter that can be obtained and is very useful in providing information regarding the severity of MGD is the periglandular inflammatory cell density\textsuperscript{2} which allows the clinician to determine the extent of inflammation occurring within the glands during ocular surface disease processes.

Optical coherence tomography (OCT) is an ultrastructural imaging technique used in ophthalmic diagnosis.\textsuperscript{16} OCT can be used to obtain three-dimensional in vivo images of the meibomian glands;\textsuperscript{2,5} it is thus possible to produce an image from reflection of light off the desired surface being viewed, allowing formation of an image of the structure.
being studied.18 OCT has an important advantage over any of the other imaging techniques, in that it is able to quantify the structure of the meibomian glands volumetrically.3 These volumetric measures can be used to diagnose and follow the progression of MGD.3

Conclusion

The production of meibum, which is responsible for forming the superficial layer of the tear film, is a complex process that can be disturbed by alterations in the structure of the meibomian glands. These changes may result in meibomian gland dysfunction which is a primary cause of evaporative dry eye. The changes occurring in the tear film can be monitored using the various imaging techniques mentioned above. Meibography has been found to be the most comprehensive procedure for imaging the morphological features of the meibomian glands. Quantitative measures can be made and the progress of ocular surface diseases can be monitored. Meibography is not generally used in routine clinical examinations; however, owing to its specificity and sensitivity in the diagnosis of MGD, it is a useful tool that could be utilised more frequently.

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors’ contributions

This article results from preliminary research towards a Master’s degree in optometry undertaken by D.L.N. (University of Johannesburg), under the supervision of Professor W.D.H.G. (University of Johannesburg).

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13. Alsuhaibani AH, Carter KD, Abramhoff MD, Nerad JA. Utility of meibography in the evaluation of meibomian glands morphologic features of the meibomian glands. Quantitative measures can be made and the progress of ocular surface diseases can be monitored. Meibography is not generally used in routine clinical examinations; however, owing to its specificity and sensitivity in the diagnosis of MGD, it is a useful tool that could be utilised more frequently.

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An investigation of the relationship between tear meniscus height and the subjective severity of ocular symptoms in keratoconus

Keratoconus is a debilitating disease in which the cornea does not develop its characteristic round shape but develops into a conical form affecting both functional vision as well as ocular comfort. Depending on the severity of the keratoconus as well as the presence of any associated conditions, keratoconic individuals may complain of various symptoms that include discomfort, irritation, dryness, reflex tearing and foreign body sensation. There are various subjective and objective measures that can be used to determine the severity of these symptoms. A subjective method that is widely used is the ocular surface disease index (OSDI) which has been shown to be fairly accurate when diagnosing dry eye disease; however, these symptoms do not correlate with objective measures of dry eye. Research has revealed the various structural and biochemical changes that take place within a keratoconic cornea; however, the tear dimensions of keratoconic subjects have not been extensively investigated. It is possible that the symptoms experienced by many keratoconic individuals might be linked to alterations within the quantity of the tears of these patients. The present study compared the symptoms experienced by keratoconic individuals with the symptoms of control patients. The differences in tear meniscus heights between keratoconic individuals and those of control individuals were also compared using the Oculus Keratograph 4 (OK4). The results of the study show the absence of a relationship between the subjective symptoms experienced and the height of the tear meniscus.

Introduction

Keratoconus, one of the most common corneal ectasias, presents with various structural and biochemical changes. Research has suggested that keratoconic patients suffer from severe symptoms of dry eye that include tearing, discomfort, irritation and foreign body sensation. Alterations can be seen within each individual layer of the cornea as well as within the tears of these patients. The exact volume of tears found within the tear meniscus, however, has not been comprehensively investigated in terms of keratoconic patients. The tear meniscus plays a vital role in maintaining ocular physiology as well as providing comfort to the anterior ocular structures. Is there a possibility that the volume of tears could be related to the dry eye symptoms being experienced? The present investigation aimed to determine whether a significant difference exists between the tear meniscus heights of keratoconic individuals and those of controls. If a difference were found, can it be related to the symptoms being experienced by these patients?

Literature review

A healthy human cornea is made up of five distinct layers, each with its own specific structural arrangement necessary to maintain the transparency of the cornea. When this precise arrangement is disturbed in any way, it could have a detrimental effect on vision. Of particular importance in the present study are the effects caused by keratoconus, whereby the cornea takes on a conical shape. Keratoconus may be defined as a non-inflammatory corneal ectasia of unknown origin that is generally bilateral but asymmetric.1 It is a progressive corneal dystrophy that can be seen by observing a protruding corneal cone and may be characterised by thinning of the corneal tissue both centrally and para-centrally.1,2 Keratoconus may severely affect the function of the visual system by inducing irregular corneal astigmatism as well as myopia which is not always correctable through the use of spectacle lenses and may require more invasive intervention – that is, surgical procedures.3 The exact aetiology of keratoconus is unknown; however, evidence has shown a genetic, biochemical as well as an environmental link to keratoconus.3 Keratoconic individuals often have associated atopy leading to symptoms of irritation. To relieve these symptoms, patients tend to rub their eyes vigorously, further contributing towards the conical shape of the cornea.
Evidence has shown that there are various structural and biochemical changes that can be observed within the corneas of keratoconic individuals versus normal corneas. Numerous studies have shown the central corneal thickness as well as the volume of the cornea to be significantly less in keratoconic corneas as opposed to normal corneas, which would be expected because of the thinning that takes place as the condition progresses. Within the individual layers of the cornea, there are numerous changes that have been revealed. One of the most predominant changes taking place within the corneal structure is apoptosis of the keratocytes situated in the stromal layer. Keratocytes are mostly responsible for maintaining the extracellular matrix as well as the structure of the collagen fibrils which is imperative for the transparency of the cornea. Apoptosis, otherwise known as programmed cell death, is thought to occur due to mechanical trauma resulting from the vigorous rubbing of the eyes occurring in the majority of keratoconic patients, usually as a result of allergy. The epithelial layer is also affected in the pathophysiology of this condition, which demonstrates degeneration, causing irregularity that can be observed along the epithelial surface with confocal microscopy. Also seen in the corneal epithelium is an accumulation of iron which may result in chemical reactions that produce highly toxic hydroxyl radicals (Fleischer’s ring). In conjunction with these changes occurring within the corneal structure, there are also changes taking place within the structure and composition of the tears. Keratoconic individuals display an alteration in tear quality as well as changes within the protein profile of the tears.

In addition to the various visual complaints and distortions experienced by keratoconic subjects, these individuals also suffer from severe symptoms of ocular discomfort, dryness, reflex tearing and foreign body sensation. Symptoms are common in keratoconus, including itching, irritation, photophobia as well as eye strain. Keratoconus has been shown to be associated with various other clinical conditions, one of which is critically important, namely atopy. Atopy is a condition that generally occurs with a history of asthma or hay fever, causing itchiness of the skin that may affect various regions of the body. To relieve these symptoms of irritation, subjects tend to rub their eyes vigorously, thereby causing further mechanical damage to an impaired cornea. Could these symptoms be related to specific changes within the tear structure of these subjects?

When trying to determine the severity of symptoms experienced, one of the most common subjective methods is known as the ocular surface disease index (OSDI). The OSDI is a questionnaire comprising 12 questions related to the severity of dry eye symptoms and how these symptoms affect visual function. Research seems to suggest a lack of correlation between subjective symptoms of dry eye and objective tests; however, the OSDI questionnaire has been shown to be a reliable and valid indicator of symptoms and the effect of these symptoms. Despite the mounting evidence of changes taking place within the corneal layers of the keratoconic patient, the tear meniscus height (TMH) has not been comprehensively investigated. The tear menisci may be observed at the margins of both the upper and lower lids and are generally a good measure of tear volume. These menisci expand horizontally and are held in place by surface tension, with gravity playing a vital role. The tear meniscus is important in terms of maintaining ocular physiology, along with upholding the comfort of the ocular system. The evaluation of TMH has been shown to be a potential diagnostic factor for aqueous-deficient dry eye. There are numerous factors that may affect the height of the tear meniscus, such as the length of the lid, the punctum location, tear secretion as well as tear drainage. TMH may be measured using various methods, one of which includes photography using the Oculus Keratograph.

With the extensive changes taking place within the structure of the keratoconic cornea, is it safe to assume that the tear meniscus height would remain unchanged? If a change in TMH could be observed, is it likely to be larger or smaller than expected?

Method

Data from 25 keratoconic and 25 control patients were obtained during the present study. Across the sample of 50 subjects, age ranged between 19 and 56, with both groups comprising women predominantly. The keratoconic subject group had a mean age of 24, and the control group a mean age of 19. The keratoconic group consisted of 15 women and 10 men, whilst the control group consisted of 20 women and 5 men. The study as well as its procedures were thoroughly explained to the potential subjects, and written informed consent was received from each before taking part in the study. Within the keratoconic group, the presence of keratoconus was confirmed through the use of corneal topography as well as slitlamp procedures to determine whether clinical signs diagnostic of keratoconus were present. The control group of subjects were screened for the presence of any ocular pathology that might result in exclusion from the study. Each subject was required to complete the OSDI questionnaire consisting of 12 questions for grading the severity of the symptoms experienced by the subject. Following completion of the questionnaire, the score from each individual questionnaire was manually calculated, yielding a percentage which gave an indication of the severity of the symptoms experienced.

TMH measurements were taken, using the OK4. With the subject sitting upright, positioned correctly against both the chin and forehead rest of the OK4, photographs were obtained of the tear meniscus situated on the surface of the lower lid. The subject was asked to blink and, directly following this blink, the photograph was taken. The height of the tear meniscus in both the right and left eyes of each subject was obtained, with 5 individual photographs per eye. In an attempt to ensure that constant conditions were maintained, all tear meniscus measurements were taken at approximately the same time in the afternoon with the air conditioning set at a standard setting of 22 degrees Celsius. After completion of the photographs using the OK4, each individual photograph was magnified to
twice its original size in order to view the height of the tear meniscus. Using the tools and software available on the OK4, individual photographs were opened and each height manually measured; these scans were saved with the measurements on them and the OK4 was calibrated so that the measurements were given in millimeters (mm) and could be converted to microns. In an attempt to ensure consistency of measurements, the same individual was responsible for measuring each scan in order to rule out variability amongst different individuals taking measurements. The scans were randomly measured to ensure that the individual taking the measurements was unaware of which subject group the specific scan belonged to.

Once the TMH measurements with the OK4 had been completed, the five measurements from each eye were averaged in order to obtain one TMH value for each eye of the 50 subjects. In an attempt to ensure consistent comparisons between values, the TMH averaged for both the right and left eyes were added together and a global mean was calculated. This was done because of the fact that the severity of the symptoms given by the OSDI scores were reported as one overall value. Symptoms cannot be recorded separately for each eye and therefore the OSDI gives one value for the symptoms experienced. For a valuable comparison to be made, one value was needed to compare the symptoms versus the TMH. To achieve this, the TMH of the right and left eyes were added and averaged (25 sets of eyes were used for each of the two subject groups, resulting in 25 means for the keratoconic subjects and 25 means for the controls). Using these values for the TMH, combined with the percentages calculated from the OSDI questionnaires, a statistical analysis could be done using the SPSS (Statistical Package for Social Sciences) software.

**Results**

Table 1 presents the results of the Kolmogorov-Smirnov and Shapiro-Wilk tests. These tests were performed for the OSDI scores as well as the TMH obtained using the OK4 for both the subject groups in order to determine whether the data were normally distributed.

Using the values obtained from the normality tests, it can be seen that both the OSDI scores and the TMH are mostly shown to be normally distributed for both sets of subjects. The Kolmogorov-Smirnov results show that the OSDI scores obtained from the keratoconic group and the TMH obtained from the control group can be seen as normally distributed. Shapiro-Wilk results suggest that only the OSDI scores obtained by the keratoconic group can be seen as normally distributed. The remainder of the results were shown not to be normally distributed; consequently, mostly non-parametric statistics could be performed on the data set.

Table 2 presents the descriptive statistics for the data used in the present study with right and left eyes combined. The descriptive data in this table are presented for both the keratoconic and the control group of subjects. These descriptive statistics are given for the OSDI scores as well as the TMH, with the OSDI scores represented as a percentage whilst the TMHs are measured in microns.

When examining the values in Table 2, it can be seen that a large difference exists between the mean and median OSDI scores of the keratoconic versus the control group. The TMH measurements, however, do not seem to exhibit such a large difference. As shown in Table 2, the mean OSDI score for the keratoconic group of 58.47% is almost six times that of the control group, signifying the severity of these symptoms. The mean TMH does not seem to exhibit a large difference between the keratoconic and the control groups, being separated by approximately 20 microns. The medians are separated by only 2 microns; however, it is essential to determine whether these differences are statistically significant.

When assessing the results obtained from the Mann-Whitney U test, it can be seen that the OSDI scores are statistically significantly different, whereas the TMH does not seem to be statistically significantly different. Therefore, although symptoms indicated by the OSDI scores are significantly different between keratoconic subjects and control subjects, with keratoconic subjects displaying a greater severity of symptoms, we cannot conclude that the TMH is significantly different between the two subject groups (Table 4).

Pearson’s correlation coefficients were calculated in order to determine whether a correlation exists between the symptoms

### Table 1: Kolmogorov-Smirnov and Shapiro-Wilk tests for the normality of data.

<table>
<thead>
<tr>
<th>Subject groups</th>
<th>Kolmogorov-Smirnov</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
<td>Significance</td>
</tr>
<tr>
<td>OSDI score</td>
<td>Keratoconic</td>
<td>0.091</td>
</tr>
<tr>
<td>Control</td>
<td>0.258</td>
<td>0.709</td>
</tr>
<tr>
<td>TMH</td>
<td>Keratoconic</td>
<td>0.161</td>
</tr>
<tr>
<td>Control</td>
<td>0.106</td>
<td>0.953</td>
</tr>
</tbody>
</table>

Note: The test statistics and the significance levels are presented for OSDI scores and TMH for both subject groups. OSDI, ocular surface disease index; TMH, tear meniscus height.

### Table 2: Descriptive statistics for the data collected from the two subject groups.

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>OSDI score (%)</th>
<th>TMH (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Keratoconics</td>
<td>Controls</td>
</tr>
<tr>
<td>Mean and standard deviation</td>
<td>58.47 ± 20.62</td>
<td>94.4 ± 11.98</td>
</tr>
<tr>
<td>95% confidence interval for the mean</td>
<td>52.61 – 63.33</td>
<td>60.4 – 12.85</td>
</tr>
<tr>
<td>Median and interquartile range</td>
<td>58.9 – 28.4</td>
<td>6.3 – 9.35</td>
</tr>
</tbody>
</table>

Note: The results for both the keratoconic and the control groups are presented. The means, standard deviations, confidence intervals, medians and interquartile ranges for both the OSDI scores and TMH readings are presented. The OSDI scores are presented as a percentage whilst the TMH values are expressed in microns (µm). OSDI, ocular surface disease index; TMH, tear meniscus height.
being experienced and the TMH. Both groups showed poor correlation between the symptoms and the TMH that was measured. From the $p$ values obtained, it can be seen that in both cases the null hypothesis cannot be rejected, and therefore the correlation is not statistically significant.

Figures 1 and 2 present the scatter plots for the OSDI scores and the TMH’s of the keratoconic versus the control group respectively. The OSDI scores are represented as a percentage, with TMHs expressed in microns.

In Figures 1 and 2, no correlation appears to exist between OSDI scores and TMH. The observed values seem to be scattered randomly, with no specific relationship between the two variables.

**Discussion and conclusion**

When calculating the statistics for the present study, it was decided that TMH values for the left and right eyes would be combined in order to obtain one value for each of the 50 subjects. One reason for this approach is that the OSDI score gives an overall percentage of the severity of symptoms experienced by a specific subject. OSDI scores cannot be given for right and left eyes separately, and therefore one percentage is calculated per subject. Previous research has indicated that no significant difference exists between the TMH acquired from right and left eyes. In the study by Shen et al., four measurements of upper and lower TMH were taken 3 hours apart, and measurements were repeated upon awakening the following morning. Shen et al. reported results indicating that there was no significant difference between the tear menisci of the right and left eyes. In a study by Karakosta et al., 161 published research articles were analysed to determine the effects of combining the right and left eyes when data measurements are analysed. The research articles were categorised according to whether one eye was used, both eyes were used, and whether criteria for eye selection were included. From the results of Karakosta et al.’s study, it was determined that combining the data values for right and left eyes might have a negative effect on the results, especially if the correlation nature of the data had not been accounted for. This combination may result in an underestimation of the true variation within the data set. In a similar study by Armstrong, where research articles were analysed, it was concluded that both eyes could be used and averaged if the correlation was found to be close to one. When calculating the statistics involved in our present study, we were aware of the limitations involved when combining right and left eyes; however, the data sets were easily comparable using one value for each subject, and calculation was therefore done in this way.

The results of the present study suggest that keratoconic individuals have symptoms of greater severity than those of the control group of subjects. As seen in Table 2, the mean OSDI score obtained from the control group is approximately one-sixth that of the keratoconic group, illustrating the severity of the symptoms experienced in keratoconus. The accuracy of these results cannot be comprehensively determined as the OSDI is a subjective technique by which to determine symptom severity. The experience of each subject is entirely

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**TABLE 3:** Mann-Whitney $U$ test presenting the test statistics and significance levels for both the keratoconic and the control groups.

<table>
<thead>
<tr>
<th>Mann-Whitney $U$ test</th>
<th>Mann-Whitney $U$ score</th>
<th>Z</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSDI</td>
<td>50</td>
<td>-8.388</td>
<td>0</td>
</tr>
<tr>
<td>TMH</td>
<td>1207</td>
<td>2482</td>
<td>-0.296</td>
</tr>
</tbody>
</table>

OSDI, ocular surface disease index; TMH, tear meniscus height.

**TABLE 4:** Pearson’s correlation coefficients for both the keratoconic and the control groups.

<table>
<thead>
<tr>
<th>Pearson’s correlation</th>
<th>Keratoconic</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.0392</td>
<td>-0.2095</td>
</tr>
<tr>
<td>$p$ value</td>
<td>0.8525</td>
<td>0.3149</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.3616 – 0.4277</td>
<td>-0.5584 – 0.2024</td>
</tr>
</tbody>
</table>

Note: The table includes the correlation coefficients, significance levels and the values representing 95% confidence intervals for the correlation coefficients.
subjective and may be linked to various other causes such as atopy or connective tissue diseases. The severity of these symptoms may be influenced by altered nerve morphology present in keratoconic corneas, changes to the tear structure, the duration of the ocular disorder resulting in increased damage to the ocular surface, as well as various associated conditions such as atopy. As demonstrated in Table 3, the results of the Mann-Whitney U test indicate that a significant difference exists between the two subject groups in terms of the OSDI scores. Therefore, as anticipated, keratoconus does play a vital role in the severity of symptoms experienced by these patients.

Sarac et al.\(^\text{21}\) stated that dry eye symptoms have been shown to be present in approximately 81% of keratoconic patients. Sarac et al.’s study\(^\text{21}\) found that some of the factors contributing towards dry eye in keratoconic patients included the release of collagen degradation products, a change in corneal sensitivity or changes occurring within the surfacing mechanism of the tears. Rabinowitz\(^\text{14}\) found the symptoms in keratoconus to be variable, depending on the progression of the disease, with symptoms being subtle in the early stages and increasing in severity as the disorder progresses. A study by Johnson,\(^\text{22}\) in which symptoms and signs of dry eye were compared, indicated that the duration of any specific ocular disease might have an effect on the severity of symptoms. Depending on the duration of the disease, corneal nerves may display an altered nerve response, leading to extreme symptoms of dry eye. When assessing the results of our study, it can be seen that, of the 25 keratoconic subjects, 16 of them (64\%) obtained an OSDI score of 50\% or more, indicating dry eye symptoms ranging between moderate and severe. In the control group of subjects, however, an OSDI score above 50\% was obtained by only one subject (4\% of the control group). This finding signifies the severity of symptoms experienced as a result of the presence of keratoconus.

As shown in Table 3, the TMHs of the keratoconic and the control group do not exhibit a statistically significant difference. Therefore, we cannot conclude that a difference in TMH could be observed owing to the presence of keratoconus. In the study by Sarac et al.,\(^\text{21}\) the TMHs of keratoconic patients were investigated, and the results indicated that no significant difference existed between the TMH of keratoconic versus control patients. According to Uchida et al.,\(^\text{23}\) TMH is a valuable component when diagnosing dry eye disease. In various other conditions, such as Sjögren’s syndrome, TMH has been shown to be significantly lower.\(^\text{24}\) Owing to the compromise in lacrimal gland function, thereby causing impairment in tear secretion, Sjögren’s syndrome patients display lower tear menisci. Shen et al.\(^\text{18}\) also reported findings of lower TMH in patients with tear-deficient dry eye. Could one therefore assume that lower TMH might correlate with dry eye symptoms? It has been thought that lower TMH may apply to keratoconus owing to the various structural and biochemical changes taking place within the anterior ocular components. The results displayed in our study, however, show that keratoconus does not seem to have a direct effect on TMH, as no significant difference could be found between the keratoconic and control groups.

Furthermore, no significant correlation exists between the severity of symptoms and the TMH measured, with correlation coefficients that deviate largely from one. These findings relate to other publications demonstrating a lack of correlation between subjective symptoms and characteristic signs of dry eye. Johnson\(^\text{22}\) also indicated that there was no statistically significant correlation between subjective symptoms and characteristic signs within the general population. Consequently, it was concluded that objective measures of dry eye cannot be used to predict the symptoms that may be experienced and vice versa.\(^\text{22}\) In a study by Morales-Fernández,\(^\text{25}\) symptoms obtained from the OSDI questionnaire specifically were compared with objective measures of dry eye to determine whether a significant correlation could be found. Morales-Fernández\(^\text{25}\) concluded that no significant correlation could be observed when comparing the symptoms reported to the characteristic signs of dry eye. Similarly, we found no link between OSDI scores and objective measures of TMH. The data points in Figures 1 and 2 appear to be distributed in a random nature with no specific pattern observable. We therefore cannot find any associations between TMHs and the severity of the symptoms experienced. No conclusions can be made regarding changes in TMH as a result of keratoconus.

Limitations of the present study could be the small size of the sample group; a larger sample group consisting of an equal distribution between race and gender might provide a more conclusive finding regarding the relationship between symptoms and TMH.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors’ contributions

D.L.N. (University of Johannesburg) was responsible for collection and acquisition of data. Owing to the nature of the data measurements, one individual was responsible for measurement of each separate scan, which was performed by D.L.N. under the supervision of W.D.H.G. (University of Johannesburg). The statistical analysis was performed by Statkon (University of Johannesburg) with the remainder of the content written by D.L.N. under the supervision of W.D.H.G.
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