Correlation between Ocular Surface Disease Index and tear meniscus height in dry eye disease at a Johannesburg tertiary eye hospital

Primary Author: Dr Daniel Erasmus; MBBCh, BEngSc (BME)

Candidate: MMed (Ophthalmology)

Supervisor: Dr Roland Höllhumer; MMed, FC Ophthal (SA), MBChB

Declaration

I, Daniel Erasmus student number 0703393W, am a student registered for the degree of Master of Medicine in Ophthalmology. I declare that the work submitted for assessment of the above degree is my own unaided work except where I have explicitly stated otherwise.

Dedication

I dedicate this work to my fiancé Robyn Williams.

Abstract

Background: Dry eye disease is a common, and potentially vision-threatening problem. The Ocular Surface Disease Index is a well-established method of subjectively assessing dry eye disease. Objective means of diagnosing dry eye disease suffer from poor reproducibility, low sensitivity and specificity, are invasive, time consuming and often require specialized equipment. It is hypothesized that optical coherence tomography of the tear meniscus may address these problems.

Aim: The primary aim of this study was to describe the correlation between the Ocular Surface Disease Index © and tear meniscus height in dry eye disease measured by optical coherence tomography. The secondary aim was to determine a useful diagnostic cut-off value for tear meniscus height in the diagnosis of dry eye disease.

Setting: The study was conducted at St John Eye Hospital, Soweto, South Africa.

Methods: This was a prospective, cross-sectional study of adults at a tertiary level eye clinic. Participants were included if they were older than 18 and excluded if they were contact lens wearers, had an established diagnosis of dry eye disease, or were known to have (or be taking any treatment for) any ophthalmological or medical condition that has the potential to influence dry eye disease. A control and investigative group was determined using the Dry Eye Ocular surface disease index. The inferior tear meniscus height of both groups was imaged using optical coherence tomography.

Results: A total of 36 right eyes of 36 patients was included in this study. Patient ages ranged from 20 – 64 years, with a median age of 43 years. Overall there were more females (n = 27) than males (n = 9). There was a moderate negative correlation between the normal/dry eye group and tear meniscus height (r_b = - 0.452, p = 0.032). Optimizing sensitivity and specificity yielded a diagnostic cut-off tear meniscus height of 296um.

Conclusion: Tear meniscus height tends to decrease between Ocular Surface Disease Index classifications of 'normal' and 'dry eye disease'. However, tear meniscus height performed poorly as an objective measure of dry eye disease in our study population limiting recommendations to adopt it as a diagnostic test.

Keywords: Dry eye disease, Ocular Surface Disease Index , Optical Coherence Tomography, tear meniscus height

Acknowledgements

Thank you to Robyn Williams for her assistance with data analysis and critical review of this manuscript.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

OSDI	Ocular Surface Disease Index
AS-OCT	Anterior Segment Optical Coherence Tomography
TMH	Tear Meniscus Height
TMD	Tear Meniscus Depth
HIV	Human Immunodeficiency Virus
ROC	Receiver Operating Curves
AUC	Area Under the Curve

Introduction

Dry eye disease is an insufficiency in the volume and/or quality of the tear film which in turn causes tear instability and subsequent ocular surface disease [1]. The aetiology of dry eye disease is multifactorial and can be classified into aqueous deficient or evaporative states with significant contribution from environmental factors [2]. Dry eye disease is a common problem with an estimated one in four patients presenting to eye clinics with characteristic symptoms, and a global prevalence of 7 – 34% [3,4]. There is a paucity of epidemiological data for South Africa and indeed the African continent with an estimated prevalence of 41 – 92% in three small population based studies [5–7]. Furthermore, dry eye disease results in a significant economic burden with an average expenditure of USD $ 11,302 per patient and a total of USD $ 55.4 billion yearly in the United States of America alone [8].

There are a number of well-validated questionnaires that have been developed to subjectively assess dry eye disease – the Ocular Surface Disease Index (OSDI©) is one of the most frequently used and performs well with a sensitivity and specificity of 60% and 83% respectively [9,10]. The OSDI© consists of 12 questions based on the recall of symptoms in the preceding week. Severity, environmental factors and the degree to which activities of daily living are affected are all scored. In contrast there is no gold standard objective measure to quantify or monitor dry eye disease. Schirmer's test, ocular surface staining, fluorophotometry, biomarker sampling and osmolarity are invasive, suffer from inaccuracy or poor reproducibility, are time consuming or require specialised equipment that may not be readily available in the clinic setting [10,11].

Non-Invasive methods are attractive as they do not induce reflex tearing which may increase the normal rate of tear secretion by 100 – 500% [1]. This combined with recent advances in Anterior Segment Optical Coherence Tomography (AS-OCT) has renewed interest in meniscometry as means of measuring tear volume as a proxy for dry eye disease [11–14]. Experimental studies have shown that the inferior tear meniscus which is formed in the angle between the cornea and the lower lid (see Figure 1) compromises 75-90 % of total tear volume. Within this the Tear Meniscus Height (TMH) varies little in absolute value terms from tear meniscus area and performs the best diagnostically in both specificity and sensitivity in predicting disease [12,13,15,16].

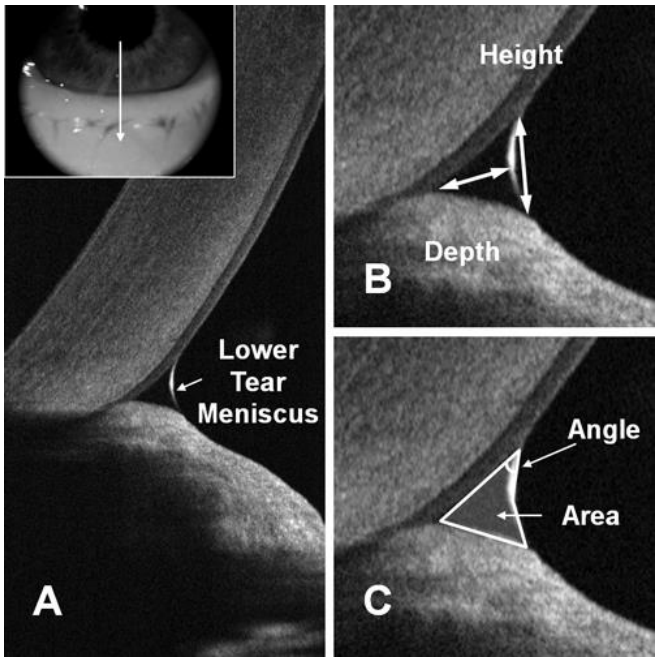


FIGURE 1: Lower lid tear meniscus shown together with infrared photo of the lower cornea and lid (frame) [A]. Tear Meniscus Height (TMH) and tear meniscus depth (TMD) [B]. Tear meniscus angle and angle alpha (α) [C] [17,18]

The primary aim of our study was to describe the correlation of TMH against the OSDI ©. Additionally we aim to determine a diagnostic cut-off of TMH for diagnosing dry eye disease.

Methodology

This was a prospective, cross-sectional, case-control study conducted at St John Eye Hospital, Soweto, Johannesburg, South Africa. Ethical clearance was approved by the Human Research Ethics Committee, University of the

Witwatersrand (M190650). The study was conducted in accordance with the declarations of Helsinki.

A sample size of 20 (17 ± 10%) for each group was determined using anticipated mean differences, a *p* value of < 0.05 was taken to be significant. Considering the strict exclusionary criteria and the anticipated difficulty in recruiting participants, especially to the control group, we decided not to power the study to perform a sub-group analysis of severity of disease, age or gender.

Patients, their family members or escorts, and staff members older than 18 years were randomly recruited to be invited to participate in the study. Participants were recruited from the general clinic and/or screening and were excluded if they were current contact lens wearers, had used topical eye drops in the preceding 14 days or had undergone any previous intraocular or extraocular surgery including laser and refractive procedures. Patients were also excluded if they were pregnant or lactating; or if they had a systemic disease or were using treatment known to be associated with dry eye disease. This includes diabetes mellitus, thyroid disease, Sjögrens syndrome, hepatitis C, vitamin A deficiency, Human Immunodeficiency Virus (HIV) infection or any hormonal dysfunction [9,17]. Patients were also excluded if they were unable to speak English, Afrikaans, Zulu or Sotho.
A direct ophthalmoscope examination of the anterior segment was performed to exclude localized ocular pathology for example blepharitis, pterygium, pinguecula, entropion and ectropion.

Demographic data including the participant's age, gender and race was collected. In addition, the total duration of dry eye symptoms was captured (if applicable). Each participant was divided into a control ('normal') or investigative ('dry eye disease') group based on the recall of symptoms of dry eye in the preceding week using the Dry Eye OSDI © iOS application (Allergan Inc., Dublin, Ireland). The investigative group was further divided into 'mild', 'moderate' and 'severe'.

Each group's TMH was measured using a SPECTRALIS ® (Heidelberg Engineering, Heidelberg, Germany) OCT with Anterior Segment capabilities. A light source 1310nm wavelength, 60nm bandwidth, scan width 15mm at eight frames per seconds, scan depth two mm in air and optical resolution less than 10um was set. Measurements were taken in a dimly lit room from 10 AM – 4 PM during the months of November 2019 to March 2020. Shen et al. showed that dry eye disease is bilateral and equally affects each eye thus the right eye was chosen by convention [13].
Each participant was asked to blink and then maintain central and steady gaze while three scans were taken approximately two seconds later. The anatomical landmark was taken to be the midpoint of the lower lid in primary gaze. Each scan was manually inspected and a quality index of greater than 20 was taken as sufficient. The TMH was measured in each scan using the caliper function at a standard magnification of 3x and averaged to mitigate random error.

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) software (IBM Corp. Released 2016. IBM SPSS Statistics for Mac, Version 24.0 Armonk, NY: IBM Corp.)

Results

Patient characteristics

A total of 36 right eyes from 36 patients was included for analysis with 14 in the control group and 22 in the dry eye disease group. Participant age ranged from 20 – 64 years, with a mean of 43 years (95 % confidence interval, 39.3 – 46.7). The age group 41-50 years accounted for 39% of all participants. Overall there were more females (*n*

= 27) than males (n = 9). All the participants were black. The mean duration of dry eye symptoms was 428 days, the median was 56 days with a range of 0 – 3640 days, standard deviation 880 days. Within the 'dry eye disease' group 55% were classified as 'mild', 13.6% as 'moderate' and 36.4% as 'severe'. Table 1 shows a breakdown of the control and investigative groups according to gender and age demographics.

Table 1: Demographic data of 'normal' (control) and 'dry eye disease' (investigative) groups

	Control (n = 14)	Dry Eye Disease (n = 22)
Gender		
Male	2	7
Female	12	15
Age		
20 – 30	4	2
31 – 40	3	7
41 – 50	4	10
51 – 60	3	2
> 61	0	1

Correlation analysis

A Pearson correlation coefficient revealed a weakly negative relationship between raw OSDI© scores and TMH (r = - 0.211, p = 0.216) as show in Figure 2. However, a bi-serial analysis performed by examining only 'normal' and 'dry eye disease' as a function of TMH showed a more strongly negative, significant relationship (r_b = - 0.452, p = 0.032). A variance analysis showed that TMH contributed only 4.45% to the overall variance observed in OSDI© scores.

There was no correlation between the duration of symptoms and TMH (r = - 0.066, p = 0.702).

An independent t test of TMH in isolation revealed that it was indeed higher in the control group (M = 659um, SD = 676um) compared to the investigative group (M = 325um, SD = 173um), however this was not found to be statistically significant (p = 0.092).

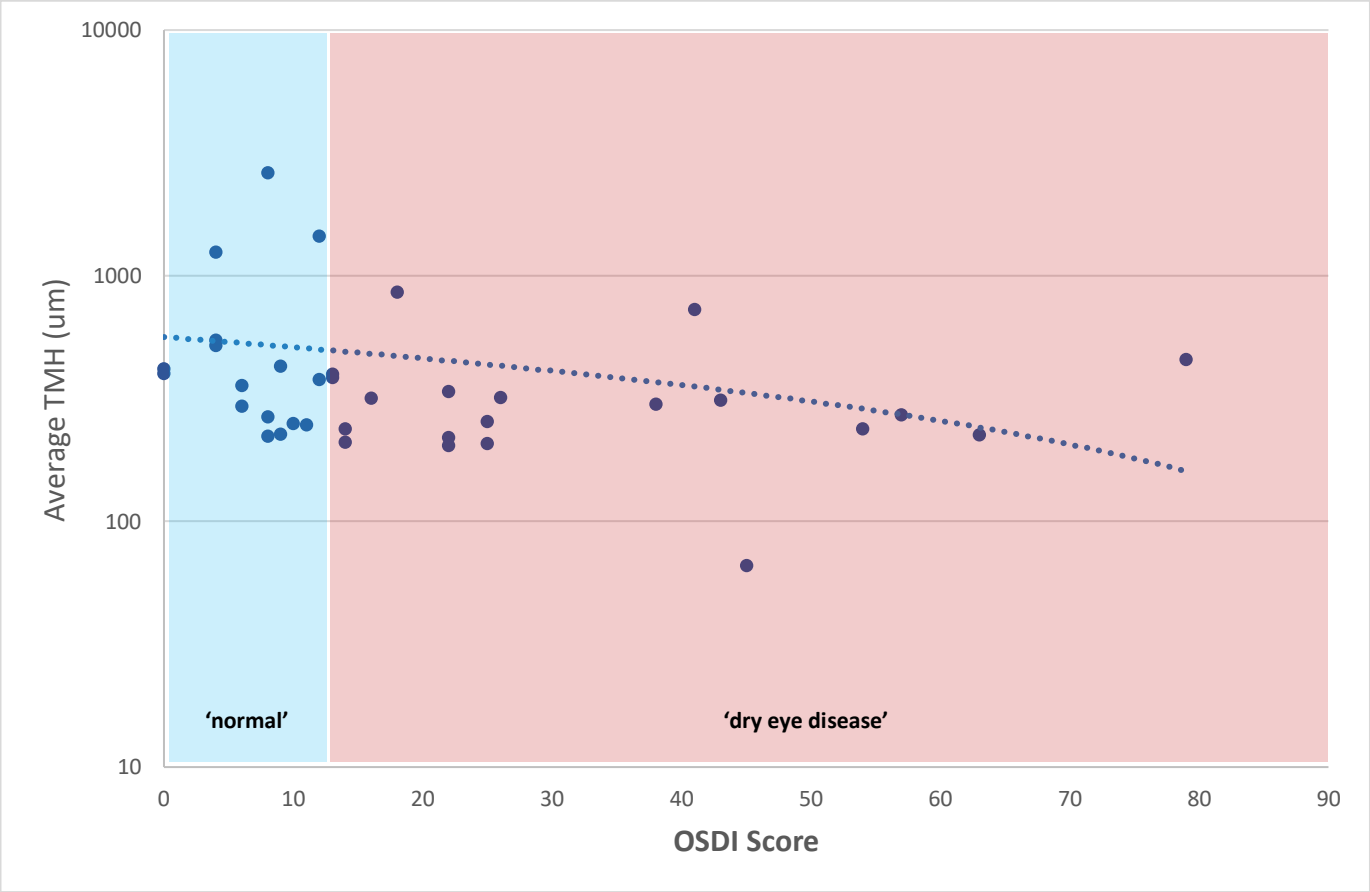


Figure 2: Correlation between Average TMH and OSDI score

Diagnostic cut-off for TMH

A diagnostic cut-off for determining 'normal' from 'dry eye disease' participants was determined by linear regression analysis of Receiver Operating Curves (ROC) as shown in Figure 3.

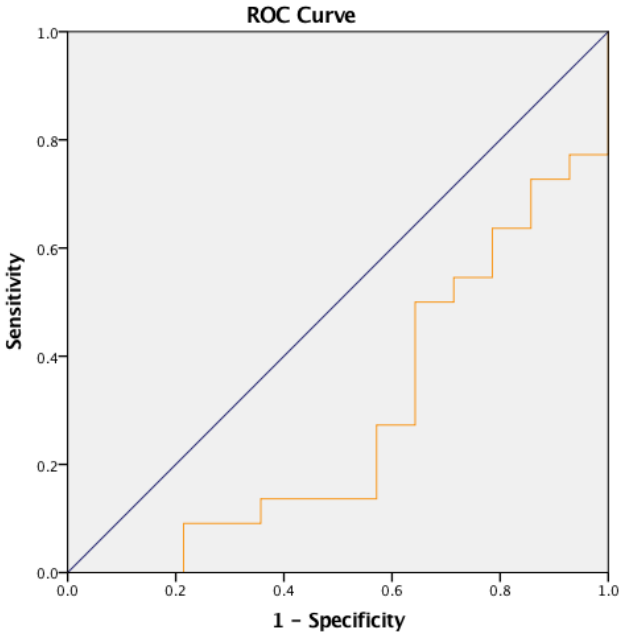


Figure 3: Receiver Operating Curve (ROC) for TMH as a diagnostic test. The area under the orange line (AUC) represents the performance of our test with a value of 0.289. Lower values of AUC indicate poor diagnostic performance. A blue reference line indicating an AUC of 0.5 is shown.

Choosing a TMH which optimises sensitivity and specificity (50% and 36% respectively) yields a TMH of 296*u*m. Maximising sensitivity at the cost of specificity (77% and 7% respectively) yields a diagnostic cut-off of 224*u*m. The Area Under the Curve (AUC) is 0.289 indicating an overall poor diagnostic performance.

Power and effect size

Our study had a Cohen effect size of 0.4 indicating a small-to-medium effect size. Retrospective calculation of a larger effect using our available data (> 0.8) would have required 36 participants in each group for a total of 72.

Discussion

Dry eye disease is a common problem and invasive methods of assessment are currently unsatisfactory, AS-OCT TMH has shown promise as a non-invasive diagnostic test that addresses these problems.

To the best of our knowledge this is the first study of its kind in South Africa attempting to compare TMH against a validated method such as the OSDI ©.

Our study found that TMH tends to decrease in those whose symptoms are sufficiently severe to have been classified as 'dry eye disease' by the OSDI©. This is consistent with the findings of other studies and may be explained by the fact that in the absence of reflex tearing the volume of tears is lower than control subjects [12,13,18]. The tear film consists of a proportionally large volume of aqueous sandwiched between an outer lipid layer and an inner mucinous layer. Regardless of whether the dry eye state is as a result of aqueous deficiency or an accelerated evaporative state the aqueous is reduced [1].

There is no consensus regarding a diagnostic cut-off value for TMH in dry eye disease. Shen et al.[13] suggested a value of 164*u*m with an associated sensitivity and specificity of 92% and 90% respectively while Tung et al.[16] calculated 210*u*m as their value. Yet another group Ibrahim et al. [15] found a higher value < 300*u*m to be statistically significant. Our study concluded that 296*u*m was the point at which one could maximise diagnostic power (sensitivity 50%, specificity 36%). However, since this test is intended primarily for screening purposes it is reasonable to maximise the sensitivity which yielded a cut-off of 224*u*m and a sensitivity and specificity of 77% and 7% respectively. While the absolute value and sensitivity of this value is similar to other author's findings our specificity and overall diagnostic power was considerably lower which limits the recommendation that this should be adopted as a diagnostic tool in our population [13,15,16]. This may reflect a limitation in our study or an undescribed intrinsic difference

in our population, the description of which exceeds the scope of this study.

Our study was limited by a small sample size as a result of stringent exclusionary criteria although it is noted that similar studies included both left and right eyes for analysis which may enhance a statistical effect.
One unmasked investigator used clinical judgement to measure the TMH which may have introduced unintended bias.
It was not possible to control for all factors which may influence the tear meniscus such as palpebral aperture height, corneal curvature and ambient conditions. The study took place mainly during the summer months which may have an influence in seasonal variation of dry eye symptoms.

Future well-designed studies should attempt to recruit a larger participant pool and sufficiently power their statistical analysis to include sub-groups such as age, gender and OSDI© severity. It may be necessary to develop automated software techniques that are able to determine tear meniscus variables with a high degree of accuracy.

Conclusion

Dry eye disease is both a commonly encountered and potentially vision-threatening disease. Despite this there is little evidence about the burden of disease in our local population or indeed the African continent. This together with the multifactorial aetiology underscores the importance of subjective and objective means of assessment. While questionnaires such as the OSDI© are well-validated, objective non-invasive methods of diagnosis are lacking. AS-OCT meniscometry is a potential solution that has shown promise in international studies. We have shown that TMH tends to decrease in dry eye sufferers compared to those without symptoms as validated by the OSDI© but this relationship has neither the same robustness nor diagnostic value as described in similar studies. This could reflect inherent limitations in our study or a broader difference in our local populace that needs to be further elucidated. As such TMH meniscometry should not be used as a diagnostic tool, or used with caution, in our local population. Further well-designed research is required to validate this tool and further describe these relationships.

What was known:

- Subjective methods of diagnosing dry eye disease such as the OSDI© are useful and well-validated.
- TMH as measured by AS-OCT shows promise as an objective tool in diagnosing dry eye disease in international studies.

What this article adds:

- Confirmation that TMH tends to decrease with increasing symptoms of dry eye disease.
- TMH performs poorly as a diagnostic test in an isolated South African population.

Acknowledgments

Thank you to Robyn Williams for her assistance with data analysis and critical review of this manuscript.

Competing Interests

The authors hereby declare that they have no financial, professional or personal relationships that may have unduly influenced them in writing this article.

Author's contributions

D.E. was responsible for the conceptualisation, study design, data collection and analysis, and final manuscript. R.H. was responsible for the supervision, conceptualisation, editing and critical analysis of the manuscript.

Funding information

This study received no financial contributions from private individuals, government, commercial or non-profit organisations.

Data availability statement

Requests for data should be addressed to D.E. (daerasmus@icloud.com).

Disclaimer

The views and opinions expressed in this article reflect those of the authors and do not represent any policy or position of the affiliated organisations of the authors.

References

1. Bowling B. Kanski's Clinical Ophthalmology: A Systematic Approach. 8th ed. Edinburgh, New York: Elsevier/Saunders; 2016. 119–130
2. Abelson M, Asbell P, Baudouin C. Report of the International Dry Eye Workshop (DEWS). Ocul Surf. 2007;5(2).
3. Osae A, Gehlsen U, Horstmann. Epidemiology of dry eye disease in Africa: The sparse information, gaps and opportunities . Ocul Surf. 2017;15:159–68.
4. Stapelton F, Garrett Q, Chan C. The Epidemiology of Dry Eye Disease. Essent Ophthalmol. 2015;2.
5. Nonkula DM, Rasengane TA, Adamjee, MF, Joubert G. The Prevalence of Dry Eye Syndrome among Patients at the Eye Clinic in Nelson Mandela Academic Hospital.Mini-dissertation Dept. of Optom. UFS, 2019.
6. Gillian W. A small-sample survey of dry eye symptoms using the Ocular Surface Disease Index. South Afr Optom. 2009;68(4):188–91.
7. Castelyn B, Majola S, Motilal R, Naidu MT, Ndebele S, Vally TA, et al. Prevalence of dry eye amongst black and Indian university students aged 18 - 30 years. Afr Vis Eye Health. 2015;74(1).
8. Yu J, Asche C, Fairchild C. The economic burden of dry eye disease in the United States: a decision tree analysis. Cornea. 2011;4:379–87.
9. Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II Diagnostic Methodology report. Ocul Surf. 2017 Jul;15(3):539–74.
10. Dougherty Wood, S. Diagnostic Tools for Dry Eye Disease. Eur Ophthalmic Rev. 2016;10(02):101.
11. Santos VA dos, Schmetterer L, Gröschl M, Garhofer G, Werkmeister RM. In vivo tear film thickness measurement and tear film dynamics visualization using spectral domain OCT and an efficient delay estimator. International Society for Optics and Photonics; 2016. p. 969-706.
12. Gumus K, Pflugfelder S. Anterior Segment Optical Coherence Tomography (AS-OCT) in the Management of Dry Eye. Int Ophthalmol Clin. 2017;57:13–22.
13. Shen M, Li J, Wang J. Upper and Lower Tear Menisci in the Diagnosis of Dry Eye. Invest Ophthalmol Vis Sci. 2009;50:2722–6.
14. Han SB, Liu Y-C, Noriega KM, Mehta JS. Applications of Anterior Segment Optical Coherence Tomography in Cornea and Ocular Surface Diseases. J Ophthalmol. 2016. p. 1-6.
15. Ibrahim OMA, Dogru M, Takano Y, Satake Y, Wakamatsu TH, Fukagawa K, et al. Application of visante optical coherence tomography tear meniscus height measurement in the diagnosis of dry eye disease. Ophthalmology. 2010 Oct;117(10):1923–9.
16. Tung C, Perin A, Gumus, K. Tear Meniscus dimensions in tear dysfunction and their correlation with clinical parameters. Am J Ophthalmol. 2008;145:795–800.
17. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo C-K, et al. TFOS DEWS II Definition and Classification Report. Ocul Surf. 2017 Jul;15(3):276–83.
18. Raj A, Dhasmana R, Nagpal CR. Anterior Segment Optical Coherence Tomography for Tear Meniscus Evaluation and its Correlation with other Tear Variables in Healthy Individuals. J Clin Diagn Res. 2016;10(5):1–4.

Appendix A: Approved Research Protocol

Correlation between Ocular Surface Disease Index and tear meniscus height in dry eye disease at a Johannesburg tertiary eye hospital

Primary Author: Dr Daniel Erasmus; MBBCh, BEngSc (BME)

Candidate: MMed (Ophthalmology)

Supervisor: Dr Roland Höllhumer; MMed, FC Ophthal (SA), MBChB

List of Abbreviations

AS-OCT	Anterior Segment Ocular Coherence Tomography
ATD	Aqueous Tear Deficiency
CHBAH	Chris Hani Baragwanath Academic Hospital
DED	Dry Eye Disease
DEWS	Dry Eye Workshop
ETD	Evaporative Tear Deficiency
OSDI	Ocular Surface Disease Index
SJEH	St John Eye Hospital
TFOS	Tear Film and Ocular Society (TFOS)
TMA	Tear Meniscus Area
TMH	Tear Meniscus Height
TMV	Tear Meniscus Volume

1. Introduction

Dry Eye Disease (DED) is defined as an insufficiency in volume and/or function of the tear film which manifests in instability and subsequent ocular surface disease [(1)]. Dry eye represents a wide spectrum of disease ranging from minor inconvenience to severe corneal scarring and irreversible loss of vision. The condition may be divided into aqueous-deficient or evaporative subtypes according to the Tear Film and Ocular Society (TFOS) and International Dry Eye Disease Workshop (DEWS). These are not mutually exclusive categorizations however and considerable overlap may occur, in addition to contribution by a variety of environmental factors [(2)].

DED is a commonly encountered problem - approximately 1 in 4 patients who present to an eye clinic complain of characteristic symptoms, and it is estimated that the global prevalence is between 7 – 34% (3,4). There is very little epidemiological data for the burden of disease in African populations, but it is believed that prevalence may be high due to secondary causes such as infections [(3)]. The scope of the problem is expanded when one considers that DED also causes a significant economic burden to health systems – it is estimated that the average cost of management is USD $ 11,302 per capita, per year and over USD $ 55 billion overall [(4)].

Thus, when one considers that DED is both common and potentially serious – it is necessary to manage patients presenting with such symptoms carefully. This takes the form of a detailed clinical history including presenting complaint, previous medical, surgical and ophthalmological history – paying particular attention to any medications and environmental factors that may be significant. However, a non-directed clinical history is by its very nature

prone to inconsistency and often the classical symptoms of DED are absent [5,6]. This has led to the development of a variety of standardised questionnaires that assist with the diagnosis and stratification of severity of DED. The Ocular Surface Disease Index (OSDI) is the most commonly used, has been validated, and offers good sensitivity and specificity of 60% and 83% respectively [6]. The OSDI consists of 12 questions (Appendix A) and is based on recall of symptoms in the preceding week, their severity, any environmental contributions and to what extent activities of daily living have been disrupted.

The OSDI is useful to stratify patients into “mild”, “moderate” or “severe dry eye disease based on their reported symptoms. However, an objective means of quantifying the problem is still necessary to decide on treatment as well as monitor the response thereof. In order to quantify the tear film changes that occur with DED it is important to note that the tear film is not a single homogenous layer but in fact consists of three layers – a outermost lipid layer secreted by the meibomian glands of the lids, a middle aqueous layer secreted primarily by the lacrimal and accessory glands, and an innermost mucinous layer secreted by the conjunctival goblet cells [1]. DED may be functionally divided into Aqueous Tear Deficiency (ATD) or Evaporative Tear Deficiency (ETD) states. Normal tear volumes are vital to maintain ocular surface physiology and are decreased in both ATD and ETD states – resulting in DED symptoms.

It is difficult to measure the total tear volume/production changes using conventional techniques as they are invasive, are prone to inaccuracy and offer poor reliability and reproducibility [7]. Traditional methods include Schirmer’s test, tear film break up time (TBUT) and ocular surface staining. Schirmer’s test measures the degree of “wetting” of a standardized piece of absorbent paper placed into the inferior fornix of the lower eyelid. By

measuring the length of filter paper that has absorbed tears an estimate of tear volume may be made. TBUT and ocular surface staining makes use of fluorescent dyes such as Rose Bengal or fluorescein to measure the time taken for instability in the tear film to develop and to stain damaged areas of epithelium, respectively. All tests suffer from inherent invasiveness (and hence reflexive tearing), poor reproducibility and low accuracy [8]. It has been experimentally shown that between 75 – 90% of the total tear volume is located in the tear meniscus at the junction between the lower lid and the cornea, with the remainder divided between the pre-ocular surface and the forniceal cul-de-sac formed by the conjunctiva [9].

The measurement of the tear film meniscus is thus an attractive, non-invasive and quantifiable means of assessing tear volume and hence DED states . Historical attempts including confocal microscopy, video and laser meniscometry have been proved either too cumbersome or impractical to use in a clinical setting and have been abandoned [8].

Recent technological advances in Anterior Segment Optical Coherence Tomography (AS-OCT) has seen renewed interest in meniscometry in the measurement of tear volume and hence ATD [7,9,10]. Four parameters of the tear meniscus of the lower lid have been earmarked for investigation – namely Tear Meniscus Height (TMH), Tear Meniscus Depth (TMD), Tear Meniscus Area (TMA) and corneo-meniscus angle (α). There is little data regarding the significance of TMD and corneo-meniscus angle (α) at this time, although TMA and TMH have both shown excellent promise in discriminating healthy and dry eyes. Indeed multiple studies have concluded a statistical significant ($P < 0.001$) decreased mean TMA and TMH values in both Sjögren and Non-Sjögren sufferers of ATD compared to control groups [7,11,12]. Furthermore sensitivity and specificity were 81% and 89% for TMH, 86% and 85% for TMA – in comparison to an abnormal Schirmer (<5mm) of 63% and 44% respectively [13]. There is

little consensus about what constitutes an acceptable cut-off value for discriminating DED from healthy eyes – one study quoted a TMH of 164um as having a sensitivity and specificity of 92% and 90% respectively [7]. Another study found selecting a cut off value of 328um yielded a sensitivity of 93% and specificity of 83% [9]. Tung and associates found 210um to be statistically significant (p = 0.0016) in a study of 128 eyes in 64 patients compared to a normal control of 345um [12].

DED is commonly encountered with potentially sight threatening complications. Clinical signs and symptoms often do not correlate well with the experience of the patient (and the reason for presentation) and hence the need for standardised and quantifiable symptoms questionnaires. The Ocular Surface Disease Index (OSDI) is well validated and frequently used for its acceptable accuracy and ease of administration - but further objective diagnostic tests are still required for deciding on the type of treatment and monitoring the response to therapies. Conventional means of evaluation are cumbersome, time consuming, prone to erroneous and reflexive responses and show poor correlation in early and asymptomatic disease. *It is therefore important to know if tear meniscus height correlates with the OSDI in dry eye disease.*

2. Study Aims and Objectives

Aim

To conduct a prospective study into whether there is a relationship between the subjective symptoms of DED measured by the OSDI questionnaire and an objective measurement of tear meniscus height using AS-OCT.

Primary objective

To Describe the correlation between OSDI score and tear meniscus height in DED.

Secondary objectives

1. Describe participant demographics of DED in a tertiary eye hospital in Johannesburg according to:
    a. Age
    b. Gender
2. Determine a diagnostic cut-off value for TMH in DED

3. Methods

The following section will detail the proposed methodology for a prospective, quantitative, cross-sectional study.

3.1 Site of Data Collection

The study will be performed at St John Eye Hospital (SJEH) - Chris Hani Baragwanath Academic Hospital (CHBAH). SJEH is a tertiary ophthalmic hospital in Soweto, Johannesburg. Hospital administration will be approached for permission to perform data collection as well as make use of the Heidelberg Engineering OCT.

3.2 Group Selection and Sampling

The study will be two-armed with the control group being formed by patient escorts who have no dry eye disease symptoms and who score as 'normal' on the OSDI. The experimental group will be patients who complain of dry eye symptoms and who score as either 'mild',

'moderate' or 'severe' dry eye disease on the OSDI. Both groups will undergo an OCT measurement of tear meniscus height on AS-OCT.

Previous investigators have noted that dry eye is a bilateral condition with no statistical difference between eyes and as such the right eye will be arbitrarily selected as the eye for examination in both the control and [9]. Where this is not possible the left eye will be used provided no exclusion criteria are present.

Participants with DED will be treated according to step-wise DEWS treatment guidelines. Initially this will take the form of lubricating drops and advice on appropriate lifestyle modification. Thereafter they will be followed up in the clinic for resolution of symptoms and or further treatment as necessary.

3.2.1 Inclusion Criteria

- For Control group:
    - Adults (≥ 18 years of age)
- For Interventional group:
    - Adults (≥ 18 years of age) and complaining of eye dryness, or characteristic symptoms such as grittiness, burning sensation or blurred vision within the preceding two weeks and causing some disruption to daily activities

3.2.2 Exclusion Criteria

- For Control and Interventional group:

- Established diagnosis of DED
- Ophthalmic medication used in preceding 14 days
- Concomitant use of systemic medications associated with DED
- Pregnant, breastfeeding or lactating
- Any previous ophthalmic surgery including laser and refractive surgery
- Established diagnosis of diabetes mellitus, thyroid disease, Sjögrens syndrome, hepatitis C, Vitamin A deficiency or Human Immunodeficiency Virus (HIV) infection, ovarian or androgen dysfunction.
- Contact lens wearers
- Localised ocular surface pathology contributing to ocular surface disease, for example pterygium, pinguecula or blepharitis.
- Does not speak English, Afrikaans, Zulu or Sotho

3.2.3 Sample Size

A sample size of 20 (17 ± 10%) for each of the control and experimental arms was calculated in consultation with a statistician. This yielded a total of 40 eyes and 40 patients. The following parameters are selected: α (two-tailed) = 0.05, β = 0.200 (yielding a power of 0.80) [3,5].

3.3 Data Collection

Patients that satisfy the inclusion and exclusion criteria will have a clinical history taken and non-invasive ophthalmological examination conducted with a slit lamp to determine if there is any local ocular pathology such as degenerative lesions or infections. They will then be invited to participate in the study and provided with an information sheet (Appendix B).

Informed consent will then be taken (Appendix C). Each participant will be assigned a numerical study identifier cross referenced to a master list that will be kept separately and password protected from raw data. Data will be captured and stored in a REDcap database that was designed for the study (Appendix D). All electronic and physical data will be destroyed 5 years from the date of publication of the study.

The OSDI questionnaire will be administered using the Allergan™ Dry Eye iPad app with the final score recorded in the REDcap database. If the patient is a non-English speaker an available nursing sister from a previously approached group of three will assist with translation in Zulu or Sotho. The nurse will be taught how to administer the OSDI questionnaire. Based on the final score the participant will be placed in either the "normal" (an OSDI score of 0 - 12) or "dry eye disease" (a score of ≥ 13) arms of the study.

3.3.1 Measurements

Participants will be tested in dimly lit consulting room between 10AM and 4 PM with the Heidelberg Engineering OCT™ instrument with a light source of 1310nm wavelength, 60nm bandwidth with anterior imaging module attached will be set up as follows: scan width 15mm at 8 frames per second, scan depth 2mm in air and optical resolution less than 10um [9,14]. The participant will be asked to maintain central and steady gaze following a blink while an anterior segment OCT is performed 2 seconds later. Using infrared light, the image is captured in less than 1 second. Immediately after acquisition the image will be assessed to confirm the quality is acceptable to analyse (greater or equal to 20 on the Heidelberg Engineering™ OCT quality index). This process will be repeated three times.

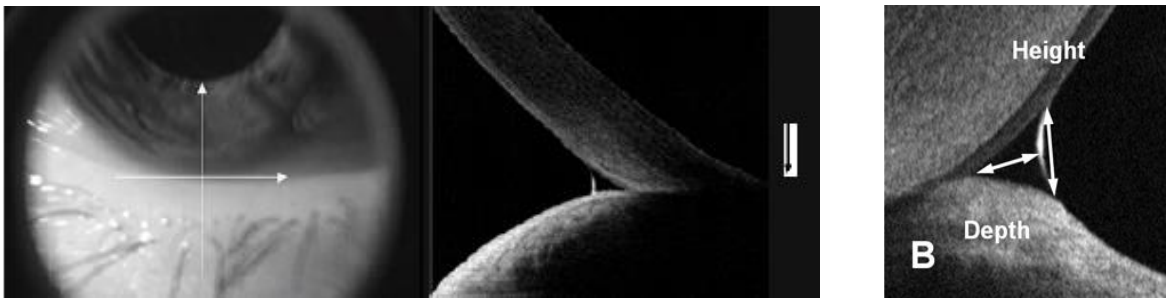


Figure 4: Infrared image of inferior limbus with tear meniscus and relevant parameters shown [10]

The participants AS-OCT will then be analysed at standardized magnification using the built-in calliper function and an average of the three readings will be taken. TMH will height will be measured as a straight line connecting the interface of the lower tear meniscus with the inferior corneal surface and the lower conjunctiva in the centre of the lid. Random error will be minimised by taking an average of the three readings. Systematic error will be avoided by setting up the parameters as described previously as well confirming the OCT has been correctly calibrated. These measurements will be entered into the database.

Participants will have standard treatment given for DED and given a follow up appointment for review in the eye clinic.

3.3.2 Potential sources of Bias

The OSDI has been developed and validated as a screening questionnaire for DED – however as with all subjective questionnaires it is subject to both respondent and researcher bias. These include acquiescence bias, social desirability bias, habituation, confirmation bias, culture bias, question-order bias and the "halo effect" [15]. This will be minimised by making use of the Allergan™ Dry Eye iPad app and administering the questionnaire in a standardised

way and using consistent technique. Where the participant is a non-English speaker a nominated translator will be used (for example a nursing sister) and instructed to administer the questions in a consistent fashion.

Since the study is not double blinded participants and their respective experimental arms will be known to the researcher and are thus subject to confirmatory bias, culture bias and the "halo effect"[15]. This will be minimised by selecting 8 participants randomly from each cohort, for a total of 16 or one in 10 of all participants and having a blinded and independent examiner review the AS-OCT findings.

3.3.3 Study Risks

This is a cross-sectional study and thus there is a small possibility that the primary objective may not show a statistically useful correlation between the OSDI and TMH as measured by AS-OCT. The risk is mitigated by the fact that multiple other studies have indeed found a statistically relevant correlation, albeit in differing degrees [7,12,14].

There is no inherent risk of mortality or morbidity to the patient, although slight discomfort may be experienced as a result of keeping the eyes open without blinking while the OCT image is taken. Wherever possible this will be mitigated.

4. Data Analysis

In consultation with a statistician all data will analysed as a mean ± standard deviation. Independent sample *t*-test will be applied to compare the TMH in control and "dry eye

disease" arms of the study provided the data is normally distributed. A cut-off value for TMH will be determined by analysing Receiver Operating Characteristics (ROC) and areas under these curves to determine optimal sensitivity and specificity.

5. Ethics

Research will be conducted in accordance with the University of Witwatersrand Steve Biko Centre for Bioethics guidelines together with the guiding principles of the Declaration of Helsinki.

Signed informed consent will be obtained from each participant which will include an explanation of why the study is being conducted, a brief description of the relevant procedures involved and a commitment that all personal data will be used for research purposes only.

There is negligible risk of morbidity with the OCT scan although in rare circumstances patients may experience mild neck and/or eye discomfort while sitting in front of the scanner and keeping the eyes open for the duration of the imaging. All efforts will be made to maximise patient comfort during the scan.

The maximum estimated time taken per patient is 20 – 25 minutes, although it is expected that this will be less once data collection is underway and optimised. Participants will be recruited directly from the clinic on the date of their schedule appointment and thus no undue monetary expenditure or time commitment is expected.

Where a translator will be used – he or she will be a single nominated nursing sister or health care provider wherever possible.

6. Timing

	2018	2019												2020							
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Apr	May	Jun
LITERATURE REVIEW																					
PROTOCOL WRITING																					
PROTOCOL ASSESSMENT																					
ETHICS APPLICATION																					
COLLECTING DATA																					
DATA ANALYSIS																					
WRITING UP – THESIS																					

Figure 5: Gantt chart for completion of dissertation

7. Funding

No additional funding will be required. Printing and stationary requirements will be kept to a minimum and be at the researchers own cost.

8. References

1. Bowling B. Kanski's Clinical Ophthalmology: A Systematic Approach. 8th ed. Edinburgh, New York: Elsevier/Saunders; 2016. 119–130 p.

2. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo C-K, et al. TFOS DEWS II Definition and Classification Report. Ocul Surf. 2017 Jul;15(3):276–83.

3. Osae A, Gehlsen U, Horstmann. Epidemiology of dry eye disease in Africa: The sparse information, gaps and oppurtunities. Ocul Surf. 2017;15:159–68.

4. Stapelton F, Garrett Q, Chan C. The Epidemiology of Dry Eye Disease. Essent Ophthalmol. 2015;2.

5. Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II Diagnostic Methodology report. Ocul Surf. 2017 Jul;15(3):539–74.

6. Dougherty Wood S, Department of Ophthalmology and Visual Sciences, Medical School, University of Michigan, Ann Arbor, Michigan, US, Mian SI, Department of Ophthalmology and Visual Sciences, Medical School, University of Michigan, Ann Arbor, Michigan, US. Diagnostic Tools for Dry Eye Disease. Eur Ophthalmic Rev. 2016;10(02):101.

7. Gumun K, Pflugfelder S. Anterior Segment Optical Coherence Tomography (AS-OCT) in the Management of Dry Eye. Int Ophthalmol Clin. 2017;57:13–22.

8. Santos VA dos, Schmetterer L, Gröschl M, Garhofer G, Werkmeister RM. In vivo tear film thickness measurement and tear film dynamics visualization using spectral domain OCT and an efficient delay estimator. In: Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XX [Internet]. International Society for Optics and Photonics; 2016 [cited 2019 Jan 23]. p. 969706. Available from: https://www.spiedigitallibrary.org/conference-proceedings-of-spie/9697/969706/In-vivo-tear-film-thickness-measurement-and-tear-film-dynamics/10.1117/12.2208338.short

9. Shen M, Li J, Wang J. Upper and Lower Tear Menisci in the Diagnosis of Dry Eye. Invest Ophthalmol Vis Sci. 2009;50:2722–6.

10. Han SB, Liu Y-C, Noriega KM, Mehta JS. Applications of Anterior Segment Optical Coherence Tomography in Cornea and Ocular Surface Diseases. J Ophthalmol [Internet]. 2016 [cited 2019 Jan 21];2016. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5046038/

11. Ibrahim OMA, Dogru M, Takano Y, Satake Y, Wakamatsu TH, Fukagawa K, et al. Application of visante optical coherence tomography tear meniscus height measurement in the diagnosis of dry eye disease. Ophthalmology. 2010 Oct;117(10):1923–9.

12. Tung C, Perin A, Gumus, K. Tear Meniscus dimensions in tear dysfunction and their correlation with clincal parameters. Am J Ophthalmol. 2008;145:795–800.

13. Czajkowski G, Kaluzny BJ, Laudencka A, Malukiewicz G, Kaluzny JJ. Tear meniscus measurement by spectral optical coherence tomography. Optom Vis Sci Off Publ Am Acad Optom. 2012 Mar;89(3):336–42.

14. Wang J, Aquavella J, Palakuru J, Chung S, Feng C. Relationships between central tear film thickness and tear menisci of the upper and lower eyelids. Invest Ophthalmol Vis Sci. 2006 Oct;47(10):4349–55.

15. Sarniak R. 9 types of research bias and how to avoid them | Articles | Quirks.com [Internet]. [cited 2019 Jan 27]. Available from: https://www.quirks.com/articles/9-types-of-research-bias-and-how-to-avoid-them

Appendix B: Ocular Surface Disease Index © (OSDI ©)

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.

Have you experienced any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . .	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9 (B)

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned? . . .	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

Add subtotals A, B, and C to obtain D
(D = sum of scores for all questions answered) (D)

Total number of questions answered
(do not include questions answered N/A) (E)

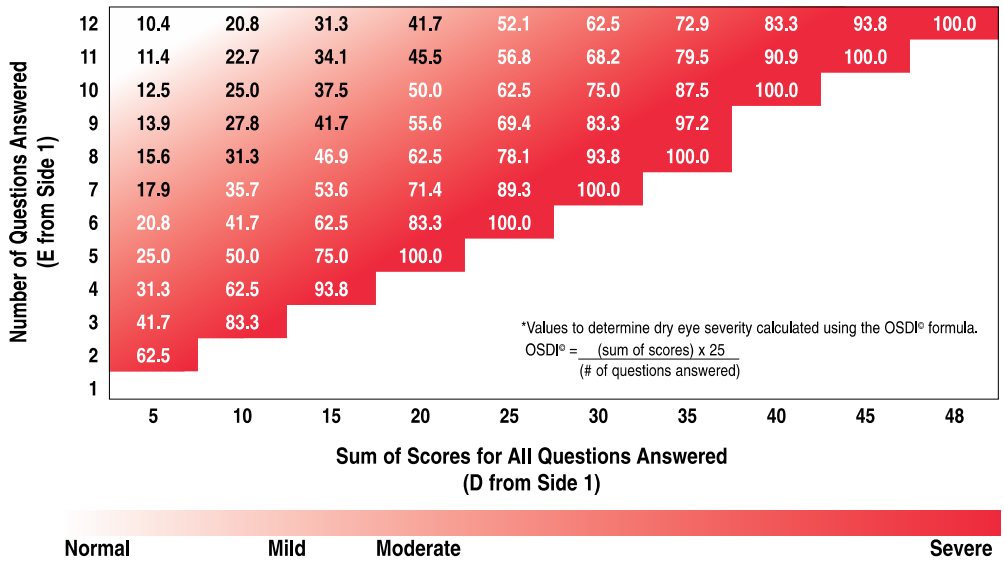
Please turn over the questionnaire to calculate the patient's final OSDI© score.

Evaluating the OSDI© Score[1]

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[1, 2]

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.



Patient's Name: _____ Date: _____

How long has the patient experienced dry eye disease? _____

Eye Care Professional's Comments: _____

1. Data on file, Allergan, Inc.
2. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615-621

Appendix C: Information Sheet

Translation of this information sheet into a language of your preference (Afrikaans, Sotho or Zulu) may be carried out by nominated nursing staff at St John Eye Hospital.

Correlation between Ocular Surface Disease Index and tear meniscus height in dry eye disease at a Johannesburg tertiary eye hospital

Good day, my name is Dr Daniel Erasmus and I am currently training to become an eye specialist doctor in the Department of Ophthalmology at the University of the Witwatersrand. Research is an important part of medicine as it allows doctors to offer better care to their patients and communities. I would like to invite you to be part of the study above. Your participation is *completely voluntary.*

In this research I would like to investigate whether there is a link between a patient experiencing symptoms of dry eyes and changes that can be measured on a machine called an OCT (Optical Coherence Tomography). This is an important question because dry eye problems are extremely common and can cause anything from minor inconvenience to severe vision loss. Furthermore the tests that we currently use to diagnose this problem have a variety of problems including taking a long time, being uncomfortable and occasionally giving inaccurate results. It is possible that the results of this study may be used to improve the way that we diagnose dry eye disease and ultimately result in sooner and better care for this condition.

If you decide to be involved in this research you will be asked a few questions about your eye and general health and undergo a brief examination of your eyes with a bright light. This is not painful at all although you might feel briefly dazzled by the light for a few seconds following the examination. I will then ask a few short questions about any symptoms of dry eyes that you may have experienced in the past two weeks. I will then perform an examination on your eyes using a machine called an OCT. This uses non visible light rays to measure the amount of tears that are present at the front of the eye. This machine is

completely safe for your eyes although you will have to remain still on the machine for a short period of time. The entire process is expected to be less than 20 minutes in total.

Your confidentiality and personal information is extremely important to me. A study number will be assigned to your data in the place of your name. I will be the only person that has access to your personal and medical information and this will be kept separately and securely. Data gathered from this study will be reviewed by myself, my supervisor, a statistician and the wider medical fraternity – at no time will this be personally linked to you. All data will be destroyed 5 years from the time it was gathered.

If you decide to withdraw at any time during the study, you may do so immediately and without any explanation. This will not impact upon any current or future medical treatment you will receive at our facility.

Please do not hesitate to contact me at any time if you require any further information.

Contact details of researcher:

Dr Daniel Erasmus

Tel: +27 (011) 933 8783

Contact details of HREC Administrative Officer

Protocol No. M190650

Mr Rhulani Mkansi

Tel: +27 717 1234/2656/2700 email: Rhulani.mkansi@wits.ac.za

Thank you for taking the time to read this information sheet.

Appendix D: Consent Form

I, _____ (name), hereby consent to voluntarily participating in this study. Dr Daniel Erasmus has fully explained the purposes of the research he will be conducting. I acknowledge that I have read and understood the information sheet I have been provided with and that I am fully aware of any potential risks that may be associated with the procedure in question.

- I am aware that I have the right to decline participating in this study, or to discontinue my participation at any time and without explanation or prejudicing my treatment for any current or future medical conditions.
- I understand that my confidentiality will be maintained at all times, and no personal identifiable data of mine will be shared. I consent to my study data being used for research and educational purposes.
- I am aware that if I have any queries or concerns they will be addressed in a timely manner.

Full name and Surname	**Date**	**Signature**
_____	_____	_____

STUDY DOCTOR

I, Dr Daniel Erasmus hereby confirm that I have fully informed the participating patient of the nature and purposes of this study as well as any potential risks that may be incurred.

Full name and Surname	**Date**	**Signature**
_____	_____	_____

WITNESS

Full name and Surname	**Date**	**Signature**
_____	_____	_____

Appendix E: Data Collection Sheet

<table>
<tr><td>Study number:</td><td>Date:</td></tr>
<tr><td colspan="2">Demographic information:

• Age:

• Gender: M / F

• Race:
o Black ☐
o Coloured ☐
o White ☐
o Indian ☐
o Other ☐</td></tr>
<tr><td colspan="2">History:

• Duration of Symptoms: (days/weeks/months)

• Symptoms (in preceding two weeks):
o Blurriness: Y / N
o “Grittiness”: Y / N
o Burning: Y / N

o Other _____

• Impact on activities daily living: Y / N</td></tr>
<tr><td>OSDI Score:</td><td>OSDI Classification:

Mild ☐
Moderate ☐
Severe ☐</td></tr>
<tr><td>OCT Findings:

TMH #1: (µm)
TMH #2: (µm)
TMH #2: (µm)</td><td>Average TMH: (µm)</td></tr>
</table>

Appendix F: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr DA Erasmus

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190650

NAME: (Principal Investigator)	Dr DA Erasmus
DEPARTMENT:	School of Clinical Medicine Department of Neurosciences Division of Ophthalmology St John Eye Hospital
PROJECT TITLE:	Correlation between Ocular Surface Disease Index and tear meniscus height in dry eye disease at a Johannesburg tertiary eye hospital
DATE CONSIDERED:	2019/06/28
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr R Höllhumer
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2019/08/26

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore reports and re-certification will be due early in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

26/08/2019
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

Appendix G: Turnitin Originality Report

Turnitin Originality Report

0703393w:Write_Up_1.4_(SAOJ_Format)_copy.docx by Daniel Erasmus

From Turnitin (b61fd313-f929-4a26-8582-2c1868ccc6dc)

- Processed on 06-May-2020 8:50 PM SAST
- ID: 1317741709
- Word Count: 2187

Similarity Index
10%
Similarity by Source

Internet Sources:
7%
Publications:
7%
Student Papers:
N/A

sources:

1 3% match (Internet from 03-May-2020)
https://journals.co.za/content/journal/aveh_v78_n1

2 1% match (publications)
Sibel Doguizi, Mehmet A Sekeroglu, Merve Inanc, Pelin Yılmazbas. "Evaluation of tear meniscus dimensions using anterior segment optical coherence tomography in video terminal display workers", Clinical and Experimental Optometry, 2019

3 1% match (Internet from 13-Apr-2020)
https://www.thieme-connect.de/products/ejournals/html/10.4103/jnrp.jnrp_38_18

4 1% match (publications)
Uchino, Yuichi, Tetsuya Kawakita, Masaki Miyazawa, Takamasa Ishii, Hiromi Onouchi, Kayo Yasuda, Yoko Ogawa, Shigeto Shimmura, Naoaki Ishii, and Kazuo Tsubota. "Oxidative Stress Induced Inflammation Initiates Functional Decline of Tear Production", PLoS ONE, 2012.

5 1% match (publications)
Jun Hyung Moon, Mee Yon Lee, Nam Ju Moon. "Association Between Video Display Terminal Use and Dry Eye Disease in School Children", Journal of Pediatric Ophthalmology & Strabismus, 2014

6 1% match (Internet from 22-Apr-2009)
http://www.icddrb.org/images/Capgan8th.pdf

7 1% match (publications)
Leilani Johnston. "Keratoconjunctivitis sicca (dry eye)", South African Family Practice, 2014

8 1% match ()
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3136145

9 < 1% match (publications)
Cho, Su-Hee, Hyun Kim, Hang Jang, and Min Park. "Modified one-day etoposide and cisplatin combination for previously untreated extensive-disease small-cell lung cancer: A retrospective evaluation of 36 cases", Molecular and Clinical Oncology, 2015.

10 < 1% match (Internet from 15-Mar-2020)
https://www.mdpi.com/1660-4601/16/16/2930/html

11 < 1% match (Internet from 10-Oct-2017)
http://files.modernmedicine.com/alfresco_images/HealthCare/2014/11/20/caa3245d-0131-4b06-85cd-92c322f7cd7c/article-722625.html

12 < 1% match (Internet from 20-Feb-2020)
https://protocolexchange.researchsquare.com/article/b0b3fa1a-f3ca-4061-b5b7-50cd107eb3e5/v1

13 < 1% match (Internet from 26-Apr-2020)
https://www.mdpi.com/2075-4418/10/4/203/scifeed_display

14 < 1% match (publications)
Mari Suzuki, Morgan L. Massingale, Fen Ye, James Godbold, Tali Elfassy, Maithreyi Vallabhajosyula, Penny A. Asbell. "Tear Osmolarity as a Biomarker for Dry Eye Disease Severity", Investigative Opthalmology & Visual Science, 2010