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# Investigation and Management of Demodex Folliculorum Blepharitis in Clinical Practice

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# Investigation and management of *Demodex folliculorum* blepharitis in clinical practice

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**Abstract**

**Introduction:** Blepharitis is a chronic inflammatory disorder of the eyelid margin. Blepharitis patients routinely present to and are managed by optometrists and ophthalmologists in practice. *Demodex folliculorum* is associated with anterior blepharitis. Presently, treatment with 50% tea tree oil is recommended by the American Academy of Ophthalmology for *Demodex* blepharitis. However, over-the-counter products have been developed and marketed at being effective for treating *Demodex* blepharitis.

**Purpose**: To examine the efficacy of over-the-counter lid hygiene products and warm compress therapy for the treatment of *Demodex* blepharitis.

**Methods**: Two hundred and forty-six participants were examined at multiple visits over four studies, for the presence and quantity of *Demodex folliculorum*. OCuSOFT® Lid Scrub® PLUS, dr.organic® tea tree face wash, Johnson's® No More Tears® baby shampoo, MGDRx EyeBag® and the OPTASE™ Moist Heat Mask were examined for treating *Demodex* blepharitis.

**Results and Conclusions:** OCuSOFT® Lid Scrub® PLUS significantly reduced the quantity of *Demodex folliculorum* when used over two and four weeks. Dr.organic® tea tree face wash significantly reduced the quantity of *Demodex folliculorum* when used over four weeks. The OPTASE™ Moist Heat Mask significantly reduced the quantity of *Demodex folliculorum* when used over eight weeks. The MGDRx EyeBag® did not demonstrate a significant reduction in the quantity of *Demodex folliculorum* over the duration of the study. Johnson's® No More Tears® baby shampoo had no effect on the quantity of *Demodex folliculorum* and demonstrated a significant increase in tear film instability when used over an eight-week treatment period.

**Declaration**

I certify that this thesis which I now submit for the examination for the award of PhD is entirely my own work and has not been taken from the work of others, save and to the extent that such work has been cited and acknowledged within the test of my work.

This thesis was prepared according to the regulations for graduate study by research of the Technological University Dublin and has not been submitted in whole or in part for another award in any other third level institution.

The work reported on in this thesis conforms to the principles and requirements of the Technological University Dublin's guidelines for ethics in research.

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Signature _____ Date: 8th November 2019

Candidate: Orla Murphy

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**Abbreviations**

| | |
|---|---|
| α | Alpha |
| AAO | American Academy of Ophthalmology |
| AIDS | Acquired immunodeficiency syndrome |
| ANOVA | Analysis of variance |
| Baby shampoo | Johnsons® No More Tears® baby shampoo |
| BPPP | Blepharitis Preferred Practice Pattern |
| CD | Cylindrical dandruff |
| DB | *Demodex brevis* |
| DED | Dry eye disease |
| DEQ-5 | 5 – item dry eye questionnaire |
| DF | *Demodex folliculorum* |
| Eyebag | MGDRx EyeBag® |
| FDA | Food and Drugs Administration |
| HIV | Human immunodeficiency virus |
| κ | Cohen's kappa |
| KW | Kruskal Wallis H |
| MGD | Meibomian gland dysfunction |
| MGO™ | Methylglyoxal |
| MWU | Mann-Whitney U |
| NITBUT | Non-invasive tear break-up time |
| OCuSOFT | OCuSOFT® Lid Scrub® PLUS |
| Optase | OPTASE™ Moist Heat Mask |
| OSDI | Ocular surface disease index |
| ROC | Receiver operator characteristic |
| $r_s$ | Spearman's correlation coefficient |
| SD | Standard deviation |
| SPSS | Statistical Package for Social Sciences |

| | |
|---|---|
| SPEED | Standard patient evaluation of eye dryness |
| TBUT | Tear break-up time |
| TTFW | dr.organic® tea tree face wash |
| TTO | Tea tree oil |
| TU Dublin | Technological University Dublin |
| UK | United Kingdom |
| WSR | Wilcoxon-signed ranks test |
| $X^2$ | Chi - square |

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# CHAPTER ONE: *DEMODEX* BLEPHARITIS

## 1.1 Background

Blepharitis is a common inflammatory disorder of the eyelid margins, that can affect patients of all ages and ethnicities.[1] It can be classified based on three factors; onset, location, and underlying aetiology.[2] Firstly blepharitis can be classified as acute or chronic. The majority of blepharitis cases tend to be chronic in nature.[2,3] Secondly, it can affect the anterior eyelid margin, eyelashes and eyelash follicles; or the posterior eyelid margin and meibomian glands. Thirdly, it can be classified as staphylococcal, seborrheic or parasitic.[1–3] Blepharitis can disrupt the tear film, leading to signs and symptoms of ocular surface disease.[1,2]

Anterior blepharitis relates to the anterior eyelid margin, eyelashes and follicles. It is typically anterior blepharitis that is classified according to underlying aetiology[2]: staphylococcal, seborrheic or parasitic. Posterior blepharitis relates to the posterior eyelid margin and meibomian glands. Meibomian gland dysfunction (MGD) is often considered a sub-type of posterior blepharitis; however, MGD can also exist separate from blepharitis. Due to their close proximity with one another, there is often overlap in signs, symptoms and aetiology between disorders of the eyelashes and meibomian glands.[4–7]

Blepharitis is one of the most common conditions presenting at ophthalmology and optometry clinics worldwide.[8–10] In a questionnaire style survey conducted by Lemp et al[10] in 2009 in the United States, 120 ophthalmologists and 84 optometrists reported blepharitis prevalence values of 37% and 47% in their respective clinics. Traditionally the majority of blepharitis cases have been treated within ophthalmology departments. However, with the development of newer ocular hygiene products, and better training and information

available, optometrists are becoming more adept and confident at treating minor eye conditions such as blepharitis. In 2008, Needle et al[11] surveyed optometrists practicing in the United Kingdom (UK) to investigate their current therapeutic practice, and ascertain their opinion on the broadening clinical role of optometrists. The results of the survey showed that optometrists were routinely managing > 70% of blepharitis and dry eye patients in-house.[11]

The clinical goal for practitioners is to identify the type of blepharitis, choose an effective treatment accordingly, and instruct and involve the patient in the long-term management of their condition.[1] The Blepharitis Preferred Practice Pattern® (BPPP) developed by the American Academy of Ophthalmology (AAO) and the College of Optometrists, recognise *Demodex folliculorum* (DF) is associated with chronic re-calcitrant blepharitis.[1,3] Ocular hygiene using a 50% tea tree oil (TTO) preparation is the recommended treatment by the BPPP and the College of Optometrists.[1,3] Briefly this involves applying the diluted TTO solution to the eyelid margin three times in 5 - 10 minute intervals.[6,12–17] The BPPP also recommend systemic ivermectin in the treatment of ocular *Demodex* infestations.[1] However, these treatments have several disadvantages (discussed in more detail in Section 1.9). Thus, the need to elucidate if less severe treatments can provide therapeutic efficacy. This thesis investigates alternative therapeutic options available to practitioners for the treatment of DF blepharitis.

### 1.2 Systematic Review

On commencing this research degree, a search of the available literature was conducted to collate information on DF. Of particular interest was research that concentrated on the prevalence of DF with respect to ophthalmology and current treatment methods. Prevalence

of DF in different cohorts was of interest as, at the time, there was limited knowledge within the optometric sector on ocular DF, and an early aim of the PhD was to investigate this further using a questionnaire. Google Scholar, PubMed, and Scopus databases were searched with the following keywords: "*Demodex folliculorum*" AND ("treatment" OR "prevalence" OR "symptoms", OR "dry eye") OR "ocular demodicosis" OR "*Demodex* blepharitis" AND ("treatment" OR "prevalence" OR "symptoms" OR "dry eye") OR ("*Demodex*" AND "meibomian gland dysfunction").

The following is a systematic review of the literature available at the start of the research project. The search strategy is presented in Figure 1.[18] Table 1 summarises the literature identified as meeting the study criterion of reporting on prevalence of ocular DF among different cohorts and relationship with signs and symptoms of dry eye. Table 2 examines treatment methods for *Demodex* blepharitis. Detailed descriptions on *Demodex*, their pathogenic potential, risk factors and associated conditions, associated ocular surface inflammation, and methods used for diagnosis and treatment are discussed in more throughout the chapter. Subsequent relevant publications have been included in the relevant sections throughout the thesis.

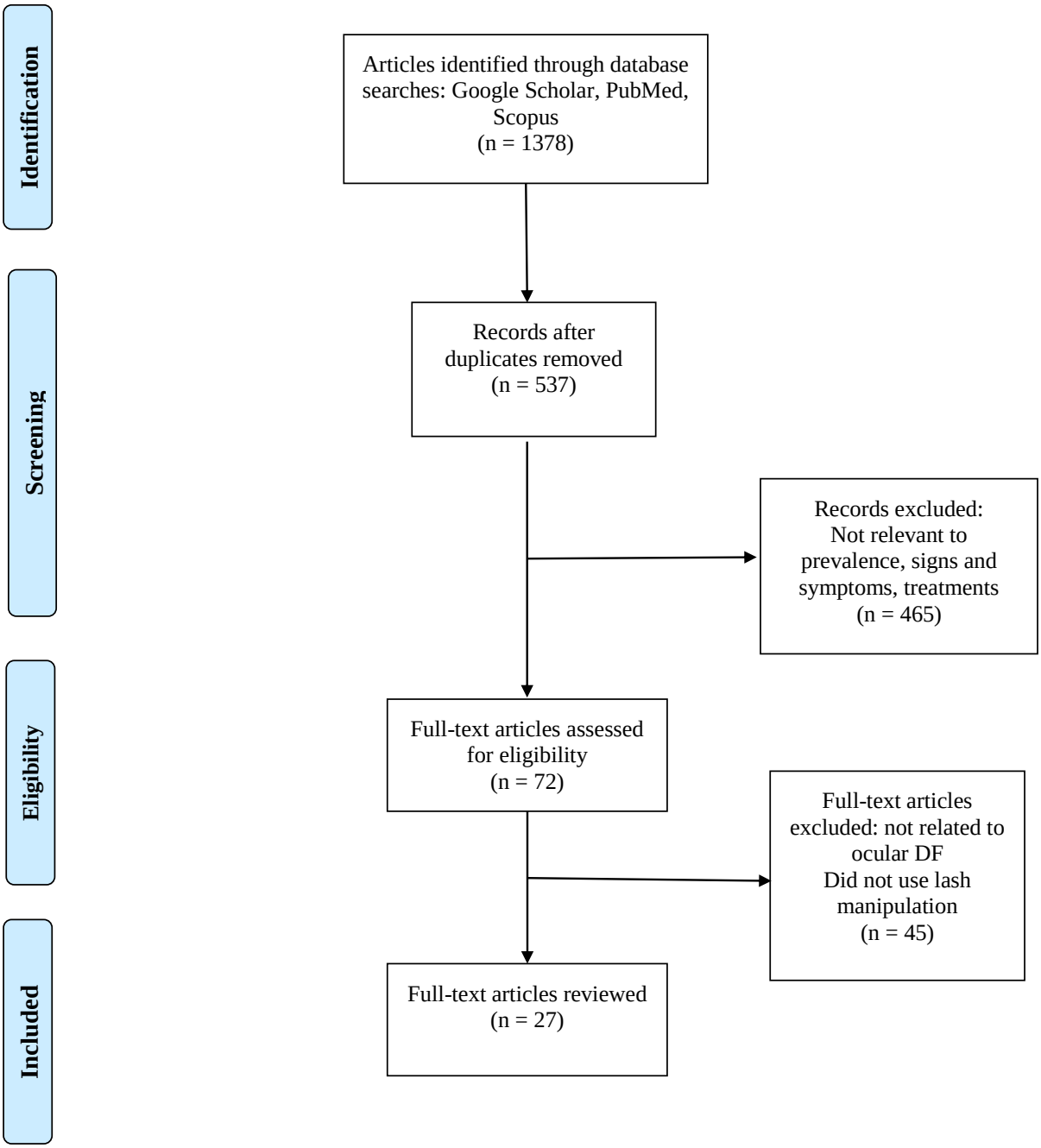


Figure 1. PRSIMA flow diagram illustrating the search strategy conducted.[18]

Table 1. Studies reporting on ocular *Demodex folliculorum* prevalence, signs and symptoms among different cohorts.

| **Study** | **Aim/Purpose** | **Population (n)** | **Clinical Examination** | **Symptom Assessment** | **Sampling Method** | **Main Results** |
|---|---|---|---|---|---|---|
| Kosik-Bogacka et al, (2013)[19] | Examination of DF and DB in healthy and immune-compromised patients | n = 1186 | Slit-lamp examination | Questionnaire – name not given | Lash epilation (2 from each upper lid) | 22.9% prevalence in controls<br>20% in immune-compromised |
| Yamashita et al, (2011)[20] | Prevalence DF in patients with proliferative diabetic retinopathy compared to healthy subjects | n = 84 | | | Modified Coston method (3 lashes from each eyelid) | 54.8% prevalence diabetic<br>38.1% healthy<br>Age and gender were not found to be significant |
| Türk M et al, (2007)[21] | Compare incidence of DF in normal versus blepharitis patients | n = 96 | | | Lash epilation | 29.72% prevalence blepharitis<br>9.09% blepharoconjunctivitis<br>4.16% healthy |
| Czepita et al (2005)[22] | Prevalence and role of Demodex in pathogenisis of chronic blepharitis | n = 435 | | | Lash epilation (4 from each eyelid) | 13% ages 3–15 years<br>34% ages 19-25 years<br>69% ages 31-50 years<br>87% ages 51-70 years<br>95% ages 71-96 years<br>58% in chronic blepharitis |
| Kemal et al, (2005)[23] | Prevalence of DF in seborrheic blepharitis patients and controls | n = 500 | | | Lash epilation (3 from each eyelid) | 28.8% in blepharitis subjects<br>26.7% in controls<br>No significant diff with age or gender |

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|---|---|---|---|---|---|---|
| Ozcelik et al, (2007)[24] | Prevalence of DF in patients with chronic kidney deficiency | n = 85 | | | Skin surface biopsy and lash epilation (8 lashes, 2 each eyelid) | 12.76% in eyelashes of patients with kidney disease<br>5.26% in controls (not significant) |
| Kim et al, (2011)[17] | Analysis of cytokine levels in lacrimal fluid to evaluate casue of ocular surface inflammation in *Demodex* blepharitis | n = 45 | Tear sampling<br>Slit lamp examination<br>TBUT<br>Schirmer II (with anaesthetic) | | Modified Coston method (8 lashes, 2 each eyelid) | Concentration of IL-17 significantly higher in DF blepharitis group than non-DF blepharitis and control groups |
| Gao et al, (2005)[25] | Prevalence of DF in eyelashes with CD | n = 55 | Routine eye examination | | Modified Coston method (8 lashes, 2 each eyelid) | 100% prevalence in CD groups<br>22% in non-CD group |
| Liang et al, (2014)[26] | Investigate correlation between *Demodex* and chalazia | n = 155 | Slit lamp examination<br>Surgical removal of chalazia | | Lash epilation (2 from each eyelid in adults + 4 from each eyelid in paediatrics) | Demodicosis significantly more prevalent in chalazia (69.2% vs 20.3%)<br>DB more prevalent than DF |
| Wesolowska et al, (2014)[27] | Prevalence of *Demodex* in eyelash follicles of different populations and its relationship with eye symptoms | n = 290 | | Specially designed questionnaire containing demographic and clinical data | Lash epilation (10 eyelashes each participant) | 54.7% prevalence in-patients<br>40.0% in health professionals<br>33.7% in medical students<br>23.5% in drug abusers<br>No difference in gender<br>Symptoms not significantly associated with *Demodex* |
| Lee et al, (2010)[28] | Relationship between the prevalence of demodex in eyelashes and the severity of ocular discomfort | n = 170 | TBUT<br>Schirmer<br>Slit-lamp | Modified OSDI | Modified Coston method (8 lashes, 2 each eyelid) | 70% prevalence.<br>No difference between sex.<br>No relationship with systemic disease. |

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| Bhandari & Reddy, (2014)[29] | Incidence and density of DF on the lashes: normal eyelids, anterior blepharitis, MGD, and mixed blepharitis | n = 200 | Standard eye examination | Irritation, itchiness, eyelid heaviness, sticky or moist sensation of the lids, mucous discharge: method not given | Lash epilation | 90% incidence in anterior blepharitis<br>60% in MGD<br>90% in mixed blepharitis<br>18% in controls |
| de Venecia & Siong, (2011)[30] | Incidence and density of DF on the lashes: normal eyelids, anterior blepharitis, MGD, and mixed blepharitis | n = 167 | | Irritation, itchiness, eyelid heaviness, sticky or moist sensation of the lids, mucous discharge, FB sensation, transient blurring of vision, redness, eye pain, tearing: method not given | Modified Coston method (8 lashes, 2 each eyelid) | 95% incidence in anterior blepharitis<br>85% in MGD<br>97% in mixed blepharitis<br>34% in controls<br>Most common symptoms: itchiness and FB sensation |
| Huang et al, (2013)[31] | Ocular demodicosis as a risk factor in pterygium recurrence | 94 | Tear sampling | | Modified Coston method (8 lashes, 2 each eyelid) | High correlation between tear<br>IL-17 levels in pterygium and demodicosis |

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|---|---|---|---|---|---|---|
| Hauswirth et al, (2014)[32] | ARVO meeting abstract: comment on symptoms associated with DF | 72 | | OSDI<br>SESoD<br>SEFoI<br>TOSS | Modified Coston method (8 lashes, 2 each eyelid) | OSDI: 36.1% classified as symptomatic for dry eye<br>SESoD: 23.6% clinically significant dryness<br>SEFoI: 20.8% clinically significant itch<br>TOSS: 27.7% clinically significant |
| Li et al, (2010)[33] | Investigate the relationship between ocular *Demodex* infestation and rosacea | 59 | Routine complete eye examination | | Modified Coston method (8 lashes, 2 each eyelid) | *Demodex* count higher in patients with positive facial rosacea.<br>Prevalence *Demodex* less in patients with aqueous deficient dry eye |

DF: *Demodex folliculorum*; DB: *Demodex brevis*; CD: cylindrical dandruff; TBUT: tear break-up time; IL: inter-leukin; MGD: meibomian gland dysfunction; FB: foreign body; OSDI: Ocular Surface Disease Index; SESoD: Subjective Evaluation of Symptom of Dryness; SEFoI: Subjective Evaluation of Frequency of Itch; TOSS: Total Ocular Surface Score

Table 2. Studies reporting on current treatment methods for ocular *Demodex folliculorum.*

| **Study** | **Aim/Purpose** | **Population (n)** | **Clinical Examination** | **Symptom Assessment** | **Sampling Method** | **Intervention** | **Main Results** |
|---|---|---|---|---|---|---|---|
| Holzchuh et al, (2011)[34] | Treatment of DF by systemic ivermectin. | n = 12 | NITBUT<br>Schirmer<br>TMH<br>corneal staining | | Lash epilation: 12 lashes (3 each eyelid) | Ivermectin | Prevalence of DF lower lid > upper lid.<br>Significant reduction in quantity DF post treatment. |
| Gao et al, (2005)[12] | Effect of TTO on ocular *Demodex.* | n = 9 | | | Modified Coston method (8 lashes, 2 each eyelid) | Weekly in-office scrubs 50% TTO.<br>Home lid scrubs tea tree shampoo | In-office scrubs with home scrubs reduce DF count to zero in 7 out of 9 patients. |
| Gao et al, (2012)[35] | Treatment of ocular itching with 5% TTO ointment. | n = 24 | Degree of itching (Graded 0 – 3 for increasing severity) | | Lash epilation | CTC<br>5% TTO ointment | No change in itching and DF counts with CTC.<br>Improvement in itching and reduced DF count with 5% TTO ointment |
| Koo et al, (2012)[13] | Relationship between ocular discomfort and DF.<br>Therapeutic effects of TTO for DF blepharitis. | n = 160 | Slit-lamp examination | OSDI | Modified Coston method (8 lashes, 2 each eyelid) | Weekly in-office scrubs 50% TTO<br>Home lid scrubs tea tree shampoo | DF in 84% patients with ocular discomfort.<br>Quantity DF associated with age and OSDI score.<br>TTO significantly reduced DF count post-treatment. |
| Salem et al, (2013)[36] | Efficacy of ivermectin and combined ivermectin-metronidazole therapy in treatment of ocular DF. | n = 120 | | | Lash epilation: 3 lashes from each lower eyelid | Metronidazole<br>Ivermectin | Combined therapy superior for decreasing DF counts to normal levels. |

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|---|---|---|---|---|---|---|---|
| Kheirkhah et al, (2007)[6] | Retrospective report on corneal manifestations associated with DF infestation. | n = 6 | Slit-lamp examination | | Modified Coston method (8 lashes, 2 each eyelid) | Weekly in-office scrubs 50% TTO<br>Home lid scrubs tea tree shampoo | Improvement in ocular surface irritation and pain.<br>Improvement in conjunctival redness.<br>DF count reduced post-treatment. |
| Liang et al, (2010)[14] | Retrospective report on DF infestation in paediatric blepharo-conjunctivitis. | n = 12 | Eye examination | | Modified Coston method (8 lashes, 2 each eyelid) | Weekly in-office lid scrubs 50% TTO<br>5% TTO ointment eyelid massages | Resolution of ocular irritation and inflammation.<br>Reduction in quantity DF |
| Fulk et al, (1996)[37] | Case series, interventional | n = 22 | | Subjects rated feelings of itch, burning, grittiness or fullness (scale 1-4) in a log | Lash epilation 6 lashes (3 from each eye) | 4% pilocarpine gel | Reduction in quantity DF with 4% pilocarpine gel |
| Filho et al, (2011)[38] | Efficacy of oral ivermectin for the treatment of chronic DF blepharitis. | n = 19 | TBUT<br>Slit-lamp examination | OSDI | Lash epilation (3 per eyelid) | oral ivermectin | Reduction in quantity DF with oral ivermectin |
| Gao et al, (2007)[15] | Retrospective review: Treating ocular demodicosis with TTO lid scrubs. | n = 11 | CD<br>MGD | Self-reported symptoms | Modified Coston method (8 lashes, 2 each eyelid) | Weekly in-office lid scrubs 50% TTO<br>Home lid scrubs with tea tree shampoo | DF associated with ocular surface inflammation – MGD/trichiasis/conjunctivitis/madarosis.<br>Reduction in DF count with TTO |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kojima et al, (2011)[16] | Use of in-vivo laser scanning confocal microscopy in the diagnosis of ocular demodicosis. | n = 23 | Slit lamp examination<br>TBUT<br>Ocular surface staining<br>Schirmer I | Visual analogue scale scores | Lash epilation (3 lashes from superior lid one eye)<br><br>Confocal laser scanning microscopy | Weekly lid scrubs 50% TTO and daily lid scrubs with tea tree shampoo | Itch and FB sensation greater in DF subjects.<br>Ocular surface staining greater in DF subjects.<br>No significant difference in mite count between methodologies.<br>DF count reduced post treatment. |
| Kim et al, (2011)[39] | Investigate clinical and immunological responses to *Demodex* on the ocular surface. | n = 10 | Slit lamp examination<br>Tear sampling | | Modified Coston method (8 lashes, 2 each eyelid) | Weekly lid scrubs with 50% TTO and daily lid scrubs with 10% TTO shampoo | Pre Tx: corneal opacities, corneal vascularization, corneal erosion and infiltration, chronic conjunctival inflammation.<br>Post Tx: *Demodex* count reduced, tear concentrations of IL-1β and IL-17 significantly reduced and clinical improvement observed in all patients. |

DF: *Demodex folliculorum*; NITBUT: non-invasive tear break-up time; TMH: tear meniscus height; TTO: tea tree oil; CTC: chlortetracycline hydrochloride; OSDI: Ocular Surface Disease Index; TBUT: tear break-up time; CD: cylindrical dandruff; MGD: meibomian gland dysfunction; IL: inter-leukin; FB: foreign body

## 1.3 Introduction to *Demodex*

*Demodex* are a group of microscopic ectoparasites that live in the pilosebaceous units of mammals. Although there are more than 100 known species of *Demodex* parasites, they are believed to be host specific. *Demodex canis*, ubiquitous to dogs, is the most well documented and investigated of the *Demodex* mites, due to its ability to cause demodectic mange in immuno-suppressed dogs. There are two known *Demodex* species that infest the pilosebaceous units of humans: DF and *Demodex brevis* (DB) (collectively referred to as *Demodex* throughout this thesis).

*Demodex folliculorum* were first described in the literature by Simon in 1842,[40] although it was not until 1963 that Akbulatova first described DB as a separate species.[41,42] In 1967, Coston first described the potential association between *Demodex* and blepharitis.[43] However, it is only in the last 15 years that significant ground has been broken in ocular associations of *Demodex* infestations: including prevalence,[23,44,45] symptoms,[35,45–47] complications,[6,46,48,49] examination,[25,49–51] and treatment.[15,52,53]

*Demodex* mites are photophobic[40] and most active at night,[54] travelling across the skin at a speed of up to 16 mm/hr.[42] They feed on skin cells and sebum, and are most commonly found in areas rich in sebaceous glands: cheeks, nose, chin and the periocular area[42,43,55]; although, they have been found in other locations on the body also.[56–59]

*Demodex* are susceptible to desiccation, and therefore cannot live for long outside of the body.[40] As a result, it is believed that direct contact is required for transference.[42,49,54] Palopoli et al[60] discovered that DNA lineages of DF were more likely to be shared within families and between spouses than between unrelated individuals; concluding that close contact was required for transmission.

## 1.4 Classification


Figure 2. *Demodex* structure: head, four pairs of legs and a long body tail (x 400 mag).

*Demodex* are translucent, spindle shaped mites, with a head, four pairs of legs and a long body tail (Figure 2).[43,57] Mating occurs at the opening of the hair follicle, and the female retreats inside the follicle or sebaceous gland to lay her eggs.[42,43] The eggs evolve to become larva and then protonypmh, inside the follicle. Finally, they move to the follicle orifice to complete maturation to deutonymph and adult.[40,42,43] The overall lifespan of *Demodex* mites is believed to be approximately 14 - 18 days.[40,42,43,61]

### 1.4.1 *Demodex folliculorum*

Adult DF is approximately 0.4 mm in length, and is larger at all developmental stages than the corresponding DB stages.[41,42] *Demodex folliculorum* reside in clusters in the

eyelash follicles,[41,43,62] and are therefore often associated with anterior blepharitis.[22,46,49,63,64] Figure 3 shows multiple DF found on one eyelash.


Figure 3. Several *Demodex folliculorum* along an epilated eyelash (x 200 mag).

### 1.4.2 *Demodex brevis*

Adult DB is smaller than DF, approximately 0.2 mm.[42] *Demodex brevis* typically resides in solidarity in the eyelash sebaceous glands and meibomian glands, and therefore has been associated with MGD.[26,41,61,62,65] Figure 4 shows a single DB found on eyelash epilation.

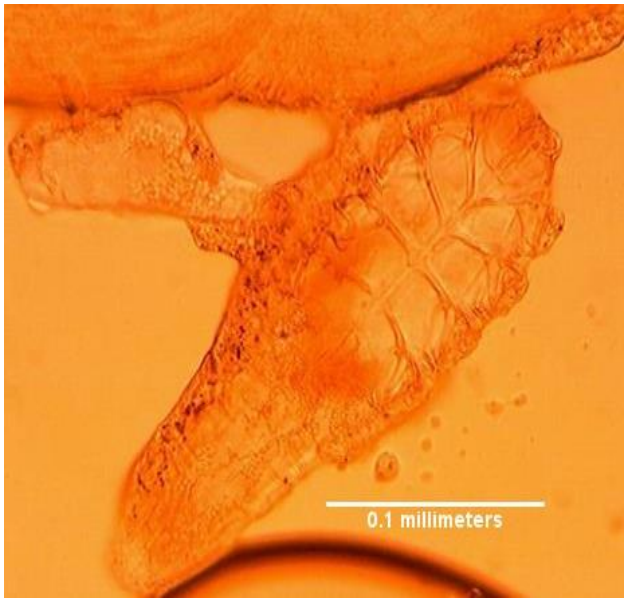



Figure 4. *Demodex brevis* (x 400 mag).

### 1.5 Pathogenicity

*Demodex* mites have a complex role to play as microflora in our cutaneous ecosystem. Several investigators believe they are simply commensal organisms[66–68]: feeding on their host, without causing damage, but without purpose. It has also been suggested that there may be a mutualistic *Demodex* – host relationship, whereby *Demodex* ingest bacteria and other micro-organisms within the follicular canal, helping the host.[66] The host's immune system then appears to regulate the quantity of *Demodex* present, preventing mite proliferation that could cause an inflammatory response. However, when *Demodex* quantities increase beyond a 'critical level', they acquire a pathogenic role, causing injury to the host.[69,70] Thus, pro-inflammatory cytokines/chemokines are released, and the immune inflammatory response follows with clinically visible cutaneous changes.[17,66,69]

Although studies have discovered the presence of *Demodex* on normal, healthy individuals[71,72]; associations have been repeatedly made between an increased presence of

*Demodex* and inflammatory conditions such as rosacea and blepharitis.[6,28,46,47,70] Thus, it has been suggested that *Demodex* mites are in fact opportunistic parasites: beginning as commensals, but have pathogenic potential in susceptible individuals.[66] It appears that the pathogenic potential of *Demodex* increases as the quantity of mites present increases.[61,66] Underlying factors that may affect *Demodex* proliferation are further discussed in detail in Section 1.6.

A third theory on the pathogenicity of *Demodex* is that they may act as vectors for bacteria. Researchers have begun to question whether it is not the presence of *Demodex* that causes problems, but whether the *Demodex* are 'ill'.[68] This hypothesis has arisen from the fact that treatment with tetracycline antibiotics resulted in clinical improvement of rosacea, even though the antibiotics had no effect on the *Demodex* mites themselves.[54] *Bacillus oleronius* is a Gram-negative bacterium that has been found on *Demodex* mites in rosacea patients.[33,73] Neutrophils are a type of white blood cell, that are released as part of our immune response to bacterial infections.[74] O' Reilly et al[75] examined the response of neutrophils to inflammatory proteins released from *Bacillus oleronius*. The authors found that neutrophils exposed to proteins from *Bacillus oleronius* increased their levels of migration, degranulation and production of inflammatory cytokines[75]: suggesting that bacteria play a role in the inflammation associated with *Demodex* infestation.

In 1993, Forton & Seys[71] examined the density of DF in skin samples of rosacea participants compared to healthy control participants. The authors discovered a mean density of 10.8 mites/$cm^2$ in rosacea participants in comparison to a mean of 0.7 mites/ $cm^2$, and < 5 mites/$cm^2$ in 98% of the healthy control skin samples; and concluded that low quantities of DF could be considered normal.[71] In 2014, Thoemmes et al[72] investigated the prevalence of *Demodex* on adults (> 18 years of age) using DNA extracted from individual

skin scrapings. Briefly, a metal laboratory spatula was gently scraped across the nose and cheek to extract sebum from the pores in the skin. After extraction, the sebum was placed in a drop of mineral oil on a cover slip and the sample was examined to note presence or absence of visible *Demodex* mites. Each sample was then transferred to a microcentrifuge for DNA extraction. The result of their study discovered the presence of *Demodex* DNA on 100% of individuals tested.[72] In the same study, the authors discovered only a 14% prevalence of *Demodex* mites based on visually observing the presence of *Demodex* within their samples.[72] These findings are in keeping with other studies that have also found low prevalence of *Demodex* among normal individuals.[23,76]

## 1.6 Risk Factors and Associated Conditions

There are several factors that may affect an individual's susceptibility to proliferation of *Demodex* to a pathogenic level. These factors, in addition to potential inhibitory factors, are outlined in detail below.

### *1.6.1 Age*

Increasing age is the most prevalent risk factor with regards to the presence of DF.[13,28,77,78] Since DF have been located on the nipple, one theory that has been proposed is that human infants acquire DF from their mothers during nursing; and as the child grows, the mites proliferate.[79] This results in a naturally higher prevalence of DF among older individuals. It has also been suggested that proliferation of DF increases with age due to a natural change in sebum composition and secretion that facilitates the growth of DF in the elderly.[27,80] Other studies have indicated a link between ocular hygiene and age: suggesting that older individuals may have a reduced ability to clean the eyelids thoroughly, thus resulting in an increased prevalence of DF.[28] This conclusion was established when Lee et

al[28] found that the prevalence of DF was higher among younger individuals with poor eyelid hygiene, in comparison to older individuals with good eyelid hygiene.

### *1.6.2 Rosacea*

The hair follicles and sebaceous glands of the skin are the main sites of involvement for DF and DB; therefore, it is not surprising that *Demodex* have been associated with several skin conditions, such as rosacea, pityrisasis folliculorum, and pustular folliculitis.[33,54,70,81,82] However, *Demodex* mites, unlike other mites such as scabies, have not been proven to cause dermatologic issues.[83] This is due to the fact that low numbers of *Demodex* mites can be found in healthy skin.[71] It is has been established that it is an increase in density of *Demodex* mites that appears to cause issues.[71]

Rosacea is a chronic, inflammatory, non-contagious skin disease that predominantly affects facial skin. It most commonly presents with various degrees of facial flushing, telangiectasia and papulo-pustular rashes.[54,84,85] The majority of cases are diagnosed after the age of 30 years, and it is consistently found to be more common in women than in men.[86–88] There are several underlying factors that are believed to play a role in the underlying pathophysiology of rosacea.[84] Genetics is considered an important factor regarding susceptibility of an individual to developing rosacea.[84] Up to one third of individuals with rosacea have a relative with rosacea.[84,89] Rosacea can affect patients of any ethnicity; however, it is more common in fair skinned individuals, with a Scandinavian/Celtic ancestory.[84,87] It has been proposed that darker skin pigmentation could conceal some of the distinguishing features, thus causing potential underdiagnosis in darker skinned individuals.[90] Exposure to UV light is considered to contribute to the development of rosacea by altering the elastic and collagen fibres of the blood vessel walls,

making them more susceptible to damage over time.[84] Heat, stress, spicy food and alcohol have all been considered secondary triggers for rosacea development in susceptible individuals.[84] However, smokers appear to have a lower risk of developing rosacea to non-smokers.[86]

The association between rosacea and *Demodex* has been well established in the literature.[69,70,81] However, the underlying aetiology remains a much debated topic. As mentioned previously in Section 1.4, one hypothesis on the aetio-pathogenicity of *Demodex* in rosacea is that *Demodex* act as a vector for bacteria and micro-organisms that cause skin inflammation, such as that seen with rosacea.[54] Another hypothesis is that *Demodex* cause perifollicular inflammation when they penetrate the dermis, stimulating the release of lymphohistiocytic infiltrates within the follicles.[71] A third hypothesis is that *Demodex* proliferate in favourable conditions: hyper-vascularised skin, lack of washing and immune status.[70] When mites proliferate, some individuals experience a type IV hypersensitivity immune reaction against the mites, causing the development of the redness and papulo-pustular rash commonly associated with rosacea.[69–71]

### *1.6.3 Immunodeficiency*

Researchers have been looking at associations between *Demodex* mites and the underlying health status of an individual. As a broad variety of patients, with a wide range of underlying health conditions, present daily to clinical practice for eye examinations and ocular health checks, it is important to understand how their systemic conditions may affect their ocular health: both internal e.g. retinopathy, and external e.g. blepharitis and dry eye.

The immune status of the individual is believed to play a major role in suppressing *Demodex* proliferation to pathogenic levels.[66] A deficient immune system cannot control

the numbers of *Demodex*, the mites proliferate, and induce an inflammatory response.[66] Several case reports in the literature have found significant DF infestation among individuals with acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus (HIV), supporting this theory.[91–94] However, larger scale studies have found no association between immune-deficiency and DF infestation.[19,27] In the study conducted by Wesolowska et al,[27] the authors inferred that the use of anti-retroviral therapy for the treatment of HIV may have improved participants immune condition to the extent where they were no longer immunocompromised enough to facilitate *Demodex* proliferation. Although DF infestation has been reported among immunocompetent children,[14,95,96] the majority of cases of paediatric demodicosis reported in the literature have been associated with leukaemia and HIV[92,97–101]; as such, practitioners should be suspicious of an underlying immune condition in children who present with severe DF infestation.

Diabetes is a group of metabolic diseases, characterised by hyperglycaemia, resulting from abnormal insulin secretion, action or both.[102] Several studies have found an increased prevalence of DF among diabetics,[20,103,104] suggesting that hyperglycemia and the immunosuppressive nature of diabetes may play a role.[104,105] A recent study examining the effect of blood glucose regulation on the presence of DF infestation in type II diabetics, showed a higher incidence of DF infestation in diabetics with poor blood glucose control; suggesting that good glucose control reduces susceptibility to DF infestation in type II diabetics.[106] In 2014, Kurt et al[107] demonstrated a significantly higher prevalence of DF infestation in participants with gestational diabetes compared to controls (pregnant participants without gestational diabetes) (24.2% versus 3.3%; $p < 0.001$). Furthermore, in agreement with earlier research,[106] participants with gestational diabetes with poor blood

glucose control were found to have a higher density of DF compared to those with good blood sugar control.[107]

Chronic kidney disease is a progressive loss of kidney function that may occur over many years. The main function of the kidneys is to maintain homeostasis; by filtration of waste products from the blood, reabsorption and transportation of nutrients from the blood, balancing electrolyte levels in the body, and controlling blood pressure. When kidney function is impaired, there is a loss to homeostasis within the body, and skin changes similar to those seen with DF infestation develop.[108] Researchers have found a positive correlation between end stage kidney disease and DF infestation.[24,108] Similar to diabetes, an underlying impairment in the immune system of such individuals, may allow proliferation of DF to pathogenic levels.[109]

Polycystic ovarian syndrome is a common, endocrine disorder affecting up to 10% of premenopausal women[110]: causing anovulation, increased androgen secretion and increased insulin resistance.[111,112] A higher prevalence of DF has been discovered in participants with polycystic ovarian syndrome.[111,113] This is likely due to the associations of polycystic ovarian syndrome with hyperglycaemia,[110] and the associations of *Demodex* infestation with uncontrolled blood sugar levels.[106]

### *1.6.4 Contact Lenses*

Jalbert and Rejab[114] found contact lens wearers were prone to higher rates of DF infestation. The reason for this is unknown, however it is postulated that increased handling of the eyelids by contact lens wearers can increase the presence of bacteria at the eyelid margin, resulting in a higher prevalence of blepharitis. Thus, making the eyelids of contact lens wearers a more desirable environment for DF to inhabit. Tarkowski et al[115] also

discovered a positive correlation between DF infestation and a significant increase in contact lens discomfort causing contact lens drop out, among previous successful comfortable contact lens wearers. Contact lens wearers were included in the preliminary epidemiological study, and the relationship found between contact lens wear and DF infestation is discussed further in Chapter 3.

### *1.6.5 Makeup*

Horváth et al[116] and Elston and Elston[79] have proposed that makeup may act as a deterrent for DF infestation; suggesting that the lipids in cosmetics may affect DF growth and therefore could be responsible for the lower presence of DF found among women. It is also possible that the use of makeup may increase good lid and facial hygiene; which has been shown to be associated with reduced numbers of DF.[28] Horváth et al[116] and Elston and Elston's[79] investigations into *Demodex* and makeup were conducted using skin surface biopsies. Currently, no data exists that examines the relationship between ocular *Demodex* and use of makeup. Chapter 3 reports on the prevalence of DF discovered amongst makeup wearers in a preliminary epidemiological study conducted during this research project.

## 1.7 Ocular Surface Inflammation

### *1.7.1 Dry Eye*

Dry eye has been defined as:

*"... a multifactorial disease of the ocular surface characterised by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage and neurosensory abnormalities play etiological roles."* [117]

Dry eye disease (DED) can be classified into two main types: aqueous deficient dry eye and evaporative dry eye. There are many underlying factors that can cause or exacerbate DED including but not limited to age, female sex, ethnicity, contact lens wear, blepharitis, refractive surgery, medications, auto-immune disease, air-conditioned dry environment, computer use, and smoking.[118–124] The prevalence of DED varies from approximately 5% - 33%,[125,126] depending on the definition of DED incorporated and study conditions; with increasing age and female sex being the predominant risk factors for disease progression.[121] *Demodex* mite infestation can be associated with DED through its close association with blepharitis and MGD.

### *1.7.2 Blepharitis*

Blepharitis has been previously defined in Section 1.1. As mentioned previously, blepharitis has been classified in different ways; however at present, the general accepted classification is by location of inflammation on the eyelids: anterior and posterior.[2,9] *Demodex folliculorum* infestation is associated with anterior blepharitis, due to its residence and effect within the eyelash follicles.[7,43,46,49,78,127] Researchers have also linked DB with posterior blepharitis,[41,61,62,65] this is discussed in more detail in Section 1.6.3 below.

In susceptible individuals, the existence of *Demodex* causes direct damage to the anterior ocular structures.[43,62] *Demodex folliculorum* use their claws to scrape at the internal walls of the lash follicles. This causes the follicles to widen, the eyelashes within to become looser, and increased hyperkeratinisation of the epithelial cells: which becomes visible as a gelatinous collar at the base of the eyelash.[62] This is clinically known as cylindrical dandruff (CD) and is now considered a pathognomonic sign for presence of DF (refer Figure 5).[25] Cylindrical dandruff is believed to be caused by the abrasive movement of the mites within

the eyelash follicles,[62] or as an inflammatory cicatrix formed from dead mites within the follicle.[128] As there is a greater quantity of eyelashes on the upper eyelids than one th lower eyelids, it may be easier to detect, and there may be a greater amount of CD present, on the upper eyelids.

Figure 5. Cylindrical dandruff:  collar at the base of the eyelashes (x24 mag).

The presence of CD at the base of the eyelash follicles is one of the methods utilised to differentially diagnose between the subtypes of blepharitis. *Demodex* blepharitis has CD: gelatinous collars at the base of the eyelashes (as shown in Figure 5).[25] By comparison, staphylococcal collarettes tend to be crusty sleeves, often stuck together, that can leave a bleeding ulcer when removed, and may be present anywhere along the length of the eyelash.[129] Seborrheic collarettes are usually greasy and soft, they don't leave a bleeding ulcer when removed, and are associated with seborrheic dermatitis.[9,129]

Despite the relatively high prevalence of blepharitis in ophthalmic clinics, the exact aetiology remains unknown, and there is still no 'cure' for chronic blepharitis.[2]

### *1.7.3 Meibomian Gland Dysfunction*

Meibomian gland dysfunction is a chronic, diffuse abnormality of the meibomian glands, that is associated with posterior blepharitis and is the most common cause of evaporative dry eye.[130]

The meibomian glands provide the main source of lipids for the human tear film. Their functions include: preventing evaporation of the aqueous layer, stabilising the tear film and providing a smooth optical surface for light refraction and improved visual acuity.[131–136] Disruption to this lipid layer causes tear film instability, hyperosmolarity and subsequent ocular surface inflammation, which further increases the instability of the tears, causing DED. Although DF typically inhabits the eyelash follicles, a high prevalence of DF has been found in patients with MGD and mixed blepharitis (anterior blepharitis and MGD).[30,127,137] As DB inhabits the sebaceous glands, often in solitude, it is suggested that DB contributes to MGD by causing granulomatous changes to the glandular cells, and physically blocking the gland orifice and preventing the flow of meibum to the ocular surface.[41,61,62,65]

At present, the most common treatment for MGD involves using compression therapies to unblock the glands.[138–141] It is postulated that frequent and regular heating of the abnormal meibum clears any obstructions allowing a smooth passage of meibum to the ocular surface. This increased availability of meibum thickens the lipid layer of the tear film, reducing evaporation of the aqueous layer, thus increasing the stability of the tears, restoring normal osmolarity and normal tear function.[138,139,141]

Although it is DB that tends to reside in the meibomian glands, a high prevalence of DF has been found in the eyelashes of MGD sufferers.[30] Chapter 7 discusses the efficacy of warm compresses in the treatment of MGD and DF blepharitis.

### *1.7.4 Symptoms*

The symptoms of DF infestation, blepharitis and DED are very similar, as they all involve the ocular adnexa and manifest on the ocular surface: dryness, itch, irritation, burning sensation and foreign body sensation have all been recorded in the literature.[6,13,15,16,45–47,49] *Demodex* can promote an inflammatory reaction on the ocular surface.[17] Kim et al[17] demonstrated that the presence of DF caused an increase in the tear protein IL-17, which is associated with lid margin inflammation.

Previous research has shown that the type and severity of symptoms can vary depending on the condition and time of day.[142] Blepharitis and MGD are often associated with a foreign body sensation and sticky eyes in the morning; while aqueous deficient dry eye tends to worsen as the day goes on.[142] Several studies have found itch to be the symptom most significantly associated with *Demodex* infestation.[16,46,47] The movement of DF within the follicle may indirectly be accountable for the signs and symptoms exhibited by many affected patients. As DF are photophobic and only active at night, one might expect patients to be most symptomatic at night or in the morning after the mites have been most active. However, at present there is no data available regarding the diurnal variation of symptoms with respect to DF infestation.

### *1.7.5 Ocular Morbidity*

Ocular surface inflammation as a result of DF infestation and subsequent DED not only causes physical symptoms but can cause functional symptoms also. Ocular morbidity associated with DED and inflammation of the ocular surface has been recognised as a public health concern.[121] As previously discussed, DF infestation can cause ocular surface inflammation which can manifest as DED and blepharitis in many patients. Subsequently, this can cause physical and functional symptoms, ranging from mild to severe, requiring differing levels of treatment and management. Chronic inflammation of the eyelids and eyelashes from DF infestation can cause loss of lashes, ocular discomfort, corneal neovascularisation, infiltration, opacities and scars.[6,143] This can impact an individuals' quality of life in several ways: physically, socially, emotionally, professionally, and financially.[121,142] Chronic inflammation from underlying DF and DED can cause physical discomfort, reduced vision and increased discomfort in contact lenses.[115] It can interfere with leisure activities and social interactions causing stress, anxiety and depression in severe cases.[144] It can also cause a reduction in productivity and time out of work and it can require many visits with a clinician, with ongoing cost of treatment resulting in increased medical bills. Early intervention and patient education could go a long way towards preserving good ocular health, comfort and vision, and preventing chronic disease that can cause ocular morbidity.

## 1.8 Diagnostic Methodologies

Diagnosis of *Demodex* infestation will often depend on the discipline. Dermatologists use skin surface biopsy techniques to assess density of DF in the skin. Whereas ophthalmologists and optometrists are concerned with DF and DB infestation of the eyelids

and eyelashes. Confirming the presence of DF, and thus diagnosis of *Demodex* blepharitis, is most commonly achieved by eyelash epilation and microscopic examination the eyelash with a light microscope,[25] or smartphone.[145] In recent years, laser confocal microscopy has also been utilised to view DF *in vivo.*[16,65,146]

Investigation of the eyelashes by epilation involves gently rotating an eyelash using sterile forceps and epilating the lash in order to count the number of DF mites present. There is no standard technique for epilating the eyelashes. However, the two most utilised methods discussed in the literature for epilating the eyelashes and counting the DF are the conventional Coston method and the modified Coston method.

### *1.8.1 Conventional Coston Method*

The conventional Coston method was described by Coston[43] in 1967 and involves the random epilation of non-adjacent eyelashes on the eyelid. The epilated eyelash is placed on a microscope slide, one drop of peanut oil is placed on the eyelash and the coverslip is placed on top. However, there are several limitations to the conventional Coston method. Firstly, randomly selecting any eyelash could result in under-counting, as there is a much better chance of detecting DF if lashes with CD are present and are selectively chosen.[25] Secondly, by adding the peanut oil before the coverslip, non-adherent DF may float away, resulting in under-counting.[25,29] Thirdly, if DF are embedded in compact CD they cannot be counted accurately.[25,29] Finally, very often not all DF get removed with the eyelash leaving some DF behind in the follicle, resulting in under-counting.[25,29] These limitations led to investigators utilising the modified Coston method in more recent studies.

### *1.8.2 Modified Coston Method*

The modified Coston method was developed by Gao et al[25] in an attempt to overcome some of the limitations outlined above. The modified Coston method firstly involves selectively choosing lashes with CD, if present, then placing the coverslip on top of the epilated eyelashes, and finally pipetting a drop (20µl) of saline at the edge of the microscope slide on lashes without CD, and alcohol and fluorescein on lashes with retained CD. By selectively choosing lashes with CD there is a greater chance of finding DF. Placing the coverslip on top of the microscope before the saline/alcohol prevents loose DF from floating away. The alcohol dissolves compact CD allowing embedded DF to become visible and easier to count. Fluorescein increases the proficiency of counting DF mites embedded in CD.[51]

The modified Coston method was utilised for counting DF after eyelash epilation in all studies discussed in this thesis. Eyelashes were prepared and examined immediately after removal.

### *1.8.3 Confocal Laser Scanning Microscopy*

Confocal laser scanning microscopy is a modern technique that has been used in recent years to detect DF *in-vivo.*[16,65,146] It is a non-invasive technique in which a probe is positioned against the area of interest and the underlying tissue structure, at different depths, can be pictured with histological resolution.[146] Confocal laser microscopy has the advantage of being less invasive than eyelash epilation, and may be more sensitive to detecting presence and quantity of *Demodex.*[147] Although, several studies comparing laser confocal microscopy to the modified Coston method have found no significant difference in prevalence or quantity of DF detected between either method.[16,65]

### *1.8.4 Eyelash Manipulation*

Previous investigators have described the use of eyelash manipulation as an adjunct procedure prior to eyelash epilation in an attempt to stimulate DF, if present, to move towards the opening of the eyelash follicle.[12,61] In 2013, Mastrota[50] indicated that it was possible to view, on the slit-lamp biomicroscope, DF tails emerging from the eyelash follicles as the eyelash was rotated *in-situ*. This involved the rotation of the eyelash in clockwise and counter-clockwise directions using sterile forceps. If DF were present, this stimulated the mites to emerge from the eyelash follicles and could be seen on slit lamp magnification and counted. Anecdotally, higher magnifications on slit-lamps (x 40 mag) have been accepted as the required magnification to identify *Demodex* within the follicle.[50] However, in the current study it is possible to identify *Demodex* tails at lower magnifications also (circa x 16-24 mag). It has been noted, that eyelash epilation alone often results in miscounting, as many DF remain within the follicle after the eyelash has been removed.[25,29,50]

Chapter 8 will discuss the clinical use of eyelash manipulation in the examination of *Demodex* blepharitis, showing that complete epilation of the eyelash is not always necessary in a clinical setting; and that eyelash manipulation may be a better indicator for severity of infestation than eyelash epilation.

## 1.9 Current Treatment Methods

### *1.9.1 Tea Tree Oil*

Tea tree oil is an essential oil that comes from the tea tree, *Melaleuca alternifolia*. It has been used historically among the Aborigines for its medicinal benefits. In more recent

years, investigators have attempted to examine the efficacy of TTO as an antibacterial, antimicrobial and anti-inflammatory agent, with promising results.[53,148,149] Terpinen-4-ol has been found to be the active ingredient in TTO effective at killing DF.[53] Studies have shown 50% TTO to be effective in reducing quantity of DF.[6,13,15] This is an important discovery in the treatment of chronic, recalcitrant blepharitis. At present 50% TTO applied in-clinic by an experienced practitioner is the recommended treatment for *Demodex* blepharitis by the AAO and the College of Optometrists.[1,3] However, the use of TTO at the eyelid margin is not without its disadvantages. It is toxic to the ocular surface, [3,150] can be irritating and uncomfortable for the patient,[13,15] and needs to be applied weekly in-house by an expert clinician resulting in increased chair-time and cost to the patient.

### *1.9.2 Ivermectin*

Ivermectin is a very effective anti-parasitic drug, and recent studies investigating the efficacy of ivermectin in treating DF infestation are showing promising results.[34,36,38] Single-dose oral ivermectin or combined therapy may be recommended treatment options for chronic, recalcitrant blepharitis or patients with poor compliance.[34,36,38] However, ivermectin is a broad-spectrum anti-parasitic drug primarily prescribed to treat human threadworm, and control river-blindness.[151] The use of ivermectin for the treatment of parasitic infections has been associated with several adverse reactions with varying degrees of severity: diarrhoea, dizziness, nausea, abdominal pain, hypotension, hepatitis, headache, paraesthesia, allergic reactions, ocular pain, skin swelling, tachycardia, breathing difficulties, fever, joint pain.[152–157] The safety of ivermectin for use by pregnant and nursing mothers, and young children has not been well established and its use is therefore contra-indicated by the Food and Drugs Administration (FDA).[151] The FDA have also advised caution in the treatment of elderly individuals with ivermectin; as it is not well established

whether older individuals respond differently to younger individuals, and in general there is an increased frequency of hepatic, renal, cardiac or concomitant disease and other drug therapy in elderly patients.[151] It does appear that the severity of adverse reactions is directly associated with the severity of parasitic infection, suggesting adverse reactions are due to the effect of dying parasites in the skin and not as a result of drug toxicity.[153] The majority of these adverse reactions have been associated with severe parasitic infections such as river blindness and Lao filariasis in developing countries and may not apply to DF infestations. The off-label use of ivermectin in the treatment of *Demodex* blepharitis has been successfully examined in clinical studies.[34,36,38] However, there is a need to be cautionary when prescribing the drug, and potentially only consider when other treatment options have been unsuccessful.

### *1.9.3 Metronidazole*

Metronidazole is a commonly used anti-protozoal agent, prescribed for the treatment of bacterial vaginosis in non-pregnant women. However, several studies have investigated its efficacy at treating DF infestation.[158–160] As with ivermectin it has some side effects which can be severe; nausea, vomiting, anorexia, dizziness, dry mouth, metallic taste, insomnia, vertigo have all been reported in the literature.[161] Metronidazole is contraindicated in patients with a previous hyper-sensitivity reaction to other nitroimidazole derivatives; in patients who have taken disulfiram concurrently; and alcohol use.[162] Its use is also cautioned in patients with kidney and liver disease, blood disorders, pregnant and nursing mothers, and paediatric and geriatric patients.[162] Metronidazole has been found to be carcinogenic in mice and rats; the FDA recommend avoiding un-necessary use of the drug.[162]

Recent studies have shown that ornidazole, an anti-amoebic agent from the same family as metronidazole, is a safer and more effective treatment option, with fewer side effects than metronidazole.[160] Nausea, headache and dizziness have been reported in the literature.[161] Also initial treatment with ornidazole and metronidazole causes an initial aggravation of facial inflammation due to foreign body reaction in the skin to dead mites; therefore anti-inflammatory therapy is also required with these treatments.[160] However, ornidazole does not feature on the FDA register, the European Medicines Register, or the Irish Health Products Regulatory Authority register at present.

### *1.9.4 Honey*

Honey has historically been used for dressing wounds due to its natural antimicrobial properties.[163] However, it was dismissed in the 1970's as harmless but ineffective,[164] and has since begun to make its comeback.[165] This is in part due to the recent growth of antibiotic resistant bacteria, promoting the need to look at 'alternative' antimicrobial options.[165] Kanuka honey has shown to be effective as a topical treatment for rosacea.[166] In 2018, researchers in New Zealand found that methylglyoxal (MGO$^{TM}$) Manuka honey is as effective at killing DF *in vivo* as 50% TTO.[167] A micro-emulsion prepared for ocular use has also shown good antimicrobial potency, with no immediate adverse reactions noted when applied to rabbit eyes; thus leading the way for future studies to look at the efficacy of MGO$^{TM}$ Manuka honey for the treatment of blepharitis in human studies.[168]

## 1.10 Conclusion

As mentioned earlier, optometrists are increasingly managing blepharitis, including *Demodex* blepharitis, in practice, often without the need for further referral.[11] As *Demodex* blepharitis gains increased recognition, practitioners are investigating for and treating it

more frequently. As mentioned in Section 1.1 and Section 1.9, the current guidelines available for practitioners recommend 50% TTO and/or ivermectin for treating *Demodex* blepharitis.[1,3] However, as 50% TTO is toxic to the ocular surface and should be used with caution, and ivermectin would require a prescription and is not currently available for medical practitioners to administer in Ireland or the UK; the aim of this post-graduate research project was to examine if any other lid hygiene products, that could be comfortably recommended by practitioners for patients to use at home, were effective at treating *Demodex* blepharitis.

On commencing this research project the vast majority of previous *Demodex* research, regarding associated risk factors and underlying health conditions, was focussed on *Demodex* within skin samples. There was limited literature available on ocular *Demodex* infestation and its associated risk factors. The main aim of the research project was to examine efficacy of treatments for *Demodex* blepharitis. However, as presence of ocular *Demodex* was being examined in participants, the opportunity was taken to look for commonalities within the population that may pre-dilect or prevent *Demodex* infestation.

Research methodologies used throughout the course of this research project are discussed in Chapter 2. The products included in each study and reasoning for each is also discussed in Chapter 2. The results on the safety and efficacy of lid hygiene products and warm compresses are discussed in Chapter 5, Chapter 6 and Chapter 7.

## CHAPTER TWO: RESEARCH METHODOLOGY

The research consisted of four recruitment phases; encompassing four prospective randomised interventional studies (discussed in Chapters 5, 6 and 7) and two observational studies (discussed in Chapters 3, 4 and 8). Inclusion and exclusion criteria varied for each phase of recruitment. Detailed inclusion and exclusion criteria are discussed for each phase in relevant chapters throughout. All participants involved in the research project were recruited through the National Optometry Centre's student and private optometry clinics in the Technological University Dublin (TU Dublin). Informed consent was obtained from all participants prior to enrolment, to permit the use of their data pseudo-anonymously (participants were assigned a number-letter code) for research purposes (refer Appendix 1). All examinations and data analysis were conducted by the PhD Candidate (Murphy, O). New participants were recruited for each stage of the research project. All participants were Caucasian. Sample size calculations were carried out for each study and are described in detail in their relevant sections. A brief outline of the four recruitment phases of this research project is outlined in Table 3. below. The recruitment phases do not follow chronological order, as the recruitment phase four was a follow-on study from recruitment phase two. As such, results from recruitment phase four are discussed in Chapter 6.

Table 3. The four recruitment phases of the PhD research project. OCuSOFT® Lid Scrub® PLUS (OCuSOFT), Johnson's® No More Tears® baby shampoo (baby shampoo), dr.organic® tea tree face wash (TTFW), MGDRx EyeBag® (Eyebag), OPTASE™ Moist Heat Mask (Optase).

| **Chapter** | **Aim** | **Phase** | **Treatment** | **Duration** | **No. of Participants** |
|---|---|---|---|---|---|
| Chapter 5 | Compare efficacy of treatments | Phase One (Pilot Study) | OCuSOFT vs baby shampoo | Two weeks | 41 |
| Chapter 5 | Compare efficacy of treatments | Phase Two | OCuSOFT vs TTFW vs BlephEx™ | Four weeks | 86 |
| Chapter 6 | Evaluate the effect of treatments on the tear film and ocular surface | Phase Four | OCuSOFT vs TTFW vs baby shampoo | Eight weeks | 48 |
| Chapter 7 | Compare efficacy of treatments | Phase Three | Eyebag vs Optase vs warm face cloth | Eight weeks | 42 |

### 2.1 Ethics Statement

All studies described in this report were conducted under the Tenets of Helsinki Declaration of Human Studies[169] after approval by the TU Dublin, formerly known as Dublin Institute of Technology, Research Ethics Committee (refer Appendix 2).

### 2.2 **Statistical Analysis**

All data was examined for normality using Shapiro Wilk statistical test. Parametric data is expressed as mean ± standard deviation (SD), non-parametric data is expressed as median and inter-quartile range (IQR) where relevant throughout the report. For all statistical tests $p \leq 0.05$ was considered statistically significant unless otherwise stated. A

brief explanation of each of the statistical tests applied throughout the thesis are outlined below.

One-way analysis of variance (ANOVA) was used on parametric data to examine for differences between the means of three or more independent groups, where the dependent variable was continuous or ordinal in nature.

Repeated measures ANOVA was used for parametric data to compare means of repeated measurements. It was used to detect change after treatment at follow-up visits.

Kruskal-Wallis (KW) statistical test was used as the non-parametric equivalent of a one-way ANOVA. It was used to examine for statistically significant difference between two or more groups of an independent variable with a continuous or ordinal dependent variable.

Friedman's was used as the non-parametric equivalent to the repeated measures ANOVA: used to detect change after treatment at follow-up visits.

Wilcoxon Signed Ranks (WSR) test was used as the non-parametric equivalent of the paired t-test; to compare two related, matched or repeated measurements to examine for differences in their population mean ranks.

Mann Whitney – U (MWU) test was used as the non-parametric equivalent of the independent t-test. It was used to compare two independent groups where the dependent variable was either continuous or ordinal.

Spearman's correlation ($r_s$) was used to assess the strength and direction of a relationship between two continuous variables.

Chi-square ($X^2$) analysis was used to examine the relationship between categorical or nominal variables.

## 2.3 Examination

The following sub-sections consist of a list of examinations that were carried out in order of least invasive to most invasive, in order to best preserve the integrity of each test, at each appointment.[170,171] As the research progressed, several procedures were included or removed as required; this is highlighted where relevant throughout.

### *2.3.1 Questionnaire*

The development and validation of the general health and lifestyle (GHL) questionnaire (refer Appendix 3) is discussed in Chapter 3. The development and validation of the modified ocular surface disease index (OSDI) symptom questionnaire (refer Appendix 4) is discussed in Chapter 4. The GHL questionnaire was completed by participants who took part in the pilot study and four-week treatment study: discussed in Chapter 5. The modified OSDI symptom questionnaire was completed by every participant, at every visit, throughout all of the studies.

### *2.3.2 Habitual Visual Acuity*

Habitual visual acuity was measured as a means of monitoring the safety of treatments; ensuring the treatments did not have a negative effect on vision. Each participant's habitual VA was measured using a Thompson logMar chart (Test Chart 2000, Thompson Software Solutions, London, UK). Habitual VA was defined as a participant's general everyday distance vision: recorded as aided or unaided as appropriate. Best acuity was recorded using

letter by letter scoring with each letter corresponding to 0.02 logMar units.[172,173] Due to time restrictions and number of appointments, refraction was not measured.

### *2.3.3 Non-Invasive Tear Break-Up Time*

Non-invasive tear break-up time (NITBUT) is a method frequently utilised to assess the quality of the tear film.[171] This was measured in seconds using the tear film analysis function on the Medmont E300 corneal topographer (Medmont International Pty Ltd., Victoria, Australia). The Medmont E300 has been shown to have a sensitivity of 81.5% and specificity of 94.4% for diagnosing DED, with good repeatability (coefficient of variation 9.4%, 95% CI 7.1% - 14.0%).[174] Looking straight ahead, participants were requested to focus on the central fixation target, blink twice gently and then to hold open their eyelids for as long as possible. An average of three readings were recorded for each eye, beginning with the right eye and alternating between them.[174] Due to availability of equipment, NITBUT has only been measured for studies conducted in phase three and four only (Chapters 8 and 6 respectively). The system was calibrated by external technicians every six months, as required.

### *2.3.4 Osmolarity*

Osmolarity refers to the concentration of dissolved particles in a solution. Hyperosmolarity of the tears occurs as a result of evaporation of aqueous tear from the ocular surface, or aqueous deficiency, or a combination of these.[117] Increased tear osmolarity has been recognised as one of the hallmark signs of DED.[175] Due to availability of equipment, the TearLab$^{TM}$ osmolarity system (TearLab Corporation, San Diego, California) was used in phases three and four of the research only (Chapter 7 and Chapter 6, respectively). One measurement from each eye was taken. Participants were asked to

gaze superior-nasally, and a measurement was taken from the lower temporal tear meniscus in each eye. The recommended threshold, using the TearLab$^{TM}$, most sensitive for detecting dry eye is 308 mOsm/L, with a sensitivity of 90.7% and specificity of 81.3%.[176] Increasing inter-eye difference has been found to correlate with increasing disease severity: variability between two eyes in normal, mild to moderate dry eye and severe dry eye patients has been found to be 6.9 ± 5.9 mOsm/L, 11.7 ±10.9 mOsm/L, and 26.5 ± 22.7 mOsm/L, respectively.[176] An inter-eye variability of ≥ 8mOsms/L is associated with tear film instability and dry eye: with the higher reading indicating greater disease severity.[176,177] The eye with the highest tear osmolarity measurement at baseline was chosen as the study eye; and this eye was used for all data analysis in each relevant study. TearLab$^{TM}$ has been shown to provide repeatable and reproducible tear osmolarity measurements (coefficients of variation 1.6% - 1.9%).[178] Quality control checks, as recommended by the manufacturer, were conducted: daily using the electronic check cards and with each new supply of test cards using the control solutions.

### *2.3.5 Ocular Surface Staining and Fluorescein Tear Break-Up Time*

Ocular surface staining was assessed using fluorescein dye and graded using the Oxford Scheme.[179] A fluorescein impregnated strip (Fluorets; Chauvin Pharmaceuticals, UK) was wetted with a single drop of saline. Excess saline was shaken off, and the tip of the strip was lightly touched off the lower bulbar conjunctiva while participants looked upwards. Fluorescein was instilled in the right eye first at each visit. Staining was assessed 30 seconds after instillation.[180] Corneal, nasal and temporal bulbar conjunctival staining were graded individually on a 6 point scale (0 - 5 for each location) to provide a composite score (0 - 15) for each eye.[179] A yellow Wrattan filter was used to enhance any ocular staining present.[181] The Oxford scheme is not widely used in clinical practice, however it

is recommended for grading ocular surface staining in clinical trials; as it uses a wider range of scores, thus allowing for the detection of smaller changes.[182]

After staining was assessed fluorescein tear break-up time (TBUT) was measured. Fluorescein TBUT is an alternative method for measuring the evaporation rate of the tears. In many practices, using fluorescein is the only way practitioners have to determine TBUT. Participants were requested to blink twice and then hold their eyes open for as long as they could, during which time the tear film was observed and the time to the 1st visible dry spot appearing was counted. This was measured in seconds using the slit lamp (x 24 mag) and a Wrattan filter. An average of three readings was recorded. Fluorescein TBUT was measured in phase one and two only (Chapter 5). As NITBUT is considered more accurate than fluorescein TBUT[171]; NITBUT was incorporated for the subsequent phases of research.

### *2.3.6 Schirmer I*

The Schirmer I test is an invasive procedure, commonly used in dry eye clinics to measure a participants' ability to produce tears. The Schirmer strip (Tear Flo; HUB Pharmaceutical, UK) was folded at the notch, and positioned into the lower lateral eyelid margin. Participants were asked to close their eyes,[183] and the score was measured as the wetting length in mm/5 min. No anaesthetic was used. This was performed for phase three, MGD warm compress treatment study only (Chapter 8). The Schirmer test has been known for its poor repeatability. However, at the time of developing study protocol, Schirmer remained on the recommended battery of dry eye tests according to DEWS I, and it had previously been used in many *Demodex* related studies (Table 1).

### *2.3.7 Cylindrical Dandruff*

As previously described in Section 1.7.2, CD is pathognomonic for DF infestation. The degree of CD present on the base of the eyelashes of each eyelid was graded as described by Milton Hom at the American Academy of Optometry annual meeting in 2013, on the diagnosis and treatment of *Demodex* infestation (Table 4).[184] '*Clumps*' refer to the joining of CD from two or more adjacent lashes to form one CD '*clump*'.

Table 4. Cylindrical dandruff grading scheme (Milton Hom)[184]

| Grade | Description |
|---|---|
| G0 | Normal, clean eyelid margin |
| G1 | Occasional fragments, 1 – 5 collarettes |
| G2 | Few fragments, 6 – 20 collarettes |
| G3 | Many fragments, 21 – 40 collarettes ± 1 – 2 clumps |
| G4 | > 3 clumps ± 40 collarettes |

### *2.3.8 Meibomian Gland Dysfunction Evaluation*

Slit-lamp bio-microscopy (Topcon SL-D701, Topcon Medical Systems Inc., Dublin, Ireland) was conducted to examine the meibomian glands in accordance with the diagnostic subcommittee of the International Workshop on Meibomian Gland Dysfunction[185]; firm digital pressure was applied to the centre of each eyelid margin, and the quality of meibum expressed and the number of glands expressible was graded on a four-point scale: Table 5. As recommended, composite scores derived from the expression of both upper and lower eyelids were generated and used for statistical analysis.[185]

Table 5. Meibomian gland dysfunction grading as recommended by the International Workshop on Meibomian Gland Dysfunction.[185]

| Grade | Quality | Expressibility |
|---|---|---|
| G0 | Clear fluid | All glands expressible |
| G1 | Cloudy fluid | 3 – 4 glands expressible |
| G2 | Cloudy particulate fluid | 1 – 2 glands expressible |
| G3 | Inspissated like toothpaste | No glands expressible |

### *2.3.9 Demodex Investigation*

Finally, each participant was assessed for the presence and quantity of DF. This involved the rotation of the eyelash in clockwise and counter-clockwise directions using sterile forceps. If DF were present, this stimulated them to emerge from the eyelash follicles and could be seen on slit lamp magnification (circa X 16 – 24 magnification). There is no gold standard method for manipulating eyelashes to investigate for the presence of DF. In an attempt to standardise eyelash manipulation, each eyelash was manipulated by rotating it four times anti-clockwise and then four times clockwise, *in situ*, using sterile forceps (Figure 6).

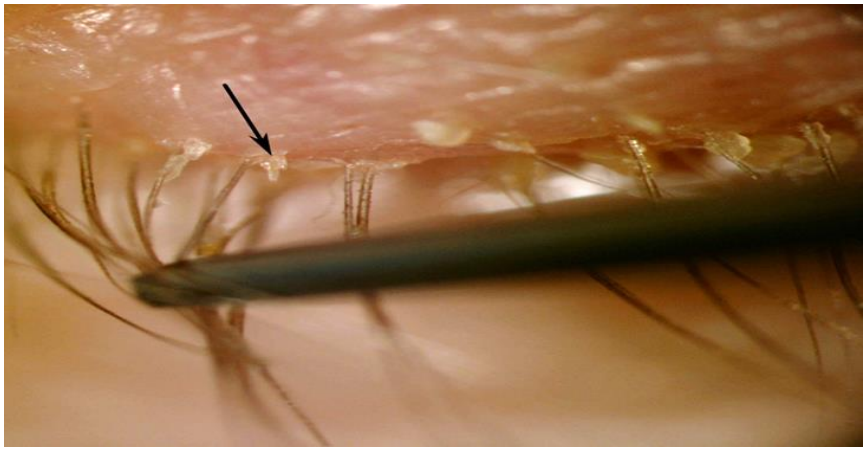


Figure 6. *Demodex folliculorum* visible emerging from the follicle during eyelash manipulation with sterile forceps, black arrow (x24 mag).

Following this, the manipulated eyelashes were removed and placed on a microscope slide and counted using the modified Coston method described earlier (Section 1.8.2). This method was used to count DF in all studies described in this thesis. Chapter 8 compares the two techniques, eyelash manipulation and eyelash epilation, and their use in the investigation of DF blepharitis.

Two eyelashes from each eyelid were removed at each visit in the pilot study (Chapter 5). Only one eyelash was removed from each eyelid at each visit in the subsequent studies. This decision was made due to an increase in number of appointments, and therefore an increase in the number of eyelashes that would need to be removed. Many of the participants were older, some with chronic MGD and blepharitis and therefore had a reduced number of eyelashes. Hence, it was difficult to get participants to agree to have double the quantity of eyelashes removed at each visit.

### 2.4 Treatments

The most recent official guidelines for the initial management and treatment of all types of blepharitis from The College of Optometrists and the AAO in 2018 are to first advise warm compresses and eyelid cleansing; which can be accomplished in several ways, including diluted baby shampoo or dedicated commercial eyelid cleansers.[1,3] If this is in-effective topical/systemic antibiotic therapy followed by topical/systemic anti-inflammatory therapy is advised. In recalcitrant cases, it is then recommended to consider *Demodex* as the underlying aetiology and treat accordingly. Products targeted at treating DF are continuously being developed and marketed. However, there is little evidence available that examines the efficacy of these treatments being administered to patients. The aim of this research is to investigate the safety and efficacy of several of these blepharitis

treatments available for the treatment of *Demodex* blepharitis. The treatments used throughout this study are described below.

### *2.4.1 Baby Shampoo*

For years, lid scrubs with diluted baby shampoo was considered the 'go-to' or 'traditional' method practitioners use to treat general blepharitis. As mentioned previously, it remains a recommendation on the guidelines developed for practitioners to use.[1] However, baby shampoo has no medicinal qualities; and furthermore, a recent study conducted by Sung et al[186] discovered that long-term use of baby shampoo could have negative effects on the goblet cell function, thus causing damage to the ocular surface. However, at the time the studies were being developed, baby shampoo remained a recommendation for blepharitis treatment on the guidelines by the College of Optometrists in the UK.

Baby shampoo was utilised in the phase one, pilot study (Chapter 5) and in phase four (Chapter 6) of this research project. Participants were provided with instructions to create a 10% solution of baby shampoo for home lid scrubs. These instructions can be seen in Appendix 5 (a) and Appendix 5 (b) for the pilot study and phase four, respectively. Guidelines given to participants for home lid scrubs with baby shampoo is described in Table 4.


Figure 7. Johnson's® No More Tears® Baby shampoo.

### *2.4.2 OCuSOFT® Lid Scrub® PLUS*

OCuSOFT wipes (Figure 8) were supplied by Scope Ophthalmics Ltd (Dublin, Ireland). The active ingredient in OCuSOFT is 1,2-Octanediol; a substance with pediculicide potential in as low as 1% concentration.[187] At higher concentrations 1,2-Octanediol is considered toxic to the ocular surface.[150] A 0.5% concentration of 1,2-Octanediol is used in OCuSOFT wipes to ensure the wipes are non-irritating, and their efficacy when used repeatedly for a period of time was examined. OCuSOFT wipes were utilised in the pilot study and the extended study to examine the efficacy against DF blepharitis (discussed in Chapter 5). OCuSOFT foam was utilised in phase four to examine the effect OCuSOFT has on the tear film and ocular surface (discussed in Chapter 6). The guidelines given to participants for home lid scrubs are described in Table 6.


Figure 8. OCuSOFT® Lid Scrub® PLUS.

### *2.4.3 dr.organic® Tea Tree Face Wash*

The TTFW utilised in phase two and four of the research project (discussed in Chapters 5 and 6, respectively) was supplied by dr.organic Ltd. (Swansea, UK) (Figure 9). The active ingredient in TTO, terpinen-4-ol, has been found to be effective at killing DF in a dose dependent manner.[12,53] At the time of study development, previous studies had shown 50% TTO applied weekly was effective at reducing DF infestation,[6,12,13] and a new lid wipe containing 0.5% terpinen-4-ol (Cliradex®) was showing promising results in the US,[188] however, it was not available for purchase in Ireland at the time. Chapter 5 examines the efficacy of daily lid scrubs with TTFW for the treatment of DF blepharitis. The TTFW used in this research project had a 38% concentration of terpinen-4-ol. The guidelines given to participants for home lid scrubs is described in Table 6.


Figure 9. dr.organic® Tea Tree Face Wash.

Table 6. Home Lid Scrub Instructions. Step-by-step instructions provided to participants for nightly lid scrubs at home. Baby shampoo: Johnson's® No More Tears®, OCuSOFT: OCuSOFT® Lid Scrub® PLUS, TTFW: dr.organic® tea tree face wash.

| | Control | OCuSOFT Foam | OCuSOFT Wipes | TTFW/Baby shampoo |
|---|---|---|---|---|
| Step 1: | Using cooled boiled water, wet one of the cotton pads provided | Place a small amount of OCuSOFT foam on a cotton pad | Remove the OCuSOFT wipe from its packet | Place a small amount of shampoo dilution/ face wash on a cotton pad |
| Step 2: | Gently but thoroughly scrub the eyelid and lash margin in circular movements, ensuring to scrub along the base of the eyelashes | | | |
| Step 3: | Begin with the eyes closed to scrub along the top of the lashes. To scrub along the inner layer of lashes, look downwards to avoid contact with the cornea and gently pull the upper eyelid upwards. To scrub along the lower eyelashes, look upwards and gently pull down on the lower lid. | | | |
| Step 4: | Using a clean cotton pad, repeat on the other eye | This is a leave-on formula, do not rinse until morning | This is a leave-on formula, do not rinse until morning | Using a clean cotton pad, rinse the shampoo/face wash from the eyelids |
| Step 5: | | Using a clean cotton pad, repeat on the other eye | Using a new wipe, repeat on other eye | Using a clean cotton pad, repeat on other eye |

### *2.4.4 Microblepharoexfoliation*

Microblepharoexfoliation is the mechanical debridement and exfoliation of the eyelash margin using a hand-held electromechanical unit[189]: BlephEx™ (Figure 10). The BlephEx™ device was utilised in phase two (Chapter 5), and was supplied by Scope Ophthalmics Ltd. (Dublin, Ireland).


Figure 10. BlephEx™ device.

BlephEx™ is a patented hand-held device, developed for the treatment of ocular surface disorders including blepharitis.[190] Manufacturing guidelines and instructions for use are described in Table 7

Table 7. In – house microblepharoexfoliation procedure with BlephEx™, as per manufacturer's guidelines.

| | **BlephEx™ Microblepharoexfoliation procedure** |
|---|---|
| **Step 1:** | Soak the sterile micro-sponge tip in cleaning solution (OCuSOFT® Lid Scrub® Plus foam was used for this study) |
| **Step 2:** | Once soaked, insert one tip into the BlephEx™ chuck |
| **Step 3:** | Instruct the patient to lean their head back. Treat one eyelid at a time, using a new tip for each lid.<br>For the upper eyelid; gently pull up on the upper eyelid and instruct the patient to look downwards.<br>For the lower eyelid; gently pull down on the lower eyelid and instruct the patient to look upwards. |
| **Step 4:** | To scrub; apply the spinning micro-sponge to the edge of the eyelid and lash line and sweep from nasal to temporal and back again in a scrubbing motion for 20-30 seconds or until as much debris as possible is removed. |
| **Step 5:** | After scrubbing with BlephEx™, clean the patient's eyelids with saline to rinse off the formula. |

### *2.4.5 Warm Face Cloth*

Face cloths were utilised in phase three as part of the MGD treatment study, to act as a control 'traditional' style warm compress (Chapter 7). Each participant received a clean, new face cloth to use for the duration of the study. Participants were instructed to pour 200ml of boiled water into a bowl and allow it to cool for 10 minutes before beginning treatment. This created a water temperature ranging from 50 °C to 39 °C over the 10-minute treatment time (tested using a HYGIPLAS Easy temperature pocket catering thermometer and porcelain bowl). Participants' were then required to re-heat the face cloth every two minutes, by immersing it in the same bowl of cooled, boiled water: to maintain temperature at therapeutic levels.[191,192] Each participant was directed to use the treatment for 10 minutes

twice a day for the first two weeks. Frequency of treatment was reduced to 10 minutes once a day from weeks three to eight. The instructions given to participants are attached in Appendix 6 (a).

### *2.4.6 MGDRx EyeBag®*

The MGDRx EyeBag® (Eyebag) is a silk and cotton microwaveable device that has been shown to be a safe and effective treatment method for MGD (Figure 11).[141] The Eyebag is filled with flax seed, providing a dry heat compress. Manufacturers recommend it for the relief of MGD, blepharitis, dry eye syndrome, and rosacea, amongst others. The Eyebags utilised in the MGD treatment study (Chapter 8) were supplied by Scope Ophthalmics Ltd. Participants were instructed to heat their compress in the microwave for 15 – 30 seconds depending on the power of their microwave, as per manufacturers' guidelines. Each participant was directed to use the treatment for 10 minutes twice a day for the first two weeks. Frequency of treatment was reduced to 10 minutes once a day from weeks three to eight. The step-by-step heating instructions given to participants are attached in Appendix 6 (b).


Figure 11. MGDRx EyeBag®

### *2.4.7 OPTASE™ Moist Heat Mask*

The OPTASE™ Moist Heat Mask (Optase) is a microwaveable warm compress (Figure 12). It contains HydroBead™ Technology, which absorbs moisture from the air, and when heated, releases the moisture to provide a natural moist heat. The manufacturers of Optase claim that the moist heat helps to soften and loosen collarettes in patients with anterior blepharitis and re-establishes moisture to the eye and surrounding area; while improving meibum flow, tear film quality and reducing tear film evaporation.[193] The Optase masks utilised in the MGD treatment study (Chapter 8) were supplied by Scope Ophthalmics Ltd. Participants' were instructed to heat their compress in the microwave for 15 – 30 seconds depending on the power of their microwave, as per manufacturers' guidelines. Each participant was directed to use the treatment for 10 minutes twice a day for the first two weeks. Frequency of treatment was reduced to 10 minutes once a day from weeks three to eight. The step-by-step heating instructions given to participants are attached in Appendix 6 (c).


Figure 12. OPTASE™ Moist Heat Mask

## 2.5 Summary

This thesis focuses specifically on DF blepharitis and the safety and efficacy of the over-the-counter treatments listed above, in the treatment of DF blepharitis. The following Chapters 3 – 8 discuss the results of the interventional and observational studies that have stemmed from the four recruitment phases of this research project.

Chapter 3 focusses on the development, validation and use of the GHL questionnaire and Chapter 4 on the development, validation and use of the modified OSDI questionnaire. A paper on the validation and use of the modified OSDI questionnaire has been published in International Ophthalmology (refer List of Publications).

Chapter 5 discusses the results of the pilot study (phase one) and the extended four-week treatment study (phase two). A paper on the results of the extended four-week study has been published in Contact Lens Anterior Eye (refer List of Publications).

Chapter 6 examines the results of phase four of the research project: an extension of phase one and two, developed from peer-reviewed feedback received throughout the research project. This study extended treatment to eight weeks, eliminated the confounding effect of age on results, and facilitated the investigation of the effect eyelid hygiene had on the tear film and ocular surface. A paper discussing the results of this study has been recently accepted for publication in Contact Lens Anterior Eye (refer List of Publications).

Chapter 7 discusses the effect of heat on DF infestation, and examines the efficacy of warm compresses in the treatment of DF blepharitis. A paper discussing the results of the effect of heat therapy on DF has been recently accepted for publication in Current Eye Research (refer List of Publications).

Finally, Chapter 8 considers the second observational finding derived from phase's one and two: comparing eyelash manipulation and eyelash epilation in the examination of DF blepharitis. These techniques are both described in detail in Section 1.8.4 and Section 1.8.2 respectively, and in Chapter 8. A paper discussing this observation has been accepted for publication in Eye & Contact Lens (refer List of Publications).

# CHAPTER THREE: DEVELOPMENT AND VALIDATION OF A GENERAL HEALTH AND LIFESTYLE QUESTIONNAIRE FOR THE INVESTIGATION OF *DEMODEX FOLLICULORUM* BLEPHARITIS

## 3.1 Abstract

**Purpose:** To develop and validate a GHL questionnaire that could be used to evaluate the relationship between general health and lifestyle choices, and DF infestation. To determine the prevalence of DF infestation in an Irish population.

**Methods:** One hundred and fifty-six participants were enrolled in an epidemiological cross-sectional prevalence study. Each participant completed the novel questionnaire on general health and lifestyle. Participants were assessed for the presence and quantity of DF. Data was analysed to search for significant links between general health and lifestyle and DF infestation.

**Results:** The overall prevalence of DF detected was 67.99%. The total median number of DF detected was 1.00 mites (IQR: 0.00 – 5.00). Significant associations were found between the presence and quantity of DF with age ($p = 0.001$ and $p < 0.001$, respectively), and makeup ($p = 0.03$ and $p = 0.04$, respectively). No significant association was demonstrated between DF infestation and contact lens wear, frequency of bed linen hygiene or frequency of cleaning eyelids. The GHL questionnaire demonstrated moderate but acceptable inter-rater reliability ($\kappa \geq 0.61$).

**Conclusion:** Increasing age remains the most significant risk factor for DF infestation. Makeup may provide a preventative effect to reduce the occurrence of DF.

## 3.2 Introduction

*Demodex* mites are common human ectoparasites, described in detail in Section 1.3 and Section 1.4. Their clinical importance and association with underlying medical conditions is discussed in Section 1.6.3. As previously described in Section 1.5, although *Demodex* have been found in larger quantities in certain individuals, there remains a question mark surrounding their pathogenicity. Therefore, researchers are attempting to explain why certain individuals appear to be more susceptible to greater proliferation of mites, and therefore pathogenic DF infestation, than others.

General health and lifestyle choices as risk factors for many ocular disorders have been well documented. Family history is a risk factor for some posterior eye diseases: age-related macular degeneration[194] and glaucoma.[195] Diabetes and high blood pressure are risk factors for potentially sight threatening vascular changes at the back of the eye.[196] Laser surgery, contact lens wear, smoking, working in an air-conditioned environment are risk factors for dry eyes.[121] *Demodex folliculorum* infestation is a relatively newly recognised condition, and researchers are currently investigating risk factors that may exist causing a predilection to higher or lower numbers of mites for an individual.

Previous research has shown that factors such as increasing age (Section 1.6.1), health factors (Section 1.6.3), and contact lens wear (Section 1.6.4) are associated with increased risk of developing pathogenic DF infestation.[13,20,24,77,108,113–115] It has also been suggested that the anatomical position of the eyelids, protected by the bony protrusion of the cheek and brow bones, creates an area that is unlikely to receive as vigorous a hygiene regime as the rest of the face: causing a potential habitat for increased DF numbers.[61] To further strengthen this hypothesis, lower numbers of DF were found among participants with better

lid hygiene regardless of age.[13,28] The routine use of makeup has also been associated with lower numbers of DF.[79,116] To investigate these areas further, questions on type and frequency of lid hygiene, and use of makeup were included in the questionnaire.

As mentioned previously in Section 1.3, DF are photophobic and most active at night while one is asleep.[40,54] Therefore, it could be expected that DF may be found on pillow cases. Thus, the longer the period between changing pillowcases, the higher the potential for a greater risk for DF proliferation. Furthermore, the kill temperature of DF is between 54 - 58 $^{o}$C.[197] Hence, in theory, cleaning bed-linen above this temperature would kill any DF present on the bed-linen and therefore potentially reduce the risk of DF proliferation. A previous study examining the role of water temperature in reducing dust mites found that temperatures below 45$^{o}$C were ineffective.[198] Questions regarding frequency and temperature of bed-linen cleaning, and method of drying bed-linen were included in the GHL questionnaire to investigate if there were any associations with DF infestation.

The GHL questionnaire was developed in an effort to gain a better understanding of potential underlying risk factors for DF infestation (Appendix 3). These may provide the basis for a screening mechanism for the presence of DF in the future.

## 3.3 Methods

### *3.3.1 Examination*

Participants attending the National Optometry Centre private and student optometry clinics, and staff and students of TU Dublin were invited to take part in a cross-sectional prevalence study for DF blepharitis. Signed informed consent was received before participation.

Minimum sample size required for statistical significance was calculated using G*Power analysis. A priori analysis for two-tailed t test, difference between two independent means was conducted; alpha = 0.05, Power = 0.8, arbitrary effect size = 0.5; minimum sample size required n = 128.

- Inclusion criteria: ≥ 18 years of age.
- Exclusion criteria: participants presently being treated for blepharitis or who had used treatment in the past 6 months, active ocular infection (excluding blepharitis) or ocular surgery within the past 6 months.

One hundred and fifty-six participants were examined between October 2014 and May 2016. Seventy males and 86 females, with a median age of 45.00 years (IQR: 28.25 – 62.00) completed the novel questionnaire and were assessed for the presence of DF. Presence of DF was defined as: positive observation of DF on eyelash manipulation (Section 1.8.4) and/or one or more DF counted on microscopic examination (Section 1.8.2). The overall prevalence of DF, and any association between general health and lifestyle choices, and symptoms, and the presence and quantity of DF was examined.

### *3.3.2 Questionnaire Development*

The GHL questionnaire was developed to observe potential correlations between DF and certain lifestyle choices and health status of participants' (refer Appendix 3). Participants were questioned about the use of contact lenses, makeup, current lid hygiene regime, the presence of any medical conditions, and several other questions, to examine for potential risk factors for DF infestation. Participants were allowed to tick multiple answers on the GHL questionnaire where relevant. Answers from these questions may provide the

foundation for a screening mechanism for the presence of DF. It was not intended that the GHL questionnaire be used to assess the severity of DF infestation, if present.

### *3.3.3 Questionnaire Validation*

Cross tabulation and Cohen's kappa (κ) co-efficient were calculated to assess the inter-rater reliability of the GHL questionnaire. These validation methods were chosen as they are appropriate for measuring agreement in categorical data.[199] This was measured by giving the GHL questionnaire to 50 individuals not included in the study and asking them to repeat the questionnaire two weeks later with no change in their general circumstances. This sample size was below the desired number for several questions, this is discussed later as a limitation (Section 3.5.1). A value of $\geq 0.6$ was desirable and considered to have a moderate level of agreement, $\geq 0.8$ considered strong level of agreement and $\geq 0.9$ was considered almost perfect agreement.[200]

### *3.3.4 Statistical analysis*

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp, Armonk, NY, USA). Data was assessed for normal distribution using Shapiro-Wilk. All outcome measures investigated were determined to have a non-normal distribution ($p < 0.001$). The data between categorical variables was assessed using $X^2$ analysis. Between group data was assessed using the MWU test and KW test where appropriate. Spearman's correlation co-efficient was used to assess scaled and continuous variables. All summarised continuous data was expressed as median and IQR, $p \leq 0.05$ was considered statistically significant.

## 3.4 Results

### *3.4.1 Questionnaire Validation*

Response frequencies and their respective κ values from the cross-tabulation of answers for the GHL questionnaire are shown in Table 8. Questions on makeup and contact lens wear showed strong agreement. For all other questions, κ values fell between 0.61 – 0.79, indicating a moderate but acceptable reliability.[199]

Table 8. Cohen's kappa co-efficient and cross-tabulation of test re-test results.

| | 1st attempt: n, (%) | | | | | | | 2nd attempt: n, (%) | | | | | | | κ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CL** | None, 45(90%) | Daily, 3 (6%) | Two Weekly, 1 (2%) | Monthly, 1 (2%) | | | | None, 46 (92%) | Daily, 2 (4%) | Two Weekly, 1 (2%) | Monthly, 1 (2%) | | | | 0.88 |
| **MU** | Yes, 23 (47%) | | | No, 26 (53%) | | | | Yes, 24 (49%) | | | No, 25 (51%) | | | | 0.92 |
| **Lid Hygiene** | None, 14 (29%) | Every Night, 24 (49%) | 3-4 times a week, 4 (8%) | 1-2 times a week, 5 (10%) | < once a week, 2 (4%) | | | None, 12 (24%) | Every Night, 25 (50%) | 3-4 times a week, 1 (2%) | 1-2 times a week, 5 (10%) | < once a week, 7 (14%) | | | 0.63 |
| **Type Lid Hygiene** | None, 16 (33%) | C/T, 7 (15%) | MUR, 4 (8%) | FW, 5 (10%) | JJ, 1 (2%) | Other, 8 (17%) | Multi, 7 (15%) | None, 12 (25%) | C/T, 7 (15%) | MUR, 1 (2%) | FW, 8 (17%) | JJ, 1 (1%) | Other, 12 (25%) | Multi, 7 (15%) | 0.79 |
| **Bed linen Freq** | > once a week, 4 (8%) | Once a week, 14 (28%) | Once a fortnight, 20 (40%) | Once a month, 11 (22%) | < once a month, 1 (2%) | | | > once a week, 3 (6%) | Once a week, 13 (26%) | Once a fortnight, 19 (38%) | Once a month, 12 (24%) | < once a month, 3 (6%) | | | 0.72 |
| **Temp No** | ≤ 30 ºC, 7 (14%) | 40 ºC, 30 (61%) | ≥ 60 ºC, 12 (25%) | | | | | ≤ 30 ºC, 7 (14%) | 40 ºC, 26 (54%) | ≥ 60 ºC, 15 (31%) | | | | | 0.66 |
| **Linen Dried** | Air Dry, 27 (54%) | Tumble Dry, 17 (34%) | Laundrette, 2 (4%) | Air + Tumble Dry, 4 (8%) | | | | Air Dry, 30 (60%) | Tumble Dry, 14 (28%) | Laundrette, 1 (2%) | Air + Tumble Dry, 5 (10%) | | | | 0.61 |

| | 1st attempt: n, (%) | | | | | | | 2nd attempt: n, (%) | | | | | | | κ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Med Cond** | Yes, 4 (8%) | | | | | | | No, 46 (92%) | | | | | | | 0.63 |
| **Meds** | Yes, 5 (10%) | | | | | | | No, 45 (90%) | | | | | | | 0.78 |
| **Allergies** | None, 22 (44%) | SL, 11 (22%) | AA, 1 (2%) | SS, 4 (8%) | DT, 4 (8%) | MTPL, 8 (16%) | | None, 23 (47%) | SL, 15 (31%) | AA, 1 (2%) | SS, 3 (6%) | DT, 1 (2%) | MTPL, 6 (12%) | | 0.652 |
| **Skin Conds** | None, 29 (58%) | ROS, 9 (18%) | ECZ, 4 (8%) | ANE, 2 (4%), | PSRS, 0 (0%) | SnS, 5 (10%) | M, 1 (2%) | None, 32 (64%) | ROS, 9 (18%) | ECZ, 1 (2%) | ANE, 2 (4%) | PSRS, 1 (2%) | SnS, 4 (8%) | M, 1 (2%) | 0.764 |

* κ = Cohen's kappa; CL = Contact Lens Modality; MU = Makeup; Lid Hygiene: C/T = cleanser/toner, MUR = makeup remover, FW = face wipes, JJ = Johnson + Johnson lid scrubs, Other = other lid scrubs, Multi = multiple lid hygiene methods; Allergies: SL = seasonal, AA = asthma, SS = skin sensitivity, DT = dust, MTPL = multiple allergies; Skin conditions: ROS = rosacea, ECZ = eczema, ANE = acne, PSRS = psoriasis, SnS = sensitive skin, M = multiple skin conditions

### *3.4.2 Questionnaire Application*

One hundred and fifty-six participants (median 45.00 years, IQR: 28.25 – 62.00) completed the questionnaire and were assessed for the presence and quantity of DF. An overall prevalence of 67.99% DF was detected amongst the study cohort. The overall quantity of DF discovered, per participant, on microscopic examination was median 1.00, IQR: 0.00 – 5.00. There was no significant difference in presence or quantity of DF between genders ($p = 0.13$ and $p = 0.17$, respectively) (refer Table 9).

Table 9. Comparison of age and presence and quantity of *Demodex folliculorum* for male and female study participants. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Mann Whitney-U: B = Chi-square.

| | **N** | **Age (yrs.)** | **Presence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| Male | 70 | 44.50 (27.00 – 59.00) | 74.29 | 2.00 (0.00 – 5.00) |
| Female | 86 | 45.50 (29.00 – 63.00) | 62.79 | 1.00 (0.00 – 6.00) |
| | | p = 0.43 (A) | p = 0.13 (B) | p = 0.17 (A) |

There was a significant increase in prevalence of DF with increasing age (MWU; $p < 0.001$). Similarly, there was also a low but significant correlation between increasing quantity of DF and increasing age ($r_s$ 0.39; $p < 0.001$) (refer Figure 13).

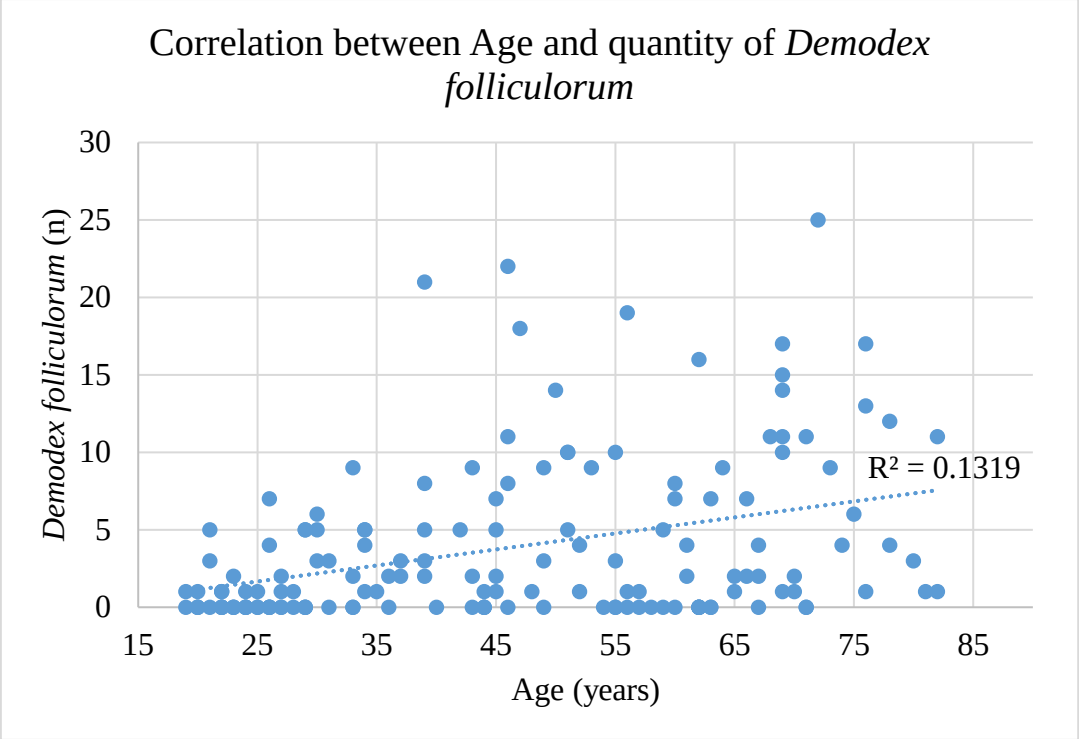



Figure 13. Correlation between increasing age and increasing quantity of *Demodex folliculorum* ($r_s$ 0.39; $p < 0.001$).

Table 10. shows the relationship between contact lens wear and DF. A slightly higher presence and quantity of DF was detected among the non-contact lens wearers. However, non-contact lens wearers were significantly older than contact lens wearers (MWU: $p = 0.046$). Furthermore, the difference in DF presence and quantity between contact lens wearers and non-contact lens wearers was not found to be significant (refer Table 10).

Table 10. Contact lens wear descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Mann Whitney-U: B = Chi-square. *Significant results highlighted in bold.

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| No | 128 | 46.00 (29.00 – 62.00) | 69.67 | 2.00 (0.00 – 5.00) |
| Yes | 28 | 34.00 (25.50 – 48.00) | 57.14 | 0.50 (0.00 – 4.00) |
| | | ***p = 0.046 (A)** | p = 0.20 (B) | p = 0.22 (A) |

Table 11 shows relationship between DF and makeup. Overall, the presence and quantity of DF was significantly lower amongst makeup wearers than non-makeup wearers ($X^2$: p = 0.03 and MWU: p = 0.04, respectively). Age was not found to be an influencing factor in the result; but both male and female participants were included in the analysis.

Table 11. Overall makeup descriptives: age, prevalence *Demodex folliculorum* and quantity *Demodex folliculorum*. Age (median and IQR), *Demodex folliculorum* quantity (median, IQR); A = Mann Whitney-U: B = Chi-square. *Significant results highlighted in bold.

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| No | 85 | 46.00 (29.00 – 62.00) | 75.29 | 2.00 (0.00 – 7.00) |
| Yes | 71 | 40.00 (28.00 – 62.00) | 59.15 | 1.00 (0.00 – 5.00) |
| | | p = 0.33 (A) | ***p = 0.03 (B)** | ***p = 0.04 (A)** |

Females accounted for 100% of makeup wearers, and 82.56% of females reported wearing makeup. When analysing for females alone, the presence and quantity was still lower amongst makeup wearers, although not significantly ($X^2$: p = 0.13 and MWU: p = 0.21, respectively). Furthermore, female makeup wearers were significantly younger than non-makeup wearers (MWU p = 0.005) (refer Table 12), which is likely to have impacted the results.

Table 12. Female-only makeup descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Mann Whitney-U: B = Chi-square. *Significant results highlighted in bold

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| No | 15 | 69.00 (55.00 – 70.00) | 80.00 | 1.00 (0.00 – 10.00) |
| Yes | 71 | 40.00 (28.00 – 62.00) | 59.15 | 1.00 (0.00 – 5.00) |
| | | ***p = 0.005 (A)** | p = 0.13 (B) | p = 0.21 (A) |

The majority of makeup wearers (63.38%) reported cleaning their eyelids every night, however, the frequency of eyeld hygiene among female makeup wearers was not found to be significant (KW p = 0.32). The most popular methods of removing makeup were: cleanser/toner (25.35%), eye makeup remover (25.35%) or a combination of methods (21.13%). The method of lid hygiene used by female makeup wearers was also not found to be significant (KW p = 0.30).

The relationship between the presence and quantity of DF and wearing mascara was found to be significant ($X^2$: p = 0.01 and KW: p = 0.01 respectively). As only females reported wearing mascara, only female participants were included in the analysis. Post-hoc analysis demonstrated: quantity DF was lowest amongst participants using waterproof

mascara. Applying Bonferroni correction $\alpha \leq 0.05/6 = 0.0083$. The only significant difference in quantity of DF was between participants not wearing mascara (median: 3.50, IQR: 1.00 – 10.00) and those wearing waterproof mascara (mean: 0.00, IQR: 0.00 – 1.00) (MWU: $p = 0.003$). Similarly, these results are significantly influenced by age: those not wearing mascara were significantly older (MWU: $p < 0.001$). When analysing for female makeup wearers only, mascara and eyeliner were not found to be significant factors (KW $p = 0.06$ and $p = 0.26$, respectively).

Table 13 illustrates the relationship found between presence and quantity of DF and reported frequency of eyelid hygiene. As can be seen from Table 13, those that reported the lowest frequency of eyelid hygiene demonstrated the highest presence (74.42%) and quantity (median: 3.00, IQR: 0.00 – 9.00) of DF, although the difference was not found to be significant ($X^2$: $p = 0.69$ and KW: $p = 0.35$, respectively). Nonetheless, participants with the lowest frequency of eyelid hygiene appeared to be significantly older than those that reported more regular eyelid hygiene (KW: $p = 0.02$), which is likely to have influenced the result. However, after post-hoc analysis (with Bonferroni correction $0.05/10 = 0.005$), none of the comparisons between the subgroups were found to be significant (MWU $p > 0.005$ in all groups). Lid hygiene frequency was significantly associated with grade of CD (KW $p = 0.02$). After post-hoc analysis (with Bonferroni correction $0.05/10 = 0.005$) the only significant difference was between participants who cleaned their eyelids nightly and those that never cleaned their eyelids (median CD grade: 0.00 IQR 0.00 – 1.00 versus 1.00 IQR 0.00 – 2.00, respectively. MWU $p = 0.001$).

Table 13. Frequency of lid hygiene descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square. * Significant results highlighted in bold

| **Nights/7** | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| 0/7 | 43 | 55.00 (37.00 – 69.00) | 74.42 | 3.00 (0.00 – 9.00) |
| < 1/7 | 17 | 36.00 (25.00 – 46.00) | 70.59 | 1.00 (0.00 – 5.00) |
| 1-2/7 | 14 | 49.00 (33.00 – 58.00) | 71.43 | 3.00 (0.00 – 7.00) |
| 3-4/7 | 20 | 35.00 (24.00 – 47.50) | 70.00 | 2.00 (0.00 – 5.00) |
| 6-7/7 | 62 | 44.00 (28.00 – 62.00) | 61.29 | 1.00 (1.00 – 2.00) |
| | | ***p = 0.02 (A)** | p = 0.69 (B) | p = 0.35 (A) |

Table 14 examines the type of lid hygiene reported by participants. As can be seen from the results in Table 14, no significant relationship was found between type of lid hygiene and presence or quantity of DF.

Table 14. Type of lid hygiene descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square.

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| None | 48 | 52.00 (30.50 – 66.00) | 75.00 | 2.50 (0.00 – 9.00) |
| Cleanser/ Toner | 20 | 35.00 (26.00 – 45.00) | 40.00 | 0.00 (0.00 – 3.50) |
| Makeup Remover | 18 | 34.00 (28.00 – 60.00) | 61.11 | 0.50 (0.00 – 2.00) |
| Face Wipes | 12 | 52.50 (25.00 – 63.00) | 83.33 | 5.00 (1.00 – 7.50) |
| J+J Lid Scrubs | 6 | 60.00 (48.00 – 69.00) | 83.33 | 9.50 (1.00 – 15.00) |
| Other Lid Scrubs | 5 | 46.00 (39.00 – 49.00) | 60.00 | 1.00 (0.00 – 21.00) |
| Other Method | 31 | 45.00 (31.00 – 59.00) | 77.42 | 2.00 (0.00 – 5.00) |
| Multiple | 16 | 36.00 (27.50 – 55.00) | 56.25 | 0.50 (0.00 – 6.50) |
| | | p = 0.24 (A) | p = 0.71 (B) | p = 0.06 (A) |

Table 15 presents the relationship between the frequency of cleaning bed linen and DF presence and quantity. One might expect higher prevalence and quantities of DF among participants who clean their bed linen less frequently. However, as can be seen from Table 15, the frequency of cleaning bed linen did not influence presence or quantity of DF.

Table 15. Frequency of bed linen cleaned descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square.

| **Freq** | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| > once a week | 38 | 45.00 (33.00 – 56.00) | 73.68 | 1.50 (0.00 – 5.00) |
| once a week | 46 | 46.50 (24.00 – 63.00) | 65.22 | 1.00 (0.00 – 7.00) |
| once a fortnight | 48 | 48.50 (31.50 – 67.50) | 66.66 | 1.00 (0.00 – 3.50) |
| once a month | 17 | 34.00 (30.00 – 51.00) | 70.59 | 2.00 (0.00 – 6.00) |
| < once a month | 7 | 62.00 (24.00 – 78.00) | 57.14 | 2.00 (0.00 – 12.00) |
| | | p = 0.062 (A) | p = 0.88 (B) | p = 0.97 (A) |

Similarly, as DF are affected by higher temperatures, one might expect that the temperature bed linen is washed at could influence DF presence or quantity. Table 16. presents the relationship between temperature of bed linen washing and DF presence and quantity. Eleven participants reported not knowing what temperature the bed linen was washed at, and they were removed from analysis. As can be seen from Table 16, as the temperature increased, the prevalence of DF decreased. However, the difference between the groups was not found to be significant ($X^2$: p = 0.06)

Table 16. Temperature bed linen washed descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square.

| **Temp (°C)** | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| 30 | 53 | 40.00 (24.00 – 60.00) | 71.70 | 1.00 (1.00 – 6.00) |
| 40 | 66 | 43.50 (29.00 – 62.00) | 66.70 | 2.00 (0.00 – 7.00) |
| 60 | 26 | 49.00 (33.00 – 62.00) | 46.15 | 0.00 (0.00 – 2.00) |
| | | p = 0.34 (A) | p = 0.06 (B) | p = 0.10 (A) |

Table 17 examines the relationship between the presence and quantity of DF with self-reported allergies: seasonal (hayfever), asthma, sensitive skin, dust or a combination of allergies. However, no significant relationship between allergies and presence or quantity of DF was detected.

Table 17. Allergies descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square.

| **Allergies** | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| None | 63 | 49.00 (31.00 – 64.00) | 69.84 | 2.00 (0.00 – 5.00) |
| Seasonal | 13 | 44.00 (28.00 – 65.00) | 76.92 | 1.00 (0.00 – 4.00) |
| Asthma | 6 | 29.50 (27.00 – 42.00) | 33.33 | 0.00 (0.00 – 3.00) |
| Skin Sensitivities | 13 | 52.00 (34.00 – 56.00) | 61.54 | 1.00 (0.00 – 9.00) |
| Dust | 40 | 44.50 (22.00 – 60.00) | 70.00 | 1.50 (0.00 – 8.00) |
| Multiple | 21 | 39.00 (33.00 – 60.00) | 66.66 | 1.00 (0.00 – 7.00) |
| | | p = 0.21 (A) | p = 0.52 (B) | p = 0.79 (A) |

Table 18 illustrates the relationship between the presence and quantity of DF and self-reported skin conditions. The current study did not find any association between DF and skin conditions. However, the numbers of individuals with skin conditions were limited (refer Table 18).

Table 18. Skin conditions descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square.

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| None | 71 | 49.00 (30.00 – 63.00) | 66.20 | 1.00 (0.00 – 4.00) |
| Rosacea | 6 | 60.00 (47.00 – 68.00) | 83.33 | 6.00 (1.00 – 11.00) |
| Dermatitis | 1 | 24.00 (24.00 – 24.00) | 100 | 1.00 (1.00 – 1.00) |
| Eczema | 4 | 31.50 (27.00 – 56.00) | 75.00 | 4.50 (2.00 – 8.50) |
| Acne | 6 | 27.50 (23.00 – 39.00) | 33.33 | 0.00 (0.00 – 1.00) |
| Sensitive Skin | 23 | 46.00 (33.00 – 59.00) | 78.26 | 2.00 (1.00 – 5.00) |
| Psoriasis | 4 | 37.00 (29.00 – 45.50) | 75.00 | 2.50 (0.00 – 5.00) |
| Multiple | 4 | 36.00 (28.00 – 54.00) | 75.00 | 5.00 (1.00 – 12.50) |
| Other | 37 | 45.00 (22.00 – 60.00) | 64.86 | 1.00 (0.00 – 8.00) |
| | | p = 0.07 (A) | p = 0.64 (B) | p = 0.54 (A) |

The current study found participants who reported an underlying systemic medical condition to have a significantly greater presence and quantity of DF ($X^2$; p = 0.03 and MWU: p = 0.01, respectively) (refer Table 19). However, on further analysis this was significantly influenced by increasing age (MWU; p < 0.001).

Table 19. Medical Conditions descriptives: age, prevalence and quantity *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square. *Significant results highlighted in bold.

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| No | 93 | 37.00 (26.00 – 49.00) | 63.44 | 1.00 (0.00 – 4.00) |
| Yes | 63 | 61.00 (40.00 – 70.00) | 74.60 | 3.00 (0.00 – 9.00) |
| | | ***p < 0.001 (A)** | ***p = 0.03 (B)** | **p* = 0.01 (A)** |

### 3.5 Discussion

The overall prevalence of DF detected in the current study (67.99%) was in good agreement with that previously reported in the literature. Similarly, Lee et al[28] reported a general prevalence of 70% in their demographic epidemiology study. Kabataş et al[46] reported a prevalence of 67.2% in participants with blepharitis compared to 54.9% in control participants. Roth[77] described a general prevalence of 84% that increased to 100% in participants over 70 years of age. The current study discovered a prevalence of 88.89% in participants over 70 years of age.

Increasing age continues to be the most dominant risk factor for DF infestation.[13,28,77] The current study is in agreement with those of previous studies regarding significant associations between both presence and quantity of DF and increasing age. As mentioned previously in Section 1.6.1, one potential reason for an increase in DF with age is the continued, progressive colonisation of DF within the epidermal hair follicles and sebaceous glands over the years. A second hypothesis is that changes in the skin and oil of older

individuals may be more favourable to mite proliferation and widening of the follicle orifice may make detection on mites easier in older individuals.[201]

The GHL questionnaire was developed to investigate potential correlations between DF and certain lifestyle choices, such as the use of makeup and contact lens wear, and the health status of participants. As mentioned earlier (Section 1.6.5), Elston and Elston[79] suggested that men were typically more heavily infested than women, due to a greater androgen–induced sebum production in men. The present study did find a higher prevalence among men, although it was not significant ($X^2$; $p = 0.13$). Elston and Elston[79] also proposed that the lipids in cosmetics may affect DF growth, and therefore could be responsible for the lower presence of DF found among women. Similarly, Horváth et al[116] studied the risk factors of *Demodex* among young adults and also found that the use of makeup reduced the likelihood of *Demodex* infestation. All makeup wearers in this study were women, and on further analysis, when gender was taken into consideration, the difference between makeup wearers and non-makeup wearers was not found to be significant. Given that 82.56% of females wore makeup, and none of the males reported wearing makeup, it cannot be ruled out that this influenced the slightly lower rate of DF infestation detected amongst females, and higher rate of DF infestation detected amongst the males in the study population.

Previous studies have looked at the relationship between eye makeup usage and ocular discomfort and found that the use of eye makeup, such as eyeliner and mascara, was associated with ocular discomfort.[202] However, to the best of the author's knowledge, no previous studies have been conducted on whether wearing eyeliner or mascara is preventative or proliferative to DF infestation. The current study found a lower quantity of DF amongst females wearing mascara. However, those that did not wear mascara were older and would be naturally more pre-disposed to higher DF infestation rates. Therefore,

further age and sex matched control studies would be warranted to investigate any potential relationships. Theoretically, those that wear mascara may be more inclined to clean their eyelids at night, thus reducing the numbers of DF present. On the other hand, wearing makeup/mascara and not removing it could help harbour DF, thus increasing the risk of DF proliferation. As mentioned in Section 1.5, previous studies have shown an association between eyelid hygiene and *Demodex* infestation.[13,28] However, the GHL questionnaire questioned participants on the frequency of eyelid hygiene, and no significant association was established between eyelid hygiene and DF infestation.

Contact lens wear is becoming an increasingly popular form of refractive correction. Previous studies have found significant links between contact lens wearers and DF infestation.[114,115] As such, the GHL questionnaire included questions regarding contact lens wear frequency and modality in order to further evaluate contact lens wear as a potential risk factor for DF proliferation. Jalbert and Rejab[114] investigated the relationship between DF and contact lens wear; observing a higher density of DF among contact lens wearers. It was suggested that contact lens wearers may be at a higher risk of DF infestation as they handle their eyelids more frequently. However, the authors could not come to any further conclusion on their finding as they were unable to establish any association between DF infestation and other signs and symptoms of discomfort and DED.[114] Conversely, Tarkowski et al[115] discovered greater quantities of DF amongst contact lens wearers with discomfort, and previously successful contact lens wearers who dropped out due to discomfort, compared to contact lens wearers with no comfort issues. Therefore, in contact lens wearers who were previously comfortable, and begin to complain of discomfort, or completely drop-out of contact lens wear as a result of discomfort; it is worth investigating for the presence of DF and treating as required. In contrast to Jalbert and Rejab[114], the

current study demonstrated lower numbers of DF among contact lens wearers. However, there were only 28 contact lens wearers involved in the study in comparison to 128 non-lens wearers, and furthermore, contact lens wearers were significantly younger than the non-wearers. Thus, no real value could be taken from this finding. Further investigation into the relationship between contact lens wearers and DF is warranted.

The GHL questionnaire also asked participants to report on frequency of cleaning bed linen and temperature at which bed linen was washed and dried, in an effort to uncover associations with DF. It was conceived that reduced frequency of cleaning bed linen, similar to lid hygiene, would be associated with increased prevalence of DF. As the kill temperature of DF is between 54-58 $^{o}$C,[197] it was also speculated that participants who commonly clean their bedlinen at temperatures $\geq$ 60 $^{o}$C would have a lower prevalence of DF. However, no association was found between frequency of cleaning bed linen or temperature at which the bed linen was washed and dried. *Demodex folliculorum* cannot survive outside of the human body for longer than a few hours.[40] Therefore, it is unlikely that they would survive on a pillow case from one night to the next. Furthermore, cleaning bed linen at $\geq$ 60 $^{o}$C is not likely to impact DF infestation on humans, as they are unlikely to survive outside of the body for prolonged periods of time.

The current study did not find any association between DF and skin conditions or allergies. However, it has been well established previously in the literature that *Demodex* mites are associated with many pustular skin conditions.[54,70,75,81,82] Therefore, it is important to consider *Demodex* when treating blepharitis in patients with a history of rosacea and papulo-pustular skin conditions.

There appears to be some discrepancy in the literature about the relationship between DF and the health status of an individual. As mentioned previously in Section 1.5.3, several studies have found an increased prevalence of DF among individuals with health conditions: a weakened immune system,[91–93] renal failure,[24,108] diabetes.[20,106,107] Several case reports have found a high prevalence of DF causing rosacea like lesions in children with leukaemia[92,97] and adults with AIDS and HIV.[71,93,94] The results of these case reports suggest that an immune-deficient state favours increased numbers of DF. However, no significant difference in density of DF between healthy and immunocompromised patients has also been reported in the literature[19,71]; suggesting no significant relationship between DF infestation and patient immune status. In an adult who is naturally going to have higher density and prevalence of DF with time, it is difficult to quantify the contribution of a reduced immune system to density/prevalence of DF. Participants were questioned about systemic conditions such as high blood pressure, diabetes, and under-active thyroid. The current study found participants who reported underlying systemic medical conditions to be at a higher risk of increased numbers of DF; however, this was significantly influenced by increasing age, and the number of participants with medical conditions was too low to take any statistically relevant meaning from the results.

### 3.5.1 Limitations

The GHL questionnaire lacked a continuous structure throughout. Response items to questions varied from two to seven choice responses. This affected the minimum sample size required for statistical significance: higher numbers were required for the dichotomous questions.[203] While further investigation into the relationship between DF infestation and for example; contact lens wear, makeup and lid hygiene is required, a new questionnaire with a solid structure and less ambiguous questions would need to be constructed. The use

of focus groups would be beneficial to check wording of questions during the design process for future questionnaires. Due to the limitations outlined above, the GHL questionnaire was not administered to participants in any of the subsequent recruitment phases. Furthermore, changes to exclusion criteria, such as excluding participants with underlying systemic conditions e.g. diabetes, meant the GHL questionnaire was no longer suitable for the study cohort being represented.

### 3.6 Conclusion

The novel questionnaire demonstrated moderate to good reliability. Several findings from the study would suggest possible associations that warrant further investigation: the association between makeup, mascara, and contact lens wear.

Chapter 3 examined the relationship between DF and participant lifestyle choices, such as contact lens wear and makeup usage; general day to day habits, such as eyelid hygiene frequency and methods, frequency and temperature of cleaning bed linen; and underlying systemic conditions, such as medical conditions, allergies or skin conditions. In practice it is important to be aware of general risk factors for problematic DF infestation, such as increasing age and rosacea. However, it is also important to be able to recognise symptoms that may be more indicative of DF infestation. The next chapter, Chapter 40, will discuss the development and validation of a modified OSDI symptom questionnaire; and will examine it's use in the detection of DF, and the association between DF infestation and symptoms, in particular the symptom 'itchy eyes'.

# CHAPTER 4: DEVELOPMENT AND VALIDATION OF A MODIFIED OCULAR SURFACE DISEASE INDEX SYMPTOM QUESTIONNAIRE FOR THE INVESTIGATION OF *DEMODEX FOLLICULORUM* BLEPHARITIS

## 4.1 Abstract

**Purpose:** To modify and validate the OSDI symptom questionnaire for use in the examination of the relationship between DF infestation and ocular symptoms.

**Methods:** One hundred and fifty-six participants were enrolled in an epidemiological cross-sectional prevalence study. Each participant completed the modified OSDI symptom questionnaire. Participants were assessed for the presence and quantity of DF. Data was analysed to examine any association between DF infestation and ocular symptoms.

**Results:** The overall prevalence of DF detected was 67.99%. The total median number of DF detected was 1.00 mites (IQR: 0.00 – 5.00). Participants with DF were more symptomatic ($p = 0.04$). The presence and quantity of DF was most significantly associated with the symptom 'itchy eyes' ($p = 0.03$ and $p = 0.04$ respectively). The modified OSDI questionnaire demonstrated good internal consistency (Cronbach's alpha; $\alpha > 0.7$) and good reliability (Intra-class Correlation Co-efficient; ICC > 0.7). A positive symptom result using the modified OSDI questionnaire exhibited a sensitivity and specificity of 70.75% and 46.00%, respectively.

**Conclusion:** Although not all participants with DF will become symptomatic, the prevalence of DF was significantly associated with an increase in symptoms, in particular the symptom 'itchy eyes'. The newly developed modified OSDI symptom questionnaire is

reliable for measuring change in symptoms over a period of time and is suitable for monitoring patient self-reported outcomes in interventional treatment studies.

## 4.2 Introduction

Anterior blepharitis, MGD, aqueous deficient and evaporative dry eye, amongst other ocular abnormalities, share similar symptoms involving the ocular surface: itchiness, grittiness, inflammation, burning and foreign body sensations.[13,49,204] This can cause difficulties for practitioners, to distinguish between each condition, if screening patients' based on symptoms alone. Furthermore, dry eye is a multifactorial disease, and the symptoms of DED and ocular surface disease fluctuate, and often do not correlate well with the degree of ocular signs present.[205–207]

As mentioned previously in Section1.7.4, individual symptoms reported by participants, and the severity of those symptoms can fluctuate depending on the underlying aetiology, and often, time of day.[142] Blepharitis and MGD have been linked with symptoms of foreign body sensation and sticky eyes, commonly in the morning; whereas participants with aqueous deficient dry eye often report worsening of symptoms towards the evening.[142] Previous studies have found itchiness to be one of the most frequently reported symptoms associated with DF infestation.[16,45–47] As suggested earlier (Section 1.6.4), this may be as a result of the movement of the mites across the surface of the skin. As *Demodex* are most active at night,[54] this could potentially cause the severity of symptoms for participants suffering with pathogenic DF infestation to worsen at night, or in the morning, subsequent to the *Demodex* being most active.

Patient reported outcomes have an increasingly important role in clinical trials.[208,209] Research has shown that observing patient reported outcomes after treatment is beneficial

for examining the effect of treatment on the patients.[209] In 2011, the International Workshop on Meibomian Gland Dysfunction considered the significance of patient reported outcomes in clinical trials. A recommendation was made to try to ascertain distinctive symptoms for specific conditions: as the difficulty in discerning between symptoms of different anterior abnormalities is a continuous challenge.[210] The modified OSDI symptom questionnaire was developed and validated, to assess the relationship between DF and dry eye symptoms. The questionnaire's ability to function as a diagnostic screener for DF blepharitis, and its ability to detect change in symptoms post treatment were investigated.

## 4.3 Methods

### *4.3.1 Questionnaire Development*

The modified OSDI symptom questionnaire was adapted from the validated OSDI symptom questionnaire. The OSDI format was chosen as it has shown suitable repeatability and validity for assessing the severity of dry eye.[211] Furthermore, it is one of the most commonly used symptom questionnaires that has been administered to participants in DF related clinical trials.[13,28,114] In keeping with Lee et al[28], the questionnaire was modified to incorporate questions connecting to blepharitis (itchy eyes and matter along the eyelid margin), in order to augment the questionnaires sensitivity to detect DF. Questions from several other validated dry eye questionnaires were also included: McMonnies (dryness, burning), 5 – item dry eye questionnaire (DEQ-5) (dryness, watery), Standard Patient Evaluation of Eye Dryness (SPEED) (dryness, burning sensation, watery eyes). Equally, questions not found in previous dry eye questionnaires, such as itch and red eyes, were included due to increased reports of such symptoms previously in the literature (refer Appendix 4).[13,49]

As recommended by Schiffman et al[211], a formula was applied to calculate the modified OSDI symptom score, described below; which in turn could be used to establish severity of symptoms.

*Total symptom number (A) x 25/number of questions answered (B).*[211,212]

The OSDI symptom score is marked from 0 – 100: increasing scores indicating increasing symptoms. Each question is marked on a 4 - point Likert scale, indicating frequency of the symptom in question: 0 = none of the time, 1 = some of the time, 2 = half of the time, 3 = most of the time, and 4 = all of the time. Since the formula takes into account the number of responses, it is possible to use the formula to get OSDI values for the separate sub-scales also.[211] Questions from different sub-scales have also previously been merged to produce separate sub-scores using the OSDI formula.[213]

To examine the relationship between DF and symptoms, the symptom results were analysed in three ways: the presence of symptoms, the total modified OSDI score, and the severity of symptoms. The presence of symptoms was described as asymptomatic or symptomatic (irrespective of severity). The total modified OSDI score was calculated using the formula described above. The severity of symptoms was categorised from the total modified OSDI symptom score as shown in Table 20. This classification was based on the minimal clinically important difference for the 12-item OSDI.[214]

Table 20. Severity of symptoms in accordance with the total modified OSDI score.

| Grade | Modified OSDI Score |
|---|---|
| G0: Asymptomatic | 0 – 12 |
| G1 Mild | 13 - 22 |
| G2: Moderate | 23 - 32 |
| G3: Severe | 33 - 100 |

The modified OSDI questionnaire has been completed by all participants who have taken part in the research project.

### *4.3.2 Questionnaire Validation*

For a symptom questionnaire to be suitable and fit for purpose it must be reliable, reproducible, and responsive and sensitive to change. That is to say, that any change in symptoms ascertained by the questionnaire is genuine, and not as a result of poor repeatability.[121]

The reliability and reproducibility of the questionnaire was calculated in two ways. Firstly, Cronbach's alpha (α) was used to determine the internal consistency of the questionnaire. In keeping with the literature, an alpha value > 0.7 was accepted.[215,216] Secondly, intra-class correlation co-efficient (ICC)[217] and the test-retest method were used to determine the inter-rater reliability of the questionnaire; $p < 0.4$ indicated poor reliability, $0.4 \leq p \geq 0.75$ indicated fair to good reliability and $p \geq 0.75$ indicated excellent reliability.[218]

The test-retest method post-treatment for both a treatment group and a non-treatment group was used to examine responsiveness and sensitivity to change. A two–tailed t–test was used to compare the means between the two groups ($p < 0.05$ significance).

Factor analysis is a validation method used on questionnaires to determine if multiple variables (questions) have similar response patterns, and therefore load onto similar sub-scales or 'factors'. Factor analysis was conducted during validation of the original OSDI and was found to have three factors or sub-scales: ocular symptoms, vision related functions and environmental triggers.[211] A factor analysis was applied to the modified questionnaire to determine if the sub-scales of the modified OSDI questionnaire were similar to the original OSDI. As the data was non-parametrically distributed, the 'principal axis factoring' extraction method was chosen.[219] Principal axis factoring gives the least number of factors that can account for the correlation within a set of variables. Cronbach's α was then applied to the overall questionnaire and to each subscale.

In distinguishing between normal subjects and 'all dry eye' subjects OSDI has sensitivity and specificity values of 60% and 83% respectively.[211] In distinguishing between normal subjects and 'severe dry eye' subjects OSDI has sensitivity and specificity values of 92% and 83% respectively.[211] A receiver operating characteristics (ROC) curve was constructed to establish the sensitivity and specificity of the modified OSDI symptom questionnaire for the diagnosis of DF infestation.

### *4.3.3 Statistical analysis*

Statistical analysis was performed using the SPSS (version 25.0). Data was assessed for normal distribution using Shapiro-Wilk. All outcome measures investigated were determined to have a non-normal distribution ($p < 0.001$). The data between categorical

variables was assessed using $X^2$. Between group data was assessed using the MWU test and KW test where appropriate. Spearman's correlation co-efficient was used to assess scaled and continuous variables. All summarised continuous data was expressed as median and IQR; $p \leq 0.05$ was considered statistically significant.

## 4.4 Results

### *4.4.1 Questionnaire Validation*

Factor analysis was applied to the results from the 156 participants who filled out the questionnaire at least once, to determine if the sub-scales of the modified OSDI questionnaire were similar to the original OSDI. Factor analysis of the modified OSDI questionnaire displayed three sub-scales similar to the original OSDI questionnaire; ocular symptoms, vision related function and environmental triggers (refer Table 21).[211] Burning sensation, discomfort in cold air and discomfort in air conditioned environments loaded on more than one factor. This was potentially due to the multi-factorial nature of dry eye and common crossover between symptoms and causes.

Table 21. Factor analysis of the modified OSDI questionnaire.

| Symptom | Factor | | |
|---|---|---|---|
| | **Ocular Symptoms** | **Vision Related Function** | **Environmental Triggers** |
| **Dryness** | 0.53 | | |
| **Gritty/Irritated** | 0.70 | | |
| **Itchy** | 0.64 | | |
| **Red Eyes** | 0.55 | | |
| **Burning Sensation** | 0.40 | 0.37 | |
| **Photophobia** | | 0.34 | |
| **Watery** | | | 0.64 |
| **Lids Stuck Together** | 0.21 | | |
| **Reading** | | 0.72 | |
| **Night Driving** | | 0.52 | |
| **Computer** | | 0.75 | |
| **Television** | | 0.50 | 0.24 |
| **Wind** | | | 0.89 |
| **Cold Air** | 0.23 | | 0.61 |
| **Air Conditioning** | 0.22 | 0.25 | 0.28 |
| Extraction method: Principal axis factoring; Rotation method: Oblimin with Kaiser normalisation; Kaiser-Meyer-Olkin measure of sampling adequacy: 0.78 | | | |

Cronbach's α was applied to each subscale, and to the questionnaire as a whole. Cronbach's α for the overall symptom questionnaire was good at 0.84, each of the subscales had a slightly lower α value, but were still > 0.7 (Table 22).[215,216]

Table 22. Reliability Analysis: Cronbach's α measuring internal consistency, and Intraclass correlation coefficient measuring repeatability for the questionnaire. Results are shown for each sub-scale and for the overall questionnaire. All values > 0.7.

| | **Internal consistency: Cronbach's α (95% confidence interval) (n=156)** | **Test-retest: Intra-class Correlation Co-efficient (95% confidence interval) (n=50)** |
|---|---|---|
| **Ocular symptoms** | 0.74 | 0.83 |
| **Vision related function** | 0.80 | 0.73 |
| **Environmental triggers** | 0.83 | 0.89 |
| **Whole questionnaire** | 0.84 | 0.90 |

Fifty separate participants, not enrolled in any interventional treatment study, completed the questionnaire twice for the test-retest method to examine the reliability of the questionnaire. Participants completed the questionnaire two weeks apart, at the same time of day, with no change to their daily routines or general circumstances between testing. The test-retest reliability of the questionnaire was established by calculating the ICC (refer Table 22). All scores surpassed 0.7 which was the desired criteria to be met.[218]

For the test-retest reliability assessment, it is expected that a participant's condition remains stable between the first test and the retest: as no intervention has taken place. This is clear from the strong ICC value ascertained for total symptom score of 0.90. Taking this into account, a post-hoc ICC was performed to compare the repeatability of the total symptom score after two weeks of treatment in a separate interventional treatment study. It

was predicted that the correlation would be much weaker: as participants' symptoms should have changed since commencing treatment. This hypothesis was confirmed with an ICC = 0.66 < 0.89. A two-tailed t-test was applied to both sets of data. There was no significant difference in total symptom score in the test-retest group ($p = 0.54$). However, there was a highly significant difference in retest total symptom score in the group that received treatment ($p < 0.001$). The placebo effect of receiving treatment must be taken into consideration when assessing how effective treatments are at improving symptoms. This is discussed in further detail in Chapter 6. However, with regards to validating the questionnaire and evaluating its ability to measure change in subjective symptoms, the placebo effect is considered extraneous.

The ROC curve was generated to assess the diagnostic capacity of the symptom questionnaire to examine for the presence of DF (refer Figure 14). The red line illustrates the ROC curve plotted from the study test results. Each point represents a sensitivity/specificity pair relating to a specific decision threshold. The closer the curve follows the y axis, especially towards the top, the greater the area under the curve and the more accurate the test. The closer the curve comes to the diagonal dotted line, the less accurate the test. The dotted line symbolises a worthless test result. A moderately flat ROC curve was formed, with an area under the curve of 0.61. A positive symptom result, that is ≥ G1, gives a sensitivity of 70.75% and a specificity of 46.00% for the modified OSDI questionnaire.

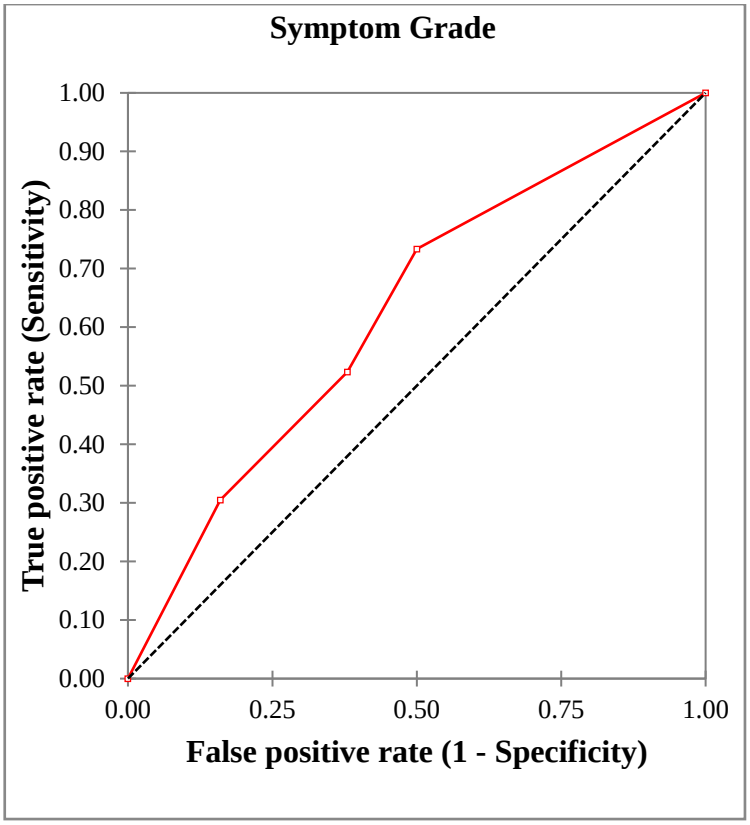



Figure 14. Receiver operator characteristics curve demonstrates the ability of the modified OSDI questionnaire to assess for presence of *Demodex folliculorum* using symptom grade (Normal – Severe: 0 – 3). Area under the curve = 0.61.

### *4.4.2 Questionnaire Application*

One hundred and fifty-six participants completed the questionnaire and were assessed for the presence and quantity of DF. An overall prevalence of 67.99% DF was detected amongst the study cohort. The median quantity of DF discovered, per participant, on microscopic examination was median 1.00, IQR 0.00 – 5.00. There was no significant difference in presence or quantity of DF between genders ($p = 0.13$ and $p = 0.17$, respectively) (refer Table 23). There was a significant increase in prevalence with

increasing age and symptoms (MWU; $p < 0.001$ and $p = 0.05$, respectively) (refer Table 23).

Table 23. Comparison of age and symptoms (presence and modified OSDI score) for subjects with and without *Demodex folliculorum*. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Mann Whitney-U: B = Chi-square. *Significant results highlighted in bold.

| ***Demodex* Present** | **N** | **Age (yrs)** | **Prevalence Symptoms (%)** | **Total Modified OSDI Score (0-100)** |
|---|---|---|---|---|
| No | 54 | 29.00 (24.00 – 56.00) | 54.00 | 13.33 (7.69 – 28.57) |
| Yes | 102 | 49.00 (34.00 – 66.00) | 70.77 | 20.83 (10.00 – 39.29) |
| | | ***p < 0.001 (A)** | ***p = 0.04 (B)** | ***p = 0.05 (A)** |

*Presence of Demodex folliculorum versus Symptoms:*

As shown in Table 23 above, and in Figure 15 below, there was a significantly higher proportion of symptomatic participants (≥ G1 modified OSDI symptom) amongst participants with DF than those without DF (70.77% versus 54.00%, $X^2$; $p = 0.04$). In keeping with this, the total modified OSDI symptom score (0 – 100) was also found to be significantly higher in participants with DF in comparison to participants without DF (refer Table 23). Regardless of DF, symptomatic participants were also found to be significantly older than asymptomatic participants (49.00, IQR 33.00 – 66.00 versus 35.00, IQR 24.00 – 57.00. MWU: $p < 0.001$).

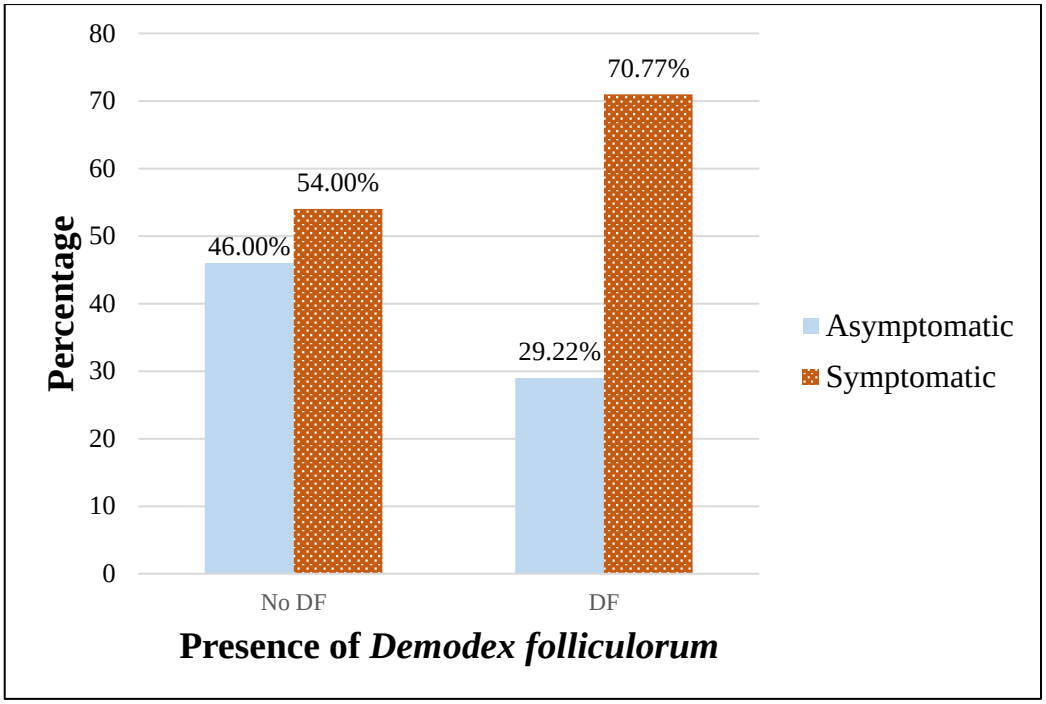



Figure 15. Percentage frequency distribution of symptomatic and asymptomatic participants, with and without DF. Participants with DF were significantly more symptomatic ($X^2$; p = 0.04).

The severity of symptoms (Grade 0 – 3) was also examined. Participants with DF were found to have a significantly greater severity of symptoms than those without DF ($X^2$; p = 0.04). As can be seen from Figure 16, this was most noticeable in the severe symptom group. Participants without DF were predominantly asymptomatic, followed by mild to moderately symptomatic, and only a small number (n = 7) were severely symptomatic. In contrast, participants with DF were predominantly symptomatic; the majority of which were severely symptomatic (n=35) (refer Figure 16). As can be seen from Figure 16, 46.00% of participants without DF were asymptomatic and only 14.00% had severe symptoms. Only 29.24% of participants with DF were asymptomatic, however 32.07% had severe symptoms. It is also evident that the majority of symptomatic participants with DF were severely symptomatic.

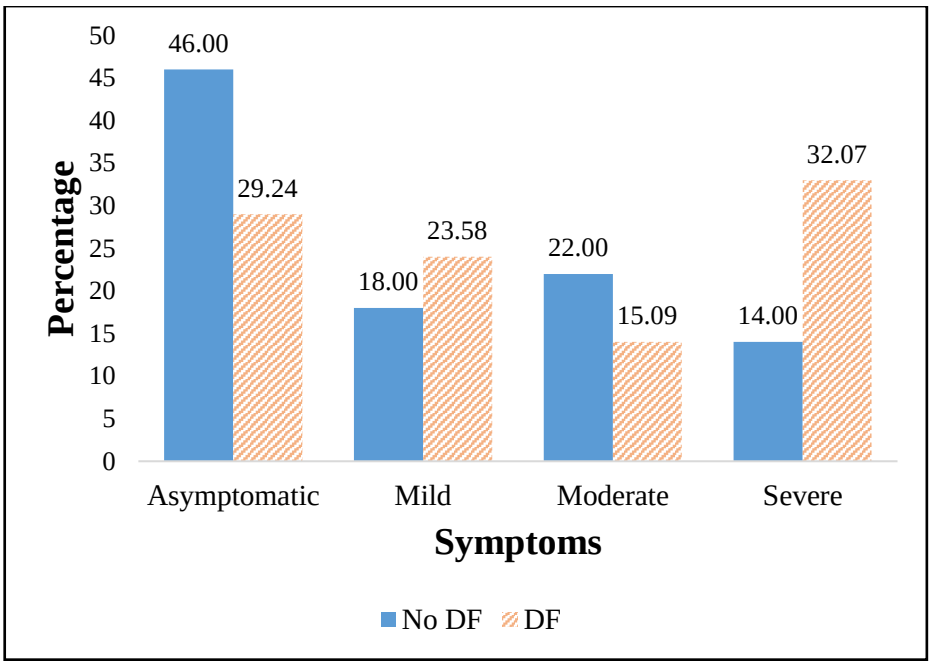



Figure 16. Percentage frequency distribution of grade of severity of symptoms amongst participants with and without *Demodex folliculorum* ($X^2$; p = 0.04).

The prevalence of individual symptoms reported by participants with and without DF is shown in Table 24. A significant association was detected between the symptom 'itchy eyes' and the presence of DF. 'Itchy eyes' was more commonly reported by participants with DF than those without (68.88% vs 52.00%) ($X^2$; p = 0.03). However, the frequency of the symptom 'itchy eyes' was not found to be significantly associated with the presence of DF ($X^2$; p = 0.13). Overall participants with 'itchy eyes' were not significantly older than those without 'itchy eyes' (KW; p = 0.83). However, participant's with 'itchy eyes' and DF were significantly older than those with 'itchy eyes' and no DF (KW; p < 0.001). Asymptomatic individuals with DF were also older, but not significantly (KW; p = 0.30).

Table 24. Prevalence of symptoms reported by participants with and without *Demodex folliculorum*; *Significant results highlighted in bold.

| Symptoms | Participants with DF (%) | Participants without DF (%) | P value ($X^2$) |
|---|---|---|---|
| **Gritty / Irritated** | 72% | 70% | 0.69 |
| **Itchy** | 69% | 52% | ***0.03** |
| **Dryness** | 68% | 68% | 0.88 |
| **Wind** | 57% | 62% | 0.46 |
| **Air Conditioning** | 55% | 44% | 0.25 |
| **Watery** | 53% | 60% | 0.35 |
| **Photophobia** | 45% | 46% | 0.96 |
| **Red Eyes** | 45% | 40% | 0.38 |
| **Computer** | 43% | 40% | 0.66 |
| **Cold Air** | 43% | 30% | 0.17 |
| **Problems Reading** | 42% | 36% | 0.30 |
| **Television** | 42% | 12% | ***< 0.001** |
| **Burning Sensation** | 28% | 18% | 0.14 |
| **Lids stuck together** | 21% | 12% | 0.18 |
| **Night Driving** | 19% | 8% | 0.06 |

The functional vision symptom of ‘discomfort watching television’ was also found to be significantly associated with the presence of DF (41.55% vs 12.00%) ($X^2$; $p < 0.001$). However, on further analysis, participants reporting symptoms of ‘discomfort when watching television’ were found to be significantly older than those without those symptoms (MWU; $p = 0.01$). Given that increasing age is one of the most significant risk factors for DF infestation; as the symptomatic group were older, they were naturally predisposed to having an increased presence of DF, and this is likely to have impacted this finding.[13,77]

*Quantity of Demodex folliculorum versus Symptoms:*

As mentioned previously, the median quantity of DF discovered, per participant, on microscopic examination was median 1.00, IQR 0.00 – 5.00. Although DF was detected on asymptomatic individuals, the median number of mites was significantly higher amongst symptomatic participants in comparison to asymptomatic participants (2.00 IQR 0.00 – 7.00 versus 1.00 IQR 0.00 – 3.00. MWU; $p = 0.02$). A low positive correlation was established between quantity of DF and the total modified OSDI score; however, it was not found to be significant ($r_s = 0.12$; $p = 0.13$). However, a low, but significant, positive correlation was ascertained between the quantity of DF and increasing severity of symptoms ($r_s = 0.16$; $p = 0.04$) (refer Figure 17).

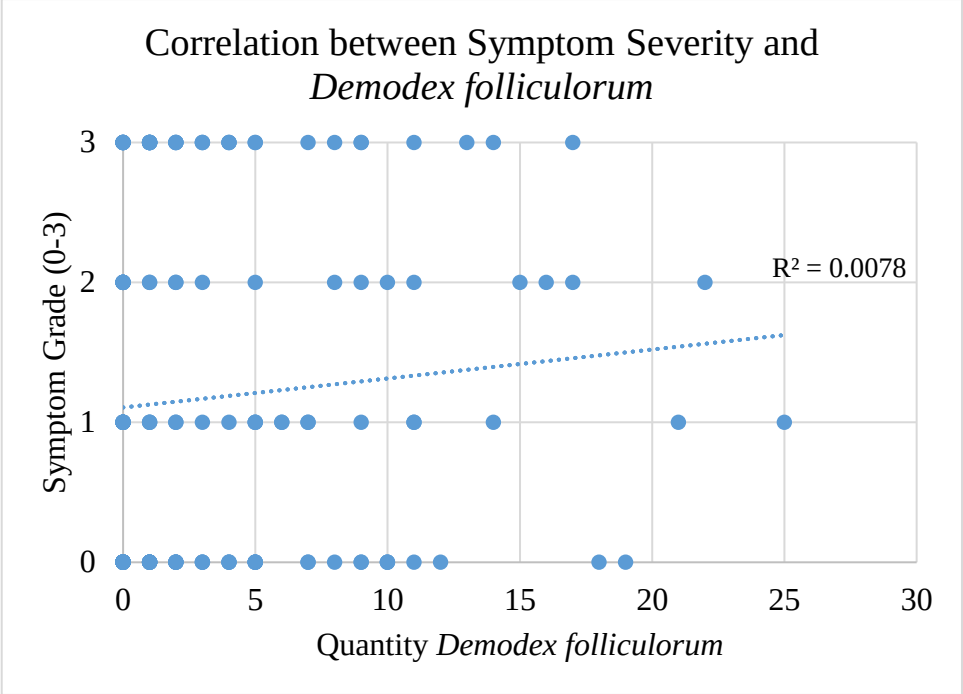



Figure 17. Scatter plot illustrating the positive correlation between symptom severity and quantity of *Demodex folliculorum* ($r_s = 0.16$; $p = 0.04$).

This correlation is expressed in the equation:

$$Y = 0.0267\ X\ (\text{number of DF}) + 1.2565$$

According to the above formula, on average 1 DF mite = G1.28 symptoms: mild symptoms. On average an additional 28 mites are required to cause moderate symptoms, and an additional 38 mites (minimum 66 mites) are required to cause severe symptoms.

A small, but significant, positive correlation was also established between the quantity of DF and the severity of 'itchy eyes' symptom ($r_s = 0.17$; $p = 0.04$) (refer Figure 18).

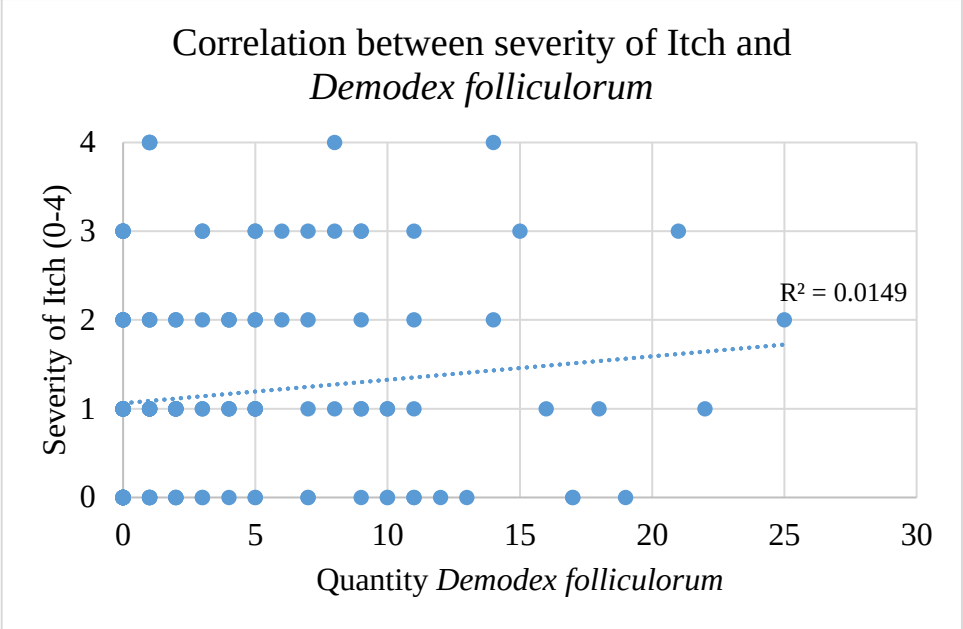



Figure 18. Scatter plot illustrating the positive correlation between severity of 'itch' and quantity of *Demodex folliculorum* ($r_s$ = 0.17; p = 0.04).

Spearman's correlation demonstrated an equation similar to the one above:

$$Y = 0.0264\ X\ (\text{number of DF}) + 1.0552$$

Similarly, this equation proposes that 1 DF = G1.08 symptoms: 'itchy eyes' some of the time. On average an additional 36 mites, respectively, are required to cause respective increases in severity of symptoms. Thus, the above equations for severity of symptoms and severity of 'itchy eyes' demonstrates that just 1 DF has the ability to produce mild symptoms. However, due to the multifactorial nature of ocular surface disease, participants can be symptomatic in the absence of DF, as has been seen previously.

A low, but significant, correlation was detected between increasing quantity of DF and increasing 'discomfort when watching television' ($r_s$ = 0.16; p = 0.04). Additional analysis demonstrated that this increase in quantity of DF was directly correlated to an increasing age for the same symptom ($r_s$ = 0.24; p = 0.003).

Time of day has been reported to have an effect on symptoms depending on the underlying aetiology.[142] However, in the current study, time of day did not appear to affect symptoms with respect to DF. No other individual symptom was found to be significantly associated with the presence or quantity of DF.

*Pathogenic Infestation*

Previous studies have proposed that the presence of DF is not necessarily pathogenic; but that an increased quantity of DF causes pathogenic infestation.[65,71] Incorporating the severity scale proposed by Randon et al[65], ≥ 4 mites per follicle, data was investigated to look at the prevalence of pathogenic DF in the current study population; and any links between pathogenic DF infestation and age and symptoms (refer Table 25).

Pathogenic DF infestation increased significantly with age. Participants with pathogenic DF infestation were found to be significantly older than participants with non-pathogenic DF infestation (MWU; $p = 0.01$), and participants with no DF (MWU; $p < 0.001$). Furthermore, participants with non-pathogenic DF infestation were older than participants with no DF (MWU; $p = 0.07$), but not significantly.

Participants with either pathogenic or non-pathogenic DF were found to be more symptomatic than participants with no DF. Although the difference was not found to be significant for either group (MWU; $p = 0.08$ and $p = 0.12$ respectively). Similarly, the greatest prevalence of the symptom ‘itchy eyes’ was detected amongst participants with pathogenic DF. However, when compared to participants with no DF, this was not found to be significant ($X^2$; $p = 0.08$).

Table 25. Comparison of quantity of *Demodex folliculorum*, age, presence of symptoms, modified OSDI score, and presence of itch for participants with; no *Demodex folliculorum*, mild/normal non-pathogenic infestation, and pathogenic infestation of *Demodex folliculorum*. Continuous variables expressed as median and IQR. A = KW P value: B = $X^2$ P value. *Significant results highlighted in bold.

| | **Frequency, n (%)** | **Quantity mites, n** | **Age, yrs** | **Presence of Symptoms (%)** | **Modified OSDI (0-100)** | **Presence of Itch (%)** |
|---|---|---|---|---|---|---|
| **No Demodex *folliculorum*** | 50 (32%) | 0.00 (0.00 – 0.00) | 29.00 (26.00 – 46.00) | 54.00 | 13.33 (11.67 – 23.22) | 52.00 |
| **Mild/Normal infestation (< 4 mites/follicle)** | 39 (25%) | 1.00 (1.00 – 2.00) | 39.00 (35.00 – 52.00) | 64.10 | 25.00 (15.00 – 35.00) | 66.67 |
| **Pathogenic infestation (≥ 4 mites/follicle)** | 60 (38.5%) | 8.00 (7.00 – 10.00) | 52.50 (47.00 – 61.00) | 75.00 | 20.00 (16.67 – 26.67 | 71.67 |
| **Mites visible on lash manipulation but not on microscope** | 7 (4.5%) | 0.00 (0.00 – 0.00) | 44.00 (40.00 – 63.00) | 71.43 | 18.33 (8.33 – 81.25) | 57.14 |
| | | | ***< 0.001 (A)** | 0.14 (B) | 0.27 (A) | 0.16 (B) |

## 4.5 Discussion

There is continued debate over the pathogenicity of the *Demodex* mites.[49,66–68] The findings in this study are in agreement with previous authors[29,30]; that DF can be found amongst asymptomatic individuals. The current study discovered a median number of 1.00 mites (IQR 1.00 – 3.00) amongst asymptomatic participants. As discussed earlier in Section 1.5, Lacey et al[66] suggested that generally DF is a commensal organism with a potentially beneficial role: consuming bacteria and other micro-organisms in the lash follicle. The presence of DF in normal healthy individuals appears to strengthen this proposal. Baima et al [67] proposed that DF becomes pathogenic to the host when quantities of DF increased beyond a critical level. With regards to dermatology and density of DF in the skin, Forton et al[70] suggested that > 5 mites/cm$^2$ was the critical level for pathogenic DF infestation. With regards to the eyelash follicles, Randon et al[65] suggested the critical level to be ≥ 4 mites per follicle. As demonstrated in previous studies[13,28,46,47] and in the current study also, there was a positive association between increasing symptoms and increasing densities of DF; which adds to the suggestion that DF has pathogenic potential as the quantity of DF present increases. Additionally, as will be discussed in Chapter 5, symptoms were found to improve following treatment, further strengthening the case that the DF do have pathogenic potential.

Sędzikowska et al[47] recently published results of a large scale study exploring the relationship between DF and presence of symptoms as stated by patients, without the use of a questionnaire. The results proposed a minimum of seven DF mites per eight epilated eyelashes was required to produce one symptom, and a further 18 mites were required to produce a second symptom. In the same study, the authors did not quantify the severity of

symptoms reported by patients: intermittent vs constant, sometimes vs all of the time; merely the presence of the symptom.[47] The present study examined the severity of symptoms reported by patients using the modified OSDI questionnaire. Applying similar statistical analysis to that applied by Sędzikowska et al[47], the current study discovered a greater quantity (33) of DF was required to produce moderate symptoms. Although the results of both studies are in good agreement, that increasing quantities of DF cause increases in symptoms, they are not directly comparable: Sędzikowska et al[47] counted DF mites on eight epilated eyelashes, whereas the present study counted DF mites on four epilated eyelashes. As previously mentioned in Section 2.3.9 only four eyelashes were epilated as most participants were requested to attend for multiple visits, and repeat tests over time, thus increasing the number of eyelashes that needed to be epilated.

Although there is a positive correlation between symptoms and presence of DF, they are not ubiquitous with one another. Participants can be symptomatic in the absence of DF; and similarly, participants can be asymptomatic in the presence of DF. A prevalence of 67.99% DF was found in the current study. Of those with DF, 70.77% (75/106) had symptoms (refer Figure 15). It is possible that chronic inflammation of the anterior ocular surface caused changes in corneal morphology,[220] leading to corneal hypoesthesia; thus resulting in reduced symptoms in the presence of severe infestation and inflammation.[175,221,222] Therefore, subjectively reported symptoms are not always in line with clinical signs of ocular surface disease; as was demonstrated in the present study. For example, of the 106 individuals with DF, the quantity of DF discovered on microscopic examination was similar between asymptomatic and symptomatic participants (3.00 IQR 1.00 – 7.00 versus 4.00 IQR 1.00 – 9.00 mites, respectively; MWU; $p = 0.19$). It is possible, that chronic infestation and inflammation caused hypoesthesia at the ocular surface,

resulting in asymptomatic participants with increased quantities of DF. Although, as DF inhabit in the eyelash follicle, the scuttling, scratching movement of DF would be expected to cause an itching sensation: which would not likely be affected by corneal hypoesthesia. It is more likely that not all DF were removed during lash epilation. This limitation of eyelash epilation has been alluded to in previous studies.[25,29,50] Throughout the present study, it became apparent, particularly in cases where lashes were loose in damaged follicles due to the presence of increased densities of DF, that the lash would fall away during eyelash rotation: leaving the DF behind inside the lash follicle. Subsequently, further investigation was conducted into the relationship between eyelash manipulation and eyelash epilation techniques used during *Demodex* investigation. This is discussed in detail in Chapter 8.

Individual symptoms commonly associated with DF have been reported previously: itch, burning sensation, foreign body sensation, redness and crusts along the lid margins, blurred vision and misdirection of eyelashes.[6,13,15,16,46,47,49] In a study conducted by Koo et al[13] investigating the relationship between ocular discomfort and *Demodex* infestation, the authors found dryness (74.7%), itching (42.78%), and irritation (39.1%) were the most commonly reported symptoms described by participants with *Demodex* infestation. Kabataş et al[46] reported redness (80%), itching (63.6%) and foreign body sensation (55.6%) as the most commonly reported symptoms in participants with DF infestation. Likewise, Sędzikowska et al[47] reported similar symptoms, but at lower prevalence values: itching (28%), redness (21%), watery eyes (15%), and dryness (6%). It is not clear which symptom questionnaire was used by Kabataş et al[46], and Sędzikowska et al[47] did not use a questionnaire. Thus, symptom reporting was not prompted by the use of a questionnaire, but depended on each participant complaining of a symptom of their own accord: the likely

cause of the lower prevalence values reported in their study.[47] The most common symptoms reported by participants with DF in the current study were gritty–irritated eyes (72%), followed by; itch (69%), dryness (68 %), watery (57%), photophobia (45%), red eyes (45%), burning sensation (28%), and lids stuck together (21%). Several of these symptoms were also commonly reported by participants that did not have any DF, and were not found to be significantly associated with DF (refer Table 24). However, in keeping with previous studies,[16,46,47] the current study found that the symptom 'itchy eyes' was associated with an increased presence of DF. Furthermore, one of the main and novel findings of the current study was that the severity of 'itchy eyes' increased as the number of DF increased. This further strengthens the basis for 'itchy eyes' as a significant symptom of DF infestation.

Dry eye is multi-factorial by nature and there can be discrepancy between signs and symptoms of dry eye.[223] The current study did not investigate the influence of non-dry eye related symptoms. For example, the symptom 'itchy eyes' was also reported by many of the participants who did not have DF. Itch is one of the hallmark symptoms of allergy. As data collection took place over two years, it is possible that a history of allergy influenced the severity of the symptom 'itchy eyes'. However, chi-square analysis did not find any significant correlation between the presence of allergy and the presence of general symptoms, or symptoms of 'itchy eyes' amongst participants that did not have DF ($X^2$; $p = 0.79$ and $p = 0.09$ respectively). The findings of the current study do not suggest that 'itchy eyes' should be considered a diagnostic symptom of DF infestation; simply that 'itchy eyes' seems to be a more common and repeatedly reported symptom amongst participants suffering with DF infestation. Furthermore, it is possible that the presence of the 'itchy eyes' is in fact an allergic reaction to the presence of DF within the eyelash follicles, which has been postulated previously.[49,69]

A low, but significant, correlation was detected between increasing quantity of DF and increasing ‘discomfort when watching television’. However, the total prevalence of ‘discomfort when watching television’ was low (32%), even amongst participants with DF; and those that reported this symptom were significantly older. Therefore, it is likely that the confounding effect of age – related dry eye changes contributed to this finding. Nevertheless, presence and quantity of DF should still be considered as it is an age-related change, and very few control participants reported discomfort.

The original OSDI questionnaire is one of the most commonly utilised symptom questionnaires in DF related clinical trials.[13,28,114] Results from the current study, and previous studies outlined above, have established that a symptom of ‘itchy eyes’ is amongst the most frequent complaint in participants with DF. However, no question exists on the original OSDI to inquire about ‘itchy eyes’. Lee et al[28] modified the OSDI questionnaire to include a question on ‘itchy eyes’, and demonstrated that the overall OSDI score was significantly associated with higher quantities of DF. Nonetheless, it was not clear if the questionnaire modified and used by Lee et al[28] had been validated. Therefore, the current questionnaire was developed to include a question about ‘itchy eyes’ and has been validated as discussed above. As such, the modified OSDI questionnaire was used to assess symptoms in all the studies discussed in this thesis.

### 4.6 Conclusion

The novel modified OSDI questionnaire demonstrated good internal consistency (Cronbach’s α was > 0.7 for both the total questionnaire and each of the subscales) and good to very good repeatability (> 0.75) for both the total questionnaire and each of the subscales in the test-retest ICC. The strong repeatability aspect of the questionnaire

demonstrated that it can be employed as a valid method of observing subjective symptoms, following treatment, over time in a clinical setting. This is progressively becoming more important as patient reported outcomes become an essential element of patient-centred management in the health sector.[208]

The questionnaire exhibited a reasonable sensitivity value of 70.75%, for correctly detecting participants with DF infestation. However, with regards to confirming the presence of DF infestation and establishing who requires further intervention, this would not be sufficient. A thorough clinical work-up, involving eyelash manipulation, will always be required for diagnosis but an awareness of risk factors for the disease will help practitioners to better diagnose, treat and advise their patients.

The validation and use of the modified OSDI questionnaire have been published in International Ophthalmology (refer List of Publications). A significant link was established between the presence and quantity of DF, and severity of symptoms, using the modified OSDI questionnaire that was developed during this research project. 'Itchy eyes' was significantly associated with the presence of DF. In clinical practice it is important to consider the presence of DF in patients reporting 'itchy eyes'. As such, it would be advisable to incorporate the modified OSDI questionnaire, or a similar questionnaire that contains questions on symptoms of itch, when managing and treating anterior ocular disorders such as blepharitis. Nonetheless, as mentioned previously, a detailed clinical work-up is still necessary for differential diagnosis between various anterior ocular disorders.

As with many anterior ocular disorders, subjective symptoms are often similar and are not always present. As demonstrated in the current study, not all participants with DF were

symptomatic, even when infestation was apparently severe. The relationship between DF infestation and corneal hypoesthesia requires investigation, including research into the triggers that cause a patient to become symptomatic.

Although not all DF infestation is symptomatic, and not all DF infestation requires intervention, it is important to be able to intervene in an effective manner when necessary. The following chapter, Chapter 5, will discuss the results of a two-week pilot treatment study and an extended four-week treatment study. The pilot study compared the efficacy of OCuSOFT with baby shampoo for treating DF blepharitis. The four-week study compared OCuSOFT, TTFW, and the effect of in-house microblepharoexfoliation treatment. The four-week treatment study has been published in Contact Lens Anterior Eye, and is adapted accordingly for Chapter 5.

# CHAPTER 5: THE EFFICACY OF BABY SHAMPOO, OCUSOFT LID SCRUB PLUS, DR. ORGANIC TEA TREE FACE WASH AND MICROBLEPHAROEXFOLIATION IN THE TREATMENT OF *DEMODEX FOLLICULORUM* BLEPHARITIS

## 5.1 Abstract

**Purpose:** To investigate and compare the efficacy of baby shampoo, OCuSOFT, TTFW and microblepharoexfoliation at treating DF blepharitis.

**Methods:** A randomised, controlled, examiner blind, two – week interventional, pilot study was conducted. Eighty-two eyes of 41 participants (21 male/20 female: median age 45.00 years) were examined for signs and symptoms of DF. Participants completed the GHL and modified OSDI symptom questionnaires and were examined for the presence of DF. Eight eyelashes, two from each eyelid, were manipulated and epilated for microscopic examination. Adult DF count was recorded using the modified Coston method. Each participant was given the treatment (OCuSOFT) for one eye, and a control lid hygiene (10% solution baby shampoo) for the contra-lateral eye. Participants were advised to clean each eye, using the relevant treatment, nightly for a fortnight.

Subsequently, 86 participants (38 males/48 females: median age 43.50 years) were enrolled in a randomised, controlled, examiner blind, four-week interventional treatment study. Participants completed the modified OSDI symptom questionnaire and were assessed for the presence of DF. One eyelash from each eyelid, right and left, were manipulated and epilated for microscopic examination, using the modified Coston method. Participants were divided into three groups according to treatment: TTFW (A) (n=28), OCuSOFT (B) (n=30), and in-house microblepharoexfoliation before nightly lid scrubs

with OCuSOFT (C) (n=28). Participants were advised to clean their eyelids nightly for four weeks with the specified treatment. Each participant was re-assessed for symptoms and presence of DF after two weeks and four weeks of treatment.

**Results:** *Demodex folliculorum* was found on 61.00% of the 41 participants tested in the pilot study. The overall total median number of DF per participant found pre-treatment 2.00 mites (IQR 0.00 – 8.00) There was no significant difference in quantity of DF pre-treatment between the treatment and control groups (1.00 IQR: 0.00 – 3.00 and 1.00 IQR 1.00 – 4.00 respectively, $p = 0.77$). The quantity of DF was significantly reduced post-treatment to median 0.00 mites (IQR 0 .00 – 0.00) in the treated eye versus median 1.00 mites (IQR 1.00 – 2.00) in the control eye ($p = 0.01$). The presence and quantity of DF was higher amongst symptomatic participants pre-treatment, but not significantly ($p = > 0.05$).

*Demodex folliculorum* was detected on 80.23% of the 86 participants tested in the extended treatment study. The overall median quantity of DF found per participant pre-treatment was 2.00 mites (IQR 2.00 – 5.00). There was no significant difference in quantity of DF between the three treatment groups pre-treatment ($p = 0.22$). The quantity of DF significantly reduced after four weeks of treatment in all three groups ($p < 0.05$). There was no difference in efficacy between the three treatments at reducing quantity of DF ($p = 0.50$). Subjective symptoms reported were significantly improved after two and four weeks of treatment in all three groups ($p < 0.05$). There was no difference in efficacy between the three treatments to reduce symptoms after two or four weeks ($p > 0.50$).

**Conclusion:** There was a relatively high prevalence of DF discovered amongst both study cohorts. OCuSOFT applied nightly for two weeks significantly reduced the quantity of DF found post-treatment in the preliminary study, but it did not eradicate the presence

completely. Similarly, when treatment was extended to four weeks, all three methods tested demonstrated good ability to reduce DF quantity, improve subjective symptoms and help treat DF blepharitis. However, complete eradication was still not achieved. Baby shampoo demonstrated no therapeutic effect on DF infestation and alternative treatment options should be considered for the treatment of DF blepharitis.

## 5.2 Introduction

The structure and classification of DF has been described previously in Section 1.4. As mentioned, DF are ubiquitous to human skin, feed on sebum and epidermal skin cells and are therefore commonly found in larger quantities on the face: cheeks, nose, chin and eyelashes.[42,43,55] Although often considered a normal saprophytic component of our biological flora and fauna,[66–68] DF have also been noted as opportunistic parasites: proliferating and causing inflammatory reactions in susceptible individuals.[69] As such, DF have been associated with inflammatory skin conditions such as rosacea,[69,70,81] and inflammatory eyelid conditions such as anterior blepharitis.[7,43,46,49]

Indications of ocular DF infestation reported in the literature include; CD, eyelash abnormalities, anterior and posterior blepharitis, MGD, conjunctival and eyelid hyperaemia, corneal superficial vascularisation and opacities.[6,25,28,49,54,143] Symptoms of ocular DF infestation are similar to dry eye symptoms; itch, irritation, redness, burning sensation, visual disturbance.[6,13,15,16,46,47] However, as was demonstrated in Chapetr 4 and previously in the literature,[25,49] not all patients with DF will be symptomatic. This can lead to difficulties in deciding who requires treatment and when to begin.

Several of the risk factors associated with DF infestation have been discussed previously in Section 1.6 and in Chapter 3. Age has consistently been found to be one of

the most significant risk factors for the presence of DF infestation.[13,28,77] Due to increasing longevity, *Demodex* blepharitis in the elderly causing anterior eyelid abnormalities and subsequent dry eye, ocular discomfort and ocular morbidity will increase, resulting in an increased burden on the health system when patients seek treatment.[121]

To date, the majority of interventional studies have researched treatment of *Demodex* skin infestation, with varying results.[94,158–160,224–227] As mentioned previously in Section 1.8.1, in recent years, researchers have found TTO to be effective at killing DF,[12,53] and its use in treating DF blepharitis is expanding.[6,13,15,228] It's effectivity as a treatment is undeniable, but it is not without its disadvantages. Even at the diluted concentration of 50%, TTO is still toxic to the ocular surface. The College of Optometrists in the UK released guidelines for the use of TTO in practice stressing that "*daily lid scrub with 50% tea tree oil … should be undertaken only by experienced practitioners as such preparations are toxic to the ocular surface*".[3] Increased chair time with specialist practitioners can be costly to patients and or the governing health board. Also, the treatment experience can be uncomfortable for patients. Additional studies have examined the efficacy of other anti-parasitic medications, such as ivermectin and metronidazole, with varying reports of success.[34,36,38] However, the use of ivermectin and other systemic anti-parasitic drugs are not without their complications,[152–157] and may not be suitable for all patients.[151] Alternative therapies need to be available for those not suitable, or in countries were the drug has not yet been licensed for human use.

There are many products available over-the-counter to consumers, marketed for the treatment of blepharitis. However, a systematic review recently carried out by Lindsley et al[2] highlights the lack of knowledge and evidence based research available to clinicians regarding the commercial products available and marketed for the treatment of blepharitis.

The aim of the current study was to investigate the efficacy of different treatment methods at reducing the quantity of DF. This was a patient-outcome focused, clinically relevant study, with the potential benefit of being a more practitioner and patient friendly treatment alternative to TTO. This study will provide evidence-based results on the performance of commercial products available to patients and practitioners for the treatment of DF blepharitis in a clinical setting; demonstrating that optometrists and ophthalmologists are ideally placed to detect and begin first line treatment in many cases of DF infestation.

## 5.3 Methods

All participants were recruited through the National Optometry Centre, TU Dublin. Written informed consent was obtained from all participants prior to enrolment. Participants were eligible to participate if they were ≥ 18 years of age. Participants were excluded if they; presented with ocular disease (apart from MGD and blepharitis), were currently using blepharitis treatment or had used such treatment within the last six months or had ocular surgery in the last six months.

### *5.3.1 Pilot Study*

Minimum sample size required for statistical significance was calculated using G*Power analysis. A priori analysis for repeated measures ANOVA between factors, two groups two measurements, was conducted; alpha = 0.05, Power = 0.8, arbitrary effect size = 0.5; minimum sample size required n = 26.

Fifty participants enrolled between October 2014 and March 2015. Following attrition, 41 participants completed the two-week treatment study. Each participant completed the GHL and modified OSDI symptom questionnaires. Severity of subjective symptoms was

graded according to the total modified OSDI symptom score (refer Table 20). Calculation of the total modified OSDI symptom score, using the formula, has been discussed in detail in Section 4.3.1. Slit lamp examination was conducted by one optometrist (the author: Murphy, O). Clinical findings recorded were: conjunctival hyperaemia, MGD grade, CD, and fluorescein TBUT. Tear break-up time was measured in seconds, approximately one minute after the instillation of fluorescein. An average of three measurements was recorded. *Demodex* investigation involved examining eight eyelashes, two from each eyelid, on a slit-lamp biomicroscope (Topcon SL-D701, Topcon Medical Systems Inc., Dublin, Ireland). Each eyelash was first manipulated (as described in Section 1.7.4) using sterile forceps and was subsequently epilated for microscopic examination. Adult DF count was recorded using the modified Coston method (described in Section 1.8.2).[25]

Each participant received a treatment pack containing both the treatment (OCuSOFT) and a control lid scrub (10% baby shampoo) to use nightly for two weeks. In order to ensure 10% was used, vials with the exact measurement of shampoo were made up by the author (Murphy, O) and instructions were given to patients on how to fill with water at home and scrub the eyes (refer Appendix 5 (a)). The treated eye was randomised and blind to the examiner. Participants returned following two weeks treatment and the process was repeated and findings were recorded.

Following peer-review feedback received on the results of the pilot study, suggested changes were incorporated, and the extended treatment study was developed. Firstly, the study was extended to four weeks, to ensure sufficient time was given to tackle DF infestation, given their lifespan is 14 – 18 days.[40,42,43,61] Secondly, both eyes were treated with the same treatment, to prevent cross-contamination through migration of DF from

control eye to the treated eye. Thirdly, reviewers suggested comparing OCuSOFT to a tea tree-based product (TTFW) as opposed to baby shampoo.

### *5.3.2 Extended Study*

Minimum sample size required for statistical significance was calculated using G*Power analysis. Effect size was calculated from the mean difference in quantity of DF, pre and post treatment, from the treatment group in the pilot study and the SD of the pre-treatment group. The pre-treatment group was chosen as it is representative of the population not affected by experimental intervention: (2.32 – 0.66)/ 3.30 = 0.50. A priori analysis for repeated measures ANOVA between factors, three groups two measurements, was conducted; alpha = 0.05, Power = 0.8, effect size = 0.5; minimum sample size required n = 33.

One hundred and six participants enrolled between May 2015 and May 2017. Following attrition, 86 participants completed the four-week extended treatment study. As with the pilot study, each participant completed the modified OSDI symptom questionnaire. Participants underwent the same slit-lamp examination described above, and likewise were examined for the presence of DF as previously described.

Participants were randomly divided into three groups according to treatment: TTFW (Group A, n = 28), OCuSOFT (Group B, n = 30) and BlephEx$^{TM}$ microblepharoexfoliation device (Group C, n = 28). Each treatment has been previously discussed in detail in Section 2.4. Randomisation was achieved using the random number generator function on Excel. Each treatment was randomly assigned a number from 1 to 108. Each participant chose a number and was subsequently given the treatment assigned to that number. The examiner (author; Murphy, O) was blind to the treatment throughout all stages of the study for Groups

A+B. The examiner performed the BlephEx$^{TM}$ treatment on participants in Group C and was therefore not blind to treatment in this group.

The lid scrub routine was previously outlined in Table 6. In house microblepharoexfoliation was carried out on Group C at the initial visit only. The procedure was conducted as per manufacturer's guidelines (refer Table 7). All participants returned for a check-up appointment at two weeks and again for a final check at four weeks.

### *5.3.3 Statistical Analysis*

Statistical analysis was performed using the SPSS (version 25.0). Data was assessed for normal distribution using Shapiro-Wilk. All outcome measures investigated were determined to have a non-normal distribution ($p < 0.001$). All summarised continuous data was expressed as median and IQR. Between group data was assessed using the MWU test and KW test where appropriate. Wilcoxon-signed ranks test (WSR) was used to analyse within group data. The data between categorical variables was assessed using $X^2$ analysis. Spearman's correlation co-efficient was used to assess scaled and continuous variables; $p \leq 0.05$ was considered statistically significant.

## 5.4 Results

### *5.4.1 Pilot Study*

Forty-one participants (21 males: 20 females) with a median age of 45.00 years enrolled in the two-week pilot treatment study. At baseline, an overall prevalence of 68.23% DF was found, with a median quantity of 2.00 mites (IQR 0.00 – 8.00) per participant detected. *Demodex folliculorum* was discovered on 14 males (66.67%) and 11 females (55.00%) ($X^2$; $p = 0.28$).

Table 26. Comparison of age, gender and symptoms for subjects with and without *Demodex folliculorum*: Pilot Study. Age (median and IQR); A = Mann Whitney-U: B = Chi-square. *Significant results highlighted in bold.

| ***Demodex* Present (n)** | **Age (yrs.)** | **Modified OSDI score (0-100)** | **Presence Symptom** |
|---|---|---|---|
| No (n = 13) | 27.00 (22.00 – 55.00) | 10.00 (6.67 – 13.89) | 40.63% |
| Yes (n = 28) | 50.50 (45.00 – 59.00) | 15.83 (11.67 – 21.67) | 58.00% |
| | ***p = 0.02 (A)** | ***p = 0.02 (A)** | p = 0.13 (B) |

As can be seen in Table 26 above, participants with DF were significantly older than those without (MWU: p = 0.02). Increasing age was also significantly associated with increasing quantity of DF ($r_s$ = 0.44, p = 0.004). Figure 19 illustrates the positive relationship between increasing quantity of DF and increasing age.

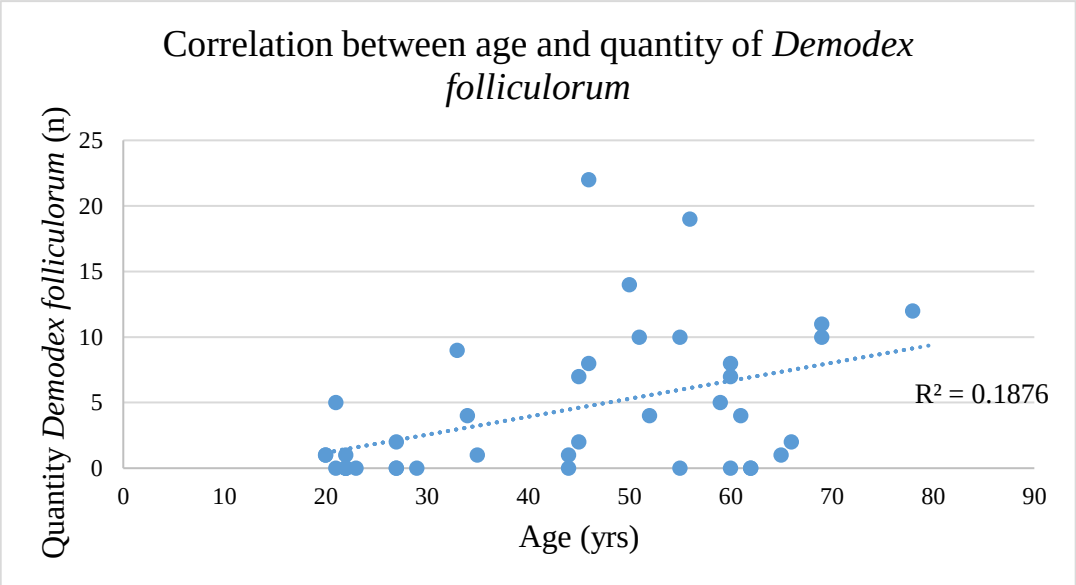



Figure 19. Scatter plot illustrating the positive correlation between increasing age and increasing quantity of *Demodex folliculorum*.

At baseline, participants with DF had a significantly greater modified OSDI score than those without (MWU: p = 0.02). (refer Table 26). However, no significant correlation was detected between increasing quantity of DF and increasing modified OSDI score ($r_s$ = 0.21: p = 0.19). Likewise, there was no association found between quantity of DF and severity of symptoms (KW: p = 0.38).

Bulbar conjunctival hyperaemia was graded using the Efron grading scale. Presence and quantity of DF were not significantly associated with conjunctival hyperaemia ($X^2$ p = 0.312 and $r_s$ = 0.074 p = 0.508). Most subjects with DF did have trace or mild conjunctival hyperaemia; however, overall most subjects had trace or mild conjunctival hyperaemia (refer Figure 20). A low positive correlation was detected between quantity of DF and severity of conjunctival hyperaemia, but it was not significant ($r_s$ = 0.074 p = 0.508).

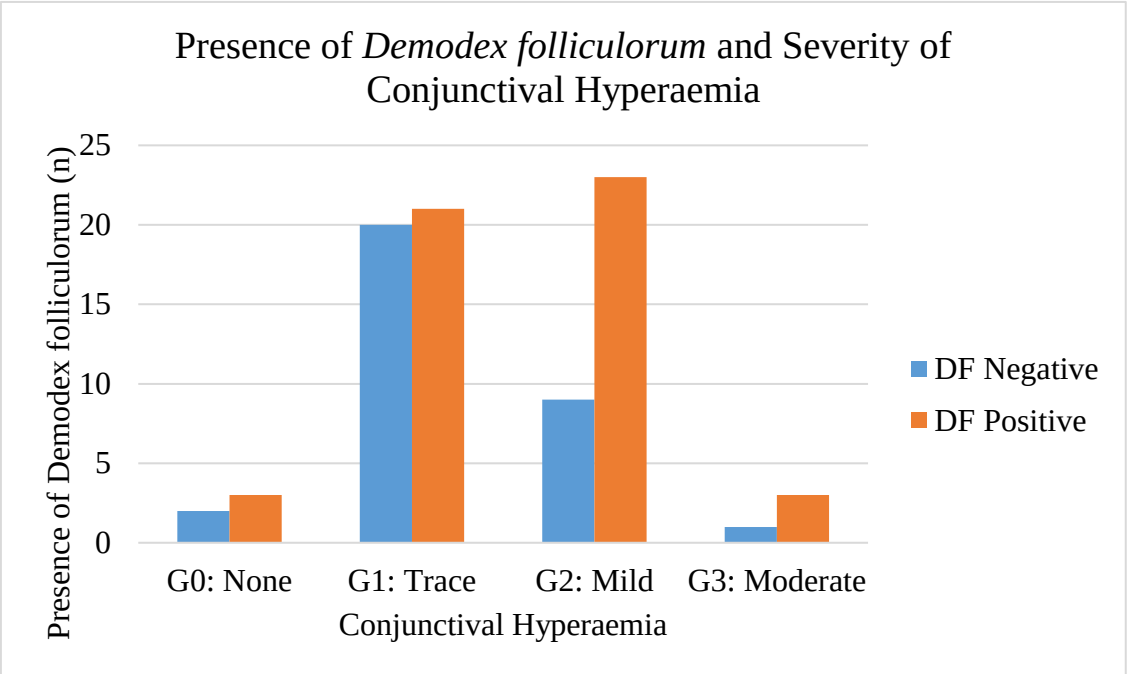



Figure 20. Bar chart illustrating the relationship between presence of *Demodex folliculorum* and severity of conjunctival hyperaemia ($X^2$ p = 0.312).

As mentioned previously, CD has been established as a pathognomonic sign for DF.[25] As expected, both the presence and quantity of DF were significantly associated with increased severity of CD ($X^2$: $p < 0.001$ and $r_s = 0.68$: $p < 0.001$ respectively). Figure 21 illustrates the significant relationship between presence of DF and grade of CD. As can be seen from Figure 11, the majority of participants without CD also had no DF, and ≥ G2 CD was considerably associated with the presence of DF. The definition of CD severity grades applied in the study can be seen in Table 4

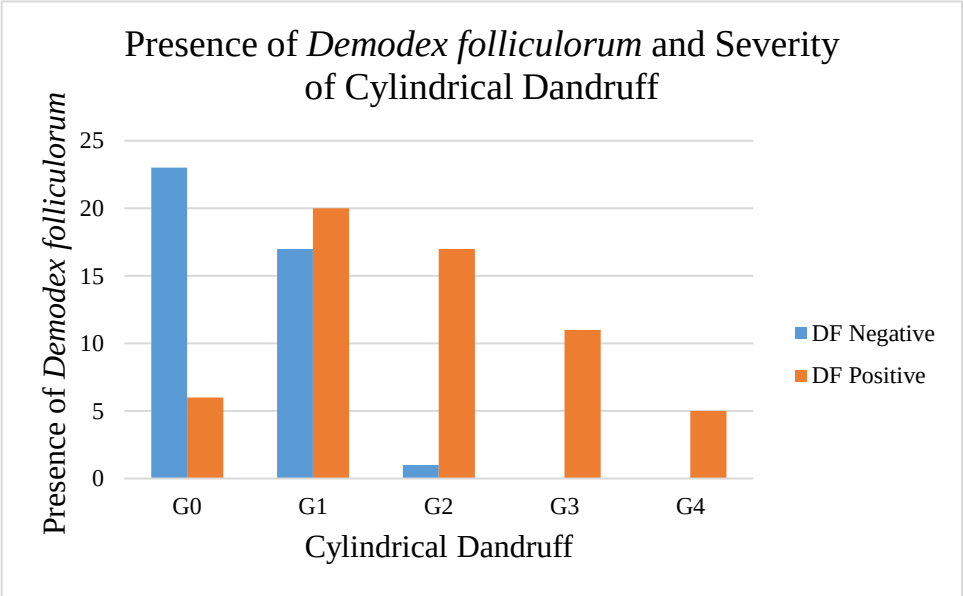



Figure 21. Bar chart illustrating the relationship between presence of *Demodex folliculorum* and severity of cylindrical dandruff ($X^2$ $p < 0.001$).

Figure 22 demonstrates the correlation between quantity of DF and severity of CD. As can be seen from Figure 22, the quantity of DF increases significantly with increasing severity of CD ($r_s = 0.68$, $p < 0.001$).

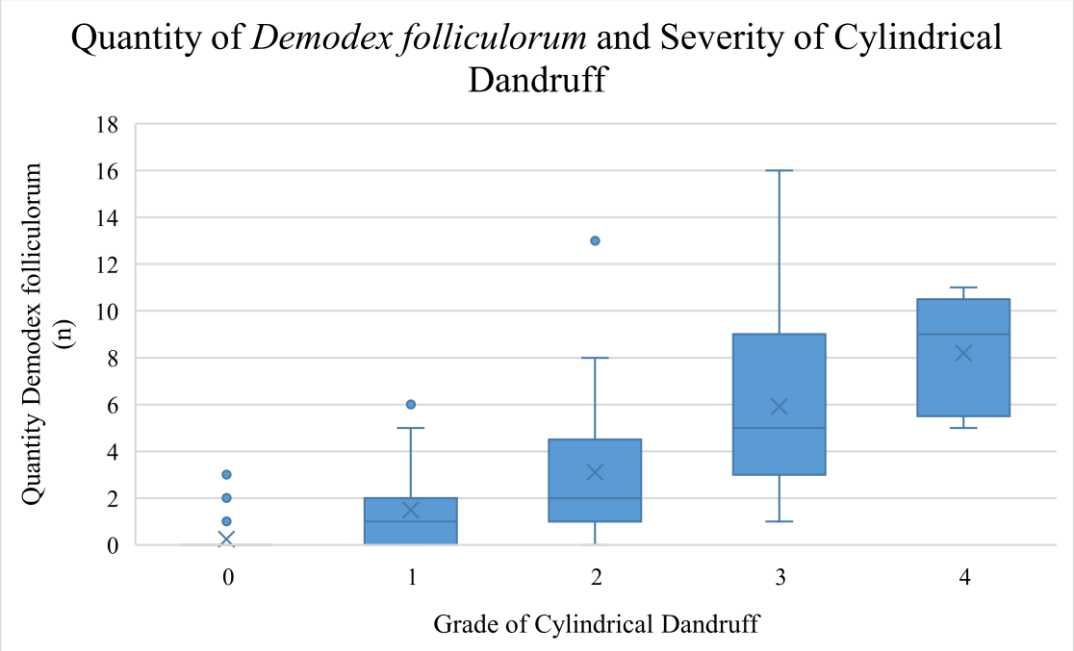



Figure 22. Box plot illustrating the relationship between quantity of *Demodex folliculorum* and severity of cylindrical dandruff ($r_s = 0.68$, $p < 0.001$).

Similarly, there was a significant relationship detected between presence and quantity of DF and MGD ($X^2$ $p = 0.01$ and $r_s = 0.23$, $p = 0.04$ respectively) (refer Figure 23). There was no significant relationship demonstrated between DF presence or quantity and TBUT (MWU: $p = 0.38$ and $r_s = 0.11$: $p = 0.50$ respectively).

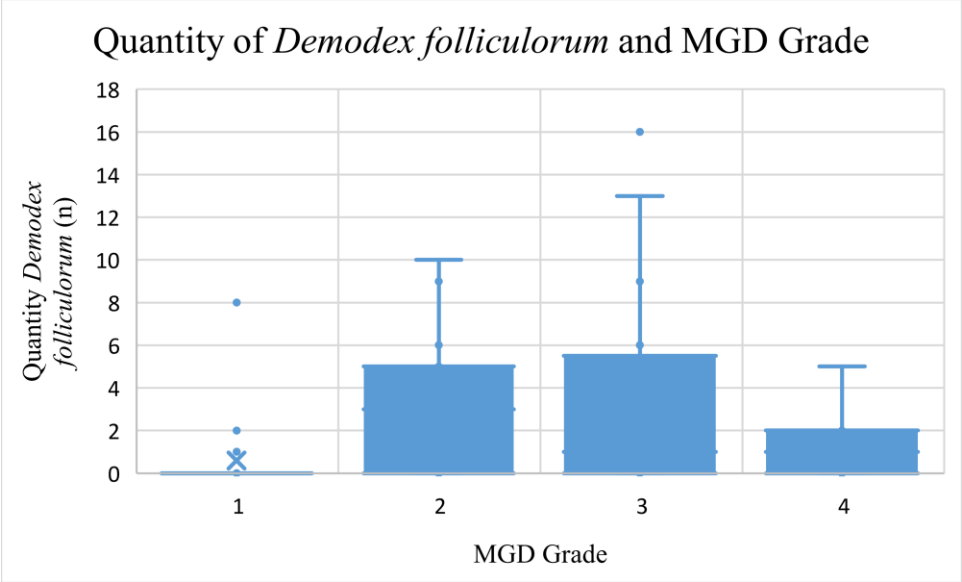



Figure 23. Box plot illustrating the relationship between quantity of *Demodex folliculorum* and meibomian gland dysfunction ($r_s$ = 0.23, p = 0.04).

Other significant findings from the pilot study included: a higher quantity of DF detected amongst participants who cleaned their bed linen more frequently (KW: p = 0.002), and a lower presence and quantity of DF amongst participants who wore makeup compared to those that didn't ($X^2$ p = 0.03 and MWU p = 0.01) (refer Figure 24). However, further analysis showed increasing age was a significant factor amongst those that wore makeup and cleaned their bed linen more frequently, which is likely to have skewed that result (MWU: p = 0.002 and KW: p = 0.03). No significant relationship was found between frequency of eyelid hygiene and presence or quantity of DF (KW p = 0.77).

Figure 24 shows a box plot illustrating the quantity of DF amongst female makeup wearers and non-makeup wearers. Males were excluded from this analysis as no males in the study reported wearing makeup. Females who wore makeup demonstrated a lower prevalence of DF infestation (45.22% versus 80.00%; $X^2$: p = 0.05) and quantity of DF

(median 0.00 IQR 0.00 – 2.00 versus median 4.00 IQR 3.25 – 6.00; MWU p = 0.006). Participants who wore makeup were younger, but not significantly (47.00 years IQR 33.00 – 56.00 versus 57.00 years IQR 51.00 – 69.00. MWU: p = 0.07).

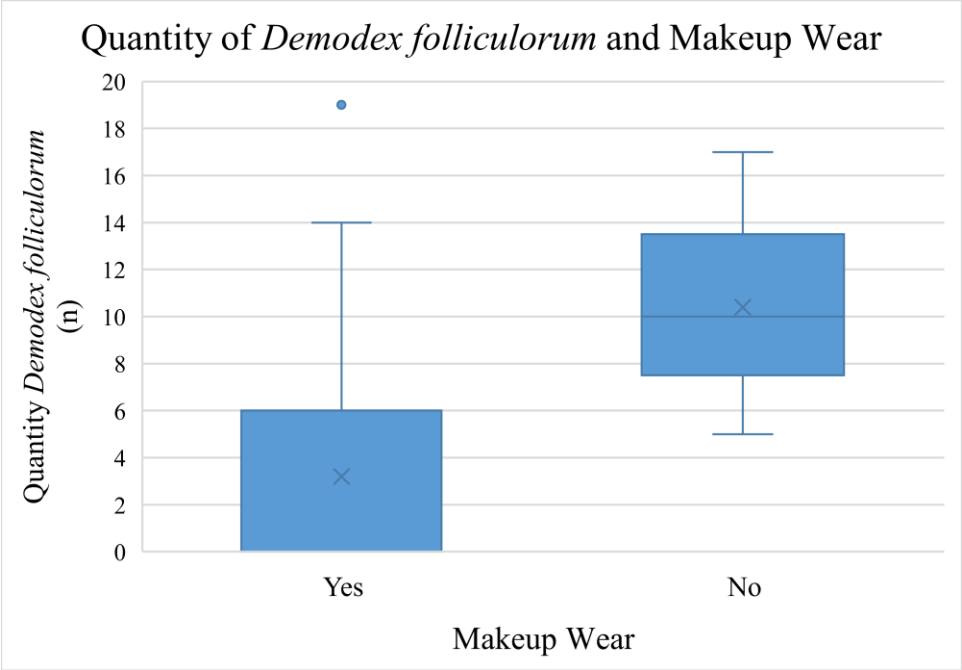



Figure 24. Box plot illustrating quantity of *Demodex folliculorum* found amongst female makeup wearers and non-makeup wearers.

Each participant was given two treatments, OCuSOFT and 10% baby shampoo, one to use on each eye nightly for two weeks, to assess the efficacy of each treatment against DF infestation. Pre and post treatment results for DF quantity can be seen in Table 27. There was no significant difference in mean number of DF pre-treatment between the treatment and control eye. OCuSOFT demonstrated better efficacy at treating DF infestation than baby shampoo (refer Table 27). The presence of DF pre-treatment in the OCuSOFT eye was 65.85%. This dropped slightly to 51.22% post-treatment, but complete eradication of

DF was not achieved. Baby shampoo had no impact on DF infestation. Presence of DF in the baby shampoo cohort was 56.10% pre-treatment and 58.54% post-treatment.

Table 27. Quantity of *Demodex folliculorum* detected pre-treatment and post-treatment for each treatment group.

| | | **OCuSOFT** | **Baby shampoo** | p - value |
|---|---|---|---|---|
| **Quantity *Demodex folliculorum*** | Pre | 1.00 (0.00 – 3.00) | 1.00 (0.00 – 4.00) | MWU p = 0.77 |
| | Post | 0.00 (0.00 – 0.00) | 1.00 (1.00 – 2.00) | **MWU p = 0.01*** |
| | p - value | **WSR p = 0.001*** | WSR p = 0.71 | |

### *5.4.2 Extended Study*

Eighty-six participants (median age 43.50 years (IQR 29.00 – 63.50), 38 male:48 female) completed the four-week extended treatment study. Each participant completed the GHL and modified OSDI questionnaires at baseline and were examined for signs of dry eye and DF. At baseline, an overall prevalence of 80.23% DF was found, with a median quantity of 2.00 mites (IQR 2.00 – 5.00) per participant detected.

There was no significant difference detected between presence or quantity of DF and: contact lens wear ($X^2$: p = 0.28 and MWU: p = 0.96, respectively), use of makeup ($X^2$: p = 0.19 and MWU: p = 0.36, respectively), frequency of lid hygiene ($X^2$: p = 0.26 and KW: p = 0.16, respectively), frequency of cleaning bed linen ($X^2$: p = 0.45 and KW: p = 0.39, respectively), medical conditions ($X^2$: p = 0.26 and KW: p = 0.12, respectively), allergies ($X^2$: p = 0.52 and KW: p = 0.58, respectively) or skin conditions ($X^2$: p = 0.76 and KW: p = 0.51, respectively).

The relationship between prevalence and quantity of DF and type of lid hygiene is shown in Table 28. As can be seen from Table 28, there was a significant difference in prevalence and quantity of DF depending on the type of lid hygiene used. Bonferroni corrected post-hoc analysis ($p \leq 0.05/28 = 0.0018$) showed that only difference between J+J lid scrubs and 'other method' was found to be significant ($p = 0.001$). However, there was considerable difference in sizes and age between those two sub-groups which is likely to have had an impact on results.

Table 28. Type of lid hygiene descriptives: age, prevalence and quantity *Demodex folliculorum*: Extended Study. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square. *Significant results highlighted in bold

| | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| None | 24 | 51.50 (46.00 – 61.00) | 87.50 | 3.00 (2.00 – 9.00) |
| Cleanser/ Toner | 14 | 31.00 (26.00 – 43.00) | 35.71 | 0.00 (0.00 – 1.75) |
| Makeup Remover | 7 | 44.00 (28.00 – 82.00) | 100.00 | 1.00 (0.00 – 5.00) |
| Face Wipes | 6 | 61.00 (26.00 – 72.00) | 100.00 | 5.00 (1.00 – 7.00) |
| J+J Lid Scrubs | 3 | 69.00 (69.00 – 69.00) | 100.00 | 15.00 (9.00 – 17.00) |
| Other Lid Scrubs | 3 | 49.00 (39.00 – 81.00) | 66.67 | 1.00 (0.00 – 21.00) |
| Other Method | 19 | 36.00 (31.00 – 57.00) | 89.47 | 2.00 (1.00 – 5.00) |
| Multiple | 10 | 34.50 (29.00 – 47.00) | 80.00 | 5.50 (0.00 – 8.00) |
| | | p = 0.17 (A) | ***p = 0.001 (B)** | ***p = 0.01 (A)** |

The relationship between prevalence and quantity of DF and temperature bed linen was washed at was also found to be significant (refer Table 29). Five participants reported not knowing what temperature the bed linen was washed at, and they were removed from analysis. As can be seen from Table 29, as the temperature increased, the prevalence and quantity of DF decreased. However, Bonferroni corrected post-hoc analysis ($p \leq 0.05/3 = 0.0167$) showed that none of the differences between the sub-groups were found to be significant (smallest $p = 0.018$ 30°C versus 60°C).

Table 29. Temperature bed linen washed descriptives: age, prevalence and quantity *Demodex folliculorum*: Extended Study. Age (median, IQR), *Demodex folliculorum* quantity (median, IQR); A = Kruskal-Wallis: B = Chi-square. *Significant results highlighted in bold.

| **Temp (°C)** | **N** | **Age (yrs.)** | **Prevalence (%)** | **Quantity *Demodex* (n)** |
|---|---|---|---|---|
| 30 | 19 | 40.00 (31.00 – 61.00) | 100.00 | 5.00 (3.00 – 11.00) |
| 40 | 43 | 43.00 (39.00 – 57.00) | 79.07 | 2.00 (2.00 – 5.00) |
| 60 | 19 | 49.00 (33.00 – 58.00) | 57.89 | 1.00 (1.00 – 5.00) |
| | | p = 0.93 (A) | ***p = 0.01 (B)** | ***p = 0.048 (A)** |

Presence of DF was not significantly associated with grade of conjunctival hyperaemia ($X^2$ $p = 0.62$). There was a significant correlation detected between quantity of DF and grade of conjunctival hyperaemia ($r_s$ 0.24 $p = 0.03$). As can be seen in Figure 25, quantity of DF appears to be associated with moderate hyperaemia (G2) but not severe (G3).

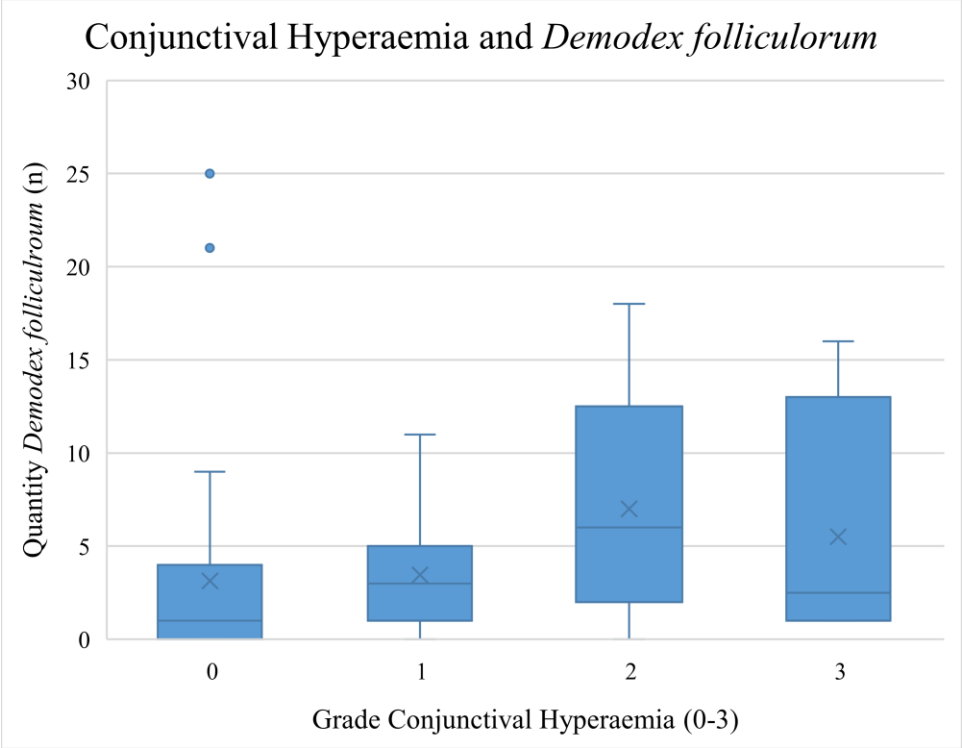



Figure 25. Box plot illustrating the relationship between quantity of *Demodex folliculorum* and conjunctival hyperaemia ($r_s$ = 0.24, p = 0.03).

There was a significant correlation between quantity DF and increasing severity of CD ($r_s$ = 0.61: p < 0.001) (refer Figure 26). There was also a significant positive correlation between age and CD ($r_s$ = 0.37: p < 0.001).

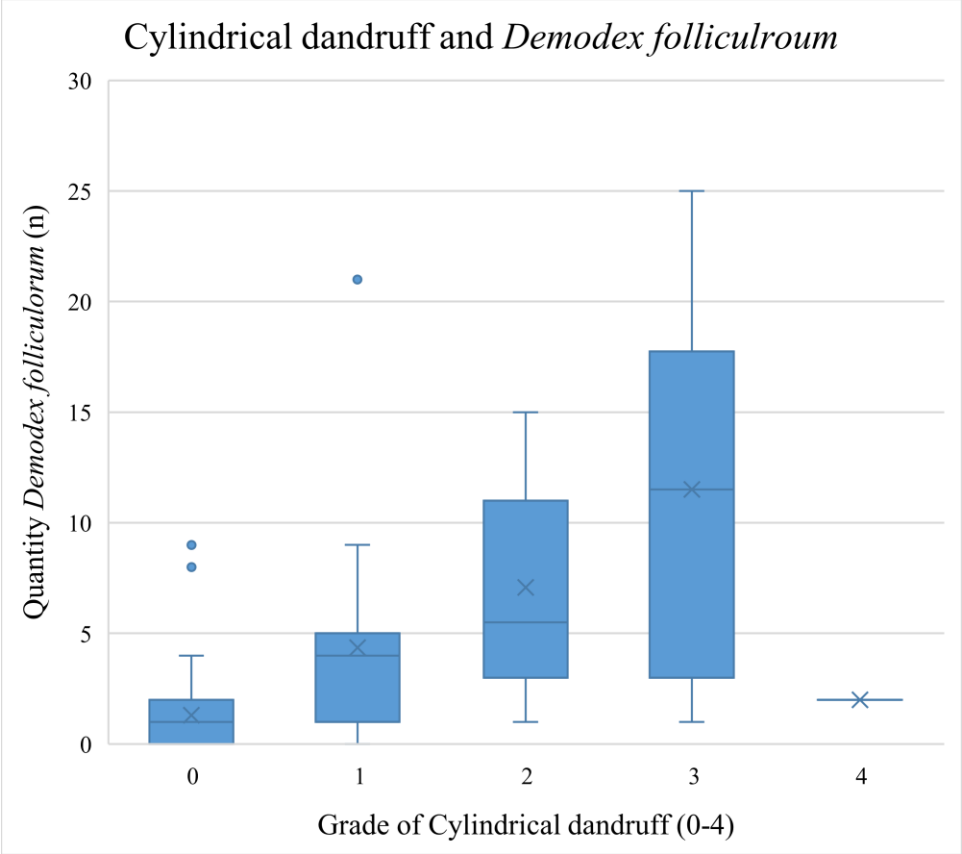



Figure 26. Box plot illustrating the positive correlation between cylindrical dandruff and quantity of *Demodex folliculorum* ($r_s = 0.61$: $p < 0.001$).

A significant correlation was also detected between quantity of DF and MGD grade ($r_s$ $p = 0.25$: $p = 0.03$). This is illustrated in Figure 27 below. Increasing age was also significantly associated with increasing grade of MGD ($r_s = 0.56$: $p < 0.001$).

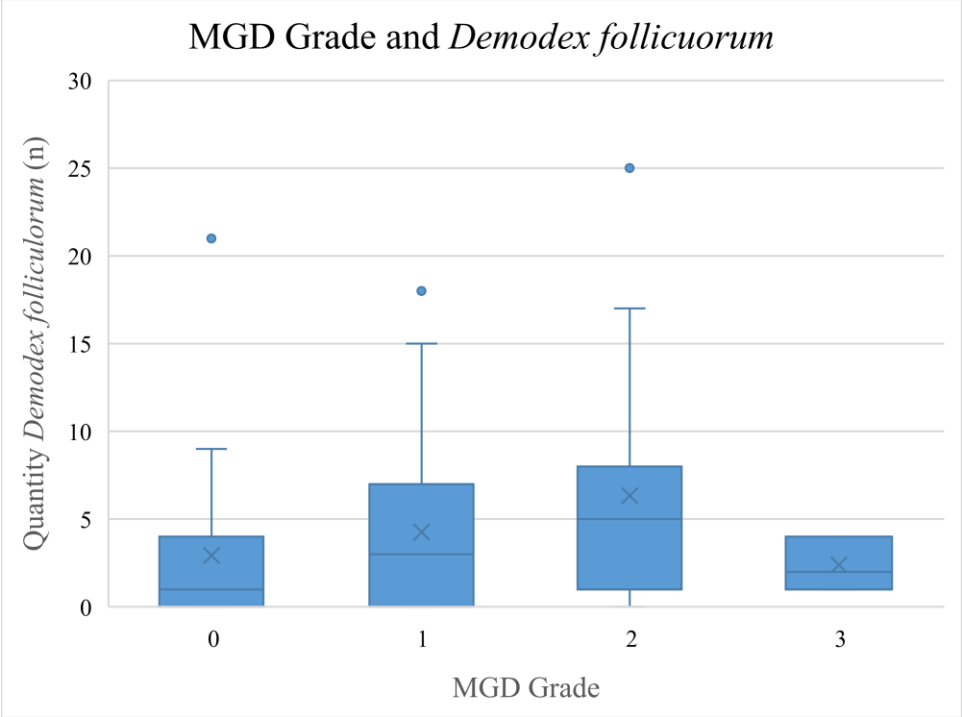



Figure 27. Box plot illustrating the positive correlation between meibomian gland dysfunction and quantity of *Demodex folliculorum* ($r_s = 0.25$: $p = 0.03$).

Participants were then divided into three groups according to treatment (refer Table 30). Participants who did not have any DF were used as controls, therefore statistical analysis on quantity of DF was only applied to individuals found positive for DF (n = 69).

Table 30. Number of participants with *Demodex folliculorum* and number of control participants in each group.

| | **Participants with DF (n)** | **Control (n)** | **Total (n)** |
|---|---|---|---|
| **Group A: TTFW** | 22 | 6 | 28 |
| **Group B: OCuSOFT** | 24 | 6 | 30 |
| **Group C: BlephEx™** | 23 | 5 | 28 |

Overall, the mean habitual logMAR visual acuity improved post-treatment (logMAR; 1.08 ± 0.26 at baseline, 1.13 ± 0.27 at two weeks, and 1.16 ± 0.26 at four weeks, Friedman's p = 0.02). Post-hoc analysis using WSR test, after alpha adjusted for Bonferroni correction ($\alpha$ = 0.016), showed that only the difference between baseline and four weeks was statistically significant (WSR p = < 0.001).

There was no significant difference in age between the three treatment groups. However, DF positive participants in group A and group B were significantly older than their respective control participants (refer Table 31).

Table 31. Age of participants with *Demodex folliculorum* and control participants in each group. *Significant results highlighted in bold.

| | **Group A TTFW (yrs)** | **Group B OCuSOFT (yrs)** | **Group C BlephEx™ (yrs)** | KW |
|---|---|---|---|---|
| **Participants with DF** | 44.00 (39.00 – 67.00) | 47.00 (37.00 – 57.00) | 49.00 (33.00 – 67.00) | p = 0.99 |
| **Control** | 27.00 (25.00 – 28.00) | 26.00 (26.00 – 67.00) | 33.00 (23.00 – 58.00) | p = 0.62 |
| MWU | **p = 0.01*** | **p = 0.01*** | p = 0.21 | |

Overall, participants with DF had a higher modified OSDI score compared to those without DF (median OSDI 26.67 IQR 20.83 – 35.00 versus 11.67 IQR 8.33 – 33.33, MWU: p = 0.03). However, no significant correlation was detected between increasing quantity of DF and increasing modified OSDI score ($r_s$ = 0.10 p = 0.35). Table 32 shows the breakdown of symptoms in all three treatment groups over the duration of the study. Total modified OSDI score reduced in all three treatment groups, however only Group B and Group C were found to be significant (Friedman's; p = 0.003 and p < 0.001, respectively). Nonetheless, there was no significant difference in symptoms or quantity of DF between each treatment

group at any visit over the duration of the study (Table 32). Results are for participants with DF only.

Table 32. Participants with *Demodex folliculorum*: Severity of symptoms and quantity of *Demodex folliculorum* in each group at baseline, two weeks, and four weeks. Severity of symptoms: Total modified OSDI number (median, IQR). Quantity of *Demodex folliculorum* (median, IQR). *Significant results highlighted in bold

| | **Group A TTFW** | **Group B OCuSOFT** | **Group C BlephEx™** | KW |
|---|---|---|---|---|
| *Symptoms* | | | | |
| **Baseline** | 25.00 (10.00 – 36.67) | 20.83 (11.67 – 33.33) | 25.83 (18.33 – 33.33) | p = 0.84 |
| **Two weeks** | 12.08 (5.00 – 18.33) | 8.33 (5.00 – 18.33) | 11.52 (8.33 – 11.67) | p = 0.63 |
| **Four weeks** | 12.02 (8.33 – 15.00) | 8.33 (3.33 – 10.71) | 8.33 (6.67 – 16.67) | p = 0.42 |
| Friedman's | p = 0.16 | **p = 0.003*** | **p = 0.001*** | |
| *Quantity DF* | | | | |
| **Baseline** | 2.00 (1.00 – 5.00) | 1.50 (1.00 – 4.00) | 3.00 (2.00 – 6.00) | p = 0.22 |
| **Two weeks** | 0.00 (0.00 – 2.00) | 0 00 (0.00 – 3.00) | 1.50 (0.00 – 3.00) | p = 0.70 |
| **Four weeks** | 0.00 (0.00 – 2.25) | 0.00 (0.00 – 3.00) | 0.50 (0.00 – 2.00) | p = 0.49 |
| Friedman's | **p < 0001*** | **p < 0001*** | **p < 0001*** | |

Although, overall, the majority of participants with DF were severely symptomatic: asymptomatic (n = 18), mild (n = 12), moderate (n = 11), and severe (n = 28): no statistically significant correlation was found between DF quantity and severity of symptom grade or modified OSDI score at baseline visit (KW: p = 0.47 and $r_s$ = -0.08: p = 0.54). As can be seen in Table 16; symptoms reduced progressively throughout the four weeks of treatment in each group. For groups B and C the reduction in symptoms over the four weeks was

significant. For group B post hoc analysis with WSR pairwise comparisons, α adjusted for Bonferroni correction ($\alpha = 0.016$), indicated that the improvement in symptoms was significant between baseline and week two ($p = 0.001$) and baseline and week four ($p = 0.001$) only. For group C post hoc analysis indicated that the improvement in symptoms was significant between baseline and week two ($p < 0.001$) and baseline and week four ($p < 0.001$).

Table 32 also demonstrates the reduction in numbers of DF over the course of the four weeks for each treatment group. Post-hoc analysis WSR test pairwise comparison, after Bonferroni correction applied, revealed: Group A significant reductions from baseline to week two ($p = 0.002$) and baseline and week four ($p < 0.001$), Group B significant reduction from baseline to week four ($p = 0.005$), and Group C significant reductions from baseline to week two ($p = 0.002$) and baseline to week four ($p = 0.001$). Similar to symptoms, the quantity of DF did continue to decrease from two weeks to four weeks, although the reduction in quantity between week two and week four was not significant (WSR; A: $p = 0.87$, B: $p = 0.94$, C: $p = 0.43$).

Participants with DF were more symptomatic than participants in the control group. However, although a significant correlation was found ($X^2$: $p = 0.005$), it was concluded that it was not a valid comparison due to the difference in sample size between the two groups. Furthermore, participants with DF were significantly older than control participants, and the impact age has on dry eye symptoms has been well established.[144] This is a confounding factor; therefore it cannot be assumed that the increased symptoms witnessed amongst participants with DF were as a result of DF alone.

There was no significant difference in control participants' symptoms at baseline between the three groups. There was no significant change in control participants' symptoms after treatment in group A and group C (Table 33). Group B did demonstrate a significant reduction in symptoms post treatment over time (Friedman's p = 0.02). However, due to the small sample size, it is difficult to take any relevance from this finding at present.

Table 33. Control participants: Severity of symptoms in each group at baseline, two weeks, and four weeks. Severity of symptoms: Total modified OSDI number (median, IQR). *Significant results highlighted in bold.

| | **Group A TTFW (n = 6)** | **Group B OCuSOFT (n = 6)** | **Group C BlephEx™ (n = 5)** | KW |
|---|---|---|---|---|
| *Symptoms* | | | | |
| **Baseline** | 7.50 (5.00 - 31.67) | 10.00 (5.00 – 33.33) | 13.33 (11.67 – 33.33) | p = 0.89 |
| **Two weeks** | 7.50 (3.33 – 15.00) | 5.28 (1.67 – 13.33) | 8.33 (3.33 – 21.67) | p = 0.97 |
| **Four weeks** | 7.50 (1.67 – 15.00) | 3.33 (0.00 – 26.67) | 8.33 (0.00 – 35.00) | p = 0.86 |
| Friedman's | p = 0.28 | **p = 0.02*** | p = 0.17 | |

## 5.5 Discussion

A reasonably high prevalence of DF was detected in both the pilot and extended study groups (61.00% and 80.23%, respectively), which is in good agreement with previous studies.[28,46,77] The overall median number of DF detected, per participant pre-treatment, was very similar between the two study groups (2.00 mites IQR 0.00 – 8.00 and 2.00 mites IQR 2.00 – 5.00 for the pilot and extended study respectively). As has been mentioned previously, the accepted consensus at present is that *Demodex* in low numbers are a normal

part of our microbiological flora and fauna.[66,229] However, when quantities of *Demodex* begin to proliferate, and the density of the mites increases beyond a critical density,[65,71] *Demodex* adopt a pathogenic role and can cause skin and ocular abnormalities. No significant relationship was detected between DF and skin conditions or allergies in either the pilot study or the extended treatment study: however, there were very few participants with skin conditions that took part to get statistically relevant results.

Increased quantities of DF have been associated with blepharitis,[7,43,46,49] chalazia,[26,230,231] corneal disturbance[6,143] and an increase in symptoms.[13,28,46,47] Similarly, both the pilot and extended studies, found significant associations between DF and increasing severity of CD, MGD and symptoms: adding further evidence to the pathogenicity of *Demodex*. Age was associated with increasing severity of CD and MGD. Cylindrical dandruff has been shown to be a bi-product of increased quantities of DF, and it is likely that age is not an influencing factor in this finding,[25,189] Age-related changes to the meibomian glands contribute to MGD,[210] and increasing age has also been repeatedly associated with increased quantities of DF.[13,28,77,78] As such, it is not possible from the results of the current studies to say whether the higher quantities of DF detected amongst subjects with MGD were as a result of participants with MGD being older, or if MGD alone is a risk factor for increased quantities of DF. Future studies should be age and sex-match controlled to avoid this.

Although the presence of DF was found to be significantly associated with increasing severity of symptoms, and the majority of participants with DF were found to be symptomatic, no correlation was detected between increasing quantity of DF and increasing symptoms in either the pilot or extended study. Again, age may be an influencer on this result[144]: it may be age-related dry eye that is causing the symptoms and not just DF.

However, treatment administered to reduce the quantity of DF significantly improved symptoms to normal levels; even though complete eradication was not achieved. In the extended study, the majority of control participants were asymptomatic, and treatment did not significantly reduce modified OSDI score. This adds further support to the theory that lower quantities of *Demodex* may be considered normal and of no immediate concern. Furthermore, as symptoms improved to normal levels in the absence of complete *Demodex* eradication; it could be argued that the aim of treatment does not need to be complete eradication, and that treatment could be considered successful when *Demodex* density is returned to normal levels.

As mentioned previously in Section 1.1, lid hygiene, using a '*variety of measures*', is the first line management recommended by both the AAO and College of Optometrists, regardless of the type of blepharitis.[1,3] The aim of lid hygiene is to reduce the bacterial load at the eyelid margin, helping to improve signs and symptoms associated with blepharitis.[3] Lid scrubs with diluted concentrations of baby shampoo have been the longstanding 'go-to' treatment for practitioners to advise their patients to use for regular home management of blepharitis. It is not entirely clear where baby shampoo as a treatment for blepharitis originated. However, in 2018, Sung et al[186] demonstrated that baby shampoo has a negative effect on goblet cell density, and thus could be more damaging to the tear film and ocular surface than therapeutic. Currently the AAO still recommend baby shampoo, or other dedicated cleansing pads, as first line management for blepharitis,[1] but the College of Optometrists have removed it from their clinical management guidelines in their most recent review.[3] At the time this study was conducted, the effect of baby shampoo on goblet cell density had not been established, and it remained on the recommended guidelines for practitioners. Furthermore, investigation into the comparative efficacy of different lid

hygiene measures had been recommended.[2] Gao et al[12] found that the survival time of DF in 50% baby shampoo solution was > 150 min, and patients treated with baby shampoo lid scrubs for up to 350 days showed no significant change in DF quantity. The results from the pilot study demonstrated a similar inadequacy by baby shampoo to treat *Demodex* blepharitis. There was no reduction in quantity of DF achieved on the eye treated with baby shampoo, compared to a significant reduction in quantity of DF achieved on the eye treated with OCuSOFT.

A '*variety of measures*' now exist for the treatment of blepharitis.[3] Over-the-counter eyelid cleansers for blepharitis have become available in recent years, but little evidence as to their ability to treat the condition currently exists.[2,3] OCuSOFT is marketed as a product for moderate to severe blepharitis sufferers, with bacterial/*Demodex* involvement. The active ingredient for killing DF used in OCuSOFT is 1,2-Octanediol. As mentioned in Section 2.3.2, 1,2-Octanediol is a surfactant with antimicrobial abilities. Burgess et al[187] investigated the efficacy of 1,2-Octanediol at treating head lice infestation, and demonstrated that a 5% solution of 1,2-Octanediol, left on for eight hours over night, effectively eliminated an established head louse infestation, with an 80% cure rate after only one use. Observations from the same study demonstrated that lower concentrations of 1,2-Octanediol solutions (1%) also killed head lice, but at a slower rate.[187] It was proposed that the chemical disrupted the cuticular lipid of the lice, causing them to become dehydrated and die.[187] It has been established previously that DF die when they become dehydrated.[40] Thus, it is possible that this proposed method works similarly on *Demodex*. The effect of pediculicides is not always instantaneous and subsequently some micro-organisms may survive long enough to lay eggs following treatment. Burgess et al[187] also found that 5% 1,2-Octanediol reduced head lice egg laying. However, previously laid eggs

were unaffected, and could potentially survive to start a new infestation.[187] OCuSOFT formula contains a 0.5% concentration of 1,2-Octanediol to ensure the product is non-irritating, yet still effective when used repeatedly for a period of time. As the formula is non-irritating, this promotes better participant compliance and willingness to use the treatment over multiple uses. The pilot and extended study both found that OCuSOFT was effective at reducing quantity of DF over both a two- and four-week period. Even after four weeks, complete elimination was not achieved. As the treatment does not appear to effect previously laid eggs, it is conceivable that these eggs hatched to give rise to the next generation. Additionally, complete coverage is required to be effective. If coverage by an applicant is incomplete, some DF mites may survive to lay and hatch more DF: although with continuously reducing quantities. However, Burgess et al[187] also specified that with 5% 1,2–Octanediol egg laying was completely inhibited and previously laid eggs did not mature to hatch. It is possible that 0.5% 1,2-Octanediol does not have the same toxic effect on eggs. Likewise, Burgess et al[187] investigated efficacy on head louse and not *Demodex.* Although both are ectoparasites, no study could be found that compared the similarities and differences between the two. Furthermore, as mentioned earlier, complete eradication may not be necessary for successful treatment.

In recent years, TTO and ivermectin have emerged as the go-to-treatment options for *Demodex* blepharitis.[6,12,13,15,34,36,38] As mentioned in Section 2.4.3, it has been established that terpinen-4-ol is the active ingredient in TTO effective at killing DF in a dose dependent manner.[12,53] Several studies have found that 50% TTO applied weekly is effective at reducing DF infestation,[6,13,15] and even at as low a concentration as 5% TTO is effective at killing *Demodex* when applied twice a day.[35] Although application of 50% TTO is the recommended treatment for *Demodex* blepharitis,[1,3] the disadvantages of this (ocular

irritation and toxicity) have been discussed in detail in Section 2.4.3. Following feedback from the pilot study, the aim was to incorporate a tea tree-based treatment that could potentially serve as a good alternative to baby shampoo. Hence, the extended study investigated the efficacy of nightly lid scrubs with TTFW for the treatment of DF blepharitis. The TTFW used in the current study had a 38% concentration of terpinen-4-ol and has shown to effectively reduce DF count over a four-week period. The extended study focussed on the use of TTFW as a treatment for blepharitis; as such, participants only scrubbed their eyelids. However, the TTFW can be used on the entire face; theoretically providing the ability to treat DF, if present, on the facial skin also. Furthermore, if *Demodex* on the face are also being treated, this reduces the risk of migration of mites back to the eyelashes again following topical treatment. The results of the extended study show that TTFW was effective at reducing signs and symptoms of *Demodex* blepharitis. An advantage of TTFW is that it can be applied at home as part of a routine facial cleaning regime, and does not require experienced practitioner application, thus reducing chair time and cost for the patient. However, irritation was still a factor with the TTFW, which could impact patient compliance in the long run.

The extended study also included a third treatment group: BlephEx$^{TM}$ was used as an adjunct therapy with OCuSOFT for Group C. BlephEx$^{TM}$ lid scrub was given to participants in-office before they began nightly home lid scrubs with OCuSOFT, similar to the way 50% TTO lid scrubs were performed in office for participants in previous studies.[6,13,15] The aim was to incorporate the BlephEx$^{TM}$ in an effort to help reduce the bacterial load prior to commencing home lid scrubs. The results of the extended treatment study found the greatest reduction in DF quantity and greatest improvement in symptoms in the BlephEx$^{TM}$ group. Even among the control participants who had no DF, they reported a significant

improvement in symptoms after two weeks. The authors' postulate that this is as a result of the scrubbing and exfoliation action of the BlephEx$^{TM}$; which leaves the eyelids feeling completely cleaned and refreshed regardless of the presence or absence of ocular disease.

### *5.5.1 Limitations*

A strength and limitation of the pilot study was that treatment was administered to only one eye. This allowed age and sex-match control for treatments and kept compliance of treatment and control the same. However, it did not prevent the possibility of cross-contamination of DF from the control eye to treated eye. Secondly, treatment was administered for a two-week period initially, which is slightly less than the lifespan of DF (14 – 18 days). This time frame was chosen as the first follow up for participants as the aim of the study was to find an effective treatment for *Demodex* blepharitis that can be easily administered and managed by optometrists in practice. Treatment non-compliance is an issue affecting efficacy of treatments in all facets of the medical profession.[232] As such, the treatment protocol was chosen to be easy to follow, as non-time consuming as possible in order to fit in with daily routines, and a short duration to help improve compliance. This is a realistic working timeframe for practitioners to administer and patients to use in practice with good compliance. A third comment made by peer-review was the lack of a tea tree-based treatment for comparative purposes.

The extended study attempted to account for these limitations and improve on them. Treatment was applied to both eyes, treatment duration was extended to four weeks, and TTFW was incorporated as a comparative treatment. However, the extended study was not without its own limitations. One such limitation of the extended study is that the group of control participants was a much smaller and younger group than the participants with DF

(Table 30). As a result, no comparisons have been made between the two groups with regards to symptoms. Given the unequal sample sizes, and the association between dry eye and increasing age, it was concluded that it would not be a valid comparison. To completely understand the relationship between DF infestation and symptoms, and the effect of treatment on those symptoms, future study cohorts should be age and sex – matched controlled.

It should also be noted that in both studies, the quantity of DF among some participants with DF pre-treatment was recorded as zero. As mentioned previously, a limitation of eyelash epilation is that sometimes DF remain within the follicle and are not removed with the eyelash, although the DF tails are clearly visible on slit lamp examination. This occurred mainly in highly infested damaged follicles where the lashes were loose. As a result, an accurate account of DF quantity that reflects severity of infestation is difficult to achieve from eyelash epilation and microscopic counting alone. Mastrota[50] describes eyelash rotation as an alternative technique to eyelash epilation to confirm DF infestation. This finding prompted investigation into incorporating eyelash manipulation to help accurately diagnose the severity of infestation and thus provide better information clinically to practitioners, in order to understand and know who and when to treat. This is discussed in detail in Chapter 8.

None of the treatment methods tested in both the pilot and extended treatment study fully eradicated DF in all participants. Potential reasons for this could be; the duration of treatment, frequency of application, participant compliance, and migration of DF. Participants scrubbed their eyelids nightly for two to four weeks. This may be too short a time frame to treat generations of DF. Similarly, treatment was only applied once a day, at night, and may be more successful if applied in the morning also. Furthermore, it is possible

that participants did not follow lid scrub instructions carefully, which could impact efficacy of treatment. Future studies could monitor compliance by requesting participants to return empty and/or unused treatments at the end of the study. Finally, DF can reside in other hair follicles on the face and body, not just the eyelashes. Therefore, it is possible that DF may have migrated back to the eyelashes from other locations; hence, total eradication of DF may not be possible using a local treatment.

## 5.6 Conclusion

These studies have demonstrated that nightly lid hygiene with both OCuSOFT and TTFW are effective at reducing DF quantity and symptoms. In-house microblepharoexfoliation has a greater impact on symptoms. Baby shampoo has no therapeutic effect on quantity of DF and can be considered ineffective for the treatment of *Demodex* blepharitis. The current study provides evidence-based results for the use of commercial products available for the treatment of DF blepharitis in a clinical setting.

The safety of using these products on the ocular surface has not been fully investigated. The following chapter examines the effect of OCuSOFT, TTFW and baby shampoo on the tear film and ocular surface.

# CHAPTER SIX: THE EFFECT OF LID HYGIENE ON THE TEAR FILM AND OCULAR SURFACE

## 6.1 Abstract

**Purpose:** To evaluate the effect blepharitis lid cleansers have on the tear film and ocular surface, and to examine the prevalence of DF in a young population.

**Methods:** Forty-eight university students completed a randomised, controlled, investigator-masked, eight-week clinical trial. Three eyelid hygiene products were investigated: blepharitis eyelid cleanser (OCuSOFT® Lid Scrub® PLUS foam), diluted baby shampoo (10% Johnson's® No More Tears®) and a TTFW (dr.organic®). Cooled boiled water was used as a control. Participants attended for four visits: baseline, two weeks, four weeks and eight weeks. At each visit, subjective symptoms, NITBUT, and ocular surface staining were assessed to evaluate any positive or negative effect on the tear film and ocular surface. DF investigation involving eyelash manipulation and epilation was conducted to examine for the presence and quantity of DF. Osmolarity was measured at baseline and week eight only.

**Results:** The overall prevalence of DF found at baseline was 14.60%. Subjective symptoms improved in all groups, including control. There was no significant difference in mean osmolarity between the groups or within each group after eight weeks. There was a significant increase in osmolarity inter-eye variability in the baby shampoo group ($p = 0.03$). There was no significant change in NITBUT or ocular surface staining after eight weeks of eyelid hygiene.

**Conclusion:** A low prevalence of DF can be found in a young student population. All blepharitis lid cleansers used in the current study demonstrated subjective improvement in symptoms, with no negative effects on TBUT or ocular surface staining. OCuSOFT and TTFW revealed no adverse effect on mean osmolarity or inter-eye variability. Baby shampoo did not cause a significant increase in mean osmolarity, but demonstrated a significant increase in inter-eye variability, signifying a possible increase in ocular surface inflammation.

### 6.2 Introduction

Blepharitis has been previously defined and classified in Section 1.1 and Section 1.7.2. This chronic inflammatory process at the eyelid margins has been shown to disrupt tear film stability, causing ocular surface irritation and dry eye.[233] Despite the relatively high prevalence of blepharitis in ophthalmology and optometry clinics, the exact aetiology remains unknown, and there is still no 'cure' for chronic blepharitis.[2] As discussed above in Chapter 5 (Section 5.5), lid hygiene remains the first line treatment for anterior blepharitis,[1,3] and manufacturers are increasingly developing lid scrubs and washes for practitioners to recommend and distribute to their patients. At present there is no 'one-for-all' treatment for blepharitis. Antibiotics have shown good efficacy against bacterial blepharitis,[1,2,234] antifungals against seborrheic blepharitis,[235–239] and TTO and anti-parasitic therapy have demonstrated notable ability to treat *Demodex* blepharitis.[15,35,52]

OCuSOFT and TTFW are two of the over-the-counter treatments that were used in the pilot and extended treatment studies (refer Chapter 5) and have shown good efficacy at treating *Demodex* blepharitis.[52] The active ingredients and potential toxicity of each product has been previously described in Section 2.4.2and Section 2.4.3 respectively. Although at

higher concentrations, terpinen-4-ol and 1,2-octanediol are considered toxic to the ocular surface,[3,150] their therapeutic abilities has meant that these chemical compounds have been incorporated into eyelid cleansers at lower concentrations reducing the risk of toxicity: TheraTears® SteriLid® (terpinen-4-ol: 0.02 mg/ml = 0.002%), Cliradex® (terpinen-4-ol: 4.61 mg/ml = 0.461%), Oust™ *Demodex*® Swabstix™ (terpinen-4-ol: 0.29 mg/ml = 0.029%) and OCuSOFT® Lid Scrub® PLUS (1,2-octanediol: 0.5%).[52,240] Several recent studies have investigated the safety and tolerability of these eyelid cleansers.[186,241,242] However, TTFW contains 38% terpinen-4-ol, and the impact of using such a high concentration of terpinen-4-ol close to the ocular surface has not been established.

As listed above, many blepharitis products are currently available for practitioners to recommend to their patients. However, as previously mentioned in Section 2.4.1, although baby shampoo has been shown to have a negative effect on goblet cell function,[186] practitioners still routinely recommend patients to use the 'traditional' method of a mild dilution of baby shampoo for eyelid hygiene in the treatment of blepharitis.

The ocular surface comprises of the combination of the cornea, conjunctiva, lacrimal glands, meibomian glands, eyelashes, eyelids and nasolacrimal duct.[243] The tear film lubricates the ocular surface, protecting it from foreign pathogens, maintaining a homeostatic environment, preventing infection and inflammation and providing a clear smooth refractive surface for vision.[244] The migratory effect of substances applied near the eyelid margins, such as makeup, to the tear film has been well established.[245–249] Topical products used for eyelid hygiene to treat blepharitis, inevitably come in close contact with the ocular surface. However, to the best of the authors knowledge, the effect the products have on the tear film and ocular surface has not been clearly established.

Therefore, it is important to investigate the effects products have on the ocular surface, to help inform practitioners in clinical practice. The primary aim of the current study was to examine and compare the effect of home use lid hygiene products on the ocular surface and tear film parameters. A secondary aim of the study was to investigate the prevalence of *Demodex* blepharitis in a young population.

### 6.3 Methods

This was a single-centre, interventional, randomised, controlled, examiner masked clinical trial. All participants were students recruited from the Department of Optometry in TU Dublin. Written informed consent was obtained from all participants prior to enrolment.

Power calculations were made with osmolarity as the designated outcome. Effect size was calculated with G*Power analysis using mean + SD of baseline osmolarity values of the three groups used in the MGD warm compress study (discussed in Chapter 8). Effect size computed was 0.518. Minimum sample size required for statistical significance was calculated using G*Power analysis. A priori analysis for repeated measures ANOVA between factors was conducted; alpha = 0.05, Power = 0.8, effect size = 0.5; minimum sample size required n = 32: 8 participants per group.

Fifty-six participants in total, 14 per group, were enrolled from February to October 2018. Following attrition, 48 participants, completed the two-month treatment study. In an effort to avoid confounding effects of age on tear film and ocular surface parameters,[250] participants aged between 18 – 24 years were included. Participants were excluded if they were using any systemic/topical medications known to affect the eyes (including artificial tears), had used any blepharitis treatment or had ocular surgery within the previous six

months. Contact lens wearers could take part, however, participants were required to wear their spectacles on examination days.

Participants attended the National Optometry Centre for four visits in total: baseline, week two, week four, and week eight. Ocular surface parameters were investigated at each visit to note any changes over time with treatment. The exception was osmolarity, which due to the associated costs was only performed at baseline and week eight only.

All examinations were performed in the following order at each visit, from least invasive to most invasive[170,171]: Modified OSDI questionnaire (validation discussed in detail in Chapter 4), NITBUT (Section 2.3.3), osmolarity (Section 2.3.4), and ocular surface staining (Section 2.3.5). Each of these examination techniques has been described in detail previously.

Previous studies in the literature have used differing parameters and cut-offs to distinguish between dry eye and non-dry eye.[176,177,251] As mentioned previously, a cut-off of 308 mOsm/L is accepted as most sensitive to distinguishing normal participants from participants with mild DED.[176,177] As such, participants within each group were also sub-divided into low tear osmolarity (< 308 mOsm/L) and high tear osmolarity (≥ 308 mOsm/L) in order to assess the correlation between common signs and symptoms of DED with increased tear osmolarity, and the effect that lid hygiene products has on participants with low and high tear osmolarity.

Each participant was finally examined for the presence of DF using the eyelash manipulation and eyelash epilation techniques described earlier (Section 2.3.9). Similar to the pilot study and extended study discussed in Chapter 5, the presence of DF was defined as one or more DF visible on eyelash manipulation and/or microscopic examination.

Participants were randomly divided into four groups according to treatment: Group 1: Cooled boiled water (control) (n = 12), Group 2: OCuSOFT (n = 12), Group 3: 10% baby shampoo (shampoo) (n = 11), and Group 4: TTFW (n = 13). A 2 ml syringe and 20 ml plastic test tube were provided to participants in Group 3 to make up the 10% shampoo solution nightly (Appendix 5b). Randomisation was achieved using the random number generator function on Excel. Each treatment was randomly assigned a number from 1 to 56. Each participant chose a number and was subsequently given the treatment assigned to that number.

Step-by-step instructions, similar to those provided in the pilot and extended treatment studies, were provided to each participant for nightly lid scrubs at home (refer Table 6). The lid scrubbing routine remained consistent between treatments. The only difference was, that as per manufacturer's guidelines, OCuSOFT formula was left on overnight; whereas the shampoo and face wash were rinsed off after scrubbing.

Participants were asked to clean their eyelids nightly with their respective treatments following the step-by-step instructions given to them and to return for repeat examinations after two, four and eight weeks. The examiner remained blind to all treatments throughout all stages of the investigation.

In an effort to monitor compliance, participants were asked to self-report, during return visits, their treatment compliance for the previous 14 or 28 nights (at week two/four, and week eight respectively). Participants were also asked to give feedback: if they would recommend the treatment for participants with dry eyes or blepharitis.

### *6.3.1 Statistical Analysis*

Statistical analysis was performed using SPSS (version 25.0). The study eye used for data analysis was chosen based on the eye with the greater tear osmolarity value. This randomised the process, and is in keeping with previous studies and manufacturer guidelines.[174,176,177] Data was assessed for normal distribution using Shapiro-Wilk. All outcome measures investigated were found to have a non-normal distribution ($p < 0.001$).

Friedman's test was used to analyse repeated measures, within each group, across different visits for non-parametric data. Post-hoc analysis was conducted, where appropriate, using WSR test for pairwise comparisons, adjusted using Bonferroni correction to avoid Type I error ($\alpha = 0.05$/number of comparisons: $\alpha = 0.05/6 = 0.008$).[252] Kruskal Wallis H test was used to analyse data between categorical variables at baseline and at different visits. Post-hoc analysis was conducted, where appropriate, using MWU test for pairwise comparison, adjusted using Bonferroni correction ($\alpha = 0.05$/number of comparisons: $\alpha = 0.05/4 = 0.013$).[252] Data was expressed as median and IQR. Alpha level $\leq 0.05$ was considered statistically significant, with the exception of Bonferroni adjusted post-hoc analysis as described above.

## 6.4 Results

Forty-eight participants, with a median age of 19.50 years (IQR 19.00 – 20.75 years), enrolled and completed the eight-week treatment study. An overall prevalence of DF of 14.60% was detected within this young study cohort. The overall median quantity of DF detected was 0.00 mites (IQR 0.00 – 0.00) and 0.00 mites (IQR 0.00 – 0.00) on eyelash manipulation and microscopic examination, respectively. As the presence and quantities of DF found were so low, no further statistical analysis was conducted in that regard.

The effect of home lid scrubs on symptoms, osmolarity, NITBUT and ocular surface staining was evaluated over eight weeks. Compliance and subjective feedback from participants were also analysed.

Median total symptom score for each treatment group at each time point is shown in Table 34. A box plot illustrating change in symptom score from baseline for each treatment groups over the duration of the study is shown in Figure 28. There was no significant difference in total symptom score between the treatment groups, at any stage, over the two months (KW $p > 0.05$). At baseline, all four treatment groups had a total symptom score > 12 and < 22, signifying 'mild symptoms' according to the OSDI classification.[214]

Table 34. Modified OSDI symptom (median, IQR) for each treatment at each time point. KW: Kruskal Wallis, F: Friedmans. *Significant results highlighted in bold.

| Treatment | Baseline | Week Two | Week Four | Week Eight | F |
|---|---|---|---|---|---|
| **Control** | 12.92 (10.83 – 16.67) | 9.17 (5.83 – 12.50) | 8.33 (4.17 – 16.67) | 9.17 (5.83 – 12.50) | ***p = 0.01** |
| **OCuSOFT** | 10.00 (5.00 – 23.33) | 9.17 (3.33 – 23.33) | 5.00 (2.50 – 19.17) | 9.17 (3.33 – 23.33) | ***p = 0.047** |
| **Baby Shampoo** | 15.00 (3.33 – 31.67) | 10.00 (3.33 – 16.67) | 6.67 (0.00 – 8.33) | 10.00 (3.33 – 16.67) | ***p < 0.001** |
| **TTFW** | 10.71 (6.67 – 16.67) | 6.67 (5.00 – 10.00) | 10.71 (1.67 – 13.33) | 6.67 (5.00 – 10.00) | ***p = 0.04** |
| KW | p = 0.78 | p = 0.80 | p = 0.61 | p = 0.82 | |

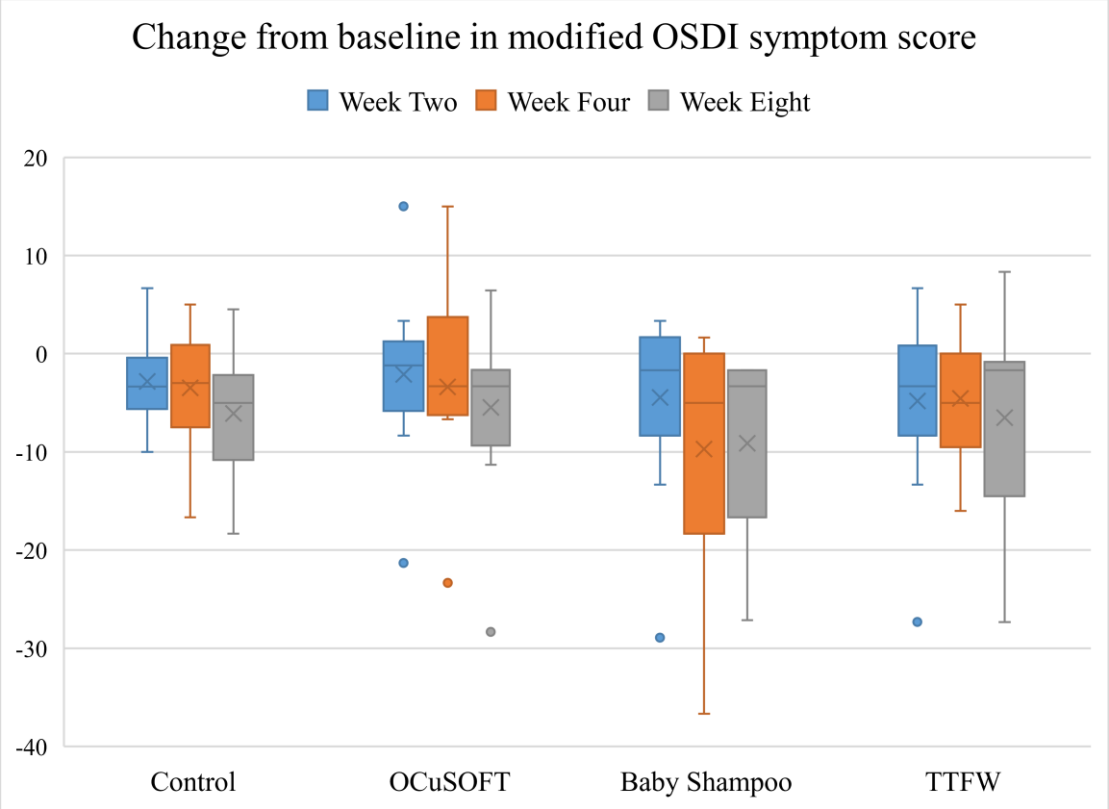



Figure 28. Box plot illustrating change in modified OSDI symptom score for each lid hygiene product at each time point. X represents the mean change in modified OSDI score, small circles represent outliers.

The three treatment groups and control group all demonstrated a reduction in total symptom score over time (Figure 28; Friedman's $p < 0.05$). Wilcoxon signed-ranks test post-hoc analysis is shown in Table 19. As can be seen from Table 35, after Bonferroni correction was applied, only the reduction in total symptom score with shampoo from baseline to week eight ($p = 0.001$) and week two to week eight ($p = 0.004$) was found to be significant.

Table 35. Total modified OSDI symptom score post-hoc Wilcoxon Signed Ranks test pair-wise comparisons. Bonferroni adjusted alpha level $\alpha$ =0.0083. Significant results highlighted in bold. B = baseline, W2 = week two, W4 = week four, W8 = week eight.

| | **B – W2** | **B – W4** | **B –W8** | **W2 – W4** | **W2 – W8** | **W4 – W8** |
|---|---|---|---|---|---|---|
| **Control (Friedmans, p = 0.01)** | 0.04 | 0.07 | 0.01 | 0.72 | 0.06 | 0.14 |
| **OCuSOFT (Friedmans, p = 0.05)** | 0.22 | 0.41 | 0.03 | 0.50 | 0.12 | 0.24 |
| **Shampoo (Friedmans, p < 0.001)** | 0.31 | 0.01 | **0.001*** | **0.004*** | 0.01 | 0.92 |
| **Face Wash (Friedmans, p = 0.04)** | 0.03 | 0.02 | 0.04 | 0.94 | 0.44 | 0.24 |

Table 36 illustrates the median and IQR of the maximum osmolarity values recorded at baseline, and the subsequent change in osmolarity value found for the same eye after eight weeks of treatment, in each group. There was no significant difference in maximum osmolarity value found between the treatment groups at baseline and week eight (KW $p > 0.05$), or within each treatment group after eight weeks of treatment (WSR $p > 0.05$).

Table 36. Osmolarity (median, IQR) and inter-eye variability (median, IQR) before and after treatment. KW: Kruskal Wallis, WSR: Wilcoxon signed ranks test, $p < 0.05$ significant. *Significant results highlighted in bold.

| Variable | Time | Control (n=12) | OCuSOFT (n=12) | Shampoo (n=11) | Face wash (n=13) | KW |
|---|---|---|---|---|---|---|
| **Osmolarity (mOsm/L)** | Baseline | 304.50 (298.00 – 309.50) | 310.50 (300.50 – 333.00) | 305.00 (300.00 – 313.00) | 308.00 (298.00 – 309.00) | p = 0.42 |
| | Week Eight | 299.50 (296.00 – 304.00) | 305.00 (301.50 – 310.50) | 303.00 (299.00 – 308.00) | 301.00 (290.00 – 311.00) | p = 0.50 |
| | WSR: | p = 0.09 | p = 0.37 | p = 0.22 | p = 0.96 | |
| **Inter-eye variability (mOsm/L)** | Baseline | 4.50 (1.00 – 10.00) | 6.00 (4.50 – 8.00) | 3.00 (2.00 – 8.00) | 4.00 (3.00 – 11.00) | p = 0.74 |
| | Week Eight | 6.00 (3.00 – 15.00) | 4.50 (2.00 – 10.50) | 15.00 (8.00 – 21.00) | 11.00 (4.00 -18.00) | p = 0.13 |
| | WSR: | p = 0.15 | p = 0.89 | **p = 0.03*** | p = 0.10 | |

Inter-eye variability is also shown in Table 36. There was no significant difference in inter-eye variability found between the groups at baseline, or at week eight (KW $p > 0.05$). Within group analysis revealed that there was no significant difference in inter-eye variability found between baseline and week eight with the control, OCuSOFT and face wash treatments (WSR $p > 0.05$). However, the inter-eye variability with shampoo was significantly greater after eight weeks of lid scrubs than it was at baseline (15.00 mOsm/L IQR 2.00 – 8.00 vs 3.00 mOsm/L IQR 8.00 – 21.00, respectively; WSR $p = 0.03$). Furthermore, with shampoo, the overall presence of tear film instability increased from 27.27% at baseline to 81.81%, resulting in a 54.54% increase in the presence of instability after eight weeks of treatment. None of the other treatments resulted in such an increase in instability. Presence of tear film instability increased by 8.34%, 16.67% and 23.08% with control, OCuSOFT and face wash, respectively.

Data was also analysed for any differences depending on low or high tear osmolarity (refer Table 37). There was no significant difference in signs and symptoms associated with DED between low and high tear osmolarity at baseline. Expectedly, there was significantly greater inter-eye variability in the high tear osmolarity group (MWU $p = 0.03$). Statistical analysis within each treatment group is not given as there was insufficient data for statistical significance. Table 37 also shows eight week results for both osmolarity groups and treatment sub groups. Inter-eye variability remained higher in the high tear osmolarity group (MWU $p = 0.004$). Symptoms were found to be significantly lower post-treatment in the high tear osmolarity groups (MWU $p = 0.047$). There was no significant difference in NITBUT or ocular surface staining between low and high tear osmolarity after eight weeks of treatment. The median and IQR for quantity of DF for all treatment groups in both low and high tear osmolarity was 0.00 (0.00 – 0.00) and has not been included in Table 37.

Table 37. Baseline and eight week descriptives for low and high tear osmolarity sub-groups. U: Mann-whitney U. *Significant results highlighted in bold.

| **Baseline Descriptives** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Low Tear Osmolarity (n = 25)** | | | | **High Tear Osmolarity (n = 23)** | | | | **P value** |
| **Osmolarity (mOsm/L)** | 299.00 (297.00 – 302.50) | | | | 312 (309.00 – 318.00) | | | | **<0.001[U]** |
| | Control (n = 8) | OCuSOFT (n = 5) | J&J (n = 6) | TTFW (n = 6) | Control (n = 4) | OCuSOFT (n = 7) | J&J (n = 5) | TTFW (n = 7) | |
| | 300.50 (295.50 – 304.50) | 299.00 (298.00 – 302.00) | 301.00 (300.00 – 303.00) | 297.50 (296.00 – 298.00) | 311.00 (309.50 – 312.50) | 331.00 (312.00 – 335.00) | 313.00 (312.00 – 315.00) | 309.00 (308.00 – 318.00) | |
| **Variability (mOsm/L)** | 3.00 (1.00 – 7.00) | | | | 7.00 (4.00 – 9.00) | | | | **0.03[U]** |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 1.50 (1.00 – 8.00) | 5.00 (2.00 – 6.00) | 6.00 (3.00 – 14.00) | 2.00 (1.00 – 3.00) | 6.50 (5.00 – 10.00) | 7.00 (6.00 – 9.00) | 2.00 (1.00 – 2.00) | 11.00 (7.00 – 18.00) | |
| **Percentage Variability (n)** | 24.00% | | | | 39.13% | | | | |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 25.00% (2) | 20.00% (1) | 50.00% (3) | 0.00% (0) | 50.00% (2) | 28.57% (2) | 0.00% (0) | 71.43% (5) | |
| **Modified OSDI (0 – 100)** | 13.33 (6.67 – 31.67) | | | | 10.00 (3.33 – 16.67) | | | | 0.06[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 13.33 (12.08 – 25.83) | 11.67 (8.33 – 11.67) | 23.33 (6.67 – 45.00) | 18.33 (6.67 – 32.69) | 10.83 (8.57 – 12.50) | 6.67 (3.33 – 35.71) | 3.33 (1.67 – 20.00) | 10.71 (6.67 - 13.33) | |
| **NITBUT (secs)** | 5.56 (3.29 – 11.82) | | | | 4.50 (3.20 – 6.47) | | | | 0.46[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 4.77 (3.04 – 14.85) | 9.70 (5.85 – 11.37) | 5.94 (4.47 – 11.73) | 3.93 (2.77 – 4.03) | 5.49 (3.90 – 9.89) | 4.93 (3.00 – 9.20) | 4.13 (3.00 – 4.23) | 4.53 (3.77 – 12.93) | |
| **Ocular Surface Staining (0-15)** | 0.00 (0.00 – 1.50) | | | | 0.00 (0.00 – 1.00) | | | | 0.74[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 0.50 (0.00 – 1.00) | 2.00 (1.00 – 2.00) | 00.50 (0.00 – 2.00) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 1.00) | 1.00 (0.00 – 1.00) | 0.00 (0.00 – 1.00) | |
| **DF Prevalence (%)** | 16.00% (4) | | | | 13.04% (3) | | | | |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 25.00% (2) | 20.00% (1) | 16.67% (1) | 0% | 0% | 28.57% (2) | 0% | 14.29% (1) | |

| Eight Week Descriptives | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Low Tear Osmolarity (n = 25)** | | | | **High Tear Osmolarity (n = 23)** | | | | **P value** |
| **Osmolarity (mOsm/L)** | 299.00 (296.00 – 303.00) | | | | 309.00 (300.00 – 319.00) | | | | **0.001**[U] |
| | Control (n = 8) | OCuSOFT (n = 5) | J&J (n = 6) | TTFW (n = 6) | Control (n = 4) | OCuSOFT (n = 7) | J&J (n = 5) | TTFW (n = 7) | |
| | 297.50 (294.50 – 300.00) | 303.00 (303.00 – 304.00) | 299.50 (299.00 – 303.00) | 299.00 (290.00 – 301.00) | 309.00 (303.50 – 312.00) | 309.0 (300.00 – 326.00) | 308.00 (306.00 – 311.00) | 310.00 (290.00 – 349.00) | |
| **Variability (mOsm/L)** | 4.00 (3.00 – 9.50) | | | | 15.00 (6.00 – 24.00) | | | | **0.004**[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 5.00 (1.50 – 12.00) | 4.00 (3.00 – 5.00) | 8.50 (4.00 – 10.00) | 3.50 (3.00 – 14.00) | 10.50 (5.50 – 16.50) | 8.00 (1.00 – 16.00) | 21.00 (18.00 – 27.00) | 13.00 (11.00 – 29.00) | |
| **Percentage Variability (n)** | 40.00% | | | | 73.91% | | | | |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 37.50% (3) | 20.00% (1) | 66.67% (4) | 33.33 (2) | 50.00% (2) | 57.14% (4) | 100.00% (5) | 85.71% (6) | |
| **Modified OSDI (0 – 100)** | 6.67 (5.00 – 15.60) | | | | 3.33 (0.00 – 11.67) | | | | **0.047**[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 9.17 (6.67 – 17.86) | 5.00 (1.67 – 10.00) | 10.00 (5.00 – 17.86) | 5.18 (5.00 – 11.67) | 4.17 (0.83 – 9.17) | 3.33 (1.67 – 28.33) | 0.00 (0.00 – 5.00) | 10.00 (0.00 – 15.00) | |
| **NITBUT (secs)** | 4.77 (3.50 – 9.85) | | | | 4.67 (2.83 – 8.90) | | | | 0.55[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 3.77 (3.32 – 4.48) | 7.10 (3.26 – 7.73) | 6.59 (3.47 – 11.30) | 7.27 (5.80 – 10.63) | 4.90 (3.38 – 8.34) | 6.67 (3.03 – 10.93) | 4.57 (2.83 – 5.10) | 4.67 (2.60 – 5.70) | |
| **Ocular Surface Staining (0-15)** | 0.00 (0.00 – 0.00) | | | | 0.00 (0.00 – 1.00) | | | | 0.38[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 0.00) | 1.00 (0..00 – 2.00) | 0.00 (0.00 – 1.00) | |
| **DF Prevalence (%)** | 20.00% (5) | | | | 4.35% (1) | | | | |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 37.50% (3) | 20.00% (1) | 16.67% (1) | 0% | 0% | 0% | 0% | 14.29% | |

Mean NITBUT values, at baseline and subsequent visits, are shown in Table 38. There was no significant difference within each treatment group (Friedmans $p > 0.05$) or between the treatments (KW $p > 0.05$), at any time over the eight weeks. With the control treatment, mean NITBUT reduced by approximately 3 seconds after eight weeks, although this drop was not found to be significant (Friedmans: $p = 0.25$). For OCuSOFT, shampoo and face wash treatments, NITBUT remained relatively stable over the eight weeks. Thus, none of the over-the-counter lid scrub treatments used in the current study appeared to have an adverse effect on NITBUT.

Table 38. Non-Invasive tear break-up time (median, IQR) and ocular surface staining (median, IQR) before and after treatment. BL: Baseline, W2: Week Two, W4: Weeks Four, W8: Week Eight, KW: Kruskal Wallis, F: Friedmans

| Variable | Time | Control (n=12) | OCuSOFT (n=12) | Shampoo (n=11) | Face wash (n=13) | KW |
|---|---|---|---|---|---|---|
| **Non-Invasive Tear Break Up Time (sec)** | BL | 5.24 (3.34 – 13.89) | 5.94 (3.12 – 10.54) | 4.47 (3.27 – 6.40) | 4.20 (3.37 – 8.85) | p = 0.92 |
| | W2 | 4.12 (3.05 – 8.04) | 5.50 (3.03 – 10.73) | 4.85 (3.30 – 7.33) | 4.33 (2.90 – 5.63) | p = 0.81 |
| | W4 | 5.30 (3.27 – 6.30) | 6.10 (3.85 – 13.98) | 6.27 (3.97 – 7.63) | 4.13 (3.37 – 6.93) | p = 0.57 |
| | W8 | 4.04 (3.32 – 5.17) | 6.94 (3.23 – 9.91) | 4.57 (2.83 – 11.30) | 5.70 (4.47 – 7.83) | p = 0.63 |
| **F** | | p = 0.25 | p = 0.54 | p = 0.81 | p = 0.87 | |
| **Ocular Surface Staining (0-15)** | BL | 0.00 (0.00 – 1.00) | 1.00 (0.00 – 2.00) | 1.00 (0.00 – 2.00) | 0.00 (0.00 – 1.00) | p = 0.34 |
| | W2 | 0.00 (0.00 – 0.50) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 0.00) | p = 0.75 |
| | W4 | 0.00 (0.00 – 0.50) | 0.50 (0.00 – 1.00) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 0.00) | p = 0.50 |
| | W8 | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 2.00) | 0.00 (0.00 – 0.00) | p = 0.12 |
| **F** | | p = 0.20 | p = 0.07 | p = 0.71 | p = 0.78 | |

Ocular surface staining median and IQR, at baseline and subsequent visits, are also shown in Table 38. Due to the nature of the study cohort; young healthy individuals with no ocular surface disease; there was very little ocular surface staining present at baseline in all groups (KW p = 0.34). The aim was to see if any of the treatments caused an adverse reaction, for e.g. increase in ocular surface staining with use. As can be seen from Table 38, there was no significant increase in ocular surface staining over the duration of the study in any group.

Contact lens wearers accounted for 40% (n = 19/48) of the study cohort. The use of contact lenses was not found to have any impact on baseline measurements (Table 39). Raw data on the breakdown of baseline measurements between contact lens wearers and non-contact lens wearers within each treatment group is shown in Table 39. Statistical analysis on contact lens wearers within each treatment group is not given, as there was insufficient data for statistical significance. The median and IQR for quantity of DF for all treatment groups in both contact lens wearers and non-contact lens weaers was 0.00 (0.00 – 0.00) and has not been included in Table 39.

Table 39. Baseline descriptives (median, IQR) for contact lens wearers and non-contact lens wearers. U: Mann-whitney U test

| **Baseline Descriptives** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **No Contact Lens Wear (n = 29)** | | | | **Contact Lens Wear (n = 19)** | | | | **P value** |
| **Osmolarity (mOsm/L)** | 307.00 (299.50 = 314.50) | | | | 305.00 (298.00 – 311.00) | | | | 0.56[U] |
| | Control (n = 7) | OCuSOFT (n = 9) | J&J (n = 6) | TTFW (n = 7) | Control (n = 4) | OCuSOFT (n = 3) | J&J (n = 5) | TTFW (n = 6) | |
| | 307.00 (299.00 – 311.00) | 315.00 (303.00 – 335.00) | 303.50 (300.00 – 308.00) | 301.00 (298.00 – 310.00) | 304.00 (297.00 – 305.00) | 302.00 (298.00 – 309.00) | 312.00 (303.00 – 313.00) | 308.00 (297.00 – 309.00) | |
| **Variability (mOsm/L)** | 6.00 (2.00 – 8.50) | | | | 4.00 (1.00 – 12.00) | | | | 0.98[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 4.00 (1.00 – 8.00) | 7.00 (6.00 – 9.00) | 3.00 (2.00 – 8.00) | 3.00 (1.00 – 9.00) | 5.00 (1.00 – 12.00) | 4.00 (2.00 – 5.00) | 4.00 (1.00 – 5.00) | 7.50 (3.00 – 18.00) | |
| **Modified OSDI (0 – 100)** | 11.67 (5.00 – 30.83) | | | | 11.67 (6.67 – 20.00) | | | | 0.84[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 13.33 (11.67 – 31.67) | 11.67 (3.33 – 15.00) | 23.33 (3.33 – 45.00) | 6.67 (3.33 – 11.67) | 11.67 (10.00 – 12.50) | 8.33 (6.67 – 31.67) | 6.67 (5.00 – 20.00) | 15.00 (10.71 – 32.69) | |
| **NIBUT (secs)** | 4.93 (3.29 – 11.67) | | | | 4.35 (2.99 – 6.14) | | | | 0.23[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 13.30 (4.50 – 15.23) | 4.93 (3.03 – 9.70) | 6.34 (4.13 – 11.73) | 4.03 (2.77 – 4.77) | 3.37 (2.70 – 3.57) | 6.03 (5.85 – 11.85) | 4.23 (3.00 – 4.47) | 4.53 (3.93-12.93) | |
| **Ocular Surface Staining (0-15)** | 0.00 (0.00 – 1.00) | | | | 0.00 (0.00 – 1.00) | | | | 0.89[U] |
| | Control | OCuSOFT | J&J | TTFW | Control | OCuSOFT | J&J | TTFW | |
| | 0.00 (0.00 – 1.00) | 1.00 (0.00 – 2.00) | 1.00 (0.00 – 1.00) | 0.00 (0.00 – 1.00) | 1.00 (0.00 – 1.00) | 1.00 (0.00 – 2.00) | 0.00 (0.00 – 2.00) | 0.00 (0.00 – 1.00) | |

Participants were asked to self-report on their compliance throughout the study. Compliance results are shown in Figure 29. Only the control group demonstrated a significant drop in compliance (Friedmans: $p = 0.04$). Post-hoc Bonferroni adjusted WSR test pairwise comparison (adjusted $\alpha = 0.05/3 = 0.0167$) found significant reduction in compliance after two weeks: from week two to week four ($p = 0.01$) and from week two to week eight ($p = 0.007$). The overall lowest compliance throughout the eight weeks was seen with the shampoo group. After two weeks the compliance within this group was < 70%. The OCuSOFT group had the highest overall compliance throughout the study and remained at > 70% over the eight weeks.

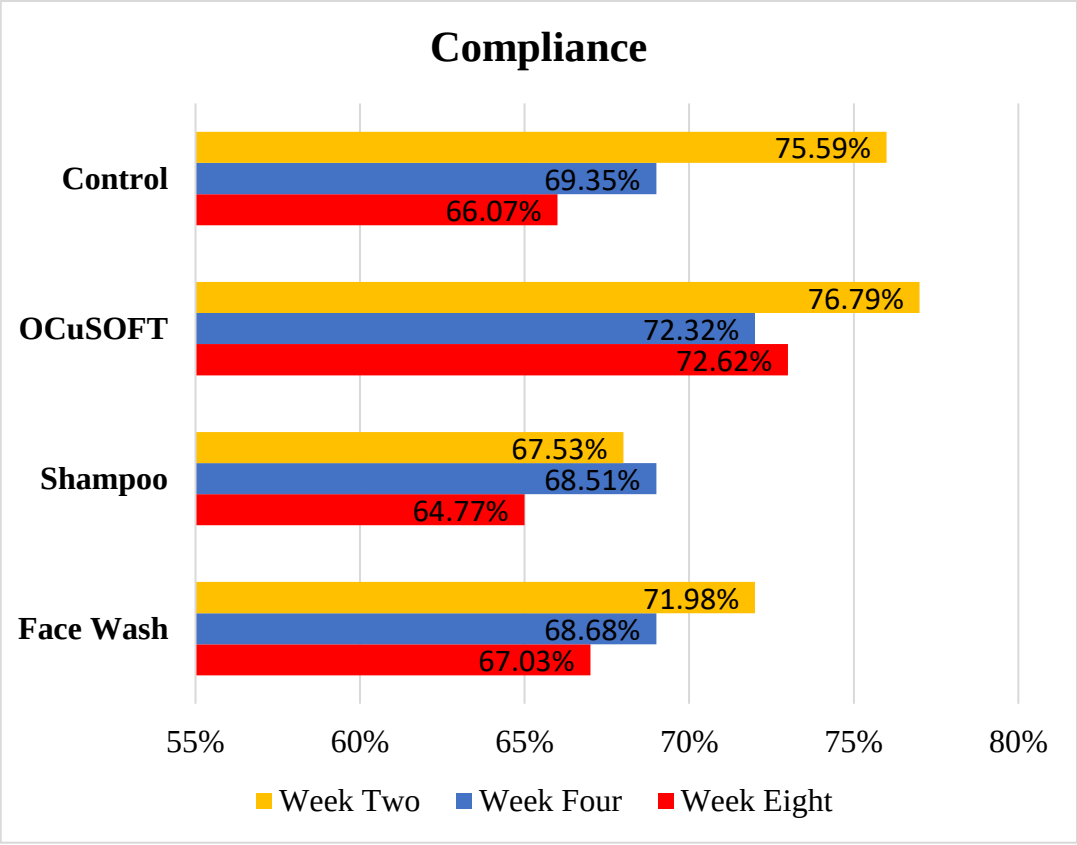



Figure 29. Self-reported percentage compliance over the duration of the study.

Participants were also asked to give feedback regarding if they would recommend the treatment for people with blepharitis or dry eyes. After eight weeks of treatment; 66.67% of participants using control treatment, 83.33% using OCuSOFT, 72.73% using shampoo, and 69.23% using face wash said they would recommend the treatment. The reasons given for not recommending the treatments were as follows: The treatment made no difference (Control n = 4, OCuSOFT n = 1, Shampoo n = 3, TTFW n = 1), the treatment was uncomfortable and irritating during use (TTFW n = 3) and their eyes felt dryer after use (OCuSOFT n = 1).

### 6.5 Discussion

As previously described in Chapter 3, Section 3.5, the overall prevalence of DF reported in the literature varies greatly; likely due to the differences in participant ages and techniques used for detecting presence of the mites. As mentioned in Chapter 1, DF has consistently been associated with increasing age (Section 1.6.1),[13,28,77,253] blepharitis (Section 1.7.2),[7,43,46,49,64,253] dermatological conditions (Section 1.6.2),[69–71,81,82] and systemic diseases (Section 1.6.3).[20,24,92,93,107,108,113] Nonetheless, higher prevalence values have also been established in normal, healthy individuals.[23,46,254,255] Kemal et al[23] discovered an overall prevalence of 26.67% on the eyelashes of normal individuals (mean age 37.5 ± 16.5 years). In the same study, in control participants < 20 years of age, the authors' discovered a 16.67% prevalence of DF,[23] which is in good agreement with the results found in the current study (14.60%). Kaḅatas et al[46] discovered a higher prevalence of 54.9% DF on the eyelashes of control participants, however they were considerably older than participants in the current study: 54.6 ± 13.4 years. Zhao et al[254] discovered an overall prevalence of 67.6% DF in the skin of a young study cohort (aged 13 – 22 years). Karaman et al[255] discovered a 37% prevalence DF among college students living in shared

accommodation. The higher prevalence values found by Zhao et al[254] and Karaman et al[255] in comparison to the current study are most likely due to the sampling methods used: skin sampling versus eyelash epilation. The low prevalence of DF discovered in the current study further re-enforces that DF can be found among young, normal, healthy individuals.

Evidence-based practice is steadily becoming a principal element of health care, including optometry.[256–259] Evidence-based health care is the clear and careful use of current 'best evidence' in clinical decision making with respect to the treatment and management of patients.[260] In recent years, several studies have examined the clinical efficacy of eyelid hygiene products with respect to *Demodex* blepharitis.[13,15,35,52] However, there is limited evidence available for practitioners on the safety of these products. The results of the current study will help guide practitioners on the safety of such products that are often used in close contact with the ocular surface.

Subjective symptoms improved in all treatment groups, including the control group with water. In a meta-analysis of placebo controlled trials it was found that there was no statistically significant difference in trials with continuous subjective outcome measures between treatment and placebo.[261] The placebo effect occurs when a participant experiences a beneficial effect from the control treatment which cannot be attributed to the properties of the treatment itself, and is therefore believed to be a psychological belief by the patient in the treatment. However, in the current study, the control treatment used was water. Participants were aware that it was water, and as predominantly students of optometry, were also aware that the likelihood of water having a therapeutic effect was small. Yet, an improvement in symptoms was demonstrated. It is possible that the control used did have some therapeutic effect, as the physical nature of rubbing the eyelids nightly, even if just using water, could help clean and remove some of the bacterial load at the eyelid margin.[262]

This therapeutic rubbing of the eyelids could also account for the subjective improvement detected across all four groups. Although subjective symptoms improved across all four groups, the only significant reduction in symptoms was with the baby shampoo treatment. The authors were surprised, as baby shampoo is a detergent and has previously been reported to have a stinging and uncomfortable sensation when used.[186,263] The authors postulate that although no significant difference in symptoms was found at baseline between the four groups, the baby shampoo group did have the highest symptoms of the four groups (OSDI = 15.00 IQR: 3.33 – 31.67). High baseline scores have been associated with high placebo responses,[262] believed to be due to a 'regression to the mean'.[264]

Tear osmolarity has been found to be one of the most effective methods for detecting ocular surface inflammation and DED.[176,206,251] The biological range of tear osmolarity values in the lower tear meniscus varies from 275 mOsm/L to 400 mOsm/L; with higher numbers indicating greater surface inflammation.[206] Tear osmolarity values between 308 mOsm/L - 316 mOsm/L have been recommended in the literature as cut-off referent values for dry eye diagnosis.[176,177,251] A cut-off of 308 mOsm/L is considered most sensitive to distinguishing normal participants from participants with mild DED.[176,177] Whereas, a cut-off of 316mOsm/L is considered to better discriminate between mild and moderate – severe dry eye, and has an overall predictive accuracy of 89%.[251] In the current study, at baseline, the mean osmolarity values for the control, shampoo and face wash treatment groups were < 308 mOsm/L, and can therefore be considered within that normal range value. The OCuSOFT treatment group had a slightly higher mean osmolarity value at baseline (315.8 mOsm/L), however it was still within the cut-off referent recommended by many previous studies.[177,251] Although the mean osmolarity in the OCuSOFT group was slightly higher at baseline, KW comparison of means found no statistical significant difference between the

four groups. Furthermore, the aim of the study was to assess if each of the treatments used had a negative impact on tear film osmolarity. As can be seen from the current study, mean tear osmolarity dropped in all four groups: although, not significantly. A study published in 2014, comparing the efficacy of thermal massagers and artificial eye drops for the treatment of DED, also found that osmolarity values improved post treatment in both groups.[265] The authors concluded that the improvement in lipids to the ocular surface helped to improve tear film stability. The use of lid hygiene could be beneficial for reducing tear osmolarity by reducing the overall bacterial load at the eyelid margin, and the pressure applied to the eyelids during scrubbing effect may also provide an element of massage to the eyelids.

A reduction in tear osmolarity suggests a reduction in ocular surface inflammation and improvement in tear film stability. Inter-eye variability of > 8mOsm/L is considered an indicator of tear film stability.[176] Lemp et al[176] found that the variability between two eyes in normal, mild to moderate dry eye and severe dry eye patients was 6.9 ± 5.9 mOsm/L, 11.7 ±10.9 mOsm/L, and 26.5 ± 22.7 mOsm/L, respectively. In the current study, variability values at baseline were in good agreement with those found by Lemp et al[176]: 4.50 (1.00 – 10.00) mOsm/L CoV 0.89% (control), 6.00 (4.50 – 8.00) mOsm/L CoV 0.45% (OCuSOFT), 3.00 (2.00 – 8.00) mOsm/L CoV 0.99% (shampoo), and 4.00 (3.00 – 11.00) mOsm/L CoV 1.36% (face wash). However, post treatment inter-eye variability was found to increase slightly in all groups, and significantly with the shampoo group: 6.00 (3.00 – 15.00) mOsm/L CoV 1.30% (control), 4.50 (2.00 – 10.50) mOsm/L CoV 1.05% (OCuSOFT), 15.00 (8.00 – 21.00) mOsm/L CoV 0.63% (shampoo), and 11.00 (4.00 – 18.00) mOsm/L CoV 1.02% (face wash). Variation in measurements detected in the current study have been found to be less than that previously reported. TearLab$^{TM}$ has previously

been shown to provide repeatable tear osmolarity measurements with CoV between 1.6% - 1.9%.[178] The increase in inter-eye variability detected in the shampoo group can be considered clinically significant, as the increase in median is >8mOsm/L.

With the exception of OCuSOFT, post-treatment inter-eye variability values found in the current study suggest mild-moderate dry eye according to Lemp et al[176] standards. Therefore, as symptoms and mean osmolarity reduced in the current study suggesting no adverse effects of the treatments used, the inter-eye variability in osmolarity values suggests the contrary. Although slight increases in inter-eye variability were detected in all groups, only the increase with shampoo was found to be significant. This significant instability in tear osmolarity with shampoo occurred even in the presence of relatively low participant compliance within that group (< 70% over the eight weeks). A recent study by Sung et al[186] discovered that the use of diluted baby shampoo appeared to negatively impact the tear film by causing a reduction in levels of MUC5AC, a goblet cell-specific mucin; suggesting that the use of baby shampoo caused a reduction in goblet cell density.[266,267] Hyperosmolarity acts as a stressor to the ocular surface, causing morphological and inflammatory changes including a reduction in mucin producing goblet cells.[268] In DED, the same is true in reverse: a reduction in mucin producing goblet cells can cause tear film instability and hyperosmolarity.[269] Findings from the current study correlate well with Sung et al[186]: The increase in tear film instability and inter-eye variability found post-treatment in the shampoo group could be as a result of adverse changes to goblet cell density caused by the baby shampoo.

Non-invasive tear break-up time and ocular surface staining can also be indicators of tear film instability and ocular surface inflammation. However, in the current study none of the eyelid hygiene products used caused negative effects on NITBUT or ocular surface

staining. This was in keeping with Sung et al[186] who found no significant change in NITBUT or ocular surface staining after four weeks of treatment with baby shampoo or TheraTears® SteriLid® cleanser. Similarly, in a recent study by Ngo et al[241] investigating the short-term responses associated with eyelid hygiene products available for the treatment of DF; the authors found a significant decrease in NITBUT using a 50% tea tree based formula, but no significant change in NITBUT using any of the other eyelid cleansers, including OCuSOFT and two other TTO based products (TheraLid® and Cliradex ®). Although the timings of repeat measurements were different, and the eyelid hygiene products investigated were different, the outcome is similar. It appears that regardless of whether NITBUT was measured after 10 minutes,[241] four weeks,[186] or eight weeks of treatment; common eyelid hygiene products do not appear to have a negative effect on NITBUT.

The current study provides practitioners with a good insight into realistic compliance from patients. It is possible that due to reduced compliance over the course of the study, potential significant adverse events have not been elucidated in the current study. However, as the current study may be more indicative of a 'real-world' blepharitis treatment scenario, the authors believe that the study provides a good representation of the safety of the blepharitis eyelid hygiene products used over the course of eight weeks. Longer studies would be required to confirm absolute safety in the long-term. The lowest reported compliance in the current study was within the shampoo group at 64.77%, and the control group at 66.07%. However, these were still greater than that reported in a recent study investigating patient compliance with eyelid hygiene over six weeks, in which self-reported compliance was only 55%.[270] In that compliance study, participants were also asked to clean their eyelids using a diluted solution of baby shampoo or warm water. Reasons given

for non-compliance included inconvenience, forgetfulness, and a belief that therapy was not required.[270] The authors believe that these underlying reasons are likely to also exist for the control group in the current study. The OCuSOFT group had the highest overall compliance throughout the study and remained at > 70% over the eight weeks. The authors believe that this may be due to the convenient nature of using this type of treatment. However, it is also possible that as the participants in this study were optometry students, they may have had a better understanding of the potential benefits of eyelid hygiene and subsequently overall compliance may have been greater as a result.

Participants were also asked if they would recommend the product to future blepharitis patients. OCuSOFT received the highest recommendation (83.33%), followed by shampoo (72.72%), face wash (69.23%) and control (66.67%). The control group received the lowest recommendation due to its presumed lack of therapeutic ability. Although the face wash was TTO based, and thus has anti-bacterial, anti-microbial and anti-inflammatory potential, it received a lower recommendation due to the discomfort associated with the product. This is in agreement with the study by Ngo et al[241] that found that tea tree based eyelid cleansers marketed to treat *Demodex* blepharitis caused varying degrees of ocular irritation. Ngo et al[241] also found that OCuSOFT caused minimal irritation which corresponded well with the higher recommendation for its use from participants in the current study.

As participants were required to make their own 10% baby shampoo solution at home, this could have caused differences in the % solution being used by the participants. It is possible that this may have impacted the results, and future studies should have a more standardised % solution to avoid this.

### 6.6 Conclusion

A low prevalence of DF was found amongst young, healthy individuals. Overall, the three eyelid hygiene products investigated were well tolerated. Symptoms improved for all groups, and there were no negative effects on NITBUT or ocular surface staining. There was a mild increase in tear film instability and inter-eye variability with both OCuSOFT and TTFW. However, this also occurred with control lid hygiene scrubs with water, and these changes were not found to be significant. In contrast, 10% baby shampoo caused a significant increase in inter-eye osmolarity variability and tear film instability, suggesting a possible increase in ocular surface inflammation. This study was conducted on healthy participants with healthy tear films and ocular surface. Future studies should consider inclusion of participants with compromised tear film and ocular surface to elicit a more magnified response to treatment.

The results of the study indicate that *Demodex* blepharitis related eyelid hygiene products OCuSOFT and TTFW, used in the pilot and extended treatment study, demonstrated no significant adverse ocular reactions. A paper on the results of this study has been recently accepted for publication in Contact Lens & Anterior Eye.

Thus far in this thesis, treatments for *Demodex* blepharitis have concentrated on the traditional lid hygiene method, and the safety and efficacy of the different products tested. As mentioned previously in Section 3.2, DF is susceptible to damage from heat. In the treatment of MGD, heat is often applied to the eyelids, to help soften and improve the flow of meibum to the tear film and ocular surface. Heat applied to the eyelids, must pass through the eyelash follicles to reach the meibomian glands underneath. Therefore, in theory, heat applied to the eyelids in the treatment of MGD, may have a dual therapeutic effect by also

killing DF present within the eyelash follicles. The following chapter, Chapter 7, will discuss the relationship between DF and MGD in more detail, and will examine the effect that heat therapy can have on treating *Demodex* blepharitis. The results of this study have been recently accepted for publication in Current Eye Research and has been adapted accordingly for Chapter 7.

# CHAPTER SEVEN: THE EFFICACY OF WARM COMPRESSES IN THE TREATMENT OF MEIBOMIAN GLAND DYSFUNCTION AND *DEMODEX FOLLICULORUM* BLEPHARITIS

## 7.1 Abstract

**Purpose:** To examine and evaluate the effect of warm compresses on MGD and DF blepharitis.

**Methods:** Forty-two participants (13 males, 29 females; median age of 59.00 years) enrolled and completed the two-month warm compress treatment study. Three warm compress treatments were compared: Warm face cloth, MGDRx EyeBag® (Eyebag) and OPTASE$^{TM}$ Moist Heat Mask (Optase). Participants attended for four visits: baseline, two weeks, four weeks, and eight weeks. Similar to previous studies, examinations at each visit included: subjective symptoms, osmolarity, NITBUT, ocular surface staining, Schirmer I, number of expressible glands and quality of expressed meibum. Eyelash manipulation and epilation were conducted to assess for the presence of DF.

**Results:** Utilising a composite score of meibum quality and expressibility, MGD grade reduced significantly with the Eyebag and the Optase ($p < 0.05$). No significant difference in efficacy for treating MGD was observed between the two devices ($p > 0.05$). The Optase was the only compress that significantly reduced the quantity of DF after eight weeks of treatment. Symptoms and ocular surface staining also improved significantly with the Eyebag and the Optase ($p < 0.05$), but not the warm face cloth ($p > 0.05$). There was no significant change detected in osmolarity, NITBUT or Schirmer I with any treatment ($p > 0.05$).

**Conclusion:** Both the Eyebag and Optase exhibited superior efficacy in treating signs and symptoms of MGD, compared to the use of a warm face cloth, over the eight-week period. The Optase demonstrated dual therapeutic abilities, treating both MGD and DF blepharitis. Repeated application of warm compresses remains an effective home-remedy for the treatment of MGD.

### 7.2 Introduction

The meibomian glands are a group of holocrine glands found in the upper and lower eyelids. Structurally, they consist of parallel rows of secretory acini organised around a central duct, which opens onto the eyelid margin.[271,272] As mentioned in Section 1.7.3, the function of the meibomian glands is to supply meibum to the ocular surface: preventing tear film evaporation, improving vision, and protecting against microbial agents and organic matter such as dust.[133–136] Disruption to this supply, often through terminal duct obstruction or changes in glandular secretion, can interfere with the homeostasis of the tear film and ocular surface: leading to inflammation and subsequent symptoms of discomfort.[135,273]

The eyelash follicles are situated within the eyelids, anterior to the meibomian glands. Due to their close proximity with one-another, anomalies of the eyelash follicles and the meibomian glands are frequently seen in combination.[4–7] For example, inhabitation of the eyelash follicles and meibomian glands with *Demodex*.

The association between DB infestation and severe MGD and keratitis has been described in the literature.[6,274] Although DF are generally associated with anterior blepharitis, the prevalence of DF in the eyelash follicles of MGD patients has been reported to vary between 46.5% to 85%.[7,30,48]

The mainstay treatment recommended for *Demodex* blepharitis is lid scrubs with diluted quantities of TTO.[15,35,52] Although lid scrubs have been indicated as an early treatment option for patients with mild MGD, warm compress therapy remains the leading treatment for MGD.[138] For warm compresses to be effective, heat must pass through the anterior eyelid structures, including the eyelashes, to warm and liquify thickened meibum within the meibomian glands. The melting temperature of normal meibum is circa 32 °C, and is higher at approximately 35 °C in obstructed glands with thickened secretions.[138,275] Hence, it is suggested that warm compresses need to heat the inner eyelid to a temperature of ≥ 40 °C, to be effective at treating MGD.[192] However, both DB and DF prefer lower temperatures, and Zhao et al[197] have shown that temperatures above 37 °C are damaging to DF. Higher temperatures cause death by protein coagulation and denaturation, and eventual paralysis of the DF nervous system.[197] Murakami et al[276] have demonstrated that although there are differences in the innermost eyelid temperatures achieved by various warm compresses, most methods do manage to reach outer eyelid temperatures of ≥ 40 °C. Therefore, as heat from the warm compress spreads through the eyelash follicles to heat the inner eyelid, it could conceivably have a killing effect on DF within the eyelash follicle.

Traditionally, home based warm compresses were carried out using a warm face cloth.[138,277] However, this method has its limitations, including poor heat retention,[278] and inconvenience leading to reduced compliance.[138] Over the years, more patient-friendly warm compresses have become available, such as the Eyebag and the Optase. Although both warm compresses are similar; they are heated in a microwave, and one heating is required to provide 10 minutes of therapy; there are fundamental differences between them. The Optase contains HydroBead™ Technology, which absorbs moisture from the air, and when heated, releases it to provide a moist heat. Optase manufacturers report temperatures

from 50 °C to 41 °C over the 10-minute duration of therapy.[279] The moist heat softens eyelash debris in patients with anterior blepharitis, and restores moisture to the eye and surrounding area, in conjunction with improving meibum flow, tear film quality and reduced tear film evaporation.[279] In contrast, the Eyebag contains flax seed and provides a dry heat when applied to the eyelids. Manufacturers recommend it for relief of, including but not limited to: MGD, blepharitis, dry eye syndrome, and rosacea. Previous research has shown that the Eyebag achieves temperatures of 46 °C dropping to 39 °C after 5 minutes.[280] However, their efficacy in the treatment of *Demodex* blepharitis had not previously been investigated. As such, the aim of the current study was to assess the therapeutic effect of these common home-based warm compresses on DF infestation in MGD patients.

### 7.3 Methods

This was a single-centre, interventional, randomised, controlled, examiner masked clinical trial. All participants were recruited through the National Optometry Centre's private and student optometry clinics. Written informed consent was obtained from all participants prior to enrolment.

Minimum sample size required for statistical significance was calculated using G*Power analysis. Effect size was calculated from the mean and SD of the difference of DF presentation on lash manipulation and microscopic examination from previous data collected: 0.84/1.59 = 0.52. A priori analysis for repeated measures ANOVA between factors was conducted ($\alpha = 0.05$, $1\text{-}\beta = 0.80$, effect size = 0.5: 3 groups, 2 measurements). The minimum total sample size required was 33 participants; 11 participants per group. Fifty participants in total enrolled between April 2017 and May 2018. Participants had to be ≥ 18 years of age and have ≥ G1 MGD based on meibomian gland expression according

to the diagnostic subcommittee of the International Workshop on Meibomian Gland Dysfunction,[185] to be eligible to participate. Participants were excluded if they: wore contact lenses, were pregnant, had a systemic disease or were using topical/systemic medication known to affect the eyes, presented with ocular disease (with the exception of MGD and blepharitis), were currently using MGD/blepharitis treatment or had used such treatment within the last six months, or had ocular surgery in the last six months.

Participants attended the National Optometry Centre for four visits in total: baseline, week two, week four, and week eight. All examinations were conducted in the same room, at the same time of day (+/- 30 minutes), by the same examiner (author OM). All examinations were conducted in the same order at each visit, from least invasive to most invasive[170,171]: modified OSDI questionnaire refer (Chapter 4), NITBUT (Section 2.3.3), osmolarity (Section 2.3.4), ocular surface staining (Section 2.3.5), Schirmer I (Section 2.3.6), MGD evaluation (Section 2.3.8) and *Demodex* investigation (Section 2.3.9). Each of these examination techniques has been described in detail previously. Participants were considered to have 'dry eye' if found to have three or more of the following parameters; modified OSDI ≥ 13, osmolarity ≥ 308 mOsm/L, inter-eye variability ≥ 8mOsm/L, NITBUT < 10 secs, ocular surface staining ≥ 3 Oxford score or Schirmer I score ≤ 5mm/5min. Cut-off values employed are in keeping with those recommended by DEWS II.[171] To grade MGD, composite scores were derived from the expressibility and quality of meibum from both upper and lower eyelids and used for statistical analysis.[185] Similarly, a composite score was derived for quantities of DF found on upper and lower eyelids and used for statistical analysis. The percentage of participants with DF in each group was also determined. Positive DF infestation was defined as the presence of ≥ 1 DF detected on either eyelash manipulation or microscopic examination. Based on work by Randon et al[65]

the prevalence of DF was further classified into non-pathogenic (≤ 3 mites) and pathogenic infestation (≥ 4 mites) per eye.

The ICC was determined to examine the agreement in pre-treatment results between the right and left eyes for each participant. A two-way mixed analysis with absolute agreement and 95% confidence intervals was conducted.[281] Results are shown in Table 40. As recommended, data analysis was conducted on one eye only for each participant.[282] As all correlations were between moderate to excellent, either eye was considered eligible for selection. Therefore, in keeping with previous studies and osmolarity measurement guidelines, the eye selected for data analysis was chosen based on the higher tear osmolarity value at baseline.[174,176,177]

Table 40. Intraclass correlation co-efficient, two-way mixed effects, absolute agreement, average of multiple measurements, 95% confidence interval. Values of less than 0.5 indicate 'poor' agreement, between 0.5 and 0.75 'moderate' agreement, between 0.75 and 0.9 'good' agreement, and greater than 0.90 'excellent' agreement.[281]

| Outcome Measure | ICC | Reliability |
|---|---|---|
| Quantity *Demodex folliculorum* | 0.71 | Moderate |
| MGD Grade | 0.93 | Excellent |
| Osmolarity | 0.68 | Moderate |
| Non-invasive Tear Brake-Up Time | 0.82 | Good |
| Ocular Surface Staining | 0.77 | Good |
| Schirmer | 0.91 | Excellent |
| Dry Eye Prevalence | 0.67 | Moderate |

Participants were randomly assigned one of three treatments to use at home: Warm face cloth (Group 1, conventional treatment: n = 12), Eyebag (Group 2, dry heat: n = 16), Optase (Group 3, moist heat: n = 14). Randomisation was achieved using the random number generator function on Excel. Each treatment was randomly assigned a number from 1 to 60. Each participant chose a number and was subsequently given the treatment assigned to that number.

In accordance with manufacturers' guidelines, each participant was provided with an instruction leaflet (refer Appendix 6 (a – c)) and was directed to use the treatment for 10 minutes twice a day for the first two weeks. Frequency of treatment was reduced to 10 minutes once a day from weeks three to eight.

Participants in Group 1 were instructed to pour 200ml of boiled water into a bowl and allow it to cool for 10 minutes before commencing treatment. This created a water temperature ranging from 50 °C to 39 °C, over the 10-minute treatment time (tested using an Easytemp thermometer (HYGIPLAS, Wellingborough, UK) and porcelain bowl). Participants' were advised to re-heat the face cloth every two minutes, by immersing it in the same bowl of cooled, boiled water: to maintain temperature at therapeutic levels.[191,192] Group 2 and Group 3 were instructed to heat their compress in the microwave for 15 – 30 seconds, depending on the power of their microwave, as per manufacturers' guidelines.

### *7.3.1 Statistical Analysis*

Statistical analysis was performed using SPSS (version 25.0). Normality was measured using Shapiro-Wilk statistical test. Repeated measures ANOVA with Bonferroni post-hoc analysis was used to analyse repeated measures within each group over time for parametric data. Friedman's test was used to analyse repeated measures, within each group, across

different visits for non-parametric data. With Friedman's test, post-hoc analysis was conducted, where appropriate, using WSR test for pairwise comparisons, adjusted using Bonferroni correction ($\alpha = 0.05$/number of comparisons: $\alpha = 0.05/6 = 0.0083$).[252] One way ANOVA with Bonferroni post-hoc analysis was used to analyse data between continuous variables at baseline and at different visits for parametric data. Kruskal Wallis H was used for non-parametric data. With KW, post-hoc analysis was conducted, where appropriate, using MWU test for pairwise comparison, adjusted using Bonferroni correction ($\alpha = 0.05$/number of comparisons: $\alpha = 0.05/3 = 0.0167$).[252] Parametric data was expressed as mean ± SD, non-parametric data was expressed as median and IQR. A p-value $\leq 0.05$ was considered statistically significant, apart from Bonferroni adjusted post-hoc analysis as described above.

### 7.4 Results

Fifty participants were enrolled between April 2017 and May 2018. The attrition rate was 16%. Four participants withdrew from the study, without any known adverse reactions, and were lost to follow-up. Two participants stopped as they felt their symptoms were worsening. A further two were removed from data analysis as their records were incomplete. Following attrition, 42 participants (13 males and 29 females) with a median age of 59.00 (IQR: 50.00 – 69.00) years completed the two-month warm compress treatment study. At baseline, the prevalence of DF detected within the entire study cohort was 57.11%, with a median quantity of 0.5 (IQR 0.00 – 3.25) and 0.00 (IQR 0.00 – 2.00) mites on lash rotation and microscopic examination respectively (WSR: p = 0.008). There was no significant difference in age (Group 1: 60.00 (IQR 52.00 – 69.00), Group 2: 59.00 (IQR 52.00 – 72.50 , Group 3: 59.50 (IQR 32.00 – 68.00), KW: p = 0.75) or quantity of DF

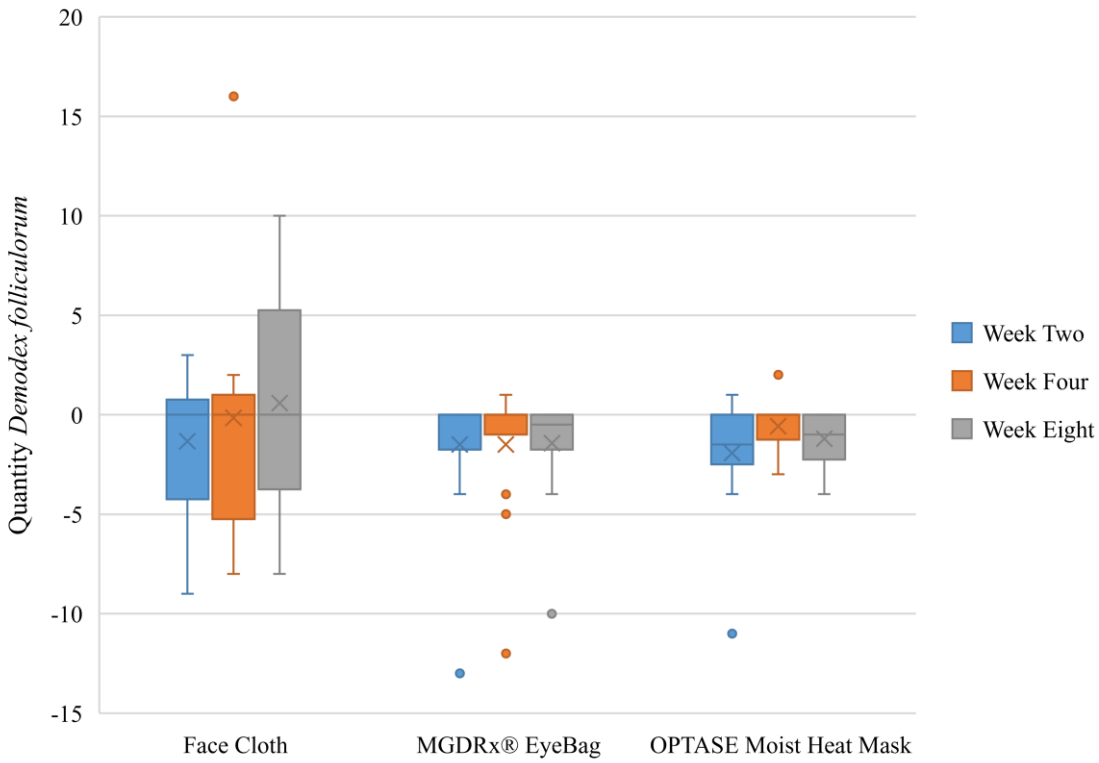
on either lash rotation or microscopic examination (KW: $p = 0.78$ and $p = 0.85$) between the three groups before treatment.

Table 41 shows median and IQR for quantity of DF at each visit for each treatment group. Figure 30 displays the change in quantity of DF detected on eyelash rotation within each group over the eight weeks. Within treatment analysis showed that the quantity of DF dropped significantly over the duration of the study in Group 3 (Optase) (Friedman's $p = 0.04$). Post-hoc pairwise comparisons using WSR test (alpha adjusted for Bonferroni correction) revealed only the change from baseline to week eight to be significant (mean: 2.64, Range: 0 – 11 versus mean: 1.42, Range: 0 – 8; WSR $p = 0.008$). There was no significant change in DF quantity in Group 1 (Warm Face Cloth) or Group 2 (Eyebag) over the eight weeks (Friedman's $p = 0.88$ and $p = 0.66$, respectively). Between treatments analysis did not show any significant difference between the treatments over the eight weeks (KW $p > 0.05$, refer Table 39).

The mean and range for quantity of DF detected on microscopic examination at each visit, for each group, are also shown in Table 41. In contrast to results detected on eyelash rotation, there was no significant change in DF quantity detected on microscopic examination over time in each group (Friedman's $p > 0.05$) or between treatments (KW $p > 0.05$).

Table 41. *Demodex folliculorum* quantity (median, IQR), and MGD grade (median, IQR) before and after treatment. B: Baseline, W2: Week Two, W4: Week 4, W8: Week Eight. Statistical Tests Applied: $\alpha \leq 0.05$ significant. *Significant results highlighted in bold.

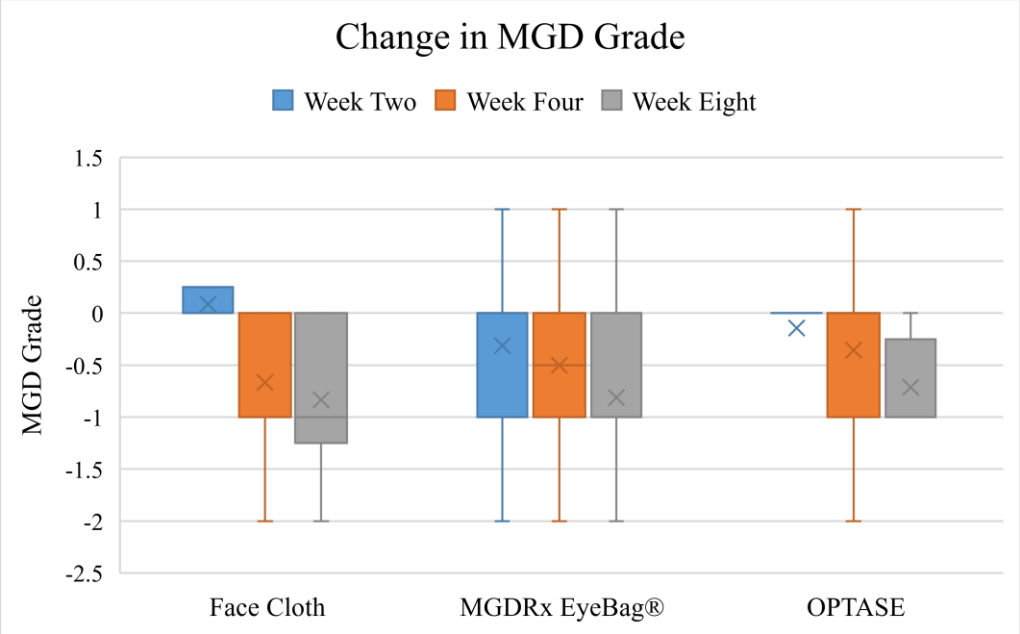
| **Variable** | **Time** | **Group 1 (Warm Face Cloth) N = 12** | **Group 2 (MGDRx Eye Bag®) N = 16** | **Group 3 (Optase Moist Heat Mask™) N = 14** | Kruskal Wallis |
|---|---|---|---|---|---|
| **Quantity *Demodex folliculorum* (n) Lash Rotation** | BL | 0.00 (0.00 – 5.25) | 0.50 (0.00 – 2.00) | 1.50 (0.00 – 4.25) | p = 0.78 |
| | W2 | 0.50 (0.00 – 1.75) | 0.00 (0.00 – 2.00) | 0.00 (0.00 – 1.00) | p = 0.69 |
| | W4 | 0.50 (0.00 – 3.50) | 0.00 (0.00 – 3.00) | 0.00 (0.00 – 4.00) | p = 0.91 |
| | W8 | 0.50 (0.00 – 8.50) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 1.50) | p = 0.28 |
| Friedman's | | p = 0.87 | p = 0.64 | **p = 0.04*** | Kruskal Wallis |
| **Quantity *Demodex folliculorum* (n) Microscope** | BL | 0.00 (0.00 – 1.75) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 2.75) | p = 0.84 |
| | W2 | 1.00 (0.00 – 2.75) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 0.00) | p = 0.02 |
| | W4 | 0.50 (0.00 – 1.00) | 0.00 (0.00 – 0.00) | 0.50 (0.00 – 1.75) | p = 0.32 |
| | W8 | 0.00 (0.00 – 1.75) | 0.00 (0.00 – 0.00) | 0.00 (0.00 – 1.00) | p = 0.49 |
| Friedman's | | [F] p = 0.72 | [F] p = 0.67 | [F] p = 0.18 | Chi-square |
| **MGD Grade (0-3)** | BL | 2.00 (1.00 – 2.00) | 2.00 (1.00 – 2.00) | 1.00 (1.00 – 2.00) | p = 0.16 |
| | W2 | 2.00 (1.00 – 2.00) | 2.00 (1.00 – 2.00) | 1.00 (1.00 – 2.00) | p = 0.22 |
| | W4 | 1.00 (1.00 – 1.50) | 1.00 (1.00 – 2.00) | 1.00 (1.00 – 1.00) | p = 0.92 |
| | W8 | 1.00 (0.00 – 1.50) | 1.00 (0.00 – 1.00) | 1.00 (0.00 – 1.00) | p = 0.33 |
| Friedman's | | **p = 0.008*** | **p = 0.002*** | **p = 0.002*** | |




| Post - hoc | Face Cloth | MGDRx EyeBag® | OPTASE™ Moist Heat Mask |
|---|---|---|---|
| B – W2 | 0.271 | 0.730 | 0.023 |
| B – W4 | 0.677 | 0.523 | 0.313 |
| B – W8 | 0.527 | 0.344 | **0.008*** |
| W2 – W4 | 0.496 | 0.713 | 0.500 |
| W2 – W8 | 0.173 | 0.713 | 1.000 |
| W4 – W8 | 0.831 | 1.000 | 0.125 |

Figure 30. Box plot illustrating the change in quantity of *Demodex folliculorum* at two, four, and eight weeks, with each treatment. Post – hoc analysis: Wilcoxon signed ranks test pairwise comparison with Bonferroni adjusted correction applied ($\alpha \leq 0.0083$ significant). X represents the mean change in quantity of *Demodex folliculorum*. B: Baseline, W2: Week Two, W4: Week 4, W8: Week Eight. *Significant results highlighted in bold.

Table 41 also displays MGD grade mean and SD at each visit, for each group. A significant improvement in MGD grade with time for each treatment was detected (Friedman's $p < 0.05$, Table 39). The box plot in Figure 31 shows MGD grade at each visit, for each group. However, post-hoc analysis using alpha adjusted WSR test pairwise comparisons revealed; only improvements from baseline to week eight in Group 2 (Eyebag) (WSR $p = 0.008$); and improvements from baseline to week eight (WSR $p = 0.002$) and week two to week eight (WSR $p = 0.003$) in Group 3 (Optase); were found to be significant. There was no significant difference found between the treatments at any time point over the eight weeks (KW $p > 0.05$, Table 41).




| | | Face Cloth | MGDRx EyeBag® | OPTASE™ Moist Heat Mask |
|---|---|---|---|---|
| Median (IQR) | W2 | 0 (0 – 0.75) | 0 (-1 – 0) | 0 (0 – 0) |
| | W4 | -1 (-1 – 0) | -0.5 (-1 – 0) | 0 (-1 – 0) |
| | W8 | -1 (-1.75 - 0) | -1 (-1 – 0) | -1 (-1 – 0) |
| p value | | **[F] p = 0.008*** | **[F] p = 0.002*** | **[F] p = 0.002*** |

| Post - hoc | Face Cloth | MGDRx EyeBag® | OPTASE™ Moist Heat Mask |
|---|---|---|---|
| BL – W2 | 0.414 | 0.527 | 1.000 |
| BL – W4 | 0.035 | 0.035 | 0.180 |
| BL – W8 | 0.030 | **0.008*** | **0.002*** |
| W2 – W4 | 0.021 | 0.025 | 0.414 |
| W2 – W8 | 0.018 | 0.024 | **0.003*** |
| W4 – W8 | 0.414 | 0.157 | 0.058 |

X represents the mean change in MGD Grade. Post – hoc analysis: Wilcoxon signed ranks test pairwise comparison with Bonferroni adjusted correction applied ($\alpha \leq 0.008$ significant). BL: Baseline, W2: Week Two, W4: Week 4, W8: Week Eight. *Significant results highlighted in bold.

Figure 31. Box plot illustrating MGD Grade, derived from composite quality and expressibility scores of both upper and lower eyelids, at baseline, and two, four, and eight weeks, with each treatment.

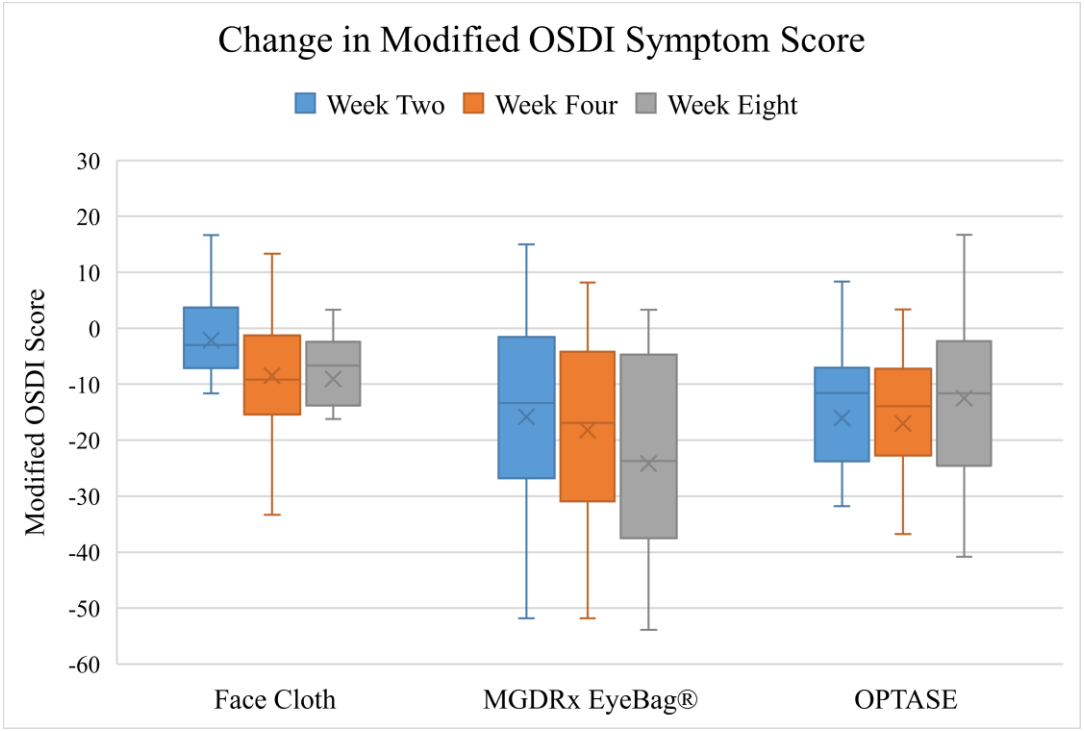
Table 42 demonstrates the prevalence of pathogenic and non-pathogenic infestation for each treatment group, at each point in time. The overall prevalence of DF in each group at baseline was: Group 1 (Warm Face Cloth) - 66.67%, Group 2 (Eyebag) – 50.00%, and Group 3 (Optase) – 57.14%. No significant difference was detected between the groups (KW $p = 0.61$). This reduced to an overall prevalence of: Group 1 – 58.33%, Group 2 – 25.00% and Group 3 – 50.00% after eight weeks. As can be seen from Table 42, Group 3 showed the greatest change in pathogenic infestation. This likely accounted for the reason Group 3 appeared to have the greatest overall effect on DF quantity. Group 2 appeared to have the greatest overall effect on DF prevalence. Nonetheless, no significant difference in prevalence was established between the groups at week eight (KW $p = 0.19$). Furthermore, no significant difference in prevalence of DF infestation was established within the groups over the eight weeks: Group 1 (Friedman's $p = 0.99$), Group 2 (Friedman's $p = 0.18$) and Group 3 (Friedman's $p = 0.49$).

Table 42. Prevalence of pathogenic and non-pathogenic *Demodex folliculorum* infestation in each treatment group, at each time point. B: Baseline, W2: Week Two, W4: Week 4, W8: Week Eight.

| | Time | No Demodex % (n) | Non-Pathogenic Infestation % (n) | Pathogenic Infestation % (n) | Overall Prevalence |
|---|---|---|---|---|---|
| **Group 1: Face Cloth (n = 12)** | BL | 33.33 (n = 4) | 33.33 (n = 4) | 33.33 (n = 4) | 66.67% (n = 8) |
| | W2 | 33.33 (n = 4) | 41.67 (n = 5) | 25.00 (n = 3) | 66.67% (n = 8) |
| | W4 | 33.33 (n = 4) | 41.67 (n = 5) | 25.00 (n = 3) | 66.67% (n = 8) |
| | W8 | 41.67 (n = 5) | 25.00 (n = 3) | 33.33 (n = 4) | 58.33% (n = 7) |
| | *Difference* | *+8.34% (n = 1)* | *-8.34% (n = -1)* | *0.00% (n = 0)* | *-8.14% (n = -1)* |
| **Group 2: MGDRx EyeBag® (n = 16)** | BL | 50.00 (n = 8) | 31.25 (n = 5) | 18.75 (n = 3) | 50.00% (n = 8) |
| | W2 | 62.50 (n = 10) | 12.50 (n = 2) | 25.00 (n = 4) | 37.50% (n = 6) |
| | W4 | 62.50 (n = 10) | 25.00 (n = 4) | 12.50 (n = 2) | 37.50% (n = 6) |
| | W8 | 75.00 (n = 12) | 12.50 (n = 2) | 12.50 (n = 2) | 25.00% (n = 4) |
| | *Difference* | *+25.00% (n = 4)* | *-18.75% (n = -3)* | *-6.25% (n = -1)* | *-25.00% (n = -4)* |
| **Group 3: OPTASE™ Moist Heat Mask (n = 14)** | BL | 42.86 (n = 6) | 28.57 (n = 4) | 28.57 (n = 4) | 57.14% (n = 8) |
| | W2 | 50.00 (n = 7) | 35.71 (n = 5) | 14.29 (n = 2) | 50.00% (n = 7) |
| | W4 | 50.00 (n = 7) | 28.57 (n = 4) | 21.43 (n = 3) | 50.00% (n = 7) |
| | W8 | 50.00 (n = 7) | 35.71 (n = 5) | 14.29 (n = 2) | 50.00% (n = 1) |
| | *Difference* | *+7.14% (n = 1)* | *+7.14% (n = 1)* | *-14.29% (n = -2)* | *-7.14% (n = -1)* |

Figure 32 illustrates a box plot of the modified OSDI score for each group at each visit. Table 43 displays the mean and SD of the modified OSDI symptoms score for each group, at each visit. There was a significant improvement in symptom score with time for each treatment (repeated ANOVA $p = 0.04$, $p = 0.02$ and $p = 0.02$ for Groups 1 – 3, respectively). As can be seen from Figure 36, the greatest reduction in symptoms appears to be in Group 2 and Group 3. Post-hoc analysis with Bonferroni pair-wise comparison revealed; only the reduction in symptoms from baseline to week two and baseline to week eight ($p = 0.03$ and $p = 0.008$, respectively) in Group 2, and reduction in symptoms from

baseline to week two and baseline to week eight (p =0.01 and p = 0.05, respectively) in Group 3, were found to be significant. There was no significant difference in modified OSDI symptom score between the treatments at any time point over the eight weeks (ANOVA p > 0.05, Table 43).




| Post - hoc | Face Cloth | MGDRx EyeBag® | OPTASE™ Moist Heat Mask |
|---|---|---|---|
| BL – W2 | 1.000 | **0.033*** | **0.013*** |
| BL – W4 | 0.266 | 0.078 | 0.064 |
| BL – W8 | 0.054 | **0.008*** | **0.047*** |
| W2 – W4 | 0.673 | 1.000 | 1.000 |
| W2 – W8 | 0.687 | 0.100 | 1.000 |
| W4 – W8 | 1.000 | 0.464 | 1.000 |

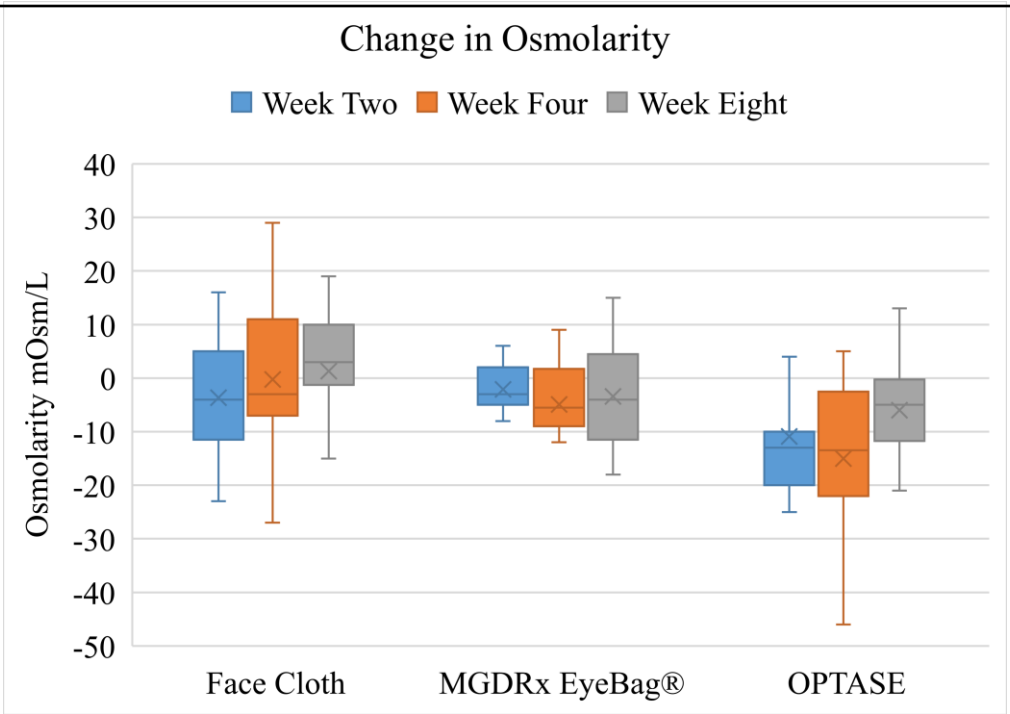
X illustrates mean change in modified OSDI score. Post – hoc analysis: Bonferroni ($\alpha \leq 0.05$ significant). BL: Baseline, W2: Week Two, W4: Week 4, W8: Week Eight. *Significant results highlighted in bold.

Figure 32. Box plot illustrating modified OSDI symptom score for each treatment groups, at each visit.

Table 43. Dry eye parameters; Modified OSDI symptom score (mean ± SD), Osmolarity (mean ± SD), tear film instability (%), NITBUT (median, IQR), ocular surface staining (median, IQR), Schirmer I (median, IQR), before and after treatment. BL: Baseline, W2: Week Two, W4: Week Four, W8: Week Eight. $\alpha \leq 0.05$ significant. *Significant results highlighted in bold.

| Variable | Time | Group 1 | Group 2 | Group 3 | ANOVA |
|---|---|---|---|---|---|
| **Modified OSDI Score (0-100)** | BL | 24.21 ± 15.27 | 39.81 ± 23.21 | 39.01 ± 20.34 | p = 0.12 |
| | W2 | 22.05 ± 15.29 | 27.40 ± 19.29 | 24.70 ± 18.87 | p = 0.69 |
| | W4 | 15.71 ± 7.86 | 23.03 ± 19.55 | 23.65 ± 19.89 | p = 0.41 |
| | W8 | 15.14 ± 12.75 | 16.67 ± 13.07 | 26.52 ± 17.15 | p = 0.10 |
| Repeated ANOVA | | **p = 0.04*** | **p = 0.02*** | **p = 0.02*** | ANOVA |
| **Osmolarity (mOsm/L)** | BL | 304.17 ± 18.10 | 303.53 ± 9.55 | 318.86 ± 13.44 | **p = 0.008*** |
| | W2 | 301.27 ± 17.47 | 304.71 ± 12.98 | 310.27 ± 16.46 | p = 0.41 |
| | W4 | 303.92 ± 18.88 | 299.93 ± 13.68 | 305.92 ± 14.69 | p = 0.69 |
| | W8 | 305.50 ± 18.82 | 303.07 ± 11.91 | 312.86 ± 13.37 | p = 0.19 |
| Repeated ANOVA | | p = 0.60 | p = 0.86 | **p = 0.01*** | Chi-square |
| **Instability (%)** | BL | 83.33% | 40.00% | 71.44% | p = 0.06 |
| | W2 | 54.55% | 50.00% | 63.67% | p = 0.91 |
| | W4 | 50.00% | 53.33% | 66.67% | p = 0.78 |
| | W8 | 41.67% | 60.00% | 64.22% | p = 0.53 |
| Cochran's Q | | p = 0.22 | p = 0.87 | p = 0.79 | Kruskal Wallis |
| **NITBUT (secs)** | BL | 4.19 (2.81 – 13.46) | 5.15 (2.98 – 11.44) | 4.13 (2.60 – 5.60) | p = 0.16 |
| | W2 | 7.63 (2.60 – 13.64) | 4.90 (2.83 – 11.32) | 3.97 (2.60 – 8.20) | p = 0.69 |
| | W4 | 6.44 (2.83 – 11.10) | 6.37 (2.64 – 8.21) | 4.17 (.60 – 6.90) | p = 0.70 |
| | W8 | 4.14 (3.14 – 9.46) | 6.78 (3.56 – 14.87) | 5.13 (2.97 – 5.13) | p = 0.34 |
| Friedman's | | p = 0.95 | p = 0.87 | p = 0.60 | Kruskal Wallis |
| **Staining (0-15)** | BL | 1.00 (0.00 – 2.00) | 1.00 (0.00 – 1.75) | 0.00 (0.00 – 2.00) | p = 0.98 |
| | W2 | 0.00 (0.00 – 2.00) | 1.00 (0.00 – 2.00) | 1.00 (0.00 – 3.25) | **p = 0.03*** |
| | W4 | 0.00 (0.00 – 0.75) | 0.00 (0.00 – 1.00) | 0.00 (0.00 – 1.00) | p = 0.72 |
| | W8 | 0.00 (0.00 – 0.75) | 0.00 (0.00 – 0.75) | 0.00 (0.00 – 0.75) | p = 0.95 |
| Friedman's | | **p = 0.04*** | **p = 0.007*** | **p = 0.04*** | Kruskal Wallis |
| **Schirmer (mm/5min)** | BL | 10.50 (8.25 - 19.00) | 32.00 (11.00 – 35.00) | 21.50 (5.25 – 28.75) | p = 0.25 |
| | W2 | 8.00 (5.25 – 15.25) | 18.00 (11.00 – 35.00) | 13.50 (6.25 – 25.25) | p = 0.06 |
| | W4 | 13.50 (5.75 – 17.75) | 28.00 (7.00 – 35.00) | 13.00 (7.00 – 27.75) | p = 0.67 |
| | W8 | 8.00 (8 – 13.75) | 20.00 (15 – 32.00) | 17.00 (6.00 – 17.00) | p = 0.07 |
| Friedman's | | p = 0.21 | p = 0.93 | p = 0.57 | |

Figure 33 displays a box plot of osmolarity values for each group at each visit. There was a significant reduction in osmolarity for participants in Group 3 over the eight weeks (repeated ANOVA $p = 0.014$, refer Table 43). Bonferroni post-hoc analysis showed that this was significant from baseline to week four only ($p = 0.017$, Figure 33). There was no significant change in osmolarity detected for participants in Groups 1 and 2 at any stage over the eight weeks (repeated ANOVA $p > 0.05$, Table 43). Overall, repeated measures of ANOVA taking treatment into consideration as a between participants' factor, showed no significant change in osmolarity overtime ($p = 0.107$).




| Post - hoc | Face Cloth | MGDRx EyeBag® | OPTASE™ Moist Heat Mask |
|---|---|---|---|
| BL – W2 | 1.000 | 1.000 | 0.220 |
| BL – W4 | 1.000 | 1.000 | **0.017*** |
| BL – W8 | 1.000 | 1.000 | 0.980 |
| W2 – W4 | 1.000 | 1.000 | 1.000 |
| W2 – W8 | 1.000 | 1.000 | 1.000 |
| W4 – W8 | 1.000 | 1.000 | 0.699 |

X denotes mean change in osmolarity. Post – hoc analysis: Bonferroni ($\alpha \leq 0.05$ significant). BL: Baseline, W2: Week Two, W4: Week 4, W8: Week Eight. *Significant results highlighted in bold.

Figure 33. Box plot illustrating osmolarity values for each treatment groups at each visit.

Tear film instability, an inter-eye difference of ≥ 8mOsm/L, was also measured for each participant. Table 43 displays the prevalence of tear film instability in each group, at each visit. As shown in Table 43, after eight weeks tear film instability had reduced in Group 1 (41.67%) and Group 3 (64.22%) but had increased slightly in Group 2 (60.00%).

However, none of these changes were found to be significant within each group over time (Friedmans $p > 0.05$) or between each group at any point in time (KW $p > 0.05$).

Table 43 also demonstrates ocular surface staining mean and range for each group at each visit. As can be seen from Table 43, there was a significant reduction in ocular surface staining over time for each treatment (Friedman's $p = 0.04$, $p = 0.007$ and $p = 0.04$ for Groups 1 – 3, respectively). Post-hoc analysis, with Bonferroni corrected alpha, using WSR test pairwise comparisons revealed; only a reduction in staining from week two to week eight in Group 2 (1.71, range 0 – 8 versus 0.33, range 0 – 2; WSR $p = 0.006$), and week two to week eight in Group 3 (mean: 1.77, range 0 – 9 versus mean: 0.50, range 0 – 4; WSR $p = 0.008$), were found to be significant. There was no significant difference in ocular surface staining detected between the treatments at any time over the eight weeks (KW $p > 0.05$).

Mean NITBUT and Schirmer I scores for each treatment group, at each time point, are also shown in Table 43 above. No significant change in NITBUT, or Schirmer I score, was detected over time in each group (Friedman's $p > 0.05$), or between treatments (KW $p > 0.05$).

Employing the dry eye classification (if found to have three or more of the following parameters; modified OSDI $\geq$ 13, osmolarity $\geq$ 308 mOsm/L, inter-eye variability $\geq$ 8mOsm/L, NITBUT < 10 secs, ocular surface staining $\geq$ 3 Oxford score or Schirmer I score $\leq$ 5mm/5min), the prevalence of dry eye for each group, at each visit, is shown in Figure 34. No significant change in proportion of subjects with dry eye was detected in any of the three treatment groups post-treatment (Group 1 – 3: Friedman's $p = 0.36$, $p = 0.77$ and $p = 0.28$, respectively).

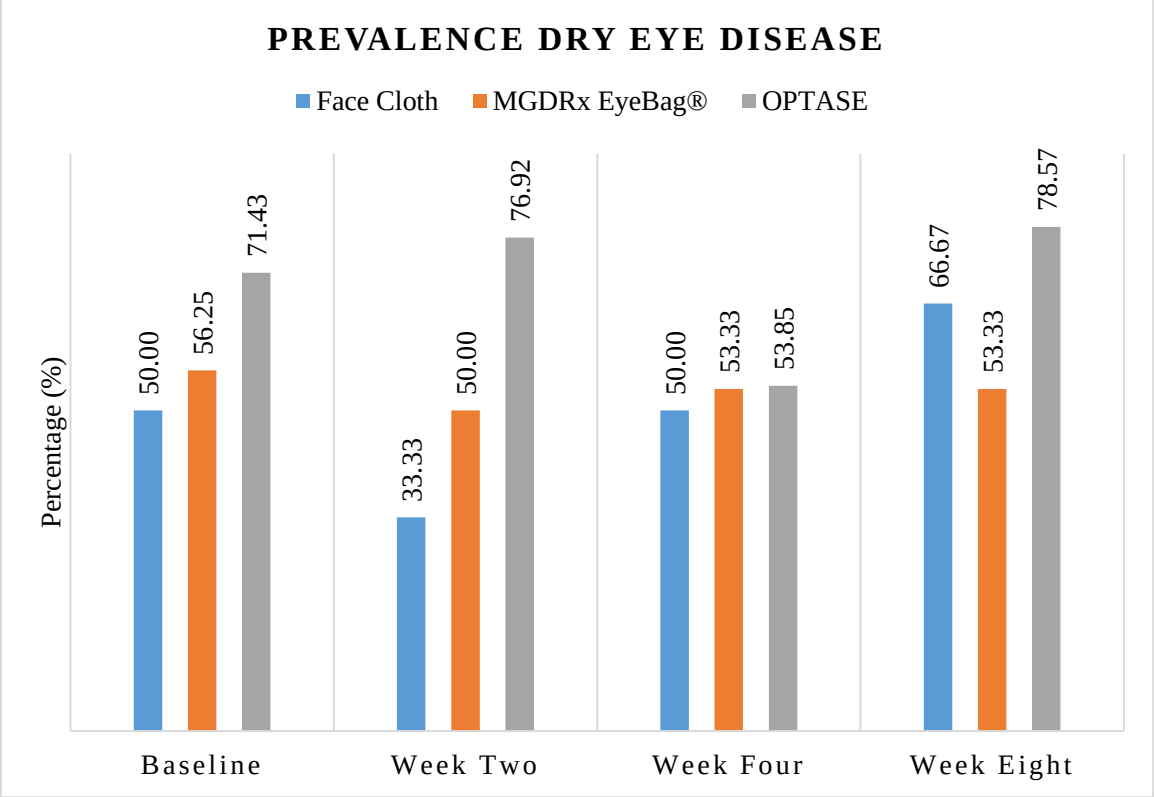



Figure 34. Prevalence of dry eye disease in each warm compress treatment group at each time point.

### 7.5 Discussion

As mentioned previously in Section 1.4.2, DB is most commonly associated with MGD; however, an association between DF and MGD has also been established in the literature.[7,30,48] Currently, lid scrubs remain the principal treatment recommended for *Demodex* blepharitis,[3] and warm compress therapy for MGD.[138] Intense pulsed light therapy has been previously used, successfully, to treat DF infestation.[283] However, to the best of the authors' knowledge, this is the first study to investigate the effect of heat therapy using warm compresses on DF infestation. This study has shown that Optase may have a double therapeutic effect, treating MGD and reducing DF in combination. Over the eight weeks, moist heat therapy from Optase significantly reduced the quantity of DF detected

using the eyelash rotation technique. No significant change was noted with the moist heat from the warm face cloth, or the dry heat from the Eyebag. The reason for this is unknown at present. It could be associated with the compresses ability to achieve a higher treatment temperature. As mentioned previously, manufacturers of Optase report temperatures ranging from 50 °C to 41 °C, over the 10-minute duration of therapy.[279] However, the Eyebag achieves temperatures from 46 °C dropping to 39 °C, which is less than the recommended 40 °C,[192] after 5 minutes.[280] Similarly, the warm face cloth does not maintain its heat for longer than two minutes, without needing to be re-heated.[191,192] This could affect the therapeutic temperature achieved by the face cloth with respect to treating DF infestation. Furthermore, greater quantities of DF have been found in dryer skin.[284,285] As Optase is a moist heat device and Eyebag is a dry heat device, it is possible that moisture may have played a role in the therapeutic efficacy demonstrated by Optase. However, further investigation is warranted for verification.

There was a considerable improvement in prevalence of DF infestation in Group 2 (Eyebag) from 50.00% at baseline to 25.00% at week eight (Table 42); yet there was no significant reduction in the quantity of DF detected on participants with DF in the same group. Although there was no significant difference in mean quantity of DF between the three treatment groups at baseline; Group 2 had the lowest quantity and lowest percentage prevalence of participants with pathogenic infestation of the three groups at baseline. Therefore, it is possible that these lower numbers of DF within Group 2 affected the compresses ability to exhibit significant changes over time. As such, a post-hoc power calculation was conducted on the data, and a low power ($1 - \beta = 0.26$) was detected; which would have affected the power and significance of the results. Future research that focusses

on pathogenic infestation (to ensure higher quantities of DF per participant), would help reduce any limitations caused by lower numbers of DF.

Similarly, no significant change was detected in quantity of DF using the traditional modified Coston method,[25] for any of the warm compresses studied. Limitations of this technique regarding under-counting of DF have been discussed previously in the literature,[25,29,50] and in Section 7.5. Similar to results from Chapter 7, the quantity of DF detected whilst rotating the eyelash *in-situ* was significantly greater than that observed on microscopic examination in the current study also (paired t-test $p = 0.01$, $p = 0.01$ and $p = 0.007$ for baseline, week four and week eight, respectively). No significant difference was observed between both techniques in week two, where overall a low quantity of DF was detected. The authors infer that the low quantity of DF detected in week two may be as a result of participants using the warm compresses for 10 minutes twice a day; thus, increasing the length of time DF were subjected to heat therapy. After week two, participants reduced treatment time to 10 minutes once a day, and the quantities of DF appeared to increase again slightly. The authors postulate that the reason for a lack of significance found using the modified Coston method was due to the limitations of the method, previously described (Section 7.5): resulting in under-counting and mis-representation of the degree of DF infestation present.

As mentioned previously in Section 2.3.4, hyperosmolarity is accepted as one of the characteristic signs of DED and ocular surface inflammation.[117] In MGD, availability of meibum to the ocular surface is decreased, either through reduced secretion (possibly due to poor expressibility, or severe meibomian gland dropout), or a poor quality secretion; thus causing quicker tear evaporation and, as one would expect, hyperosmolarity.[286] Although, reports in the literature differ with regards to this.[286–289] In the current study, participants in

Group 3 (Optase) demonstrated a significant improvement in osmolarity overtime. However, participants in Group 3 had a greater osmolarity at baseline in comparison to participants in the other two groups, which is likely to have influenced the overall reduction in osmolarity values detected in Group 3. Comparable to the current study, Kim et al[290] found a significant improvement in osmolarity post-treatment in participants with a baseline osmolarity of > 307mOsm/L, but no significant improvement in osmolarity post-treatment in participants with an osmolarity of < 307mOsm/L at baseline. The authors deduced that heat treatment with a thermal pulsation device was effective at improving osmolarity in participants with abnormal tear osmolarity, but did not have an effect on those with normal osmolarity.[290] In contrast, Godin et al[287] discovered that treatment of MGD using thermal pulsation on a cohort of participants with Sjogrens syndrome initially caused an increase in osmolarity two months after treatment (305.2 vs. 315.6, $p = 0.026$), but no significant increase one year after treatment (305.2 vs 311.0, $p = 0.86$). Baseline osmolarity values in Group 1 (Warm Face Cloth) and Group 2 (Eyebag), in the current study, were similar to those found by Godin et al.[287] However, neither the heat therapy from the warm face cloth or the Eyebag caused an increase in osmolarity values after two months of treatment. Giannaccare et al[288] investigated the performance of an ocular surface work-up, using modern automated non-invasive techniques for diagnosing MGD: such as NITBUT, osmolarity, lipid layer thickness and non-contact meibography. The authors discovered significant differences between MGD participants and controls for NITBUT, OSDI symptom score and meibomian gland loss. However, they found no significant difference in osmolarity values between the two groups. Similar to the current study, and the study by Godin et al[287], Giannaccare et al[288] found a low mean osmolarity value within their MGD participants using the TearLab$^{TM}$ (303.5 ± 9.8 mOsm/L). The lack of significance

discovered in the present study may be related to the low osmolarity found amongst MGD patients.[287,288] It has been proposed that MGD disease may not cause hyperosmolarity, as the disease alone may not be enough to alter the homeostatic control in many participants.[288,289] In the present study, the authors have been unable to find an explanation for the higher osmolarity values observed in Group 3 (Optase). TearLab™ has been shown to provide repeatable and reproducible tear osmolarity measurements on a healthy ocular surface,[178] but becomes less repeatable and shows increased variability with increasing disease severity.[171,291] Participants in Group 3 were not significantly older, or more symptomatic, and did not have a significantly greater quantity of DF, MGD, ocular surface staining, reduced TBUT or tear secretion at baseline. Furthermore, repeated measures of ANOVA, with treatment as a between participants' factor, showed no significant change in osmolarity overtime. Therefore, the authors infer that the hyperosmolarity observed at baseline in Group 3 may be coincidence, or may be due to measurement errors that can occur with TearLab™.

Heat therapy increases the availability of meibum to the tear film and ocular surface, helping to improve the stability of the tears, and thus, increase TBUT.[191,280,292–294] In the current study, the greatest improvement in NITBUT was with the Eyebag, a dry warming device. These results are in keeping with Arita et al[292] who found that only dry warming devices were able to significantly improve the oily tear film layer, and reduce evaporation. In the same study, no improvement was observed after the repeated use of a hot towel compress, and the authors' concluded that moisture on the surface of the eyelid skin could give rise to evaporative cooling; thus limiting the beneficial effects of warming.[292] There was no significant increase in NITBUT demonstrated in the current study. This may be accredited to the differences in measurement techniques and timings of measurements post-

treatment. In previous studies, TBUT has been evaluated invasively using fluorescein,[294,295] or non-invasively using a TearScope Plus.[280,293] Fluorescein dye is invasive and has been shown to alter the tear film and affect the natural TBUT.[296,297] TearScope Plus is a non-invasive method; however, it is a subjective measurement, and depends on the examiner detecting the first perceptible break in the fine line pattern. In the current study, NITBUT was measured using the automated Medmont E300 Corneal Topographer; which has demonstrated good repeatability with a high sensitivity and specificity for diagnosing moderate to severe DED.[174] Therefore, all measurements were objectively taken by the topographer, which is more sensitive to small tear film instabilities. Furthermore, in previous studies, many TBUT measurements have been taken immediately after, 5-10 minutes after, or up to 1 hour after heat therapy has been applied.[280,292,293] However, in the current study, participants applied the warm compresses at home in the evenings. Therefore, there was a longer time period between last warm compress and time of measurement. While immediate effect of treatment has not been shown in the current study, the results do demonstrate the 'realistic effect' of each treatment on participants' tear film and ocular surface.

Another shortcoming of the current study is that participants were applying the heat therapy at home. As such, it was not possible to measure the temperature of the compress each time it was used. Inadequate lid warming of warm compresses have been noted previously.[298,299] Although all participants were given written instructions, it is possible that participants may not have heated the compress sufficiently, or did not complete the full 10-minute therapy requested of them. To monitor compliance, at each aftercare, participants were asked to report on how many evenings and for how long they used the warm compress as instructed. At baseline overall reported compliance was 83.61%: 81.14% warm face

cloth, 84.02% Eyebag, and 85.16% Optase. This remained relatively stable over the duration of the study. The overall reported compliance at the end of the study was 83.74%: 77.92% warm face cloth, 86.81% Eyebag, and 85.45% Optase. Poor compliance is a problem with examining efficacy of treatments; thus, it would be preferable to have 100% compliance and treatment delivered in-house by the examiner. However, the use of warm compresses in the current study is likely a better indicator of warm compress use in 'real world' environments. Nonetheless, the warm face cloth group had the lowest overall compliance, and the potential impact that this may have had on the compresses ability to demonstrate significant results for treating MGD of DF blepharitis cannot be excluded. It is difficult to monitor compliance for use of warm compresses in the home. However, following-up with participants at return visits, requesting they demonstrate how they use the compresses, may be a good way to remind participants of the protocol, correct any mistakes and overall help improve compliance.

The Eyebag and Optase significantly reduced the presence of MGD over the duration of the study. Although some improvement in MGD was seen with the warm face cloth, these changes were not found to be significant. Furthermore, the warm face cloth compress had to be re-dipped every two minutes, as it lost its heat quickly. Therefore, compresses that can be heated once and used for 10 minutes at a time are more efficient and convenient for patients. A higher rate of attrition was also found in the warm face cloth group compared to either of the other two groups, and overall compliance was lowest in this group. The authors feel that this was due to the inconvenient nature of the treatment. As the study was conducted on participants with a relatively low grade of MGD and DF infestation, this may have impacted on the ability of the compresses to elicit change. Future studies should

examine participants with a greater severity of MGD and DF infestation to demonstrate a more magnified response to treatment.

### 7.6 Conclusion

The microwaveable compresses, Eyebag and Optase, exhibited a greater ability to treat MGD, reduce symptoms and reduce ocular surface staining, compared to the more 'traditional' warm face cloth compress. Optase demonstrated the ability to provide dual treatment to patients with MGD and *Demodex* blepharitis. Further research is required to investigate whether moisture, heat, or a combination of both are the underlying therapeutic forces at play.

It has previously been reported that DF tails are visible within the eyelash follicle while manipulating the eyelash *in situ* during a slit-lamp biomicroscope examination.[50] In the early stages of this research project it became evident that counting DF mites on microscopic examination of the epilated eyelash alone was resulting in under counting and mis-representing the degree of infestation present. In an effort to counter-act this, from recruitment phase two onwards DF quantity on lash manipulation and lash epilation were counted. Chapter 8 discusses the comparison of the two investigative methods used throughout this research project. The results of this observational study have been accepted for publication in Eye and Contact Lens and has been adapted accordingly for Chapter 8.

# CHAPTER EIGHT: THE CLINICAL USE OF EYELASH MANIPULATION IN THE EXAMINATION OF *DEMODEX FOLLICULORUM* BLEPHARITIS

## 8.1 Abstract

**Purpose:** To compare the efficacy of using an eyelash manipulation technique to the traditional eyelash epilation and subsequent microscopic examination technique, when investigating for the presence of DF in a clinical setting.

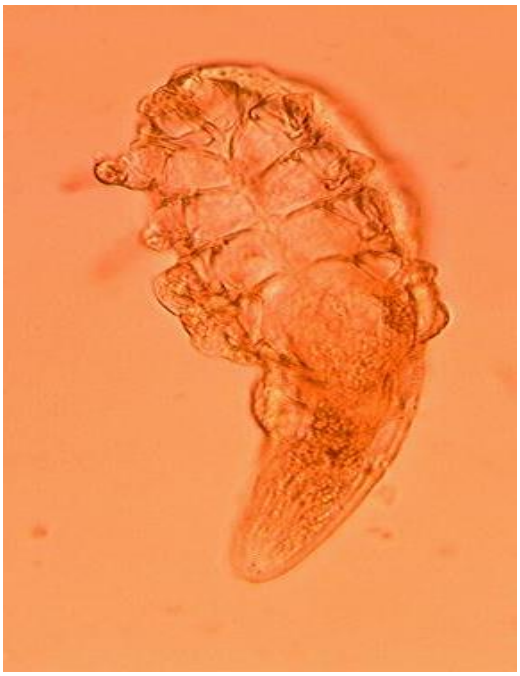
**Methods:** Four hundred and twenty-eight eyelashes of 107 participants were selected to evaluate the association between the quantity of DF visible on eyelash manipulation to that counted on microscopic examination of the same epilated eyelash. Eyelash manipulation was conducted as described in Section 2.2.9. As the eyelash was manipulated, the number of DF seen emerging from the follicle was counted. The same eyelash was then epilated and the number of DF on the epilated eyelash was counted using the modified Coston method (Section 1.7.2). Data was analysed to check for agreement between the two techniques.

**Results:** Intra-class correlation co-efficient showed moderately good agreement for assessing the quantity of DF (0.78) between both techniques. However, the Bland-Altman plot suggested consistently higher quantities were discovered on eyelash manipulation. The overall median quantity of DF was also greater on eyelash manipulation than on microscopic examination of the epilated eyelash (0.00 mites, IQR: 0.00 – 2.00 and 0.00 mites, IQR 0.00 – 1.00, respectively) ($p = < 0.001$). Weighted kappa ($\kappa_w = 0.56$) indicated weak levels of agreement between the two methods for addressing severity of infestation.

**Conclusion:** Eyelash manipulation exhibited larger quantities of DF than complete epilation of the eyelash with microscopic examination. In a clinical setting, complete eyelash epilation is not necessary to accurately detect *Demodex* blepharitis requiring treatment.

### 8.2 Introduction

As mentioned in Section 1.4, DF typically reside in clusters within the eyelash follicles[41,43,62] (Figure 35 (a)) and DB typically reside in solitude, deeper in the sebaceous and meibomian glands (Figure 35 (b)).[41,61,62,65] The scraping movement of the mites within the eyelash follicles damages the follicles,[43,62] causing hyperplasia and hyperkeratinisation of the epithelial cells, which becomes visible as CD at the base of the eyelashes (Figure 35(c)). Chronic *Demodex* infestation also causes the eyelash follicles to widen and the eyelashes within to become looser, which can lead to trichiasis, madarosis, and eyelash misdirection.[49,62]

(a)  (b) 

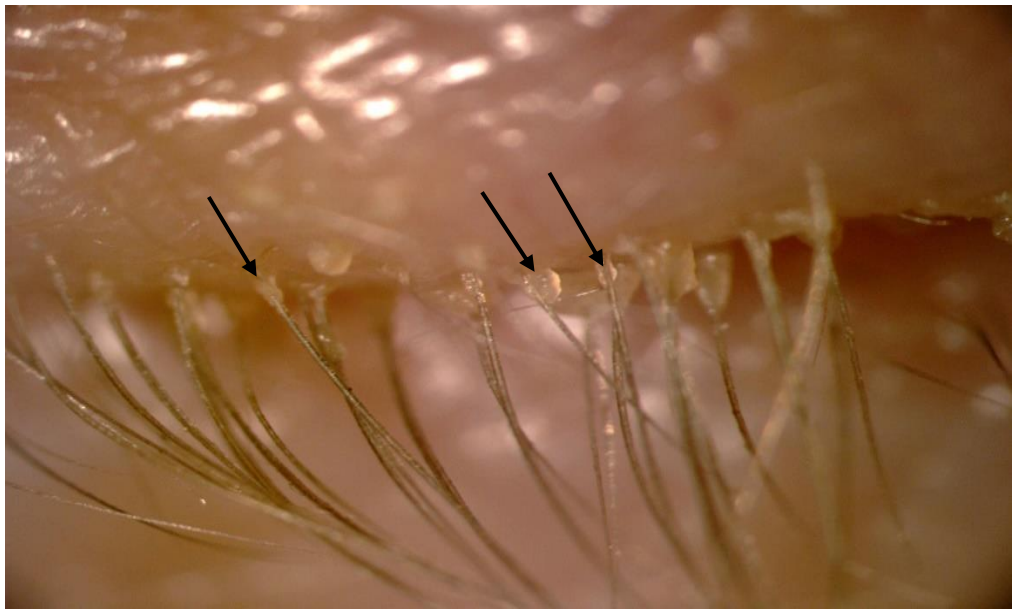
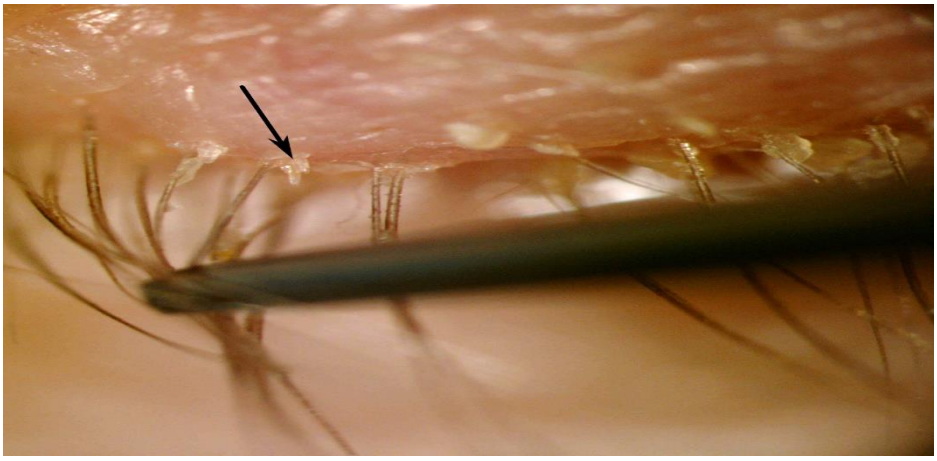
(c) 

Figure 35. (a) *Demodex folliculorum* cluster on an epilated eyelash; (b) Single *Demodex brevis*; (c) Cylindrical dandruff visible as a translucent cuff along the base of the eyelash, black arrows.

Confirming the presence of DF and thus diagnosis of *Demodex* blepharitis can be achieved by eyelash epilation,[25] and inspecting the eyelash under a light microscope, or smartphone[145]; or laser confocal microscopy, to view DF *in-vivo*.[16,65,146] Previous investigators have described the use of eyelash manipulation as an adjunct procedure prior to eyelash epilation in an attempt to stimulate DF to move towards the opening of the eyelash follicle.[12,61] In 2013, Mastrota indicated that it was possible to view, on the slit-lamp biomicroscope, DF tails emerging from the eyelash follicles as the eyelash was rotated *in-situ* (as can be seen in Figure 36(a)).[50]

Eyelash epilation alone often results in miscounting, as many DF remain within the follicle after the eyelash has been removed (Figure 36(b)).[25,29,50] This became particularly apparent during data collection for the pilot study. Clearly infested eyelash follicles were being given a recorded count of zero DF on microscopic examination: as the eyelash would fall out leaving all the DF behind within the eyelash follicle. This recurrent outcome prompted this investigation into evaluating the benefit of incorporating eyelash manipulation into the *Demodex* investigation routine. Furthermore, eyelash manipulation removes the stress and discomfort for patients that can be associated with having the eyelash epilated.

(a)



(b)

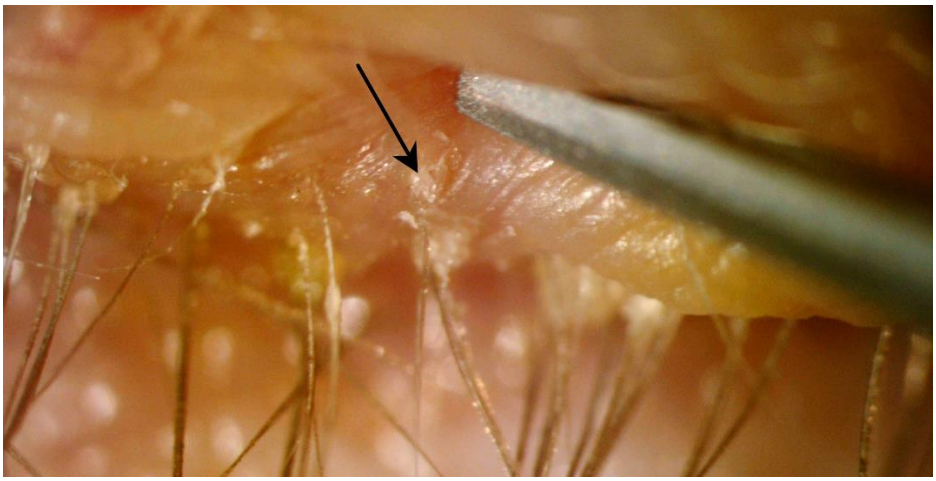


Figure 36. Illustrates manipulation of the eyelash within the follicle to observe *Demodex folliculorum* infestation *in-situ* (a) *Demodex folliculorum* visible emerging from the follicle during eyelash manipulation, black arrow. (b) *Demodex folliculorum* remaining within the follicle after the eyelash has been epilated, black arrow.

The current study aimed to show that complete epilation of the eyelash is not always necessary in a clinical setting, and eyelash manipulation may be a better indicator for severity of infestation than eyelash epilation.

### 8.3 Methods

Four hundred and twenty-eight eyelashes of 107 participants, one from each eyelid, were chosen to compare the quantity of DF detected using the two techniques previously described: eyelash manipulation (Section 1.8.4) and microscopic examination (Section

1.8.2) of the same epilated eyelash. Participants were recruited through the National Optometry Centre, TU Dublin.

Each participant was examined for the presence of DF on the slit-lamp biomicroscope as previously described (Section 2.3.9). In keeping with the modified Coston method, lashes with CD, if present, were selectively chosen to maximize the chance of finding DF.[25] In the absence of CD, eyelashes were chosen at random. As described previously, eyelash manipulation stimulates DF within the follicle, to emerge from the follicle opening (arrow Figure 36 (a)). However, often in severely infested follicles, after the eyelash was epilated, all or most of the DF remained within the follicle (Figure 36 (b)). This resulted in an inaccurately low count on microscopic examination of the eyelashes.

The quantity of DF visible on eyelash manipulation was compared to the quantity of DF visible on the microscope. It was difficult to count precise numbers in highly infested follicles, due to the greater quantities of DF present. Therefore, a 'severity of infestation' was categorised based on the quantity of DF counted on eyelash manipulation, as can be seen in Table 44. This system was based on work by Randon et al[65] using *in-vivo* confocal microscopy, who distinguished ≤ 3 mites per follicle as a low rate of infestation, and deemed non-pathogenic; and ≥ 4 mites per follicle as a high rate of infestation, and considered pathogenic. In the current study, it was discovered that several follicles were extremely infested (~ 10 mites visible in the follicle). As such, a second pathogenic group was included for analysis: severely infested (≥ 7 mites).

Table 44. Severity of *Demodex folliculorum* infestation of the eyelash follicle.

| Grade | Description |
|---|---|
| 0 | No mites present |
| 1 | Mild: Non-Pathogenic (1 – 3 mites present) |
| 2 | Moderate: Pathogenic (4 – 6 mites present) |
| 3 | Severe: Pathogenic (≥ 7 mites present) |

### *8.3.1 Statistical Analysis*

There are no previous studies that have directly compared the quantity of DF mites visible on eyelash manipulation and microscopic examination. As a result, expected mean and SD were not known. Data from the first 100 eyelashes were used to calculate the minimum number of pairs required. This was calculated using MedCalc® (ver.18.9.1), alpha 0.05, beta 0.8. From this, a minimum number of 255 pairs was required for a method comparison study using the Bland-Altman plot.

Statistical analysis was performed using SPSS version 25.0. Wilcoxon signed-rank test was used to compare the means of the two groups. Agreement between the two techniques was measured using ICC and Bland Altman's limits of agreement method[300] for continuous variables (quantity of DF) and $\kappa_w$ for ordinal variables (severity of infestation).

## 8.4 Results

Four hundred and twenty-eight eyelashes of 107 participants (39 males: 68 females, median age 54.00 years, IQR 33.00 – 65.00 years) were assessed for the presence of DF by

means of eyelash manipulation *in-situ* and subsequent epilation of the same eyelash for microscopic examination, by the modified Coston method.[25] *Demodex folliculorum* was detected on 44.16% of the eyelashes tested.

*Quantity of Demodex folliculorum*

Intra-class correlation co-efficient analysis was used to establish the level of agreement between both investigation techniques. With regards to examining quantity of DF detected using both techniques, the ICC was in moderately good agreement (ICC = 0.78: 95% Confidence Intervals 0.69 – 0.84). A cross-tabulation of the quantities of DF found using both techniques is shown in Table 45. Both techniques found no *Demodex folliculorum* on 239 eyelashes, non-pathogenic infestation (≤ 3 *Demodex folliculorum*) on 111 eyelashes and pathogenic infestation on 29 eyelashes. However, pathogenic infestation was missed on six eyelashes using eyelash manipulation in comparison to 43 eyelashes using microscopic examination (WSR $p < 0.001$).

Table 45. Cross tabulation of the quantity of *Demodex folliculorum* detected on eyelash manipulation versus microscopic examination. Green numbers indicate the quantity of eyelashes on manipulation that failed to detect pathogenic infestation (≥ 4 *Demodex folliculorum*). Purple numbers indicate the quantity of eyelashes on microscopic examination that failed to detect pathogenic infestation.

| **Eyelash Manipulation Quantity** | **Microscope Quantity** | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **9** | **16** | |
| | **0** | 239 | 10 | 3 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 253 |
| | **1** | 21 | 22 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 47 |
| | **2** | 13 | 10 | 8 | 2 | 0 | **1** | 0 | 0 | 0 | 0 | 34 |
| | **3** | 8 | 4 | 3 | 3 | **3** | 0 | **1** | 0 | 0 | 0 | 22 |
| | **4** | **3** | **3** | **5** | **5** | 5 | 1 | 0 | 0 | 0 | 0 | 22 |
| | **5** | **1** | 0 | **2** | **3** | 1 | 2 | 1 | 0 | 0 | 0 | 10 |
| | **6** | **4** | **4** | **3** | **2** | 4 | 0 | 2 | 1 | 0 | 0 | 20 |
| | **7** | **2** | **1** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |
| | **8** | 0 | **2** | 0 | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 8 |
| | **9** | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | **10** | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 2 |
| | **11** | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | **12** | **1** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| | **13** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Total | | 292 | 56 | 27 | 18 | 18 | 7 | 7 | 1 | 1 | 1 | 428 |

The level of agreement between both techniques was assessed using the Bland-Altman method by examining the mean difference and constructing limits of agreement.[300] As data was not normally distributed (Shapiro-Wilk $p < 0.001$), a non-parametric form of limits of agreement method was incorporated. This was achieved by ordering the data, and placing the upper and lower limits of agreement at the top and bottom 5% of the ordered data respectively.[301] The difference between both techniques, eyelash manipulation (A) and microscopic examination (B) (A - B), was plotted on the y-axis against the average of both techniques (A + B)/2 on the x-axis (refer Figure 37). The mean difference value was 0.64 mites, with a 95% confidence interval of 0.45 and 0.83 mites. A clear positive trend can be seen in Figure 37: as the average quantity of DF increases, the greater the difference between the two methods. This implies that eyelash manipulation (method A) presents higher quantities of DF than microscopic examination (method B), especially in severe infestations. In agreement with this interpretation, the overall median quantity of DF detected was significantly greater using eyelash manipulation (0.00 mites, IQR: 0.00 – 2.00) compared to microscopic examination (0.00 mites, IQR: 0.00 – 1.00) ($p = < 0.001$).

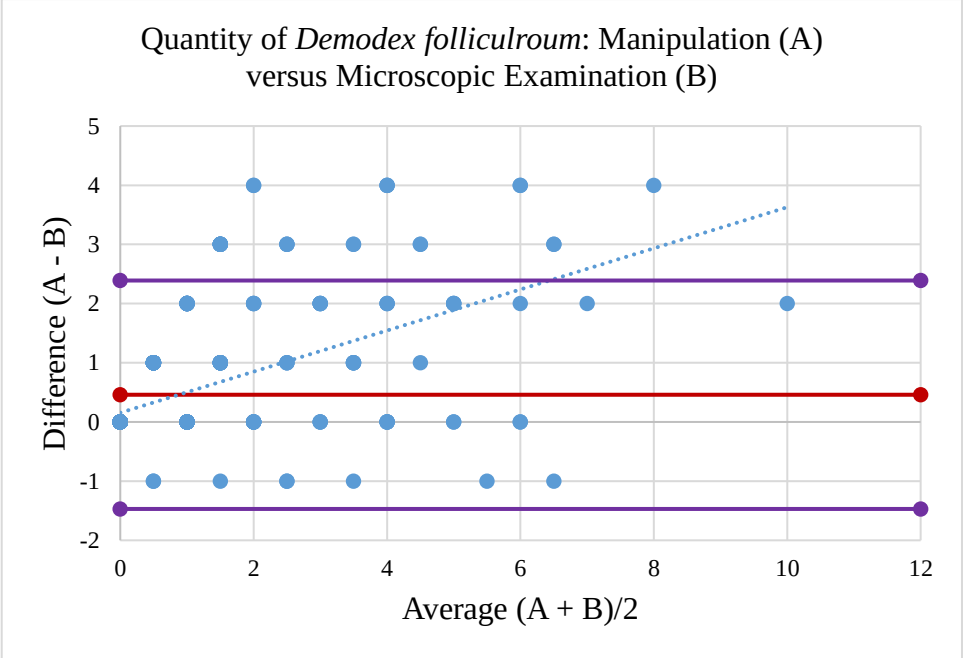



Figure 37. Bland Altman plot illustrating the 95% limits of agreement between the quantity of *Demodex folliculorum* on eyelash manipulation (A) and microscopic examination (B). Purple lines show limits of agreement (Upper 4.0, Lower -1.00), and the red line shows the mean value (0.64) of the differences.

To examine the repeatability of the eyelash manipulation versus microscopic examination techniques, participants were asked to return for a subsequent check two weeks later. Data from 280 eyelashes was analysed (70 participants, four eyelashes from each). The results of the second analysis were in good agreement with the original examination. A mean difference of 0.5 mites (95% confidence interval of 0.32 to 0.68 mites) with upper and lower limits of agreement at 4.0 and -1.0 as previously were found. Figure 38 illustrates the Bland-Altman plot constructed, which is very similar to that displayed above in Figure 37.

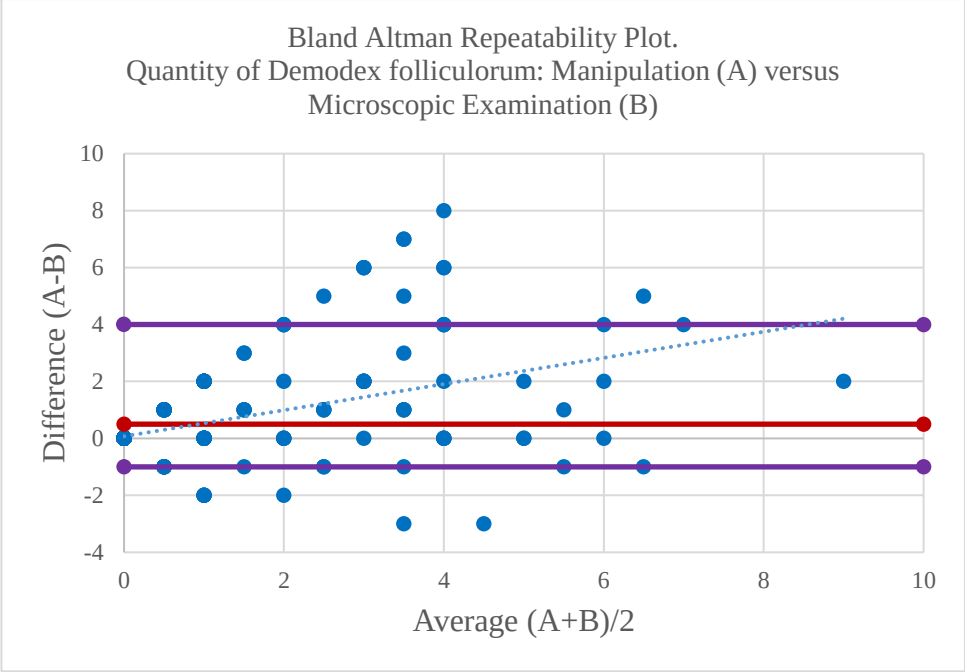



Figure 38. Bland Altman repeatability plot illustrating the 95% limits of agreement between the quantity of *Demodex folliculorum* on eyelash manipulation (A) and microscopic examination (B). Purple lines show limits of agreement (Upper 4.0, Lower -1.0), and the red line shows the mean value (0.5) of the differences.

*Severity of Infestation*

In an attempt to counteract estimating DF quantity during eyelash manipulation, each eyelash was graded from 0 – 3 according to severity of infestation. The frequency distribution of severity of infestation detected using both techniques are presented in Table 46. For eyelash manipulation and microscopic examination respectively, the majority of eyelashes were classified as Grade 0 (59.11% and 68.22%) or Grade 1 (24.06% and 23.69%). However, the percentage of eyelashes demonstrating pathogenic infestation, either Grade 2 (12.15% and 7.47%) or Grade 3 (4.67% and 0.77%) was greater using eyelash manipulation compared to microscopic examination. Additionally, eyelash

manipulation identified a greater severity of infestation on 95 eyelashes, in comparison to only 20 eyelashes for microscopic examination (WSR $p < 0.001$).

Table 46. Severity of infestation frequency distribution.

| | | **Severity of Infestation** | | | |
|---|---|---|---|---|---|
| | | **Eyelash Manipulation** | | **Microscopic Examination** | |
| **Grade** | **Description** | **Frequency (n)** | **Percent (%)** | **Frequency (n)** | **Percent (%)** |
| 0 | No *D. folliculorum* | 253 | 59.11 | 292 | 68.22 |
| 1 | Mild, non-pathogenic infestation | 103 | 24.06 | 101 | 23.59 |
| 2 | Moderate, pathogenic Infestation | 52 | 12.15 | 32 | 7.47 |
| 3 | Severe, pathogenic Infestation | 20 | 4.67 | 3 | 0.77 |

Table 47 illustrates a cross-tabulation of the 'severity of infestation' groups. Using the accepted technique of examining DF on microscopic examination, 292 eyelashes were classified as having no DF on microscopic examination. However, of those 292 eyelashes; 42 (14.33%) were classified as mildly infested, eight (2.77%) moderately infested, and three (1%) severely infested, when examined using eyelash manipulation. By comparison, 253 eyelashes were classified as having no DF on eyelash manipulation. Of those 253 eyelashes; only 13 (5%) were classified as mildly infested, one (0.33%) moderately infested (grade 2), and none were severely infested (grade 3) on microscopic examination.

Table 47. Cross tabulation of the severity of infestation detected on eyelash manipulation versus microscopic examination. Green numbers indicate the quantity of eyelashes on manipulation that failed to detect pathogenic infestation (≥ 4 *Demodex folliculorum*). Purple numbers indicate the quantity of eyelashes on microscopic examination that failed to detect pathogenic infestation.

| | | **Microscope** | | | | Total |
|---|---|---|---|---|---|---|
| | | **No *D. folliculorum*** | **Mild, Non-Pathogenic** | **Moderate, Pathogenic** | **Severe, Pathogenic** | |
| **Eyelash Manipulation** | **No *Demodex folliculorum*** | 239 | 13 | **1** | 0 | 253 |
| | **Mild, Non-Pathogenic** | 42 | 56 | **5** | 0 | 103 |
| | **Moderate, Pathogenic** | **8** | **27** | 16 | 1 | 52 |
| | **Severe, Pathogenic** | **3** | **5** | 10 | 2 | 20 |
| Total | | 292 | 101 | 32 | 3 | 428 |

To examine the level of agreement between both methods on an ordinal scale,[302] $\kappa_w$ statistics were used. There were 313 (73.13%) observed agreements and 201 (46.96%) agreements expected by chance. Weighted kappa ($\kappa_w$ = 0.56) was slightly greater than unweighted kappa ($\kappa$ = 0.49). Nonetheless, the level of agreement between both techniques for measuring severity of infestation appears to be relatively weak.[200]

### 8.5 Discussion

Infestation of the eyelash follicle with large quantities of *Demodex* has become a well-known underlying cause of recalcitrant blepharitis.[7,43,46,49,63,64] Clinically in practice, the importance of ascertaining the quantity of DF is to establish if there is pathogenic infestation (≥ 4 mites per follicle) or non-pathogenic infestation present. This will help a practitioner in deciding whether to treat or monitor a patient. Currently, the most common

method of investigation for infestation has required epilating the eyelash in order to count any DF present. This technique, the Coston method,[43] has been previously described in detail in Section 1.8.1. However, there were several limitations to this method. The modified Coston method (described in Section 1.8.2) was developed to account for these limitations, which is the technique predominantly used by investigators today.[25,30,32,45,52,303] However, even after modifications have been made to these epilation methods, as has been noted previously[25,29,50] and in the current study, often DF remain within the follicle after the eyelash has been removed; resulting in under-counting on microscopic examination.

The present study has shown that eyelash manipulation without epilation is better at detecting pathogenic levels of infestation than the modified Coston method.[25] This manipulation technique would work well in a clinical setting, and may help improve practitioners' confidence and ability to detect pathogenic DF infestation requiring treatment. Although ICC revealed a moderately good level of agreement (0.78) between both methods, the Bland-Altman method suggested consistently greater quantities of DF visible on eyelash manipulation than on microscopic examination. This analysis was further strengthened, as the overall median quantity of DF detected was greater on eyelash manipulation and repeated examination on a second visit showed similar results. The authors believe that the strong agreement between both methods with respect to no DF present and low non-pathogenic levels of infestation was responsible for the higher level of agreement detected with ICC. However, the eyelash manipulation method appeared to be more effective with greater severity of infestation.

An overall greater range of DF was detected on microscopic examination (range 0 – 16) compared to eyelash manipulation (range 0 – 13). This was on account of the subjective nature of observing the number of DF tails discernable on eyelash manipulation. By

comparison, it is possible to precisely count the number of DF visible on a microscope slide. However, as mentioned above, the recognised method of eyelash epilation and counting of DF on the microscope can be imprecise and often leads to miscounting.[25,50] Moreover, using the eyelash manipulation technique, the differentiation between detecting the presence of no DF or one to three tails compared with four to six tails, or greater than seven tails, is quite unmistakeable; as can be seen from the strong agreement between both techniques in non-pathogenic infestation (refer Table 40). Therefore, in a clinical setting where pathogenic infestation can be considered ≥ 4 mites per follicle, exact numbers for large quantities are not necessary: as it is evident that the follicle is severely infested.[65]

Furthermore, as can be seen from Table 39and Table 41, only six eyelashes (1.44% of the eyelashes included in the study) detected pathogenic DF on the microscope and not on eyelash manipulation. By comparison, 43 eyelashes (10.01%) detected pathogenic DF infestation on eyelash manipulation but not on the microscope. Hence, there appears to be almost a 10-fold greater likelihood of identifying pathogenic DF infestation on eyelash manipulation than on microscopic examination of the epilated eyelash. This clearly illustrates the limitation of using eyelash epilation and microscopic examination in isolation when examining for DF infestation. These damaged eyelash follicles were visibly infested. However, as the eyelashes were removed without removing all the DF within the damaged follicle, the severity of infestation present was erroneous. There was an increased discrepancy between both methods for detecting severity of infestation as the severity of infestation increased, confirmed by the weak level of agreement detected with weighted kappa ($\kappa_w$ = 0.56). In clinical studies, agreement analysis between two methods is conducted to assess if a new method is good enough to replace an old one; with a recommendation of 80% as the minimum acceptable agreement value.[200] This high level of

agreement was not achieved in the current study. However, it is believed that the eyelash manipulation technique outperformed the accepted eyelash epilation technique: thus, causing the observed disagreement between the two techniques. As eyelash manipulation appears a better indicator of pathogenic infestation, it should be considered more often in clinical and research settings.

Apart from under-counting, a second disadvantage of eyelash epilation is the discomfort and anxiety associated with epilating several eyelashes at each examination. Manipulating the eyelash without epilation is less stressful and more comfortable for the patient. Furthermore, in the current study, this technique was found to be more accurate for determining quantity and assessing severity of infestation. Additionally, madarosis as a result of chronic blepharitis has been previously reported in the literature.[25,304] It was noted that several patients declined to part-take in the study; they were apprehensive about having their eyelashes removed when they felt they had so few eyelashes remaining. As such, from a cosmetic viewpoint, eyelash manipulation is a preferred technique.

As mentioned in Section 1.8.3, confocal laser scanning microscopy is a relatively new, non-invasive technique that has been used to detect DF *in-vivo*.[16,65,146] Studies have compared laser confocal microscopy to the modified Coston method, but found no significant difference in prevalence or quantity of DF detected between either method.[16,65] However, these studies had much smaller patient cohorts than the current study (n = 25[65] and n = 15[16]), which may account for the lack of significance found. Additionally, confocal laser scanning microscopes are expensive, specialised pieces of equipment, mainly utilised in specialised clinics and research laboratories. General optical practices are not likely to invest in such technology. On the other hand, all optical practices have access to a slit-lamp biomicroscope, and the likelihood is that all optometrists will encounter patients attending

with blepharitis and CD. As such, eyelash manipulation as part of a slit-lamp biomicroscope routine to examine for presence of DF and assess severity of infestation may be more clinic and practitioner friendly than a confocal laser microscope.

### 8.6 Conclusion

The results of this study confirm the early observations in the pilot and extended studies (Chapter 5), that counting DF visible on microscopic examination alone was often mis-representing the degree of infestation present. Eyelash manipulation demonstrated greater quantities of DF than complete epilation of the eyelash and was superior for identifying moderate to severe pathogenic levels of infestation. However, eyelash epilation and microscopic examination will still be required in certain investigative settings. Epilation is still required to distinguish between DF and DB, or in certain specialised clinics/laboratories where isolation of bacteria from *Demodex* may be required. However, in clinical practice, eyelash epilation is not essential to accurately identify pathogenic DF infestation and *Demodex* blepharitis necessitating treatment. Hence, going forward, practitioners should feel confident in being able to detect *Demodex* blepharitis in practice without the need to epilate patients eyelashes.

# CHAPTER NINE: SUMMARY, CONCLUSIONS AND FUTURE RESEARCH

## 9.1 Summary and Conclusions

The research presented in this thesis provides optometrists and ophthalmologists with evidence-based results into the safety and efficacy of over-the-counter products available for the treatment of *Demodex* blepharitis. This provides practitioners effective alternatives to TTO and ivermectin, which can be managed in-house without the need for further referral. Furthermore, the current study highlights the ability to detect pathogenic infestation requiring treatment without the need for eyelash epilation and microscopic counting of *Demodex* mites on slides. In clinic, eyelash manipulation at the slit-lamp is a more patient- and practitioner- friendly technique that is more effective at demonstrating pathogenic DF infestation requiring treatment.

### *9.1.1 Development and validation of a General Health and Lifestyle questionnaire*

Chapter 3 discussed the development and validation of the GHL questionnaire. Results found that age remains the most significant risk factor for *Demodex* blepharitis, which was in good agreement with previous research.[13,28,77] An interesting potential association between DF and makeup was detected. It appears that makeup may have a protective mechanism against DF infestation. The questionnaire demonstrated moderate to good repeatability. However, there were several limitations, especially with the variety in number of response items to each question. As such, the questionnaire was not utilised after the second recruitment phase. Instead, the research concentrated on the efficacy of treatment products going forward.

### *9.1.2 Development and validation of a modified Ocular Surface Disease Index questionnaire*

Chapter 4 discussed the development and validation of the modified OSDI symptom questionnaire. The questionnaire was modified to include questions such as itch and debris at the base of the eyelashes, which are commonly associated with blepharitis.[16,28,46,47] The modified OSDI questionnaire demonstrated good internal consistency and repeatability. Good repeatability validated the use of the questionnaire to observe subjective symptoms over time in a clinical setting. The questionnaire exhibited a sensitivity value of 70.75%. This means that there is a 70.75% chance that a positive result using the questionnaire alone would correctly identify the presence of DF. However, with regards to confirming the presence of DF infestation and establishing which participants require further intervention, this would not be sufficient. To the best of the author's knowledge, this was the first study to examine the prevalence of ocular DF infestation and its associated symptoms in an Irish population. Furthermore, it was the first study to show an association between increasing quantity of DF and increasing severity of the symptom 'itchy eyes'. Results of the study discussed in Chapter 4 have been published in International Ophthalmology.[45]

### *9.1.3 The efficacy of baby shampoo, OCuSOFT, TTFW and microblepharoexfoliation in the treatment of Demodex folliculorum blepharitis*

Chapter 5 discussed the results of the pilot study and the extended treatment study. The pilot study compared the efficacy of OCuSOFT to 10% baby shampoo in a two-week treatment study. Results showed that OCuSOFT significantly reduced the quantity of DF, but not presence; and baby shampoo had no effect on DF quantity or presence. The extended treatment study was conducted to improve on limitations of the pilot study following peer-

review. Firstly, the study was extended to four weeks, to ensure sufficient time was given to treat DF infestation, given their lifespan is 14–18 days.[40,42,43,61] Secondly, both eyes were treated with the same treatment, to prevent cross-contamination through migration of DF from the control eye to the treated eye. Thirdly, reviewers suggested comparing OCuSOFT to a tea tree-based product (TTFW) as opposed to baby shampoo. The BlephEx$^{TM}$ device was also used on one group of participants to evaluate if any additional benefit was attained from microblepharoexfoliation of the eyelids prior to home lid scrubs. Results of the extended study demonstrated that both OCuSOFT and TTFW were effective at reducing the quantity of DF. Tea tree-based face wash also has the added benefit of being used as a face wash and treating DF in hair follicles all over the face and not just the eyelashes. The use of BlephEx$^{TM}$ demonstrated a greater reduction in symptoms although it was not significantly better. Results of the extended study discussed in Chapter 5 have been published in Contact Lens Anterior Eye.[52]

### *9.1.4 Effect of lid hygiene on the tear film and ocular surface*

Chapter 6 evaluated the effect blepharitis lid cleansers have on the tear film and ocular surface and examined the prevalence of DF in a young population. No adverse ocular events were detected following the use of OCuSOFT or TTFW for up to eight weeks. However, a significant increase in tear film instability was detected after eight weeks of lid scrubs with 10% baby shampoo. The findings from this study are in keeping with recent studies that have found that baby shampoo could have a damaging effect on goblet cell density,[186] and provides further evidence to practitioners to move away from recommending lid scrubs with baby shampoo when treating blepharitis. In agreement with previous research,[23] a low prevalence of DF was observed amongst the young study population. A paper on the results

of the study discussed in Chapter 6 has been accepted for publication in Contact Lens & Anterior Eye.

### *9.1.5 Effect of warm compress therapy on Demodex folliculorum infestation*

Finally, Chapter 7 examined the efficacy of using heat from warm compresses to treat DF blepharitis. Three warm compress treatments were compared: Warm face cloth, MGDRx EyeBag® and OPTASE™ Moist Heat Mask. Warm compress therapy was conducted for eight weeks. Both the MGDRx EyeBag® and OPTASE™ Moist Heat Mask exhibited superior efficacy in treating signs and symptoms of MGD, compared to the use of a warm face cloth, over the eight-week period. The OPTASE™ Moist Heat Mask demonstrated dual therapeutic abilities, treating both MGD and DF blepharitis. A paper on the results of the study discussed in Chapter 7 has been recently accepted for publication in Current Eye Research.

### *9.1.6 Clinical use of eyelash manipulation versus microscopic examination*

Chapter 8 compared the efficacy of using an eyelash manipulation technique to the traditional eyelash epilation and subsequent microscopic examination technique, when investigating for the presence of DF in a clinical setting. A moderately good agreement for assessing quantity of DF was detected between both techniques. However, the Bland-Altman plot suggested consistently higher quantities were discovered on eyelash manipulation. The overall mean quantity of DF was also greater on eyelash manipulation than on microscopic examination of the epilated eyelash. A weak level of agreement was detected between the two methods for addressing severity of infestation. This was caused by the superior ability of eyelash manipulation to detect pathogenic infestation in comparison to microscopic examination of the epilated eyelash. As such, complete eyelash

epilation is not necessary to accurately detect *Demodex* blepharitis requiring treatment, in a clinical setting. Results of the extended study discussed in Chapter 8 have been accepted for publication in Eye & Contact Lens.[305]

### *9.1.7 Conclusions*

Based on the results of the research conducted throughout this research project, DF blepharitis can be effectively diagnosed and treated by practitioners in-house, without the need for further referral in many cases. Investigation to assess presence and severity of infestation should be performed using eyelash manipulation. The results from Chapter 7 have proven that there is no clinical requirement to epilate an eyelash when examining for the severity of DF infestation. Practitioners can feel confident that recommending nightly lid scrubs with OCuSOFT® Lid Scrub® Plus or a tea tree-based product will reduce quantities of DF and improve patients' symptoms. The use of BlephEx$^{TM}$ is advised to help start the lid scrubbing regime by reducing the extent of CD and bacterial load at the eyelid margin. The benefit of using a tea tree-based face wash, is that it can be used to clean the entire face, not just the eyelids and eyelashes. Hence, treating DF infestation in other locations on the face. Practitioners should refrain from recommending baby shampoo for lid scrubs going forward, as it has no impact on *Demodex* blepharitis, but also may be harmful to the ocular surface in the long run.

## 9.2 Future research

*Demodex* remains a relatively novel research area within ophthalmology. Future research regarding treatment such as: effect of BlephEx$^{TM}$ on lid hygiene; effect of OCuSOFT applied overnight versus washed off; effect of face cleansers compared to TTFW; comparison between in-house lid scrubs scrubs with TTO versus TTFW at home;

and the effect of lid scrubs used twice a day versus once a day; are all areas that require further investigation. However, any future treatment-based investigation should make a considerable effort to quantify participant compliance. Methods to improve participant compliance in all treatment-based studies is a field that also requires further research and investigation.

Over the course of this research project, it has become clear that there are several topics that require further clarification with regards to *Demodex*, helping to improve practitioner's knowledge of underlying risk factors and associations.

There has been limited research conducted on the relationship between *Demodex* and contact lenses. The majority of references to contact lens wear and *Demodex* is anecdotal, and based around the work of Jalbert and Rejab[114] and Tarkowski et al[115]. Both hard and soft contact lens wearers were included in the study conducted by Jalbert and Rejab[114], and although this included a mix of daily, fortnightly and monthly soft contact lenses, and rigid gas permeable lenses; no inter-lens modality analysis was conducted: just contact lens wear and non-contact lens wear groups. Similarly, Tarkowski et al[115] focussed on DF as a cause of drop-out in previously successful contact lens wearers, but did not discriminate between contact lens modality. Jalbert and Rejab[114] found a 90% prevalence of DF within contact lens wearers in their study. The authors' suggested the higher prevalence may be due to increased handling of and presence of bacteria, such as *Staphylococcus epidermis* and *Corynebacteria*, on the eyelids of contact lens wearers,[306] concluding that contact lenses may provide a route for micro-organisms to grow and create an environment that favours *Demodex* proliferation. However, it would be interesting to examine the relationship between DF and contact lens modality, as re-usable contact lenses, such as fortnightly or monthly lenses, are cleaned and stored in solutions that contain anti-microbial agents to

help prevent infection. Hence, in theory, the use of re-usable contact lenses may reduce the risk of DF infestation. Furthermore, if the quantity of DF is increased, handling of the eyelids may make contact lens wearers more susceptible to DF infestation. A comparison of participants who wear their contact lenses on a full-time basis compared to participants who wear contact lenses occasionally could be carried out to investigate which cohort has a higher risk of DF infestation. Contact lens modality was evaluated during the pilot study. In contrast to Jalbert and Rejab[114], non-contact lens wearers, and daily disposable soft lens wearers, had higher quantities of DF than re-usable contact lens wearers. However, the results were significantly impacted by age. As such, no reliable results could be ascertained from the data. Future studies should have equal groups of contact lens wearers in the different modalities, with age matched control non-contact lens wearers.

The use of makeup appeared to have a protective effect against DF infestation in the pilot study. Similar to contact lenses, there has been very little research conducted on the relationship between DF and makeup. Although several hypotheses exist, nothing confirmatory has been established. As mentioned in Section 1.6.5, Horváth et al[116] and Elston and Elston[79] suggested that makeup may reduce colonisation of DF in the hair follicles of the skin and eyelashes. Elston and Elston[79] suggested that it may be the presence of exogenous lipids in cosmetics that could impact the proliferation of *Demodex*. Horváth et al[116] found a lower prevalence of DF among makeup wearers compared to non-makeup wearers and provided three theories for their results. Firstly, the authors suggested that makeup may obstruct follicles, inhibiting migration and proliferation of *Demodex*.[116] Secondly, the authors suggested that makeup may contain chemicals that are toxic to *Demodex*, thus preventing them from inhabiting skin covered with makeup.[116] Thirdly, the authors suggested that patients who wear makeup regularly are more inclined to remove

makeup and clean their faces. Thus, the increased hygiene could help prevent *Demodex* proliferation, or chemicals in the products used to clean the face may be toxic to *Demodex*: preventing proliferation.[116] Providing some consensus with regards to improved hygiene, Koo et al[13] did find that older participants with good eyelid hygiene had lower prevalence of DF compared to younger participants with poor eyelid hygiene. However, further studies are required to confirm any relationship between makeup and *Demodex* infestation and establish the underlying mechanisms if such a relationship exists.

In Chapter 8, a low overall quantity of DF detected over the duration of the study affected the power of some of the study results, namely the quantity of DF on microscopic examination and MGDRx EyeBag®. Further investigation that concentrates on pathogenic infestation, to ensure larger quantities of DF when testing, is required. Similarly, with regards to the MGDRx EyeBag® and OPTASE™ Moist Heat Mask, further research is required to examine the therapeutic temperature achieved, and duration of such, at the inner eyelid by each compress. Further investigation is required to evaluate any potential role that moisture may have played in the therapeutic abilities demonstrated by the OPTASE™ Moist Heat Mask.

### 9.3 Dissemination to date

Dissemination of research findings is recognised as an integral aspect of the research process. Within public health, dissemination of research findings provides evidence-based results to practitioners, which can improve and develop their clinical management skills: thus, providing best-practice care to their patients.[307,308] Research results can be disseminated in several different ways: peer-reviewed publications, research reports, professional magazine articles, workshops, conference proceedings and social media

platforms, to name a few.[309] The research findings outlined in the above thesis have been disseminated in peer-reviewed journals, optometry magazine articles, conference posters, conference workshops at home and abroad, and in online lecture material.

### *9.3.1 Peer-reviewed publications*

Results of the development and validation of the modified OSDI questionnaire discussed in Chapter 4 have been published in International Ophthalmology (refer List of Publications 1).

Results of the efficacy of OCuSOFT® Lid Scrub® Plus and dr.organic® tea tree face wash for treating DF infestation discussed in Chapter 5 have been published in Contact Lens Anterior Eye (refer List of Publications 2).

Results of the effect of OCuSOFT® Lid Scrub® Plus, dr.organic tea tree face wash and Johnson's® No More Tears® baby shampoo on the tear film and ocular surface, discussed in Chapter 6, have been recently accepted for publication in Contact Lens & Anterior Eye (refer List of Publications 3).

Results of the study examining the efficacy of warm compresses for treating DF infestation, discussed in Chapter 7, have been recently accepted for publication in Current Eye Research (refer List of Publications 4).

Finally, results of the observational finding that eyelash manipulation is better than microscopic examination for detecting severity of DF infestation and is suffice in a clinical setting, discussed in Chapter 8, have been published in Eye and Contact Lens (refer List of Publications 5).

### *9.3.2 Conferences and workshops*

Murphy O. *Demodex* Blepharitis, Workshop, DIT, Dec 2014.

Murphy O, et al., Prevalence of *Demodex folliculorum* in symptomatic and asymptomatic individuals and the efficacy of 1,2-Octanediol at treating *Demodex* infestation, Poster Presentation, British Contact Lens Association, May 2015.

Murphy O, et al. The effectiveness of topical treatment with OCuSOFT Plus on ocular *Demodex folliculorum*, Poster Presentation, European Academy of Optometry and Optics, May 2015.

Murphy O. Dry Eye: Blepharitis – Meibomian gland dysfunction – *Demodex,* Lecture Presentation, Association of Optometrists Ireland AGM, November 2015.

Murphy O. Dry Eye: Blepharitis – Meibomian gland dysfunction – *Demodex,* Lecture Presentation, Irish Association of Dispensing Optometrists AGM, April 2016.

Murphy O. *Demodex* blepharitis: Diagnosis and Treatment, Workshop, Optometry and Eye Health Conference, Sofia, Bulgaria, Oct 2018.

### *9.3.3 Other publications*

Murphy O. *Demodex* blepharitis. Online Lecture. Wales Optometry Postgraduate Education Centre. April 2018

Murphy O. *Demodex* blepharitis in practice. Optometry Today. May 2018, p.77-80.

## REFERENCES


1. Amescua G, Akpek EK, Farid M, et al. Blepharitis Preferred Practice Pattern®. *Ophthalmology*. 2018;126:P56-P93. doi:10.1016/j.ophtha.2018.10.019

2. Lindsley K, Matsumura S, Hatef E, Akpek EK. Interventions for chronic blepharitis. *Cochrane database Syst Rev*. 2012;5:CD005556. doi:10.1002/14651858.CD005556.pub2

3. The College of Optometrists. Blepharitis (Lid Margin Disease). Clinical Management Guidelines. https://www.college-optometrists.org/guidance/clinical-management-guidelines/blepharitis-lid-margin-disease.html. Published 2018. Accessed June 1, 2018.

4. Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester J V. Meibomian gland dysfunction in chronic blepharitis. *Cornea*. 1991;10(4):277-285.

5. McCulley JP, Shine WE. Eyelid disorders: the meibomian gland, blepharitis, and contact lenses. *Eye Contact Lens*. 2003;29(1 Suppl):S93-5; discussion S115-8, S192-4.

6. Kheirkhah A, Casas V, Li W, Raju VK, Tseng SCG. Corneal Manifestations of Ocular Demodex Infestation. *Am J Ophthalmol*. 2007;143(5):743-749.

7. Czepita D, Kuźna-Grygiel W, Czepita M, Grobelny A. Demodex folliculorum and Demodex brevis as a cause of chronic marginal blepharitis. *Ann Acad Med Stetin*. 2007;53(1):63-67.

8. Edwards RS. Ophthalmic emergencies in a district general hospital casualty

department. *Br J Ophthalmol*. 1987;71:938-942.

9. Nijm LM. Blepharitis: Classification. In: Holland E, Mannis M, Lee W, eds. *Ocular Surface Disease: Cornea, Conjunctiva and Tear Film*. Elsevier Inc.; 2013:55-60.

10. Lemp MA, Nichols KK. Blepharitis in the United States 2009: a survey-based perspective on prevalence and treatment. *Ocul Surf*. 2009;7(2 Suppl):S1-S14. doi:10.1016/S1542-0124(12)70620-1

11. Needle JJ, Petchey R, Lawrenson JG. A survey of the scope of therapeutic practice by UK optometrists and their attitudes to an extended prescribing role. *Ophthalmic Physiol Opt*. 2008;28(3):193-203. doi:10.1111/j.1475-1313.2008.00551.x

12. Gao YY, Di Pascuale MA, Li W, et al. In vitro and in vivo killing of ocular Demodex by tea tree oil. *Br J Ophthalmol*. 2005;89(11):1468-1473. doi:10.1136/bjo.2005.072363

13. Koo H, Kim TH, Kim KW, Wee SW, Chun YS, Kim JC. Ocular surface discomfort and Demodex: effect of tea tree oil eyelid scrub in Demodex blepharitis. *J Korean Med Sci*. 2012;27(12):1574-1579.

14. Liang L, Safran S, Gao Y, Sheha H, Raju VK, Tseng SCG. Ocular Demodicosis as a Potential Cause of Pediatric Blepharoconjunctivitis. *Cornea*. 2010;29(12):1386-1391. doi:10.1097/ICO.0b013e3181e2eac5

15. Gao YY, Di Pascuale MA, Elizondo A, Tseng SC. Clinical treatment of ocular demodecosis by lid scrub with tea tree oil. *Cornea*. 2007;26(2):136-143.

16. Kojima T, Ishida R, Sato EA, et al. In Vivo Evaluation of Ocular Demodicosis Using

Laser Scanning Confocal Microscopy. *Invest Ophthalmol Vis Sci*. 2011;52:565-569.

17. Kim JT, Lee H, Chun YS, Kim JC. Tear cytokines and chemokines in patients with Demodex blepharitis. *Cytokine*. 2011;53:94-99.

18. Moher D, Liberati A, Tetzlaff J, Altman DG, Group TP. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med*. 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097

19. Kosik-Bogacka DI, Łanocha N, Łanocha A, et al. Demodex folliculorum and Demodex brevis in healthy and immunocompromised patients. *Ophthalmic Epidemiol*. 2013;20(3):159-163. doi:10.3109/09286586.2013.789532

20. Yamashita LS, Cariello AJ, Geha NM, Yu MC, Hofling-Lima AL. Demodex folliculorum on the eyelash follicle of diabetic patients. *Arq Bras Oftalmol*. 2011;74(6):422-424.

21. Türk M, Oztürk I, Sener AG, Küçükbay S, Afşar I, Maden A. Comparison of incidence of Demodex folliculorum on the eyelash follicule in normal people and blepharitis patients. *Turkiye Parazitol Derg*. 2007;31(4):296-297.

22. Czepita D, Kuźna-Grygiel W, Kosik-Bogacka D. Demodex as an etiological factor in chronic blepharitis. *Klin Oczna*. 2005;107(10-12):722-724.

23. Kemal M, Sümer Z, Toker MI, Erdoğan H, Topalkara A, Akbulut M. The Prevalence of Demodex folliculorum in Blepharitis Patients and the Normal Population. *Ophthalmic Epidemiol*. 2005;12(4):287-290.

24. Ozçelik S, Sümer Z, Değerli S, et al. The incidence of Demodex folliculorum in

patients with chronic kidney deficiency. *Turkiye Parazitol Derg*. 2007;31(1):66-68.

25. Gao YY, Di Pascuale MA, Li W, et al. High prevalence of Demodex in eyelashes with cylindrical dandruff. *Invest Ophthalmol Vis Sci*. 2005;46(9):3089-3094.

26. Liang L, Ding X, Tseng SCG. High prevalence of demodex brevis infestation in chalazia. *Am J Ophthalmol*. 2014;157(2):342-348.e1. doi:10.1016/j.ajo.2013.09.031

27. Wesolowska M, Knysz B, Reich A, et al. Prevalence of Demodex spp. in eyelash follicles in different populations. *Arch Med Sci*. 2014;10(2):319-324. doi:10.5114/aoms.2014.42585

28. Lee SH, Chun YS, Kim JH, Kim ES, Kim JC. The relationship between demodex and ocular discomfort. *Invest Ophthalmol Vis Sci*. 2010;51(6):2906-2911.

29. Bhandari V, Reddy JK. Blepharitis: always remember demodex. *Middle East Afr J Ophthalmol*. 2014;21(4):317-320.

30. de Venecia AB, Lim Bon Siong R. Demodex sp. infestation in anterior blepharitis, meibomian-gland dysfunction, and mixed blepharitis. *Philipp J Ophthalmol*. 2011;36(1):15-22.

31. Huang Y, He H, Sheha H, Tseng SCG. Ocular Demodicosis as a Risk Factor of Pterygium Recurrence. *Ophthalmology*. 2013;120(7):1341-1347. doi:10.1016/j.ophtha.2013.01.001

32. Hauswirth SG, Schachter SE, Hom MM. Symptoms Associated with the Presence of Demodex folliculorum. *Invest Ophthalmol Vis Sci*. 2014;55(13):1996.

33. Li J, O'Reilly N, Sheha H, et al. Correlation between ocular Demodex infestation and

serum immunoreactivity to Bacillus proteins in patients with Facial rosacea. *Ophthalmology*. 2010;117(5):870-877.e1. doi:10.1016/j.ophtha.2009.09.057

34. Holzchuh FG, Hida RY, Moscovici BK, et al. Clinical Treatment of Ocular Demodex folliculorum by Systemic Ivermectin. *Am J Ophthalmol*. 2011;151(6):1030-1034.e1. doi:10.1016/j.ajo.2010.11.024

35. Gao YY, Xu DL, Huang LJ, Wang R, Tseng SCG. Treatment of Ocular Itching Associated With Ocular Demodicosis by 5% Tea Tree Oil Ointment. *Cornea*. 2012;31(1):14-17. doi:10.1097/ICO.0b013e31820ce56c

36. Salem DA-B, El-Shazly A, Nabih N, El-Bayoumy Y, Saleh S. Evaluation of the efficacy of oral ivermectin in comparison with ivermectin-metronidazole combined therapy in the treatment of ocular and skin lesions of Demodex folliculorum. *Int J Infect Dis*. 2013;17(5):e343-7. doi:10.1016/j.ijid.2012.11.022

37. Fulk GW, Murphy B, Robins MD. Pilocarpine gel for the treatment of demodicosis--a case series. *Optom Vis Sci*. 1996;73(12):742-745. doi:10.1097/00006324-199612000-00004

38. Filho PAN, Hazarbassanov RM, Grisolia ABD, Pazos HB, Kaiserman I, Gomes JÁP. The efficacy of oral ivermectin for the treatment of chronic blepharitis in patients tested positive for Demodex spp. *Br J Ophthalmol*. 2011;95(6):893-895. doi:10.1136/bjo.2010.201194

39. Kim JH, Chun YS, Kim JC. Clinical and Immunological Responses in Ocular Demodecosis. *J Korean Med Sci*. 2011;26(9):1231. doi:10.3346/jkms.2011.26.9.1231

40. Spickett SG. Studies on Demodex folliculorum Simon (1842). I. Life history.

*Parasitology*. 1961;51:181-192.

41. Desch C, Nutting WB. Demodex folliculorum (Simon) and D. brevis Akbulatova of Man: Redescription and Reevaluation. *J Parasitol*. 1972;58(1):169. doi:10.2307/3278267

42. Rufli T, Mumcuoglu Y. The Hair Follicle Mites Demodex folliculorum and Demodex brevis : Biology and Medical Importance. *Dermatologica*. 1981;162:1-11.

43. Coston T. *Demodex folliculorum* blepharitis. *Trans Am Ophthalmol Soc*. 1967;65:361-392.

44. Chen D, Li R, Liu X, Li Y. Prevalence and treatment effects of Demodex species in eyelash follicles in patients with meibomian gland dysfunction. *Chinese J Ophthalmol*. 2017;53(3). doi:10.3760/cma.j.issn.0412-4081.2017.03.009

45. Murphy O, O'Dwyer V, Lloyd-McKernan A. Ocular *Demodex folliculorum* : prevalence and associated symptoms in an Irish population. *Int Ophthalmol*. January 2018:1-13. doi:10.1007/s10792-018-0826-1

46. Kabataş N, Doğan AŞ, Kabataş EU, Acar M, Biçer T, Gürdal C. The Effect of Demodex Infestation on Blepharitis and the Ocular Symptoms. *Eye Contact Lens*. 2017;43(1):64-67.

47. Sędzikowska A, Osęka M, Grytner-Zięcina B. Ocular symptoms reported by patients infested with Demodex mites. *Acta Parasitol*. 2016;61(4):808-814.

48. Zhang X-B, Ding Y-H, He W. The association between demodex infestation and ocular surface manifestations in meibomian gland dysfunction. *Int J Ophthalmol*.

2018;11(4):589-592. doi:10.18240/ijo.2018.04.08

49. Liu J, Sheha H, Tseng SCG. Pathogenic role of Demodex mites in blepharitis. *Curr Opin Allergy Clin Immunol*. 2010;10(5):505-510.

50. Mastrota KM. Method to Identify Demodex in the Eyelash Follicle Without Epilation. *Optom Vis Sci*. 2013;90(6):e172-e174. doi:10.1097/OPX.0b013e318294c2c0

51. Kheirkhah A, Blanco G, Casas V, Tseng SC. Fluorescein dye improves microscopic evaluation and counting of demodex in blepharitis with cylindrical dandruff. *Cornea*. 2007;26(6):697-700. doi:10.1097/ICO.0b013e31805b7eaf

52. Murphy O, O'Dwyer V, Lloyd-McKernan A. The efficacy of tea tree face wash, 1, 2-Octanediol and microblepharoexfoliation in treating Demodex folliculorum blepharitis. *Contact Lens Anterior Eye*. 2018;41(1):77-82. doi:10.1016/j.clae.2017.10.012

53. Tighe S, Gao YY, Tseng SCG. Terpinen-4-ol is the Most Active Ingredient of Tea Tree Oil to Kill Demodex Mites. *Transl Vis Sci Technol*. 2013;2(7):2. doi:10.1167/tvst.2.7.2

54. Jarmuda S, O'Reilly N, Zaba R, Jakubowicz O, Szkaradkiewicz A, Kavanagh K. Potential role of Demodex mites and bacteria in the induction of rosacea. *J Med Microbiol*. 2012;61(PART 11):1504-1510.

55. Aylesworth R, Vance JC. Demodex folliculorum and Demodex brevis in cutaneous biopsies. *J Am Acad Dermatol*. 1982;7(5):583-589. doi:10.1016/S0190-9622(82)70137-9

56. Beerman H, Stokes JH. Rosacea Complex and *Demodex folliculorum*. *Arch Dermatol*.

1934;29(6):874.

57. Breckenridge RL. Infestation of the Skin With Demodex Folliculorum. *Am J Clin Pathol*. 1953;23(4):348-352.

58. Jansen T, Bechara FG, Stücker M, Altmeyer P. Demodicidosis of the nipple. *Acta Derm Venereol*. 2005;85(2):186-187.

59. Wesołowska M, Baran W, Szepietowski J, Hirschberg L, Jankowski S. [Demodicidosis in humans as a current problem in dermatology]. *Wiad Parazytol*. 2005;51(3):253-256.

60. Palopoli MF, Fergus DJ, Minot S, et al. Global divergence of the human follicle mite Demodex folliculorum: Persistent associations between host ancestry and mite lineages. *Proc Natl Acad Sci U S A*. 2015;112(52):15958-15963. doi:10.1073/pnas.1512609112

61. Lacey N, Kavanagh K, Tseng S. Under the lash: Demodex mites in human diseases. *Biochem (Lond)*. 2009;31(4):2-6.

62. English FP, Nutting WB. Demodicosis of ophthalmic concern. *Am J Ophthalmol*. 1981;91(3):362-372.

63. Sędzikowska A, Osęka M, Skopiński P. The impact of age, sex, blepharitis, rosacea and rheumatoid arthritis on Demodex mite infection. *Arch Med Sci*. 2018;14(2):353-356. doi:10.5114/aoms.2016.60663

64. Zhao Y-E, Wu L-P, Hu L, Xu J-R. Association of Blepharitis with *Demodex* : A Meta-analysis. *Ophthalmic Epidemiol*. 2012;19(2):95-102. doi:10.3109/09286586.2011.642052

65. Randon M, Liang H, El Hamdaoui M, et al. *In vivo* confocal microscopy as a novel and reliable tool for the diagnosis of *Demodex* eyelid infestation. *Br J Ophthalmol*. 2015;99(3):336-341. doi:10.1136/bjophthalmol-2014-305671

66. Lacey N, Ní Raghallaigh S, Powell FC. Demodex mites--commensals, parasites or mutualistic organisms? *Dermatology*. 2011;222(2):128-130.

67. Baima B, Sticherling M. Demodicidosis revisited. *Acta Derm Venereol*. 2002;82(1):3-6.

68. Elston DM. Demodex mites: Facts and controversies. *Clin Dermatol*. 2010;28(5):502-504.

69. Georgala S, Katoulis AC, Kylafis GD, Koumantaki-Mathioudaki E, Georgala C, Aroni K. Increased density of Demodex folliculorum and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. *J Eur Acad Dermatol Venereol*. 2001;15(5):441-444.

70. Forton F, Germaux M-A, Brasseur T, et al. Demodicosis and rosacea: Epidemiology and significance in daily dermatologic practice. *J Am Acad Dermatol*. 2005;52(1):74-87.

71. Forton F, Seys B. Density of Demodex folliculorum in rosacea: a case-control study using standardized skin-surface biopsy. *Br J Dermatol*. 1993;128(6):650-659.

72. Thoemmes MS, Fergus DJ, Urban J, Trautwein M, Dunn RR. Ubiquity and diversity of human-associated Demodex mites. *PLoS One*. 2014;9(8):e106265. doi:10.1371/journal.pone.0106265

73. Lacey N, Delaney S, Kavanagh K, Powell FC. Mite-related bacterial antigens stimulate inflammatory cells in rosacea. *Br J Dermatol*. 2007;157(3):474-481. doi:10.1111/j.1365-2133.2007.08028.x

74. Wenisch C, Patruta S, Daxböck F, Krause R, Hörl W. Effect of age on human neutrophil function. *J Leukoc Biol*. 2000;67(1):40-45.

75. O'Reilly N, Bergin D, Reeves EP, McElvaney NG, Kavanagh K. Demodex-associated bacterial proteins induce neutrophil activation. *Br J Dermatol*. 2012;166(4):753-760. doi:10.1111/j.1365-2133.2011.10746.x

76. Norn MS. Incidence of Demodex folliculorum on skin of lids and nose. *Acta Ophthalmol*. 1982;60(4):575-583.

77. Roth AM. Demodex folliculorum in hair follicles of eyelid skin. *Ann Ophthalmol*. 1979;11(1):37-40.

78. Biernat MM, Rusiecka-Ziółkowska J, Piątkowska E, Helemejko I, Biernat P, Gościniak G. Occurrence of Demodex species in patients with blepharitis and in healthy individuals: a 10-year observational study. *Jpn J Ophthalmol*. 2018;62(6):628-633. doi:10.1007/s10384-018-0624-3

79. Elston CA, Elston DM. Demodex mites. *Clin Dermatol*. 2014;32(6):739-743. doi:10.1016/j.clindermatol.2014.02.012

80. Dhingra KK, Saroha V, Gupta P, Khurana N. Demodex-associated dermatologic conditions – A coincidence or an etiological correlate. Review with a report of a rare case of sebaceous adenoma. *Pathol - Res Pract*. 2009;205(6):423-426. doi:10.1016/j.prp.2008.11.013

81. Bonnar E, Eustace P, Powell FC. The Demodex mite population in rosacea. *J Am Acad Dermatol*. 1993;28(3):443-448. doi:10.1016/0190-9622(93)70065-2

82. Karincaoglu Y, Bayram N, Aycan O, Esrefoglu M. The Clinical Importance of *Demodex folliculorum* Presenting with Nonspecific Facial Signs and Symptoms. *J Dermatol*. 2004;31(8):618-626. doi:10.1111/j.1346-8138.2004.tb00567.x

83. Moran EM, Foley R, Powell FC. Demodex and rosacea revisited. *Clin Dermatol*. 2017;35(2):195-200. doi:10.1016/j.clindermatol.2016.10.014

84. Bamford JTM. Rosacea: Current thoughts on origin. *Semin Cutan Med Surg*. 2001;20(3):199-206. doi:10.1053/sder.2001.27553

85. Buechner SA. Rosacea: An Update. *Dermatology*. 2005;210(2):100-108. doi:10.1159/000082564

86. Spoendlin J, Voegel JJ, Jick SS, Meier CR. A study on the epidemiology of rosacea in the U.K. *Br J Dermatol*. 2012;167(3):598-605. doi:10.1111/j.1365-2133.2012.11037.x

87. Tan J, Schöfer H, Araviiskaia E, et al. Prevalence of rosacea in the general population of Germany and Russia - The RISE study. *J Eur Acad Dermatol Venereol*. 2016;30(3):428-434. doi:10.1111/jdv.13556

88. Berg M, Lidén S. An epidemiological study of rosacea. *Acta Derm Venereol*. 1989;69(5):419-423.

89. Elsaie ML, Choudhary S. Updates on the Pathophysiology and Management of Acne Rosacea. *Postgrad Med*. 2009;121(5):178-186. doi:10.3810/pgm.2009.09.2066

90. Browning DJ, Rosenwasser G, Lugo M. Ocular rosacea in blacks. *Am J Ophthalmol*.

1986;101(4):441-444. doi:10.1016/0002-9394(86)90644-6

91. Sarro RA, Hong JJ, Elgart ML. An unusual demodicidosis manifestation in a patient with AIDS. *J Am Acad Dermatol*. 1998;38(1):120-121.

92. Morras PG, Santos SP, Imedio IL, Echeverria ML, Hermosa JMH. Rosacea-Like Demodicidosis in an Immunocompromised Child. *Pediatr Dermatol*. 2003;20(1):28-30. doi:10.1046/j.1525-1470.2003.03006.x

93. Clyti E, Sayavong K, Chanthavisouk K. [Demodecidosis in a patient infected by HIV: successful treatment with ivermectin]. *Ann Dermatol Venereol*. 2005;132(5):459-461.

94. Jansen T, Kastner U, Kreuter A, Altmeyer P. Rosacea-like demodicidosis associated with acquired immunodeficiency syndrome. *Br J Dermatol*. 2001;144(1):139-142.

95. Patrizi A, Neri I, Chieregato C, Misciali M. Demodicidosis in Immunocompetent Young Children: Report of Eight Cases. *Dermatology*. 1997;195(3):239-242. doi:10.1159/000245951

96. Guerrero-González GA, Herz-Ruelas ME, Gómez-Flores M, Ocampo-Candiani J. Crusted demodicosis in an immunocompetent pediatric patient. *Case Rep Dermatol Med*. 2014;2014:458046. doi:10.1155/2014/458046

97. Damian D, Rogers M. Demodex infestation in a child with leukaemia: treatment with ivermectin and permethrin. *Int J Dermatol*. 2003;42(9):724-726. doi:10.1046/j.1365-4362.2003.01916.x

98. Sahn EE, Sheridan DM. Demodicidosis in a child with leukemia. *J Am Acad Dermatol*. 1992;27(5 Pt 2):799-801.

99. Castanet J, Monpoux F, Mariani R, Ortonne JP, Lacour JP. Demodicidosis in an immunodeficient child. *Pediatr Dermatol*. 14(3):219-220.

100. Barrio J, Lecona M, Hernanz JM, et al. Rosacea-Like Demodicosis in an HIV-Positive Child. *Dermatology*. 1996;192(2):143-145. doi:10.1159/000246341

101. Sanchez-Viera M, Hernanz JM, Sampelayo T, Gurbindo MD, Lecona M, Soto-Melo J. Granulomatous rosacea in a child infected with the human immunodeficiency virus. *J Am Acad Dermatol*. 1992;27(6 Pt 1):1010-1011.

102. American Diabetes Association AD. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33 Suppl 1(Suppl 1):S62-9. doi:10.2337/dc10-S062

103. Clifford CW, Fulk GW. Association of diabetes, lash loss, and Staphylococcus aureus with infestation of eyelids by Demodex folliculorum (Acari: Demodicidae). *J Med Entomol*. 1990;27(4):467-470.

104. Akdeniz S, Bahceci M, Tuzcu A, Harman M, Alp S, Bahceci S. Is demodex folliculorum larger in diabetic patients? *J Eur Acad Dermatology Venereol*. 2002;16(5):539-541. doi:10.1046/j.1468-3083.2002.00545_7.x

105. Moutschen MP, Scheen AJ, Lefebvre PJ. Impaired immune responses in diabetes mellitus: analysis of the factors and mechanisms involved. Relevance to the increased susceptibility of diabetic patients to specific infections. *Diabete Metab*. 1992;18(3):187-201.

106. Gökçe C, Aycan-Kaya Ö, Yula E, et al. The effect of blood glucose regulation on the presence of opportunistic Demodex folliculorum mites in patients with type 2 diabetes mellitus. *J Int Med Res*. 2013;41(5):1752-1758. doi:10.1177/0300060513494730

107. Kurt RK, Kaya OA, Karateke A, et al. Increased density of Demodex folliculorum mites in pregnancies with gestational diabetes. *Med Princ Pract*. 2014;23(4):369-372. doi:10.1159/000363244

108. Karincaoglu Y, Esrefoglu Seyhan M, Bayram N, Aycan O, Taskapan H. Incidence of Demodex folliculorum in patients with end stage chronic renal failure. *Ren Fail*. 2005;27(5):495-499.

109. Kurts C, Panzer U, Anders H-J, Rees AJ. The immune system and kidney disease: basic concepts and clinical implications. *Nat Rev Immunol*. 2013;13(10):738-753. doi:10.1038/nri3523

110. Dunaif A. Insulin Resistance and the Polycystic Ovary Syndrome: Mechanism and Implications for Pathogenesis*. *Endocr Rev*. 1997;18(6):774-800. doi:10.1210/edrv.18.6.0318

111. Eser A, Erpolat S, Kaygusuz I, Balci H, Kosus A. Investigation of Demodex folliculorum frequency inpatients with polycystic ovary syndrome. *An Bras Dermatol*. 2017;92(6):807. doi:10.1590/ABD1806-4841.20176043

112. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The Prevalence and Features of the Polycystic Ovary Syndrome in an Unselected Population. *J Clin Endocrinol Metab*. 2004;89(6):2745-2749. doi:10.1210/jc.2003-032046

113. Silfeler D, Keskin K, Kaya O, et al. Demodex folliculorum in polycystic ovary syndrome patients. *Eur Rev Med Pharmacol Sci*. 2015;19(7):1141-1145.

114. Jalbert I, Rejab S. Increased numbers of demodex in contact lens wearers. *Optom Vis Sci*. 2015;92(6):671-678.

115. Tarkowski W, Moneta-Wielgoś J, Młocicki D, M&#x142, ocicki D. Demodex sp. as a Potential Cause of the Abandonment of Soft Contact Lenses by Their Existing Users. *Biomed Res Int*. 2015;2015:259109. doi:10.1155/2015/259109

116. Horváth A, Neubrandt DM, Ghidán Á, Nagy K. Risk factors and prevalence of Demodex mites in young adults. *Acta Microbiol Immunol Hung*. 2011;58(2):145-155. doi:10.1556/AMicr.58.2011.2.7

117. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II Definition and Classification Report. *Ocul Surf*. 2017;15(3):276-283. doi:10.1016/j.jtos.2017.05.008

118. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol*. 2000;118(9):1264-1268.

119. Sahai, Malik P, Sahai A, Malik P. Dry eye: prevalence and attributable risk factors in a hospital-based population. *Indian J Ophthalmol*. 2005;53(2):87-91. doi:10.4103/0301-4738.16170

120. De Paiva CS, Chen Z, Koch DD, et al. The Incidence and Risk Factors for Developing Dry Eye After Myopic LASIK. *Am J Ophthalmol*. 2006;141(3):438-445. doi:10.1016/j.ajo.2005.10.006

121. Smith JA, Albenz J, Begley C, et al. The Epidemiology of Dry Eye Disease: Report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5(2):93-107.

122. Gayton JL. Etiology, Prevalence, and Treatment of Dry Eye. *Clin Ophthalmol*. 2009;3:405-412.

123. Galor A, Feuer W, Lee DJ, et al. Prevalence and Risk Factors of Dry Eye Syndrome in a United States Veterans Affairs Population. *Am J Ophthalmol*. 2011;152(3):377-384.e2. doi:10.1016/j.ajo.2011.02.026

124. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol*. 2003;136(2):318-326. doi:10.1016/S0002-9394(03)00218-6

125. Lin P-Y, Tsai S-Y, Cheng C-Y, Liu J-H, Chou P, Hsu W-M. Prevalence of dry eye among an elderly Chinese population in Taiwan. *Ophthalmology*. 2003;110(6):1096-1101. doi:10.1016/S0161-6420(03)00262-8

126. McCarty C, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia, Historical image. *Ophthalmology*. 1998;105(6):1114-1119. doi:10.1016/S0161-6420(98)96016-X

127. Wu M, Wang X, Han J, Shao T, Wang Y. Evaluation of the ocular surface characteristics and Demodex infestation in paediatric and adult blepharokeratoconjunctivitis. *BMC Ophthalmol*. 2019;19(1):67. doi:10.1186/s12886-019-1074-5

128. Nicholls SG, Oakley CL, Tan A, Vote BJ. Demodex species in human ocular disease: new clinicopathological aspects. *Int Ophthalmol*. 2017;37(1):303-312. doi:10.1007/s10792-016-0249-9

129. Efron N, Efron N. Eyelash disorders. *Contact Lens Complicat*. January 2012:67-75. doi:10.1016/B978-0-7020-4269-0.00007-9

130. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on

meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci*. 2011;52(4):1930-1937. doi:10.1167/iovs.10-6997b

131. Green-Church KB, Butovich IA, Willcox M, et al. The international workshop on meibomian gland dysfunction: Report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease. *Invest Ophthalmol Vis Sci*. 2011;52:1979-1993. doi:10.1167/iovs.10-6997d

132. Rolando M, Zierhut M. The ocular surface and tear film and their dysfunction in dry eye disease. *Surv Ophthalmol*. 2001;45 Suppl 2:S203-10.

133. Holly F, Lemp M. Tear physiology and dry eyes. *Surv Ophthalmol*. 1977;22(2):69-87. doi:10.1016/0039-6257(77)90087-X

134. Bron A, Tiffany J, Gouveia S, Yokoi N, Voon L. Functional aspects of the tear film lipid layer. *Exp Eye Res*. 2004;78(3):347-360. doi:10.1016/J.EXER.2003.09.019

135. Jeyalatha M, Qu Y, Liu Z, et al. Function of meibomian gland: Contribution of proteins. *Exp Eye Res*. 2017;163:29-36. doi:10.1016/j.exer.2017.06.009

136. Foulks GN. The Correlation Between the Tear Film Lipid Layer and Dry Eye Disease. *Surv Ophthalmol*. 2007;52(4):369-374. doi:10.1016/j.survophthal.2007.04.009

137. Luo X, Li J, Chen C, Tseng S, Liang L. Ocular Demodicosis as a Potential Cause of Ocular Surface Inflammation. *Cornea*. 2017;36 Suppl 1(Suppl 1):S9-S14. doi:10.1097/ICO.0000000000001361

138. Geerling G, Tauber J, Baudouin C, et al. The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Management and Treatment of

Meibomian Gland Dysfunction. *Invest Ophthalmol Vis Sci*. 2011;52(4):2050. doi:10.1167/iovs.10-6997g

139. Geerling G, Baudouin C, Aragona P, et al. Emerging strategies for the diagnosis and treatment of meibomian gland dysfunction: Proceedings of the OCEAN group meeting. *Ocul Surf*. 2017;15(2):179-192. doi:10.1016/j.jtos.2017.01.006

140. Baumann A, Cochener B. Meibomian gland dysfunction: A comparative study of modern treatments. *J Fr Ophthalmol*. 2014;37(1773-0597 (Electronic)):303-312.

141. Bilkhu PS, Naroo SA, Wolffsohn JS. Randomised masked clinical trial of the MGDRx eyebag for the treatment of meibomian gland dysfunction-related evaporative dry eye. *Br J Ophthalmol*. 2014:1-5. doi:10.1136/bjophthalmol-2014-305220

142. Achtsidis V, Kozanidou E, Bournas P, Tentolouris N, Theodossiadis PG. Dry Eye and Clinical Disease of Tear Film, Diagnosis and Management. *Eur Ophthalmic Rev*. 2014;8(1):17-22. doi:10.17925/EOR.2014.08.01.17

143. Kamoun B, Fourati M, Feki J, et al. Blepharitis due to Demodex: myth or reality? *J Fr Ophthalmol*. 1999;22(5):525-527.

144. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II Epidemiology Report. *Ocul Surf*. 2017;15(3):334-365. doi:10.1016/j.jtos.2017.05.003

145. Kaya A, Gürdal C. Office-Based Diagnosis of Demodex Using Smartphone. *Eye Contact Lens Sci Clin Pract*. June 2018:1. doi:10.1097/ICL.0000000000000507

146. Sattler EC, Maier T, Hoffmann VS, Hegyi J, Ruzicka T, Berking C. Noninvasive *in vivo* detection and quantification of *Demodex* mites by confocal laser scanning

microscopy. *Br J Dermatol*. 2012;167(5):1042-1047. doi:10.1111/j.1365-2133.2012.11096.x

147. Wang Y-J, Ke M, Chen X-M. Prospective Study of the Diagnostic Accuracy of the In Vivo Laser Scanning Confocal Microscopy for Ocular Demodicosis. *Am J Ophthalmol*. 2019;203:46-52. doi:10.1016/j.ajo.2019.02.026

148. Carson CF, Riley T V. Safety, efficacy and provenance of tea tree ( Melaleuca alternifolia ) oil. *Contact Dermatitis*. 2001;45(2):65-67. doi:10.1034/j.1600-0536.2001.045002065.x

149. Carson CF, Hammer KA, Riley T V. Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 2006;19(1):50-62. doi:10.1128/CMR.19.1.50-62.2006

150. National Center for Biotechnology Information. PubChem Compound Database. https://pubchem.ncbi.nlm.nih.gov/compound/14231.

151. Food and Drugs Administration. STROMECTOL ®. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/050742s026lbl.pdf. Published 2009. Accessed May 23, 2017.

152. Chouela EN, Abeldaño AM, Pellerano G, et al. Equivalent therapeutic efficacy and safety of ivermectin and lindane in the treatment of human scabies. *Arch Dermatol*. 1999;135(6):651-655.

153. De Sole G, Remme J, Awadzi K, et al. Adverse reactions after large-scale treatment of onchocerciasis with ivermectin: combined results from eight community trials. *Bull World Health Organ*. 1989;67(6):707-719.

154. Heukelbach J, Winter B, Wilcke T, et al. Selective mass treatment with ivermectin to control intestinal helminthiases and parasitic skin diseases in a severely affected population. *Bull World Health Organ*. 2004;82(8):563-571. doi:/S0042-96862004000800005

155. Pacqué M, Munoz B, Taylor H., Dukuly Z, Greene B., White A. Safety of and compliance with community-based ivermectin therapy. *Lancet*. 1990;335(8702):1377-1380. doi:10.1016/0140-6736(90)91253-7

156. Sparsa A, Bonnetblanc J-M, Peyrot I, Loustaud-Ratti V, Vidal E, Bédane C. [Systemic adverse reactions with ivermectin treatment of scabies]. *Ann Dermatol Venereol*. 2006;133(10):784-787.

157. Thomas J, Peterson GM, Walton SF, Carson CF, Naunton M, Baby KE. Scabies: An ancient global disease with a need for new therapies. *BMC Infect Dis*. 2015;15(1). doi:10.1186/s12879-015-0983-z

158. Hoekzema R, Hulsebosch HJ, Bos JD. Demodicidosis or rosacea: what did we treat? *Br J Dermatol*. 1995;133(2):294-299.

159. Forton, Seys, Marchal, Song. Demodex folliculorum and topical treatment: acaricidal action evaluated by standardized skin surface biopsy. *Br J Dermatol*. 1998;138(3):461-466. doi:10.1046/j.1365-2133.1998.02125.x

160. Luo Y, Sun Y-J, Zhang L, Luan X-L. Treatment of mites folliculitis with an ornidazole-based sequential therapy. *Medicine (Baltimore)*. 2016;95(27):e4173. doi:10.1097/MD.0000000000004173

161. Kurt O, Girginkardeşler N, Balcioğlu IC, Ozbilgin A, Ok UZ. A comparison of

metronidazole and single-dose ornidazole for the treatment of dientamoebiasis. *Clin Microbiol Infect*. 2008;14(6):601-604. doi:10.1111/j.1469-0691.2008.02002.x

162. Food and Drugs Administration. Flagyl®. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/020868s011lbl.pdf. Published 2015. Accessed May 25, 2017.

163. Carter DA, Blair SE, Cokcetin NN, et al. Therapeutic Manuka Honey: No Longer So Alternative. *Front Microbiol*. 2016;7:569. doi:10.3389/fmicb.2016.00569

164. Soffer A. Chihuahuas and Laetrile, Chelation Therapy, and Honey From Boulder, Colo. *Arch Intern Med*. 1976;136(8):865. doi:10.1001/archinte.1976.03630080007003

165. Lu J, Carter DA, Turnbull L, et al. The effect of New Zealand kanuka, manuka and clover honeys on bacterial growth dynamics and cellular morphology varies according to the species. *PLoS One*. 2013;8(2):e55898. doi:10.1371/journal.pone.0055898

166. Braithwaite I, Hunt A, Riley J, et al. Randomised controlled trial of topical kanuka honey for the treatment of rosacea. *BMJ Open*. 2015;5(6):e007651. doi:10.1136/bmjopen-2015-007651

167. Frame K, Cheung IMY, Wang MTM, Turnbull PR, Watters GA, Craig JP. Comparing the in vitro effects of MGO$^{TM}$ Manuka honey and tea tree oil on ocular Demodex viability. *Contact Lens Anterior Eye*. July 2018. doi:10.1016/J.CLAE.2018.06.006

168. Craig JP, Rupenthal ID, Seyfoddin A, et al. Preclinical development of MGO Manuka Honey microemulsion for blepharitis management. *BMJ open Ophthalmol*. 2017;1(1):e000065. doi:10.1136/bmjophth-2016-000065

169. World Medical Association. World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. *JAMA*. 2013;310(20):2191-2194.

170. Foulks GN. Challenges and pitfalls in clinical trials of treatments for dry eye. *Ocul Surf*. 2003;1(1):20-30. doi:10.1016/S1542-0124(12)70004-6

171. Wolffsohn JS, Arita R, Chalmers R, et al. TFOS DEWS II Diagnostic Methodology report. *Ocul Surf*. 2017;15(3):539-574. doi:10.1016/j.jtos.2017.05.001

172. Lovie-Kitchin JE. Validity and reliability of viscual acuity measurements. *Ophthalmic Physiol Opt*. 1988;8(4):363-370. doi:10.1111/j.1475-1313.1988.tb01170.x

173. Oduntan OA, Mashige KP, Raliavhegwa-Makhado M. A comparison of two methods of logMAR visual acuity data scoring for statistical analysis. *African Vis Eye Heal*. 2009;68(3):155-163. doi:10.4102/aveh.v68i3.162

174. Downie LE. Automated Tear Film Surface Quality Breakup Time as a Novel Clinical Marker for Tear Hyperosmolarity in Dry Eye Disease. *Invest Ophthalmol Vis Sci*. 2015;56(12):7260. doi:10.1167/iovs.15-17772

175. Lemp MA, Baudouin C, Baum J, et al. The Definition and Classification of Dry Eye Disease: Report of the Definition and Classification Subcommittee of the International Dry Eye Work Shop (2007). *Ocul Surf*. 2007;55(22):75-92.

176. Lemp MA, Bron AJ, Baudouin C, et al. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol*. 2011;151(5):792-798.e1. doi:10.1016/j.ajo.2010.10.032

177. Jacobi C, Jacobi A, Kruse FE, Cursiefen C. Tear Film Osmolarity Measurements in Dry Eye Disease Using Electrical Impedance Technology. *Cornea*. 2011;30(12):1289-1292. doi:10.1097/ICO.0b013e31821de383

178. Gillan W. Repeatability and reproducibility of TearLab measurements. *S Afr Optom*. 2013;72(1):19-24.

179. Bron AJ, Evans VE, Smith JA. Grading Of corneal and conjunctival staining in the context of other dry eye tests. *Cornea*. 2003;22(7):640-650.

180. Stevens S. Ophthalmic practice. *Community eye Heal*. 2005;18(53):79.

181. Bron AJ, Argüeso P, Irkec M, Bright FV. Clinical staining of the ocular surface: Mechanisms and interpretations. *Prog Retin Eye Res*. 2015;44:36-61. doi:10.1016/j.preteyeres.2014.10.001

182. DEWS. Methodologies to Diagnose and Monitor Dry Eye Disease: Report of the Diagnostic Methodology Subcommittee of the International Dry Eye Workshop (2007). *Ocul Surf*. 2007;5(2):108-152.

183. Serin D, Karsloğlu Ş, Kyan A, Alagöz G. A Simple Approach to the Repeatability of the Schirmer Test Without Anesthesia. *Cornea*. 2007;26(8):903-906. doi:10.1097/ICO.0b013e3180950083

184. Hom M. Mites that eat and live on your lids. In: *American Academy of Optometry Annual Meeting*. Seattle; 2013.

185. Tomlinson A, Bron AJ, Korb DR, et al. The International Workshop on Meibomian Gland Dysfunction: Report of the Diagnosis Subcommittee. *Invest Ophthalmol Vis Sci*.

2011;52(4):2006-2049.

186. Sung J, Wang MTM, Lee SH, et al. Randomized double-masked trial of eyelid cleansing treatments for blepharitis. *Ocul Surf*. 2018;16(1):77-83. doi:10.1016/J.JTOS.2017.10.005

187. Burgess IF, Lee PN, Kay K, Jones R, Brunton ER. 1,2-Octanediol, a novel surfactant, for treating head louse infestation: identification of activity, formulation, and randomised, controlled trials. *PLoS One*. 2012;7(4):e35419. doi:10.1371/journal.pone.0035419

188. Cheng AM, Sheha H, Tseng SC. Recent advances on ocular Demodex infestation. *Curr Opin Ophthalmol*. 2015;26(4):295-300. doi:10.1097/ICU.0000000000000168

189. Rynerson JM, Perry HD. DEBS - a unification theory for dry eye and blepharitis. *Clin Ophthalmol*. 2016;10:2455-2467. doi:10.2147/OPTH.S114674

190. Rynerson JM. Method and device for treating an ocular disorder. 2012.

191. Olson MC, Korb DR, Greiner J V. Increase in tear film lipid layer thickness following treatment with warm compresses in patients with meibomian gland dysfunction. *Eye Contact Lens*. 2003;29(2):96-99. doi:10.1097/01.ICL.0000060998.20142.8D

192. Blackie CA, Solomon JD, Greiner J V., Holmes M, Korb DR. Inner Eyelid Surface Temperature as a Function of Warm Compress Methodology. *Optom Vis Sci*. 2008;85(8):675-683. doi:10.1097/OPX.0b013e318181adef

193. OPTASE Moist Heat Mask | Daily Lid Hygiene | Scope Ophthalmics. http://www.scopeophthalmics.com/optase/optase-moist-heat-mask. Accessed

November 6, 2019.

194. Shahid H, Khan JC, Cipriani V, et al. Age-related macular degeneration: the importance of family history as a risk factor. *Br J Ophthalmol*. 2012;96(3):427-431. doi:10.1136/bjophthalmol-2011-300193

195. Gramer G, Weber BHF, Gramer E, et al. Results of a Patient-Directed Survey on Frequency of Family History of Glaucoma in 2170 Patients. *Invest Ophthalmol Vis Sci*. 2014;55(1):259. doi:10.1167/iovs.13-13020

196. Fiebai B, Ejimadu CS, Komolafe RD. Incidence and risk factors for retinal vein occlusion at the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. *Niger J Clin Pract*. 2014;17(4):462-466. doi:10.4103/1119-3077.134040

197. Zhao YE, Guo N, Wu LP. The effect of temperature on the viability of Demodex folliculorum and Demodex brevis. *Parasitol Res*. 2009;105(6):1623-1628. doi:10.1007/s00436-009-1603-x

198. McDonald LG, Tovey E. The role of water temperature and laundry procedures in reducing house dust mite populations and allergen content of bedding. *J Allergy Clin Immunol*. 1992;90(4):599-608. doi:10.1016/0091-6749(92)90132-L

199. Patton N, Aslam T, Murray G. Statistical strategies to assess reliability in ophthalmology. *Eye*. 2006;20(7):749-754. doi:10.1038/sj.eye.6702097

200. McHugh ML. Interrater reliability: the kappa statistic. *Biochem medica*. 2012;22(3):276-282.

201. Sengbusch HG, Hauswirth JW. Prevalence of hair follicle mites, Demodex

folliculorum and d. brevis (Acari: Demodicidae), in a selected human population in western New York, USA. *J Med Entomol*. 1986;23(4):384-388.

202. Ng A, Evans K, North R, Purslow C. Eye cosmetic usage and associated ocular comfort. *Ophthalmic Physiol Opt*. 2012;32(6):501-507. doi:10.1111/j.1475-1313.2012.00944.x

203. Bujang M, Nurakmal B. Guidelines of the minimum sample size requirements for Cohen's Kappa. *Epidemiol Biostat Public Heal*. 2017;14(2).

204. Savini G, Prabhawasat P, Kojima T, Grueterich M, Espana E, Goto E. The challenge of dry eye diagnosis. *Clin Ophthalmol*. 2008;2(1):31-55.

205. Nichols K, Mitchell G, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea*. 2004;23(3):272-285. doi:10.1097/00003226-200404000-00010

206. Sullivan BDBD, Whitmer D, Nichols KKK, et al. An objective approach to dry eye disease severity. *Invest Ophthalmol Vis Sci*. 2010;51(12):6125-6130. doi:10.1167/iovs.10-5390

207. Pult H, Purslow C, Murphy PJ. The relationship between clinical signs and dry eye symptoms. *Eye (Lond)*. 2011;25(4):502-510. doi:10.1038/eye.2010.228

208. Pesudovs K, Burr JM, Harley C, Elliott DB. The Development, Assessment, and Selection of Questionnaires. *Optom Vis Sci*. 2007;84(8):663-674.

209. Willke RJ. Measuring the value of treatment to patients: patient-reported outcomes in drug development. *Am Heal drug benefits*. 2008;1(1):34-40.

210. Schaumberg DA, Nichols JJ, Papas EB, Tong L, Uchino M, Nichols KK. The

International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on the Epidemiology of, and Associated Risk Factors for, MGD. *Invest Ophthalmol Vis Sci*. 2011;52(4):1994-2005.

211. Schiffman RM, Christianson MD, Jacobsen G, et al. Reliability and Validity of the Ocular Surface Disease Index. *Arch Ophthalmol*. 2000;118(5):615. doi:10.1001/archopht.118.5.615

212. Walt J. *Ocular Surface Disease Index (OSDI) Administration and Scoring Manual*. Irvine, CA; 2004.

213. Mathews PM, Ramulu PY, Friedman DS, Utine CA, Akpek EK. Evaluation of ocular surface disease in patients with glaucoma. *Ophthalmology*. 2013;120(11):2241-2248. doi:10.1016/j.ophtha.2013.03.045

214. Miller KL, Walt JG, Mink DR, et al. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol (Chicago, Ill 1960)*. 2010;128(1):94-101. doi:10.1001/archophthalmol.2009.356

215. Bland JM, Altman DG. Statistics notes: Cronbach's alpha. *BMJ*. 1997;314(7080). doi:10.1136/bmj.314.7080.572

216. Tavakol M, Dennick R. Making sense of Cronbach's alpha. *Int J Med Educ*. 2011;2:53-55.

217. Hallgren KA. Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. *Tutor Quant Methods Psychol*. 2012;8(1):23-34.

218. Rosner B. *Fundamentals Of Biostatistics*. 7th ed. Boston: Brooks/Cole; 2011.

219. Costello AB, Osborne JW, Costello AB. Best Practices in Exploratory Factor Analysis: Four Recommendations for Getting the Most From Your Analysis. *Pan-Pacific Manag Rev*. 2009;12(2):131-146.

220. Villani E, Magnani F, Viola F, et al. In Vivo Confocal Evaluation of the Ocular Surface Morpho-Functional Unit in Dry Eye. *Optom Vis Sci*. 2013;90(6):576-586.

221. Hoşal BM, Örnek N, Zilelioğlu G, Elhan AH. Morphology of corneal nerves and corneal sensation in dry eye: a preliminary study. *Eye*. 2005;19(12):1276-1279.

222. Bourcier T, Acosta MC, Borderie V, et al. Decreased Corneal Sensitivity in Patients with Dry Eye. *Invest Ophthalmol Vis Sci*. 2005;46(7):2341.

223. Nichols K, Nichols J, Mitchell G. The lack of association between signs and symptoms in patients with dry eye disease. *Cornea*. 2004;23(8):762-770.

224. Ashack RJ, Frost ML, Norins AL. Papular pruritic eruption of Demodex folliculitis in patients with acquired immunodeficiency syndrome. *J Am Acad Dermatol*. 1989;21(2):306-307. doi:10.1016/S0190-9622(89)70182-1

225. Dominey A, Rosen T, Tschen J. Papulonodular demodicidosis associated with acquired immunodeficiency syndrome. *J Am Acad Dermatol*. 1989;20(2):197-201. doi:10.1016/S0190-9622(89)70021-9

226. Rufli T, Mumcuoglu Y, Cajacob A, Büchner S. *Demodex folliculorum*: aetiopathogenesis and therapy of rosacea and perioral dermatitis. *Dermatologica*. 1981;162(1):12-26.

227. Siddiqui K, Stein Gold L, Gill J. The efficacy, safety, and tolerability of ivermectin

compared with current topical treatments for the inflammatory lesions of rosacea: a network meta-analysis. *Springerplus*. 2016;5(1151).

228. Nicholls SG, Oakley CL, Tan A, Vote BJ. Demodex treatment in external ocular disease: the outcomes of a Tasmanian case series. *Int Ophthalmol*. 2016;36(5):691-696. doi:10.1007/s10792-016-0188-5

229. Lacey N, Russell-Hallinan A, Powell FC. Study of Demodex mites: Challenges and Solutions. *J Eur Acad Dermatol Venereol*. 2016;30(5):764-775. doi:10.1111/jdv.13517

230. Schear MJ, Milman T, Steiner T, Shih C, Udell IJ, Steiner A. The Association of Demodex with Chalazia: A Histopathologic Study of the Eyelid. *Ophthal Plast Reconstr Surg*. 2015;32(4):275-278. doi:10.1097/IOP.0000000000000500

231. Guvendi Akcinar U, Unal E, Akpinar M. Demodex spp. Infestation Associated with Treatment-Resistant Chalazia and Folliculitis. *Turkish J Parasitol*. 2017;40(4):208-210. doi:10.5152/tpd.2016.4869

232. Jin J, Sklar GE, Min Sen Oh V, Chuen Li S. Factors affecting therapeutic compliance: A review from the patient's perspective. *Ther Clin Risk Manag*. 2008;4(1):269-286.

233. Jackson B. Blepharitis: current strategies for diagnosis and management. *Can J Ophthalmol*. 2008;43(2):170-179. doi:10.3129/i08-016

234. Haque RM, Torkildsen GL, Brubaker K, et al. Multicenter Open-Label Study Evaluating the Efficacy of Azithromycin Ophthalmic Solution 1% on the Signs and Symptoms of Subjects With Blepharitis. *Cornea*. 2010;29(8):871-877. doi:10.1097/ICO.0b013e3181ca38a0

235. Ford GP, Farr PM, Ive FA, Shuster S. The response of seborrhoeic dermatitis to ketoconazole. *Br J Dermatol*. 1984;111(5):603-607.

236. Comert A, Bekiroglu N, Gurbuz O, Ergun T. Efficacy of Oral Fluconazole in the Treatment of Seborrheic Dermatitis. *Am J Clin Dermatol*. 2007;8(4):235-238. doi:10.2165/00128071-200708040-00005

237. Zisova LG. Fluconazole and its place in the treatment of seborrheic dermatitis--new therapeutic possibilities. *Folia Med (Plovdiv)*. 2006;48(1):39-45.

238. Baysal V, Yildirim M, Ozcanli C, Ceyhan AM. Itraconazole in the treatment of seborrheic dermatitis: a new treatment modality. *Int J Dermatol*. 2004;43(1):63-66.

239. Kose O, Erbil H, Gur A. Oral itraconazole for the treatment of seborrhoeic dermatitis: an open, noncomparative trial. *J Eur Acad Dermatol Venereol*. 2005;19(2):172-175. doi:10.1111/j.1468-3083.2005.01090.x

240. Cheung IMY, Xue AL, Kim A, Ammundsen K, Wang MTM, Craig JP. In vitro anti-demodectic effects and terpinen-4-ol content of commercial eyelid cleansers. *Contact Lens Anterior Eye*. 2018;41(6):513-517. doi:10.1016/j.clae.2018.08.003

241. Ngo W, Jones L, Bitton E. Short-Term Comfort Responses Associated With the Use of Eyelid Cleansing Products to Manage Demodex folliculorum. *Eye Contact Lens Sci Clin Pract*. 2018;44:S87-S92. doi:10.1097/ICL.0000000000000415

242. Qiu TY, Yeo S, Tong L. Satisfaction and convenience of using terpenoid-impregnated eyelid wipes and teaching method in people without blepharitis. *Clin Ophthalmol*. 2018;12:91-98. doi:10.2147/OPTH.S144483

243. Gipson IK. The ocular surface: the challenge to enable and protect vision: the Friedenwald lecture. *Invest Ophthalmol Vis Sci*. 2007;48(10):4390; 4391-4398. doi:10.1167/iovs.07-0770

244. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. *Exp Eye Res*. 2004;78(3):409-416.

245. Ng A, Evans K, North R V., Purslow C. Migration of Cosmetic Products into the Tear Film. *Eye Contact Lens Sci Clin Pract*. 2015;41(5):304-309. doi:10.1097/ICL.0000000000000124

246. Ng A, Evans K, North R V., Jones L, Purslow C. Impact of Eye Cosmetics on the Eye, Adnexa, and Ocular Surface. *Eye Contact Lens Sci Clin Pract*. 2016;42(4):211-220. doi:10.1097/ICL.0000000000000181

247. Malik A, Claoué C. Transport and interaction of cosmetic product material within the ocular surface: Beauty and the beastly symptoms of toxic tears. *Contact Lens Anterior Eye*. 2012;35(6):247-259. doi:10.1016/j.clae.2012.07.005

248. Goto T, Zheng X, Gibbon L, Ohashi Y. Cosmetic Product Migration Onto the Ocular Surface: Exacerbation of Migration After Eyedrop Instillation. *Cornea*. 2010;29(4):400-403. doi:10.1097/ICO.0b013e3181bd4756

249. Purslow C, Conaty C, Ng A. Migration of Substances Applied Around the Eyelid Margin. *Invest Ophthalmol Vis Sci*. 2013;54(15):954-954.

250. de Paiva CS. Effects of Aging in Dry Eye. *Int Ophthalmol Clin*. 2017;57(2):47-64. doi:10.1097/IIO.0000000000000170

251. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: Determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci*. 2006;47(10):4309-4315. doi:10.1167/iovs.05-1504

252. Kim H-Y. Statistical notes for clinical researchers: Nonparametric statistical methods: 2. Nonparametric methods for comparing three or more groups and repeated measures. *Restor Dent Endod*. 2014;39(4):329-332. doi:10.5395/rde.2014.39.4.329

253. Rabensteiner DF, Aminfar H, Boldin I, et al. Demodex mite infestation and its associations with tear film and ocular surface parameters in patients with ocular discomfort. *Am J Ophthalmol*. 2019;Article in Press. doi:10.1016/j.ajo.2019.03.007

254. Zhao Y, Guo N, Xun M, Xu J, Wang M, Wang D. Sociodemographic characteristics and risk factor analysis of Demodex infestation (Acari: Demodicidae). *J Zhejiang Univ Sci B*. 2011;12(12):998-1007. doi:10.1631/jzus.B1100079

255. Karaman Ü, Kolören Z, Enginyurt Ö, Özer A. [The epidemiology of demodex mites at the college students living in dormitories in the city of Ordu]. *Turkiye Parazitol Derg*. 2014;38(3):166-171. doi:10.5152/tpd.2014.3517

256. Anderton PJ. Implementation of evidence-based practice in optometry. *Clin Exp Optom*. 2007;90(4):238-243. doi:10.1111/j.1444-0938.2007.00153.x

257. Adams AJ. Whither Goes Evidence-Based Optometry? *Optom Vis Sci*. 2008;85(4):219-220. doi:10.1097/OPX.0b013e3181719a7a

258. Elliott DB. Evidence-based optometry and in-practice research. *Ophthalmic Physiol Opt*. 2012;32(2):81-82. doi:10.1111/j.1475-1313.2012.00899.x

259. Suttle CM, Challinor KL, Thompson RE, et al. Attitudes and Barriers to Evidence-Based Practice in Optometry Educators. *Optom Vis Sci*. 2015;92(4):514-523. doi:10.1097/OPX.0000000000000550

260. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. *BMJ*. 1996;312(7023):71-72. doi:10.1136/BMJ.312.7023.71

261. Howick J, Friedemann C, Tsakok M, et al. Are treatments more effective than placebos? A systematic review and meta-analysis. *PLoS One*. 2013;8(5):e62599. doi:10.1371/journal.pone.0062599

262. Imanaka T, Sato I, Tanaka S, Kawakami K. Predictive factors for the placebo effect in clinical trials for dry eye: a pooled analysis of three clinical trials. *Br J Ophthalmol*. 2017;101(11):1471-1474. doi:10.1136/bjophthalmol-2016-309887

263. Key J. A Comparative Study of Eyelid Cleaning Regimens in Chronic Blepharitis. *Contact Lens Assoc Ophthalmol*. 1996;22(3).

264. McDonald CJ, Mazzuca SA, McCabe GP. How much of the placebo 'effect' is really statistical regression? *Stat Med*. 2(4):417-427.

265. Lee J-E, Kim NM, Yang JW, Kim SJ, Lee JS, Lee JE. A randomised controlled trial comparing a thermal massager with artificial teardrops for the treatment of dry eye. *Br J Ophthalmol*. 2014;98(1):46-51. doi:10.1136/bjophthalmol-2013-303742

266. Argüeso P, Balaram M, Spurr-Michaud S, Keutmann HT, Dana MR, Gipson IK. Decreased levels of the goblet cell mucin MUC5AC in tears of patients with Sjögren syndrome. *Invest Ophthalmol Vis Sci*. 2002;43(4):1004-1011.

267. Jumblatt MM, McKenzie RW, Jumblatt JE. MUC5AC mucin is a component of the human precorneal tear film. *Invest Ophthalmol Vis Sci*. 1999;40(1):43-49.

268. Baudouin C, Aragona P, Messmer EM, et al. Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the OCEAN group meeting. *Ocul Surf*. 2013;11(4):246-258. doi:10.1016/j.jtos.2013.07.003

269. Baudouin C. A new approach for better comprehension of diseases of the ocular surface. *J Fr Ophthalmol*. 2007;30(3):239-246.

270. Alghamdi YA, Camp A, Feuer W, Karp CL, Wellik S, Galor A. Compliance and Subjective Patient Responses to Eyelid Hygiene. *Eye Contact Lens*. 2017;43(4):213-217. doi:10.1097/ICL.0000000000000258

271. Jester J, Nicolaides N, Smith R. Meibomian gland studies: histologic and ultrastructural investigations. *Invest Ophthalmol Vis Sci*. 1977;20(4):537-547.

272. Knop E, Knop N, Millar T, Obata H, Sullivan D. The international workshop on meibomian gland dysfunction: report of the subcomittee on anatomy, physiology and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci*. 2011;52(4):1938-1978. doi:10.1167/iovs.10-6997c

273. Nichols KK, Foulks GN, Bron AJ, et al. The International Workshop on Meibomian Gland Dysfunction: Executive Summary. *Invest Ophthalmol Vis Sci*. 2011;52(4):1922. doi:10.1167/iovs.10-6997a

274. Liang L, Liu Y, Ding X, Ke H, Chen C, Tseng SCG. Significant correlation between meibomian gland dysfunction and keratitis in young patients with *Demodex brevis* infestation. *Br J Ophthalmol*. October 2017:bjophthalmol-2017-310302.

doi:10.1136/bjophthalmol-2017-310302

275. McCulley JP, Shine WE. Meibomian Secretions in Chronic Blepharitis. In: Springer, Boston, MA; 1998:319-326. doi:10.1007/978-1-4615-5359-5_45

276. Murakami DK, Blackie CA, Korb DR. All Warm Compresses Are Not Equally Efficacious. *Optom Vis Sci*. 2015;92(9):e327-e333. doi:10.1097/OPX.0000000000000675

277. Smith G, Dart J. External eye disease. In: Jackson T, ed. *Moorfields Manual of Ophthalmology*. Philadelphia: Mosby Elsevier; 2008.

278. Lacroix Z, Léger S, Bitton E. Ex vivo heat retention of different eyelid warming masks. *Contact Lens Anterior Eye*. 2015;38(3):152-156. doi:10.1016/J.CLAE.2015.01.005

279. Rogers P. *A Technical Report for Scope Ophthalmics (OPTASE Moist Heat Mask)*. Milton Keynes: Intertek Testing & Certification Ltd.; 2016.

280. Bilkhu PS, Naroo SA, Wolffsohn JS. Effect of a Commercially Available Warm Compress on Eyelid Temperature and Tear Film in Healthy Eyes. *Optom Vis Sci*. 2013;91(2):1. doi:10.1097/OPX.0000000000000134

281. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med*. 2016;15(2):155-163. doi:10.1016/j.jcm.2016.02.012

282. Armstrong RA. Statistical guidelines for the analysis of data obtained from one or both eyes. *Ophthalmic Physiol Opt*. 2013;33(1):7-14. doi:10.1111/opo.12009

283. Zhang X, Song N, Gong L. Therapeutic Effect of Intense Pulsed Light on Ocular

Demodicosis. *Curr Eye Res*. October 2018:1-7. doi:10.1080/02713683.2018.1536217

284. Turan N, Kapicioglu Y, Sarac G. The Effect of Skin Sebum, pH, and Moisture on Demodex Infestation in Acne Vulgaris and Rosacea Patients. *Turkish J Parasitol*. 2017;41(3):143-147. doi:10.5152/tpd.2017.5068

285. Demirdağ HG, Özcan H, Görsoy Ş, Beker Akbulut G. The effects of sebum configuration on Demodex spp. density. *Turkish Med J Sci*. 2016;46(5):1415-1421. doi:10.3906/sag-1504-77

286. McMonnies CW. Conjunctival Tear Layer Temperature, Evaporation, Hyperosmolarity, Inflammation, Hyperemia, Tissue Damage, and Symptoms: A Review of an Amplifying Cascade. *Curr Eye Res*. 2017;42(12):1574-1584. doi:10.1080/02713683.2017.1377261

287. Godin MR, Stinnett SS, Gupta PK. Outcomes of Thermal Pulsation Treatment for Dry Eye Syndrome in Patients With Sjogren Disease. *Cornea*. 2018;37(9):1155-1158. doi:10.1097/ICO.0000000000001621

288. Giannaccare G, Vigo L, Pellegrini M, Sebastiani S, Carones F. Ocular Surface Workup With Automated Noninvasive Measurements for the Diagnosis of Meibomian Gland Dysfunction. *Cornea*. 2018;37(6):740-745. doi:10.1097/ICO.0000000000001500

289. Potvin R, Makari S, Rapuano CJ. Tear film osmolarity and dry eye disease: a review of the literature. *Clin Ophthalmol*. 2015;9:2039-2047. doi:10.2147/OPTH.S95242

290. Kim MJ, Stinnett SS, Gupta PK. Effect of thermal pulsation treatment on tear film parameters in dry eye disease patients. *Clin Ophthalmol*. 2017;11:883-886. doi:10.2147/OPTH.S136203

291. Keech A, Senchyna M, Jones L. Impact of time between collection and collection method on human tear fluid osmolarity. *Curr Eye Res*. 2013;38(4):428-436. doi:10.3109/02713683.2013.763987

292. Arita R, Morishige N, Shirakawa R, Sato Y, Amano S. Effects of Eyelid Warming Devices on Tear Film Parameters in Normal Subjects and Patients with Meibomian Gland Dysfunction. *Ocul Surf*. 2015;13(4):321-330. doi:10.1016/j.jtos.2015.04.005

293. Wang MTM, Jaitley Z, Lord SM, Craig JP. Comparison of Self-applied Heat Therapy for Meibomian Gland Dysfunction. *Optom Vis Sci*. 2015;92(9):e321-e326. doi:10.1097/OPX.0000000000000601

294. Matsumoto Y, Dogru M, Goto E, et al. Efficacy of a new warm moist air device on tear functions of patients with simple meibomian gland dysfunction. *Cornea*. 2006;25(6):644-650. doi:10.1097/01.ico.0000208822.70732.25

295. Goto E, Monden Y, Takano Y, et al. Treatment of non-inflamed obstructive meibomian gland dysfunction by an infrared warm compression device. *Br J Ophthalmol*. 2002;86(12):1403-1407.

296. Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. Effect of fluorescein instillation on the pre-corneal tear film stability. *Curr Eye Res*. 1985;4(1):9-12. doi:10.3109/02713688508999961

297. Mooi JK, Wang MTM, Lim J, Müller A, Craig JP. Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability. *Contact Lens Anterior Eye*. 2017;40(3):170-174. doi:10.1016/J.CLAE.2017.01.004

298. Lane SS, DuBiner HB, Epstein RJ, et al. A New System, the LipiFlow, for the

Treatment of Meibomian Gland Dysfunction. *Cornea*. 2012;31(4):396-404. doi:10.1097/ICO.0b013e318239aaea

299. Pult H, Riede-Pult BH, Purslow C. A Comparison of an Eyelid-Warming Device to Traditional Compress Therapy. *Optom Vis Sci*. 2012;89(7):E1035-E1041. doi:10.1097/OPX.0b013e31825c3479

300. Altman DG, Bland JM. *Measurement in Medicine: The Analysis of Method Comparison Studies †*. Vol 32.; 1983.

301. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res*. 1999;8(2):135-160. doi:10.1177/096228029900800204

302. Watson PF, Petrie A. Method agreement analysis: A review of correct methodology. *Theriogenology*. 2010;73(9):1167-1179. doi:10.1016/J.THERIOGENOLOGY.2010.01.003

303. Tiuseco KAL, Lim R, Siong B, Reyes JM, Iguban EB. Petroleum Jelly Versus Tea Tree Oil and Tea Tree Facial Wash Lid Scrub in Patients with Blepharitis Associated with Above-normal Demodex Count. *Philipp J Ophthalmol*. 2012;37(2):73-82.

304. Kumar A, Karthikeyan K. Madarosis: a marker of many maladies. *Int J Trichology*. 2012;4(1):3-18. doi:10.4103/0974-7753.96079

305. Murphy O, O' Dwyer V, Lloyd-McKernan A. The clinical use of eyelash manipulation in the diagnosis of Demodex folliculorum blepharitis. *Eye Contact Lens*. 2019;In Press.

306. Stapleton F, Willcox MD, Fleming CM, Hickson S, Sweeney DF, Holden BA. Changes to the ocular biota with time in extended- and daily-wear disposable contact lens use.

*Infect Immun*. 1995;63(11):4501-4505.

307. Edwards DJ. Dissemination of Research Results: On the Path to Practice Change. *Can J Hosp Pharm*. 2015;68(6):465.

308. McVay AB, Stamatakis KA, Jacobs JA, Tabak RG, Brownson RC. The role of researchers in disseminating evidence to public health practice settings: a cross-sectional study. *Heal Res Policy Syst*. 2016;14. doi:10.1186/S12961-016-0113-4

309. Brownson RC, Eyler AA, Harris JK, Moore JB, Tabak RG. Getting the Word Out: New Approaches for Disseminating Public Health Science. *J Public Heal Manag Pract*. 2018;24(2):102-111. doi:10.1097/PHH.0000000000000673

## APPENDICES

### Appendix 1: Informed Consent Letter

**Patient Information Sheet**

**Project title: Comparison of traditional treatment methods and new techniques including OCuSOFT® Lid Scrub® PLUS, BlephEx™ and warm compress treatment on Demodex folliculorum blepharitis and meibomian gland dysfunction.**

You are being asked to consent to taking part in a post-graduate student clinical trial, comparing traditional treatment methods for blepharitis with newer techniques.

Each participant will be asked complete a dry eye symptom questionnaire and will undergo a series of dry eye tests and thorough examination of the front surface of the eye. Each visit should take approximately 30 minutes.

One eyelash from each eyelid will be epilated using sterile forceps to confirm presence or absence of Demodex. This procedure will be done using sterile forceps and is generally painless. It is common for eye lashes to fall out and re-grow.

You may be allocated any treatment. Treatment will be administered for home use for up to 8 weeks. You will be asked to return to the clinic for mid-treatment and post-treatment check-ups.

1. **Dry Eye: Blepharitis and *Demodex***

Blepharitis is a common condition of the eyelids where debris (crusts/scales similar to dandruff) can build up around the base of the eyelash. It may be present with a common mite which is found in skin and hair follicles called *Demodex*. This may result in symptoms of itching and irritation around the eyelid margins.

Traditional treatment includes cleaning the eyelids with a diluted shampoo. New treatment includes OCuSOFT® Lid Scrub® PLUS and tea tree face wash, both of which containing antibacterial ingredients that has been shown to be effective against bacteria commonly found on the eyelid.

BlephEx™ is a handheld device with a spinning micro-sponge that is used to remove scruff and debris by exfoliating along the base of the lashes. (BlephEx™ will only be administered in the clinic).

2. **Dry Eye: Meibomian Gland Dysfunction (MGD)**

MGD is a common condition which affects the glands around the eyelid margins. These glands become blocked and cannot release oily secretions into the tears sufficiently. This results in a reduced quality tear film which can result in gritty/dry eyes and blurred vision. Traditional treatment includes applying heat compresses with a warm face cloth and

massage to try to unblock the glands. Newer treatment includes wearing microwaveable heat masks, MGDRx EyeBag® and OPTASE™ Moist Heat Mask, which apply dry and moist heat respectively to the glands over a longer time period.

*A patch test will be carried out before proceeding with lid scrubs to ensure you have no adverse reactions to the treatment. This will involve applying treatment to a small area near the eyebrow with samples of each treatment. After 24hours if you have had no reaction you may proceed with the treatment as instructed. In the rare event that you may experience an adverse reaction; e.g. redness, itching, irritation, rash etc… - use cool compresses to help soothe and do not proceed with treatment. We ask that you contact us to inform us if this occurs.*

CONSENT FORM

| **Researcher's Name**: ORLA MURPHY | **Title:** MS |
|---|---|
| **Faculty/School/Department**:<br>SCIENCE/PHYSICS/OPTOMETRY | |
| **Title of Study**: COMPARISON OF TRADITIONAL TREATMENT METHODS AND NEW TECHNIQUES OCuSOFT® PLUS, BlephEx™ AND WARM COMPRESS THERAPY ON DEMODEX FOLLICULORUM BLEPHARITS AND MEIBOMIAN GLAND DYSFUNCTION. | |
| **To be completed by the: PATIENT** | |

3.1 Have you been fully informed/read the information sheet about this study? YES/NO

3.2 Have you had an opportunity to ask questions and discuss this study? YES/NO

3.3. Have you received satisfactory answers to all your questions? YES/NO

3.4 Have you received enough information about this study and any associated health and safety implications if applicable? YES/NO

3.5 Do you understand that you are free to withdraw from this study?

- at any time
- without giving a reason for withdrawing
- without affecting your future relationship with the Institute YES/NO

3.6 Do you agree to take part in this study the results of which are likely to be published? YES/NO

3.7 Have you been informed that this consent form shall be kept in the confidence of the researcher? YES/NO

Signed_____ Date _____

Name in Block Letters _____

Signature of Researcher _____ Date _____

## Appendix 2: Ethics Approval Letter

Institiúid Teicneolaíochta Átha Cliath, Sráid Caoimhín, Baile Átha Cliath 8, Éire
Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland
t www.dit.ie/graduateresearchschool

SCOIL TAIGHDE IARCHÉIME / GRADUATE RESEARCH SCHOOL
Professor Mary McNamara

15th September 2014

**Ms Aoife Lloyd**

**Re: Ethical Clearance Ref 14-45**

Dear Aoife

I am pleased to inform you that the following project:

***Comparison of traditional treatment methods and new techniques including OCuSOFT® PLUS and Tranquileyes on Demodex folliculitis and Meibomian gland dysfunction (MGD).***

Which you submitted to the Research Ethical Committee has been approved. The committee would like to wish you very best of luck with the rest of research project. If you have any further queries, please do not hesitate to contact me on (01) 402 7920 or at conor.mccague@dit.ie.

Yours sincerely

**Conor McCague**
**Research Graduate School**

**Appendix 3: General Health and Lifestyle Questionnaire**

Name: _____ Reference: _____

DOB: _____ Age: _____ Sex:_____

The following questions relate to your general health and lifestyle. Please tick the appropriate box:

1. Do you wear contact lenses?

   Dailies [] Two weekly [] Monthly [] Extended wear []

   I don't wear contact lenses []

2. Do you wear make – up? Yes [] No []
   a. What type of mascara do you use? Regular [] Waterproof [] None []
   b. What type of eyeliner do you use? Liquid [] Pencil [] None []

3. How often do you clean your eyelids/lashes?

   Every night [] 3-4 times/week [] 1-2times/week [] <once a week []

   Never []

4. What type of lid hygiene regime do you mainly use?

   Cleanser/Toner [] Eye makeup remover [] Face wipes []

   J+J Lid scrubs [] Other lid scrubs [] Other [] None []

   If other, please specify: _____

5. How often do you currently wash your pillowcase/bed linen?

   > Once a week [] Once a week [] Once a fortnight []

   Once a month [] < Once a month []

6. At what temperature do you currently wash your pillowcase/bed linen?

   ≤30°C []  40°C []  ≥55 °C []

7. How do you currently dry your bed linen?

   Air dry []  Tumble dry []  Launderette []  Dry Cleaner []

8. Do you suffer with any underlying medical conditions?

   Diabetes []  High Blood Pressure []  Thyroid []  Arthritis []  Other []

   None []

   If other, please specify: _____

9. Are you taking any systemic medications?  Yes []  No []

   If Yes, please specify: _____

10. Do you suffer with any allergies?

    Seasonal (e.g. hay fever) []  Asthma []  Dust []  Skin sensitivities []  None []

11. Do you currently suffer with any of the following skin conditions?

    Rosacea []  Dermatitis []  Eczema []  Acne []  Sensitivity skin []

    Psoriasis []  None []

If you do not understand any of the above questions, please ask the Optometrist to help clarify.

**Appendix 4: Modified OSDI symptom questionnaire**

Answer the questions below in relation to dry eye symptoms. Please circle/tick appropriately.

Please use this frequency list as a reference guide.

4: All of the time: All day, every day.
3: Most of the time: At least once a day, every day.
2: Half the time: e.g. At least once every second day
1: Some of the time: e.g. at least once every 2-3days
0: Rare/Not at all: Very rarely affected, practically not at all, never.

1. In the past **fortnight** have you experienced any of the following? Please circle the most appropriate:

| | All of the time | Most of the time | Half of the time | Some of the time | Rare/Not at all |
|---|---|---|---|---|---|
| Dry eyes? | 4 | 3 | 2 | 1 | 0 |
| Gritty/Irritated eyes? | 4 | 3 | 2 | 1 | 0 |
| Itchy eyes? | 4 | 3 | 2 | 1 | 0 |
| Red eyes? | 4 | 3 | 2 | 1 | 0 |
| Burning sensation? | 4 | 3 | 2 | 1 | 0 |
| Sensitivity to light? | 4 | 3 | 2 | 1 | 0 |
| Watery eyes? | 4 | 3 | 2 | 1 | 0 |
| Eyelids stuck together in the mornings? | 4 | 3 | 2 | 1 | 0 |

2. With regards to the symptoms above, what time of the day are your symptoms worst?

   Tick all that apply.

   I don't have any symptoms []  In morning on waking []  In the afternoon []

   At night []  All day long []

3. In the past **fortnight,** have problems with your eyes limited your ability in performing any of the following? Please circle the most appropriate:

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time |
|---|---|---|---|---|---|
| Reading? | 4 | 3 | 2 | 1 | 0 |
| Driving at night? | 4 | 3 | 2 | 1 | 0 |
| Using a computer? | 4 | 3 | 2 | 1 | 0 |
| Watching television? | 4 | 3 | 2 | 1 | 0 |

4. In the past **fortnight,** have your eyes felt uncomfortable in any of the following situations? Please circle the most appropriate:

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time |
|---|---|---|---|---|---|
| Windy Conditions | 4 | 3 | 2 | 1 | 0 |
| Cold Conditions | 4 | 3 | 2 | 1 | 0 |
| Air-conditioned environments | 4 | 3 | 2 | 1 | 0 |

## Appendix 5: Instruction Leaflet for 10% solution of baby shampoo

### (a) Pilot Study

Blepharitis is a common condition of the eyelids which can result in symptoms of eye irritation (itchy, gritty, stinging eyes), intermittent blurred vision or you may have no symptoms in the early stages. Demodex is a mite which commonly lives in the base of the eyelashes and skin follicles, it becomes increasingly prevalent as we get older and can sometimes cause irritation around the eyes.

You have been recommended the following treatment by your optometrist in order to alleviate any symptoms or signs of this condition. Your progress will be monitored at a check-up in two weeks' time.

Ensure you wash and dry your hands thoroughly before beginning.

Right / Left Eye:

A. **Traditional method: Diluted solution of Johnson's and Johnson's Baby Shampoo**

1. Fill the vial to the point shown (see arrow) with boiled water that has cooled.
2. Shake well to thoroughly mix the solution.
3. Pour the solution onto one of the cotton pads provided.
4. Squeeze out excess liquid from the cotton bud to prevent drips getting into your eyes, which may irritate.
5. Gently clean down across the eye in circular movements. Try to ensure you rub along the base of the lashes.

6. With eyes still closed use side – to – side strokes to gently scrub the eyelid. Again, paying particular attention, try to clean off any crusts at the base of the eyelids.
7. Open the eyes, wrap the cotton pad around your index finger, as instructed, look up and still using side to side strokes at the base of the bottom eyelashes make sure all crusts have been removed and the lid is fully clean.
8. For the top lashes use side to side strokes in an upward movement as instructed to ensure lashes are completely clean. Be careful not to scratch the ocular surface.
9. After cleaning the eyelids, rinse off the shampoo from the eyelids, using a new clean cotton wool pad.
10. Please do not dispose of vials. Keep and return them to the National Optometry Centre on your aftercare visit.

(b**) Phase Four**

Blepharitis is a common condition of the eyelids which can result in symptoms of eye irritation (itchy, gritty, stinging eyes), intermittent blurred vision or you may have no symptoms in the early stages. *Demodex* is a mite which commonly lives in the base of the eyelashes and skin follicles, it becomes increasingly prevalent as we get older and can sometimes cause irritation around the eyes.

Ensure you wash and dry your hands thoroughly before beginning.

**Johnson's and Johnson's Baby Shampoo:**

1. Using the syringe provided, insert 2ml of baby shampoo into the test vial provided.
2. Fill the vial with boiled water that has cooled.
3. Shake well to thoroughly mix the solution.
4. Pour half the solution onto one of the cotton pads provided.
5. Squeeze out excess liquid from the cotton bud to prevent drips getting into your eyes, which may irritate.
6. Gently clean down across the eye in circular movements. Try to ensure you rub along the base of the lashes.
7. With eyes still closed use side – to – side strokes to gently scrub the eyelid. Again, paying particular attention, try to clean off any crusts at the base of the eyelids.
8. Open the eyes, wrap the cotton pad around your index finger, as instructed, look up and still using side to side strokes at the base of the bottom eyelashes make sure all crusts have been removed and the lid is fully clean.
9. For the top lashes, look down, and gently pull up on the upper lid. Using side to side strokes in an upward movement as instructed to ensure lashes are completely clean. Be careful not to scratch the ocular surface.
10. For the lower lid, look up, and still using side to side strokes at the base of the bottom eyelashes make sure all crusts have been removed and the lid is fully clean.
11. After cleaning the eyelids, rinse off the shampoo from the eyelids, using a new clean cotton wool pad.
12. Repeat from 4-11 for the other eye.

Repeat this routine at night for two weeks and then return to the National Optometry Centre for a follow-up appointment.

If you have any questions or need to re-arrange please contact:
Orla Murphy
E-mail: orla.murphy@dit.ie
*Please note: Should you notice any discomfort or irritation of the eyes or skin around the eyes please cease treatment and contact your Orla in the National Optometry Centre.*

## Appendix 6: Warm Compress Instructions

### (a) Warm Face Cloth

# Warm Face Cloth Compress

**Read the instructions carefully before using the Warm Face Cloth Compress and keep them for reference. Always follow these instructions**

1. Bring the kettle to the boil.
2. Pour boiling water into a bowl. Allow to cool for approximately 10 mins.
3. Place flannel in the water.
4. Remove flannel and squeeze excess water out. Ensure it is not too hot.
5. Laying in a comfortable position place the warm flannel over the eyes.
6. Keep in place for 10 minutes. Re-dip every two mins to keep flannel as hot as possible (re-dip at 2 min, 4 min, 6 min, and 8 min).
7. Immediately after warming, massage the closed eyelids – upper and lower, to express oil from the Meibomian glands. Gently but firmly sweep your clean finger, in circular movements, over the skin at the edge of the closed eyelid from the nose outwards. Repeat this several times for about 30 seconds.
8. Clean eyes of any loose debris from the eyelids.

**Weeks 1+2: Twice a day**

**Weeks 3-8: Once a day**

**(c) MGDRx EyeBag®**

**Read instructions carefully before use and keep them for reference**

1. Remove the EyeBag® from packaging and place on a clean, microwaveable plate. Do not use the metal griddle supplied with some microwaves as this may burn your EyeBag®
2. Place in microwave and heat on full power according to the table below

Caution: If your microwave is greater than 1000w, you may need to reduce the time.

| Power | Heating Duration |
|---|---|
| Above 750W | 30 seconds |
| 750W and below | 40 seconds |

3. Check the EyeBag® is comfortably warm but not too hot before placing it over the closed eyelids and relaxing with the EyeBag® in place for 10 minutes.
4. Use the silk side as this is warmer and will stay warmer for the duration of the therapy.
5. Immediately after warming, massage the closed eyelids – upper and lower, to express oil from the Meibomian glands. Gently but firmly sweep your clean finger over the skin at the edge of the closed eyelid from the nose outwards. Repeat this several times for about 30 seconds.
6. Clean the eyelids of any loose debris.

**Weeks 1+2: Twice a day**

**Weeks 3-8: Once a day**

**(c) OPTASE™ Moist Heat Mask**

**Read the instructions carefully before using the OPTASE™ Moist Heat Mask and keep them for reference. Always follow these instructions**

1. Remove OPTASE™ Moist Heat Mask from all packaging and place on a clean, microwaveable plate. Do not use the metal griddle supplied with some microwaves as this may burn and damage the heat mask.
2. Place in microwave and heat on full power according the table below.

| Power | Heating Duration |
|---|---|
| 900W and above | 15 seconds |
| 800W | 25 seconds |

**Do not exceed a maximum of 30 seconds of heating**

3. Check the heat mask is comfortably warm but not too hot before placing it over closed eyelids.
4. Relax and keep the heat mask in place for 10 minutes.
5. Immediately after warming, massage the closed eyelids – upper and lower, to express oil from the Meibomian glands. Gently but firmly sweep your clean finger in circular movements, over the skin at the edge of the closed eyelid from the nose outwards. Repeat this several times for about 30 seconds.
6. Clean the eyelids of any loose debris.

**Weeks 1+2: Twice a day**

**Weeks 3-8: Once a day**

**LIST OF PUBLICATIONS**

1. Murphy O, O'Dwyer V, Lloyd-McKernan A. Ocular *Demodex folliculorum* : prevalence and associated symptoms in an Irish population. *Int Ophthalmol* 2018:1–13. doi:10.1007/s10792-018-0826-1.

2. Murphy O, O'Dwyer V, Lloyd-McKernan A. The efficacy of tea tree face wash, 1, 2-Octanediol and microblepharoexfoliation in treating Demodex folliculorum blepharitis. *Contact Lens Anterior Eye* 2018; 41(1).77-82. doi:10.1016/j.clae.2017.10.012.

3. Murphy O, O' Dwyer V, Lloyd-McKernan A. The effect of lid hygiene on the tear film and ocular surface, and the prevalence of *Demodex* blepharitis in university students. *Contact Lens & Anterior Eye* 2019; [Epub ahead of print]. doi: 10.1016/j.clae.2019.09.003

4. Murphy O, O' Dwyer V, Lloyd-McKernan A. The efficacy of warm compresses in the treatment of meibomian gland dysfunction and *Demodex folliculorum* blepharitis, *Current Eye Research*. Accepted for publication.

5. Murphy O, O' Dwyer V, Lloyd-McKernan A. The clinical use of eyelash manipulation in the diagnosis of *Demodex folliculorum* blepharitis. *Eye Contact Lens* 2019;In Press.