DOCTOR OF PHILOSOPHY

Management of allergic conjunctivitis and dry eye

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MANAGEMENT OF ALLERGIC CONJUNCTIVITIS AND DRY EYE

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Thesis Summary

Ocular allergy is a common eye condition encountered in clinical practice. However, little is known how seasonal allergic conjunctivitis (SAC), the most common subtype, is managed in clinical practice. Further, dry eye, another common eye condition, may be misdiagnosed as SAC and vice-versa as they share similar signs and symptoms. In addition, despite the frequent recommendation of non-pharmacological treatments for SAC, evidence to support their use has not been identified in the scientific literature. The aim of this thesis was therefore to determine the actual diagnosis and management of SAC and dry eye in clinical practice and investigate the efficacy of non-pharmacological treatments for these conditions.

The diagnostic and management strategies for SAC and dry eye employed by pharmacy staff are found to be inconsistent with current guidelines and scientific evidence based upon a mystery shopper design. Cluster analysis of tear film metrics in normal and dry eye patients identified several clinically relevant groups of patients that may allow for targeted treatment recommendations. Using a novel environmental chamber model of SAC, the use of artificial tears and cold compresses, either alone or combined is an effective treatment modality for acute and symptomatic SAC, on a par with topical anti-allergic medication, and has been demonstrated for the first time. In addition, eyelid warming therapy with a simple, readily available, seed filled device is an effective method of treating meibomian gland dysfunction (MGD) related evaporative dry eye, perhaps the most common dry eye subtype.

A greater focus on ophthalmology must be implemented as part of the formal education and training of pharmacy staff, while greater professional communication between community pharmacists, optometrists and the population they serve is required. Artificial tears and cold compresses may be considered as front line agents for acute SAC by pharmacy staff and optometrists, to whom pharmacological treatment options are limited.

Keywords: Meibomian Gland Dysfunction
Eyelid Warming Therapy
Cluster Analysis
Tear Film
Ocular Allergy Model

Dedication

For my dear and loving parents – Dad Tirlochan and Mum Susheel – thank you for being there for me over the past three years. I am truly grateful for your help, support, and love; I am forever in your debt.

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List of Abbreviations

AAO: American Academy of Ophthalmology

AC: Allergic Conjunctivitis

AKC: Atopic Keratoconjunctivitis

ANOVA: Analysis Of Variance

AT: Artificial Tears

CAC: Conjunctival Allergen Challenge

CC: Cold Compress

CCT: Conjunctival Challenge Test

CL: Contact Lens

CLGPC: Contact Lens related Giant Papillary Conjunctivitis

DEQ: Dry Eye Questionnaire

EEU: Environmental Exposure Unit

ELISA: Enzyme Linked Immunosorbent Assay

ELL: External Lower Eyelid

EQ-5D: EuroQol-5 Dimension

EUL: External Upper Eyelid

FBUT: Fluorescein Break-Up Time

GP: General Practitioner

GPC: Giant Papillary Conjunctivitis

HEDQ: Health Economic and Demographic Questionnaire

ILL: Internal Lower Eyelid

IUL: Internal Upper Eyelid

LFU: Lacrimal Functional Unit

MAP: Mitogen Activated Protein

MGD: Meibomian Gland Dysfunction

NEI: National eye Institute

NFkB: Nuclear Factor kappa-light-chain-enhancer of activated B cells

NITBUT: Non-Invasive Tear film Break-Up Time

NITMH: Non-Invasive Tear film Meniscus Height

NSAID: Non-Steroidal Anti-inflammatory Drug

NT: No Treatment

OSDI: Ocular Surface Disease Index

PAC: Perennial Allergic Conjunctivitis

POM: Prescription Only Medicine

PPP: Preferred Practice Pattern

PRT: Phenol Red-thread Test

RAST: Radioallergosorbent Test

RGP: Rigid Gas Permeable

RQLQ: Rhinoconjunctivitis Quality of Life Questionnaire

SAC: Seasonal Allergic Conjunctivitis

SPT: Skin Prick Test

TBUT: Tear film Break-Up Time

UK: United Kingdom

VFQ-25: Visual Functioning Questionnaire-25

VKC: Vernal Keratoconjunctivitis

VCC: Vienna Challenge Chamber

Chapter 1

Literature Review

1.1 Allergic Eye Disease

Allergic eye disease is the ocular manifestation of allergy, where the immune system produces an over-reaction or hypersensitivity to normally harmless substances known as allergens. The prevalence of allergy in Europe is between 15 and 20% (Williams *et al*, 2008) and is expected to increase with estimates as high as 50% by 2015 (Savage & Roy, 2005; Asher et al, 2006). Although genetics plays an important role in susceptibility, the increase in prevalence is suggested to be the result of improved hygiene practices and increased antibiotic use as part of modern lifestyle and healthcare (Cohet *et al*, 2004; Romagnani *et al*, 2004; Bresciani *et al*, 2005), in addition to environmental factors such as increased air pollution, climate change and increased planting and importation of allergenic plant species (Linneberg *et al*, 2000; Alcazar *et al*, 2004; D'Amato *et al*, 2007).

Of those who suffer from allergy, approximately 15-20% experiences a form of ocular allergy (Abelson *et al*, 2002a; Leonardi, 2005a). The prevalence of ocular allergy in patients attending optometric practice in the UK has been estimated at 8% (Wolffsohn *et al*, 2011). However, several authors have pointed out that ocular allergies may be under-diagnosed and under-treated, particularly seasonal allergic conjunctivitis (SAC) where the ocular symptoms may fall under the umbrella of seasonal hay fever which may underestimate its true prevalence (Cox, 2007; Bielory, 2008a; Berdy & Berdy, 2009). A recent study by Wolffsohn et al (2011) highlights the current poor management of ocular allergies in the UK, where patients often self-medicate and rarely undergo an ophthalmic examination (Wolffsohn *et al*, 2011). Ocular allergy encompasses a group of distinct clinical entities, typically confined to the conjunctiva, and includes allergic conjunctivitis (AC) which is subdivided into seasonal (SAC) and perennial forms (PAC), vernal keratoconjunctivitis

(VKC), atopic keratoconjunctivitis (AKC) and giant papillary conjunctivitis (GPC) (Abelson & Schaefer, 1993; Friedlaender, 1993; Bielory, 2000). Both VKC and AKC are not normally treated in primary optometric practice owing to their sight threatening potential.

Although not fully understood, VKC is considered a non-classic type I IgE mediated, Th2-lymphocyte driven allergic disorder involving mast cells and eosinophils (Bonini et al, 2004; Chigbu, 2009a; Kumar, 2009). An increased presence of sex hormone receptors in the conjunctiva of VKC patients and involvement of neural factors suggest that the pathogenesis of VKC is complex and multi-factorial in origin (Bonini et al, 2004; Kumar, 2009). VKC is an uncommon condition that mainly affects young males (male to female ratio 2:1 to 4:1) in hot and dry countries occurring on a seasonal (spring) basis although it may persist year round (23% of cases) (Kumar, 2009). Significant proportions recur during the winter (60% of cases) and 16% of seasonal cases develop in to perennial disease (Kumar, 2009). The exact prevalence is unknown. VKC usually occurs before 10 years of age, but resolves 4-10 years after initial onset (Bonini et al, 2000). Predisposing factors include a history of atopy (Bonini *et al*, 2004; Kumar, 2009). VKC is often bilateral (98% of cases) and signs include giant papillae on the palpebral conjunctiva (>1mm, with cobblestone appearance) or at the limbus, Tranta's dots (accumulation of eosinophils and epithelial cells), diffuse conjunctival hyperaemia, chemosis, and thick, white, stringy mucous discharge (Leonardi, 2002; Kumar, 2009). Frequently the cornea is involved due to the close apposition of the palpebral conjunctiva thus VKC has sight threatening potential. Corneal signs include superficial punctuate keratopathy, macro-erosion, shield ulcer, plaque formations and neovascularisation (Bonini *et al*, 2004). Occasionally conjunctival fibrosis and symblepharon may develop in severe cases (Bonin et al, 2004). Symptoms include intense itching, burning, watering, blurred vision, and difficulty opening eyes upon waking as eyelashes are matted together and photophobia (Bonini *et al*, 2004; Kumar, 2009). Complications of VKC include irregular astigmatism, keratoconus, hydrops, limbal hyperplasia (Tabarra, 1999). Cataract

and glaucoma may also occur following long term topical steroid therapy (Tabarra, 1999; Bonini *et al*, 2004).

Given the sight threatening potential of VKC, optometric treatment is limited to advising the use of artificial tears and cold compresses to bring about symptomatic relief and use of sunglasses to minimise photophobia and protect the eyes from exposure to sun, wind and dust (Bonini *et al*, 2004; Chigbu, 2009b; Kumar, 2009). The presence of limbal or corneal disease necessitates urgent referral to an ophthalmologist otherwise patients with suspected VKC should be referred routinely. The mast cell stabilisers sodium cromoglycate and lodoxamide, have been shown to be effective for the treatment of VKC and are considered first line ophthalmological therapy as they can be used long term and have excellent safety profiles (Mantelli *et al*, 2007). Mucolytics such as acetylcysteine is useful in breaking down the mucous discharge (Kumar, 2009). Bandage contact lenses are indicated for corneal ulcers to aid re-epithelialisation – preservative free topical anti-allergic medications, prophylactic antibiotics and ocular lubricants are necessary in these cases (Chigbu, 2009b).

In severe cases topical steroid therapy is required but patients must be closely monitored due the potential risk of steroid induced cataract and glaucoma (Tabarra, 1999; Kumar, 2009). Alternative medications in steroid responders that have proved effective include immunomodulators such as cyclosporine and non-steroidal anti-inflammatory drugs such as ketorolac tromethamine, with the former helpful in re-epithelialisation of the cornea (Pucci *et al*, 2002; Mantelli *et al*, 2007). Surgical intervention is necessary where papillae are unresponsive to topical therapy and causing corneal ulcers or plaque formation (Bonini *et al*, 2004; Kumar, 2009). Papillae may be removed using CO_2 laser or cryotherapy (Leonardi, 2002; Kumar, 2009).

The cause of atopic keratoconjunctivitis (AKC) is not well understood. However, histopathogical and laboratory findings in AKC patients suggest that the pathogenesis of AKC is a complex non-classic type I IgE and eosinophil mediated allergic reaction involving

mast cells and Th2-lymphocytes (Bonini, 2004; Chigbu, 2009a; Bielory & Bielory, 2010). AKC may follow from childhood VKC (Chigbu, 2009a). Although uncommon, the exact prevalence is unknown, but one study estimated that 4.4% of over 1000 patients with ocular allergy had AKC (Uchio et al, 2008). AKC is considered to be the ocular component of atopic dermatitis (AD), with 20-40% of AD patients suffering from AKC (Bielory & Bielory, 2010). AKC is more common in males and often occurs between the ages of 18 to 40 years (Bonini, 2004). Predisposing factors include a history of atopy, atopic dermatitis and VKC in childhood (Bonini, 2004; Bielory & Bielory, 2010).

AKC is a chronic, potentially blinding, bilateral condition. Signs include giant papillary hypertrophy and scarring of the palpebral conjunctiva (typically superior), chemosis and diffuse conjunctival hyperaemia (Tuft *et al*, 1991; Bonini, 2004). Limbal papillae may also occur and the eyelids of AKC patients are often thickened, erythmatous, fissured and crusting, and blepharitis (chronic staphylococcal) may be present (Tuft *et al*, 1991; Bonini, 2004). Corneal involvement includes superficial punctuate keratopathy, ulcer and plaque formation and as the condition progresses, corneal scarring and neovascularisation may develop (Tuft *et al*, 1991, Bonini, 2004). In addition the cornea may become thinned and atopic cataract may occur (Foster & Calonge, 1990; Tuft *et al*, 1991). Symptoms include intense itching, burning, watering and photophobia (Chigbu, 2009b, Tuft *et al*, 1991; Bonini, 2004).

Treatment of AKC is similar to VKC. Long term therapy with topical mast cell stabilisers such as sodium cromoglycate, lodoxamide and nedocromil is required to treat the allergic inflammation in mild cases (Power *et al*, 1998; Chigbu, 2009b; Bonini, 2004). Cold compresses and artificial tears may help relieve the intense symptoms between doses, although evidence is lacking (Chigbu, 2009b). In addition, systemic antihistamines may also be used particularly if other allergies are present (Foster & Calonge, 1990). Any blepharitis should be treated with regular eyelid hygiene measures to remove bacteria and deposits from eyelids; and warm compresses to express the meibomian glands and help remove

collarettes and crusting (Jackson, 2008). Topical antibiotic ointment such as chloramphenicol is required if infection is present, but referral for long term systemic tetracyclines may be required if conventional treatment is ineffective (Jackson, 2008). However, corneal or limbal disease is often present which requires urgent ophthalmological referral owing to sight threatening potential. As with VKC, bandage contact lenses, ocular lubricants and prophylactic antibiotics are required for corneal ulcers (Lemp, 2008a). Most cases of AKC require additional topical steroid therapy to relieve inflammation and symptoms – patients are closely monitored owing to the risk of steroid induced cataract and glaucoma (Foster & Calonge, 1990; Bonini, 2004; Bielory & Bielory, 2010). However, the immunomodulator cyclosporine may be used as an effective anti-inflammatory alternative in steroid responders (Daniell *et al*, 2006; Guglielmetti *et al*, 2010). Thus, both VKC and AKC are likely to be managed in the hospital eye service under close supervision due to frequent topical steroid therapy, sight threatening potential and concomitant atopic disease in AKC (Bielory, 2000; Bonini et al, 2004; Chigbu, 2009b; Kumar, 2009).

Giant papillary conjunctivitis (GPC) is caused by a complex series of immunological inflammatory events and mechanical trauma to the palpebral conjunctiva typically by contact lenses (Donshik & Ehlers, 1997; Donshik *et al*, 2008). Ocular prostheses, extruding sclera buckles, exposed sutures and corneal deposits may also cause mechanical trauma, but are uncommon (Donshik & Ehlers, 1997; Donshik *et al*, 2008). Although not fully understood, inflammation is reported to be caused by type I hypersensitivity (with contact lens protein deposits and bacterial cell wall components as potential antigens), type IV delayed hypersensitivity mediated by T-cells which increases the inflammatory response, and mechanical trauma which releases chemotactic factors attracting neutrophils to the site of inflammation (Chigbu, 2009a; Donshik *et al*, 2008). The prevalence of GPC is reported to be 1-5% in contact lens wearers, but is reported to be as high as 20% (Donshik, 1994). It is more common in soft compared to rigid gas permeable (RGP) lenses (85% versus 15%) (Donshik & Ehlers, 1997; Donshik, 2003; Donshik *et al*, 2008). Risk factors include history of

atopy, meibomian gland dysfunction, and the presence of contact lens deposits, poor lens fitting and design (Donshik *et al*, 2008). GPC is often bilateral and is characterised by the presence of macropapillae (0.3-1mm in diameter) or giant papillae (>1mm) and hyperaemia on the superior palpebral conjunctiva (Donshik & Ehlers, 1997; Donshik *et al*, 2008). Other signs include increased mucous production and conjunctival oedema (Donshik & Ehlers, 1997; Donshik *et al*, 2008). Symptoms include burning, irritation and itching, of which the latter may increase on lens removal following manipulation of the eyelids causing mechanical stimulation of mast cells (Donshik, 1994; Donshik & Ehlers, 1997; Donshik *et al*, 2008). With a contact lens in situ, there may be increased lens movement, and reduced comfort, vision and lens tolerance (Donshik, 2003; Donshik *et al*, 2008).

Removal of the source of mechanical trauma and ceasing contact lens wear often brings about resolution within 4 weeks in moderate cases, but severe cases may take longer (Donshik *et al*, 2008). Patients should also be advised to avoid eye rubbing. In contact lens related GPC (CLGPC), patients may continue to wear contact lenses following resolution if signs and symptoms allow and the cornea is not compromised (Donshik, 1994; Donshik *et al*, 2008). Indeed, refitting after a period of lens wear cessation rather than during an episode of CLGPC is more successful in preventing recurrence (94% versus 78%) (Donshik, 1994). Refitting with new lenses of the same material has been found to reduce the recurrence of CLGPC by 61-66% but the refitting with a lens of different material or increasing the replacement frequency reduces the recurrence by 77-95% (Donshik, 1994, 2003; Porazinski & Ehlers, 1999). Furthermore, changing to an RGP lens reduces recurrence by 80% - the smaller diameter reduces the area in contact with the palpebral conjunctiva and is easier to clean (Donshik, 1994). It is essential to instruct a careful rub and rinse lens cleaning regime to minimise the build up of surface deposits and in some cases enzymatic treatment may be required (Donshik, 1994; Donshik *et al*, 2008). Topical anti-allergic medication is not usually necessary but mast cell stabilisers such as sodium cromoglycate is effective in moderate to severe CLGPC (Kruger *et al*, 1992; Donshik *et al*,

2008). Approximately 75% of patients with moderate to severe CLGPC treated with sodium cromoglycate 2% or 4% (after a period of cessation) may continue contact lens wear (Lustine *et al*, 1991; Kruger *et al*, 1992). Referral to an ophthalmologist is necessary in cases resistant to conventional treatment for initiating steroid therapy or where surgery is required to remove the source of mechanical trauma (Donshik *et al*, 2008). Steroid therapy is also indicated where therapeutic (bandage) contact lenses are required for corneal complications, such as ulcer formation, but these are uncommon in GPC (Chibu, 2009b). The multi action anti-allergic drug olopatadine may be used as an alternative to steroid therapy with recent studies highlighting comparable efficacy compared to the topical steroid fluorometholone (Khurana *et al*, 2010).

Other allergic eye conditions include contact allergy to topical medication (medicamentosa) such as anti-glaucoma agents (Wilson, 1979; Schuman, 2000) and microbial allergy such as phlyctenulosis caused by hypersensitivity to tuberculoprotein (Helm & Holland, 1993).

1.2 Seasonal and Perennial Allergic Conjunctivitis

AC is a classic type I, IgE-mast cell mediated hypersensitivity disorder and the most common form of ocular allergy. The prevalence of AC is estimated to range from 15-20%, although recent studies suggest it may be as high as 40% (Rosario & Bielory, 2011). However, SAC is far more common than PAC, making up 90% of all ocular allergic cases (Chigbu, 2009b). SAC is frequently associated with allergic rhinitis as part of hay fever (seasonal rhinoconjunctivitis) and is more common in children (Rosario & Bielory, 2011). Predisposing risk factors include a history of atopy including eczema, asthma, and rhinitis (Bielory, 2008a; Leonardi *et al*, 2008; Rosario & Bielory, 2011).

SAC occurs on a seasonal basis, often as part of seasonal rhinoconjunctivitis (hay fever) and is most frequently caused by grass, tree and weed pollens and outdoor moulds which peak at different times of the year (Bielory, 2008a; Chigbu, 2009a). PAC occurs year round

and is caused by house dust mites, animal dander, insects and indoor moulds (Chigbu, 2009a). Signs and symptoms of SAC typically develop on a gradual basis but can also develop suddenly following contact with the offending allergen (Bielory, 2000; Bielory, 2008a). They are often bilateral and include itching, tearing, eyelid oedema and conjunctival hyperaemia, chemosis and papillary reaction, with the severity often varying with pollen counts (Cox, 2007). Signs and symptoms of PAC are similar to SAC but are milder and chronic in nature and may have occasional seasonal exacerbations (Buckley, 1998; Bielory, 2008a).

Although the signs of symptoms of SAC and PAC are relatively mild, the impact of allergic eye disease on the quality of life can be profound (Bielory, 2006), affecting daily activities, productivity at work, school performance and even on an economic level (Pitt *et al*, 2004; Smith *et al*, 2005; Palmares *et al*, 2010). Comparing between confirmed SAC patients and age and sex matched healthy controls from 4 private and 1 public hospitals, Smith *et al* (2005) found that SAC patients experienced reduced quality of life, general health and increased visual symptoms (assessed using EQ-5D Health Questionnaire, National Eye Institute Visual Functioning Questionnaire (VFQ-25), the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) and Health Economic and Demographic Questionnaire (HEDQ)) compared to age and sex matched controls in Spain, with a mean cost per patient of 348.50 Euros (Smith *et al*, 2005). In the UK, Pitt *et al* (2004) found that SAC patients experienced statistically significantly greater level of ocular pain and discomfort using the EQ-5D, worse scores in all domains using the VFQ-25 except for general and colour vision, and worse RQLQ scores compared to age matched healthy controls (Pitt *et al*, 2004). The mean cost per patient, including both private and public healthcare costs, was calculated at £64.61 for a pensioner and £123.69 for those in paid employment (Pitt *et al*, 2004). However, the SAC patients included in this study were those with self-reported symptoms of itching, red and watery eyes each year and who considered these to be caused by seasonal allergens, rather than by clinical diagnosis. More recently, a cross-sectional study of 220 patients with a

diagnosis of allergic conjunctivitis (both SAC and PAC) from 16 ophthalmology departments in Portugal was found to have significantly reduced quality of life during acute episodes (Palmares *et al*, 2010).

Complications of SAC are limited to severity of presentation and linked to steroid use in cases refractory to conventional treatment (Hingorani & Lightman, 1995; Joss & Craig, 1998; Bielory *et al*, 2010). Currently there is no cure for SAC or indeed for any allergy, so treatment is aimed at preventing and alleviating symptoms, but immunotherapy shows promise (Bielory, 2008b).

1.3 Pathophysiology of SAC and PAC

Both SAC and PAC are IgE-mast cell mediated hypersensitivity reactions, divided into two phases with the mast cell playing a central role (Bacon *et al*, 2000; Choi & Bielory 2008). The reaction involves a very complex series of immunological events coordinated by various mediators initiated by an allergen (Ono & Abelson, 2005; Hodges & Kean-Myers, 2007). An allergen such as pollen reacts with specific IgE antibodies bound to a sensitised mast cell, triggering cross linkage of the IgE molecules and an influx of calcium ions into the mast cell. This causes the mast cell to degranulate and release preformed inflammatory mediators such as histamine which cause the signs and symptoms associated with the early phase response in sensitised individuals (Bielory, 2000; Ono & Abelson, 2005). The early phase response is immediate and lasts clinically for 20-30 minutes (Leonardi *et al*, 2008).

Mast cell degranulaion also initiates a series of cellular and extracellular events which lead to the late phase response, including production of prostaglandins, thromboxanes and leukotrienes derived from arachidonic acid (Ono & Abelson, 2005; Chigbu, 2009a). Mast cells also release cytokines and chemotactic factors which induce the production of IgE form B-cells, enhance production of Th2-lymphocytes, attract eosinophils and activate vascular endothelial corneal and conjunctival cells to release chemokines (chemotactic cytokines)

and adhesion molecules (Ono & Abelson, 2005; Chigbu, 2009a). The chemokines and adhesion molecules mediate the infiltration of eosinophils, basophils, neutrophils and Th2-lymphocytes to the site of inflammation and coupled with the newly formed mediators and sustained mast cell activation they result in the late phase response (Stahl *et al*, 2002; Ono & Abelson, 2005; Chigbu, 2009a). This may occur 3-12 hours after the initial reaction (Bacon *et al*, 2000), and symptoms can continue up to 24 hours (Bonini *et al*, 1989; Bonini *et al*, 1990). The year round symptoms associated with PAC are the result of chronic mast cell activation and Th2-lymphocyte infiltration (Leonardi *et al*, 2008; Chigbu, 2009a).

1.4 Diagnosis of SAC and PAC

The diagnosis of SAC and PAC is mainly clinical, based upon the signs and symptoms upon presentation and detailed history (Friedlaender, 1993, 2011; Bielory, 2008a; Wong *et al*, 2009). Allergic tests and laboratory tests can be used to confirm diagnosis, particularly those who do not exhibit the classic signs and symptoms upon examination, and where the causative allergen is not known (Bielory & Friedlaender, 2008; Leonardi *et al*, 2008). Conventional in-vivo allergic tests include skin prick tests and measurement of total and specific IgE levels in the serum (Leonardi, 2005b). Skin prick tests involve exposing patients to a number of common allergen in solution as suggested by the patient's history, where they are directly applied to the skin forearm or back and the underlying skin is pricked lightly with a sterile needle. A positive control, usually histamine, and a negative control (sterile saline) are also applied (Asbell & Ahmed, 2003). A positive result occurs when the skin produces a wheal and flare response greater than 2mm in diameter within 15 minutes (Asbell & Ahmed, 2003).

Measurement of IgE in serum can be performed using radioimmunoassy, enzyme-linked immunosorbent assay or most commonly with the radio-allergosorbent test (RAST) (Asbell & Ahmed, 2003). Here, a sample of the patient's serum is mixed with a specific allergen

chemically bound to an insoluble matrix (usually made of paper). IgE antibodies within the serum specific to the allergen will bind to the allergen coated matrix, and after washing the matrix a radio-labelled anti-IgE specific to the bound IgE is added – the amount of anti-IgE-IgE-antibody complexes are then counted using a gamma counter, and results presented as a score or severity of reactivity category (Asbell & Ahmed, 2003). However, negative skin prick tests and normal serum IgE levels do not preclude diagnosis as conjunctival tissue may be sensitized alone in absence of systemic sensitisation (Leonardi *et al*, 1990; Leonardi *et al* 1993). Indeed, although skin prick tests have a high sensitivity for systemic allergies, positive results in patients with ocular allergy have been reported to be as low as 20% (Asbell & Ahmed, 2003). Studies have also shown significant correlation between specific IgE in tears (typically measured using RAST) and ocular allergy but a poor correlation between specific and total serum IgE and ocular allergy (Hoffman-Sommersgruber *et al*, 1996); and poor correlation between specific serum IgE and specific tear IgE, where 30% of SAC and PAC patients were positive to tear tests only (Leonardi *et al*, 1993). This suggests that allergen specific IgE is produced locally (the eye) and is responsible for allergic conjunctivitis (Hoffman-Sommergruber *et al*, 1996) and conjunctival tissue can be uniquely sensitised (Leonardi *et al*, 1993).

The conjunctival provocation test can be used to confirm conjunctival sensitivity to allergens which give a positive skin prick test. It is particularly useful to establish diagnosis in those with a history of allergic conjunctivitis (signs and symptoms) but negative skin prick and serum IgE tests (Leonardi, 2005b). Here, doses of allergen solution, suggested from history and or skin prick tests, are applied in increasing concentration to the eye at 10 minute intervals until a standardised composite scoring method evaluating itching, chemosis, hyeraemia and watering is greater than or equal to 5 (Bonini *et al*, 1989; Abelson *et al*, 1990). However, only one allergen can be tested per eye per day, and the contralateral eye is exposed to a negative control. In addition, conjunctival provocation tests allow the identification of specific allergens responsible for the local (ocular) allergic response,

enhancing the investigation of the patient's allergic profile and management decisions as the patient can be given specific allergen avoidance advice (Ousler *et al*, 2005). Measurement of specific IgE in tears can also be performed if systemic (skin prick test, serum IgE measures) tests yield negative results. However, the test is difficult to perform as large tear samples are required (minimum 50µl) and reflex lacrimation may occur (and dilute sample) (Leonardi, 2005b). Instead, measures of total IgE in tears are preferred as the amount of IgE in the tears is small, but this test is only recommended for patients unable to undergo skin tests (Abelson *et al*, 2003).

Other laboratory based diagnostic tests include conjunctival cytological analysis (Bonini *et al*, 1988; Asbell & Ahmed, 2003; Leonardi, 2005b). The simplest of these tests is performing a scraping of the conjunctiva for the presence of eosinophils with a spatula. Eosinophils have been found in 20%-80% of patients with allergic conjunctivitis (Abelson *et al*, 1983; Bonini *et al*, 1988; Asbell & Ahmed, 2003). However, this technique produces highly variable results and a negative eosinophil scraping does not preclude diagnosis, as the technique is difficult to perform and eosinophils may lie deep within the conjunctival tissue (Abelson *et al*, 1983; Asbell & ahmed, 2003). In a study by Abelson *et al* (1983), eosinophils have been found only occasionally in patients with allergic conjunctivitis, whereas more recent studies found an absence of eosinophils in patients with SAC (Anderson *et al*, 1997). Although SAC and PAC are not normally associated with elevated blood eosinophils, serum eosinophil cationic protein levels were found to be significantly elevated compared to healthy controls (Leonardi *et al*, 2000). Scraping for mast cells can also be performed as they are normally absent from the normal conjunctival epithelium, although tryptase and chymase containing mast cells are present in the substantia propria. A study by Anderson *et al* (1997) showed that the median number of mast cells obtained from scraping during the pollen season was significantly greater in 61% of patients with SAC compared to healthy controls.

Other cytological tests include impression cytology, conjunctival biopsy and flow cytofluorimetry, although impression cytology is better suited to tear film pathology, and

biopsy required where a neoplastic or autoimmune pathology is suspected (Leonardi, 2005b). Tear chemical mediator assessment is another method for diagnosing allergic conjunctivitis, where chemical mediators such as tryptase, an enzyme specific for mast cell activation, are measured using immunoassays, typically the enzyme-linked immunosorbent assay (ELISA) (Butrus *et al*, 1990; Leonardi, 2005b). Histamine, the primary mediator released following mast cell degranulation, can also be detected with ELISA but it is quickly broken down by histaminases enzymes within the tears (Leonardi *et al*, 1996, 2005b). Thus, it can only be detected following massive mast cell degranulation induced by conjunctival provocation test (Leonardi, 2005b). A wide range of cytokines, chemokines and adhesion molecules within the tears have been also found to be significantly elevated in patients with severe ocular allergy (AKC, VKC), but only IL-4 was found to be significantly greater in SAC compared to normal (Fujishima *et al*, 1995; Uchio *et al*, 2000). However, although studied in the pathophysiology of allergic eye disease, these inflammatory markers are not yet utilised for ocular allergy diagnosis as not only are large quantities of tears are needed; they may also be produced and expressed by cells other than lymphocytes and inflammatory cells such as ocular surface epithelial and connective tissue cells (Leonardi, 2005b). Therefore, it is not currently possible to identify the origin of these inflammatory markers in order to reliably diagnose ocular allergy.

Some authors have suggested that history, signs and symptoms alone are insufficient for a diagnosis of allergic conjunctivitis and determination of the patient's allergic profile, tear IgE measurement and cytological analysis is warranted (Martin *et al*, 2003; Wolffsohn, 2009). However, despite the wide range of diagnostic tests available, the consensus remains to base diagnosis of allergic conjunctivitis on signs, symptoms and a detailed history as many of these tests are currently not applicable in optometric practice owing to the level of training, cost and equipment required to perform these tests. Further, emergency resuscitative equipment, adrenaline injections (epi-pen) and rapid escape routes must be available owing

to the risk of anaphylaxis during skin prick and conjunctival provocation tests (Leonardi, 2005b).

1.5 Non-pharmacological Treatment of SAC and PAC

The most important and most effective step in treating allergic eye disease is avoiding the offending allergen to prevent the hypersensitivity reaction from being triggered, but this necessitates the identification of the offending allergen and complete avoidance is not always possible (Ciprandi *et al*, 1996; Friedlaender, 2001; Bielory, 2008b). In SAC a detailed history is essential as knowledge of the period of time of year symptoms occur can allow identification using a pollen calendar to some extent but peak levels of common causative pollens often overlap. However, effective measures for allergen avoidance in SAC and PAC are based upon control of the environment. Given that pollens are the main cause of SAC, preventative measures include limiting outdoor activity during the symptomatic period, closing windows and using air conditioning when in a car or indoors, avoid touching/rubbing eyes after being outdoors, wash hands after being outdoors and wearing close fitting or wrap around style sunglasses when outdoors (Ciprandi *et al*, 1996; Veys, 2004). As PAC can affect the patient all year round, more thorough avoidance measures are necessary. Dust mite levels in the home can be reduced by using and regularly replacing protective pillow, mattress and duvet covers; washing bedding regularly at least at 60°C; vacuum and damp dust entire house on weekly basis; reduce humidity to between 35-50% and remove or regularly clean carpets, upholstery, curtains and any other areas that gather dust (Veys, 2004; Gotzche & Johansen, 2008; Sheikh *et al*, 2010). Animal dander can be reduced by eliminating all pets/animals from the home or keep them outdoors; regular vacuuming; minimising exposure to areas that gather animal dander; avoid touching animals; washing hands and avoid eye touching/rubbing after contact with animals; and washing all clothes that have come into contact with animals (Eggleston & Wood, 1992;

Veys, 2004; Bush, 2008). Washing hair before going to bed can help remove any allergens trapped in the hair and prevent transfer to the pillow. Table 1.5.1 provides a summary of practical avoidance measures for common allergens implicated in SAC and PAC.

Other non-pharmacological interventions include the use of cold compresses, cooled preservative free artificial tears or saline, which help to wash out the allergens in the conjunctiva and encourage vaso-constriction of the blood vessels to reduce eyelid swelling, chemosis and hyperaemia (Bielory, 2008b; Wong *et al*, 2009). In addition, the artificial tears may act as a barrier to the pollen allergens to prevent the hypersensitivity response (Chigbu, 2009b). Although there is a lack of evidence regarding their efficacy, their use appears plausible and these measures should be encouraged as supportive therapy, where they can be used between topical anti-allergic doses when symptoms persist or prior to the initiation of medication.

Allergen	Control Measures
Pollen and Outdoor Moulds	Limit outdoor activities during symptomatic period
	Monitor pollen levels using internet and media broadcast resources to plan outdoor activities
	Avoid rubbing eyes and nose and wash hands after being outdoors
	Wear closely fitting wrap around style sunglasses when outdoors
	Close windows and use air conditioning when in a vehicle and doors leading outside when in the home
House Dust Mites	Use protective pillow, mattress and duvet covers
	Regularly wash bedding at 60°C (at least)
	Vacuum and damp dust entire house on a weekly basis
	Remove or regularly clean carpets, upholstery, curtains and any other areas or objects that can gather dust
	Use a de-humidifier to reduce humidity in the home to between 35% and 50%
Animal Dander	Avoid contact with animals
	Keep pets outdoors or none at all
	Regularly vacuum the home and clean areas that gather animal dander
	Avoid rubbing eyes or nose after being in contact with animals
	Wash hands after and clothes which have been in contact with animals

Table 1.5.1: Allergen avoidance measures

1.6 Pharmacological Management of SAC and PAC

Despite implementing the above measures, complete avoidance is not always possible so use of anti-allergic medication may become necessary to prevent and alleviate symptoms. With increased knowledge of the pathophysiology of the hypersensitivity reaction in SAC and PAC over the years, there has been a rapid increase in the number of anti-allergic medications that target the immunological cells and inflammatory mediators involved in the allergic expression (Abelson *et al*, 2002a; Schultz, 2006; Bielory, 2008b). Ophthalmic

anti-allergic medications include topical mast cell stabilisers, antihistamines, antihistamine-vasoconstrictor combinations and dual action agents with combined mast cell stabilising and antihistaminic properties (Leonardi, 2005a; Leonardi *et al*, 2008).

The most common agent used in the pharmacological management of allergic conjunctivitis is antihistamines and are available topically for use alone, in combination with vasoconstrictors, and in oral form. Antihistamines are competitive antagonists of histamine receptors (H1 and H2) on effector cells in the conjunctiva and eyelids (Leonardi, 2005a; Bielory & Ghafoor, 2005). When stimulated by the main preformed mediator histamine, H1 receptors cause capillary dilation and increased vascular permeability which results in symptoms of itching and localised oedema typical of the hypersensitivity reaction (Abelson *et a*l, 2002a; Ono & Abelson, 2005). Therefore binding of these receptors by antihistamine the inflammatory events normally initiated by histamine are prevented (Friedlaender, 2001; Schultz, 2006). Topical ophthalmic preparations include azelastine hydrochloride 0.05% and emadastine hydrochloride 0.05%, which demonstrate efficacy in alleviating signs and symptoms of SAC with good safety profiles (Ciprandi *et al*, 1997; Giede-Tuch *et al*, 1998; Friedlaender *et al*, 2000; Netland *et al*, 2000). Azelastine is also efficacious in PAC (Canonica *et al*, 2003).

The only combination preparation available in the UK is Otrivine-Antistin, containing the antihistamine antazoline 0.5% and the sympathomimetic (vasoconstrictor) xylometazoline 0.05%. Sympathomimetics stimulate adrenergic receptors causing capillary constriction and reduced blood flow and therefore reduce hyperaemia, chemosis and eyelid swelling associated with the allergic response (Bielory *et al*, 2005). Naphazoline, another vasoconstrictor, either alone (Murine irritation & redness relief) or in combination with witch hazel (Optrex Bloodshot Eye Drops) a plant with reported astringent properties, is also available. Oral antihistamines should be considered alongside topical agents in SAC where it is associated with seasonal hay fever as the nose and throat are also affected in this condition (Bielory *et al*, 2005; Wong *et al*, 2009). However, they are not as safe or effective

as topically applied agents in treating allergic conjunctivitis (Bielory, 2002; Bielory *et al*, 2005), and can cause drying of mucous membranes such as the conjunctiva, which may exacerbate symptoms and counter the washing action of the tears from removing allergens from the ocular surface (Ousler *et al*, 2007). However, several studies have demonstrated improved efficacy when they are combined with topical anti-allergic agents (Lanier *et al*, 2001; Abelson & Kaplan, 2002; Crampton, 2003).

The mast cell stabilisers indicated for the treatment of allergic conjunctivitis include sodium cromoglycate 2%, nedocromil 2% and lodoxamide 0.1% and have demonstrated efficacy in alleviating the signs and symptoms of SAC and PAC compared to placebo (Leino *et al*, 1992; Blumenthal *et al*, 1992; Cerqueti *et al*, 1994). Mast cell stabilisers work by preventing the degranulation of the sensitised mast cells thus inhibits the release of inflammatory mediators and repressing the type 1 hypersensitivity reaction (Abelson *et al*, 2002a; Schultz, 2006). This action results from preventing the calcium ion influx into the mast cell after antigen stimulation (Abelson *et al*, 2003; Leonardi, 2005a). As mast cell stabilisers act on the mast cell before degranulation occurs, they will have no effect on the inflammatory mediators once they have been released (Chigbu, 2009b). Therefore mast cell stabilisers require a loading time of 10 to 14 days before symptoms are known to occur and are used topically as prophylactic agents (Bielory, 2008b; Chigbu, 2009b).

The dual action anti-allergic topical medications azelastine hydrochloride 0.05%, epinastine hydrochloride 500mg/mL, olopatadine hydrochloride 1mg/mL and ketotifen fumarate 250mg/mL combine both mast cell stabilising and antihistaminic properties and have demonstrated good efficacy and safety in treating SAC compared to placebo (Giede-Tuch *et al*, 1998; Abelson, 1998; Kidd *et al*, 2003; Abelson *et al*, 2004; Avunduk *et al*, 2005) and can therefore have the advantage as both a prophylactic to prevent mast cell degranulation and a therapeutic agent to bring about symptomatic relief following the onset of symptoms (Chigbu, 2009b). Table 1.6.1 below provides a summary of the medications available in the UK to treat allergic conjunctivitis.

Medication Class	Name	Formulation	Optometrist Availability
Mast Cell Stabiliser	Sodium Cromoglycate (non-proprietary)	Sodium Cromoglycate 2%	Entry level but in maximum pack size of 10mL
	Catacrom (Moorfields Pharmceuticals)	Sodium Cromoglycate 2% single use	At least AS level
	Rapitil (Sanofi-Aventis)	Nedocromil Sodium 2%	At least AS level
	Alomide (Alcon)	Lodoxamide 0.1%	Entry level
Antihistamine	Otrivine-Antistin (Novartis)	Antazoline Sulphate 0.5% and xylometazoline Hydrochloride 0.05% eye drops	Entry level
	Emadine (Alcon)	Emedastine Hydrochloride 0.05%	At least AS level
Dual Action	Opatanol (Alcon)	Olopatadine Hydrochloride 1mg/mL	At least AS level
	Optilast (Meda)	Azelastine Hydrochloride 0.05%	At least AS level
	Relestat (Allergan)	Epinastine Hydrochloride 500µg/mL	At least AS level
	Zaditen (Novartis)	Ketotifen Fumarate 250µg/mL	At least AS level
NSAID	Voltarol Ophtha Multidose (Novartis)	Diclofenac Sodium 0.1%	At least AS level
	Voltarol Ophtha (Novartis)	Diclofenac Sodium 0.1% single use	At least AS level
Vasoconstrictor	Murine irritation and redness relief (Prestige Brands)	Naphazoline Hydrochloride 0.012%w/v	Entry Level (Pharmacy only medicine)
	Optrex Bloodshot Eye Drops (Reckitt Benckiser)	Napahazoline Hydrochloride 0.01%w/v and Witch Hazel (Hamamelis water) 12.5%v/v	Entry Level (Pharmacy only medicine)

Table 1.6.1: Topical ophthalmic medications available for the treatment of allergic conjunctivitis in the UK

In severe SAC and PAC and cases unresponsive/refractory to conventional anti-allergic medications described above, anti-inflammatory agents may be necessary such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids (Bielory, 2008b; Leonardi *et al*, 2008; Wong *et al*, 2009). NSAIDs work by preventing the formation of prostaglandins responsible for itching by blocking the cyclooxygenase pathway in the hypersensitivity response (Chigbu, 2009b). Corticosteroids reduce inflammation by a variety of actions - they suppress the activation, recruitment and production of late phase inflammatory mediators; increase the availability of histaminases (enzymes which break down histamine); prevent histamine production in mast cells; inhibit T-cell activity and reduce the permeability of conjunctival blood vessels (Chigbu, 2009b).

The efficacy of the NSAIDs ketorolac trometamol 0.5% and diclofenac sodium 0.1% in treating allergic conjunctivitis has been demonstrated in several studies (Tauber *et al*, 1998; Swamy *et al*, 2007), but only the latter has been licensed for use as treatment for SAC in the UK. Treatment with corticosteroids requires careful monitoring owing to the potential increase in intraocular pressure and cataract formation (Hingorani & Lightman, 1995; Friedlaender, 1995). Immunomodulators such as cyclosporine are gaining increasing popularity in the long term treatment of severe allergic eye disease as an alternative to topical steroid therapy, particularly in VKC steroid responders (Miyazaki *et al*, 2008; Ebihara *et al*, 2009). In severe, persistent cases of allergic conjunctivitis and those with systemic allergic associations, referral to an allergologist/immunologist may be necessary to initiate immunotherapy. It is a well-established therapy with proven efficacy in a range of allergic conditions including allergic conjunctivitis (Pradalier *et al*, 1999; Bielory & Mongia, 2002; Mortemousque *et al*, 2003; Skoner *et al*, 2010). Here, small doses of the offending allergen identified by skin prick and conjunctival provocation tests are given over time to desensitise mast cells, essentially inoculating the patient (Bielory, 2008b).

It is clear that there is a wide range of anti-allergic available to manage SAC and PAC. However, no topical mast cell stabiliser or antihistamine medications have been shown to be

superior to the other in terms of efficacy and onset of action, although topical antihistamines provided relief sooner (Owen *et al*, 2004). Interestingly, there appears to be no studies in the scientific literature examining the safety and efficacy of mast cell stabilisers and antihistamines in combination versus use alone and placebo where the results may prove useful to entry level optometrists who have access to few anti-allergic agents. Recent studies comparing the efficacy of dual action agents have shown conflicting results. Borazan *et al* (2009) compared the efficacy of olopatadine, ketotifen, epinastine, emedastine and the steroid fluorometholone in an environmental model and found no significant difference between the anti-allergic medications but they were all superior to the steroid (Borazan et al, 2009). However, Lanier *et al* (2004) and Mah *et al* (2007) showed olopatadine superior to epinastine in conjunctival allergen challenge models (Lanier *et al*, 2004; Mah *et al*, 2007), but these conflicting results may be due to the different methodologies employed (Ousler *et al*, 2005). Based on current evidence, the choice of which drug to prescribe should relate to the frequency of applications, cost and patient preference in addition to contraindications and potential interactions, rather than onset of action (Owen *et al*, 2004). It is likely that dual action medications will become first line agents for the treatment of allergic conjunctivitis as they demonstrate both prophylactic and therapeutic efficacy and require fewer applications. Simplifying the treatment regimen compared to using antihistamines and mast cell stabilisers separately serves to encourage patient compliance and offer patient convenience.

1.7 Management of Allergic Conjunctivitis in the UK

Given that seasonal and perennial allergic conjunctivitis (SAC and PAC) are the most common forms of ocular allergy, most studies investigating the management of ocular allergy in the UK relate to this ocular allergy subtype. Although the management of allergic conjunctivitis in UK primary care is not well studied, a few scientific papers have shown it to be a common condition that often results in medical treatment. In addition, it is reported that

only 10-12% of seasonal allergic conjunctivitis patients seek medical attention for their symptoms (Malone *et al*, 1997), so the information presented here may only reflect the practices relating to care seeking sufferers. The College of Optometrists, the educational and scientific body for optometry in the UK, publishes clinical management guidelines relating to allergic conjunctivitis, but as with dry eye, there is no literature relating to adherence to these guidelines or correlation between adherence and clinical outcome.

In 2009, Wolffsohn performed a survey on optometrists, pharmacists and general practitioners (GP) to determine how they managed ocular allergy related to pollen, such as SAC and PAC. None of the respondents stated all of the appropriate criteria (9) for differential diagnosis, but on average optometrists did cover more criteria compared to pharmacists and GPs (4.1±1.9 versus 2.9±1.3 and 1.3±0.9 respectively). Furthermore, few GPs and even fewer pharmacists performed an examination of the eyes, compared to approximately 70% of optometrists. The most common primary management strategy by optometrists mainly consisted of non-pharmacological interventions, such as general advice, antigen avoidance and cold compresses, followed by topical anti-allergic medications (antihistamines and mast cell stabilisers). In the study by Wolffsohn (2009), pharmacologic treatments, mainly in the form of topical mast cell stabilisers were recommended first by GPs and pharmacists. Some GPs and pharmacists also prescribed oral antihistamines, whereas optometrists did not. However, the findings from the healthcare provider survey may not be representative of the diagnosis and management strategies of all healthcare providers in the UK as the data was collected from the West Midlands area only, and therefore may have been subject to selection bias and social desirability bias. However, these findings are in contrast with a survey of the scope of therapeutic practice completed by 1288 optometrists in the UK, where topical antihistamines and mast cell stabilisers were frequently prescribed by 31% and 32% of optometrists and 10% frequently recommended oral antihistamines (Needle *et al*, 2008). Consistent with the frequent usage of these medications, in the same study

allergic conjunctivitis was one the most common conditions treated (86% optometrists either frequently or occasionally managing).

The most common secondary management strategy by optometrists and pharmacists was referral to the patients GP, but GPs preferred to review and trying a different pharmacologic agent before considering referral (Wolffsohn, 2009). Approximately 10% of GPs even prescribed steroids, the use of which requires close supervision owing to potential ocular complications such as cataract and raised intraocular pressure despite lacking equipment to examine the eyes and measure intraocular pressure (Carnahan & Goldstein, 2000). It has been acknowledged that corticosteroid containing eye drops should not be used unless the practitioner is trained in measuring intraocular pressure, examining the eye with a slit lamp with considerable understanding in ocular differential diagnosis and treatment (Baratz & Hattehauer, 1999).

In a study investigating the ophthalmic disease management by GPs, the main treatment for patients diagnosed with allergic conjunctivitis (prevalence of 13%) were also pharmacologic, with such as mast cell stabilisers (sodium cromoglycate, 64.1%), oral and topical antihistamines (21.4% and 3.9%), but 37 of the 206 diagnosed had more than one treatment (Sheldrick *et al*, 1993). Another study into the management of eye disease in general practice by McDonnell (1988) shows similar results, with sodium cromoglycate the most common treatment for allergic conjunctivitis and a prevalence of 15%, but only 2.9% were prescribed steroids (McDonnell, 1988). In addition, the proportion of patients with allergic conjunctivitis prescribed corticosteroids in the study by Sheldrick *et al* (1993) was 6.8% (eye drops n=12, oral n=2), similar to that found by Wolffsohn (2009). Sheldrick *et al* (1993) also found that allergic conjunctivitis and dry eye were the most commonly misdiagnosed conditions by GPs (Shledrick *et al*, 1993). All three studies, however, collected data from practices in specific regions which may not be representative of the management of allergic conjunctivitis in GP practice in the UK as a whole.

Wolffsohn (2009) also found that pharmacists and GPs rarely referred to optometrists, despite lacking equipment to examine the eyes and relatively short consultation time, lasting 7.6 minutes on average for GPs (Kernick & Netten, 2002) and 5 minutes for pharmacists (Sclar *et al*, 2008) compared to 30 minutes by optometrists (The College of Optometrists, 2011). The frequent referral by optometrists to GPs may reflect the level of competence or training that the optometrist has in dealing with ocular allergy. However, lack of access to an appropriate medication may be sole reason for referral as the majority of topical anti-allergic agents are prescription only medications (Wolffsohn, 2009).

The differences between the management of ocular allergy by different healthcare professionals shown by Wolffsohn (2009) are interesting given that the primary treatment strategy reported in the scientific literature for ocular allergy is allergen avoidance to prevent the hypersensitivity response, and the use of cold compresses or artificial tears to provide symptomatic relief (Bielory, 2008b; Chigbu, 2009b). Even though optometrists have access to ophthalmic examination equipment and training specific to the eye, their confidence in management appears low compared to GPs and pharmacists (Wolffsohn, 2009). However, in the study by Needle *et al* (2008) examining the scope of optometric therapeutics, with further training 96.4% felt they could manage allergic conjunctivitis in their practice.

Patient reports on how different healthcare professionals manage their allergic conjunctivitis were also assessed by Wolffsohn (2009) using a detailed survey. Advice on medications mostly came from GPs (53%), followed by pharmacists (41%) and friends or relatives (18%) compared to only 11% by optometrists. This does not fit in with the results from the healthcare provider survey in the same study, where optometrists reported giving advice far more often than GPs or pharmacists – either type of patients that attend each healthcare provider differ, patients place more emphasis on advice from physicians or advice is not given at every appointment (Wolffsohn, 2009). Again, this is surprising given that optometrists have access to advanced ophthalmic examination equipment and training specific to the eyes. It is suggested optometrists may not be promoting their skills and

expertise effectively and patients perceive their condition as part of hay fever, a systemic condition rather than a distinct clinical entity (Wolfssohn, 2009). This is supported by the finding that over 80% of patients used medication in oral and nasal form compared to 40% using eye drops; and 82% of patients with ocular allergy identifying their condition as hay fever (Wolffsohn, 2009). However, this may be due to additional nasal symptoms experienced. One hundred and fifty of the patients taking part in this phase of the study were invited to undergo skin prick and conjunctival provocation testing to reduce the potential unreliability in self-reported disease accuracy by determining the patient's systemic and ocular allergic profile. The patient survey questionnaire was found to have a sensitivity of 80% for systemic allergy and 54% for ocular allergy, and a specificity of 70% for systemic allergy and 29% for ocular allergy (Wolffsohn, 2009).

Nearly all of the respondents were taking medication for their ocular symptoms (94%), but 73% obtained medication over the counter compared to 42% on prescription and only 15% using both sources (Wolffsohn, 2009). The majority of the medications were in the form of oral antihistamines (71%); followed by nasal sprays (40%), eye drops (40%) and steroids (4%). Mast cell stabilisers were the most common eye drops (53%), followed by ocular lubricants (48%), antihistamines (18%) and dual action agents (7%). The greater number of medicines obtained over the counter concurs with the greater number of patients consulting pharmacists compared to GPs but the finding that the majority of medications taken by ocular allergy patients are in the form of oral antihistamines does not fit with the stated prescribing habits of healthcare providers as determined from the healthcare provider survey (Wolffsohn, 2009). Although nearly all ocular allergy patients stated they felt their treatment was effective (93%), many still experienced symptoms, rated as severe in 19% of cases and moderate and mild in 30% and 35% respectively (Wolffsohn, 2009). However, reports of medication effectiveness in seasonal allergic conjunctivitis patients in the UK showed only 70% of participants displayed satisfaction with their medication, with the remaining 30% dissatisfied, although 82% of seasonal allergic conjunctivitis patients who consulted GPs

stated they were satisfied with the outcome of their GP appointment (Pitt *et al*, 2004). In a cross-sectional study of allergic conjunctivitis patients in Portugal, 56.1% self-treated and only 37.2% had previous allergy diagnostic testing (Palmares *et al*, 2010).

1.8 Dry Eye Disease

Dry eye is a condition of various aetiologies, acting through common mechanisms of tear hyperosmolarity and instability (Lemp, 2007). This ultimately causes ocular surface inflammation, epithelial (corneal and conjunctival goblet) cell damage and the subsequent symptoms associated with the condition (Lemp, 2007; Foulks, 2008). Dry eye can be broadly divided in to two types, aqueous deficiency and evaporative dry eye (Lemp, 2007). Dry eye can affect one or both eyes, and symptoms include irritation, grittiness, burning, soreness, watery eyes and visual disturbances (Begley *et al*, 2003; Vitale *et al*, 2004; Reiger, 1992; Goto *et al*, 2002a). Further, a compromised corneal epithelium may increase the risk of ocular infection. Although the different types of dry eye may exist in isolation, more commonly they often co-exist and can interact with or even initiate other forms of dry eye (Lemp, 2007; Bron *et al*, 2009).

In the normal eye, the lacrimal functional unit (LFU) consisting of the lacrimal glands, cornea, conjunctiva, meibomian glands, eyelids, and the sensory and motor nerves connecting these structures which all serve to control the tear film (Stern *et al*, 1998). The LFU functions to preserve the integrity of the tear film, and maintain corneal transparency and retinal image quality by responding to environmental, endocrinological and cortical influences (Stern *et al*, 1998, 2004). Dry eye is considered as a disturbance or breakdown of the LFU, where disease or damage to the sensory and motor nerves and glands (which contribute to the tear film can) can destabilise the tear film through decreased tear secretion, altered tear composition, and delayed tear clearance (Stern *et al*, 1998, 2004, Lemp, 2007).

The prevalence of dry eye is reported from large epidemiological studies to range between 5% to over 35% at different ages, with a greater prevalence in the older population (over 50 years of age) and those of Asian and Hispanic races (McCarty *et al*, 1998; Moss *et al*, 2000; Lee *et al*, 2002; Chia *et al*, 2003; Lin *et al*, 2003; Schaumberg *et al*, 2003). Dry eye, much like allergic conjunctivitis, is often a relatively mild condition but also has a significant impact on the quality of life and visual function (Smith, 2007). Indeed, utility assessment scores (using time trade off method where patients were asked to trade years of life for disease free years) for dry eye have been reported to be similar to moderate angina (Schiffman *et al*, 2003). More recent studies investigating the impact of dry eye on daily activities found that dry eye patients were more likely to experience difficulties with reading, professional work, driving, watching television and using a computer compared to those without dry eye (Miljanovic *et al*, 2007). Compared to those without dry eye, the same cohort of dry eye patients were also found to be three times more likely to experience difficulties with common daily activities (Miljanovic *et al*, 2007). The impact of dry eye on visual function includes reduction in contrast sensitivity, visual acuity, and vision related quality of life (Schiffman *et al*, 2000; Tutt *et al*, 2000; Goto *et al*, 2002a). Substantiated and consistent risk factors for dry eye are shown in Table 1.8.1.

Risk Factor	Reference
Female sex	Schaumberg *et al*, 2001
Older age	Schaumberg *et al*, 2001
Postmenopausal oestrogen therapy	Schaumberg *et al*, 2001
Androgen deficiency (including anti-androgen therapy)	Sullivan *et al*, 2002; Smith *et al*, 2004
Diet low in Omega-3 essential fatty acid	Miljanovic *et al*, 2005
Diet with high Omega-6 to Omeg-3 essential fatty acid ratio	Miljanovic *et al*, 2005
Refractive surgery	Hovanesian *et al*, 2001
Vitamin A deficiency	Sommer, 2003
Radiation therapy	Thomas *et al*, 2001
Bone marrow transplantation	Bray, 1991
Hepatitis C	Zegans *et al*, 2002
Antihistamines	Ousler *et al*, 2007

Table 1.8.1: Major risk factors for dry eye (adapted from Smith, 2007).

Other risk factors include low humidity environments such as aircraft cabins and office environments, where symptoms of ocular dryness and irritation may also be experienced as a result of an increase in tear evaporation from the ocular surface (Wolkoff *et al*, 2005, 2006; McCulley *et al*, 2006). Evaporation from the ocular surface may also be caused by high room temperature and high air flow velocity; and irritative symptoms may be worsened by indoor pollution and poor air quality (Wolkoff *et al*, 2005, 2006). The evaporation may be exacerbated in office environments where prolonged concentration and computer use may reduce the blink rate (Tsubota *et al*, 1993; Nakamori *et al*, 1997). Symptoms of dry eye are frequently reported by contact lens wearers with 50-75% of wearers experiencing ocular irritation (Doughty *et al*, 1997; Begley *et al*, 2000; Nichols *et al*, 2005a). Indeed, contact lens wear has often been associated with dry eye and these symptoms are a very common factor in contact lens wear discontinuation (Pritchard *et al*, 1999; Nichols & Sinnott, 2006; Richdale *et al*, 2007). Diabetes, HIV, human T-cell lymphotropic virus-1 infection, connective tissue disease, and systemic chemotherapy have also been associated with dry eye, but evidence for these risk factors is lacking (Smith, 2007).

1.9 Tear Film Structure and Function

The tear film is traditionally considered to be a complex structure composed of three layers or phases, although the thickness and boundaries between these layers are not yet fully established (Prydal *et al*, 1992; Dilly *et al*, 1994; Johnson & Murphy, 2004). However, recent studies suggest that the tear film consists of two layers, where a superficial lipid layer overlies an aqueous-mucin gel, with an increasing mucin concentration toward to the ocular surface epithelium (Dilly *et al*, 1994; Johnson & Murphy, 2004).

The most posterior layer is the mucous component, produced and secreted by the conjunctival goblet cells and epithelial cells of the cornea and conjunctiva (Gipson & Inatomi, 1998; Rolando & Zeirhut, 2001; Gipson, 2004). These cells also produce a mucin-like transmembrane glycoprotein on the apical surface of the epithelium, constituting the glycocalyx – this structure renders the entire ocular surface wettable (hydrophilic) and allows the overlying watery aqueous phase to spread over the ocular surface (Rolando & Zeirhut, 2001; Johnson & Murphy, 2004). The secreted mucins form a gel consisting of a network of entangled polymers that help lubricate the ocular surface, avoid shearing stresses during blinking and eye movements, and maintain the dioptric integrity if the tear film (Tiffany, 1994; Corfield *et al*, 1997). They also contribute to tear film viscosity (Tiffany, 1994; Corfield *et al*, 1997; Rolando & Zeirhut, 2001). The glycocalyx and gel mucins also serve to as a barrier to pathogens (Corfield *et al*, 1997; Johnson & Murphy, 2004).

The aqueous component forms the majority of the tear film and is secreted by the lacrimal glands and accessory glands of Krause and Wolfring, consisting of proteins, peptide growth factors, vitamins, cytokines, immunoglobulins and hormones (Johnson & Murphy, 2004). This watery layer not only allows for lubrication, but protects the ocular surface and creates the necessary conditions for normal epithelial cell function, carries nutrients and oxygen to the cornea, and cell movement over ocular surface (Rolando & Zeirhut, 2001). The aqueous also consists of electrolytes, which act as a buffer to maintain pH and help to ensure

epithelial integrity, and are largely responsible for tear film osmolarity (Johnson & Murphy, 2004). Proteins such as lysozyme, lactoferrin and lipocalin are abundant within the tear film, with the former two central to antimicrobial activity of the ocular surface (Johnson & Murphy, 2004). Peptide growth factors and vitamin A within the aqueous regulate epithelial cell activity (proliferation, motility and differentiation), and contribute to corneal wound healing (Sommer, 1983; Shultz *et al*, 1994).

The lipid layer forms the most anterior component of the tear film, and is produced and secreted by the meibomian glands, with a small contribution from the accessory glands of Zeis and Moll and serves to prevent evaporation of water from the ocular surface, overspill of the tears, and contamination by sebaceous lipids from the skin which may otherwise destabilise the tear film (Bron *et al*, 2004; Green-Church *et al*, 2011). The secretion, termed meibum, is a complex mixture of polar and non-polar lipids, consisting of cholesterol esters, triacylglycerol, free cholesterol, free fatty acids, phospholipids, wax esters and diesters (Butovich, 2009; Knop *et al*, 2011). The meibum is normally liquid at eyelid temperature and forms a reservoir alog the eyelid margin from which the tear film lipid layer is spread and formed (Tomlinson *et al*, 2011). The tear film lipid layer is considered to be a two layered structure, with a non-polar hydrophobic layer, consisting of cholesterol, sterol and wax esters in contact with the air which prevents water evaporation; necessitating an underlying polar, hydrophilic, phospholipid layer which is in contact with the aqueous (McCulley & Shine, 1997, 2003, 2004; Shine & McCulley, 2003a). This lipid bi-layer may also be intercalated by proteins from the aqueous phase, given that recent models of the tear film have shown that lysozyme and lipocalin may penetrate lipid layer (Green-Church *et al*, 2011). However, recent studies have found a relatively low proportion of phospholipids within meibum secretions, suggesting that a new lipid layer structure may be necessary to account for the interaction between the non-polar lipids and the aqueous (Butovich, 2009).

1.10 Classification of Dry Eye

The recent Dry Eye Workshop (2007) has developed an aetiopathogenic classification of dry eye, dividing each subtype under two broad groups, aqueous deficient dry eye and evaporative dry eye (Lemp, 2007). Although dry eye can be initiated in any of these subtypes, it may coexist with or lead the development of another dry eye subtype, even from a different group (Lemp, 2007; Bron *et al*, 2009). Further, each subtype may be influenced by physiologic variations between individuals and the environmental conditions they are subject to (Lemp, 2007).

Physiologic variations include low blink rate, where the increased time interval between each blink may increase evaporation of the tears from the ocular surface (Nakamori *et al*, 1997). Evaporative loss is also increased in those with wider palpebral apertures and upgaze (Cho *et al*, 2000). The presence of low androgen levels and or high oestrogen levels is considered major risk factors for dry eye. Dry eye has been found to occur in those with androgen deficiency, those on anti-androgen medication, and those with complete androgen insensitivity syndrome (Krenzer *et al*, 2000; Sullivan *et al*, 2002; Sullivan, 2004). Environmental conditions that influence the development of dry eye include conditions of low relative humidity resulting in increased evaporative loss, which can be caused by natural variations in geographic location and artificial settings, such as air-conditioned rooms (Paschides *et al*, 1998). Occupational settings may also increase evaporative loss, where those using video display terminals have been found to have reduced blink rates due to increased periods of concentration and prolonged upgaze (Tsubota *et al*, 1993).

Aqueous deficiency dry eye is characterised by reduced lacrimal secretion and volume, caused by lacrimal gland dysfunction or damage is subdivided into Sjogren syndrome dry eye, and non-Sjogren syndrome dry eye. (Behrens *et al*, 2006; Lemp, 2007; Bron *et al*, 2009). Although evaporative loss may be normal, tear film and conjunctival hyperosmolarity develops as the aqueous component of the tear film is reduced (Behrens *et al*, 2006; Lemp,

2007). This leads to a series of inflammatory events directed by MAP kinases and NFkB signalling pathways, resulting in the generation of inflammatory cytokines, tumour necrosis factor, and matrix metalloproteinases (Baudouin, 2001; Johnson & Murphy, 2004; Luo *et al*, 2005). This process may be exacerbated by the presence of lacrimal gland inflammation and lymphocyte infiltration, where the inflammatory mediators and cells are presumed to migrate to the ocular surface (Pflugfelder *et al*, 1986; Lemp, 2007).

Sjogren syndrome is an autoimmune disease where the lacrimal glands and salivary glands, among other exocrine glands, are affected. Although not well understood, the pathophysiology of Sjogren syndrome dry eye involves the infiltration of T-cells within these glands which causes acinar and ductal cell death and subsequent gland hyposecretion (Yamamoto, 2003; Larche, 2006; Jonsson *et al* 2011). Dry eye is caused by the combination of lacrimal hyposecretion, lacrimal gland inflammation, and inflammatory mediators in the tears and conjunctiva in these patients (Jones *et al*, 1994; Jonsson *et al*, 2011). Non-Sjogren syndrome dry eye is due to lacrimal gland dysfunction and hyposecretion in the absence of the autoimmune features of Sjogren syndrome, and is further subdivided into primary and secondary lacrimal gland deficiencies, lacrimal gland obstruction, and reflex hyposecretion groups (Lemp, 2007). Primary non-Sjogren syndrome dry eye includes age-related dry eye. With increasing age, an increase in periductal and interacinar fibrosis, paraductal blood vessel loss and acinar atrophy has been observed in otherwise normal healthy subjects (Obata, 2006). Thus, lacrimal dysfunction and reduction in lacrimal secretion and subsequent dry eye is brought about through an obstructive lacrimal pathology (Obata, 2006). In addition, subclinical conjunctivitis may cause stenosis of the lacrimal gland secretory ducts (Obata, 2006). However, there are conflicting reports on the effect of aging on the tear film. Although Mathers *et al* (1996) found significant age related correlations with tear evaporation, tear volume, tear flow and tear film osmolarity, other studies have not (Tomlinson & Geisbrecht, 1993; Tomlinson & Craig, 1998). However, these contrasting findings may relate to the dry eye tests themselves, owing to their variable nature particularly

within the normal population (Sullivan *et al*, 2012a). Other primary non-Sjogren syndrome dry eye subtypes include congenital alacrima, where patients may experience complete absence or significantly reduced tears, with the latter involving a lack tearing due to emotional stimulation but normal tearing due to mechanical stimulation (Lemp, 2007); and familial dysautonomia, characterised by general insensitivity to pain and lack of emotional and reflex tearing due to abnormal innervation of the lacrimal gland and ocular surface (Axelrod *et al*, 2006). However, both these syndromes are rare (Axelrod *et al*, 2006; Lemp, 2007). Secondary lacrimal gland deficiencies are associated with inflammation and infiltration, causing lacrimal gland dysfunction and destruction – causes include sarcoidosis, lymphoma, AIDS, graft versus host disease (Lemp, 2007). Partial or complete lacrimal gland ablation may also cause dry eye, but secretion, although low, may be compensated for by the accessory glands and conjunctiva (Lemp, 2007).

Any form of cicatrising conjunctivitis may cause scarring and obstruction of the ducts of the lacrimal and accessory glands, leading to aqueous deficiency dry eye. Further, tear spreading may be affected by abnormal eyelid apposition to the globe and blinking caused by scarring, leading to increased evaporation and ocular surface exposure (Lemp, 2007). Cicatrising conjunctivitis may be caused by trachoma, cicatrical pemphigoid, mucous membrane pemphigoid, erythema multiforme, and chemical and thermal burns (Power *et al*, 1995; Guzey *et al*, 2000; Thorne *et al*, 2002; Hingorani & Lightman, 2006). Further, cicatrising conjunctivitis may also cause obstruction of the meibomian glands, resulting in a deficient tear film lipid layer and increased evaporative loss (Lemp, 2007; Bron *et al*, 2009).

Reflex hyposecretion may be caused by a block or reduction in sensory drive to reflex lacrimation from the ocular surface, and may lead to aqueous deficiency dry eye. However, reflex sensory block may also cause a reduction in blink rate, leading to increased evaporative loss, thus exacerbating dry eye (Battat *et al*, 2001). Causes of reflex sensory block include herpes infection, corneal surgery, neurotrophic keratitis, topical anaesthesia and medications such as beta blockers and mydriatic drugs (Lemp, 2007). Contact lens wear

may also cause a reduction in corneal sensitivity (Bruce & Brennan, 1990; Lum *et al*, 2013) – indeed, dry eye symptoms are a major cause of contact lens wear dropout (Pritchard *et al*, 1999; Nichols & Sinnott, 2006). An increased prevalence of dry eye has been reported in patients with diabetes (Moss *et al*, 2000), with the association possibly due to diabetic neuropathy causing reduced corneal sensitivity and or microvasculature changes within the lacrimal gland (Kaiserman *et al*, 2005). Reflex hyposecretion may also be caused by reflex motor block, where damage to the 7th cranial nerve causes loss of lacrimal secreto-motor function and lagophthalmos, leading to aqueous deficiency dry eye with an evaporative component due to incomplete lid closure (Lemp, 2007).

Whereas aqueous deficiency dry eye relates to lack of tear production, evaporative dry eye relates to excessive water loss via evaporation from the ocular surface in the presence of normal lacrimal function (Lemp, 2007). The causes of evaporative dry eye may be intrinsic, where the patient exhibits abnormal eyelid structure, function or dynamics; and extrinsic causes where the ocular surface is exposed to adverse environmental conditions or pre-existing disease (Lemp, 2007). The most common intrinsic cause of evaporative dry eye is meibomian gland dysfunction (Foulks & Bron, 2003; Bron & Tiffany, 2004). Although meibomian gland dysfunction (MGD) is considered a specific disease entity in its own right with a range of causes and subtypes, in all cases of MGD of sufficient extent and severity, the meibomian glands ultimately cannot deliver sufficient lipid containing secretion (meibum) to the ocular surface and tear film (Bron & Tiffany, 2004). This may result in a deficient tear film lipid layer that can no longer minimise evaporation effectively, leading to evaporative dry eye (Bron & Tiffany, 2004). The clinical features, epidemiology, classification, diagnosis and treatment of MGD are discussed in detail in sections 1.17 to 1.22.

As described earlier, increased palpebral aperture may increase evaporation from the ocular surface (Rolando *et al*, 1985). Thus, diseases that cause proptosis such as craniostenosis and thyroid eye disease, may lead to evaporative dry eye as the eye is displaced anteriorly resulting in increased palpebral aperture width (Gilbard & Farris, 1983). Intrinsic causes also

include poor eyelid apposition and eyelid deformity may also lead to evaporative dry eye as the ocular surface may become exposed and or the tear film cannot be retained or spread effectively (Lemp, 2007). Another intrinsic cause is a low blink rate, which may be the result of periods of increased concentration (physiological phenomenon) (Nakamori et al, 1997), or by extrapyramidal disorders (causing involuntary movements) such as Parkinson's disease (Karson *et al*, 1984).

Extrinsic causes of evaporative dry eye include vitamin A deficiency (Lemp, 2007). Vitamin A is crucial for the expression of glycocalyx mucins and goblet cell development in the conjunctival epithelium, which allows ocular surface lubrication and creation of a smooth surface over which the aqueous may spread (Hori *et al*, 2004). In vitamin A deficiency, these features are reduced, leading to exposure and increased evaporation, although an element of aqueous deficiency dry eye may also be present as lacrimal acinar cells can be damaged in this form of dry eye, known as xerophthalmia (Lemp, 2007). Prolonged use of preserved topical drugs may lead to a toxic response causing epithelial cell damage (particularly those containing benzalkonium chloride), observed as diffuse punctate epithelial keratitis, which affect ocular surface wettability and may therefore lead to dry eye as the tear film spreading is inhibited (Lemp, 2007).

1.11 Pathophysiology of Dry Eye

The causative mechanisms of all forms of dry eye are centred on tear film hyperosmolarity and tear film instability, which can initiate and exacerbate dry eye or even change the nature of dry eye over time (Stern *et al*, 2004; Lemp, 2007). However, there is a complex interplay between the presence of physiologic variations, adverse environmental conditions, and risk factors or causes of dry eye that lead to break down of the LFU, followed by a cascade of biological responses (tear film hyperosmolarity and tear film instability) expressed as dry eye (Stern *et al*, 2004; Lemp, 2007). Tear film hyperosmolarity is caused

by concentration of the tear film solutes due to reduced lacrimation (aqueous flow), and or increased evaporation of tears from the ocular surface (Bron *et al*, 2009). As described earlier, tear film hyperosmolarity leads to the development of inflammatory events in the ocular surface epithelial cells (involving MAP kinases and NFkB signalling pathways) and the production of inflammatory cytokines and matrix metalloproteases which arise from and recruit inflammatory cells to the ocular surface (Baudouin, 2001; Calonge *et al*, 2010). These inflammatory events lead to apoptotic death of the ocular surface epithelial and goblet cells – in experimentally induced dry eye in mice (aqueous production was inhibited scopolamine, and were exposed to an air draft), Yeh *et al* (2003) found significantly increased levels of apoptotic cells within the central and peripheral cornea, and bulbar and tarsal conjunctiva. Indeed, goblet cell loss is present in all forms of dry eye, as evidenced by reduced levels of mucin in patients with dry eye compared to normal healthy subjects (Argueso *et al*, 2002). Further, in a study investigating the presence of inflammatory markers in patients with and without dry eye (Sjogren syndrome and non-Sjogren syndrome subtypes) using flow cytometry from impression cytology samples, there was a significantly greater level of apoptotic and inflammatory markers in those with dry eye (Brignole *et al*, 2000). Thus, the ocular surface in predisposed individuals may become compromised/damages by hyperosmolarity, inflammation and mechanical stress due to a loss of lubrication by goblet cell mucin, leading to an initial reflex lacrimation.

However, the reflex lacrimation may be insufficient to compensate for the for the tear film hyperosmolarity, leading to aqueous deficiency dry eye (Lemp, 2007; Bron *et al*, 2009). In evaporative dry eye, although lacrimation may remain normal or increase to compensate initially, the effect of evaporation may eventually outweigh the reflex response (Lemp, 2007; Foulks, 2007; Bron *et al*, 2009). However, excessive reflex lacrimation may lead to lacrimal exhaustion or induce an inflammatory response within the lacrimal gland itself, followed by release of inflammatory mediators within the tear film, exacerbating the disease (Tsubota, 1998; Tang *et al*, 2000). In chronic dry eye, corneal sensitivity may reduce due to long term

inflammation and hyperosmolar stress on the sensory nerve terminals of the ocular surface, causing the sensory drive to reflex lacrimation to decrease (Bourcier, 2005; Benitez-Del-Castillo *et al*, 2007). Thus, any compensatory reflex lacrimation would be reversed at this stage of disease, exacerbating both aqueous deficiency dry eye by further reducing lacrimal secretion and evaporative dry eye by the addition of an aqueous deficiency component (Bron *et al*, 2009). Therefore a clear clinical separation between aqueous deficiency dry eye and evaporative dry eye may be difficult, or in cases where both forms co-exist, establishing the initial causative mechanism may not be possible based upon current clinical dry eye tests. Indeed, some patients with MGD may have normal evaporation rates (Shimazaki *et al*, 1995), or even reduced tear flow (Tomlinson & Khanal, 2005); whereas patients with aqueous deficiency dry eye have been found to have increased evaporation rates (Tsubota & Yamada, 1992; Mathers, 1993), particularly those with Sjogren syndrome who have been found to have greater prevalence of MGD than the normal population, possibly due to changes in androgen levels (Shimazaki *et al*, 1998). However, the increased evaporation observed in these patients may relate to the poor spreading of the tear film lipid layer as the aqueous phase is reduced (Lemp, 2007).

Tear film instability, represented by the tear film break up time, is also causative mechanism of dry eye, and may occur in the absence of prior tear film hyperosmolarity (Lemp, 2007; Bron *et al*, 2009). If the tear film break up time is less than the blink interval (time between consecutive blinks), or less than 10 seconds (even though the blink interval maybe shorter), the tear film is regarded as unstable, causing local drying and subsequent hyperosmolarity at the site of tear film break up, leading to the inflammatory events and epithelial and goblet cell damage described above (Lemp, 2007; Bron *et al*, 2009). Thus, reduced mucin may levels negatively reinforce tear film instability as tear film spreading becomes impaired.

1.12 Diagnosis of Dry Eye

The diagnosis of dry eye is typically based upon a combination of patient symptom assessment and dry eye tests, but there is no uniform set criteria to fulfil and no gold standard (Bron *et al*, 2007; Sullivan *et al*, 2012b). Performance data of commonly used tests, described below, often suffer from selection bias, where the efficacy of a test is evaluated in a population who were defined as having a disease or not by the same test, resulting in artificially high sensitivity and specificity values (Bron *et al*, 2007). Further, the inclusion of very select patients may cause spectrum bias, compromising the data which may not be applicable to different dry eye disease subtypes or severities (Bron *et al*, 2007).

However, despite these limitations, these dry eye tests are frequently and remain used in practice for diagnosis and monitoring disease; and research to select patients for clinical trials and as outcome measures for treatment efficacy owing to a lack of commercially available and cost-effective tests and equipment (Bron *et al*, 2007). The Dry Eye Workshop (2007) recommends the use of a dry eye questionnaire, tear evaluation via tear film osmolarity, non-invasive tear film break up time and tear function, ocular surface staining assessment, tear volume assessment via Schirmer test, and evaluation of eyelid morphology and meibomian gland function in combination with a clinical history (Bron *et al*, 2007). The use of a wide range of different tests is necessary as each test evaluates specific characteristics of the state of the tear film and ocular surface, and it is unlikely that a single test could capture the multifactorial nature of dry eye (Khanal *et al*, 2008).

However, given the key role of tear film hyperosmolarity in the pathophysiology of dry eye, the measurement of tear film osmolarity offers the ability to capture a single biophysical value that provides information about the balance of tear production, evaporation and retention (Tomlinson *et al*, 2006). Thus, tear film osmolarity measurement is considered a potential gold standard measurement for dry eye diagnosis (Tomlinson *et al*, 2006; Sullivan *et al*, 2012a; 2012b). Recent studies have found that tear film osmolarity measurement

demonstrated significant correlation to dry eye disease severity (Sullivan *et al*, 2010; Versura *et al*, 2010), had the lowest variability in measurement over time (Sullivan *et al*, 2012b), and was responsive to treatment where reductions in osmolarity preceded reductions in dry eye symptoms (Sullivan *et al*, 2012b). The diagnostic performance of tear film osmolarity measurement has been well studied, demonstrating superior sensitivity and specificity values compared to traditional dry eye tests such as Schimer test, ocular surface staining, meibomian gland assessment and tear film break up time in independent samples of dry eye patients of varying severity (Tomlinison *et al*, 2006; Versura *et al*, 2010; Lemp *et al*, 2011).

Tear film osmolarity can be measured using several techniques (Bron *et al*, 2007). Traditional methods include freezing point depression, and vapour pressure testing. Freezing point depression is a highly accurate technique where tear samples are cooled and the freezing point is measured, which is then calibrated to the osmolarity of the tears using a standardised scale – higher osmolarity solutions exhibit lower freezing points compared to water (Tomlinson *et al*, 2010). However, although only small tear samples are required (0.2µL), this technique is expensive, technically challenging and is confined mainly to research settings, thus limiting its utility in clinical practice (Bron *et al*, 2007). The vapour pressure method relies on the principal that solutions (such as the tears) have lower vapour pressure than the solvent (such as water) at the same temperature and pressure – thus, solutions take longer to evaporate, and osmolarity of the solution can be inferred from the time taken to do so (Tiffany *et al*, 1994). However, the large tear sample required (5µL) has limited its use (Lemp, 1995, 2007). More recent methods to measure tear film hyperosmolarity are based upon electrical impedence, such as the TearLab osmometer, where the electrical conductivity of the tears (influenced by its ionic content) is measured and calibrated to an osmolarity measurement based on a standardised scale, visible as a digital readout (Ogasawara *et al*, 1996; Eperjesi *et al*, 2012). This device not only requires a very small quantity of tears (50nL), but provides a quick measurement, is simple to operate and is commercially available (Eperjesi *et al*, 2012; Versura & Campos, 2013). A recent

study by Tomlinson *et al* (2010) showed that the TearLab osmometer demonstrated strong correlation and better diagnostic performance than the Clifton osmometer (freezing point depression), suggesting that the TearLab is a suitable instrument to measure tear film osmolarity in a clinical and research setting.

Common tests for dry eye include meaurement of tear film break up time (TBUT), representing the stability of the tear film, another key feature involved in the pathophysiology of dry eye (Bron *et al*, 2007; Lemp, 2007). The tear film break up time is defined as the time interval between the last complete blink and the first appearance of a dry spot or disruption in the tear film (Lemp, 1995). The visualisation of the tear film is enabled by the application of fluorescein sodium to the ocular surface, or the imaging of mires via specular reflection over the cornea (Mengher *et al*, 1986; Johnson & Murphy, 2005). The addition of fluorescein to the ocular surface may itself influence the measurement, given that application may induce reflex lacrimation which artificially lengthens TBUT, and is thus considered an invasive technique (Abelson *et al*, 2002b). Further, as fluorescein is typically applied in solution or via a wetted filter paper strip, this may add volume to the tear film and again artificially lengthen TBUT (Abelson *et al*, 2002b; Johnson & Murphy, 2005). Thus, measurement of TBUT with fluorescein is denoted as the fluorescein break up time (FBUT). The imaging of mires, usually Placido rings or grid patterns, allows non-invasive measurement of the tear film break up time (NITBUT) that is not influenced by reflex lacrimation or addition of fluid to the ocular surface, and therefore provides a more accurate measure (Mengher *et al*, 1986). However, heat given off from the light source may cause the tear film to evaporate and artificially reduce NTIBUT, particularly during periods of measurement where the eyes are kept open (Guillon, 1998).

The Schirmer test is long established estimate of tear flow, and therefore a measure of aqueous production (Van Bijsterveld, 1969; Vitali *et al*, 1994, 2002). The test may be performed with (Schirmer test-1) and without anaesthesia, and involves the insertion of a filter paper strip, usually with a millimetre scale, over the eyelid margin, and the amount of

wetting is then read off the scale after 5 minutes (Vitali *et al*, 1994, 2002). However, the application of the filter paper strip to the eye may induce reflex lacrimation, thus overestimating the results; whereas the application of topical anaesthesia may inhibit the sensory drive for normal lacrimation, underestimating the results, although the transient stinging induced by the topical anaesthetic may cause reflex lacrimation initially (Bron *et al*, 2007). The phenol red thread (PRT) test also measures tear flow in a similar fashion (the length of thread that has changed colour is measured off), but as a small portion of the thread is applied over the eyelid margin and remains in place for 15 seconds there is less reflex lacrimation and improved patient tolerability (Little & Bruce, 1994).

The volume of tears within the ocular surface may be inferred by measurement of the tear film meniscus height (Mainstone *et al*, 1996; Yokoi & Komuro, 2004). Traditionally, the height of the tear film meniscus is performed by varying the height or width of the slit beam produced by a slit lamp, and reading off the corresponding scale, with or without the aid of fluorescein to visualise the tear film (Mainstone *et al*, 1996; Oguz *et al*, 2000; Farrell *et al*, 2003). The addition of fluorescein may however induce reflex lacrimation and or add volume to the tear film thus overestimating tear film meniscus height (Oguz *et al*, 2000). More recently, tear meniscometry has enabled evaluation of the radius of curvature and height of the meniscus using computer software analysis of specular images (Yokoi & Komuro, 2004), but this requires highly specialised equipment.

Determination of the extent of any corneal and conjunctival damage can be assessed by grading the degree of staining of the ocular surface following instillation of topical diagnostic dyes, such as fluorescein sodium and lissamine green (Bron *et al*, 2003, 2007). Fluorescein is believed to be absorbed by damaged ocular surface epithelial cells, such as those affected by the inflammation induced by tear film hyperosmolarity in dry eye, but does not penetrate intact tissue (Feenstra & Tseng, 1992; Wilson *et al*, 1995; Bron *et al*, 2003). Rose Bengal is a dye that stains dead and devitalised/degenerate epithelial cells, but has reduced in popularity given that it causes stinging on application, and is intrinsically toxic,

suppressing human conjunctival epithelial cells in vitro (Feenstra & Tseng, 1992; Kim & Foulks, 1999). Lissamine green stains also dead and devitailsed/degenerate cells in a similar manner to Rose Bengal, but is far less toxic and irritating to the eye and has therefore superseded Rose Bengal for the assessment of ocular surface staining (Feenstra *et al*, 1992; Manning *et al*, 1995; Bron *et al*, 2003; Machado *et al*, 2009). The choice of dye relates to the visibility of the dye on the ocular surface (Bron *et al*, 2003). Corneal staining is best observed using fluorescein given that it may be viewed over both dark and light irises, whereas lissamine green and Rose Bengal are difficult to view over dark irides (Bron *et al*, 2003). However, large amounts of fluorescein or thick tear films may obscure the underlying staining (Bron *et al*, 2003). Conjunctival staining may also be observed with fluorescein, but lissamine green and Rose Bengal show up well against light scattered by the white sclera (Bron *et al*, 2003). As they are poorly visible within the tear film, lissamine green and Rose Bengal do not obscure the underlying staining pattern, which is visible for longer as they do diffuse into the conjunctival substantia propria, whereas fluorescein diffuses rapidly into the surrounding tissue (Bron *et al*, 2003). Staining may be graded using the Oxford system, van Bijsterveld system, and the National Eye Institue/Industry Workshop (NEI; Lemp, 1995; Bron *et al*, 2007). Both the Oxford and NEI systems offer for a wider range of scoring by dividing the ocular surface into different corneal and conjunctival areas, allowing for the detection of small changes in staining (more sensitive). However, no staining system has been demonstrated to be superior to another (Bron *et al*, 2007).

There are many dry eye questionnaires are in common use to evaluate dry eye symptoms, such as the McMonnies Questionnaire (McMonnies, 1987), Schein Questionnaire (Schein et al, 1997, Dry Eye Questionnaire (DEQ; Begley *et al*, 2002), and the Ocular Surface Disease Index (OSDI; Schiffman *et al*, 2000), but few have been validated in dry eye populations (Smith, 2007). Although these tests differ in their length, they explore different aspects of dry eye ranging from diagnosis alone to the impact on the quality of life and determination of risk factors for dry eye (Smith, 2007). However, there appears to be no advantage of one

questionnaire over the other, with selection of a specific questionnaire manily relating to practical factors such as time to administer the test, length of the test, and whether it will be used for diagnosis, clinical trial recruitment or treatment guide (Bron *et al*, 2007; Lemp, 2007; Khanal *et al*, 2008).

With the exception of tear film hyperosmolarity index, the dry eye tests described herein are commonly performed in routine clinical practice (Korb *et al*, 2000). Objective dry eye tests such as meibometry to assess the meibum volume for the diagnosis of MGD (Yokoi *et al*, 1999), cytological assessment (brush or impression) to assess conjunctival inflammation (Brignole *et al*, 2000), fluorophotometry to assess changes in tear flow from measures of tear turnover and volume (Pearce *et al*, 2001), have been confined to research settings as they require considerable technical skill to perform, and expensive and often lab based equipment.

1.13 Treatment of Dry Eye

The need to treat the condition is based upon improving symptoms, quality of life and normalising the ocular surface and tear film in particular as a compromised corneal epithelium may increase the risk of ocular infection (Pflugfelder, 2007). The traditional approach to managing dry eye is to provide symptomatic relief through the application of topical lubricants, and modifying risk factors (Pflugfelder, 2000; Calonge, 2001; Lemp, 2007; Lemp, 2008b). Dietary changes such as vitamin A and fatty acid (such as omega 3) supplementation have been associated with improved dry eye symptoms and lissamine green staining of the ocular surface (Barabino *et al*, 2003; Miljanovic *et al*, 2005). There are a wide variety of topical lubricants, differing according to their composition and in form – drops, ointments, gels and sprays. Some have been produced to provide protection of the ocular surface epithelium, increase goblet cell sensity, and reduce tear film hypeosmolarity rather than lubrication alone, such as TheraTears, which mimic the natural electrolyte

balance of the tear film, and HypoTears, a hypoosmolarity agent (Bernal & Ubels, 1993; Pflugfelder, 2007).

In cases where the lipid layer of the tear film is ineffective such as in evaporative dry eye, liposomal sprays maybe useful (Craig et al, 2010). Topical lubricants may also contain lipids by the addition of castor oil or mineral oil, and have been found to increase the thickness of the tear film lipid layer in patients with dry eye (Di Pascuale *et al*, 2004; Korb *et al*, 2005). All of these are available for sale and supply by the entry level optometrist, with the exception of "Ilube", a POM combination product containing hypromellose and acetylcysteine (an astringent), and celluvisc 1%. Of note is that the evidence to support their use is based upon improvements in patient symptoms and not on resolution of the underlying inflammatory mechanism although some ocular protective effects have been demonstrated (Calonge, 2001; Lemp, 2007; Lemp 2008b). However, it is predicted that ocular lubricants can bring about 25% overall improvement in Rose Bengal staining of the ocular surface after 30 days treatment, assuming no improvement without treatment (Doughty & Glavin, 2009). It may be that topical ocular lubricants help to wash away and or dilute irritating and toxic substances from the tear film but only temporarily, necessitating frequent application and longterm therapy. Thus, macromolecular complexes have been added to ocular lubricants to increase viscocity and retention time on the ocular surface, to prolong this effect. The viscocity agent carboxylmethylcellulose in solution has been shown to have significantly slower clearance rate from the eye compared to neutral hydroxymethylcellulose solution (Pflugfelder, 2007). Further, viscocity agents have been shown to decrease Rose Bengal staining in dry eye patients, suggesting a protective effect of the ocular surface epithelium by coating the ocular surface; and some may even preferentially bind to more hydrophobic, dessicated or damaged areas of epithelium, enhancing this effect (Versura *et al*, 1989; Christiansen *et al*, 2004). It is unclear whether one formulation is superior to another despite the large number of preparations available, and is not often any better clinically than their vehicle or non-preserved counterparts (Doughty & Glavin, 2009; Alves *et al*, 2013). Perhaps more

importantly it is not known which type ocular lubricant can be predicted to be successful based upon clinical findings and symptoms, which has been complicated by the frequent lack of correlation between objective tests and symptoms of dry eye (Pflugfelder, 2007).

Given that dry eye is an inflammatory condition, medical treatment for cases of dry eye unresponsive to lubrication and risk factor modification includes anti-inflammatory agents such as topical corticosteroids and topical immuno-modulation (Perry, 2004; Lemp, 2008b). The immunomodulatory agent topical cyclosporine A 0.05%, called Restasis, is approved for dry eye in the US and has demonstrated significant improvements in dry eye signs and symptoms compared to its vehicle (Perry, 2004; Pflugfelder, 2004; Lemp, 2008b; Kim, 2009). In a randomised, double-masked, vehicle controlled clinical trial, topical cyclosporine was found to significantly reduce Rose Bengal staining, punctate keratitis and symptoms of ocular irritation in patients with moderate to severe dry eye (Stevenson *et al*, 2000). Other clinical trials with cyclosporine have demonstrated similar findings, with significant improvements in corneal fluorescein staining, Schirmer test scores, dry eye symptoms, and need for concomitant ocular lubrication accompanied by a large increase in conjunctival goblet cell density (Sall *et al*, 2000).

The antibacterial and anti-inflammatory properties of tetracyclines may also be of use in treating dry eye associated with eyelid disorders, such as ocular rosacea, posterior blepharitis and meibomian gland dysfunction, often implicated in evaporative dry eye (Lemp, 2007), where the bacterial flora present may otherwise produce lipases, enzymes that cause the lipid layer to breakdown (Dougherty *et al*, 1991; Shine *et al*, 2003b; Ta *et al*, 2003).

In those with aqueous deficiency dry eye, particularly those of moderate to severe forms, patients may benefit from punctal occlusion, where the puncta which drain the tears are blocked with punctal plugs to increase the tear volume and retain the tears on the ocular surface (Plugfelder, 2007). Many clinical studies have demonstrated the efficacy of punctal occlusion in dry eye, with improvements in corneal staining, Schirmer test scores, tear film

break up time, tear film osmolarity and goblet cell density (Balaram *et al*, 2001; Baxter & Laibson, 2004). Other treatment modalities to increase tear aqueous levels in the tear film include tear stimulation via secretagogues and cholinergic agonists, but are not commonly used (Plfugfelder, 2007; Tauber *et al*, 2004). The secretagogue diaquafosol has been found to significantly improve corneal and conjunctival staining, Schirmer test scores, and dry eye symptoms in a randomised, double-masked, placebo (vehicle) controlled parallel group study (Tauber *et al*, 2004). The cholinergic agonist pilocarpine has been evaluated in patients with Sjogren syndrome related dry eye, with one clinical trial reporting improvements in subjective global dry eye assessment and Rose Bengal staining with oral administration compared to ocular lubrication and punctual occlusion, however patients frequently reported, headaches, increased sweating and vomiting with pilocarpine (Tsifetaki *et al*, 2004). Other clinical trials show similar improvements in dry eye signs and symtoms with pilocarpine treatment, including increases in conjunctival goblet cell density (Papas *et al*, 2004; Aragona *et al*, 2006), but significant side effects associated with this treatment has limited its safety profile and therefore is not considered an effective dry eye treatment (Pflugfelder, 2007). Moisture chamber spectacles may also be used to treat dry eye by increasing periocular humidity to reduce evaporation from the ocular surface by increasing the thickness of the tear film lipid layer (Korb *et al*, 1996), but clinical trials to support their use in treating dry eye effectively are lacking (Pflugfelder, 2007).

Environmental risk factors for dry eye can be modified to increase tear production and to decrease evaporation from the ocular surface, such as avoiding anticholinergic medications such as antihistamines and antidepressants; and removing desiccating conditions such as low humidity, air drafts, and high temperatures (Moss *et al*, 2000; Bielory *et al*, 2005). Patients should also be advised to lower display screens below eye level to reduce interpalpebral diameter and encouraged to blink and take breaks regularly when performing near tasks to help decrease evaporative loss from the eyes (Tsubota & Nakamori, 1993).

1.14 Management of Dry Eye in the UK

There appears to be very little research in the scientific literature relating to the actual management of dry eye by primary healthcare professionals in the UK or indeed elsewhere. A survey on therapeutic prescribing in UK optometry by Needle *et al* (2008) showed that dry eye was among the most common conditions seen in practice and was frequently managed by 75% of respondents respectively. Nearly all respondents (99.3%) felt they could manage the condition if given full therapeutic training. This finding was supported by the frequent use of ocular lubricants by optometrists (87%). However, this study did not examine how UK optometrists managed dry eye; identify which therapeutic agents were for a particular condition and the clinical outcomes of optometric management. It is likely that the level of optometric management of dry eye is high, as the Primary Eye care Assessment and Referral Service (PEARS) designed to diagnose and treat relatively minor eye conditions (such as dry eye) in Wales has been shown to be clinically effective and highly accessible by Sheen *et al* (2009). Furthermore, a study comparing the ability of optometrists to correctly identify and manage patients in an accident and emergency department with ophthalmologists demonstrated good agreement between the two groups (Hau *et al* 2007) but the clinical outcomes were not measured and the standards to which they treated dry eye were not described or compared.

Guidelines and standards of dry eye management in the UK are provided for by the College of Optometrists, but no research has been conducted to examine adherence to these recommendations by UK optometrists or the correlation between adherence and clinical outcomes. Recently a study by Lin *et al* (2010) looked at the level of adherence by physicians, including ophthalmologists, in the USA to preferred practice patterns (PPP) of dry eye issued by the American Academy of Ophthalmology (AAO) in 1998 and 2003. The investigators found that although some areas of dry eye did conform to the PPP, the level of conformity to patient education and care management was substandard. However, only one medical centre was assessed and the study did not measure clinical outcomes although the

paper highlights the need to investigate the correlation between adherence to established guidelines and clinical outcomes (Lin *et al*, 2010). Since 2003, the AAO PPP has been updated (2008) and newer guidelines in relation to dry eye have been published with treatment based upon the level of dry eye severity such as the reports by the Management and Therapy Subcommittee of the International Dry Eye Workshop (Pflugfelder, 2007). No research has been conducted with regard to adherence by eye care professionals and measurement of and correlation with clinical outcomes to these newer guidelines. Investigating the current level of practice is important if the impact and burden of dry eye is to be reduced effectively (Lin *et al*, 2010) and data from other healthcare settings such as optometry, pharmacy and general practitioners in primary care is needed to better evaluate the current management of dry eye.

As part of a study investigating the cost of dry eye in a range of European countries (including the UK) the management practices of 23 consultant ophthalmologists were assessed, albeit from interviews and questionnaires (Clegg *et al*, 2006). The investigators found a wide range of diagnostic techniques and significant variations in diagnostic assessments between each country. For example, 50% of mild 47% of moderate and 44% of severe dry eye patients would undergo meibometry and inferometry in Italy but 0% in all severity groups in the UK. In addition, the management of patients based upon differing levels of severity also varied, for example a patient with mild dry eye in the UK would not undergo surgery but approximately 5% may receive temporary or permanent punctal plugs in the other countries (Clegg *et al*, 2006) but the prescribed treatments, mostly ocular lubricants, were similar. In the UK, the prescribed treatments and duration of treatment for all 3 severity groups were nearly identical and included hypromellose, carbomer, polyvinyl alcohol and paraffin, agents which can be sold and supplied to a patient by an entry level optometrist. Furthermore, very few dry eye patients undergo surgical interventions in the European countries surveyed but punctal occlusion was most popular, with 94% of patients who underwent surgery receiving this treatment in the UK. The data however is limited owing

to the low number of ophthalmologists taking part; the definition of dry eye severity differing between the ophthalmologists; and that the opinions of a few ophthalmologists cannot be extended to capture those of all and indeed to all regions in a particular country. The study also limited to reflecting the management of patients by ophthalmologists, who in the UK are likely to represent those with severe forms of dry eye as the majority are managed in primary care or self treat (Clegg *et al*, 2006).

In a survey of worldwide optometrist and ophthalmologist dry eye diagnostic test preference (including the UK), a wide variation in the number and type of tests were reported, suggesting no clear tests are available to diagnose dry eye and highlights the lack of a gold standard (Korb, 2000; Bron *et al*, 2007; Smith *et al*, 2008). History and symptom assessment and fluorescein break up time were the top two preferred tests (28% and 19%) when both optometrist and ophthalmologist data was combined. However, the top four preferred tests did differ between the two groups, such as Schirmer test third most popular for optometrists but most popular for ophthalmologists. This difference may reflect the different types of patients seen, as more severe aqueous deficiency dry eye such as in Sjogren's syndrome is associated with reduced Schirmer values and forms part of the diagnostic criteria for ocular involvement in the disease (Vitali *et al*, 1994; 2002). In a qualitative survey assessing the attitudes toward diagnostic tests of 32 ophthalmologists and 6 optometrists from the UK and Australia, Turner et al (2005) found that many displayed low satisfaction with current tests and current treatment options (Turner *et al*, 2005). A more objective study examining the actual practice of dry eye diagnosis by different ophthalmic professionals in the United States was carried out by Nichols *et al* (2001), where patient charts were retrospectively reviewed. Again, history and symptom assessment was the most commonly performed procedure (82.8%) and frequently two tests were conducted, with symptom assessment and fluorescein staining the most common diagnostic combination (Nichols *et al*, 2001).

Dry eye may also present to GP surgeries, with tear film disorders estimated to make up 4.5% of eye related appointments based upon a study of GPs in the Nottingham area, the fourth most common condition after infective and allergic conjunctivitis and cataract (Sheldrick *et al*, 1993). However, of the investigations made by the GPs taking part in the study, only 9.3% checked visual acuity and 1.1% performed fluorescein staining before making a diagnosis (Sheldrick *et al*, 1993). In addition, 70.3% of consultations resulted in a prescription but no data relating to medications prescribed for tear film disorders is described, although topical corticosteroids was prescribed in one case (Sheldrick *et al*, 1993). The data in this study also suggests GPs are uncertain when dealing with eye problems as the majority of referrals to ophthalmology were to ascertain diagnosis (59.7% of referrals). This is supported by a study investigating the diagnostic agreement between GPs and ophthalmologists where in only 58% of cases was the diagnosis the same, and the most common cause of confusion was between infective and allergic conjunctivitis, dry eye and blepharitis (Sheldrick *et al*, 1992). Furthermore, in 1992 Featherstone and colleagues also report that many GPs in the Devon area display low confidence in diagnosing and managing eye conditions and have a low threshold for referral even when equipment to examine the eyes is available (Featherstone *et al*, 1992). Despite many GPs in the study stating they were confident in diagnosing “watery eyes” (81%), which could be a variety of conditions including dry eye, very few displayed confidence in managing this condition (19%) but 77% would refer this condition later if necessary and many do not have access to specialist ophthalmic equipment such as a slit lamp and tonometer (Featherstone *et al*, 1992). The establishment of eye care centres and hospital eye casualty departments is reported by McDonnell (1988) to reflect this lack of confidence in ophthalmology.

A potential explanation for the lack of diagnostic accord and low confidence in diagnosis and management of eye disease including dry eye may be the result of a lack of ophthalmology training. The limited curriculum in and time allocation in ophthalmology at undergraduate level for physicians has been highlighted by Vernon (1988), and recently Baylis *et al* (2011)

discovered that there is wide variation in the level of ophthalmology training and education between UK medical schools, with some students even receiving no clinical placement. A survey by Shuttleworth and Marsh (1997) into the efficacy of undergraduate and postgraduate ophthalmology training for GPs in the Bristol area showed that GPs felt that their undergraduate training was inadequate, with only 22% feeling it was adequate. Although 83% of GPs found their postgraduate update courses were satisfactory, it did not appear to alter facilities, confidence or understanding in ophthalmology (Shuttleworth & Marsh, 1997). It is clear that GPs do encounter dry eye in practice (McDonnell, 1988; Featherstone *et al*, 1992; Sheldrick *et al*, 1993; Pierscionek *et al*, 2009) but often the investigations made appear inadequate, diagnoses are uncertain or lack agreement with ophthalmologists, confusion surrounds common anterior conditions such as dry eye and they display low confidence when dealing with eye conditions in general. However, this appears to be related to the variability and inadequacy of current undergraduate and postgraduate ophthalmology training, lack of time and the availability of appropriate equipment and resources.

It is also important to consider how patients report the management of their dry eye condition by healthcare professionals in order to determine differences in views on the adequacy of current practice. Again, very little research has assessed the patient reported outcomes of dry eye management but a study by Parry *et al* (2004) examined the management of adult dry eye patients at an eye clinic via questionnaire. The investigators found that despite daily or frequent use of artificial tear substitutes, many patients still exhibited high levels of symptoms, with the mean Ocular Surface Disease Index (OSDI) score of 46.6 (classed as severe) and signs of dry eye disease. However, the patients were from a hospital eye clinic suggesting their dry eye was severe enough to warrant ophthalmological care and this is supported by the majority of patients having Sjogren's syndrome (71.8%), a disease associated with severe aqueous deficiency dry eye (Lemp, 2007). A similar finding was demonstrated by Wojcik and Walt (2002) who looked at the outcomes of dry eye

management in patients with Sjogren's syndrome attending a Sjogren's syndrome Society meeting. They found that many Sjogren's syndrome patients utilised considerable healthcare resources for dry eye but many patients despite frequent use of ocular lubricants (54% > 15 days per month) reported their symptoms to have stayed the same or became worse over last 12 months (78% of respondents). However, these patients may not represent the range of disease severity and it may be that patients differ to those who do not attend these meetings.

1.15 Dry Eye and Allergic Conjunctivitis

Ocular surface diseases such as allergic conjunctivitis may also cause dry eye (Abelson et al, 2003). As described earlier, allergen exposure leads to degranulation of sensitised mast cells mediated by IgE, causing them to release inflammatory mediators and cytokines – this leads to a Th2-lymphocyte within the conjunctival and corneal epithelium, causing sub-mucosal changes (Fujishima *et al*, 1996a; Abelson *et al*, 2003). In a mouse model of allergic conjunctivitis, the mean goblet cell density identified via confocal microscopy and mucin gene expression after 7 days ocular allergen exposure (drops twice a day onto ocular surface) in sensitised mice was found to be significantly reduced compared to control mice eyes after the final allergen exposure (Kunert *et al*, 2003). The loss of goblet cells and inflammation may lead to corneal and conjunctival epithelial damage, which in turn may cause local tear film instability, drying and hyperosmolarity and the development of dry eye (Fujishima *et al*, 1996a; Abelson *et al*, 2003). Indeed, the Beaver Dam epidemiological study identified the presence of ocular allergy as a risk factor for dry eye (Moss *et al*, 2004). Poor tear clearance from the ocular surface in dry eye may cause allergen to build up until an allergic response develops in sensitised individuals, much like where allergen must be added to the eye in increasing concentrations until an allergic response is detected when testing for ocular allergen sensitivity (Fujishima *et al*, 1996a).

Systemic antihistamine therapy for allergy has also been associated with ocular surface dryness (Abelson *et al*, 2003; Ousler *et al*, 2004; Bielory *et al*, 2005). A study by Nally *et al* (2002) found that tear flow and tear volume as measured by fluorophotometry reduced from baseline following four days of oral loratadine treatment in normal healthy subjects. More recently, a study comparing the ocular drying effects of topical epinastine hydrochloride and systemic loratadine in patients with a history of SAC in an investigator masked cross-over study found loratadine produced a significant reduction in tear volume, flow and turnover rate compared to baseline, whereas epinastine produced no significant change in these ocular drying parameters (Ousler *et al*, 2007).

1.16 Meibomian Gland Dysfunction

Meibomian gland dysfunction (MGD) is a condition that encompasses functional abnormalities of the meibomian glands (Nelson *et al*, 2011). The normal function of the meibomian glands secretion is to provide a smooth optical surface, minimise evaporation of the aqueous phase of the tear film, enhance the stability and spread of the tear film, prevent spill-over of the tear film from the lid margin and contamination by sebum, and seal the eyelids during sleep (Foulks & Bron, 2003; Bron *et al*, 2004). Abnormalities of the meibomian glands, specifically anatomic and those relating to the secretion (meibum), lead to tear film instability and or symptoms of ocular irritation and discomfort, typical of dry eye Foulks, 2007). Studies have shown that patient's with dry eye symptoms frequently present with clinical signs of MGD. In a study examining the ocular surface in patients with dry eye, Shimazaki *et al* (1995) reported that 64.6% of patients with dry eye symptoms had obstructed meibomian glands and or glandular tissue loss and Horwath-Winter *et al* (2003) observed MGD in 78% of dry eye patients. More recently, population based studies have found over 60% of those with dry eye had clinical signs of MGD (Lin *et al*, 2003; Lekhanont

et al, 2006). Thus, MGD is considered to be the most common cause of evaporative dry eye (Bron & Tiffany, 2004).

1.17 Epidemiology of MGD

The prevalence of MGD varies considerably, ranging from 3.5% to 69.3% in population based studies with the higher estimates reported in Asian populations (Lin *et al*, 2003, Lekhanont *et al*, 2006; Uchino *et al*, 2006; Jie *et al*, 2008) compared to those with a majority of Caucasian participants (Schein *et al*, 1997; McCarty *et al*, 1998). The reported estimates are difficult to compare owing to significant differences in age and the definitions used to identify MGD (Schaumberg *et al*, 2011). This has been complicated by a lack of universal standard criteria or grading scales for some clinical signs of MGD, such as telangiectasia – thus subjective assessments of these clinical features may also affect these figures (Miljanovic *et al*, 2005; Schaumberg *et al*, 2011). In addition, methodological discrepancies may have introduced bias such as recruiting small numbers which may not be representative of the target population or via invitation where more severe forms of MGD may be over-represented (Schaumberg *et al*, 2011). Clinic based studies reveal a prevalence of between 20% and approximately 60%, but the information they yield are limited as they involve specific or carefully selected patient cohorts making comparison between them difficult (Schaumberg *et al*, 2011).

Causative risk factors for MGD have not been fully established owing to the lack of studies investigating possible particular risk factors of MGD (Schaumberg *et al*, 2011). Instead, the following ocular and systemic associations, shown in table 1.1.1, are hypothesized to occur more frequently in patients with MGD.

Risk Factor	References
Ocular	
Aniridia	Jasteneiah & Al-Rajhi, 2005
Chronic blepharitis	Mathers *et al*, 1991
Contact lens wear	Ong & Larke, 1990 Marren, 1994 Arita, 2009
Floppy eyelid syndrome	Gonnering & Sonneland, 1987
Giant papillary conjunctivitis	Molinari & Stanek, 2000
Icthyosis	Baden & Imber, 1989
Salzmann's nodular corneal degeneration	Farjo *et al*, 2006
Trachoma	Bron & Tiffany, 2004
Systemic	
Aging	Hykin & Bron, 1992 Den *et al*, 2006 Schaumberg *et al*, 2003, 2009 Lemp, 2007
Androgen deficiency	Krenzer *et al*, 2000 Sullivan *et al*, 2002
Atopy	Bron *et al*, 1991
Benign prostate hyperplasia	Schaumberg *et al*, 2009
Cicatrical pemphigoid	Bron & Tiffany, 2004
Complete androgen insensitivity syndrome	Cermak *et al*, 2003
Discoid lupus erythematosus	Ena *et al*, 2006
Ectodermal dysplasia syndrome	Kaercher, 2004
Hematopoietic stem cell transplantation	Ogawa *et al*, 1999
Hypertension	Schaumberg *et al*, 2009
Menopause	Sullivan *et al*, 2002
Parkinson's disease	Tamer *et al*, 2005
Pemphigoid	Kharfi *et al*, 2010
Polycystic ovary syndrome	Yavas *et al*, 2008
Psoriasis	Zengin *et al*, 1996
Rosacea	Zengin *et al*, 1995
Sjogren syndrome	Shimazaki *et al*, 1998
Stevens-Johnson syndrome	Sotozono *et al*, 2009
Toxic epidermal necrolysis	Sotozono *et al*, 2009
Turner syndrome	Bron & Tiffany, 2004

Table 1.17.1: Ocular and systemic risk factors for meibomian gland dysfunction. Adapted from Schaumberg *et al* (2011).

The presence of clinical features of MGD, such as lid margin abnormalities, meibomian gland loss, meibum opacification, has also been found to increase with age suggesting that aging results in meibomian gland atrophy, meibomian gland dropout, and reduced meibum

secretion and quality (Hykin & Bron, 1992; Obata *et al*, 1994; Obata, 2002; Den *et al*, 2006; Sullivan *et al*, 2006; Arita *et al*, 2008). A histopathologic study by Sullivan *et al* (2006) found significant variations in the lipid profiles of meibum between young and older eyes, which may contribute to increased prevalence of dry eye symptoms with age (Sullivan *et al*, 2006). The altered lipid profiles, reduction in meibum quality and meibomian gland orifice metaplasia observed by Sullivan *et al* (2006) in their patient cohort (ages 37-70) is strongly associated with a marked reduction in androgen levels in both sexes of the same age group (Labrie *et al*, 1997). More recently, Nien *et al* (2011) found reduced meibocyte differentiation and cell cycling from eyelid tissue samples supporting the supposition of meibomian gland atrophy with aging (Nien *et al*, 2011). Using infrared meibography to measure meibomian gland dropout, Arita *et al* (2008) found a strong age dependant increase in meibomian gland dropout which was also associated with a reduction in tear film stability (Arita *et al*, 2008). Tear film stability and thickness declines significantly with age, and the observed differences are more marked in females (Maissa & Guillon, 2010).

Although medications such as 13-cis retinoic acid for the treatment of acne has been associated with meibomian gland atrophy, abnormal meibum, reduced tear film stability, and increased tear film osmolarity and dry eye symptoms (Mathers et al, 1991; Egger *et al*, 1995), there are no other reports investigating drug effects specifically on the meibomian glands. Environmental factors such as geography, climate (temperature, humidity) and visual tasks may also be associated with MGD, however it not clear if these factors are causative or exacerbate MGD (Schaumberg *et al*, 2011). Recently, a study investigating MGD and ocular discomfort in video display terminal users found that over 70% had MGD (Fenga *et al*, 2007). The impact of contact lens (CL) wear on the prevalence of MGD is unclear. Using the force required to express meibum from the glands as a definition of MGD, Korb and Henriquez (1980) found a significantly higher proportion of MGD in CL wearers compared to non CL wearers, and Ong and Larke (1990) reported a higher prevalence of MGD in CL wearers after 6 months use (30%) compared to the non CL wearing population (20%).

However, using cloudy or absent meibum secretion upon firm digital expression as a definition of MGD, Hom *et al* (1990) found no statistically significant difference in MGD frequency between CL and non CL wearers. This finding has been found elsewhere, albeit with smaller sample sizes and different definition of MGD (Marren, 1994; Ong, 1996). A sub-analysis of the data in studies also revealed no significant difference between these groups (Schaumberg *et al*, 2011). However, more recently, using meibography to grade meibomian gland loss, Arita *et al* (2009a) found CL wearers to have significantly higher grade compared to non CL wearers. The discrepancy between the sub-analysis and the study by Arita *et al* (2009a) is difficult to resolve owing to different definitions used to identify MGD (Schaumberg *et al*, 2011).

1.18 Classification of MGD

The recent Meibomian Gland Dysfunction Workshop (2011) has recommended a classification based upon the level of meibomian gland secretion (Nelson *et al*, 2011). Low delivery states of meibomian gland secretion are subdivided into hypo-secretive and obstructive causes. Although primary causes of hypo-secretory MGD have not been established, it is associated with meibomian gland atrophy and subsequent loss of function (Nelson *et al*, 2011). However, obstructive MGD, is considered the most common form of MGD (Shimazaki *et al*, 1995; Foulks & Bron, 2003) and is further subdivided into cicatrical and non-cicatrical forms, where the obstruction is caused by either glandular obstruction and or altered secretion (Nelson *et al*, 2011). Cicatrical obstructive MGD is where the meibomian gland ducts and orifices are dragged posteriorly as a result of conjunctival scarring, whereas in non-cicatrical obstructive MGD the ducts and orifices remain in their normal position (Foulks & Bron 2003; Bron & Tiffany, 2004; Nelson *et al*, 2011). In contrast, high delivery or hyper-secretory levels of secretion is characterised by large volumes of meibum expression (Nelson *et al*, 2011). The causes of each subtype of MGD are shown in Figure 5.1.2.

<table>
<tr><td rowspan="7">Meibomian gland dysfunction</td><td rowspan="5">Low delivery</td><td rowspan="2">Hyposecretory</td><td>Primary</td><td></td></tr>
<tr><td>Secondary
Medications</td><td></td></tr>
<tr><td rowspan="4">Obstructive</td><td rowspan="2">Cicatrical</td><td>Primary</td></tr>
<tr><td>Secondary
Trachoma
Ocular pemphigoid
Erythema multiforme
Atopy</td></tr>
<tr><td rowspan="2">Non-cicatrical</td><td>Primary</td></tr>
<tr><td>Secondary
Seborrheic dermatitis
Acne rosacea
Atopy
Psoriasis</td></tr>
<tr><td rowspan="2">High Delivery</td><td rowspan="2">Hypersecretory</td><td>Primary</td><td></td></tr>
<tr><td>Secondary
Seborrheic dermatitis
Acne rosacea</td><td></td></tr>
</table>

Figure 1.18.1: Classification of meibomian gland dysfunction. Adapted from Nelson *et al* (2011).

1.19 Pathophysiology of MGD

The pathophysiology of obstructive MGD is complex, multifactorial and centred on two core mechanisms, hyperkeratinisation and increased meibum viscosity (Knop *et al*, 2011). Hyperkeratinisation is the formation and presence of desquamated epithelial cells within the meibomian gland (Korb & Henriquez, 1980; Henriquez & Korb, 1981). Since the meibomian glands share structural and embryological developmental features common to hair follicles (Knop *et al*, 2011), this process may be the result of the removal of a developmental block that normally prevents keratinisation of the duct due to wide variety of endogenous and exogenous factors such as advancing age, hormonal changes and contact lens wear (Hykin & Bron, 1992; Krenzer *et al*, 2000; Cermak *et al*, 2003; Arita *et al*, 2009a).

Histologic investigations confirmed the presence of this material within the glands and dilated central ducts caused by this obstruction in contact lens wearing patients with MGD (Korb & Henriquez, 1980; Henriquez & Korb, 1981). Obstruction via keratinisation, dilation of the central ducts, cystic degeneration and loss of secretory meibocytes (cells which produce meibum) was also observed in patients with dry eye symptoms, blocked meibomian glands and thickened meibum (Gutgesell *et al*, 1982). Thus, it has been suggested that these obstructions caused by hyperkeratinsation is followed by dilation of the meibomian gland ducts and acinar degeneration and atrophy, observed clinically and meibomian gland loss (Obata, 2002; Knop *et al*, 2011). This is further supported by the presence of keratinised cell material in expressed meibum of MGD patients in biological and immunologic assays (Ong *et al*, 1991). The atrophic degeneration is purported to occur as a result of increased pressure within the gland due to accumulation and stasis of continually produced meibum, and the limitation of duct dilation by the more rigid tarsal plate (Arita *et al*, 2008; Obata *et al*, 1994; Obata, 2002; Knop *et al*, 2011). The obstructions of the meibomian gland via hyperkeratinisation causes reduced delivery of meibum to the lid margin and tear film (Knop *et al*, 2011). In addition, the loss of meibocytes following acinar atrophy results in secondary hyposecretion (Knop *et al*, 2011). Thus, obstruction of the meibomian glands causes low delivery and low secretion of meibum to the lid margin, resulting in reduced lipid availability to the tear film which may cause evaporative dry eye (Knop *et al*, 2011)

Increased viscosity is considered the other most important pathologic mechanism in MGD (Knop *et al*, 2011). This may be either primary, due to increasing age and or hormonal changes (Sullivan *et al*, 2006), or secondary, due to the stasis of the meibum within the obstructed gland and possibly lipid degrading enzymes (Knop *et al*, 2011). Recent studies have found that compositional changes in meibum of MGD patients results in a meibum which is more viscous meibum of normal donors (Borchman *et al*, 2010; Foulks *et al*, 2010). Using infrared spectroscopy to study meibum samples, Borchman *et al* (2010) found that meibum from MGD patients contained more protein and less cholesterol esters and

hydrocarbon groups, resulting in a more ordered and viscous lipid with a higher phase transition temperature (Borchman *et al*, 2010; Foulks *et al*, 2010). This is supported by the decrease in monounsaturated fatty acid in meibum of patients with chronic blepharitis (including MGD) compared to normal samples observed by Shine and McCulley (2000), where a decreased desaturation of lipid may increase it melting temperature and viscosity (McCulley & Shine, 2004). Indeed, the meibum of MGD patients has been reported to have higher melting temperatures compared to normal subjects (McCulley & Shine, 1998; Terada *et al*, 2004; Mitra *et al*, 2005). Thus, it has been suggested that the more viscous lipid would adversely affect the ability of the meibomian glands to secrete meibum, resulting in blockage and obstruction observed clinically (Knop *et al*, 2011).

It is widely accepted that meibomian gland regulation, gene expression, and function (lipid production) is dependent on hormonal control through androgens and oestrogen (Sullivan *et al*, 2002; Knop *et al*, 2011). Thus, it has been suggested that androgen deficiency, caused by either menopause, aging, autoimmune disease, complete androgen insufficiency syndrome, and anti-androgen therapy can lead to or exacerbate existing MGD and subsequent dry eye signs or and symptoms through alteration of the fatty acid profiles of neutral lipids in meibum (Krenzer *et al*, 2000; Sullivan *et al*, 2002; Cermak *et al*, 2003).

The presence of bacterial growth on the lid margin may also influence meibum composition and therefore the pathophysiology of MGD (Knop *et al*, 2011). Studies have shown that commensal bacteria present on the lid margin and within the meibum of patient with chronic blepharitis, the most common being *P. acnes* (Dougherty & McCulley, 1984), can degrade meibomian lipid by their lipases and esterases (lipid degrading enzymes) and lead to significant changes of free fatty acids within the meibum (Dougherty & McCulley, 1986; Knop *et al*, 2011). These free fatty acids may irritate the lid margin epithelium and stimulate keratinisation (Dougherty & McCulley, 1991; Shine & McCulley, 1993). Furthermore, the increased commensal bacteria levels observed in patients with chronic blepharitis may be exacerbated by the increased cholesterol availability by bacterial cholesterol esterase

activity (Shine & McCulley, 1993). Seborrhoea, the generalised increase in sebum production, typically manifested as seborrheic dermatitis, is associated with epithelial hyperkeratinisation of sebaceous glands (Gupta & Bluhm, 2004). In addition, seborrheic blepharitis, which shares similar features to obstructive MGD, is also associated with increased bacterial presence on the lid margin and which can affect the lipid composition of meibum as described above (Jackson, 2008; Knop *et al*, 2011). Therefore, the presence of seborrhoea may also influence the pathophysiology of obstructive MGD (Knop *et al*, 2011).

The role of inflammation in the pathophysiology of obstructive MGD is less clear, given that inflammatory leukocytes were not observed in the majority of meibum samples in histopathologic studies (Korb & Henriquez, 1980; Henriquez & Korb, 1981; Gutgusell *et al*, 1982; Ong *et al*, 1991; Obata *et al*, 1994). However, in-vivo confocal microscopy studies found significantly higher inflammatory cell infiltration in the tarsal tissue of patients with blepharitis and meibomitis, and periglandular inflammatory cell infiltration in patients with MGD compared to healthy controls (Matsumoto *et al*, 2009). However, in-vivo confocal microscopy cannot reliably differentiate between inflammatory cell types, and thus inflammation, as clearly as histopathology (Knop *et al*, 2011). Therefore, based upon the general absence of infiltration of inflammatory leukocytes as observed in present studies, inflammation may not contribute significantly to the pathophysiology of obstructive MGD (Knop *et al*, 2011). However, increased bacterial presence is associated with the release of pro-inflammatory lipids (free fatty acids) which may irritate the eyelid tissue (Dougherty & McCulley, 1986). The inflammatory mediator phospholipase A2 has been detected in increased amounts in meibum of patients with blepharitis, which can lead to the formation of other pro-inflammatory mediators such as arachidonic acid, leukotrines and prostaglandins which can affect both the ocular surface epithelium and possibly tear film stability (Dougherty & McCulley, 1986; Dougherty *et al*, 1991; Shine & McCulley, 2003b).

1.20 Diagnosis of MGD

Diagnosis of MGD is based upon observation and demonstration of abnormal features and function of the meibomian glands (Tomlinson *et al*, 2011). Clinical features of MGD maybe intrinsic and or extrinsic. Intrinsic features relate to those affecting the meibomian gland alone and local eyelid tissue such as meibomian gland dropout, duct obstruction, orifice plugging and qualitative meibum changes, whereas extrinsic features refer to the secondary changes caused by the presence of MGD such as ocular surface damage, tear film instability, and lid margin hyperaemia (Tomlinson *et al*, 2011). The extrinsic features are not exclusive to MGD, but are observed in other ocular surface disorders such as dry eye (Bron *et al*, 2007; Tomlinson *et al*, 2011). Given that MGD may exist alone or cause ocular surface damage and evaporative dry eye, the diagnostic workup must include assessment of the ocular surface and tear film to detect any secondary effects and lacrimal function to differentiate between aqueous deficiency and MGD relates evaporative dry eye (Tomlinson *et al*, 2011). The recent Meibomian Gland Dysfunction Workshop (2011) has categorised MGD in four subtypes, which requires clinical assessment of MGD firstly, followed by clinical assessment of the presence of features found in ocular surface disease in general (Tomlinson *et al*, 2011).

MGD alone may be asymptomatic, representing a preclinical phase which may not be detectable without examination of qualitative meibum changes upon expression (Tomlinson *et al*, 2011). Asymptomatic patients with abnormal meibum secretion and meibomian gland dropout have previously been observed (Korb & Henriquez, 1980; Hykin & Bron, 1992). The remaining subtypes include symptomatic MGD alone, MGD with ocular surface damage, MGD related evaporative dry eye, and MGD associated with other ocular disorders (Tomlinson *et al*, 2011). Although studies have shown that a significant proportion of patients with dry eye symptoms have MGD (Shimazaki *et al*, 1995; Lin *et al*, 2005; Lekhanont *et al*, 2006), current questionnaires do not have the ability to differentiate dry eye symptoms caused by MGD and other causes of ocular surface disease, even between aqueous

deficiency and evaporative dry eye (Tomlinson *et al*, 2011). However, due to the subjective nature of symptoms, the frequent ocular surface damage and dry eye observed in MGD and other ocular surface disorders in general, it is likely that questionnaires alone may not be sufficient to do so (Tomlinson *et al*, 2011). Many questionnaires to assess ocular symptoms associated dry eye are available, and the most common demonstrate good agreement (Simpson *et al*, 2008).

Clinical signs of MGD include meibomian gland dropout, representing total or partial loss of acinar tissue, and is hypothesised to increase with MGD severity (Tomlinson *et al*, 2011). Techniques used to observe meibomian gland dropout include meiboscopy, where a light source is placed on the skin of an everted eyelid to trans-illuminate the meibomian glands and allow clinical observation and grading of dropout of this tissue on the mucosal side of the everted eyelid by silhouette (Robin *et al*, 1985). Meibography represents the same technique but infrared images are captured digitally (near infrared or infrared video camera) or with film (black and white film or infrared film) and can therefore be examined in a masked fashion to permit a more objectivity (Mathers *et al*, 1994; Nichols *et al*, 2005b; Tomlinson *et al*, 2011). However, the trans-illumination technique is difficult to perform in those with thickened tarsal plates, development of infrared film is also considerably time consuming and the light source in contact with the eyelids may cause discomfort (Tomlinson *et al*, 2011; Ngo *et al*, 2012). Recently, non-invasive meibography techniques have been developed where infrared digital photography of the everted eyelids is performed (Arita *et al*, 2008). Arita *et al* (2008) first described this technique using a slit lamp combined with an infrared transmitting filter and infrared sensitive camera to provide images of the meibomian glands such that they appear light compared to a dark background on the everted eyelid (Arita *et al*, 2008). Since then instruments utilising infrared light sources and infrared sensitive cameras have become commercially available such as the Oculus Keratograph 4M and 5M (Oculus Optikgeraete GmbH, Wetzlar, Germany) and Cobra Fundus Camera (BiB Ophthalmic

Instruments, Stevenage, UK). Using the Oculus Keratograph 4M, Srinavasan *et al* (2012) found a significant positive correlation between meibomian gland dropout and OSDI scores.

Other techniques to observe meibomian gland drop out include confocal microscopy, which allows high resolution, in vivo, real time viewing of the meibomian gland acini using image analysis software, although it is invasive in nature as it requires anaesthesia of and contact with the ocular surface (Matsumoto *et al*, 2008; Ibrahim *et al*, 2010; Ngo *et al*, 2013). This instrumentation allows measurement of meibomian gland density per square millimetre and Matsumoto *et al* (2008) showed that loss of meibomian gland density correlated with with MGD severity. Other parameters that allow evaluation of the morphologic changes of meibomian glands using this method include the longest and shortest acinar unit diameter and periglandular inflammatory cell density (Matsumoto *et al*, 2008). Testing of these parameters in patients with simple MGD and healthy control subjects showed that these parameters demonstrated acceptable diagnostic performance (sensitivity and specificity) using receiver-operator curve technique (Ibrahim *et al*, 2010). Optical coherence tomography and ultrasound have also been used to image meibomian gland structure (Bizheva *et al*, 2010; Peyman *et al*, 2012).

Although there are no agreed established standards to quantify meibomian gland dropout, several grading scales have been reported, based upon gland visibility on the lower eyelid using a 0-4 scale (Jester *et al*, 1982); presence or absence of dropout on the lower eyelid using a crude 0-1 scale (Den *et al*, 2006); percentage of gland dropout on the nasal and temporal lower eyelid graded separately using a 0-4 scale (Pflugfelder *et al*, 1998); the number of meibomian glands lost from the central eight on the lower eyelid (Mathers *et al*, 1991); percentage of glands lost on the lower eyelid using a 0-2 scale (Shimazaki *et al*, 1995); percentage of dropout from the nasal half of lower eyelid using a 0-4 scale as part of composite score with eyelid signs and gland expressibility (de Paiva *et al*, 2003); percentage of dropout from upper and lower lid graded separately using a 0-3 scale (“mieboscore”; Arita *et al*, 2008); and based upon acinar density on the lower and or upper eyelid (Matsumoto *et*

al, 2008). The “meiboscore”, ranging from 0-6 after summing the dropout scores from the upper and lower eyelids (0-3) as developed by Arita *et al* (2008), has shown promising diagnostic performance when discriminating between normal eyes and those with obstructive MGD (Arita *et al*, 2009b). Nichols *et al* (2005b) compared a gestalt system (percentage of gland dropout lower eyelid on a 1-4 scale) to individual gland counting after infrared trans-illumination meibography in a validation study and showed that meibography is an effective clinical tool for quantifying meibomian gland dropout. More recently, Pult and Reide-Pult (2012b) compared a computerised grading system of digital infrared images of the meibomian glands in the upper and lower eyelids with subjective assessment using a four and five point grading scale, and found both inter and intra observer repeatability was best with the objective computerised system (Pult & Reide-Pult, 2012b).

Another key clinical feature of MGD is altered meibomian gland secretion, where the quality and expressibility of the secretion is used to quantify these changes (Tomlinson *et al*, 2011). Normally, the quality of meibum expressed via digital pressure is clear and fluid, but in MGD it may become cloudy, viscous, contain particulate matter or opaque and toothpaste like (Tomlinson *et al*, 2011). Expressibility refers to the ease or difficulty to express meibum from the meibomian glands, and in normal eyes, light pressure is expected to express meibum from the ducts (Tomlinson *et al*, 2011). Several grading scales have been developed to quantify these changes, and often combine these characteristics of meibum. Bron *et al* (1991) suggested a 0-3 scale for meibum quailty, with increasing scores reflecting a decrease in transparency of the normally clear meibum after applying firm digital pressure, but the number of glands and location was not stated. A similar meibum quality grading scale is reported by Mathers *et al* (1991), ranging from 1-4 after applying firm digital pressure to the central eight glands on the lower eyelid. Meibum expressibility has also been graded using ordinal scales – after applying firm digital pressure to 5 glands of the upper or lower eyelid, Pflugfelder *et al* (1998) graded the number of glands expressible on a 0-3 scale. Korb and Blackie (2008) suggest application of a standard level of force using a

specially designed instrument to the nasal, temporal and central portion of the eyelids (approximately 8 glands each) and grading based upon the number of glands yielding liquid secretion. However, Shimazaki *et al* (1995) suggest applying varying degrees of pressure to determine how much pressure is required to express the meibum and grading on a 0-3 scale, incorporating the quality of the meibum. Similarly, Arita *et al* (2009) developed a pressure and meibum quality based system on a 0-3 scale.

Finally, changes in eyelid morphology must also be examined, and have also been incorporated in to grading schemes (Tomlinson *et al*, 2011). These include plugging of the meibomian orifices, which appears as an elevation of the orifice above the eyelid and caused by the build-up of meibum and keratinised epithelial cell debris obstructing the meibomian gland ducts (Tomlinson *et al*, 2011). In normal eyes and those with non-cicatrical MGD, the orifices are level with the eyelid margin and lie anterior to the muco-cutaneous junction which separates the external eyelid skin from the palpebral conjunctiva. However, with age, the muco-cutaneous junction advances anteriorly over time such that the orifices now lie behind it and is correlated with MGD (Yamaguchi *et al*, 2006). This process can cause meibomian gland orifice stenosis or obliteration and periductal fibrosis such that meibum cannot be secreted (Tomlinson *et al*, 2011). Other lid margin features of MGD include telangiectasia, lid margin hyperaemia, epithelial ridging between orifices, cystoid gland changes, concretion and chalazia formation, and notching, dimpling and rounding of the lid margin (Tomlinson *et al*, 2011). Arita *et al* (2008, 2009b) utilised a grading scale which scores the presence or absence of lid abnormalities as part of a composite score. Similar composite grading scale has also been developed (Foulks & Bron, 2003).

The recent Meibomian Gland Dysfucntion Workshop (2011) has proposed a diagnostic structure of tests in order to identify MGD followed by diagnostic tests to include or exclude the presence of ocular surface damage and dry eye. The diagnostic subcommittee of the MGD Workshop recommend (Tomlinson *et al*, 2011):

- In asymptomatic patients, gland expression by the application of digital pressure to the eyelids should be added to routine clinical examination to detect non-obvious MGD
- In symptomatic patients or those with morphologic features of MGD (as described above), assessment of the quality and expressibility of meibum following digital pressure to the central and or nasal third of the lower and or upper eyelid should be performed to determine the extent and severity of disease
- The diagnosis of MGD indicates the need to assess for ocular surface damage and dry eye using appropriate tests. The tests to diagnose dry eye and examine ocular surface damage have been described in Chapter 1.
- If MGD related evaporative dry eye is suspected, patients should undergo tests which differentiate between normal and those with general dry eye; followed by tests to distinguish between aqueous deficiency and evaporative dry eye. Thus, if tests of tear flow and or volume are normal, evaporative dry eye is implied.

A sequence of tests has also been suggested – examination of the eyelid and quantification of morphologic features of MGD; expression to assess meibum quality and expressibility; quantification of meibomian gland dropout via meibography; assessment of ocular symptomology using an appropriate established questionnaire such as the OSDI; measurement of blink rate and blink interval; measurement of tear film meniscus height; measurement of tear film osmolarity; measurement of tear film instability via tear film break up time; and measurement of tear film volume via Schirmer test or phenol red thread test (Tomlinson *et al*, 2011).

1.21 Treatment of MGD

The current management of MGD in clinical practice is not well understood, given that MGD is often confused with anterior or posterior blepharitis or may exist alongside these

conditions (Nelson *et al*, 2011; Geerling *et al*, 2011). Recently, a study investigating the prevalence and treatment strategies of blepharitis by selected ophthalmologists (n=120) and optometrists (n=84) in the United States using a survey found that it was very frequently encountered in clinical practice, with over two thirds of patients requiring treatment, and half of this group received prescription medication (Lemp & Nichols, 2009). Treatment for the majority of cases involves eyelid hygiene including eyelid warming therapy, massage, and expression to clear the obstruction within the meibomian glands and restore meibum delivery to the eyelid margin and tear film (Geerling *et al*, 2011).

Topical ocular lubricants are also recommended where dry eye is present (Geerling *et al*, 2011). Although there are currently no published clinical trials demonstrating efficacy in the treatment of MGD, the beneficial effects of topical lubricants can be inferred from studies investigating the efficacy of a wide range of topical ocular lubricants in dry eye (Geerling *et al*, 2011; Pflugfelder, 2007). In Chapter 1, the evidence basis for topical ocular lubrication in dry eye is discussed - given that the core mechanisms of dry eye involve tear film hyperosmolarity due to increased aqueous evaporation and or reduced aqueous production, supplementation of the tear film with topical ocular lubricants serves to reduce tear film osmolarity, reduce friction between the palpebral conjunctiva and ocular surface, and improve the spread of the tear film lipid layer (Korb et al, 2005; Pflugfelder, 2007; Yokoi *et al*, 2008). Furthermore, the application of topical lubricants may also wash away and or dilute toxins, debris and inflammatory material from the ocular surface (Pflugfelder, 2007; Sanchez *et al*, 2010).

As the tear film lipid layer is unable to effectively prevent evaporation of the tear film in evaporative dry eye, topical lipid based treatments have been investigated, but few have been conducted in patients with MGD or even dry eye. Reiger (1990) found statistically significant improvement in Schirmer test scores, tear film break up time and symptoms 1 week and 3 weeks after treatment with lipid containing drops in dry eye patients. However, this study was uncontrolled and patients received different formulations of the treatment

under investigation thus limiting the clinical usefulness of this data. In a double masked, randomised, controlled study where one eye received a liposomal spray and the other saline spray in healthy subjects, Craig *et al* (2010) found statistically significant increase in lipid layer thickness grade at 30 and 60 minutes post application and increase in non-invasive tear film break up time at 30, 60 and 90 minutes in eyes treated with the liposomal spray, but not control eyes. In a randomised, double masked study where one eye received Optrex Actimist (Optima-Pharma, Germany) and the other Dry Eyes Mist (Boots, UK) or Tear Mist (Tesco, UK), Pult *et al* (2012a) found that Optrex Actimist demonstrated a statistically significant increase in comfort and non-invasive break up time in healthy subjects whereas the other liposomal sprays worsened these criteria. The use of lipid based eye drops have also been investigated in patients with MGD – in a randomised, double blind, placebo controlled crossover study, where patients with obstructive MGD (with and without aqueous deficiency dry eye) received a lipid eye drop (2% castor oil and 5% polyoxyethylene castor oil) or placebo for 2 periods of 2 weeks each, Goto *et al* (2002c) found that the lipid eye drop produced a statistically significant improvement in symptom scores, tear film interference pattern, tear film evaporation rate, tear film break up time, ocular surface staining scores, and orifice obstruction scores compared to the placebo eye drop. In an interventional case series, oflaxacin eye ointment, which contains both polar and non-polar lipids, was applied along the lid margin 3 times a day for 2 weeks in patients with refractory dry eye and MGD (Goto *et al*, 2006). Compared to baseline, there was a statistically significant improvement in symptoms of ocular dryness, lipid layer thickness, tear film break up time and meibomian gland expressibility grading scores (Goto *et al*, 2006). However, it is not clear from this type of study if the improvement was attributable to the lipid component alone or the antibiotic effect of ofloxacin, and no control was used (Geerling *et al*, 2011).

Mechanical lid hygiene techniques such as physical expression of the meibomian glands, lid scrubs and cleansing solutions are considered the mainstay of treatment to help unblock and clear the meibomian gland of the obstruction. Although frequently recommended,

randomised, controlled double blind studies of mechanical lid hygiene techniques are lacking, and there is no universally agreed standardised method and patient compliance with treatment remains difficult to monitor. After 2 weeks treatment with lib scrubs and eyelid massage in patients with MGD in a randomised controlled trial, Paugh *et al* (1990) found that this treatment significantly increased the tear film break up time and clinical resolution rates compared to those who did not receive treatment. In a randomised, controlled study to evaluate the effect of topical anti-inflammatory treatment compared to topical ocular lubrication (both groups also performed lid hygiene methods) Matsumoto *et al* (2009) found statistically significant improvement in tear film break up time, ocular surface staining and inflammatory cell density (observed using confocal microscopy) in those receiving anti-inflammatory (steroid) therapy, but surprisingly none of the parameters measured (also Schirmer test 1) improved with ocular lubrication. However, no attempt was made to investigate the effect of lid hygiene alone and symptoms were not measured in this study. In an interventional case series, Romero *et al* (2004) investigated the effect of lid hygiene and heated saline combined with a preservative topical ocular lubricant in MGD patients for 6 weeks – compared to baseline, treatment improved tear break up time and relieved ocular symptoms but these findings were not compared to control subjects.

As discussed earlier, the altered meibum composition in MGD may increase the viscosity and melting temperature of the meibum which contributes to the pathological obstruction of the meibomian glands. Therefore, eyelid warming therapy to melt the pathologically altered meibum to unblock the meibomian gland and restore delivery of meibum to the eyelid margin has been recommended to resume normal tear film lipid layer function and prevent tear film evaporation (Geerling *et al*, 2011). In support of this treatment, Nagymihalyi *et al* (2004) measured the external eyelid surface temperature of normal subjects following application of a 250W infra-red lamp at 50cm and showed increases in eyelid temperature (of 4.9±0.3°C from a baseline reading of 33.4±0.1°C) which significantly increased meibum secretion (of 49±9 Instrumental Units from 154±2IU at baseline). Warming is traditionally achieved using

warm moist compresses (Olson *et al*, 2003; Blackie *et al*, 2008), but alternative sources of heat include warm moist air (Mitra *et al*, 2004; Matsumoto *et al*, 2006), warm compression devices (Goto *et al*, 2002b), light emitting diode and chemical reaction based eye masks (Mori *et al*, 2003; Ishida *et al*, 2008), and heat and pulsatile pressure combination therapy (Lane *et al*, 2012). In a randomised controlled study where one eye received warm moist compress and the contralateral eye received room temperature moist compress (control), Olson *et al* (2003) showed warm moist compresses heated to 40°C applied for 5 minutes resulted in 80% immediate increase in lipid layer thickness (60nm or less to mean of 111nm), with an additional 20% increase after 15 minutes which was associated with significant improvement in dry eye symptoms in MGD patients. However, no increase in lipid layer thickness was observed in control eyes (Olson *et al*, 2003). Using an infra-red warming device twice daily for 5 minutes in MGD patients in an interventional case series, Goto *et al* (2002b) demonstrated significantly improved tear film stability, ocular surface staining, meibomian gland orifice obstruction scores and improved dry eye symptoms compared to baseline. Mori *et al* (2003) reported significant improvements in tear film stability, meibomian gland obstruction, and reduced dry eye symptoms following use of a chemical reaction based disposable eye mask once daily for 5 minutes for 2 weeks in MGD patients compared to baseline, whereas untreated controls did not demonstrate any improvements over the same period. In a small randomised, controlled clinical trial where patients received either warm moist treatment or traditional warm compress treatment (control) for 10 minutes twice daily for two weeks, Matsumoto *et al* (2006) found that the warm moist air treated eyes significantly improved tear film break up time from baseline compared to controls, but both treatments produced a thickening of the tear film lipid layer. Mitra *et al* (2004) reported increased lipid layer thickness and more stable tear film in normal subjects with a warm moist air device. In a study investigating the efficacy of eyelid warming masks in patients with MGD and healthy controls after 10 minutes treatment for 2 weeks, Ishida *et al* (2008) found that the eyelid warming masks produced a significant improvement in tear function (tear film break up time, schirmer test, lipid layer thickness), ocular surface status (via slit

lamp examination) and dry eye symptoms in those with MGD. More recently, a device which heats and applies pulsatile pressure simultaneously, was found to improve tear film stability and signs and symptoms of MGD after one 12 minute treatment session, even lasting up to 12 months (Friedland *et al*, 2011; Greiner 2012; Lane *et al*, 2012; Greiner 2013). Another device which applies warm moist air to the eyelids using goggles has recently been developed (Pult & Reide-Pult, 2012a; Purslow, 2013). This device was investigated in a recent study where it was applied to the eyes of normal subjects – after 10 minutes, although bulbar conjunctival hyperaemia decreased significantly from baseline, limbal and palpebral hyperaemia remained similar to baseline and there was no significant change in tear film stability and ocular surface staining from baseline (Purslow, 2013). In another study, normal subjects received the warm moist air goggles device followed by traditional warm compress therapy in randomised order – the device produced significant improvements in limbal and eyelid redness, and ocular surface staining compared to pre-treatment; whereas only conjunctival redness improved with the warm compress (Pult & Reide-Pult, 2012a).

Despite these studies supporting the use of eyelid warming therapy, it remains a poorly standardised patient performed treatment owing to variations in temperature achieved, duration of application and patient compliance. Furthermore, there is a lack of randomised placebo controlled blinded trials in patients with MGD, and those which compare eyelid warming devices or treatment protocols (Geerling *et al*, 2011). In an attempt to optimise warm moist compress treatment, Blackie *et al* (2008) recommended applying a warm compress heated to 45°C for at least 4 minutes while replacing the warm compress every 2 minutes to maintain adequate warming of the meibomian lipid, suggesting the need for precise and intense treatment with this form of eyelid warming. However, patients are unlikely to heat the warm compress to this temperature at home due to the lack or absence of an appropriate thermometer capable of reliably determining accurate measurements and such intense treatment may be difficult to perform.

Although the role of bacteria in the pathophysiology of MGD, as discussed earlier, is not clear, bacteria may disrupt the lipid layer and cause inflammation through the release of lipases and pro-inflammatory cytokines (Knop *et al*, 2011; Geerling *et al*, 2011).). It may be that condition of the eyelid margin and meibomian glands in MGD are favourable to bacterial growth compared to the normal eyelid, and the increased bacterial presence may trigger the release of these toxic products, as observed in blepharitis (Geerling *et al*, 2011). Thus, antibiotics may be beneficial in treating MGD but it must be effective against those that are implicated in MGD, which currently remains unknown. Therefore, their use in MGD remains speculative (Geerling *et al*, 2011). Few clinical studies of antibiotics in the treatment of MGD have been performed. In patients with ocular rosacea and MGD, the efficacy of metronidazole combined with lid hygiene compared to lid hygiene alone was investigated in a small randomised controlled examiner masked study – the investigators found a statistically significant improvement in eyelid scores in both treated and control groups, but only an improvement in the ocular surface score (pre and post treatment combined) in treated eyes (Barnhorst *et al*, 1996). The effectiveness of azithromycin compared to placebo has been demonstrated in an open label study to produce statistically significant improvements in signs, symptoms, and lipid ordering and phase transition temperature of meibum in patients with MGD (Foulks *et al*, 2010). In an interventional case series study of topical azithromycin in patients with blepharitis, significant improvements in meibomian gland plugging, eyelid margin and palpebral conjunctival redness, and discharge were observed from baseline (Haque *et al*, 2010). In an open label study, patients with posterior blepharitis (including MGD) were randomised to receive azithromycin combined with warm compresses or warm compresses alone – after 2 weeks, only those in the combined treatment group demonstrated significant improvements in meibomian gland plugging, secretion and eyelid redness (Luchs, 2008). Despite these studies showing improvement in clinical signs and symptoms, evidence from randomised, controlled, double blind studies to support antibiotic therapy in MGD are lacking (Geerling *et al*, 2011).

The use of systemic tetracycline and its derivatives (bacteriostatic antimicrobials) in the treatment of MGD and related eyelid disorders is more established, based upon their anti-inflammatory and lipid regulating properties (Geerling *et al*, 2011). The mechanism of this class of antimicrobial in the treatment of MGD is related to the suppression of bacterial lipase production and prevention of the subsequent release of free fatty acid and diglycerides observed in patients with blepharitis, meibomianitis and MGD (Dougherty *et al*, 1991; Shine *et al*, 2003a; Souchier *et al*, 2008). However, oral minocycline has also been shown to reduce the bacterial load on the eyelid margin (Ta *et al*, 2003; Aronowicz *et al*, 2006). The majority of studies demonstrating improvements in clinical signs (eyelid margin and ocular surface inflammation and tear film stability) and symptoms with tetracycline and its derivatives are related to ocular rosacea (which may involve MGD) and MGD but most are not placebo controlled (Frucht-Pery *et al*, 1993; Quaterman *et al*, 1997; Yoo *et al*, 2005). Yoo *et al* (2005) investigated the effect of doxycycline in patients with MGD (refractory to conventional treatment with eyelid hygiene and topical therapy) where they were randomised to receive either high dose, low dose or placebo (control) in an open label study – the high and low dose group demonstrated significant improvements in tear film break up time, Schirmer test and the number and severity of symptoms compared to the control group but no difference between the low dose and high dose in any of the outcome measures (Yoo *et al*, 2005). In another randomised controlled study, tear film break up time improved and branched fatty acid levels decreased significantly in MGD patients treated with minocycline combined with eyelid hygiene compared to eyelid hygiene alone (Souchier *et al*, 2008). Despite the lack of double-blind, randomised controlled studies of these medications, other objective measurements such as lipase activity, meibomian lipid composition and matrix metalloproteinases (molecules that interact with and activate inflammatory cytokines) activity have been shown to improve with their use (Shine *et al*, 2003a; Souchier *et al*, 2008). Therefore, tetracyclines and its derivatives may be considered in more severe cases of MGD and those associated with other eyelid disease (Geerling *et al*, 2011)

The use of steroids to MGD remains controversial, given the uncertain role of inflammation as described earlier in the pathophysiology of MGD (Geerling *et al*, 2011). As the potential for sight threatening complications in MGD is low, it is considered inappropriate to use topical steroid in MGD owing to the risk of cataract formation and intraocular pressure elevation associated with long term use (Geerling *et al*, 2011). Although one short term study has found an improvement in signs (tear break up tie, ocular surface staining, and inflammatory cell density) of MGD using topical steroid therapy combined with eyelid hygiene compared to topical ocular lubricants (Matsumoto *et al*, 2009), none have been performed to evaluate the long term effect and safety of topical steroids in MGD patients (Geerling *et al*, 2011). However, steroid has been found to be beneficial in inflammatory complications of MGD such as chalazia formation (Watson & Austin, 1984). Topical non-steroidal anti-inflammatories are not recommended for long term treatment as required in MGD due to the increased risk of corneal epithelial damage (Gaynes & Fiscella, 2002). However, the topical calinuerin inhibitor cyclosporine, licensed in the US for increasing tear film production in inflammatory dry eye, has been demonstrated to be effective in patients with MGD – after 3 months use in patients with MGD in a randomised, double blind, placebo controlled trial, Perry *et al* (2006) found that cyclosporine produced a statistically significant improvement in Schirmer scores, lid margin hyperaemia, telangiectasia, and ocular surface staining and meibomian gland inclusions compared to placebo, but no improvement in symptoms. In another study where patients with posterior blepharitis (including MGD) were randomised to receive either cyclosporine or tobramycin combined with dexamethasone for 3 months, Rubin and Rao (2006) found that although both treatments resulted in improved signs and symptoms compared to baseline, cyclosporine treatment produced statistically significant improvement in Schirmer scores, tear film break up time, meibum quality compared to the combined treatment. Thus, topical cyclosporine may be considered in MGD associated with aqueous deficiency dry eye unresponsive to conventional therapy (Geerling *et al*, 2011).

Recently, the use of dietary essential fatty acid supplements has been associated with a reduced incidence of dry eye in women (Miljanovic *et al*, 2005). Thus, many clinical trials of essential fatty acid supplements to treat dry eye have been investigated (Kokke *et al*, 2008; Rashid *et al*, 2008; Wojtowicz *et al*, 2011; Kangari *et al*, 2013). However, very few studies of essential fatty acid supplementation have been conducted in patients with MGD (Geerling *et a*l, 2011). In a small double masked, randomised clinical trial, patients with MGD were randomised to receive linoleic acid and gamma-linoleic acid (essential fatty acids), warm compresses alone, or essential fatty acids combined with warm compresses – after 180 days treatment, the combined treatment group was superior to the other groups in reducing symptoms and eyelid inflammation (Pinna *et al*, 2007). However, more recently, in a randomised controlled double blind trial where patients received either essential fatty acid or placebo 3 times a day for 1 year, Macsai (2008) found that both groups demonstrated improvement in symptoms, tear break up time and meibum expressibility and quality scores compared to baseline. There was no statistically significant difference between each group although the improvement was greater in the treatment group. Thus, further evidence with larger scale clinical trials are required to determine if essential fatty acid supplementation is beneficial in MGD (Geerling *et al*, 2011).

1.22 MGD and Evaporative Dry Eye

In obstructive MGD, abnormalities in the delivery of meibum to the eyelid reservoir and or composition and viscosity of meibum may affect the normal functioning of the tear film lipid layer (Foulks & Bron, 2003; Bron & Tiffany, 2004; Foulks 2007). The reduced levels and or altered of meibum may lead to a functionally underperforming tear film lipid layer which can no longer prevent evaporation effectively, causing tear film instability and hyper-osmolarity leading to evaporative dry eye (Bron & Tiffany, 2004; Foulks, 2007). Abnormalities of the tear film lipid layer in MGD also include slow spreading patterns of this

layer over the aqueous component of the tear film (Goto & Tseng, 2003a). In addition, poor spreading of the lipid layer in severe aqueous deficiency dry eye may due to thinning of the aqueous layer may lead to functional evaporative dry eye in the absence of MGD but if present would exacerbate signs and symptoms of dry eye (Goto & Tseng, 2003b; Yokoi *et al*, 2008; Bron *et al*, 2009).

Although the thickness of the tear film lipid layer influences evaporation, a stable lipid layer, regardless of its thickness, has been found to reduce evaporation (Craig & Tomlinson, 1997) suggesting the chemical composition of the meibum is an important factor in retarding evaporation (Bron & Tiffany, 2004; King-Smith *et al*, 2010). The thinning rate of the tear film lipid layer, rather than its thickness, is considered to influence evaporation such that slower rates are associated with reduced evaporation rates and vice versa in a dichotomous fashion (King-Smith *et al*, 2010). It is currently unknown how meibum composition affects the thickness of the tear film lipid layer, tear film stability, and evaporation from the ocular surface (Bron & Tiffany, 2004; Foulks, 2007). However, the tear film lipid layer thickness has been found to significantly positively correlate with tear film break up time, a surrogate measure of tear film stability (Nichols *et al*, 2002; Isreb *et al*, 2003); in addition increased meibum secretion and tear film stability has been found to significantly positively correlate with evaporation rates (Mathers, 1993; Mathers, 2004).

Recent studies have shown significantly thinner tear film lipid layers in patients with MGD compared to normal eyes (Eom *et al*, 2013), and lipid layer thickness is significantly negatively correlated with dry eye symptoms (Blackie *et al*, 2009). Furthermore, meibomian gland loss, characteristic of MGD, has been found to be significantly correlated with lipid layer thickness (Pult *et al*, 2012b; Pult & Reide-Pult, 2012b; Eom *et al*, 2013), evaporation rates (Mathers, 1993; Shimazaki *et al*, 1995; Goto *et al*, 2003d) and tear film stability (Pult *et al*, 2012b; Pult & Reide-Pult, 2012b) and dry eye symptoms (Matsumoto *et al*, 2008; Pult & Reide-Pult, 2012b; Srinavasan *et al*, 2012).

1.23 Measuring the Tear Film Lipid Layer

Although methods to investigate the tear film in relation to dry eye have also been discussed in Chapter 1, the tear film lipid layer, which plays an important role in MGD and evaporative dry eye, may also be investigated using a technique called interferometry. Given that the tear film lipid layer is very thin, light reflected from the anterior and posterior surface produces interference fringes dependant on the thickness of the tear film lipid layer, which can be observed as specular reflection images (Tomlinson *et al*, 2011; Guillon, 1998). Interferometers utilise these optical principles to visualise and analyse the tear film lipid layer pattern, movement and thickness non-invasively, and several types have been developed over the years, typically involving an interference camera (Doane, 1989; Korb *et al*, 1994; Yokoi *et al*, 1996; King-Smith *et al*, 1999) or specially designed hand held device (Guillon, 1998). Based upon the observed difference in pattern between normal and dry eyes, interference images have been graded depending on dry eye severity in Sjogren's syndrome using a modified specular microscope (Danjo & Hamano, 1995), dry eye severity (Goto & Tseng, 2003a), and colour and pattern (Guillon, 1998; Yokoi *et al*, 1996; Goto *et al*, 2003). Interferometers have also been used to investigate the spread of the tear film lipid layer, by measuring the lipid layer spread time, representing the time taken for the lipid layer to stabilise after a complete blink (Goto & Tseng, 2003a). Goto and Tseng (2003a) examined the lipid spread time using interference images in both normal and dry eye subjects, and found the lipid spread time to be significantly slower in dry eye patients (3.54±1.86 seconds) compared to normal eyes (0.36±0.22 seconds). Post blink, the tear film lipid layer moves upwards before stabilising (King-Smith *et al*, 2009)

Interferometry devices have traditionally measured the thickness of the tear film lipid layer using a colour comparison method based upon the colour and pattern of the interference images using Newton's Colour Scale, providing a rough estimate or semi-quantitative value (Guillon, 1998; Korb *et al*, 1994). However, using the DR-1 interferometer (video camera), Goto *et al* (2003d) developed a method to measure lipid layer thickness more precisely

using a sophisticated colorimetric approach, where a new colour chart was created that corresponded to lipid layer thickness ranging from 0-1000nm. Using interferometry, the thickness of the tear film lipid layer normal eyes is reported to range from 20nm-160nm (King-Smith *et al*, 1999; Bron *et al*, 2004; King-Smith *et al*, 2010). In the studies presented herein, the Tearscope (Tearscope Plus, Keeler, UK) was used to non-invasively measure the tear film break up and tear film lipid layer thickness.

The Tearscope consists of a tubular, cold cathode light source housed in a casing that produces even illumination for 360° viewing of the tear film lipid layer over the entire cornea (Guillon, 1998). The cold cathode light source and it position away from the ocular surface helps to minimise any heating and subsequent evaporation of the tear film, and any heat that is produced is mostly within the handle, away from the patient's eyes (Guillion, 1998). The tubular design allows the insertion of filters to observe any fluorescein instilled in the eyes, concentric ring patterns to observe corneal topography, and grid patterns to measure non-invasive break up time over the cornea with a built in digital 0.1 second interval timer (Guillon, 1998). The Tearscope can also be mounted on a slit lamp with a specially designed adaptor to help stabilise the device and make use of its magnification function to aid observation of the tear film lipid layer (Guillon, 1998).

1.24 Ocular Surface Temperature

Human body temperature has long been recognised as a fundamental parameter of tissue metabolism given that all metabolic events within the body produce heat, and has been measured to investigate human physiology in health and disease (Purslow & Wolffsohn, 2005; Tan *et al*, 2009). Thus, measurement of ocular temperature has been widely studied to help understand ocular physiology in health and disease (Purslow & Wolffsohn, 2005). Early methods to measure ocular temperature were invasive such that contact was made between the measurement device and the ocular surface, typically the

cornea (Purslow & Wolffsohn, 2005). However, the development of non-contact infrared sensitive cameras to detect the heat radiated from the human body has made temperature measurement more patient friendly, potentially more accurate, and able to measure in real time over a wider area (Purslow & Wolffsohn, 2005; Tan *et al*, 2009). This method, termed infrared thermography, has been widely used to examine pathological changes in ophthalmology where differences in inter-ocular temperature may indicate the presence of disease (Morgan *et al*, 1993). In medical practice, infrared thermography is mainly used to detect abnormal tissue growth and inflammation in a wide range of conditions such as breast cancer (Ng, 2009) rheumatic disease (Ring, 1998) thyroid disease (Helmy *et al*, 2008), and in the field of dentistry (Gratt & Anbar, 1998).

The measured temperature of the ocular surface at a particular point or area is considered to primarily relate to the overlying tear film (Hamano *et al*, 1969, Fatt & Chaston, 1980; Morgan *et al*, 1993). Hamano *et al* (1969) suggested that at tear film thicknesses greater than 20µm, 100% of the infrared radiation emitted by the cornea will be absorbed by the tear film. However, tear film thickness measurements have been reported in the scientific literature to range from 3µm-40µm in healthy eyes (King-Smith *et al*, 2004), and may therefore affect the extent to which the tear film contributes to the measured ocular surface temperature (Purslow & Wolffsohn, 2007). Within the eye, however, infrared radiation is emitted by an ocular structure will be absorbed by an ocular structure anterior to it (at wavelengths above 3µm) as they have similar absorption and emission characteristics to water (Mapstone, 1968; Van den Berg, 1997) – therefore, posterior ocular structures contribute very little if at all to the measured ocular temperature (Mapstone, 1968). More recently, in a study to investigate the relationship between anterior eye physical properties and ocular surface temperature, ocular surface temperature was mainly associated with tear film stability (measured indirectly via non-invasive break up time; correlation coefficient $r=-0.68$, $p<0.005$) rather than corneal thickness, curvature and anterior chamber depth, thus supporting the

concept that the measured ocular surface temperature is principally related to the tear film (Purslow & Wolffsohn, 2007).

The measurement of ocular surface temperature is influenced by a variety of factors. These include heat transfer via conduction and convection from adjacent structures (Mapstone, 1970), warming of the ocular surface due to the spread of tears over the ocular surface with each blink (Mapstone, 1968), cooling of the ocular surface due to tear film thinning (Morgan et al, 1996; Craig *et al*, 2000), and cooling or warming due changes in blood flow to the eye and or head (Mapstone, 1968; 1970; Morgan *et al*, 1999). Both room and body temperature demonstrates a positive correlation with ocular surface temperature (Freeman & Fatt, 1973; Morgan *et al*, 1993; Girardin *et al*, 1999), with reported increases of 0.15-0.2°C per 1°C increase in room temperature (Mapstone, 1968; Morgan *et al*, 1993). Ocular surface temperature has been shown to decrease in the presence of increased air flow as the transfer of heat from the eye to the air increases under these conditions (Freeman & Fatt, 1973).

Studies of ocular thermography in the normal healthy eye demonstrate a characteristic thermal profile across the ocular surface where the warmer limbal and conjunctival area, (nasal>temporal) surrounds the cooler cornea (coolest centrally). This is due to the differential heating of the overlying tear film by the vascular limbal and conjunctival tissue and avascular cornea (Morgan *et al*, 1993; Efron *et al*, 1989). The central cornea is coolest as it is the most anterior projection of the eye and thus more readily exposed to heat loss to the air – indeed, steeper corneas demonstrate a steeper temperature gradient from the periphery to the centre (Morgan *et al*, 1993). In addition, as the anterior chamber is deeper in the centre than the periphery, the influence of infrared radiation from deeper tissue may be reduced (Purslow & Wolffsohn, 2005). Also, the temperature distribution over the cornea in healthy eyes has been found to be elliptical in shape, due to the horizontal shape of the palpebral aperture (Efron *et al*, 1989). In the normal eye, after the warm tears have spread over the ocular surface post blink, the ocular surface temperature begins to decrease as the

heat transfers to the air immediately (Mapstone, 1968; Efron *et al*, 1989) – the continued reduction in the open eye state may be due to tear film destabilization and or thinning as the tear film evaporates (Efron *et al*, 1989; Morgan *et al*, 1996; Craig *et al*, 2000). However, periods of eye closure have been found to increase ocular surface temperature (Mapstone, 1968).

Ocular surface temperature has been found to be higher in the affected eye than the fellow eye in anterior uveitis caused by increased local blood flow to the limbal and conjunctival blood vessels in response to inflammation (Mapstone, 1968, 1970). Using an infrared bolometer, Efron *et al* (1988) reported a statistically significant positive correlation between conjunctival hyperaemia and ocular surface temperature, confirming the association with inflammation, hyperaemia and temperature and supporting the findings observed in conditions associated with ocular hyperaemia. In their study, conjunctival hyperaemia was induced by instilling hypertonic saline in to the eyes of healthy subjects to observe the change in ocular surface temperature over time (Efron *et al*, 1988). Higher temperatures have also been observed on the lower eyelid, caruncle, nasal and temporal conjunctiva in patients with Grave's ophthalmopathy compared to normal controls, and temperature variation positively correlated with clinical activity score (Chang *et al*, 2008). After the patients were treated with methylprednisolone, temperature of the lower eyelid, caruncle and nasal conjunctiva decreased close to those of normal controls (Chang *et al*, 2008). However, a study by Wachtmeister (1970) found no significant increases in ocular surface temperature due to posterior eye disease - Mapstone (1968) suggests that the increased metabolic heat generated due to posterior segment disease is unlikely to influence the ocular surface temperature as will be quickly dissipated by the choroid, the highly vascular tissue lying between the retina and sclera (Nickla & Wallman, 2010).

On the contrary, lower ocular surface temperature were found in patients with central retinal vein occlusion (and their fellow unaffected eyes) compared to healthy controls, and were lower still in those with ischaemic central retinal vein occlusion – the authors suggest that the

observed reduction in ocular surface temperature in affected eyes may be caused by local blood stasis whereas the reduction in temperature observed in the fellow unaffected eye may be caused by general impairment of blood flow (Sodi *et al*, 2007). In glaucoma, the ocular surface temperature at 5 anatomical points across the ocular surface were also found to be lower in eyes with primary open angle glaucoma compared to healthy controls, and ocular surface temperature significantly negatively correlated with blood resistivity index (Galassi *et al*, 2007), supporting the role of blood flow dysregulation and reduced ocular perfusion pressure in the vascular pathogenesis theory of glaucoma (Flammer *et al*, 2002). Indeed, other studies have demonstrated an association between ocular surface temperature and ocular blood flow. Horven (1975) found that the corneal temperatures in eyes of patients with occlusive arterial disease (polymyalgia rheumatic, central retinal artery embolism or temporal arteritis) were markedly lower than the fellow unaffected or most affected eye. In 1982, Auker *et al* measured temperature of the choroid, scleral surface and conjunctiva in cats and monkeys before and after deliberately increasing intraocular pressure (to decrease choroidal blood flow) and found a reduction in temperature at all locations from baseline. More recently, Morgan *et al* (1999) found that the degree of carotid artery stenosis significantly negatively correlated with ocular surface temperature. The same finding was observed between the relative difference in carotid artery stenosis and relative difference in ocular surface temperature, suggesting that the reduction in blood flow caused by carotid artery stenosis on the affected side could not be compensated for and leads to a reduction in ocular surface temperature on the same side (Morgan *et al*, 1999). In patients with ophthalmic post herpetic neuralgia in one eye, Cardona *et al* (1996) found that the ocular surface temperature of the affected eye was significantly cooler than the fellow eye, with ocular ischaemia and sympathetic nervous system up-regulation suggested as possible causes.

Infrared thermography has also been used to investigate dry eye. In 1995, Morgan *et al* (1995) measured ocular surface temperature at different locations on the eyes of dry eye

patients (with poor tear break up time or Schirmer test) and healthy age and gender matched controls – mean ocular surface temperature and temperature difference between the limbus and cornea was statistically significantly greater in dry eye patients compared to controls. The authors suggested that the higher mean ocular surface temperature in dry eye patients was due to the warming effect of the increased conjunctival and limbal hyperaemia outweighing the cooling effect caused by tear film evaporation in dry eye patients. In a pilot study, Morgan *et al* (1996) found that the cooling rate was statistically significantly faster in dry eye patients compared to healthy controls, and cited the combined effect of increased evaporation rate and ocular surface temperature (which increases evaporisation) observed in the dry eye patients as a potential cause of this faster cooling rate. More recently, Craig *et al* (2000) found higher evaporation rates, osmolarities, and temperature variation factor (describing the variation in temperature across the ocular surface) and reduced tear film stability and central corneal temperature in dry eye patients compared to healthy control subjects. A statistically significant correlation between temperature variation factor and evaporation rate was also found, thus supporting the contention that the faster cooling rates in dry eye patients is caused by increased tear film evaporation (Craig *et al*, 2000). However, higher central corneal temperatures have been measured in dry eye patients elsewhere by Fujishima *et al* (1996b) and Mori *et al* (1997) - it may be that the dry eye patients measured in these studies represented those with normal evaporation rates and therefore a different dry eye subtype (Craig *et al*, 2000).

In MGD, infrared thermography has been used to measure temperature changes of the eyelids following eyelid warming therapy to demonstrate the transfer of heat to the eyelids and meibomian gland tissue contained within to melt the pathologically altered meibum (Mori *et al*, 2003). Changes in eyelid temperature following eyelid warming therapy have also been measured using infrared radiation thermometers (Goto *et al*, 2002b; Matsumoto *et al*, 2006; Blackie *et al*, 2008). Studies examining eyelid warming therapies have often investigated the eyelid temperature during and after treatment, both externally (Mori *et al*, 2003; Matsumoto

et al, 2006) and internally (Blackie *et al*, 2008) to demonstrate heat transfer to the meibomian glands and to avoid thermal injury. Much of the reported literature relates to the temperature of the external eyelids, but Balckie *et al* (2008) argue that measurement of the internal eyelid would serve as a better correlate of the meibomian gland temperature as they are imbedded in the tarsal plate closer to the inner eyelid surface than the external eyelid surface.

In the study presented in this chapter and others, ocular surface temperature was measured using the Thermo Tracer TH7102MX (NEC, Tokyo, Japan), a self-calibrating infrared video camera. This device consists of a lens coated with germanium to permit a specific portion (8-14µm) of infrared radiation to pass to the infrared sensitive detector (focal plane array micro-bolometer), and following signal amplification and processing the output is displayed as a colour coded or greyscale image with corresponding temperature scale (capable of measuring from -20°C to 100°C) on a built in monitor (320X240 pixel display, up to 60Hz frame speed) visible through the eyepiece. The instrument has a thermal resolution of 0.1°C, with an accuracy of ±2% over its full range. This instrument was mounted on to a slit lamp with a specially designed fitting to help stabilise the camera and allow for fine focussing adjustment, and was fitted with a close up lens to view close objects (up to 60cm with 50µm spatial resolution).

1.25 Summary and Aims

Allergic conjunctivitis and dry eye are common eye conditions that produce similar signs and symptoms, may often coexist or even initiate one another. Although both are relatively mild conditions, the impact on quality of life can be profound, affecting vision, ocular comfort, school and work performance, at a considerable economic cost to the patient.

Dry eye is a multifactorial disease, and a range of tests to measure the tear film and ocular surface to diagnose dry eye are available. However, establishing the cause of dry eye remains difficult owing to conflicting results from these tests, but whether doing so affects treatment decisions remains unknown. Evidence to support the use of commercially available eyelid warming devices for the most common form of dry eye, meibomian gland dysfuction related dry eye, appears to be absent from the scientific literature although previous studies of this from of therapy demonstrate efficacy.

Seasonal allergic conjunctivitis (SAC), the most common subtype of allergic conjunctivitis, may often be overlooked as part of general hay fever, thus its true prevalence may be underestimated and treatment may be suboptimal. Non-pharmacological treatments to help alleviate the signs and symptoms of SAC are readily available in optometry and pharmacy practices, but lack evidence to support their use.

Understanding the actual management of these conditions within clinical practice may help identify areas of practice that require improvement to deliver better care for such patients. In addition, clinical studies with randomised controlled trial deisgns targeting patients affected by the disease under investigation would determine the efficacy of the aforementioned non-pharmacological treatments, thus providing robust scientific evidence to aid clinical treatment decisions.

Therefore, the aims of this thesis were to investigate the diagnostic and management capabilities of pharmacy staff in the UK for allergic conjunctivitis and dry eye, and conduct clinical trials to determine the efficacy of eyelid warming therapy for meibomian gland dysfunction related dry eye and non-pharmacological treatments (cold compresses and artificial tears) for SAC. Further, cluster analysis was applied to common dry eye tests performed in both normal and dry eye patients to determine whether distinct clinically relevant groups of dry eye exist, which may help guide treatment decisions.

Chapter 2

The Management of Ocular Allergy in UK Community Pharmacies

2.1 Introduction

In the United Kingdom (UK), as pharmacists do not have access or training in using ophthalmic examination instruments, it is necessary for pharmacists and pharmacy staff (under supervision of pharmacist) to differentially diagnose ocular allergies based upon history and symptoms alone in order to provide correct treatment and management advice. Furthermore, the ability to perform thorough history and symptom questioning is essential for differential diagnosis as the signs and symptoms ocular allergic disease (Bielory, 2000; Bielory, 2011; Friedlaender, 2011), particularly SAC, may not be present at the time of a consultation (Fonacier *et al*, 2001). It has been estimated that 3% of patients consulting a pharmacist suffer from ocular allergy (Wolffsohn, 2009), and combined with the availability of many anti-allergic preparations over the counter, pharmacists and pharmacy staff are frequently in a position to differentially diagnose and manage ocular allergy. However, research into the actual diagnosis and management of ocular allergy by healthcare professionals, such as pharmacists, has not been widely studied (Wolffsohn, 2009).

Methods to assess the management of certain diseases or scenarios by health care professionals have traditionally focussed on practitioner self-completion or interviewer administered questionnaires to evaluate the quality of clinical care and safety. However, self-completion questionnaires often have a low response rate and is subject to self-selection bias, where those with strong opinions or substantial knowledge in the study area are more likely to respond leading to misrepresentation of the intended population; and non-response bias, where the answers of those that did respond may be different than potential answers of those that did not respond (Moriarty *et al*, 2003; Watson *et al*, 2006). These forms of bias are related, have a greater influence in studies with low response rates, and may lead to

polarised views or opinions. Indeed, response rates of healthcare professionals to surveys are considerably low (Watson *et al*, 2006; Rhodes, 2011). Furthermore, self-completion questionnaires are also subject to social desirability bias that may produce an overestimation of ability and an inaccurate perspective of true clinical performance, despite the anonymity they can provide (Rhodes, 2011).

Personal or telephone interviewer administered questionnaires are also prone to self-selection, non-response and social desirability bias, but also interviewer bias where the interviewer may record only part of the answer or interpret the answer incorrectly (Watson *et al*, 2006; Rhodes, 2011). Thus, the ability of these tests to reflect the clinical practice of the entire population may be undermined. More recently, mystery shopper techniques, typically used in market research, are being increasingly utilised in healthcare evaluation and guidelines have been established for their use (Watson *et al*, 2006). In market research, this technique involves the use of trained participants to measure service quality, compliance with specific protocols or regulations, or to obtain information about products and services – this is achieved by simulating a consumer episode based upon a standardised routine (with predetermined responses or questions) with the establishment being evaluated (which does not know the true identity or purpose of the mystery shopper), followed by providing timely feedback (written and/or audiotape recording) about the episode to the investigators (Wilson, 2001; Wiele *et al*, 2005). In healthcare, the mystery shopper refers to a simulated patient who presents and interacts with the healthcare service or professional to test their behaviour (Gaba, 2004; Watson *et al*, 2006; Rhodes & Miller, 2011). Simulated patients have been used extensively in pharmacy practice research to investigate counselling and advice provision, treatment for a range of disease severities (from head lice to HIV and tuberculosis), and public health activities (Watson et al, 2006). Mystery shopper studies are considered to be a robust and reliable tool for investigating patient experiences and actual healthcare professional conduct and behaviour (Gaba, 2004; Watson *et al*, 2006), as they can be designed to not suffer from social desirability bias (the individual is unaware of the

identity of the mystery shopper or that the study is taking place), self-selection bias and non-response bias (the individual investigated does not have choice in taking part or not) (Gaba, 2004; Watson *et al*, 2006). However, no mystery shopper studies have been conducted to investigate the assessment and treatment of ocular allergy in community pharmacies, where treatment is readily available.

2.2 Study Aim

This study aimed to determine and quantify the history and symptom questioning used for diagnosis and management strategies for allergic conjunctivitis employed by pharmacists and pharmacy staff in community pharmacy practices across the UK using a mystery shopper methodology.

2.3 Methods

A mystery shopper technique was used in 100 pharmacy practices across the UK by 4 investigators each visiting different practices alone from October 2011 to January 2012. This was a prospective, observational study as no previous data on the management of ocular allergy by pharmacy staff has been identified – as such, 100 pharmacies were deemed sufficient. Pharmacies were a mixture of independent (n=38) and chain practices (n=62). The same mystery shopper scenario was used by all 4 investigators (PSB, DT, BH, EG). Upon entering the pharmacy practice, the investigators approached the counter and when acknowledged by a staff member made the following opening statement to begin the consultation:

"My brother's eyes are red and itchy. What would you recommend?"

The scenario answers to the subsequent questions on patient history and symptoms indicating a diagnosis of allergic conjunctivitis based on the scientific literature (Chapter 1) are given in Table 2.3.1.

Question	Scenario Answer
Age of patient?	16
Duration of symptoms?	2 days
History of allergies?	Mild dust allergy
Severity of symptoms?	Moderate but not lifestyle changing or debilitating
Bilateral or unilateral?	Bilateral – both eyes affected equally
Stickiness or crusting?	No
Watering or tearing?	Sometimes
Pain?	No
Foreign body sensation?	No
Burning?	No
Headaches?	No
Dryness?	No
Visual disturbance/changes?	No
Contact lens wearer?	No
History of eye problems?	No
Previous treatment used?	Not known
Concurrent medication?	No
GP appointment taken place?	No
Other	No

Table 2.3.1: History and symptom questions and scenario answers

The investigators answered the above questions only when asked but did not volunteer any information other than the opening statement. Inevitable variations in the exact phrase of each question were accepted and answered appropriately. At each consultation attention was paid to whether or not the above questions were asked, the subsequent diagnosis and management strategy. Immediately after leaving each pharmacy practice these details were recorded in a table by hand. All data including the frequency of each history and symptom question asked; the number of questions asked by the pharmacy staff member approached

and management responses were then transferred into Microsoft Excel (Microsoft, USA) spreadsheets for analysis.

Pharmacy practices were not informed that the study was taking place and were visited in semi-random order in a particular location. The order of the locations visited was according to practical and logistical convenience. This study received ethical approval by institutional ethics committee and the research conformed to the Tenets of the Declaration of Helsinki.

2.4 Results

Out of a total of 100 pharmacy practices, 11% of consultations took place with a pharmacist, 21% with a pharmacy assistant or trainee dispenser, 8% with staff with ambiguous job titles where the professional status was unclear (healthcare advisor, healthcare consultant, pharmacy manager, team manager) but in the remaining 60% of consultations it was not possible to determine the professional status without questioning as identification badges or labels were not visible or not worn. All consultations took place with the same person, and none delegated to another staff member. Figure 2.4.1 (below) shows the locations of the towns and cities where the pharmacies were visited.

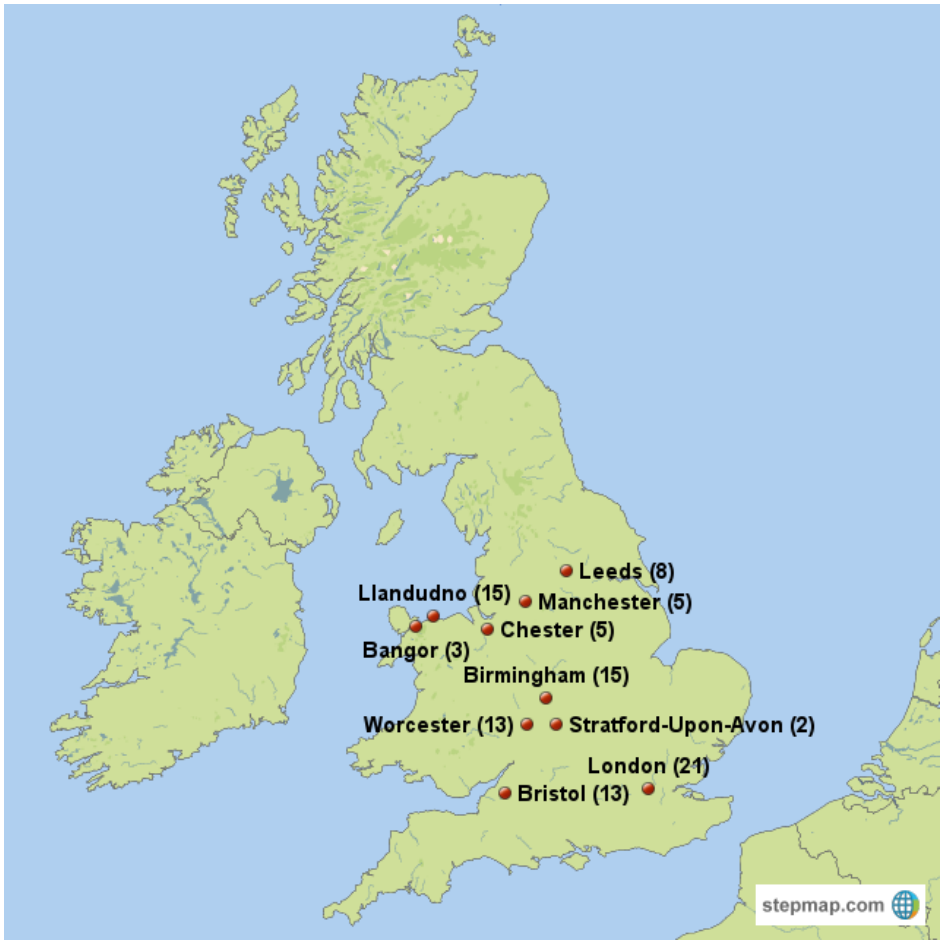

Figure 2.4.1: Image showing the distribution of the pharmacies visited across the UK. Numbers in brackets represent the number of pharmacies visited in that location. Image courtesy of StepMap (www.stepmap.com).

The mean number of history and symptom questions asked by pharmacy staff was 3.5±2.6, ranging from 0-10. At a few practices (7%) pharmacy staff asked no questions at all. Only 11% of pharmacy staff asked additional questions not on the original list (other symptoms, see Table 2.3.1) such as if there was blood in or on the eye; the patient had a cold, used cosmetics; the eyes felt gritty; any eyelid swelling; whether a personal computer was used frequently; and if a new laundry powder had been used. The most common question asked was whether the patient had a history of allergies (45%) followed by any stickiness or crusting of the eyes or eyelid margin (42%). The percentage of each question asked by the pharmacy staff members performing the consultation is presented in Figure 2.4.2.

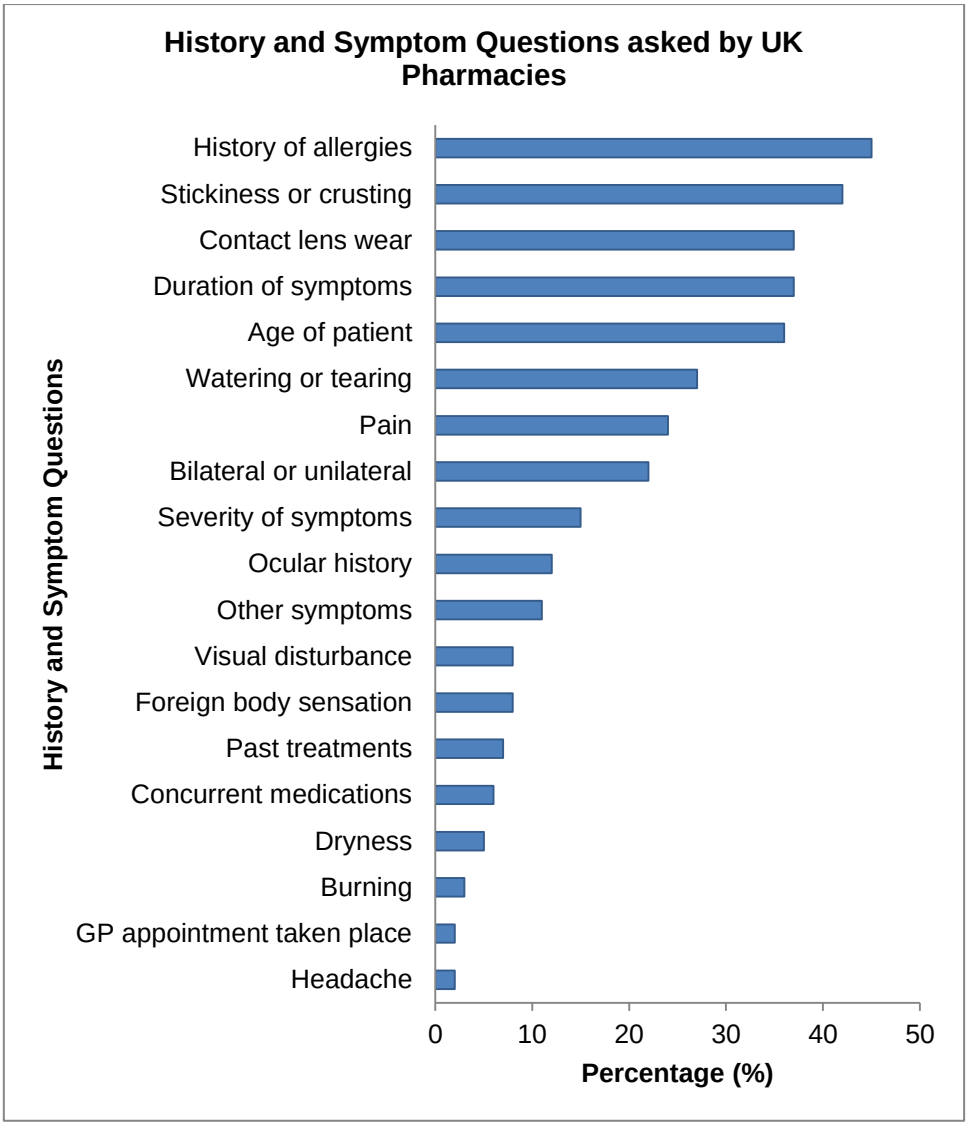

Figure 2.4.2: History and symptom questions asked by the pharmacy staff member performing the consultation following mystery shopper scenario opening statement. No information was volunteered but was given as detailed in Table 2.3.1 when asked by the pharmacy staff member performing the scenario consultation (n=100 pharmacies).

The majority of practices advised treatment (91.0%, n=91), with most of these recommending pharmacological treatments (96%, n=87) in the form of topical eye drops (97%, n=84) and oral medications (3%, n=3). Only 4% (n=4) of the treatments were non-pharmacological, and included allergen avoidance and use of hypromellose (artificial tear supplement). Only 37% (n=32) of pharmacies recommending pharmacological treatment asked the patient's age and only 43% (n=37) asked whether the patient wore contact lenses

when recommending topical eye drops. In addition, pharmacies recommending pharmacological treatment only gave dosage advice in 42% (n=37) of consultations. Those pharmacies that did not recommend treatment (9.0%, n=9) instead referred the patient to their GP (n=4) or for a pharmacist consultation n=4) but only one referred to an optometrist.

Figure 2.4.3: Treatment types and advice combinations recommended by the pharmacy staff member performing the scenario consultation**.**

The most common treatment recommended was topical sodium cromoglycate 2% (50%, n=50) followed by witch hazel (hamamelis virginiana) (28%, n=28). Only 62% (n=31) of pharmacies recommending sodium cromoglycate offered dosage advice. Three percent (n=3) of pharmacies recommended antazoline sulphate 0.5% and xylometazoline hydrochloride 0.05% antihistamine vasoconstrictor combination product (Otrivine-Antistin) and 1% (n=1) recommended naphazoline hydrochloride 0.1% (vasoconstrictor). Only one of

these pharmacies asked about the patient's age, history of eye disease or questions pertaining to systemic health. The antibacterial agent Brolene (propamidine isetionate 0.1%) was recommended in 6% (n=6) of consultations although 67% (n=4) of these pharmacies did not ask if the patient had stickiness or crusting of the eyes. Three percent (n=3) of pharmacies recommended systemic and topical treatment, combining oral antihistamine with hypromellose (artificial tear substitute) or sodium cromoglycate. Only 5% (n=5) of pharmacies advised follow up where the investigator was asked to bring the patient to the pharmacy. Fourteen percent (n=13) of pharmacies recommending treatment advised making an appointment with the patient's GP either to confirm diagnosis or if treatment was ineffective or symptoms became worse. However, only one pharmacy advised referral to an optometrist for diagnosis confirmation or further investigation if symptoms did not resolve with treatment or worsened (Figure 2.4.4).

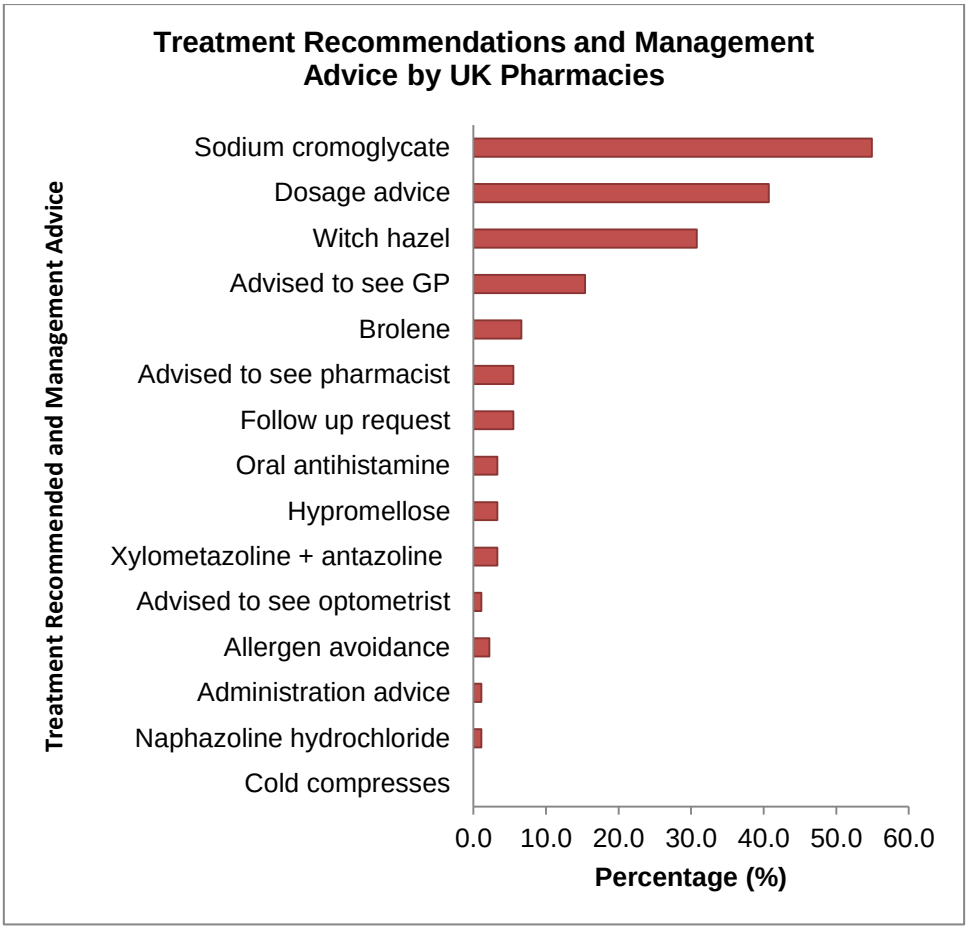

Figure 2.4.4: Treatment recommendations and management advice given by the pharmacy staff member performing to the presented patient scenario.

2.5 Discussion

A mystery shopper technique was used to simulate a realistic consultation scenario in order to determine the history and symptoms questions asked and management strategy recommended by pharmacy staff in practices across the UK. Pharmacies were not informed that the study was being conducted and the pharmacy staff were unaware that the consultation was simulated – thus eliminating the possibility of the Hawthorne effect influencing the outcome of the consultation. Although some authors have suggested mystery shopping techniques are unethical (Ng Kwet Shing & Spence, 2002), such criticisms relate to competitive intelligence gathering between competing businesses whereas this study aims to identify the ability of pharmacy staff to identify and manage allergic conjunctivitis in

the interest of educational needs, cross referral necessity and public safety (Rhodes & Miller, 2012). Patient satisfaction with healthcare services is insufficient to fully evaluate healthcare service provision and safety alone as they often lack the expertise to formulate criteria to measure performance (Moriarty *et al*, 2003; Rhodes, 2011). In addition, some authors argue that impossible to fully evaluate healthcare, such as psychiatric services, without using deceptive methods such as mystery shopping (Gaba, 2004; Walker & George, 2010). Analysis of patient records may be used to evaluate diagnostic and management performance, but pharmacies do not generally offer private consultations, or keep patient records. Mystery shopping techniques have previously been employed extensively in healthcare research including pharmacies to evaluate service provision, such as counselling and detection of drug interactions (Moriarty *et al*, 2003; Watson *et al*, 2006), but none have investigated the management of eye disease, such as ocular allergy, in pharmacy practice.

The methodology required the investigator to begin the scenario with the first member of staff to acknowledge them in order to simulate a realistic query by a member of public, rather than request the pharmacist directly. The opening scenario statement and answers to 19 key history and symptom questions were selected to indicate a diagnosis of allergic conjunctivitis in a 16 year old male. Allergic conjunctivitis is characterised by signs of conjunctival hyperaemia, chemosis, eyelid swelling, watering and symptoms of itching that are typically bilateral and mild to moderate in severity (Cox, 2007; Bielory 2008a; Berdy & Berdy, 2009). Treatment in this case should involve advice on allergen avoidance and treatment with artificial tears, cool compresses, topical mast cell stabilisers, anti-histamines or antihistamine-vasoconstrictor combination products (Bielory 2008b; Chigbu, 2009b).

No pharmacy visited in the study asked all of the 19 history and symptom questions designed to elicit the above signs and symptoms to differentially diagnose allergic conjunctivitis. Instead, pharmacists and pharmacy staff elicited only a few questions (3.5±2.6) for diagnosis. The low number of questions asked on average by pharmacists and pharmacy staff identified in the present study are consistent with low number of criteria

covered on average by pharmacists when diagnosing ocular allergy in the study by Wolffsohn (2009). The high proportion of treatments offered (91%, n=91) compared to referral (9%, n=9) suggest that pharmacists and pharmacy staff are perhaps confident in their abilities to diagnose and treat allergic conjunctivitis. This is supported by very few pharmacies advising referral to another healthcare professional alongside treatment to confirm diagnosis or for further investigation (8.8%, n=8). This finding is also consistent with the study by Wolffsohn (2009) where all pharmacists recommended pharmacological treatments. Only 2 of the pharmacies in this study recommended referral to an optometrist (including one directly without offering treatment) despite these healthcare professionals having training and knowledge specific to the eye and specialist ophthalmic equipment to examine the eyes. Very low levels of referrals to optometrists by pharmacists were also found in the study by Wolffsohn (2009). This may be due to a lack of awareness of the abilities and role in primary eye care optometrists provide by the public and amongst other healthcare professionals, such as pharmacists. Conversely, the optometric profession may not be promoting or communicating their abilities effectively. Instead, pharmacies referred to either GPs or the pharmacist working in the same pharmacy. It has been highlighted in one study that GPs are often confused between dry eye, blepharitis, infective conjunctivitis and allergic conjunctivitis when comparing ocular diagnoses with ophthalmologists (Sheldrick *et al*, 1992). In addition GPs were found to demonstrate low confidence in ophthalmology in general from a GP survey (Featherstone *et al*, 1992). The low diagnostic accord and confidence in eye disease by GPs may be due to the lack of undergraduate ophthalmology training (Bayliss *et al*, 2011) and or ineffective postgraduate ophthalmology training (Shuttleworth & Marsh, 1997). Perhaps pharmacists and pharmacy staff perceive ocular symptoms as part of a systemic allergy that requires GP investigation rather than a local allergy of the eyes.

Many of the differential diagnoses of allergic conjunctivitis described above (Table 2.3.1) have features that may only be visible upon ophthalmic examination in order to arrive at a

correct diagnosis. However, it was not surprising that the pharmacist/pharmacy staff did not perform an examination as the patient was obviously not present (simulated) and they do not possess the training or specialist equipment to examine the eyes. Furthermore, history and symptom questioning alone may not be sufficient for the differential diagnosis of ocular allergic conditions. In the study by Wolffsohn (2009), 150 patients with a history indicative of ocular allergy determined from a questionnaire, blood and ocular sensitivity were examined to confirm the diagnosis – the author found that the questionnaire, which elicited thorough history and symptoms, only had a sensitivity of 54% and specificity of 29% for diagnosing ocular allergy to grass pollen (Wolffsohn, 2009).

Allergen avoidance strategies have been identified as the primary treatment for all allergic conditions as preventing or minimising exposure to the causative allergen prevents the initiation of a hypersensitivity response (Abelson *et al*, 2002a; Bielory 2008b; Chigbu, 2009b). However, this advice was given in less than 2% of pharmacy consultations. The low level of advice given to patients regarding ocular allergies by pharmacists was also reported by Wolffsohn (2009).

Half of pharmacies advised the use of sodium cromoglycate 2%, a mast cell stabiliser which is available as an OTC preparation in the UK. Mast cell stabilisers work by preventing the degranulation of sensitised mast cells and therefore inhibit the release of pre-formed inflammatory mediators (Abelson *et al*, 2002a; Leonardi, 2005a; Schultz, 2006). The efficacy of sodium cromoglycate 2% compared to placebo in the treatment of allergic conjunctivitis has been demonstrated in both environmental studies (Lindsay-Miller, 1979; Leino *et al*, 1992) and conjunctival challenge models (Montan *et al*, 1994). Interestingly, none of the pharmacies recommended lodoxamide trometamine 0.1%, a mast cell stabiliser (OTC) which has been demonstrated to act faster and produce a greater improvement in signs and symptoms of ocular allergy than sodium cromoglycate 2% (Fahy *et al*, 1992). The second most common treatment was topical witch hazel (hamamelis virginiana), a plant extract with purported astringent properties was recommended in just over one quarter of consultations.

Despite a number of products available, there is no evidence in the scientific literature regarding the pharmacological action of witch hazel or its efficacy in treating ocular allergy.

Less than 5% of pharmacies recommended a topical vasoconstrictor or vasoconstrictor-antihistamine combination product. The vasoconstrictor naphazoline hydrochloride and has been shown to be more effective in treating allergic conjunctivitis compared to placebo (Miller & Wolf, 1975). There appears to be no scientific literature on the efficacy of the vasoconstrictor-antihistamine xylometazoline-antazoline in treating allergic conjunctivitis but one study found mild sympathomimetic responses (vasoconstriction of conjunctival blood vessels and pupil dilation) in the eyes of 16 healthy volunteers (Trew *et al*, 1989). Several other vasoconstrictor-antihistamine combinations, including antazoline sulphate, were found to be effective in allergic conjunctivitis, although no significant difference was found between each treatment in alleviating the signs and symptoms (Lanier *et al*, 1983). Naphazoline and xylometazoline work owing to their sympathomimetic activity - stimulation of adrenergic receptors in blood vessels causes vasoconstriction and reduced blood flow, resulting in reduced hyperaemia, eyelid swelling and chemosis (Bielory *et al*, 2005; Schultz, 2006). H1 antihistamines, such as antazoline sulphate, are competitive antagonists of H1 histamine receptors in the conjunctiva and eyelids which prevent binding of histamine, an inflammatory mediator released upon mast cell degranulation (Bielory *et al*, 2005; Bielory & Ghafoor, 2005; Schultz, 2006). This prevents vasodilation and increased vascular permeability which would otherwise cause itching, hyperaemia and swelling (Bielory & Ghafoor, 2005; Schultz, 2006). Of concern is that only one of the pharmacies recommending these products asked the patient's age or whether they had concurrent medical conditions or eye disease. Topical sympathomimetics are not selective and are therefore contraindicated in patients with glaucoma (owing to pupil dilation), and should be used with caution in patients with heart disease, high blood pressure and diabetes (Chigbu, 2009b). Furthermore, xylometazoline-antazoline may only be used in children from 12 years onwards.

Oral antihistamines were recommended in less than 5% of pharmacies alongside a topical treatment, either hypromellose or sodium cromoglycate. Oral antihistamines are indicated where ocular and systemic allergies are present such as hay fever where the eyes, nose and throat is involved (Bielory, 2002; Bielory et al, 2005). Although they are not as effective or safe as topical anti-allergic medications in treating allergic conjunctivitis (Davies *et al*, 1996; Bielory *et al*, 2005), several studies have demonstrated improved efficacy when they are combined (Lanier *et al*, 2001; Abelson & Kaplan, 2002; Crampton, 2003). Another interesting finding is that none of the pharmacies recommended the use of multi-action anti-allergic drugs which combine mast cell stabilising and antihistaminic properties, such as olopatadine hydrochloride, despite being superior to mast cell stabilisers and antihistamines in treating ocular allergies and requiring fewer daily doses (Butrus *et al*, 2000). However, these are only available with a prescription and this may have been the intention of pharmacy referrals to GPs.

Hypromellose, a tear supplement for dry eye (Pflugfelder, 2007), may aid removal of allergens by flushing them from the ocular surface and act as barrier to further allergen exposure (Bielory 2008b; Chigbu, 2009b). Cooled artificial tears and cold compresses may also cause vasoconstriction of the conjunctival blood vessels and bring about symptomatic relief (Bielory 2008b; Chigbu, 2009b). Therefore, the use of non-pharmacological treatments should come first to minimise exposure and the initiation of the hypersensitivity response. However, despite several authors recommending their use as adjunct therapy (Abelson *et al*, 2003; Bielory 2008b; Chigbu, 2009b; Wong *et al*, 2009), evidence of efficacy, although plausible, is lacking. Treatment advice was therefore evidence based in only 59.3% of consultations where eye drops were recommended.

Incorrect treatment advice was given by 6% of pharmacies, in the form of brolene (propamidine isetionate 0.1%), an antibacterial mediation with no anti-allergic therapeutic effect – indicating a diagnosis of bacterial rather than allergic conjunctivitis. However, only 2 out of 6 of these pharmacies asked if the patient had stickiness or crusting of the

eyelids/lashes and if they had any discharge from the eye, key questions in the differential diagnosis between bacterial and allergic conjunctivitis (Granet, 2008). Treatment with this agent would have no therapeutic effect and may have prolonged signs and symptoms unnecessarily. Although administration of the eye drop may help to flush out allergens from the ocular surface, the patient may mistakenly believe the antibacterial reduced their symptoms and may use or request the same agent when similar symptoms develop. Furthermore, topical administration of medications requires removal of contact lenses as the active ingredient and preservatives can bind to the contact lens, causing build-up and prolonged exposure which may result in ocular toxicity or drug-induced allergic inflammation (Lemp, 2008a). Sodium cromoglycate requires 4 times daily dosage to produce a therapeutic effect; therefore contact lens wear is not suitable. Olopatadine hydrochloride and epinastine hydrochloride may be useful in contact lens wearers with ocular allergy as it requires twice daily dosage which can be applied before insertion and after removal (Brodsky *et al*, 2003). However, less than half of pharmacies recommending topical medications asked about contact lens wear. In addition, less than half of these pharmacies gave dosage advice. Thus a significant proportion of patients may suffer from toxicity reaction or drug induced inflammation from build-up of the medication in their contact lenses or from incorrect usage. In addition, these patients may not be compliant with the required dosing and administration which may prolong their symptoms and cause them to suffer unnecessarily.

This study is limited to the conduct and behaviour of pharmacy staff in general, and cannot be extrapolated to pharmacists as the professional identity of over half of staff was unknown. However, the study was intended to reflect a real world scenario where a patient attends and encounters a member of staff that acknowledges them and did not exclude cases where the initial staff member delegated the scenario to another staff member, although this happened very rarely. The use of audiotape recording may have helped to minimise interviewer bias and validate the mystery shopper report (Watson *et al*, 2006), but the present study utilised a standardised report form where information could be checked off based upon the expected

outcomes and space was available to write any additional information that was offered. Consistency of reporting and how much information was provided by mystery shoppers have limited some previous pharmacy practice studies (Yelland *et al*, 1998; Watson *et al*, 2004; 2006), but the present study used only well trained mystery shoppers (n=4) and a standardised scenario with predetermined answers where no further information was volunteered.

2.6 Conclusions

The history and symptoms questioning by UK pharmacists and pharmacy staff for the differential diagnosis of allergic conjunctivitis, a common form of ocular allergy, is poor, given the number of eye conditions which have similar presentation. In addition, many of these conditions require different treatments strategies for effective management. Pharmacists and pharmacy staff place their confidence in diagnosing allergic conjunctivitis in only a few questions, relating to allergy history and eyelid signs. Although treatments were recommended in most of consultations, just over half were based on evidence in the scientific literature. Furthermore, dosage and administration advice and questioning of contact lens wear was low, which may place patients at risk of further complications and unnecessary suffering. In cases where referral was advised, only 2 referred to optometrists, despite them possessing training, knowledge and equipment specific to the eyes. This study has highlighted the need for improved ophthalmology training for both pharmacists and pharmacy staff and the need for increased awareness of the optometric profession. Optometrists also need to improve communication and promote their skills and examination equipment better to the public and healthcare professionals.

Given that approximately 1 in 30 pharmacy patients have ocular allergy (Wolffsohn, 2009), unless there is no doubt about the diagnosis, pharmacists should refer patients with suspected ocular allergy to healthcare professionals with training, knowledge and equipment

available to examine the eyes, such as optometrists, to confirm diagnosis or further investigation in order to develop an effective management plan. If pharmacological treatments are advised, only those which demonstrate efficacy should be recommended on an individual patient basis, although artificial tears and cold compresses may also be effective. Oral antihistamines should be recommended when other allergies occur concurrently.

From the present methodology it was difficult to distinguish between pharmacists and the different types of pharmacy staff, so comparisons between them were not possible. Future mystery shopper studies could investigate the knowledge of pharmacists by specifically requesting a consultation. In addition, scenarios where the patient presents with symptoms indicative of different ocular conditions would allow investigation of how pharmacies manage a wide range of eye disease and identify areas of ophthalmology training which may require improvement.

Chapter 3

The Management of Dry Eye in UK Pharmacies

3.1 Introduction

The traditional approach to managing dry eye is to provide symptomatic relief through the application of topical lubricants (Pflugfelder, 2007). There are a wide variety of topical lubricants, differing according to their composition and form, including drops, ointments, gels and sprays (Pflugfelder, 2007; Doughty & Glavin, 2009). Many of these treatments are available as over the counter preparations in UK pharmacy practices. Despite the wide range of topical lubricants available, there is a paucity of research relating to how dry eye is managed by healthcare professional services in the UK, with most relating only to optometrists and ophthalmologists (Korb, 2000; Turner *et al*, 2005; Clegg *et al*, 2006; Graham *et al*, 2010). As qualified and trained professionals in safe and effective medicine use and prescribing in primary care, pharmacists are in a key position to interact with patients. Further, over the counter and pharmacy only medications can be given to patients without prescription. Topical anti-allergic medications fall into this category. Thus, the pharmacist has a duty to give advice about medicines, their use and indication, thus necessitating a diagnosis.

3.2 Study Aim

Given the large number of over-the-counter dry eye treatments available in pharmacy practice and the lack of objective dry eye management research, the aim of this study was to investigate the management of dry eye by community pharmacy practices across the UK.

3.3 Methods

A mystery shopper technique was used in 50 community pharmacy practices across the UK by 2 investigators (GLT, PSB) each visiting different practices alone in order of convenience from October 2012 to January 2013, usin the same methodology as Chapter 2. This was a prospective, observational study as no previous data on the management of dry eye by pharmacy staff has been identified – as such, 50 pharmacies were deemed sufficient based upon practical and logistical constratints. The pharmacies were a mixture of independent (n=12) and chain practices (n=38), selected at random from major towns and cities from www.yell.com (a business and service address listing website) using a random number generator. These pharmacies were different to those visited in Chapter 2. The same mystery shopper scenario was used by both investigators. Upon entering the p harmacy practice, the investigators approached the counter and when acknowledged by the staff (pharmacist or pharmacy staff under supervision of a pharmacist) made the following opening statement to begin the consultation:

"My mother's eyes are sore and gritty. What would you recommend?"

The scenario answers to the subsequent questions on patient history and symptoms indicating a diagnosis of dry eye based upon the International Dry Eye WorkShop (Lemp *et al*, 2007) are given in Table 3.3.1.

Question	Scenario Answer
Age of patient?	50
Duration of symptoms?	2 weeks
History of allergies?	No
Severity of symptoms?	Moderate but not lifestyle changing or debilitating
Bilateral or unilateral?	Bilateral – both eyes affected equally
Stickiness or crusting?	No
Watering or tearing?	Sometimes
Itching?	No
Discharge?	No
Pain?	No
Foreign body sensation?	Yes, occasionally
Burning?	Yes, occasionally but mild
Headaches?	No
Dryness?	Yes, worse toward end of the day
Visual disturbance/changes?	Vision improves after blink
Contact lens wearer?	No
History of eye problems?	No
Previous treatment used?	Not known
Concurrent medication?	No
GP appointment taken place?	No
Other	No

Table 3.3.1: Mystery shopper scenario answers to the questions on patient history and symptoms indicating a diagnosis of dry eye.

The investigators answered the above questions only when asked but did not volunteer any information other than the opening statement. Inevitable variations in the exact phrase of each question were accepted and answered appropriately. At each consultation attention was paid to whether or not the above questions were asked, the subsequent diagnosis and management strategy. Immediately after leaving each pharmacy practice these details were recorded in a table by hand. All data was then transferred into Microsoft Excel (Microsoft, USA) spread sheets for analysis.

Pharmacy practices were not informed that the study was taking place and were visited in random order in a particular location. The order of the locations visited was according to practical and logistical convenience. This study received ethical approval by the institutional review board and conformed to the Tenets of the Declaration of Helsinki.

3.4 Results

A total of 50 pharmacies were visited between July 2012 and January 2013 in major cities across the UK, including London (n=12), Birmingham (n=5), Manchester (n=9), Worcester (n=7), Bristol (n=7), Leeds (n=3) and Nottingham (n=7). Of these, 38 were chain and 12 were independent businesses. Staff types from name badges or identification included pharmacists (n=34), pharmacy assistants (n=3), dispensing assistants (n=4), pharmacy manager (n=3), and an unknown group (unidentifiable from name badges or identification, n=6).

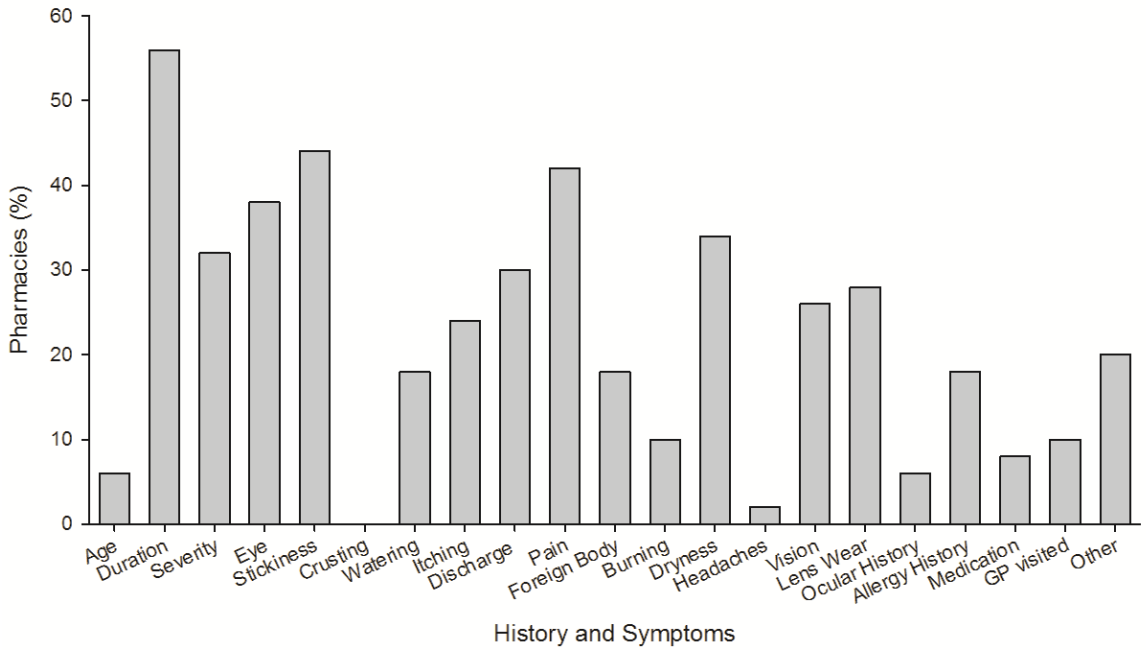

Figure 3.4.1: Percentage of each history and symptom questions asked by UK pharmacy staff (n=50).

The mean number of history and symptom questions asked by UK pharmacy staff was 4.5 (SD 1.7; range 1 to 10), with the most common being duration of symptoms (56%, n=28). The least common was whether the patient had a history of headaches (2%, n=1), whereas no staff member asked for the presence of crusting (Figure 3.4.1). Twenty-per cent (n=10) of

staff asked additional questions (“other”), including whether there was a history of computer use (n=2), when and if symptoms increased in severity (n=3), a history of inflammatory disease (n=1), symptoms of dry mouth (n=1), symptoms of poor concentration (n=1), symptoms of diplopia (n=1) and whether the patient had seen an optometrist (n=1).

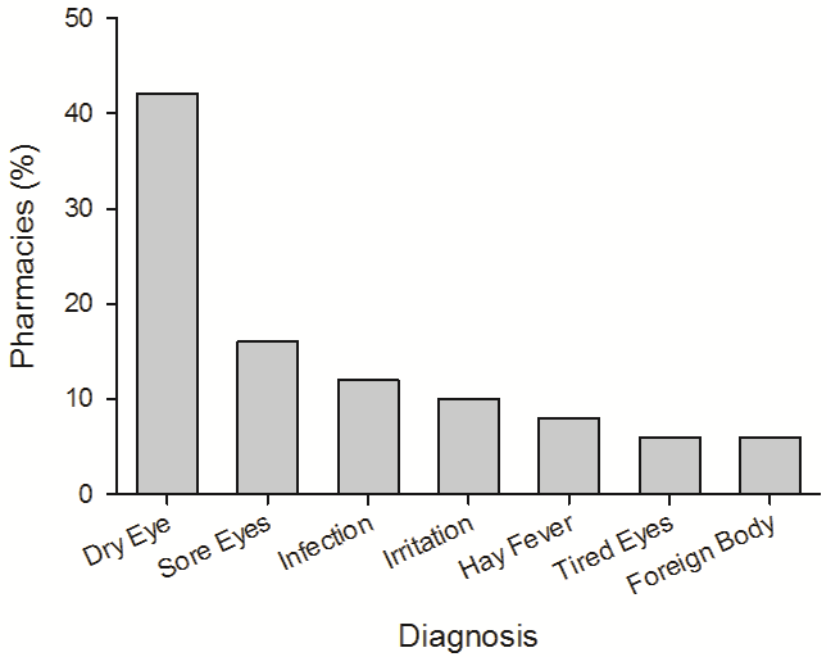

Figure 3.4.2: Diagnoses given by UK pharmacy staff (n=50).

All pharmacy staff gave a diagnosis following history and symptom questioning (Figure 3.4.2). Although the most common diagnosis was dry eye (42%, n=21), the majority were incorrect with 16% (n=8) giving a diagnosis of sore eyes, 12% (n=6) infection, 10% (n=5) irritation, 8% (n=4) hay fever, 6% (n=3) tired eyes and 6% (n=3) foreign body.

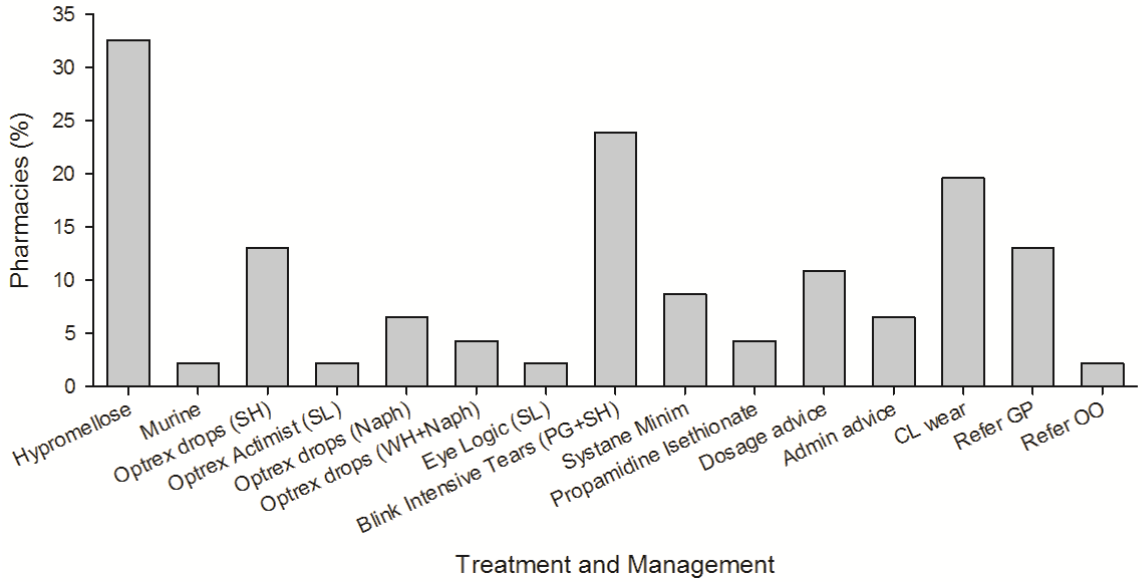

Figure 3.4.3: Treatment advised by UK pharmacy staff (n=46). SL=sodium hyaluronate, SL=soy lecithin, Naph=naphazoline, WH=witch hazel, PG=polythene glycol, CL=contact lens, GP=general practitioner, OO=ophthalmic optician (optometrist).

Treatment was advised by 92% (n=46) pharmacy staff in topical ocular form, with the remaining 8% advising referral directly to the patient's GP (n=2) or optometrist (n=2) alone. Of those advising treatment, 96% (n=44) were indicated for dry eye. Propamidine isethionate, a topical antibiotic drug, was advised by the remaining 4% (n=2) pharmacy staff. However, only 11% (n=5) offered dosage advice, 7% (n=3) offered administration advice, and only 20% (n=9) offered advice relating the use of contact lens wear during treatment. The most common treatment advised was hypromellose (33%, n=15, Figure 3.4.3). Fifteen per cent (n=7) referred to another healthcare professional (n=6 to the patient's GP, n=1 to an optometrist) in addition to advising treatment. Figure 3.4.4 shows the treatment and management advice given by pharmacy staff who gave a diagnosis of dry eye.

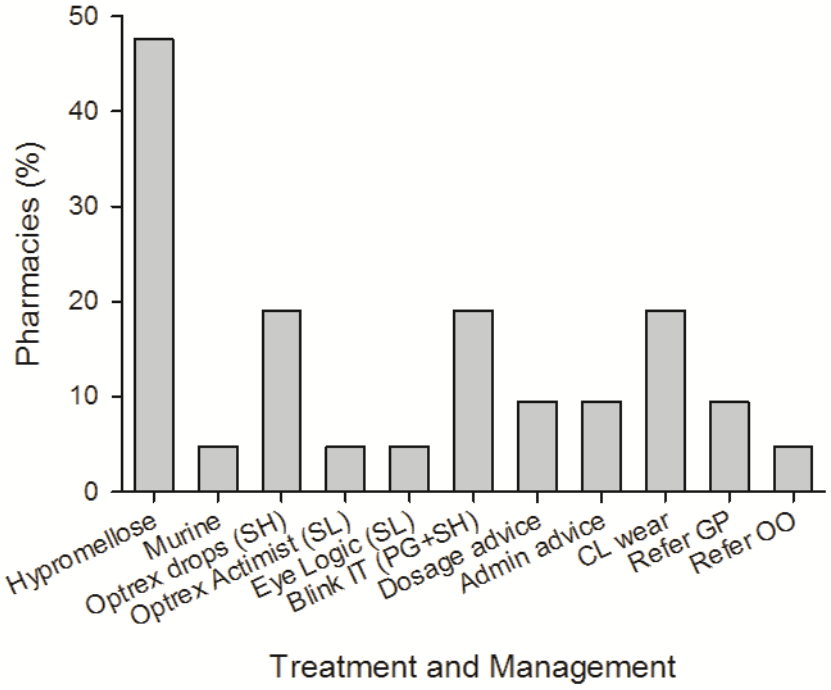

Figure 3.4.4: Treatment and management decisions made by pharmacy staff who gave a diagnosis of dry eye (n=21). SL=sodium hyaluronate, SL=soy lecithin, CL=contact lens, GP=general practitioner, OO=ophthalmic optician (optometrist).

3.5 Discussion

A mystery shopper technique was used to determine the history and symptoms questions asked to elicit a diagnosis of dry eye and subsequent treatment and management advice recommended by pharmacy staff in community practices across the UK. As pharmacies were not informed that the study was being conducted and pharmacy staff was unaware that the consultation was simulated, the possibility of the Hawthorne effect/social desirability bias influencing the outcome of the consultation was eliminated. As there were very uneven proportions of each staff subtype, it was not possible to compare between them.

Although a diagnosis was given at each consultation suggesting confidence in their ability to identify eye disease, the correct diagnosis of dry eye was only given by 42% of pharmacy

staff. Interestingly, the mean number of questions asked by those giving a correct diagnosis was not dissimilar to those who gave an incorrect diagnosis (4.2±1.9, n=21 versus 3.8±1.4 respectively, n=29) but notably there was large difference in the frequency of asking about the duration of symptoms (85.7% versus 34.5%), severity (52.4% versus 17.2%), laterality (61.9% versus 20.7%) and whether or not there was any “dryness” between the correct and incorrect diagnostic groups (71.4% versus 6.9%). Thus it appears that the specific questions asked rather than the number of questions is more important in formulating the correct diagnosis and that certain answers carry more weight than others. This finding is supported by the item by item symptom strategy investigated by Julio *et al* (2012), where specific dry eye questions were found to predict particular clinical signs, whereas global symptoms scores did not (Julio *et al*, 2012). However, as the patient was not present and no examination took place, it may be that dry eye cannot be reliably diagnosed based on symptoms alone. The order of asking each question may also have a role to play in arriving at a particular diagnosis, but this data was not captured by the present study. The apparent confidence in the ability to differentially diagnose dry eye is supported by the lack of referral to optometrists.

Although dry eye symptoms are well described, it remains difficult to manage as not only is there is considerable overlap in signs and symptoms between normal and dry eye patients, there is poor correlation between most traditional diagnostic tests which suffer from poor reliability as a result of environmental factors, patient characteristics and the dynamic nature of the tear film (Nichols *et al*, 2004a; Nichols *et al*, 2004b; Moore *et al*, 2009; Cuevas *et al*, 2012; Sullivan *et al*, 2012a, 2012b). Thus, current international consensus panels advise that symptoms should form the basis of diagnosis and treatment decisions with objective tests providing supportive information (Bron *et al*, 2007). This is supported by the widespread reliance on symptom assessment in clinical practice to diagnose dry eye rather than traditional dry eye tests such as tear film break up time, Schirmer’s test and ocular surface staining (Korb, 2000; Clegg *et al*, 2006; Smith *et al*, 2008; Graham *et al*, 2010). Indeed,

several questionnaires specifically designed for dry eye diagnosis have been validated such as the Ocular Suface Disease Index (OSDI), McMonnies Dry Eye Index and Dry Eye Questionaire (DEQ), but although none of these were utilised by pharmacy staff, this was not expected as they are usually administered in research settings rather than clinical practice (Smith, 2007). However, it may be that dry eye, particularly mild cases, is characterised by a lack of association between different clinical signs and symptoms due to the wider range of values in mild and moderate cases (Sullivan *et al*, 2010).

Treatment was advised by all pharmacy staff providing a diagnosis of dry eye (n=21), with 100% recommending topical treatments suitable for dry eye. The most common treatment advised was hypromellose (48%, n=10), followed by topical dry eye drops containing sodium hyaluronate and or polyethylene glycol (Optrex Dry Eye Drops; Blink Intensive Tears; 43%, n=9) and lipid based sprays (Optrex Actimist; Eye Logic; 10%, n=2). However, very few of these pharmacies gave advice on dosage, administration and other prescribing factors such as age and contact lens wear (Figure 4) and none advised follow up – although mild to moderate dry eye may not be sight threatening, one quality of life study reported dry eye as being similar to suffering from moderate angina (Schiffman *et al*, 2003). Thus the lack of follow up and low level of referral to primary eye care practitioners such as optometrists if symptoms persist or become worse is a cause for concern. However, it may be that optometrists do not promote their clinical services well enough. Topical ocular lubricants are recommended in dry eye to improve comfort and supplement the tear film; however, despite the wide range of treatments available, none have demonstrated a significant advantage over one another in clinical trials (Pflugfelder, 2007; Doughty & Glavin, 2009; Alves *et al*, 2013). The viscosity agents sodium hyaluronate and polyethylene glycol are often added to ocular lubricants to increase retention time of the lubricant on the ocular surface, and subsequently prolong ocular comfort (Pflugfelder, 2007). However, clinical trials of these agents compared to other ocular lubricants produce mixed results, with no clearly superior eye drop (Doughty & Glavin, 2009; Alves *et al*, 2013). It may be that these eye drops do not

have any clinical effect apart from lubrication or that traditional dry eye tests are not capable of capturing any differences between treatments and in addition clinical trials are often poorly designed (Pflugfelder, 2007; Alves *et al*, 2013). More recent studies have shown that Blink Intensive Tears produced a reduction in tear film osmolarity after 30 days usage, but this was not significant (Benelli *et al*, 2010). However, another study reported a significant reduction in tear film osmolarity after 21 days use (3 times a day) to a greater extent than traditional dry eye drops, but this study was unmasked and non-randomised (Montani, 2013). Furthermore, although Blink Intensive Tears produced a significant increase in tear film meniscus volume as measured by optical coherence tomography, results were similar to traditional hypromellose (Garcia-Lazaro *et al*, 2011). Lipid based sprays, such as Optrex Actimist, have been shown to produce a significant improvement comfort, tear film stability and lipid layer thickness compared to saline spray after one 1 hour in a randomised, double masked study and more recently was found to be superior to other commercially available sprays (Craig *et al*, 2010; Pult *et al*, 2012a). However, no clinical trials have yet been published to evaluate their effect in dry eye patients.

Previous research in to the actual management of dry eye disease relates to eye care professionals such as optometrists and ophthalmologists, but none in community pharmacy practice, where dry eye treatments are available to purchase over the counter. In a study by Clegg *et al* (2006), the management practices of 23 consultant ophthalmologists across Europe (including the UK) were assessed using interviews and questionnaires (Clegg *et al*, 2006). The investigators found a wide range of diagnostic techniques and significant variations in diagnostic assessments between each country, but symptom assessment remained a key element in dry eye diagnosis; this finding has also been reported in other surveys of dry eye test preference amongst optometrists and ophthalmologists (Korb, 2000; Turner *et al*, 2005; Graham *et al*, 2010). In the UK, the prescribed treatments and duration of treatment for all severity groups were nearly identical and included hypromellose, carbomer, polyvinyl alcohol and paraffin, many of which can be obtained from pharmacy practices, and

are similar to those recommended by the pharmacies in this study (Clegg *et al*, 2006). However, the data from the study by Clegg *et al* (2006) is limited owing to the low number of ophthalmologists taking part; the definition of dry eye severity differing between the ophthalmologists; and that patients in the UK are likely to represent those with severe forms of dry eye as the majority are managed in primary care or self-treat (Clegg *et al*, 2006). Furthermore, the diagnostic and management strategies described may not be representative of healthcare professionals such as optometrists and community pharmacies where patients may attend prior to ophthalmologist referral (Clegg *et al*, 2006). However, more recent international surveys by optometrists and ophthalmologists have reported similar findings where ocular lubrication and lid hygiene methods were the most common treatment methods employed (Graham *et al*, 2010). Although these surveys and questionnaires provide valuable information relating to the diagnosis and management of dry eye disease, the data may not be representative of the professions examined on the whole due to the relatively poor levels of participation, self-selection bias and that the data may not reflect the participant's actual practice as knew that they were being examined (Hawthorne Effect). Furthermore, these surveys and questionnaires were only based on eye care professionals and were not specific to the UK.

A more objective study examining the actual practice of dry eye diagnosis by different ophthalmic professionals in the US was carried out where patient charts were retrospectively reviewed (Nichols *et al*, 2001). History and symptom assessment was the most commonly performed procedure (82.8%) with symptom assessment and fluorescein staining the most frequently used diagnostic combination (Nichols *et al*, 2001). Again, this data may only reflect management of a population with medical records who are likely to have a severe form of dry eye disease. In the UK, pharmacists generally do not keep medical records of a patient consultation so a retrospective approach to understanding eye disease management by these professionals is not currently possible. More recently, a study examining the management of ocular allergy by UK pharmacies using the same mystery shopper

methodology showed similar results as the present study (Bilkhu *et al*, 2013). The mean number of questions asked were low (n=3.5±2.6) and nearly all advised treatment (91%) again suggesting pharmacy staff is confident in diagnosing and managing eye disease (Bilkhu *et al*, 2013). However, although 50% advised topical anti-allergy medication, a high proportion of treatments without evidence to support their use were advised, and many did not offer any additional treatment and prescribing advice (Bilkhu *et al*, 2013).

The diagnoses given by pharmacy staff also included sore eyes, tired eyes, hay fever, irritation foreign body and infection. However, sore eyes, tired eyes and irritation are not considered diagnoses but symptoms of dry eye that may be present other eye conditions. Nonetheless, treatment was advised by 92% (n=46) pharmacy staff – of those providing a diagnosis of sore eyes, tired eyes and irritation (n=13), 62% (n=8) were suitable for dry eye, 31% (n=4) were non-specific eye drops and the remaining staff member advised the patient to attend their GP. Thus, it may be that pharmacy staff may be unsure of the correct terminology relating to dry eye, use these terms interchangeably with dry eye, or consider them specific diagnoses with the same treatment approach as dry eye. However, the packaging on some of the non-specific treatments (Optrex Refreshing Eye Drops; Optrex Eye Brightener) uses language such as “suitable for tired, uncomfortable and irritated eyes”, symptoms typical of dry eye, which may contribute to this terminology confusion and lead to the use of inappropriate treatments. Indeed, Optrex Eye Brightener contains naphazoline hydrochloride, a vasoconstrictor that must not be used in the long term due to the risk of rebound hyperaemia and is contraindicated in those with glaucoma (Bielory *et al*, 2005; Bielory, 2008b). In addition, Optrex Refreshing Eye Drops contain witch hazel, an ingredient with reported astringent properties, but no research has been identified in the scientific literature confirming this or demonstrating efficacy in any ocular disease. Furthermore, of those that did recommend these treatments, none provided dosage advice and only 1 provided administration and contact lens wear advice.

Of those that diagnosed hayfever (n=4), none advised the use of topical ocular or systemic anti-allergic medications; instead artificial tear supplements, indicated for dry eye, was recommended by 75% (n=3) and the remaining staff member advised the patient to attend an optometrist. However, the use of topical lubricants are advised in the scientific literature to help wash out allergens from and prevent them binding to the ocular surface to relieve ocular allergic symptoms, although currently there is no clinical evidence support this recommendation (Bielory, 2008b; Wong *et al*, 2009). Three pharmacy staff diagnosed foreign body ("grit" n=2; "eyelash" n=1) as the cause of the patient symptoms, with 2 advising the use of artificial tears and the other referring to an optometrist. This diagnosis is somewhat surprising given that the patient was not present and therefore could not be examined, and that a foreign body may such as "grit" may damage the ocular surface, particularly the cornea, rendering the eye prone to infection such that an eye examination is necessary to locate and remove the foreign body (Wirbelauer, 2006; Cronau *et al*, 2010). The remaining 6 pharmacy staff gave ocular infection as a diagnosis, but did not state which tissue was suspected to be infected. Of these, 84% (n=5) advised treatment; the use of topical treatments suggests that the suspected infection was anterior in location with 60% (n=3) recommending artificial tear supplements and the remaining 40% (n=2) advising the use of Brolene (propamidine isethionate), a topical anti-infective medication, combined with referral to the patient's GP. Despite these pharmacy staff asking questions pertinent to an ocular infection (laterality, stickiness, pain, duration, discharge, visual change), the predetermined answers (table 1) do not suggest this diagnosis. The proportion of incorrect diagnoses in the present study (26%) is higher than that reported for allergic conjunctivitis by Bilkhu *et al* (2013), where only 6% of pharmacy staff misdiagnosed (Bilkhu *et al*, 2013). It may be that dry eye lacks a defining set of symptoms, such that the potential to misdiagnose is higher compared to allergic conjunctivitis, where bilateral ocular itching is pathognomic of the condition (Bielory 2008a; Friedlaender, 2011). However, symptoms of itching and soreness were already volunteered by the mystery shopper in the opening statement by Bilkhu *et al* (2013) and may have directed further questioning relating to allergy.

As the patient was not present, an examination could not be performed but this was not expected as pharmacies do not have equipment specific to the examination of the eyes nor do the staff, including pharmacists, have training to do so. However, given the lack of correlation between different dry eye signs and with symptoms, particularly mild dry eye, such tests may only provide information about the ocular surface on an individual patient basis (Moore *et al*, 2009; Cuevas *et al*, 2012; Sullivan *et al*, 2010; Sullivan *et al*, 2012a). Furthermore, treatment of mild dry eye, regardless of sub-type based upon a particular set of signs, remains tear film supplementation and ocular surface lubrication (Pflugfelder, 2007; Alves *et al*, 2013). Thus, although it is unlikely that an eye examination in this case would yield information supportive of dry eye and influence treatment choice, it may help to exclude other differential diagnoses. The absence of the patient appears to be a limitation of the study in that it may invite other diagnoses, but given that all pharmacy staff asked relatively few questions, provided a diagnosis and very few referred to another colleague or healthcare practitioner, this suggests that they are perhaps confident in their abilities to perform history and symptom questioning and differentially diagnose eye disease, even in the absence of the patient. However, with the range of diagnoses given based upon the same predetermined scenario answers, disparity between the suggested diagnosis and treatment advice, this study has highlighted that the actual ability to manage dry eye disease by pharmacy staff may need enhancing. Another potential limitation was the small number of pharmacies visited (n=50) and the results from which may not be representative of all community pharmacies. The inclusion of non-pharmacists may have affected the results, but of the 29 giving an incorrect or ambiguous diagnosis, 76% were identified as pharmacists. It could have been possible to request to see the pharmacist only, but it was not unreasonable to expect the staff member to defer the case to the pharmacist if they felt they were unable to provide advice, and although this was a possibility with the present methodology, none referred to another another staff member. Future studies may overcome this by specifically asking to speak with a particular staff member before initiating the scenario. Mystery shopping techniques have been used extensively in healthcare research to help evaluate

service provision, and many authors argue that it may be impossible to fully do so without deceptive methods given the limitations of surveys and questionnaires by healthcare professionals, the lack or absence of patient records, and that patients satisfaction measures may not reflect actual healthcare service performance (Moriarty *et al*, 2003).

3.6 Conclusions

The ability to diagnose dry eye in UK community pharmacies is generally poor, with less than half giving the correct diagnosis, although some appear to be unsure of terminology and confuse symptoms with diagnoses. However, the lack of distinct symptoms for dry eye may have contributed to this and account for the higher proportion of incorrect diagnoses compared to allergic conjunctivitis. The absence of equipment to examine the eyes may not hinder the ability to diagnose mild cases of dry eye, but may help exclude other diagnoses that were offered here. Despite the lack of equipment, pharmacy staff appear confident in their ability differentially diagnose due to the low number of history and symptom questions asked, low referral rates and that all staff offered a diagnosis without prompt. Of those that correctly identified dry eye, treatments advised were suitable. However, there is a lack of treatment advice given relating to dosage, administration, contraindications and follow up when recommending treatments not only for dry eye but also other diagnoses. Research is also much needed to identify which dry eye treatments work best for different individuals to overcome the need for trial and error, or patient dissatisfaction with treatments. Therefore there is clear need for improved training relating to the differential diagnosis and management of dry eye and other common eye disease which may present at community pharmacies and the establishment of cross referral relationships with optometry practices. An improvement in ophthalmological training is required at undergraduate pharmacy level and continuing professional education and training for qualified pharmacists. In addition, it is suggested that non-pharmacist staff should recognise

the limits of their competence and refer suspected eye disease to an appropriately trained member of staff for management advice.

Chapter 4

Dry Eye Cluster Analysis

4.1 Introduction

In spite of the vast progress made in the understanding of dry eye with respect to its effects on visual function (Rieger, 1992; Goto *et al*, 2002a), tear osmolarity (Tomlinson et al, 2006) and ocular surface inflammation (Pflugfelder *et al*, 1999; Brignole, 2000), no single diagnostic test has proven to reliably distinguish those with or without dry eye (Bron, 2007; Smith, 2007; Khanal *et al*, 2008). Currently there is no consensus as to which tests to perform to diagnose the disease (a gold standard) in a clinical setting or in research despite the variety of tests in common usage (Korb, 2000; Smith, 2007).

Many of the tests used to diagnose and monitor dry eye demonstrate a lack of repeatability (Nichols *et al*, 2004a). In addition, the majority of repeatability studies on dry eye tests have been performed on normal (non-dry eye) individuals whereas few have been performed on actual dry eye patients (Lemp, 1995; Bron *et al*, 2007). It can be argued that dry eye test repeatability studies need to be performed on dry eye patients, as studies on normal individuals only give the overall repeatability of the test examined rather than on a specific disease (Nichols *et al*, 2004a). In addition, measures of tear film breakup time, tear flow, tear osmolarity, tear meniscus height, tear flim lipid layer interferometry, all common dry eye tests, are influenced by or are assumed to be influenced by environmental factors such as time of day, humidity, temperature (Johnson & Murphy, 2006; Bron *et al*, 2007), and therefore it is not unexpected that repeated measures of the same test on the same subject at different times may vary widely (Bron *et al*, 2007). It has been suggested that current dry eye tests may be truly unrepeatable or that dry eye is highly variable in the short term (Nichols *et al*, 2004a).

The challenge of producing diagnostic criteria for dry eye has been complicated by the controversial lack of correlation between clinical signs identified by traditional tests and symptoms of dry eye (Schein *et al*, 1997), and the presence of clinical signs in normal, asymptomatic patients (Lemp, 1995; Smith, 2007).

Nichols *et al* (2004b) reported no statistically significant correlations between patient self-reported symptoms and any common diagnostic dry eye tests, even after adjusting for patient age and artificial tear use (Nichols *et al*, 2004b). Begley *et al* (2003) found statistically significant but low to moderate correlation between many of the clinical signs and dry eye symptoms. More recently, in a study consisting of patients with only non-aqueous deficient mild to moderate dry eye, only a few dry eye tests correlated significantly with each other, including biomicroscopic examination of meibomian gland function with tear break up time (TBUT) and dry eye symptom score (McMonnies Questionnaire), and between goblet cell density, TBUT, and dry eye symptoms score, but associations were also low to moderate based upon McNemar's test (Moore *et al*, 2009). However, In a study by Mizuno *et al* (2010), although it included dry eye patients with and without Sjogrens syndrome, dry eye symptoms did not significantly differ between each group and when the data was combined, no statistically significant correlations were found between any of the ocular surface findings (Schirmer test, ocular surface staining with fluorescein and Rose Bengal) and dry eye symptom scores (Mizuno *et al*, 2010). Sullivan *et al* (2012a) also found no significant correlation between any dry eye tests (tear osmolarity, TBUT, Schirmer test, ocular surface staining, meibomian gland assessment) and between each dry eye test and symptoms in patients representing a primary care clinical practice cohort (Sullivan *et al*, 2012a).

In a study investigating the relationship between signs and symptoms before and after treatment in a more specific dry eye cohort (meibomian gland dysfunction related evaporative dry eye) utilised only worse eye data based upon symptom scores – however, visual function scores and clinical signs did not differ significantly between the better and worse eyes (Cuevas *et al*, 2012). At the initial visit, only TBUT correlated significantly with

one type of dry eye questionnaire, whereas conjunctival hyperaemia, Rose Bengal staining and TBUT correlated significantly with another (Cuevas *et al*, 2012). However, after treatment, none of these correlations reached statistical significance – thus, it appears that the type of questionnaire used affects the ability of clinical tests to reflect any relationship with dry eye symptom scores, and that clinical signs may not be responsive to treatment despite improvement in symptoms. This is supported by the significant variability in dry eye test values over time and the lack of significant change in dry eye test values after treatment with topical cyclosporine A, with the exception of tear osmolarity in another study evaluating the correlation between dry eye signs and symptoms (Sullivan *et al*, 2012b).

The lack of correlation between signs and symptoms of dry eye using common clinical tests may be largely explained by the poor repeatability of the tests used, as described above. However, the subjective nature of symptoms, variability of pain thresholds and variability of cognitive responses to questions about physical sensations in the eyes may contribute at least in part to the observed lack of correlation (Smith, 2007; Bron *et al*, 2007). With advancing age and worsening dry eye disease, the development of reduced corneal sensitivity) may also explain the disparity between symptoms and clinical signs of dry eye (Xu *et al*, 1996; Bourcier *et al*, 2005). It may be that current dry eye tests are not measuring or capturing a parameter related to the causative symptoms (Lemp, 2007; Bron *et al*, 2007). Many of the common objective dry eye tests are dynamic measures which are subject to measurement error (although contributing a small amount the variability within signs) and examiner interpretation so the high level of scatter is predictable when attempting to correlate dry eye symptoms, already known for their variable nature (Schaumberg *et al*, 2007; Blackie *et al*, 2009; Lemp *et al*, 2012). Therefore, the variability within common dry eye tests is likely due to the inherent pathophysiology of dry eye – tear film instability or lack of tear film homeostasis, inherent to all dry eye subtypes, may cause transient fluctuations in ocular surface findings and tear film parameters that contribute to each measurement, in addition to methodological errors in test measurement (Bron *et al*, 2007; 2009; Sullivan *et al*,

2012b). Thus, the lack of correlation and wide range of values observed may characterise the disease, particularly in early stages and mild to moderate cases where compensatory mechanisms may temporarily ameliorate the effects of the environment on the ocular surface (Bron *et al*, 2009; Sullivan *et al*, 2010; 2012a). Measures of ocular surface staining, TBUT, Schirmer test, and meibomian gland function have been found to produce values across a wide range in mild to moderate dry eye but the range of values narrow as severity increases (Sullivan *et al*, 2010). Hence, the range of values obtained for a particular sign or test is likely to be strongly influenced by severity (Sullivan *et al*, 2010). Indeed, signs of dry eye tend to worsen with increases in severity (Begley *et al*, 2003; Mizuno *et al*, 2010; Versura *et al*, 2010; Sullivan *et al*, 2010; 2012a). The inclusion of a range of severities and subtypes of dry eye may therefore explain why some studies investigating the relationship between signs and symptoms report correlations between some tests and not others, and also the lack of consistency between studies of which tests do correlate.

Interestingly, self-reported patient symptoms of dry eye are found to be more repeatable and more able to accurately grade severity compared to objective dry eye tests (Nichols *et al*, 1999; Schiffman *et al*, 2000), resulting in symptoms becoming key for diagnosis and monitoring of dry eye disease (Nichols *et al*, 2001; Smith *et al*, 2008). Symptoms questionnaires such as the ocular surface disease index (OSDI) have demonstrate excellent performance data and test-retest reliability (Schiffman *et al*, 2000). Given the higher levels of repeatability compared to other dry eye tests, dry eye is therefore considered to be a symptom based disease (Smith *et al*, 2008). Recent international panels aimed at developing a consensus for dry eye have highlighted symptoms as the primary component of diagnosis and treatment decisions, with objective dry eye tests taking a secondary position (Behrens *et al*, 2006; Bron *et al* 2007; Mizuno *et al*, 2010). Smith *et al* (2008) attempted to clarify the roles of signs, symptoms and objective tests with respect to the contrasting findings of Begley *et al* (2003) and Nichols *et al* (2004b) where an expert panel rated the tests according to when they would be used in hypothetical dry eye cases -

symptom assessment was found to be the most prominent test in diagnosis and management (Smith *et al*, 2008). This is supported by the widespread use of dry eye symptom assessment in clinical practice to diagnose dry eye rather than traditional dry eye tests (Korb *et al*, 2000; Turner *et al*, 2005; Clegg *et al*, 2006).

In clinical practice, the overreliance on symptoms as an indicator of possible dry eye disease and stimulus for further investigation may represent a serious oversight – patients with no symptoms suggestive of dry eye may not be evaluated further, and if only those with symptoms of dry eye were evaluated the true spectrum of disease may be underestimated (Sullivan et al, 2012a). Sullivan et al (2012a) found that of patients showing evidence of dry eye, only 57% had symptoms suggestive of dry eye. Further, a recent study on the prevalence of meibomian gland dysfunction (MGD), perhaps the most common cause of dry eye, has found that asymptomatic meibomian gland dysfunction (MGD) is more common than symptomatic MGD, and conclude that a symptom based approach is inappropriate for MGD diagnosis and prevalence estimation (Viso *et al*, 2012). It is therefore unlikely that patients with symptoms inconsistent with a diagnosis of dry eye would be evaluated further by dry eye tests in a clinical setting (Sullivan *et al*, 2012a). Recent studies have found that symptom questionnaires also exhibit variability over time, such that initially normal patients at baseline may have symptoms similar to moderate to severe dry eye a few weeks later (Miller *et al*, 2010). Thus, it may not be mathematically possible to correlate clinical signs with absolute dry eye symptom scores (Sullivan *et al*, 2012b). Further, symptoms of dry eye are common to a range of anterior eye diseases, such as ocular allergy and MGD (Bielory, 2004; Granet, 2008). In Chapter 2 an appropriate diagnosis was only provided by 42% of pharmacy staff. In the remaining cases, diagnoses given were sore eyes, irritation, tired eyes, foreign body, infection and hay fever. Thus, the diagnosis and management of dry eye may not be sufficient using symptom assessment alone, but that a group of clinical tests including symptom assessment is required (Mizuno *et al*, 2010; Sullivan *et al*, 2010, 2012a).

Based upon the reviewed scientific literature, dry eye may therefore be considered on a spectrum of disease severity rather than a defined cut-off between normal and diseased states based upon individual clinical test thresholds. In support of this, a recent study by Sullivan *et al* (2010) classified patients into different dry eye severity groups using a continuous composite severity index based upon the relative contribution of each clinical test (Schirmer test, TBUT, ocular surface staining, OSDI, meibomian gland function, tear osmolarity) value to the level of evidence for dry eye as recommended by the Dry Eye WorkShop severity scale (Lemp, 2007) – comparison of the composite severity index to a standard clinical threshold based severity classification showed significant overlap (63% poorly classified) between the severity of prospectively defined normal and dry eye groups (Sullivan *et al*, 2010). However, correlation between signs and symptoms is considered highly desirable as quantifying symptoms and comparing them to a well correlated sign(s) capable of indicating problems in an individual patient allows improvement in diagnosis, monitoring and determination of a treatment effect in dry eye (Behrens, 2006; Bron *et al*, 2007). However, it remains unknown whether identification of a specific subtype of dry eye, based upon the results in dry eye tests, helps to guide treatment decisions.

4.2 Study Aim

Given that dry eye tests do not often correlate with each other, with dry eye symptoms or dry eye severity, the aim of the present study was to determine if different subgroups of dry eye patients could be identified based upon dry eye tests themselves into clinically relevant groups that may help guide and respond to treatment using a cluster analysis methodology.

4.3 Methods

Subjects were recruited from three sites across the UK were recruited in the study, including patients from two primary care community optometry clinical practices (Laika Essa/Nigel Best) and staff, students and optometry clinic patients from Aston University. Subjects were required to be at least 18 years of age, non-contact lens wearers with no ocular medications, no systemic medications known to affect the eyes, no history of eye surgery in the last 3 months; and were excluded if they had a history of diabetes, Sjogren's syndrome, hay fever, recent ocular infection, or were pregnant. Subjects were enrolled following written informed consent. The study was approved by the Institutional Review Board and conformed to the Tenets of the Declaration of Helsinki.

Subjects attended for one visit where measures of ocular symptomology, non-invasive tear film meniscus height (NITMH), non-invasive tear film break up time (NITBUT), fluorescein tear film break up time (FBUT), ocular surface staining (OST, corneal and conjunctival), and Schirmer test or phenol red thread test (PRT) were performed in sequence on the right eye only (except for OST) by an unmasked examiner (PSB, LA, or NB).

- Ocular symptomology was assessed using the Ocular Surface Disease Index (OSDI), a validated dry eye questionnaire consisting of 12 questions graded on a scale of 0-4 (Schiffman *et al*, 2000). The OSDI score was calculated by multiplying the total score by 25 and dividing by the total number of questions answered yielding a result between 0 and 100 (Schiffman *et al*, 2000). Subjects were assigned normal (≤10) or symptomatic (>10) status based upon the OSDI score.

- NITMH was measured using a slit lamp bio-microscope (X25 magnification) where the slit beam was rotated to align parallel to the lower eyelid margin, and the height of the slit beam was adjusted to match the tear meniscus located directly below the pupil while the patient was looking in primary gaze. The NITMH was defined at the distance

between the lower eyelid margin and the upper limit of the reflected zone of the tear meniscus (Farrell *et al*, 2003). The height of the NITMH was recorded from the built in, calibrated, slit beam width scale. This process was repeated 3 times in total and was averaged to give a mean NITMH value.

- NITBUT was measured using the TearScope Plus (Keeler Ltd, Windsor, UK) combined with a fine grid pattern insert mounted on a slit lamp bio-microscope to produce an image of the fine grid pattern over the entire cornea via specular reflection. Subjects were instructed to blink normally and then keep their eyes open for as long as possible. The NITBUT was defined as the time period between the last complete blink and the appearance of a break or distortion in the fine grid pattern (Guillon, 1998), measured using a digital stop-clock. This was repeated 3 times in total and was averaged to give a mean NITBUT value.

- FBUT was measured with the slit lamp bio-microscope set at a magnification of X10 with a diffuse cobalt blue light at maximum brightness. A single drop of sterile saline was applied to a fluorescein sodium impregnated paper strip (Fluorets, 1mg fluorescein sodium, Chauvin Pharmaceuticals, Essex, UK) and the excess shaken off in to a bowl. The lower lid of the right eye was everted and the wetted strip was quickly but gently applied to the lower tarsal conjunctiva. The subject was then instructed to blink normally after application to distribute the fluorescein. Subjects were then asked to look in primary gaze without blinking for as long as possible. FBUT was defined as the interval of time between the last complete blink and the first appearance of a dry spot or disruption (black/dark blue area) in the tear film or the next complete blink (whichever came first) measured with a digital stop-clock (Lemp, 1995). A yellow filter (Kodak Wratten 12) was used to enhance contrast and improve visibility of breaks in the tear film (Bron *et al*, 2003). This process of measurement (blinking followed by primary gaze

without blinking) was repeated 3 times in total and were averaged to give a mean FBUT value.

- Ocular surface staining was assessed using fluorescein sodium (Fluorets) and lissamine green (Green Glo, 1.5mg lissamine green, HUB Pharmaceuticals, USA) impregnated paper strips. Lissamine green was used to examine the conjunctiva, where a single drop of sterile saline was applied to the strip with the excess shaken off in to a bowl. The lower lid of each eye was everted and the wetted strip was quickly but gently applied to the lower tarsal conjunctiva. The subject was then instructed to blink normally after application to distribute the lissamine green, before the conjunctiva of each eye was examined using a slit lamp bio-microscope (white light, X10 magnification). After 5 minutes, corneal staining was assessed using Fluorets, applied in the same fashion as described above for FBUT to each eye (Petersen *et al*, 2006). The cornea of each eye was examined use a cobalt blue light source at X10 magnification with contrast enhanced using a yellow filter. The presence of staining on the cornea and conjunctiva in one or both eyes was recorded.

- Schirmer test (ST) or PRT was used to measure tear flow. The variation of Schirmer test used herein was conducted without anaesthesia (ST1), where a filter paper strip with a printed millimetre scale (Tear Flo, HUB Pharmaceuticals, USA) was bent at the notch and gently inserted along the lower temporal eyelid margin of the right eye. Immediately after insertion the subject was instructed to close their eyes for 5 minutes, timed with a digital stop-clock. After 5 minutes the subject is instructed to open their eyes and the filter paper strip was removed. The amount of wetting (identified by translucence of the filter paper) was measured by reading from the millimetre scale to the nearest 0.5mm. Similarly, 3mm of the yellow phenol (phenolsulfonphthalein) impregnated thread was inserted along the lower temporal eyelid margin of the right eye. Immediately after insertion the subject was instructed to blink normally while looking in primary gaze for 15 seconds, timed with a digital stop-clock. After 15 seconds, the thread was removed and

the portion of the thread changed in colour from yellow to red was measured using a steel rule to the nearest 0.5mm (Little & Bruce, 1994).

Statistical analysis was performed using SPSS (IBM Corportation, USA) for Microsoft Windows. Cluster analysis to identify any subgroups of dry eye based upon clinical tests/tear metrics was performed using a “k-means” clustering algorithm. A cluster is defined as a group of data that is separated from another based upon the data points distance from the cluster centre. The cluster centre is a data point which is well separated (mean distance similar to) from another data point. Initial cluster centres (k-centres) were identified from cases that were well separated. Cases closest to an initial cluster based upon the distance from the cluster centre are then assigned to that cluster. Once cases have been assigned, the cluster centres are recalculated and cases reassigned to the new cluster centres. This process is repeated until 10 iterations fail to converge or less than 10% of the cases are within each cluster, whichever came first. Outliers were not excluded as they aid cluster separation. Statistical significance was tested using ANOVA. As the clusters have been chosen to maximize the differences among cases between clusters, statistical significance levels are used for descriptive purposes only – increasing levels of significance indicate that it is more likely that a variable contributes to cluster separation.

4.4 Results

A total of 354 subjects (mean age: 45.4±21.9 years, age range: 19-91 years, 223 female, 131 male) from the three sites completed the study.

Cluster Analysis

K-means cluster analysis was performed for 2 and 3-way clusters. No further cluster analysis with higher number of clusters was performed as iterations failed to converge for the 3-way cluster. The number of cases in each group is shown in Table 4.4.1.

	Number of Cases in each Cluster			
Number of Clusters	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Convergence Achieved?**
2	65	289		Yes
3	53	180	121	No

Table 4.4.1: Number of cases within each cluster for 2 and 3-way cluster analysis. Convergence was achieved for 2-way cluster, but not 3-way clusters. There were at least 10% of the cases in each cluster.

The 2 way cluster was selected for statistical analysis as convergence was achieved and at least 10% of cases were in each group. Cluster 1 had a mean age of 57.9±21.1 years (75.4% female); cluster 2 had a mean age of 42.6±21.1 years (60.2% female). The final cluster centres (median values) for each variable is shown in Table 4.4.2.

	Cluster	
Dry Eye Test (Variable)	**1 (n=65)**	**2 (n=289)**
OSDI Score	45.89*	11.72*
NITMH (mm)	0.12*	0.17*
NITBUT (seconds)	11.55*	12.77*
FBUT (seconds)	11.64	12.22
Schirmer Test 1/PRT (mm)	13.38*	18.01*
Corneal Staining (0,1 or 2 eyes affected)	0.83*	0.36*
Conjunctival Staining (0, 1 or 2 eyes affected)	1.60*	0.67*

Table 4.4.2: Median values (final cluster centres) for each dry eye test using 2-way cluster analysis. Asterisks (*) represent variables that significantly contributed to cluster separation.

The data set for cases in cluster 1 (n=65) of the 2-way cluster analysis was then selected to for additional K-means cluster analysis. Two and 3-way cluster analysis was performed. No further cluster analysis was performed as less than 10% of cases were in one of the groups in the 3-way cluster analysis, although convergence was achieved. The number of cases in each group is shown in Table 4.4.3.

	Number of Cases in each Cluster			
Number of Clusters	**Cluster 1a**	**Cluster 1b**	**Cluster 1c**	**Convergence Achieved?**
2	20	45		Yes
3	16	43	6	Yes

Table 4.4.3: Number of cases within each cluster for 2 and 3-way cluster analysis. Convergence was achieved for both 2-way and not 3-way clusters, but cluster 3 of the 3-way cluster analysis had less than 10% of cases.

The 2-way cluster was selected for statistical analysis as convergence was achieved and at least 10% of cases were in each group. Cluster 1a had a mean age of 54.6±22.1 years (70.0% female); cluster 1b had a mean age of 59.4±20.8 years (77.8% female). The final cluster centres (median values) for each variable is shown in Table 4.4.4.

	Cluster	
Dry Eye Test (Variable)	**1a (n=20)**	**1b (n=45)**
OSDI Score	59.38*	39.90*
NITMH (mm)	0.11	0.12
NITBUT (seconds)	12.29	11.22
FBUT (seconds)	11.82	11.55
Schirmer Test 1/PRT (mm)	12.90	13.60
Corneal Staining (0,1 or 2 eyes affected)	0.35*	1.04*
Conjunctival Staining (0, 1 or 2 eyes affected)	1.60	1.60

Table 4.4.4: Median values (final cluster centres) for each dry eye test using 2-way cluster analysis. Asterisks (*) represent variables that significantly contributed to cluster separation.

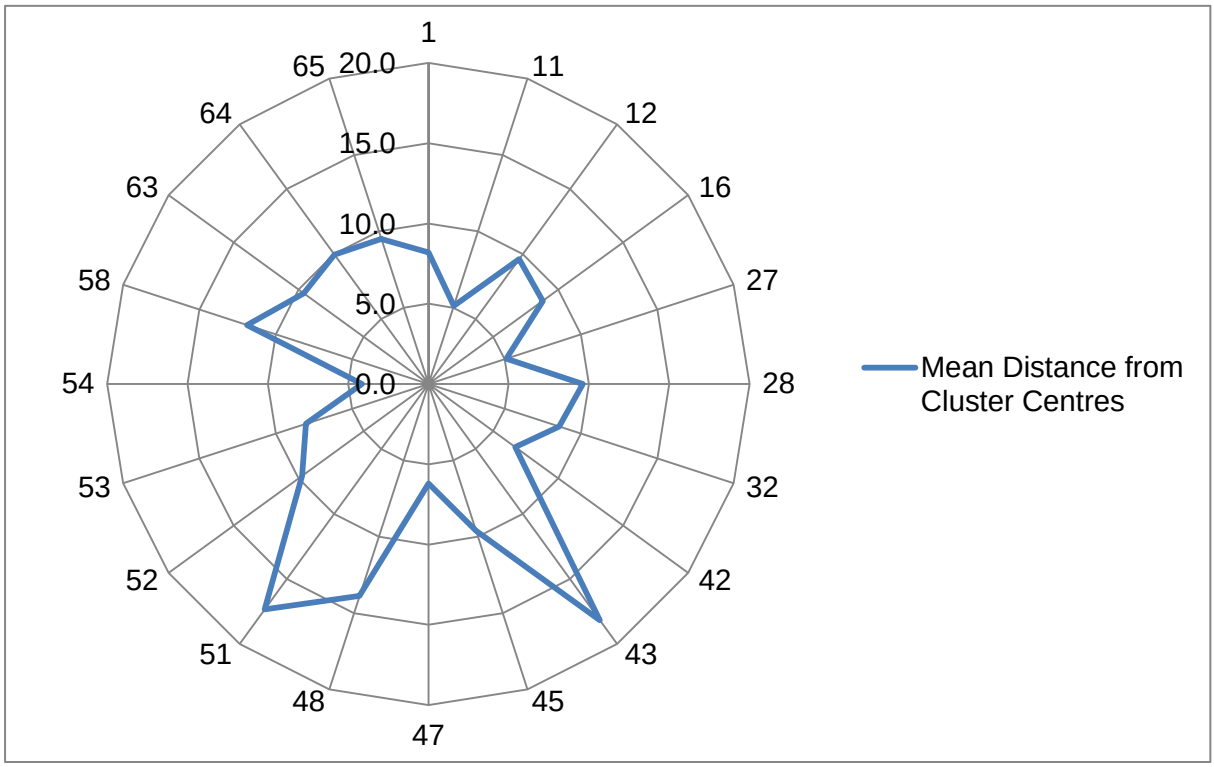

Figure 4.4.1: Mean distance from the cluster centres in cluster 1a for each case (n=20).

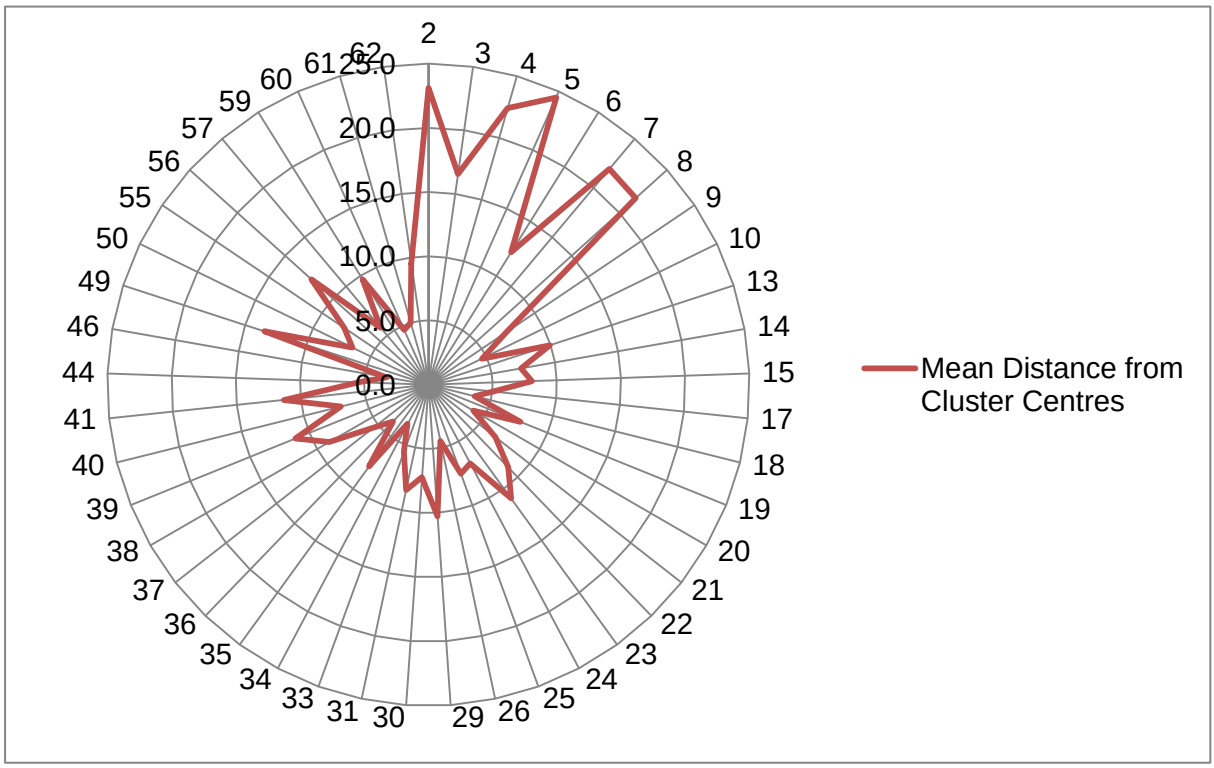

Figure 4.4.2: Mean distance from the cluster centres in cluster 1b for each case (n=45)

The proportion of normal and symptomatic patients based upon the OSDI score in each cluster is shown in Table 4.4.5.

Cluster	Normal	Symptomatic
1	0.0% (n=0)	100% (n=65)
2	50.5% (n=146)	49.5% (n=143)

Table 4.4.5: Proportion (n=number) of normal and symptomatic patients in clusters 1 and 2. Clusters 1a and 1b consist of patients from cluster 1 only.

4.5 Discussion

In clinical practice, dry eye is often classified into either aqueous deficient and evaporative forms corresponding to disorders of the meibomian and lacrimal glands, yet many in cases both are likely to be present (Lemp, 2007, 2012; Bron *et al*, 2009). As discussed earlier, most dry eye tests, even those specific to a dry eye subtype, often fail to correlate and conflict with each other, exhibit significant variation, and overlap both normal and dry eye patients making classification into traditional subtypes difficult. In contrast, the present study sought to identify and subsequently classify patients into clinically relevant dry eye subtypes based upon on dry eye tests that are measurable and commonly used in clinical practice, which may help decide which dry eye treatment is appropriate. Cluster analysis is used for grouping a set of objctes, in this case tear film paramters, in to a group or cluster more similar compared to another cluster based upon the distance from the cluster centre, thus allowing grouping of patients according to their individual values and therefore applicable to the requirements of the aim of this study.The initial cluster analysis (2-way) identified cluster 1 consisting of older patients with significantly worse symptoms and signs compared to cluster 2, with the exception of FBUT which was similar. This group consisted of 100% symptomatic patients, with a median OSDI score of 45.89 corresponding to moderate dry eye, although the median values for NITBUT, FBUT and Schirmer test 1/PRT classify these patients as normal based upon currently accepted clinical thresholds in the scientific literature (Mengher *et al*, 1986; Vitali *et al*, 1994; Lemp, 1995; Patel *et al*, 1998; Abelson *et al*, 2002b). In contrast, NITMH was indicative of dry eye, given a diagnostic cut-off of <0.18mm (Farrell *et al*, 2003). However, no single diagnostic test has proven to reliably distinguish between those with or without dry eye (Smith, 2007; Khanal *et al*, 2008; Sullivan *et al*, 2010, 2012b). Moreover, cut-off values for established dry eye diagnostic tests such as those used herein often suffer from both selection and spectrum bias (Bron *et al*, 2007). Selection bias refers to the measurement of efficacy of a test in population who are categorised as affected or not affected by the test under scrutiny whereas spectrum bias

refers to the selection of patients with a certain level of disease severity. These forms of bias result in artificially high sensitivity and specificity values (higher than that expected from and independent population) and therefore compromise test performance data (Knottnerus *et al*, 2002). In addition, increased sensitivity and specificity may result when the cut-off value for diagnosis is derived from the same study sample. Ideally, cut-off values should be calculated to maximise sensitivity and specificity from independent populations consisting of normal and dry eye patients of varying severity as part of an iterative process (Bron *et al*, 2007). Nonetheless, each clinical test provides important information about the state of ocular surface on an individual basis, although the relative importance of each test is not clearly defined (Bron *et al*, 2007; Sullivan *et al*, 2010).

Thus, it may be that these patients represent those with normal aqueous production but increased rates of evaporation, accounting for the low meniscus height and the presence of corneal and conjunctival staining. However, the tear film of these patients were relatively stable, suggesting the composition of the tear film lipid layer may influence evaporation rate, although neither evaporation rate was measured or tear film lipid samples taken for analysis. It is currently unknown how lipid composition affects evaporation rates, but recent models of the tear film lipid layer suggest that those consisting of higher proportions of wax esters retard evaporation significantly (Rantamaki *et al*, 2012). A reduced blink rate, extending the period during which the ocular surface is exposed to evaporation, may also account for the reduced meniscus height observed in these patients (Tsubota *et al*, 1995; Nakamori *et al*, 1997). However, due to the reduced tear meniscus observed in this group it may also be that these patients represent those with aqueous deficiency dry eye rather than increased evaporation. Here, the reduced lacrimal secretion leads to a reduced tear volume, causing tear film hyperosmolarity which stimulates the generation of inflammatory mediators that lead to apoptotic death of epithelial and goblet cells thus accounting for the reduced tear meniscus height and increased presence of ocular surface staining (Brignole *et al*, 2000; Kunert *et al*, 2002; Yeh *et al*, 2003; Bron *et al*, 2009).

Cluster 2 was far larger (n=289) compared to cluster 1 (n=65), and based upon OSDI scores, and NITBUT (>10 seconds), FBUT (>10 seconds) and Schirmer test 1 (>5.5mm after 5 minutes)/PRT (>9mm after 15 seconds) diagnostic cut-offs, patients were considered normal. However, NITMH was below the clinical threshold for distinguishing between normal and dry eye patients and although minimal, both corneal and conjunctival staining was present. Cluster 2 may therefore consist of healthy patients, or those with subclinical evaporative dry eye without significant symptoms where a compensatory mechanism such as increased tear production (Schirmer test 1/PRT significantly greater in cluster 2 than cluster 1, see Table 4.4.2) is temporarily alleviating symptoms, or both. This is supported by the near equal proportion of normal and symptomatic patients in this cluster, and is in agreement with the considerable overlap between signs and symptoms between normal and dry eye patients, particularly those in early stages of and mild dry eye observed in previous studies (Lemp, 1995; Bron *et al*, 2007; Sullivan *et al*, 2012b). This group of patients may benefit from early treatment with lipid sprays to help reduce evaporation from the ocular surface prevent or delay the onset of overt symptoms.

Given that Cluster 1 was suggestive of symptomatic dry eye patients but without a certain etiopathological cause, cases in this cluster were selected for further K-means cluster analysis where two clusters (1a and 1b) were identified. However these clusters were only separated by OSDI score and the presence of corneal staining. Interestingly, cluster 1b had lower OSDI scores but greater presence of corneal staining than cluster 1a. It may be that this cluster paradoxically consists of more severe aqueous deficient dry eye patients, where the increased hyperosmolarity causes corneal staining; but the reduction in corneal sensitivity in chronic disease, caused by longterm effects of inflammation on the sensory nerve terminals supplying the ocular surface, may result in reduced dry eye symptoms (Xu *et al*, 1995; Bourcier *et al*, 2005; Benitez-Del-Castillo *et al*, 2007). In patients with Sjogren's syndrome, Adatia et al (2004) found that corneal staining (fluorescein and lissamine green) correlated negatively with corneal sensitivity (measured using Cochet-Bonnet

aesthesiometer) and overall symptom scores (including OSDI measurement), suggesting that those with advanced dry eye and corneal staining tend to have fewer dry eye symptoms (Adatia *et al*, 2004). In turn, the reduced sensory input, may lead to reduced lacrimal gland secretion and tear film volume as evidenced by the low meniscus height and Schirmer test 1/PRT scores (Lemp, 2007; Bron *et al*, 2009). Further, the reduced aqueous secretion and subsequent tear volume may inhibit the spread of the tear film lipid layer, resulting in a concurrent functional evaporative dry eye that may exacerbate tear film hypersomolarity (Bron *et al*, 2009). The patients in cluster 1a may represent those at an earlier stage of aqueous deficiency dry eye, where hyperosmolarity results in staining of the ocular surface but is insufficient to affect corneal sensitivity, thus accounting for the reduced tear meniscus and higher OSDI scores. The patients in clusters 1a and 1b may therefore benefit from more viscous artificial tear substitutes with hypoosmoloarity electrolytes to help replenish the aqueous component and protect against inflammation and epithelial damage caused by hyperosmolority of the tears.

Other dry eye tests such as tear film osmolarity and fluorophotometry may have helped identify different dry eye subgroups. However, a previous cluster analysis study found that these tests were not needed to classify blepharitis, dry eye and ocular surface disease patients into clinically relevant clusters (Mathers & Choi, 2004). This study divided these patients into 9 categories based upon 6 initial clusters, where, in order of utility, meibomian gland dropout, lipid viscosity, evaporation, Schirmer test (1), and lipid volume was able to separate cases (Mathers & Choi, 2004). Interestingly, normal subjects were distributed across 8 of the 9 categories, suggesting the overlap between signs and symptoms is not confined to dry eye but a range of ocular surface and eyelid margin disease (Mathers & Choi, 2004). Since meibomian gland dysfunction is considered the most common cause of evaporative dry eye (Bron & Tiffany, 2004), measurement of meibomian gland function and evaporimetry may have helped separate patients in to more clinically relevant groups in the present cohort. However, with the exception of the Schirmer test, these tests are not often

performed in primary care clinics as they require specialised equipment which is often expensive.

4.6 Conclusions

Since tear film stability is a key mechanism of dry eye common to all forms of dry eye (Lemp, 2007), it is therefore not unexpected that NITBUT and FBUT failed to distinguish between cases in each of the dry eye clusters. Indeed, this study may have captured patients at different stages of dry eye development where compensatory mechanisms or functional exacerbations have occurred. However, cluster analysis appears to have identified three distinct groups, such that an etiopathological explanation may be offered for each based upon the result of common objective tests. Cluster 2 consists of normal and potentially borderline dry eye patients; cluster 1a consists of early stage aqueous deficiency dry eye; and cluster 1b consists of late stage aqueous deficiency dry eye. Therefore, it appears that current and commonly used clinical tests in primary care for dry eye are able to separate patients and identify different and clinically relevant dry eye subtypes to help guide treatment, rather than relying on trial and error to determine the most effective treatment.

Chapter 5

The Effect of Eyelid Warming Therapy on Eyelid Temperature and Tear Film in Healthy Eyes

5.1 Introduction

Meibomian gland dysfunction (MGD) is a common disorder representing a condition of various aetiologies (Bron *et al*, 2004). Obstructive meibomian gland disease is considered to be the most common subtype (Foulks *et al*, 2003), typically caused by altered meibomian secretion and obstruction of the terminal ducts or plugging of the orifices (Foulks *et al*, 2003; Nelson *et al*, 2011). Normally the meibomian gland secretion forms reservoirs along the upper and lower lid margin from which the tear film lipid layer is formed, which acts to stabilise and prevent evaporation of the tear film (Foulks *et al*, 2003). In obstructive MGD, the secretion delivered to the reservoir may be reduced to a point where the lipid layer is insufficient to prevent evaporation effectively, thus causing dry eye (Foulks, 2007).

There is considerable variation in the temperature at which the meibomian secretion begins to melt in the scientific literature, owing to the significant variation in chemical composition of the meibum between individuals and even between glands of the same eye (Bron *et al*, 2004). However higher lipid melting points have been found in MGD compared to normal eyes (McCulley & Shine, 1998; Terada *et al*, 2004; Mitra *et al*, 2005). Therefore, eyelid warming therapies have been recommended as a treatment for MGD to clear the obstructed glands (Mitra *et al*, 2005; Goto *et al*, 2002; Mori *et al*, 2003; Olson *et al*, 2003; Matsumoto *et al*, 2006; Ishida *et al*, 2008; Lane *et al*, 2012). Here, the heat transferred melts the pathologically altered lipids that have become inspissated and stagnant and relieve the dry eye symptoms associated with MGD (Goto *et al*, 2002; Geerling *et al*, 2011). Warming can be achieved by a variety of means, such as warm moist compresses, warm moist air, warm compression devices, light emitting diode and chemical reaction based eye masks, and heat and pulsatile pressure combination therapy.

5.2 Study Aim

Since 2004 an eyelid warming device called the MGDRx EyeBag (The EyeBag Company, Halifax, UK) has been commercially available to treat MGD, composed of one silk and one cotton surface, filled with flax seed (Figure 5.2.1). However there appears to be no scientific evidence to demonstrate eyelid warming or support the efficacy of this device. The aim of this study was to determine the temperature of the external and internal upper and lower eyelids following a warm compress with the MGDRx EyeBag. In addition, the short term effects of warm compresses with the MGDRx EyeBag on the tear film lipid layer thickness and tear film stability were investigated in order toaddress the purported therapeutic actions of the MGDRX EyeBag.

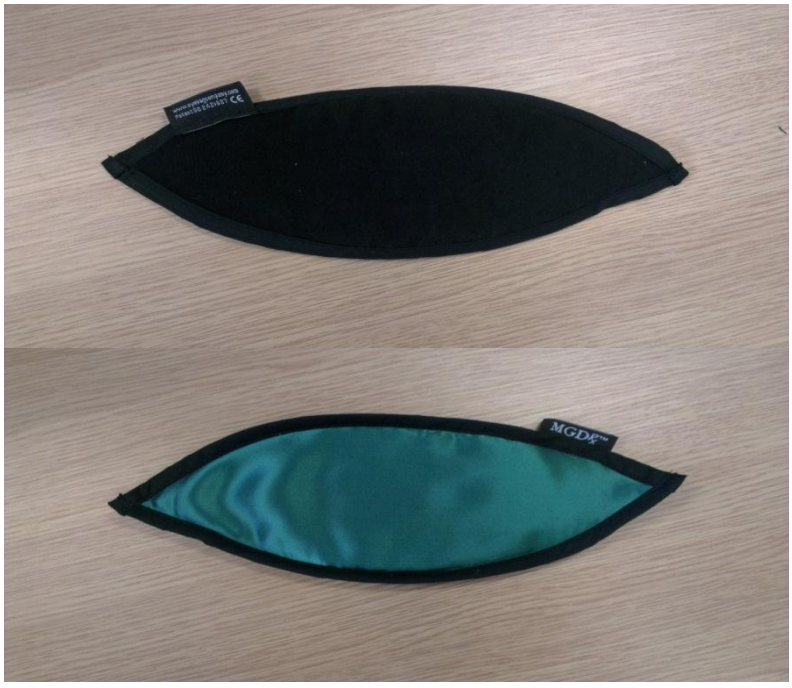

Figure 5.2.1: Images of the MGDRx EyeBag eyelid warming device showing the silk (top) and cotton (bottom) surfaces. It is approximately 11cm at its widest section, 25cm in length and 1.5cm in height when lying flat, with an approximate weight of 130g.

5.3 Methods

Subjects recruited consisted of staff, students and optometry clinic patients from Aston University (Birmingham, UK) through advertisement and email correspondence. The study required subjects to be at least 18 years of age, asymptomatic, non-contact lens wearers with no active eye disease, no ocular medications, no systemic medications known to affect the eyes and no history of eye surgery in the last 3 months. Subjects enrolled following written informed consent and underwent slit lamp bio-microscope examination to ensure there was no active eye disease present, including MGD. The primary outcome measure of the study was measurement of ocular surface temperature. To detect a treatment effect of 1°C change in temperature with 80% power at the 5% level of statistical significance (α=0.05), 22 subjects were required as each subject acted as their own control (one test eye, contralateral control eye) (standard deviation of normal values=±1.1°C) (Purslow & Wolffsohn, 2005). The study was approved by the Institutional Review Board and conformed to the Tenets of the Declaration of Helsinki.

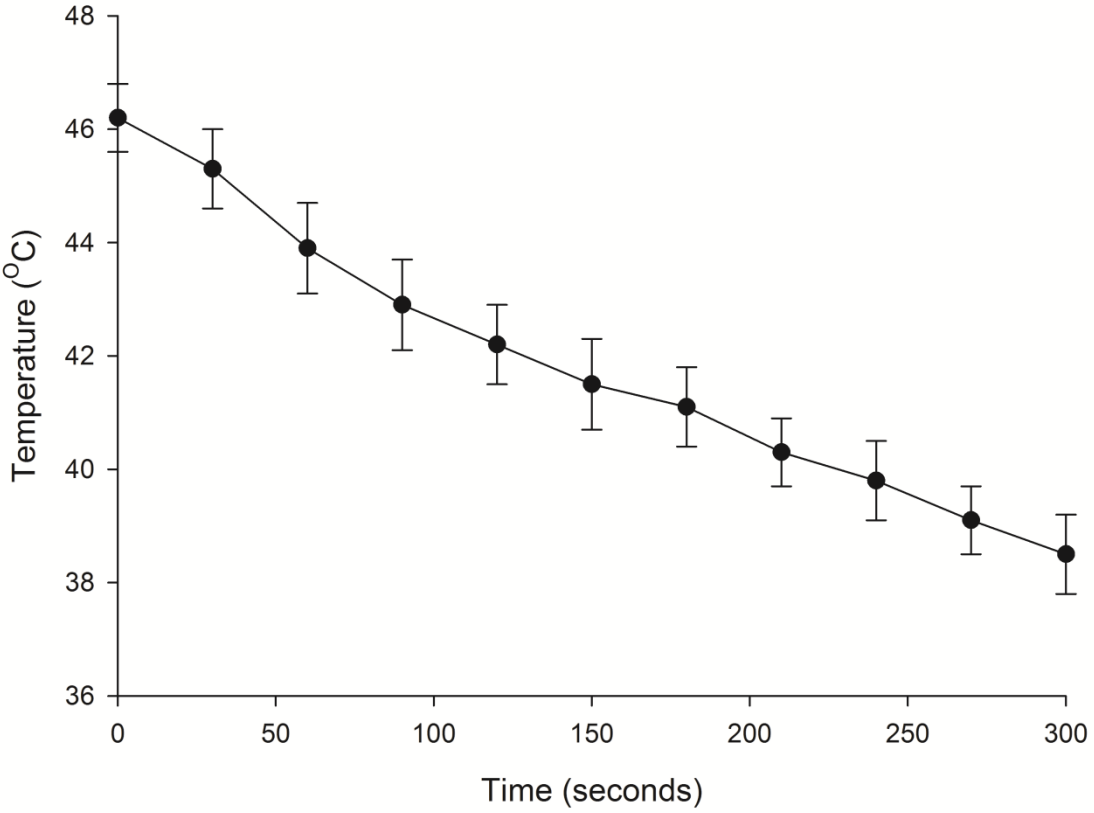

Figure 5.3.1: Mean temperature (°C) of the MGDRx EyeBag at 30 second intervals immediately after heating for 5 minutes. Error bars represent ±1 SD.

An eyebag was heated for 40 seconds in an 800W microwave oven at full power as recommended in the manufacturer's instructions. The region of the heated eyebag intended to be in contact with the eyes was measured to be 46.2±0.6°C (115.2±1.1°F) immediately after heating using a thermal camera (ThermoTracer 7102MX, NEC, Japan) on 5 eyebags, repeated on 3 occasions at least 24 hours apart. After 5 minutes, the eyebag cooled to a mean surface temperature of 38.5±0.7°C (101.3±1.3°F; Figure 5.3.1). The eyebag was applied with the silk surface in contact with the eyelids of one eye selected at random (using a random number generator) for 5 minutes immediately after heating by an unmasked researcher (SN). At the same time, a non-heated eyebag (mean surface temperature=18.1±1.0°C; 64.6±1.8°F) was applied (silk side) to the contralateral eye as a

control (Figure 5.3.2) by the same researcher. Care was taken to ensure that the eyebags did not touch. Timing was kept using a digital stop-clock. A second but masked researcher conducted the study measurements (PSB). Measurements were taken before (baseline), immediately after and 10 minutes after the removal of the eyebags and the order of which eye was measured first was randomised using a random number generator. The test room was the same for all subjects and had a temperature of 20-22°C (68.0-71.6°F), 10-20% humidity and no air circulation/wind.

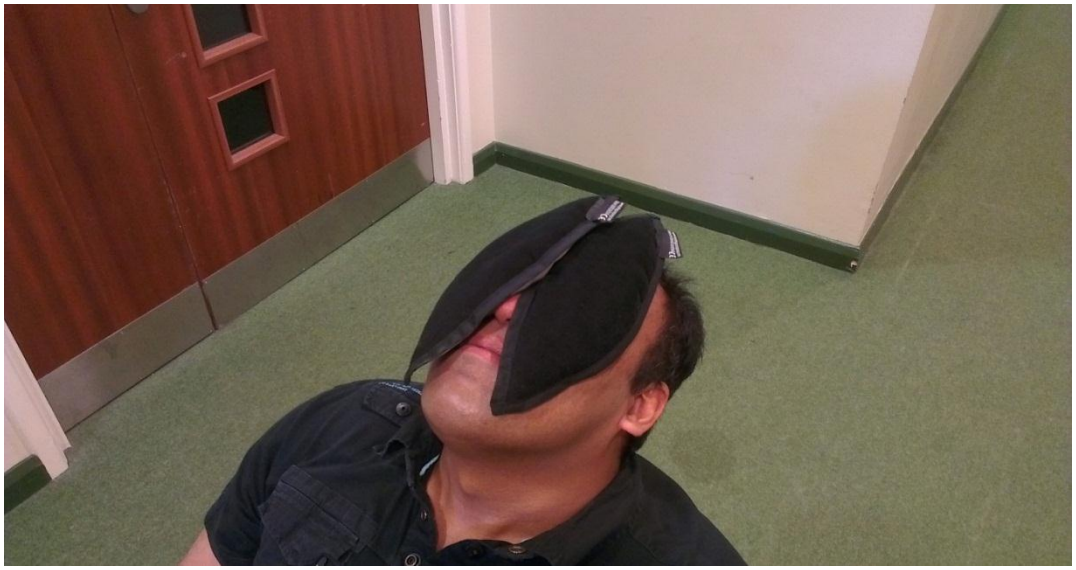

Figure 5.3.2: Position of the MGDRx EyeBags applied to the right and left eyes.

The surface temperature of the central internal and external upper and lower eyelids (external upper eyelid=EUL; external lower eyelid=ELL; internal upper eyelid=IUL; internal lower eyelid=ILL) of both eyes was measured using the thermal camera (as above) mounted on a slit lamp bio-microscope (Purslow & Wolffsohn, 2005; Giraldez *et al*, 2009). The external upper and lower eyelids were measured by asking the subject to look down and up; the internal upper and lower eyelids were measured by everting the upper and lower eyelids with a sterile cotton bud. At each location a thermal image was captured immediately to

provide a static temperature reading averaged over a 10mm by 10mm area shown on the camera display. Central, nasal and temporal markers on the thermal camera display were used to ensure that the same central region of the eyelid surfaces was measured to maintain consistency. Temperature measurements of the external eyelid surfaces were made at baseline, within 10 seconds after eyebag removal (immediately after) and 10 minutes after treatment. The inner eyelid surfaces were measured within 10 seconds of eyelid eversion using sterile cotton buds.

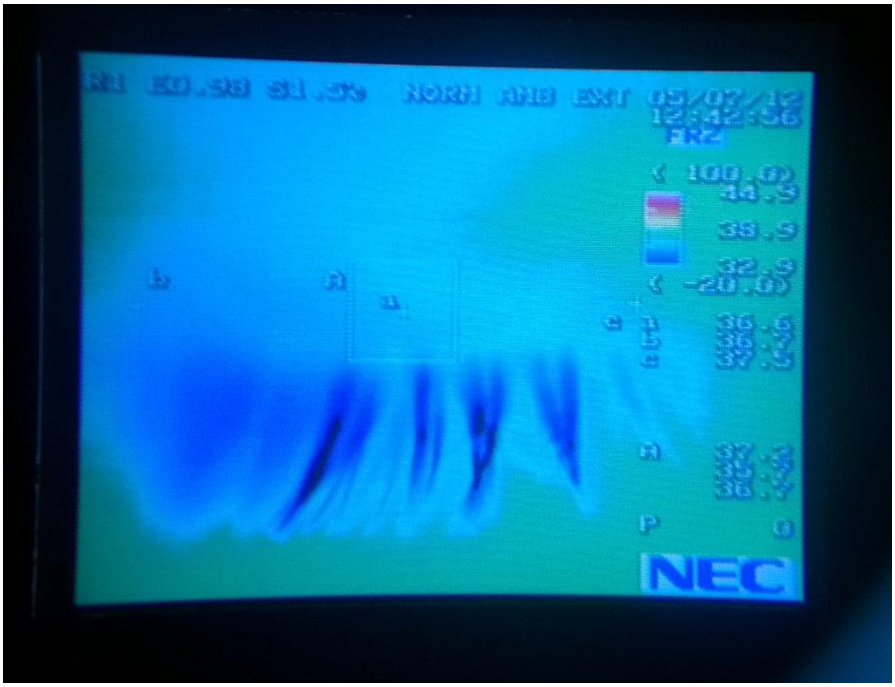

Figure 5.3.3: Thermal image of the external upper eyelid. Temperature was taken as the average temperature within the 10mm by 10mm box “A”. Central (a), temporal (b), and nasal (c) markers were used to ensure the same area of the eyelid was measured.

The thickness of the tear film lipid layer and tear film stability were measured in both eyes non-invasively using the TearScope Plus (Keeler Ltd, Windsor, UK) mounted on a slit lamp bio-microscope. An interference image of the lipid layer was produced over the cornea as subjects looked at a distant target (spot light 6m away) while blinking naturally. Based upon

the interference pattern observed, the thickness range of the lipid layer was deduced using a grading scale developed by Guillon (1998) where grade 1=open meshwork, lipid layer 13-50nm thick; 2=closed meshwork, 30-50nm; 3=wave, 50-70nm; 4=amorphous, 80-90nm; 5=coloured fringes, 90-180nm; 6=globular, >200nm (Guillon, 1998).

Open meshwork, wave and amorphous patterns represent a normal tear film lipid layer. A grid pattern insert was used to assess tear film stability by measuring non-invasive tear film break up time (NITBUT) as described in Chapter 4.3. Measurements were made at baseline, immediately after and 10 minutes after treatment.

Statistical analysis was performed using SPSS for Microsoft Windows. Differences between control and test eyes for normally distributed data (Kolmogorov-Smirnov Test $p>0.05$; eyelid temperature and NITBUT) over time were evaluated by repeated measures ANOVA, and where significant, post-hoc analysis was performed using t=tests with a Bonferroni correction applied (significance level set at $p<0.05$).

Differences between control and test eyes for non-normally distributed data (lipid layer thickness) were evaluated by the Freidman test as the classifications are non-linear and post-hoc analysis where significant, was performed using Wilcoxon signed-rank tests with a Bonferroni correction applied (significance level set at $p<0.05$).

5.4 Results

A total of 22 healthy subjects (50% male) were assessed and had a mean age of 22.0±2.7 years (range 18-27 years) aged matched by gender. No adverse events were reported during or after the study.

Eyelid Temperature

At baseline there was no statistically significant difference between control and test eyelid temperature at all locations (EUL: 35.0±1.2°C versus 35.1±1.1°C, p=0.514; ELL: 35.0±1.2°C versus 35.0±1.1°C, p=0.920; IUL: 35.6±1.1°C versus 35.6±1.1°C, p=0.275; ILL: 35.6±1.0°C versus 35.6±1.0°C, p=1.000). There was a statistically significant change in temperature over time from baseline in test eyes (F=46.451, p<0.001), but not control eyes (F=0.872, p=0.426). Immediately after removal of the eyebag there was a statistically significant increase in eyelid temperature at all locations from baseline in test eyes (F=20.533, p<0.001) with mean increases of 2.3±1.2°C on EUL, 2.0±1.0°C on ELL, 1.4±1.0°C on IUL and 1.3±1.0°C on ILL (Figure 5.6.1). The difference in temperature between control eyes and test eyes was statistically significant at all eyelid locations (EUL, ELL, IUL, ILL: p<0.001).

Ten minutes post application, the increase in eyelid temperature from baseline in test eyes remained statistically significant at all locations (EUL: 1.0±0.7°C; ELL: 0.9±0.6°C; IUL: 0.7±0.6°C; ILL: 0.5±0.6°C; F=14.247, p=0.001), but had decreased from immediately after removal (Figure 5.4.1). The difference in temperature between control and test eyes was also statistically significant at all eyelid locations (EUL, ELL, IUL, ILL; p<0.001).

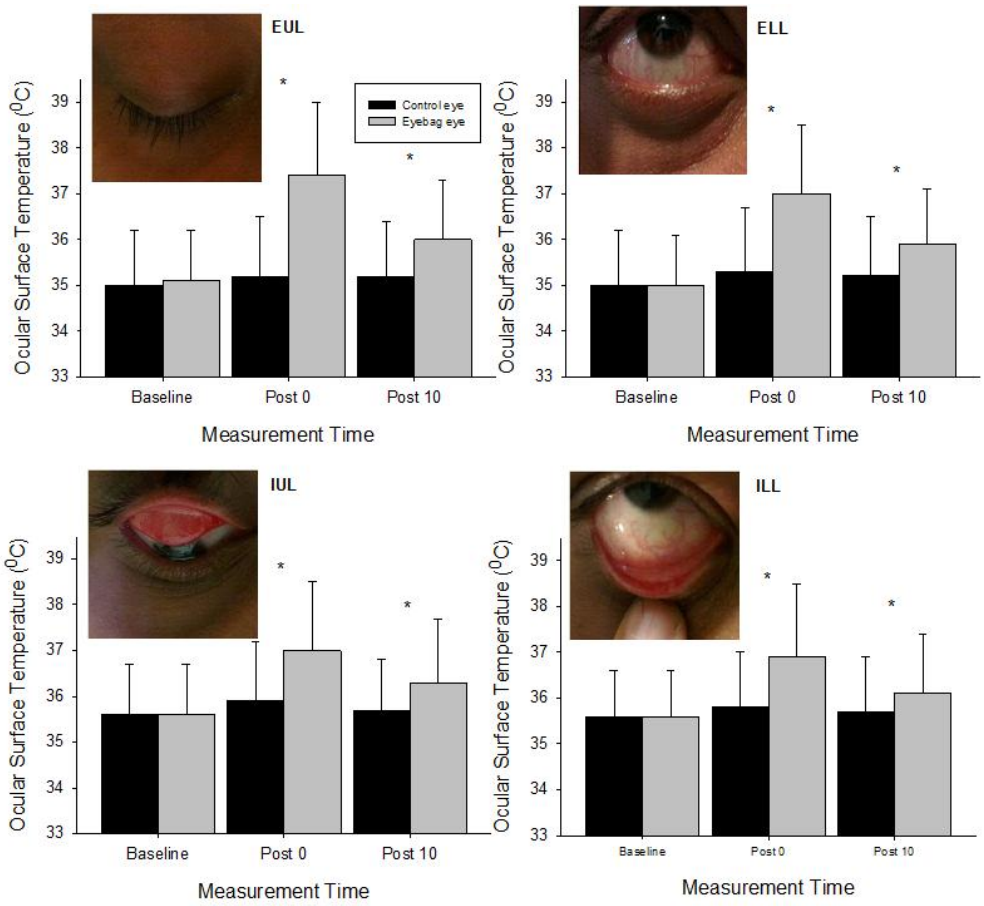

Figure 5.4.1: Mean temperature (°C) at baseline (pre), immediately after (Post 0) and 10 minutes after (Post 10) MGDRx EyeBag removal on the external upper and lower eyelids (EUL, chart A; ELL, chart B) and internal upper and lower eyelids (IUL, chart C; ILL chart D) of control and test eyes. n=22. Error bars represent ±1 SD. *=statistically significant difference between control and test eyes.

Non-Invasive Tear Film Break Up Time

At baseline there was no statistically significant difference in NITBUT between control and test eyes (9.2±2.4 seconds versus 9.4±1.8 seconds, p=0.468). There was a statistically significant increase in NITBUT in test eyes (F=47.904, p<0.001), but not control eyes (F=0.625, p=0.540), over time from baseline. The difference in NITBUT between control and test eyes immediately and 10 minutes after eyebag removal was also significant (p<0.001). There was a statistically significant increase in NITBUT from baseline in test eyes (pairwise comparison p<0.001) with a mean increase of 4.0±2.3 seconds immediately after removal.

Ten minutes post removal, the difference in NITBUT from baseline in test eyes was also statistically significant (difference 3.6±2.1 seconds, pairwise comparison p<0.001). Although the NITBUT reduced slightly from immediate to 10 minutes after eyebag removal (Figure 5.4.2), this was not statistically significant (pairwise comparison p=0.322).

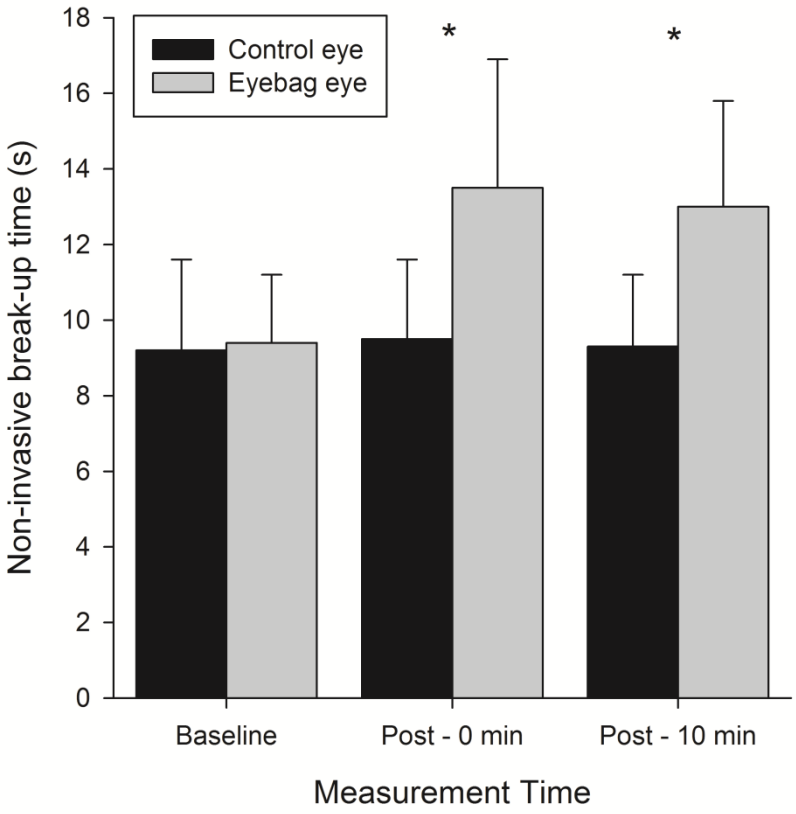

Figure 5.4.2: Mean non-invasive tear film break up time (seconds) at baseline (pre), immediately after (Post 0) and 10 minutes after (Post 10) MGDRx EyeBag removal on control and test eyes. n=22. Error bars represent ±1 SD. *=statistically significant difference between control and test eyes.

Tear Film Lipid Layer Thickness

At baseline there was no statistically significant difference in lipid layer thickness grade between control and test eyes, with only one subject showing a difference in lipid appearance between the eyes (grade 2.4±0.7 versus 2.4±0.8, Z=0.000, p=1.000). There was

a statistically significant increase in lipid layer thickness in test eyes (X^2=35.313, p<0.001), but not in control eyes (X^2=5.200, p=0.074), over time from baseline. Post-hoc analysis confirmed there was a statistically significant increase in lipid layer thickness from baseline in test eyes (Z=-4.035, p<0.001), with a mean grade increase of 2.0±0.9 immediately after removal. The difference in lipid layer thickness between control and test eyes was statistically significant (Z=-4.102, p<0.001) at this stage. Ten minutes after application the increase in lipid layer thickness from baseline in test eyes remained statistically significant (Z=-3.835, p<0.001), however, the mean increase was smaller than immediately after removal (1.5±0.9 grades higher than baseline). After 10 minutes the difference between control and test eyes also remained statistically significant (Z=-3.759, p<0.001). The distribution of lipid layer thickness grade at baseline, immediately after and 10 minutes after eyebag application is shown in Figure 5.4.3.

Figure 5.4.3: Frequency distribution of tear film lipid layer thickness patterns for control and test eyes at baseline (chart A), immediately after (Post 0; B) and 10 minutes after (Post 10; C) application of the MGDRx EyeBag. n=22. Grade 1 (pattern: open meshwork) = 13-50nm; 2 (closed meshwork) = 30-50nm; 3 (wave) = 50-70nm; 4 (amorphous) = 80-90nm; 5 (coloured fringes) = 90-180nm; 6 (globular) = >200nm (Guillon, 1998).

5.5 Discussion

The temperature of test eyes covered with the heated eyebag increased significantly at all measurement locations from baseline immediately after removal, demonstrating the transfer of heat from the eyebag to the eyelid tissue, including the meibomian glands. The maximum mean temperature measurement was 37.4±1.6°C (99.3±2.9°F) which occurred on the external upper eyelid immediately after eyebag removal. This was not unexpected given the relatively closer and larger surface area in contact with the eyebag compared to the lower external eyelid when the eyes were closed. The temperatures presented here are

lower and the treatment duration shorter than those reported to cause thermal injury to the eyelid tissue (Despa *et al*, 2005). In addition, heat (infra-red) radiation is also known to cause cataract (Lydahl & Philipson, 1984) - in an animal model it has been shown that lens protein changes start to develop at 40°C (104°F) following 2 minutes direct infra-red exposure (Okuno, 1994). Eyebag therapy applies heat through the closed lids so the crystalline lens will be protected to some extent. It was not possible in our study to measure the maximum eyelid temperature as the eyebag obscured the view of the eye - it is likely that the eyelids were initially warmer during earlier stages of heating as the eyebag naturally decreased in temperature over the 5 minute treatment protocol (Figure 5.6.1). Thus the maximum eyelid temperature achieved during heating is unknown and it is unclear if thermal injury is possible. However, no adverse events were reported during or after the study. Eyelid warming therapies have also been associated with transient visual blur, caused by pressure exerted on the eyelids. Solomon *et al* (2007) found the corneal polygonal reflex of Fischer-Schweitzer in experimental eyes receiving warm compress therapy combined with gentle eyelid pressure was significantly positively correlated to visual blur and visual acuity decrease (Solomon *et al*, 2007). More recently increases in corneal temperature following eyelid rubbing or massage has been associated with corneal deformation and subsequent visual degradation (McMonnies *et al*, 2012). It is purported that eyelid warming therapy where heat is applied directly to the eyelids combined with massage may exacerbate this effect (McMonnies *et al*, 2012). Therefore we did not apply any additional pressure to the eyelids other than to stabilise the device and our treatment protocol did not require massage. Until the safety of eyelid warming therapy with devices making contact with the eyelids such as the eyebag is fully established, patients should be instructed carefully as to their use and advised not to rub the eyes after treatment (McMonnies *et al*, 2012). The internal lower eyelid was less warm immediately after eyebag removal, and the internal upper eyelid temperature was also less warm than the external upper eyelid, providing further support for non-complete heat transfer between external and internal eyelid surfaces (Blackie *et al*, 2008). This is likely to be due to blood flow within the eyelid vasculature which carries heat

away via convection as well as the different insulating properties of the ocular tissues (Despa *et al*, 2005; Huang *et al*, 2010). However, the temperature of the internal eyelid, an area of tissue in closer apposition to the meibomian glands than the external eyelid, was significantly warmer compared to baseline in test eyes. Although not statistically significant, the small increase in temperature in control eyes immediately after removal is somewhat surprising given that the non-heated eyebag was much cooler than eyelid temperature. It is likely to have been caused by the insulating properties of the non-heated eyebag and or the creation of a layer of air between the eyelid and eyebag surfaces minimising heat loss.

Ten minutes post removal the temperature of test eyes remained significantly warmer at all test locations from baseline ($F=20.533$, $p<0.001$), but lower than immediately after application ($F=24.889$, $p<0.001$). The heat source had been removed 10 minutes earlier and heat energy dissipated to neighbouring tissues and the surrounding air, causing the surfaces to cool. The rate of cooling appeared to be slightly faster for the external eyelid surfaces compared to the inner eyelid surfaces in test eyes. This finding could be explained by the larger temperature gradient between the external eyelid surface and air compared to the inner eyelid surface and globe. In addition, the inner eyelid surface is insulated by the eyelid tissue from exposure to the surrounding air.

A potential source of error in our measurements could have resulted from everting the upper and lower eyelids to obtain internal eyelid surface temperature – although brief (approximately 5 seconds), the time taken from everting the eyelids to measurement may have allowed heat loss via convection following exposure to the air. In addition, the time taken to measure one eye may have impacted on the contralateral eye measurement in a similar fashion, but the order of which eye was measured first was randomised, negating any systematic bias. Manipulating the eyelids during eversion may have also caused changes in vascular blood flow and subsequent changes in heat loss (Mori *et al*, 1999). In addition, although cotton buds were used to evert the upper and lower eyelids, the heat radiated from the eyes following treatment may have been detected by the examiner so they were no

longer masked to the treatment eye. Therefore bias may have been introduced in our measurements. However, given the nature of eyelid warming therapies it is not possible to completely eliminate this limiting factor where measurements are taken by human examiners. Tear film break up time was measured non-invasively rather, than using fluorescein sodium as the latter may disrupt the tear film by artificially increasing the volume of the tear film and the application technique inducing reflex tearing, all of which may affect the measurement (Mengher *et al*, 1986). In addition, the TearScope utilises a cold light source which prevents/minimises any additional evaporative loss (Guillon, 1998).

Tear film stability has been demonstrated to correlate significantly with the thickness of the tear film lipid layer, derived from secretions of the meibomian glands (Bron *et al*, 2004; Nichols *et al*, 2002; Isreb *et al*, 2003). Increasing the tear break up time and lipid layer thickness may therefore help prevent dry eye symptoms associated with MGD (Foulks, 2007; Mori *et al*, 2003). In our study, immediately after removal of the heated eyebag, a significant increase in NITBUT from baseline was detected ($p<0.001$), which was sustained at 10 minutes post application ($p=0.965$). Previous studies on eyelid warming therapies have also demonstrated significant increases in tear film stability and improved symptoms in MGD patients and normal subjects, although the treatment methodology differed with respect to eyelid warming device used, and the frequency and duration of application (Mitra *et al*, 2005; Goto *et al*, 2002b; Mori *et al*, 2003; Matsumoto *et al*, 2006; Lane *et al*, 2012).

Tear film lipid layer thickness also increased significantly in test eyes and this increase from baseline was sustained at 10 minutes post application, albeit at a slightly smaller thickness than immediately after eyebag removal. Increases in tear film lipid layer thickness following eyelid warming therapy in healthy subjects and MGD patients have been demonstrated in previous studies, although the treatment methodology differed again with respect to eyelid warming device used, and the frequency and duration of application (Mitra *et al*, 2005; Mori *et al*, 2003; Olson *et al*, 2003; Matsumoto *et al*, 2006). Increases in tear film lipid layer thickness following eyelid warming therapy have also been associated with improved signs

and symptoms in patients with MGD and normal subjects (Mitra *et al*, 2005; Mori *et al*, 2003; Matsumoto *et al*, 2006; Ishida *et al*, 2008). Although statistically insignificant, the increase in lipid layer thickness observed in control eyes may hev been due to the increase in temperature in control eyes, where the heat may have caused the meibum to become less viscous and subsequently increasing the availablity of lipid to the eyelid margin and tear film.

5.6 Conclusions

Based on the present data, the short-term increases in tear film stability and tear film lipid layer thickness in healthy subjects can be attributed to the heat transferred across the eyelids, consistent with previous studies and supports the use of eyelid warming therapy for MGD treatment. The eyebag is simple to heat and handle compared to traditional moist warm compress, which are reported to require a methodical and labour intensive protocol to optimise treatment (Blackie *et al*, 2008). Furthermore, the eyebag is relatively inexpensive and can be reused by the same person, whereas many devices providing alternative sources of heat are not commercially available (Mitra *et al*, 2005; Goto *et al*, 2002b; Mori *et al*, 2003; Matsumoto *et al*, 2006; Mori *et al*, 1999). More recently, the LipiFlow system, which combines eyelid heating and massage, has demonstrated short and long term improvements in meibomian gland secretion and tear break up time after a single in-office treatment including in severe cases of MGD (Lane *et al*, 2012). In comparison the eyebag can be recommended by optometrists for home therapy in milder cases, or as adjunctive therapy following in-office procedures in severe cases where more intensive treatment is required. Although the eyebag requires the eyes to be closed during application which minimises exposure to direct heat, further research is required to determine the maximum eyelid temperature induced to evaluate the safety of this eyelid warming device. After 5 minutes application, the heat transferred from MGDRx EyeBag produces increases in tear film stability and lipid layer thickness that are maintained up to 10 minutes post application.

These effects on the tear film have improved the signs the signs and symptoms of MGD in previous studies and support the use of eyelid warming therapy in MGD. However, these short term effects were observed in healthy subjects without MGD, who are likely to have lower meibomian secretion melting points and few, if any, obstructed glands. In addition, long term and frequent treatment may be necessary to produce a clinically significant improvement in signs and symptoms of MGD owing to its chronic nature, although short term effects are encouraging. Future longer treatment duration clinical studies are therefore required to determine the efficacy and safety of this eyelid warming device in patients with MGD, which is investigated in the next Chapter.

Chapter 6

Efficacy of the MGDRx EyeBag for the Treatment of Meibomian Gland Dysfunction Related Evaporative Dry Eye

6.1 Introduction

The effect of eyelid warming therapy with the MGDRx EyeBag in healthy subjects was investigated in Chapter 4. In a randomised, internally controlled, examiner masked study, the MGDRx EyeBag produced a significant increase in both tear film lipid layer thickness and tear film break up time. The transfer of heat to the eyelids following treatment with the MGDRx EyeBag was demonstrated using infrared thermography, where a significant increase in temperature was observed on the upper and lower internal and external eyelids.

Previous studies have demonstrated that eyelid warming therapy, using a variety of devices, are efficacious in the treatment of MGD but many are not commercially available in the UK (Goto *et al*, 2002b; Olson *et al*, 2003; Mori *et al*, 2003; Matsumoto *et al*, 2006; Ishida *et al*, 2008). More recent studies focussed on in-office treatment procedures and have found long term improvements in signs and symptoms of MGD (Friedland *et al*, 2011; Greiner 2012; Lane *et al*, 2012; Greiner 2013), but these may be reserved for severe cases or those unresponsive to conventional eyelid hygiene and warming procedures. In addition, there are no agreed upon or standardised eyelid warming procedure in the scientific literature, complicated with patient compliance issues where they may not heat the device sufficiently, use the device inconsistently, or use the device over an insufficient amount of time (Geerling *et al*, 2011). Studies which have attempted to optimise treatment using conventional warm moist compresses suggest that precise and intense treatment is required to maintain sufficient eyelid warming to melt the pathologically altered lipid in MGD (Blackie *et al*, 2008), but this process may not be feasible for certain patients such as the young and elderly owing to a complex treatment protocol (heating to 45°C, optimise contact with warmcompress and

eyelid, reheat the warm compress frequently and have another warm conpress at 45°C to hand to exchange while the other is reheating; and applying for at least 4 minutes per eye) and dexterity and cognitive ability issues. Thus, the MGDRx EyeBag, with its simple design and treatment instructions, may improve patient compliance and combined with the positive findings in Chapter 4, suggest that it may be beneficial in the treatment of MGD and cases associated with evaporative dry eye.

6.2 Study Aim

The aim of this study was to investigate the efficacy of the MGDRx EyeBag in patients with MGD related evaporative dry eye. An additional aim was to compare the non-invasive tear film break up time measurements using 3 different techniques.

6.3 Methods

This study was designed as a randomised, internally controlled (one control eye, fellow eye test/experimental eye), examiner masked clinical trial.

Study participants were identified during routine eye examination in a local optometry practice by KB. Subjects were required to be ≥18 years old, with no systemic disease or medications known to affect the eyes and no other active eye disease except symptomatic MGD related evaporative dry eye. Diagnosis of at least mild severity was based upon recommendation by the diagnostic subcommittee of the International Workshop on Meibomian Gland Dysfunction (Tomlinson *et al*, 2011): Ocular Surface Disease Index (OSDI) questionnaire score of >12; presence of meibomian gland orifice plugging/obstruction on the lower or upper eyelids of both eyes; abnormal meibomian gland function in at least one eye (≤20 years old: quality or expressibility score of >1; >20 years old: quality and expressibility score ≥1; see below). Non-invasive tear film break up time

(NITBUT) of <10 seconds in at least one eye (Mengher *et al*, 1986) and a negative Schirmer-1 Test result (>5.5mm after 5 minutes; van Bijsterveld, 1969) in at least one eye was required to differentiate between evaporative and aqueous deficiency dry eye (Tomlinson *et al*, 2011). Eligible subjects attended for a baseline visit (Day 0) where the diagnosis and entry criteria was confirmed and the following battery of tests was performed in sequence:

Visual acuity: best corrected monocular and binocular visual acuity was measured using a digital logMAR chart at 6m, randomised between presentations. Best corrected logMAR visual acuity was defined as last line on which 3 out of the 5 letters were read correctly. The score was determined using letter by letter scoring with each letter corresponding to -0.02 logMAR units.

Ocular symptomology: dry eye symptoms were assessed for each eye using the validated Ocular Surface Disease Index (Schiffman *et al*, 2000). It consists of 12 questions graded on a scale of 0-4. The OSDI score is calculated by multiplying the total score by 25 and dividing by the total number of questions answered yielding a result between 0 and 100.

Tear film osmolarity: Osmolarity of the tear film in each eye was measured via electrical impedance using the OcuSense TearLab (San Diego, USA). Two measurements were taken in random order from each eye and were averaged to give a mean osmolarity value for each eye (Tomlinson *et al*, 2010).

Corneal topography: Anterior corneal curvature was measured on each eye using the Keratograph 5M (Oculus Optikgeraete GmbH, Wetzlar, Germany).

Tear film lipid layer thickness: was measured non-invasively by assessing the ocular surface interference images produced by a slit lamp mounted TearScope (Keeler Ltd, Windsor, UK) as described in section 4.3 and the Keratograph 5M for each eye, performed in random order.

Tear film stability: was determined by assessing the non-invasive tear film break up time (NITBUT) using a slit lamp mounted TearScope combined with a fine grid pattern insert and (Guillon, 1998) the Keratograph 5M (using the built in software) and human Video Observation (video of Placido ring mires imaged on the tear film via specular reflection from the Oculus Keratograph 5M) on each eye, performed in random order. For the TearScope and Video Observation method, NITBUT was defined as the time period between the last complete blink and the appearance of a break or distortion in the fine grid pattern (Placido Ring (Video Observation) mires (Mengher *et al*, 1986; Guillon, 1998) and was measured using the built in digital stop-clock - this was repeated 2 more times and the values were averaged to give a mean NITBUT value.

Tear Film Meniscus Height: the height of the tear film meniscus was measured using image analysis software on the Keratograph 5M. Patients were instructed to look in primary gaze and specular reflective images of the right and left eyes were captured immediately post blink. Digital callipers were used to measure the height of the tear film meniscus located directly below the centre of the pupil and was defined as the distance between the lower eyelid margin and the upper limit of the reflective zone (Farrell *et al*, 2003).

Conjunctival hyperaemia: digital images of the nasal and temporal bulbar conjunctiva were captured and analysed using image analysis software to provide an objective measurement of conjunctival hyperaemia via edge detection and colour extraction (red) (Wolffsohn & Purslow, 2003). This objective technique has shown sensitivity and repeatability better than subjective grading methods (Wolffsohn & Purslow, 2003).

Meibomian gland function: based upon meibum quality and expressibility (Tomlinson et al, 2011) using a slit lamp. The quality and expressibility of meibum secreted from the central 8 meibomian glands on the lower and upper eyelid was graded on a four-point scale (quality: 0=clear fluid, 1=cloudy fluid, 2=cloudy particulate fluid, 3=inspissated like toothpaste; expressibility: 0=all glands expressible, 1=3-4 glands expressible, 2=1-2 glands expressible,

3=no glands expressible) following firm digital pressure to the eyelid margins. The scores from the upper and lower eyelids were added to give a composite value for quality and expressibility.

Meibomian gland dropout: was measured via infrared meibography using the Keratograph 5M for the central 15 meibomian glands in the upper and lower everted eyelids of each eye (Arita et al, 2013). Meibomian gland dropout in the image was grade using a 4 point scale where 1=no partial glands; 2=<25% partial glands; 3=25-75% partial glands; 4=>75% partial glands (Nichols *et al*, 2005b). A partial gland was defined as one that is incomplete (presumed relative to the length of neighbouring intact meibomian glands) and present in lumps or clusters (Tomlinson *et al*, 2011). The scores from the upper and lower eyelids were added to give a composite value.

Ocular surface staining – damage to the conjunctival and corneal epithelium was assessed via instillation of lissamine green (GreenGlo, Sigma Pharmaceuticals, Monticello, USA) and fluorescein sodium (Fluorets, Chauvin Pharmaceuticals Ltd, London, UK) diagnostic dyes to each eye. Corneal, nasal and temporal bulbar conjunctival staining was graded individually using the Oxford Grading Scale on a 6 point scale (0-5 for each location) to provide a composite score (0-15) for each eye (Bron *et al*, 2003). Intraobserver repeatability (k) has been reported as 0.86 for the cornea and 0.69 for the conjunctiva (Bron *et al*, 2003).

Subjects were then randomised to receive a heated eyebag (40 seconds in microwave on full power) treatment on either the left or right eye (experimental eye). The contralateral eye received a non-heated eyebag treatment (control eye). Subjects were instructed (oral and written) to use the eyebags at the same time twice a day (morning and evening, separated by at least 12 hours) for 2 weeks (Day1-14) beginning the day after baseline measurements and were required to provide feedback on a daily basis – subjects were texted twice a day (morning and evening) to grade ocular comfort for each eye on a 1-10 scale immediately before applying the eyebags (where 1=poor, 10=excellent). This score was also obtained at

baseline (Day 0). The examiner (PSB) was masked to which eye received the heat treatment. Subjects then attended for a follow up visit (Day 15) where they did not use the eyebag and the above measurements were repeated.

Statistical analysis was performed using SPSS for Microsoft Windows. Differences between control and test eyes for normally distributed data (Kolmogorov-Smirnov Test $p>0.05$; visual acuity, ocular symptomology, corneal topography, conjunctival hyperaemia, tear film stability, tear film meniscus height, and tear film osmolarity) were evaluated by paired t-tests with a Bonferroni correction applied (significance level set at $p<0.05$). Differences between control and test eyes for non-normally distributed data (tear film lipid layer thickness, meibomian gland function, meibomian gland dropout and ocular surface staining) were evaluated by the Wilcoxon signed-rank test with a Bonferroni correction applied (significance level set at $p<0.05$). The change in ocular comfort scores over time was evaluated using Friedman's Test and post-hoc analysis was performed using Wilcoxon signed-rank tests with a Bonferroni correction applied.

Sample size was determined using power calculation (80% power at the 5% level of statistical significance, $\alpha=0.05$) To detect a treatment effect of 1 unit change in meibomian gland dropout with 80% power ($\beta=0.2$) at the 5% level of statistical significance ($\alpha=0.05$), 23 subjects were required as each subject acted as their own control (one test eye, contralateral control eye) based upon published data (standard deviation in MGD patients $=\pm1.7$ per eight glands) (Mathers *et al*, 1991).

A total of 25 patients with confirmed MGD related evaporative dry eye (16 female, 4 male) were enrolled and completed the clinical trial with a mean age of 28.7±7.8 years (range 19-42 years).

6.4 Results

Visual Acuity

At baseline, there was no statistically significant difference in LogMAR visual acuity between each eye (control eye (mean ± standard deviation): -0.04±0.03, test eye: -0.04±0.03, p=0.79). After the 2 week treatment period, there was no statistically significant difference between control and test eyes (-0.05±0.03, -0.05±0.03, p=0.42) or compared to baseline measures for test eyes (p=0.06) or control eyes (p=0.08).

Ocular Symptomology and Comfort

There was no statistically significant difference in OSDI scores between control and test eyes at baseline (43.0±14.4, 43.9±13.4, p=0.36). After the 2 week treatment period, there was a statistically significant improvement in OSDI scores in test eyes from baseline (20.7±8.7, p<0.001) but not control eyes (39.8±12.7, p=0.30). The difference between control and test eyes after the 2 week treatment period was also statistically significant (p<0.001; Figure 6.4.1).

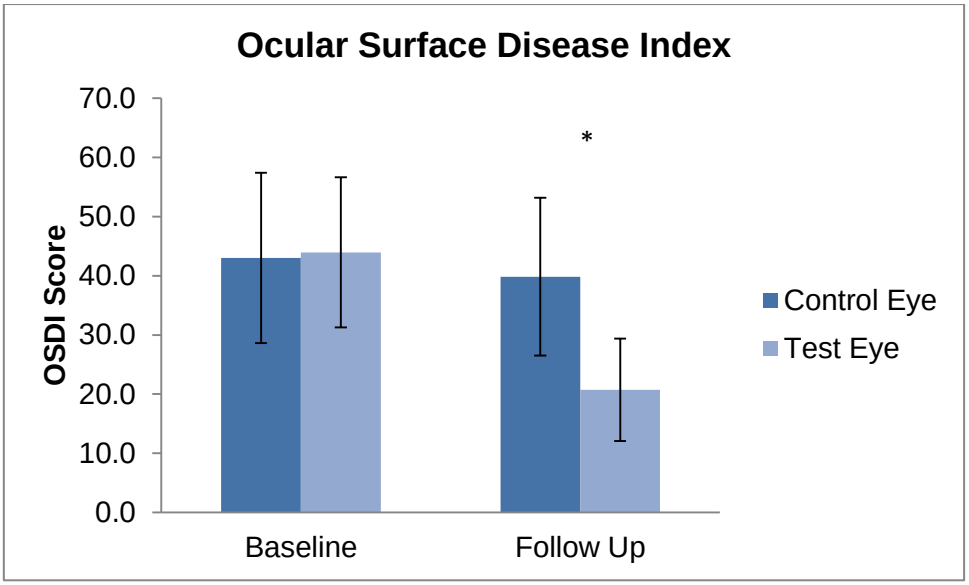

Figure 6.4.1: Mean ocular surface disease index (OSDI) scores for control and test eyes at baseline and follow up (after 2 weeks treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

At baseline (Day 0), there was no statistically significant difference in ocular comfort scores between control and test eyes (mean score control eye: 5.15±1.57; test eye 5.25±1.33; Z=-0.30; p=0.76). An improvement in ocular comfort scores over time was observed over the 2 week treatment period in test eyes (X^2=340.88, p<0.001) but not control eyes (X^2=27.78, p=0.42), with peak mean ocular comfort score in the test eye on the morning of Day 14 (control eye Day 14 morning: 4.95±1.36; test eye: 8.55±0.89; Z=-3.96; p<0.001). Post-hoc analysis showed that a statistically significant difference in comfort scores between control and test eyes occurred from the evening on treatment Day 1 (control eye Day 1 evening: 5.00±1.45; test eye: 6.40±1.35; Z=-3.72, p<0.001), and this difference continued to remain statistically significant in the morning and evenings (p<0.001) for the entire duration of the 2 week treatment period (Figure 6.4.2).

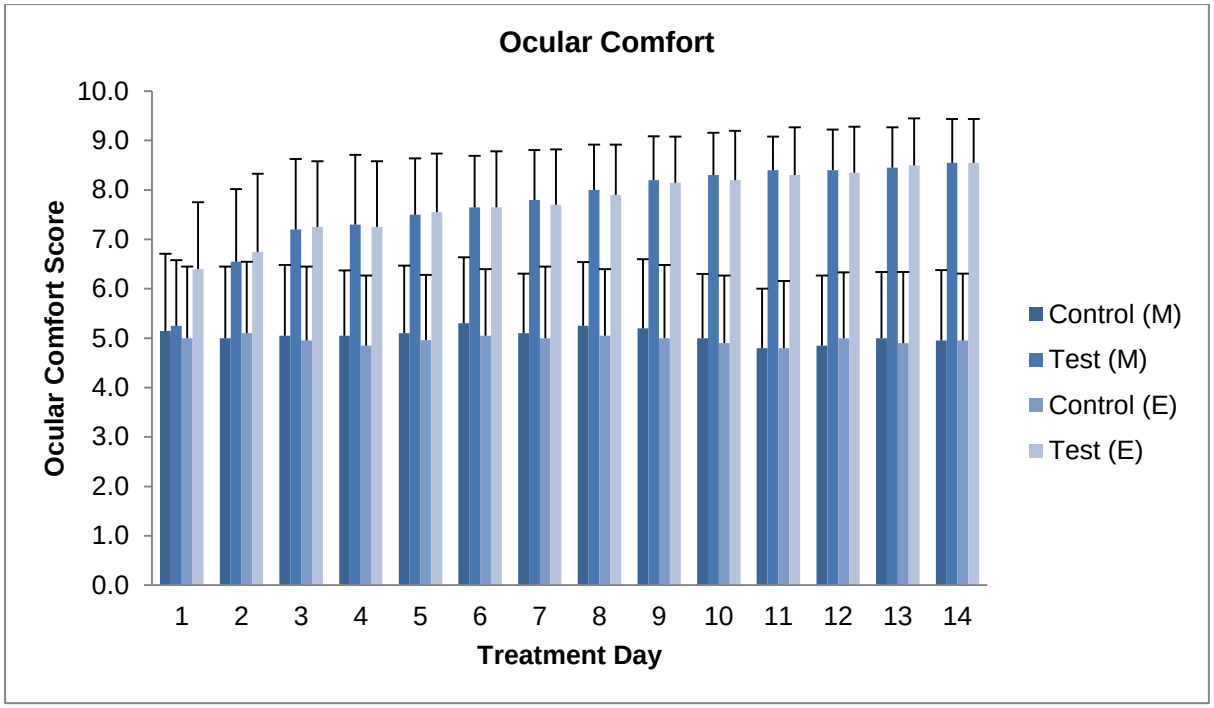

Figure 6.4.2: Mean ocular comfort scores for control eyes and test eyes in the morning (M) and evening (E) from during the treatment period (Day 1 to Day 14). Error bars represent 1 standard deviation. The difference in ocular comfort scores between control and test eyes was statistically significant ($p<0.001$) from the evening of Day 1 onwards.

Tear Film Osmolarity

There was no statistically significant difference in osmolarity between control and test eyes at baseline (310.3±10.6mOsm/L, 310.9±9.5mOsm/L, $p=0.43$). After the 2 week treatment period, there was a statistically significant improvement in osmolarity in test eyes from baseline (305.0±9.1mOsm/L, $p<0.001$) but not control eyes (308.9±11.6mOsm/L, $p=0.14$). The difference between control and test eyes after the 2 week treatment period was also statistically significant ($p=0.03$; Figure 6.4.3).

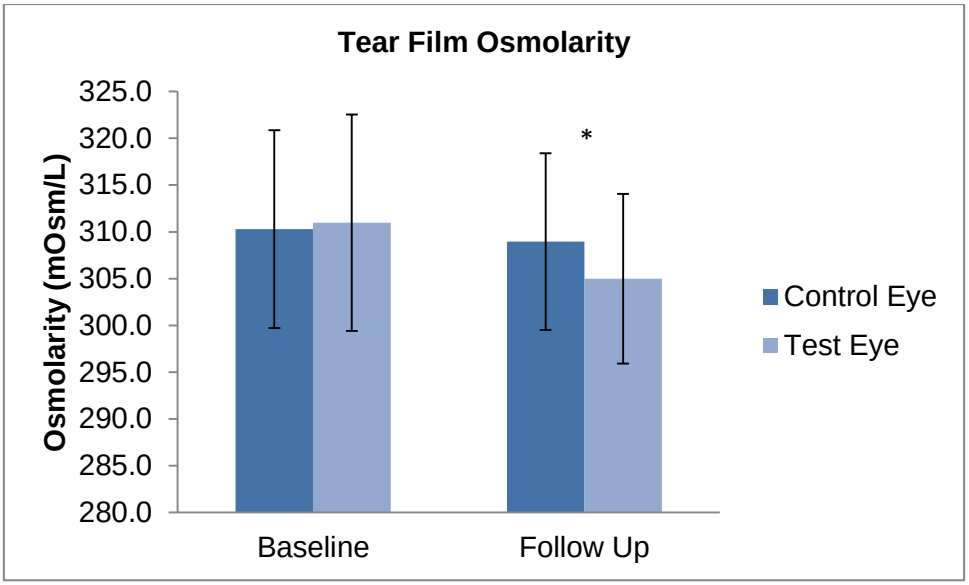

Figure 6.4.3: Mean tear film osmolority for control and test eyes at baseline and follow up (after 2 weeks treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Corneal Topography

At baseline, there was no statistically significant difference in corneal eccentricity between each eye (control eye: 0.37±0.15, test eye: 0.37±0.16, p=0.89). After the 2 week treatment period, there was no statistically significant difference between control and test eyes (0.37±0.15, 0.37±0.17, p=0.91) or compared to baseline measures for test eyes (p=0.65) or control eyes (p=0.73).

Tear Film Stability

Non-invasive tear film break up time (NITBUT) was measured using 3 techniques which showed the same pattern of results (Table 6.4.1 and 6.4.2). There was no statistically significant difference between control and test eyes at baseline ($p>0.05$). After the 2 week treatment period, there was a statistically significant increase in NITBUT in test eyes from baseline, but not control eyes ($p<0.05$). The difference between control and test eyes after the 2 week treatment period was also statistically significant ($p<0.001$).

	NITBUT (seconds)			
	Baseline		Follow Up	
Method	Control	Test	Control	Test
Keratograph	9.36±3.67	9.29±4.24	9.65±3.56	11.38±3.78
Tearscope	9.29±3.64	9.48±3.74	9.32±3.56	11.37±3.45
Video Observation	8.76±3.20	9.19±3.54	9.12±3.25	11.19±3.16

Table 6.4.1: Mean NITBUT values for control and test eyes at baseline and after 2 weeks treatment period (Follow Up) for each testing method.

	p value			
Method	$Control_B$ vs $Test_B$	$Control_F$ vs $Test_F$	$Control_B$ vs $Control_F$	$Test_B$ vs $Test_F$
Keratograph	0.86	<0.001*	0.17	<0.001*
Tearscope	0.12	<0.001*	0.07	<0.001*
Video Observation	0.61	<0.001*	0.89	<0.001*

Table 6.4.2: Statistical significance (p value) between control and test eyes at baseline ($_B$) and follow up ($_F$) for each NITBUT testing method. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

A statistically significant liner relationship was found between the NITBUT measured by the Keratograph 5M and video observation (r=0.92, p<0.001); Keratograph 5M and Tearscope (r=0.94, p<0.001); and Tearscope and Video Observation (r=0.96, p<0.001). There was no statistically significant difference in NITBUT measurements between the Keratograph 5M and Video Observation (p=0.16), and the Keratograph 5M and Tearscope (p=0.73) but was statistically significant between and the Tearscope and Video Observation (p<0.05). Agreement between the instruments was evaluated using Bland-Altman analysis (Table 6.4.3)

	NITBUT Bland-Altman Analysis				
	Bias	**SD**	**LoA**	**LL**	**UL**
T vs K	-0.06	1.46	2.85	-2.91	2.80
T vs VO	0.30	0.97	1.90	-1.60	2.20
K vs VO	0.35	1.48	2.90	-2.55	3.26

Table 6.4.3: Bland-Altman analysis to determine the degree of agreement between between the Tearscope (T), Keratograph 5M (K) and Video Observation (VO), where SD=standard deviation, LoA=Limits of Agreement, LL=Lower Limit, and UL=Upper Limit.

Tear Film Lipid Layer Thickness

At baseline, there was no statistically significant difference in tear film lipid layer thickness grade between control and test eyes (2.10±0.72, 2.05±0.89, Z=-0.45, p=0.66). After 2 weeks treatment period, there was a statistically significant increase in test eyes from baseline (3.1±0.91, Z=-3.52, p<0.001), but not control eyes (2.2±0.62, Z=-1.00, p=0.32). The difference between control and test eyes after the 2 week treatment period was also significant (Z=-3.11, p<0.05; Figure 6.4.4).

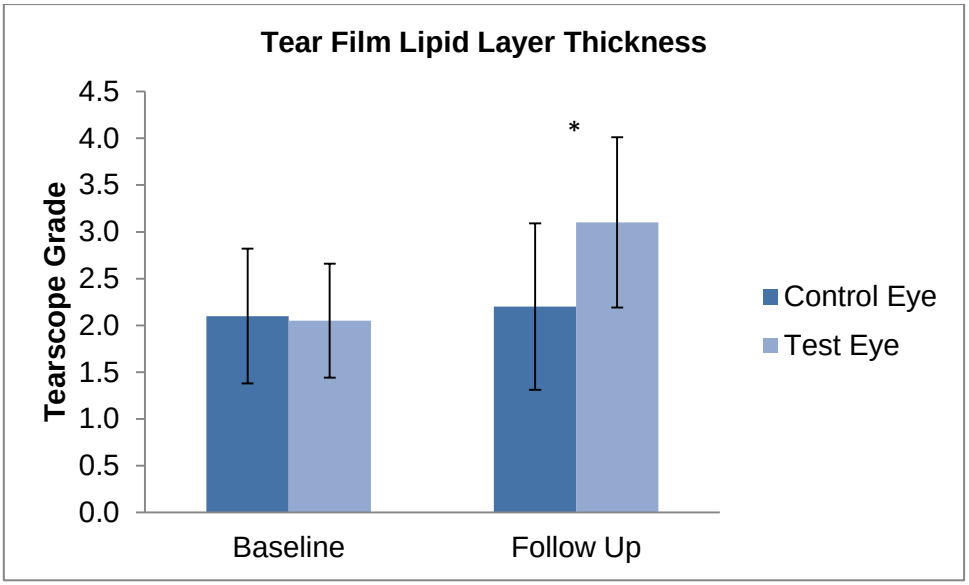

Figure 6.4.4: Mean tear film lipid layer thickness grade for control and test eyes at baseline and follow up (after 2 weeks treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Tear Film Meniscus Height

There was no statistically significant difference in non-invasive tear film meniscus height (NITMH) between control and test eyes at baseline (0.34±0.06mm, 0.34±0.07mm, p=0.69). After the 2 week treatment period, there was a statistically significant improvement in tear film meniscus height in test eyes from baseline (0.36±0.06mm, $p<0.05$), but not control eyes (0.34±0.06mm, p=0.29). The difference between control and test eyes after the 2 week treatment period was also statistically significant ($p<0.001$; Figure 6.4.5).

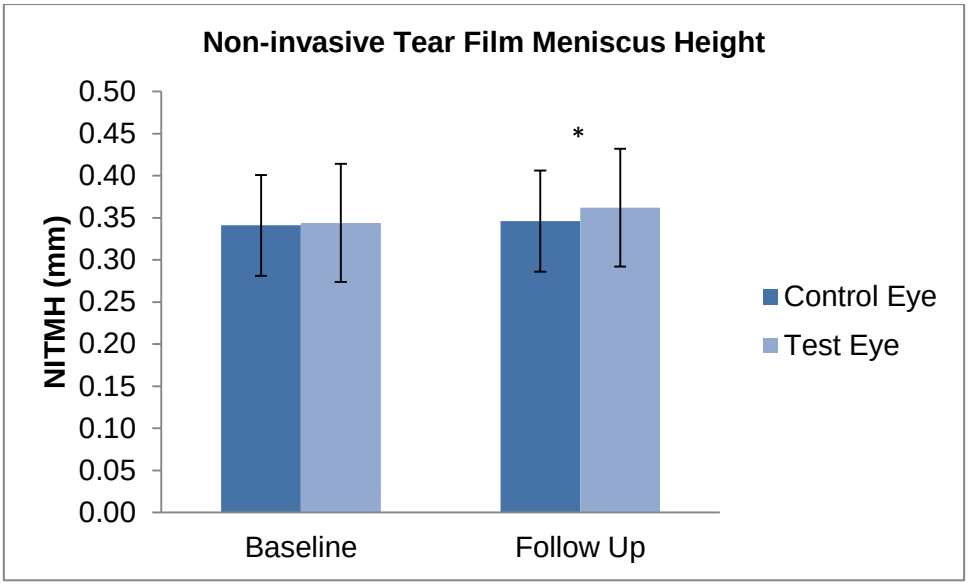

Figure 6.4.5: Mean NITMH for control and test eyes at baseline and follow up (after 2 weeks treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Conjunctival Hyperaemia

At baseline there was no statistically significant difference in blood vessel edge detection between control and test eyes for both nasal (1.94±1.41%, 1.67±0.99%, p=0.40) and temporal (1.78±1.62%, 1.55±1.38%, p=0.15) locations. After the 2 week treatment period, there was statistically significant reduction in blood vessel edge detection in test eyes at both locations (nasal: 0.99±0.65%, p<0.05; temporal: 0.89±0.64%, p<0.05), but not control eyes (nasal: 1.45±0.95%, p=0.11; temporal: 1.42±1.17%, p=0.12). The difference between control and test eyes after the 2 week treatment period was statistically significant at both nasal (p<0.05) and temporal (p<0.05) locations (Figure 6.4.6).

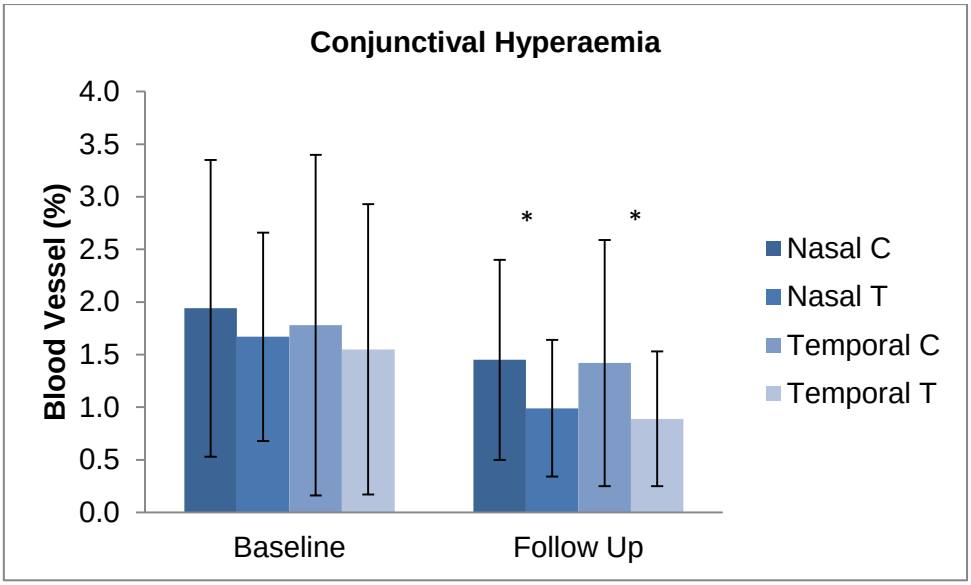

Figure 6.4.6: Mean blood vessel percentage for nasal and temporal locations on control (C) and test (T) eyes at baseline and follow up (after 2 weeks treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

At baseline there was no statistically significant difference in red colour saturation between control and test eyes for both nasal (38.96±1.50%, 38.8±1.58%, p=0.37) and temporal (38.65±1.74%, 38.26±1.73%, p=0.08) locations. After the 2 week treatment period, there was statistically significant reduction in red colour saturation in test eyes at both locations (nasal: 38.27±1.46%, p<0.05; temporal: 37.73±1.35%, p<0.05) but not control eyes (nasal: 38.71±1.50%, p=0.06; temporal: 38.26±1.34%, p=0.07). The difference between control and test eyes after the 2 week treatment period was statistically significant at both nasal (p<0.05) and temporal (p<0.05) locations (Figure 6.4.7).

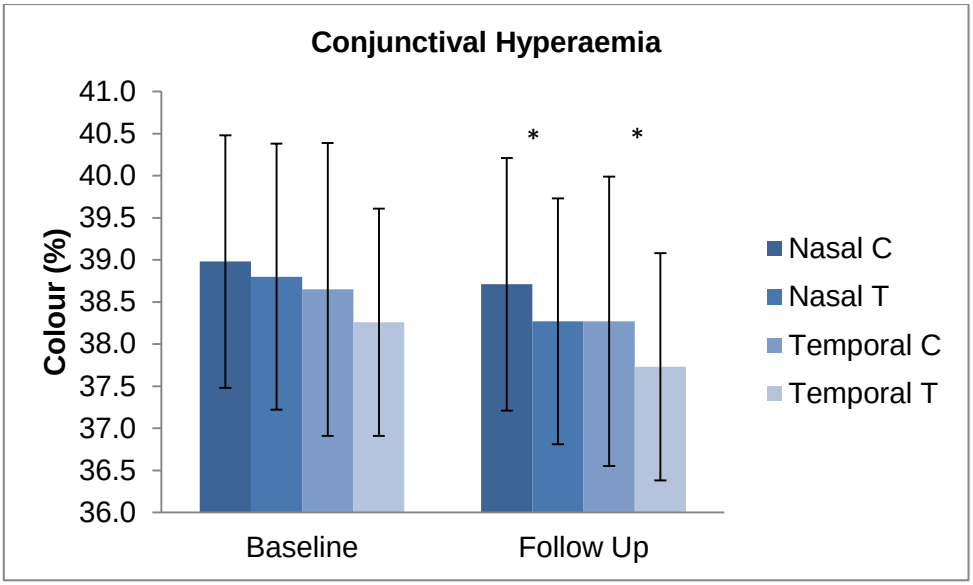

Figure 6.4.7: Mean colour percentage for nasal and temporal locations on control (C) and test (T) eyes at baseline and follow up (after 2 weeks treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Meibomian Gland Function

At baseline, there was no statistically significant difference in composite (upper eyelid score combined with lower eyelid score) meibomian gland secretion quality grade between control and test eyes (2.40±1.09, 2.35±0.99, Z=-0.30, p=0.76). After 2 weeks treatment period, there was a statistically significant decrease in test eyes from baseline (composite: 1.3±0.92, Z=-3.52, p<0.001), improving in both the lower (baseline: 1.55±0.60, follow up: 0.90±0.55, Z=-2.83, p<0.05) and upper eyelids (baseline: 0.80±0.52, follow up 0.40±0.50, Z=-3.61, p<0.001) but not control eyes (composite: 2.05±1.23, Z=-1.54, p=0.12). The difference between control and test eyes after the 2 week treatment period was also significant (Z=-2.27, p<0.05; Figure 6.4.8), but the improvement was observed to be statistically significant between the lower eyelids of the control and test eyes only (control: 1.45±0.69, test: 0.90±0.55, Z=-2.67, p<0.05).

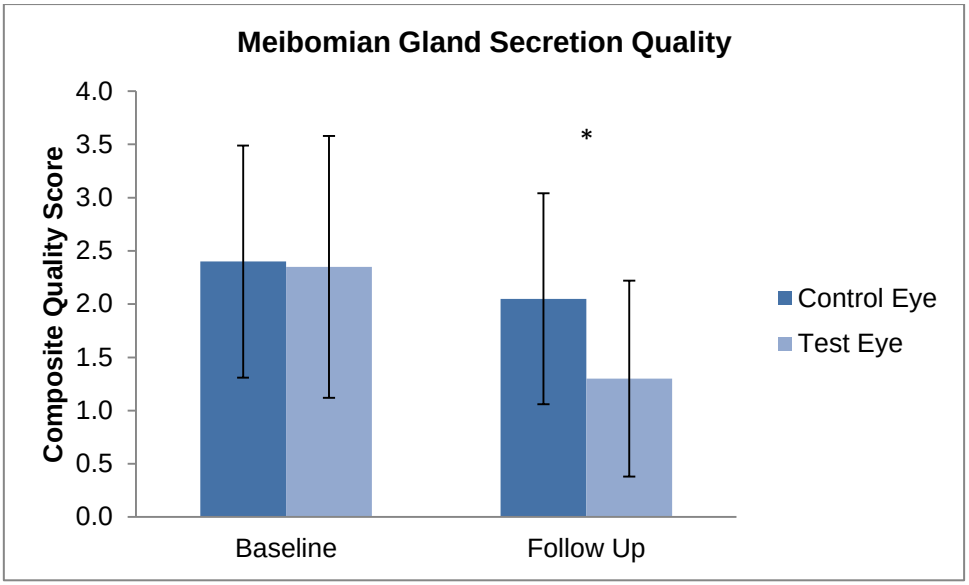

Figure 6.4.8: Mean composite meibomian gland secretion quality score for control and test eyes at baseline and follow up (after 2 week treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

For composite meibomian gland expressibility grade, there was also no significant difference between control and test eyes at baseline (composite control: 2.00±1.08, composite test: 2.15±0.89, Z=-1.134, p=0.26). There was a significant improvement in composite expressibility for both test (1.35±0.82, Z=-3.39, p=0.001) and control eyes (1.70±1.03, Z=-2.00, p<0.05) after the 2 week treatment period. However, the improvement observed in control eyes only occurred in the lower eyelid (baseline: 1.4±0.68, follow up: 1.2±0.62, Z=-2.646, p=0.008), whereas the improvement observed in test eyes improved in both upper (baseline: 0.70±0.47, follow up: 0.55±0.51, Z=-2.00, p<0.05) and lower eyelids (baseline: 1.45±0.51, follow up: 0.8±0.52, Z=-3.60, p<0.001). Thus, the difference between the composite scores for control and test eyes after the 2 week treatment period was statistically significant (control: 1.70±1.03, test: 1.35±0.82, Z=-2.65, p<0.05; Figure 6.4.9) but only between the lower eyelid of the control and test eyes (control: 1.2±0.62, test: 0.80±0.52, Z=-2.00, p<0.05).

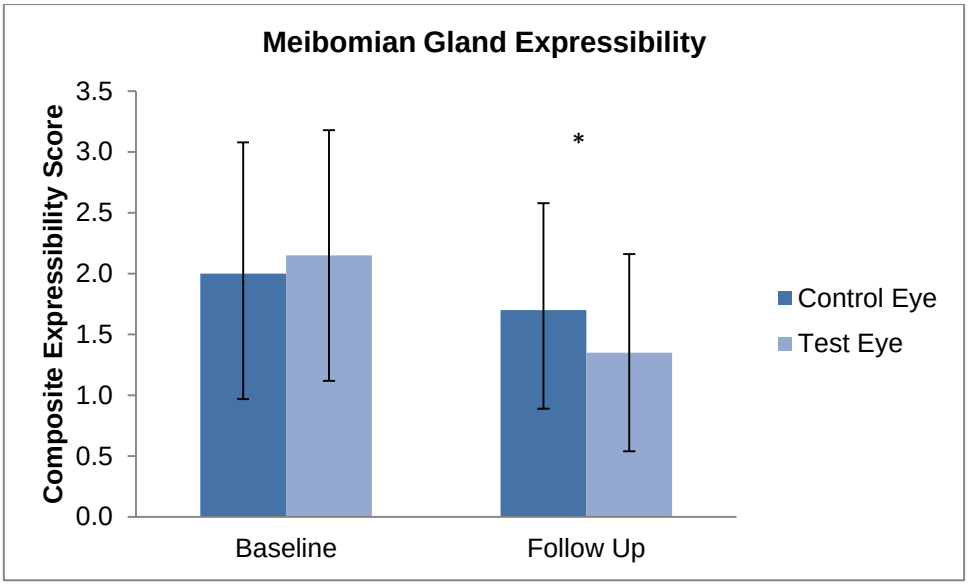

Figure 6.4.9: Mean composite meibomian gland secretion expressibility score for control and test eyes at baseline and follow up (after 2 week treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Meibomian Gland Dropout

At baseline, there was no statistically significant difference in composite meibomian gland dropout scores between control and test eyes (2.80±1.24, 2.90±1.25, Z=-0.82, p=0.41). After 2 weeks treatment period, there was a statistically significant decrease in test eyes from baseline (2.05±1.15, Z=-3.17, p<0.05), but not control eyes (2.65±1.04, Z=-1.73, p=0.08). The difference between composite meibomian gland dropout scores for control and test eyes after the 2 week treatment period was also significant (Z=-2.13, p=0.03; Figure 6.4.10), where both upper (control: 1.1±0.64, test: 0.90±0.69, Z=-2.64, p<0.05) and lower (control: 1.5±0.61, test: 1.1±0.72, Z=--2.65, p<0.05) eyelids demonstrated improvement compared to control eyes at the same location.

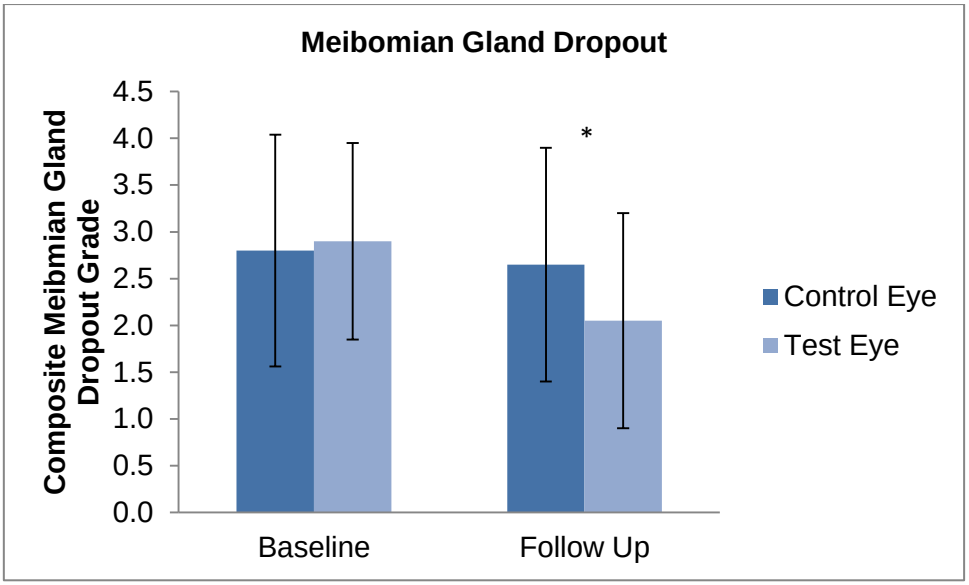

Figure 6.4.10: Mean composite meibomian gland dropout score for control and test eyes at baseline and follow up (after 2 week treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Ocular Surface Staining

At baseline, there was no statistically significant difference in composite ocular surface staining score (Oxford Grading Scale) between control and test eyes (3.2±0.2.06, 3.75±1.71, Z=-1.79, p=0.07). After 2 weeks treatment period, there was a statistically significant decrease in test eyes from baseline (1.85±0.99, Z=-3.98, p<0.001), but not control eyes (2.95±1.39, Z=-1.41, p=0.16). The difference between composite ocular surface staining for control and test eyes after the 2 week treatment period was also significant (Z=-2.00, p<0.05; Figure 6.4.11), but a significant difference was only found between the staining observed on the cornea (control: 0.95±0.75, test: 0.65±0.45, Z=-2.22, p=0.03) rather than the temporal (control: 0.70±0.66, test: 0.45±0.51, Z=-1.73, p=0.08) or nasal (control: 0.95±0.60, test: 0.75±0.44, Z=-1.79, p=0.07) locations.

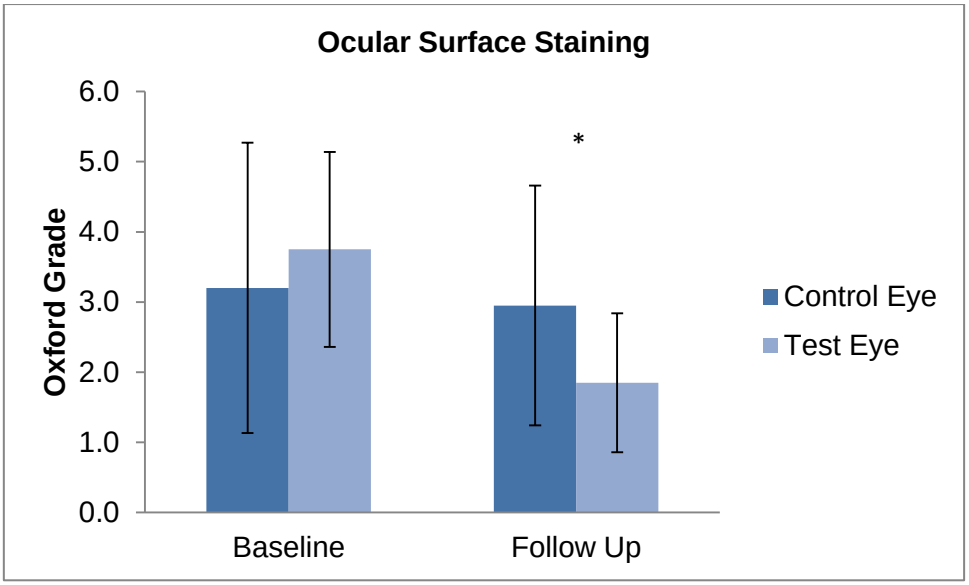

Figure 6.4.11: Mean composite ocular surface staining grade (Oxford) for control and test eyes at baseline and follow up (after 2 week treatment period). Error bars represent ±1 standard deviation. Asterisks (*) represent a statistically significant difference between control and test eyes (α level=0.05).

Adverse Events

One patient reported a transient stinging sensation at the follow up visit when the heated MGDRx EyeBag was placed on the upper eyelid on the first 4 occasions. No other adverse event was reported by this patient or the other patients for the duration of the 2 week treatment period.

6.5 Discussion

The efficacy of the MGDRx EyeBag was evaluated in a randomized, controlled, single masked clinical trial in patients with confirmed MGD related evaporative dry eye of at least mild severity. A battery of tests to evaluate both the meibomian glands and dry eye were performed at baseline and after 2 weeks treatment with a heated MGDRx EyeBag,

used twice a day for 5 minutes on one randomly selected eye (test eye). The fellow eye received a non-heated MGDRx EyeBag and served as a control.

As hypothesised in Chapter 4, the MGDRx EyeBag produced a statistically significant increase in Tearscope NITBUT by an average of 1.9±1.3 seconds in patients with MGD related evaporative dry eye. Although the increase in Tearscope NITBUT was not as large as that measured in the healthy cohort, this comparison is limited in that measurements were taken within a short period of time in the healthy cohort (immediately after removal: 4.0±.2.3 seconds; 10 minutes after removal: 3.6±2.1 seconds) whereas in the present study measurements were taken 2 weeks later at varying times in the day after the last application. Thus, It may be that the MGDRx EyeBag produces an initial increase in NITBUT immediately after single use, which reduces over time (as observed in Chapter 5) to either stabilise at higher NTIBUT compared to baseline, or reduce to baseline levels sometime in the near future. However, after longer term use as prescribed in the present study, habitual NITBUT may increase gradually in small increments after successive use rather than return to initial baseline levels. The increase in NITBUT also measured with the Keratograph 5M and Video Observation were similar (2.1±1.3 seconds and 2.0±1.7 seconds) and there was no statistically significant difference between the increases observed between the Tearscope and Keratograph ($p=0.42$), Tearscope and Video Observation ($p=0.74$), and Keratograph 5M and Video Observation ($p=0.83$).

The correlation between each of the NITBUT measurement techniques demonstrated a very strong positive statistically significant relationship. Further, the limits of agreement (95% of any difference between the instruments may occur by chance within these limits) for each method were small, suggesting they may be considered to demonstrate good agreement clinically. However, there was a statistically significant difference between the measurements obtained by the Tearscope and Video Observation techniques, where the latter produced NTIBUT values 0.29±0.90 seconds shorter on average. As the Video Observation method involved retrospectively observing the video of the tear film, the larger

display and time available to carefully review the tear film over the entire cornea may have allowed the examiner to detect signs of tear film break up in the Placido ring mires at an earlier stage compared to the Tearscope, where measurements were taken immediately after viewing a relatively smaller area. Therefore, although the increases in NIBUT measured over time with the Tearscope and Video Observation methods were similar, the individual values obtained with the latter were of shorter duration. Although this difference is very small and may not be clinically significant, when Video Observation method is used as a diagnostic tool, classification of whether or not the patient has healthy and diseased eyes based upon current cut-off values may be affected and may need to be adjusted. However, although this method allows the patients and examiners to be masked to minimise any bias in measurement, Video Observation NITBUT is unlikely to be employed in clinical practice as it involves the use of specialised video recording equipment, such as that utilised by the Keratograph 5M which incidentally provides a quicker, objective, yet very similar NITBUT value.

The thickness of the tear film lipid layer also demonstrated a statistically significant increase increased with following use of the heated MGDRx EyeBag, with a mean increase of 1.05±0.82 grades. As with NITBUT, the increase observed here was less than that observed immediately and 10 minutes after removal of the MGDRx EyeBag as demonstrated in Chapter 4. Again, the larger changes in tear film lipid layer thickness observed in the short term with single use may be transient whereas the process of repeated eyelid warming may produce a gradual increase in habitual tear film lipid layer thickness over time. Furthermore, the meibum of the healthy subjects used in Chapter 5 was already clear and expressible, whereas the patients with MGD had by definition poor quality meibum with impaired expressibility. Thus, the larger changes in NITBUT and tear film lipid layer thickness observed in the short term may be due in part to the normal functionality of the mebomian glands and the relative ease of expressing meibum in these subjects. In MGD patients, it may be that these short term improvements after single use of the MGDRx EyeBag are not

as great or even apparent as the functionality of the meibomian glands is affected unless long term, repeated eyelid warming treatment is advised. The evidence to support this hypothesis is conflicting - a recent study investigating the effect of warm moist air goggles in MGD patients found no statistically significant increase in tear film stability 10 minutes after use (Purslow, 2013). However, Olson *et al* (2003) found an 80% increase in lipid layer thickness in MGD related dry eye patients after 5 minutes into treatment with warm moist compress heated to 40°C for 30 minutes, and 66% increase 5 minutes after removal. Thus, it may be that improvements in tear film stability and lipid layer thickness in MGD patients can only be achieved where eyelid warming devices make contact with the eyelids, where the force of weight alone may be sufficient to help express the meibomian glands and subsequently release the melted lipid to the tear film. However, although Nagymihalyi *et al* (2004) demonstrated a statistically significant increase in meibum secretion after exposing subjects to a 250W infrared lamp at 50cm for 5 minutes, the subjects were healthy without MGD.

Meibomian gland function also improved with the heated eyebag after the 2 week treatment period, where there were statistically significant improvements in meibomian gland secretion quality and expressibility in test eyes. However, the improvement in meibum quality compared to control eyes was only statistically significant between the lower eyelids. At baseline, the meibum quality grade on the lower eyelid was significantly greater than the upper eyelid of the test eyes and control eyes, such that the upper eyelid was considered normal – thus, the improvement in meibum quality compared to control eyes may only have been detected in the lower eyelids based upon the limited resolution of the grading scale. Of interest was the statistically significant improvement in composite expressibility grade in control eyes after the 2 week treatment period (composite reduction of 0.35±0.81). As observed in Chapter 5, the non-heated eyebag may have warmed the eyelids by insulating and preventing heat loss of the closed eyes, which subsequently brought about an improvement in the abnormally viscous meibum. In addition, the frequent application of the

eyebag with the hands may have also applied a force which massaged the eyelids to help clear the obstructed glands. Since the heated and non-heated eyebags were applied at the same time and for the same duration, this massaging effect may have contributed in at least in part to the greater improvement in expressibility observed in the test eyes (composite reduction of 0.8±0.70). With respect to the semi-quantitative nature of the expressibility grading scale, eyelids with 5, 6 or 7 expressible glands were recorded as “grade 1” as the resolution of the scale was not sufficient to capture these changes – thus, the improvements observed in both test and control eyes may have been underestimated.

Statistically significant improvements in meibomian gland dropout, tear film meniscus height, tear film osmolarity, and conjunctival hyperaemia and ocular surface (corneal) staining was also observed in test eyes, but not control eyes. Given that measurements were taken a two time points separated by two weeks, it was not possible to determine which order these improvements took place to provide a natural history of the treatment approach. Based upon the pathophysiological observations of MGD described in Chapter 5, it is likely that the frequent and regular heating provided by the MGDRx EyeBag melted the abnormally viscous and thickened meibum, clearing the hyperkeratinsed material obstruction within the meibomian gland and allowing the meibum to be secreted on to the eyelid margin. The increased quantity of meibum (and lipid) available may therefore help thicken and restore normal tear film lipid layer function such that the evaporation of the underlying aqueous layer is prevented, tear film stability is improved, tear film osmolarity and ocular surface staining reduced and tear film meniscus height increased (Bron *et al*, 2004; Foulks, 2007). This is supported by the significant positive correlation observed between lipid layer thickness and tear film stability (Nichols *et al*, 2002; Isreb *et al*, 2003). Although we did not measure meibum quantity or evaporation rates, previous studies have found a significant positive correlation between these measurements and tear film stability (Mathers, 1993; Creech *et al*, 1998; Mathers, 2004). Indeed, evaporation of the tear film is considered a major cause of tear film instability (King-Smith *et al*, 2008; Kimball *et al*, 2010).

The improvement in clinical signs was associated with an improvement in ocular symptoms in test eyes, with a statistically significant improvement in OSDI score after the 2 week treatment period. Ocular comfort was also evaluated twice daily throughout this period using a 1-10 scale. Surprisingly, there was a statistically significant improvement in ocular comfort scores as early as the evening of treatment Day 1 (which was sustained for the remaining treatment period). Although the follow up took place at the end of the treatment period, it is not clear which clinical sign the improvement in ocular comfort was associated with, if at all. However, these findings suggest that the MGDRx EyeBag produces an increase in ocular comfort for at least 12 hours following single use. It should be noted that subjects were not masked to the control eye and therefore some placebo effect could be possible, although the effect was marked and occurred in all subjects.

Eyelid warming therapy associated with eyelid massage to treat MGD has been suggested to induce to corneal deformation due increases in corneal temperature (McMonnies *et al*, 2012). During eyelid closure, corneal temperature may increase as the vascular palpebral conjunctiva on the inner eyelids is in close apposition to the cornea for an extended period of time, thus warming the underlying tear film and corneal tissue. In addition, evaporation of the tear film from the ocular surface is reduced during eyelid closure. Thus, the application of eyelid massage, which necessitates eyelid closure and increases contact between the palpebral conjunctiva and cornea, may exacerbate the increase in corneal temperature caused by eyelid warming therapy. The mechanism by which massage induces corneal deformation may be through a reduction in mechanical resistance to corneal bending (and deformation) as a result of a reduction in viscosity of the proteoglycan ground substance of the cornea during massage (McMonnies, 2009) – the reduction in viscosity may also occur with increases in temperature, and is associated with a reduction in shear modulus and glue function (McMonnies *et al*, 2012). Importantly, failure to recover from corneal bending may lead to the development of corneal ectasia, and an associated reduction in visual acuity (McMonnies, 2009). Other factors which may increase susceptibility to corneal deformation

include accelerated abnormal corneal cell enzyme activity and corneal collagen denaturation caused by increases in corneal temperature, and reduction in collagen denaturation temperature caused by periods of sinusoidal fluctuating strain which may be induced during massage (McMonnies *et al*, 2012). Furthermore, the distending forces caused by transient increase in intraocular pressure observed following digital pressure to the globe (McMonnies & Boneham, 2007), which may occur during eyelid massage, may contribute to the development corneal deformation – particularly those with thinner, less rigid corneas (McMonnies *et al*, 2012). Thus, due to the delayed cooling of the cornea (due to it being avascular), and the possible persistence of ocular surface hyperaemia (due to the existing MGD and response to increases in temperature), eyelid warming therapy may place patients at risk of corneal deformation, which may be increased with concurrent massage (McMonnies *et al*, 2012). Therefore, in the present study, we did not advise patients to massage the eyelids after removal of the MGDRx EyeBag.

However, only one case of corneal deformation following eyelid warming therapy and mechanical eyelid hygiene has been described in the scientific literature. Here, a patient with obstructive MGD developed 1 dioptre of oblique corneal astigmatism associated with a reduction of visual acuity in one eye after 7 weeks bilateral treatment of daily warm compresses (15 minute applications twice a day) followed by manual meibomian gland expression (McMonnies *et al*, 2012). The authors reported that the patient also had a habit of rubbing the eyes after experiencing itching following warm compress therapy (McMonnies *et al*, 2012), and the corneal changes observed in the one eye may be associated with the greater force applied by the more dominant hand being on that side (McMonnies & Boneham, 2003). Interestingly, after cessation of eyelid warming and massage, and the use of cold compresses/ice and opatonol (olopatadine; anti-allergic medication) to prevent itching and rubbing, visual acuity and corneal astigmatism retuned to baseline (McMonnies *et al*, 2012). The duration of the warm compress application in this case is far longer than that prescribed in the present study, where eyelid massage/mechanical meibomian gland

expression was also not advised. Transient visual degradation without changes in corneal topography has been observed after warm compresses (45°C) were applied every 2 minutes for 30 minutes without massage (Solomon *et al*, 2007), but again the duration of application and peak temperature sustained for longer than the present study and the experimental treatment methodology is not typically advised to MGD patients. In addition, changes in corneal astigmatism (0.50-0.75 dioptres) has been observed after horizontal eye rubbing for 1 minute, suggested to be caused by transient changes in corneal epithelial thickness rather than corneal distortion (Mansour & Haddad, 2002). Therefore, it appears that corneal deformation and/or visual changes, may only occur following unusually long and intense treatment application durations and long term therapy associated with eyelid rubbing.

6.6 Conclusions

Given that there was no reduction in visual acuity and no change in corneal topography (as evaluated by eccentricity) measurements from baseline after the 2 week treatment period, the risk of corneal deformation with the MGDRx EyeBag, as prescribed here, appears to be very low. In addition adverse events were only experienced by one patient, where they experienced transient stinging sensation on the eyelids after removal of the heated eyebag on the first four occasions, but were not experienced thereafter on continued use. Further, there was a statistically significant improvement in ocular surface staining in test eyes, particularly the cornea. Thus, when used based upon the treatment regimen prescribed herein, the MGDRx EyeBag may be considered a safe and effective eyelid warming device with a low risk of corneal deformation and visual changes; and results in improved comfort and tear film parameters involved in evaporative dry eye in patients with MGD. Longer term bilateral studies where MGD patients are followed for at least 8 weeks (as symptoms may return within a few weeks post treatment due to the chronic nature of the disease) and followed up post treatment cessation are required to determine the course of

chronic treatment with the MGDRx EyeBag, and whether this causes corneal deformation and a reduction in visual acuity and to confirm the long term safety profile of this eyelid warming device.

Chapter 7

Effectiveness of Non-Pharmacological Treatments in Acute Seasonal Allergic Conjunctivitis

7.1 Introduction

As discussed in Chapter 1, the primary treatment strategy for SAC involves avoidance of the offending allergen to prevent the initiation of the allergic response. However, complete avoidance is not often possible and use of topical anti-allergic medications is required when signs and symptoms occur (Friedlaender 2001; Bielory, 2002, 2008b). It has been suggested that non-pharmacological treatments such as artificial tears and cold compresses (cooled gele eye mask, frozen goods wrapped in towel) may be used in conjunction with allergen avoidance strategies and anti-allergic medications to help bring about symptomatic relief (Hingorani & Lightman, 1995; Bielory, 2002, 2008b; Chigbu, 2009b). However, there appears to be no evidence in the scientific literature to demonstrate the efficacy of using artificial tears or cold compresses for treating SAC.

Given that SAC is the most common form of ocular allergy and causes significant impact on quality of life, productivity, visual tasks, social interactions and increased economic costs, there is a definite need to develop effective treatments. Moreover, the models used to identify effective treatments must be accurate and reliable (Abelson & Loeffler, 2003). Indeed, the relatively benign and self-limiting nature of SAC lends itself to be investigated and modelled in human subjects (Ousler *et al*, 2005). Models of ocular allergy currently include the environmental challenge and conjunctival allergen challenge designs, but allergen challenge to the eye has long been studied. In the early 20th century, allergens such as pollen have been applied to the eye to test sensitisation when skin tests were found to be negative to help diagnose allergy (Peshkin, 1931; Abram, 1949; Ousler *et al*, 2005). Rather than for diagnostic purposes, the effect of various mediators and cytokines released during the IgE mediated mast cell degranulation have also been investigated by instilling them into

the eye and observing the both the clinical and histological responses (Abelson & Loeffler, 2003, Leonardi, 2005b). Of these, histamine is the only mediator that once instilled in to the eye can produce all of the clinical signs and symptoms of an allergic reaction in the eye (Abelson & Loeffler, 2003). Instillation of histamine in the eye produces a dose dependant increase in itching, conjunctival hyperaemia and oedema, watering and eyelid swelling (Abelson & Loeffler, 2003). Therefore, the simulation of the ocular allergic reaction with histamine has been used as a model to investigate the efficacy of anti-allergic treatments but is limited to only antihistaminic drugs and vasoconstrictors as mast cell degranulation is not stimulated directly (Abelson *et al*, 1980; Abelson & Smith, 1988; Abelson & Loeffler, 2003). Further, the measuring of histamine to determine the efficacy of a particular treatment is difficult owing to the concurrent release of histaminase upon mast cell degranulation, an enzyme which serves to break down histamine (Abelson & Loeffler, 2003; Ousler *et al*, 2005). However, Leonardi and Abelson (2003) found that tear histamine levels reduced following use of olopatadine twice daily for 5 days, and correlated positively with signs and symptoms.

Other methods to model ocular allergy include the instillation of secretagogues such as compound 40/80 which also stimulate mast cell degranulation (Butrus *et al*, 1990), but not through an IgE mediated pathway as required to accurately reflect an ocular allergic reaction (Abelson & Loeffler, 2003). Thus, instillation of actual allergen to the eye in a controlled fashion to stimulate IgE mediated mast cell degranulation and therefore mimic allergic conjunctivitis has been developed as the conjunctival allergen challenge (CAC) model (Moller *et al*, 1984; Abelson *et al*, 1990). Here, asymptomatic subjects with a history of allergic eye disease are recruited and undergo a series of screening visits prior to drug testing – on the first visit; an allergen is selected and instilled in to both eyes at increasing concentrations until a predetermined clinical threshold is met (titration), such that the reaction closely resembles a natural allergic reaction (Abelson *et al*, 1990; Abelson & Loeffler, 2003; Ousler *et al*, 2005). However, if patients do not respond to a particular

allergen after the final dose, another may be tested in the same fashion. Those who meet the minimum level of response are then challenged at a second visit with the same dose that produced the threshold response on the first visit to confirm the consistency and reproducibility of the allergic reaction (Abelson *et al*, 1990; Abelson & Loeffler, 2003; Ousler *et al*, 2005). Those who produce a consistent and reproducible minimum level of response then proceed to the experimental phase of the study (Abelson *et al*, 1990; Abelson & Loeffler, 2003; Ousler *et al*, 2005). Thus, this CAC model allows the creation of a homogenous allergic response at baseline for a given population that can be used to evaluate drug efficacy (Ousler *et al*, 2005). At the experimental visit, drug can be instilled in both eyes, drug in one eye and placebo or active control in the other – after which the threshold allergen dose is then instilled (Abelson & Loeffler, 2003; Ousler *et al*, 2005). The onset and duration of the drug can be evaluated by varying the time intervals at which the signs and symptoms are evaluated.

The main advantage of the CAC model is that it produces a homogenous, consistent and reproducible IgE mediated ocular allergic reaction in a controlled setting – only those with ocular allergy are enrolled, titration allows the threshold dose to be established and combined with the follow up confirmation visit ensures reproducibility (Abelson & Loeffler, 2003). Thus, patients are exposed to a known level of allergen that causes an ocular allergic response in a controlled fashion - the CAC is performed in office in patients who are asymptomatic without the influence of other allergens and variable exposure which may otherwise confound the results (Abelson & Loeffler, 2003; Ousler *et al*, 2005). The in-office setting also ensures compliance with the treatment, allows evaluation of clinical signs by trained, masked examiners, while standardised scales can be carefully explained to patients when grading symptoms (Abelson & Loeffler, 2003; Ousler *et al*, 2005). Further, instilling the drug in one eye and placebo/active control in the fellow eye serves as a highly reproducible internal control (Abelson & Loeffler, 2003; Ousler *et al*, 2005).

The limitation of the CAC model is the lack of natural exposure and the inability to evaluate drug efficacy and safety in the long term (Abelson & Loeffler, 2003; Ousler *et al*, 2005). The high allergen doses used may initiate a severe ocular allergic response, provoking the late phase response and cellular infiltration atypical of SAC (and PAC). The environmental model of allergic conjunctivitis may overcome this to an extent, but is limited by several important factors that may confound the results. Typically, patients with a history of ocular allergy are randomised in a masked fashion to receive either drug or placebo, or drug in one eye and placebo/active control in the fellow eye to act as a control, during the allergy season (Abelson & Loeffler, 2003; Ousler *et al*, 2005). Patients are given a diary and standardised grading scale to record signs and symptoms, and to record treatment usage/dosing – at predetermined times during the treatment period, which may span several weeks to months, patients attend in office to evaluate efficacy, safety and ensure compliance with the treatment and diary keeping protocol (Abelson & Loeffler, 2003; Ousler *et al*, 2005).

Chiefly among the limitations of the environmental model is the inability to control exposure to the offending allergen or allergens during the study – each patient is exposed to different types and concentrations of allergen owing to variations in natural pollen counts, work habits, lifestyle, environment at work and in the home. This may be complicated by behavioural modifications such as the deliberate avoidance of allergen exposure to prevent the onset of signs and symptoms. Thus, if the patient does not experience these signs and symptoms, it may not be possible to identify any drug effect, or it may be that the efficacy of the drug and or placebo is artificially improved. Although the variability of environmental pollen counts can be monitored daily by local pollen counting stations, and coupled with tracking of patients diaries on a daily basis can help provide a more accurate measure of exposure and actual drug efficacy, patients may be allergic to indoor allergens and pollen counting stations can vary within the same area (Abelson & Loeffler, 2003; Ousler *et al*, 2005). Thus, pollen counting stations may not provide a measure of an individual's true allergen exposure. Further, studies have found that clinical signs and symptoms may not

always correlate with absolute pollen counts – here, patients with a positive history of ocular allergy were monitored for symptoms of itching during the pollen season at 6 different locations, and although there was a general trend of increased itching scores with increasing pollen levels, this relationship was not significant and pollen counts varied significantly between sites (Rosner et al, 2003). The CAC model overcomes this variable exposure issue by instilling the exact dose of allergen in to the eyes which is known to produce a bilaterally homogenous and sufficient ocular allergic reaction without influence of other allergens.

The utilisation of patient diaries to measure drug efficacy over time may be affected by a high level of subjectivity, owing to variations in each patient's experience and interpretation of their symptoms. Although standardised grading scales can be used, the quality of the data may be affected by lack of compliance with the grading protocol where patients may forget to record their signs and symptoms in a timely fashion, only to then complete it at a later date and therefore relying on memory rather than actual experience at that specific time point (Abelson & Loeffler, 2003; Ousler *et al*, 2005). By instilling the intervention and measuring primary outcome variables in office by a masked examiner, the CAC model however ensures compliance with treatment and timely assessment of signs and symptoms.

Another key issue in environmental study design is the enrolment of patients who truly have an ocular allergy, and if so whether they are allergic to the allergens (pollen) present in the environment during the period of study. Although subjects may be skin tested prior to enrolment to determine systemic sensitivity to a particular allergen, ocular tissue may be sensitised in the absence of a systemic allergic response (Leonardi *et al*, 1990). Therefore, those who do exhibit ocular allergic reaction only may be excluded, and those included may not exhibit an ocular allergic reaction despite positive skin testing (Abelson & Loeffler, 2003) Further, each individual may demonstrate significant variations in sensitivity to the airborne allergens, which is complicated by the limited duration of the active pollen season which naturally gradually reduces over time – thus, a suitable baseline from which a drug effect can be detected may not be established and the patient may improve over time regardless of the

intervention (Abelson & Loeffler, 2003; Ousler *et al*, 2005). In contrast, the CAC model may be used outside the pollen season and those who exhibit ocular allergy at baseline are excluded, ensuring each subject exhibits the same minimum level of response such that a homogenous baseline is produced from which a drug effect can be detected (Abelson & Loeffler, 2003; Ousler *et al*, 2005). However, this environmental study design issue can be resolved by performing both skin and ocular challenge tests to ensure those who suffer ocular allergy at a sufficient level to the airborne allergens during the period of study are included.

More recently, environmental chamber models of ocular allergy has been used to help overcome inter and intra subject variability affecting the environmental model (Day *et al*, 2006). Instead of allowing patients free movement within their normal environment, the environmental chamber model condenses the environment in to a specific location to expose patients to airborne allergens in a more controlled manner (Day *et al*, 2006). The location or chamber is typically a room that circulates airborne allergen, allowing control over the type of allergen, its concentration and the duration to which each patient is exposed. Therefore, this model can be used outside the allergy season like the CAC model, but allows exposure to allergens known to cause an ocular allergic reaction in a natural manner and concentration. However, very few studies evaluating ocular anti-allergic medication performance in an environmental chamber model have been performed. After exposing subjects to cat dander in a room where cats were present, patients exhibiting at least mild symptoms of ocular itching were randomised to receive nedocromil 2% in one eye and olopatadine 0.1% in the fellow eye and signs and symptoms were measured at predetermined time intervals after drug instillation – although no significant difference was found between the two treatments, signs and symptoms of significantly reduced over time (Monson *et al*, 2004). However, in this study there were no pre-treatment baseline evaluations to compare any reduction in signs and symptoms except for ocular itching. Further, it is not clear if the patients were asymptomatic prior to exposure, or if they were tested for sensitivity to cat dander, either by

skin test or ocular challenge. In a similar study, patients were pre-treated with pemirolast 0.1% in one eye and olopatadine 0.1% in the fellow eye, exposed in the cat room, followed by a series of measurements at predetermined time intervals – no significant difference in itching scores and clinical signs were found between the two treatments (Rothman & Raizman, 2004). In a second clinical trial arising from the same study cohort, patients were first exposed to the cat room, but only those experiencing at least mild itching were then dosed with the same anti-allergic medications in the same manner before being assessed for signs and symptoms of ocular allergy at predetermined time intervals – again, no significant difference was found between the two treatments in alleviating ocular allergy (Rothman & Raizman, 2004). Again, it is not clear in this study if patients were confirmed to be sensitive to cat dander, whether they were asymptomatic prior to exposure, and no baseline examinations with the exception of itching assessment were conducted.

Another environmental chamber model, The Vienna Challenge Chamber (VCC), not only allows control of the allergen but also the temperature, air circulation and humidity to reflect a more natural simulation of the environment. Although this model and the similar Environmental Exposure Unit (EEU) have been used to investigate ocular anti-allergic medications, most studies have focussed on allergic rhinitis and its treatment (Day & Briscoe, 1999; Day *et al*, 2006). In a double masked, randomised, crossover study, patients with grass pollen allergy were exposed in the VCC before instillation of emedastine 0.05% or ketotifen 0.025% in each eye, followed by evaluation of nasal and ocular symptoms 0-2 and 5-8 hours after initial exposure – although both significantly reduced the symptoms ocular allergy at during both time periods, ketotifen had a faster onset of action (mean of 15 minutes) and greater symptom relief during the first 2 hours (Horak *et al*, 2003). However, this study did not examine ocular signs and patients were only skin tested to determine systemic sensitivity to the grass pollen. Further, a minimum response level to the grass pollen was not established so that patients may have experienced varying degrees of ocular allergy severity. The efficacy of different concentrations of azelastine has also been

investigated in the VCC, where subjects with a history of allergic conjunctivitis received different concentrations of azelsatine or placebo to each eye (in random order on separate visits as part of a randomised, double masked, crossover study design) before exposure to airborne allergen in the VCC where signs and symptoms were monitored. Azelastine produced a significant dose dependent reduction in itching, lacrimation, conjnunctival redness and chemosis compared to placebo, with an optimal effect at 0.05% (Horak *et al*, 1998). However, patients were not tested for ocular or even systemic sensitivity to the airborne pollen prior to exposure, thus it is not clear if the drug or simply the lack of ocular sensitivity truly prevented or caused the reduction in signs or symptoms observed. Another limitation of these studies described here is the lack of allergen concentration monitoring, given that local variations in the distribution of the allergen may occur within the chamber that may give rise to variations in allergic responses, particularly in large rooms where multiple patients are tested such as the VCC and EEU (Ousler *et al*, 2005). Thus, despite the potential of the environmental chamber model, which allows controlled, natural allergen exposure (unlike the CAC model), it appears that current study designs do not utilise the advantages of the CAC model to help ensure patient ocular sensitivity prior to enrolment and that a homogenous baseline of ocular allergic reactivity is produced to elicit statistically and clinical significant differences between interventions. Further, the lack of monitoring allergen concentration may lead to insufficient exposure.

7.2 Study Aims

The aim of this study was to investigate the efficacy of instillation of artificial tear substitutes (AT) and application of cold compresses (CC) alone and in combination in patients with confirmed ocular allergic sensitivity to grass pollen using an environmental chamber model. In addition, the effectiveness of these treatments compared to a dual action

antihistamine-mast cell stabiliser licenced for the treatment of SAC alone and in combination with CC was investigated.

7.3 Methods

The study received ethical approval from the Aston University Research Ethics Committee and was registered as a clinical trial. The research was conducted in accordance with the principles expressed in the Declaration of Helsinki.

Subjects

All participants were ≥18 years old with no history of asthma, any active eye pathology and were not using ocular or systemic medications known to affect the eye. None of the participants experienced any form of allergic conjunctivitis at least 1 month before the study took place or used anti-allergic medication over the 14 days prior to testing.

Screening Protocol

Subjects underwent skin prick (SPT) and bilateral conjunctival challenge tests (CCT) to confirm systemic and ocular allergic sensitivity to grass pollen (Abelson et al, 1990; Leonardi, 2005b; Leonardi et al 2012). SPT was performed on the forearm using grass pollen solution (10 HEP, Soluprick SQ, ALK-ABELLO, Denmark) and positive (histamine solution) and negative (saline) controls by LR. After 20 minutes, the size of the wheal response was measured and a positive result was recorded for diameters ≥3mm. CCT was performed by PSB by applying 20µL of grass pollen (Soluprick SQ, ALK-ABELLO, Denmark) solution in two-fold increasing concentrations from 3IR/mL to 100IR/mL to one eye (selected at random to be the experimental eye) and saline solution to the contralateral (control) eye every 10 minutes until a composite score of ≥5 using a standardized scoring method was reached (Abelson *et al*, 1990; Fauqert *et al*, 2004; Ousler *et al*, 2005). Eligible subjects who had a positive SPT and CCT proved sensitivity to grass pollen were enrolled into the study with written informed consent.

Eighteen subjects (12 females, 6 males) took part in the study with a mean age of 29.5±11.0 years (age range 20-65 years). At each visit subjects underwent slit lamp bio-microscopy to ensure signs and symptoms of SAC were not present prior to testing. This was followed by a series of measurements on both eyes including grading of nasal and temporal bulbar conjunctival hyperaemia using a grading scale (Jenvis Research, Germany), and ocular surface temperature of the cornea and temporal and nasal bulbar conjunctiva (5mm^2 area, 2 seconds post-blink) using an infra-red camera (Thermo Tracer TH7102, NEC, Japan) where a series of digital markers were used to ensure the temperature was measured at the same location for each subject (Wolffsohn *et al*, 2010). Ocular allergy symptomology was also measured using the eye symptom section from the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) on a 0 to 6 scale, summed score for itching, watering, swelling and soreness 0 to 24 (Juniper *et al*, 1999).

Subjects were exposed to between 251 and 500 grains/m^3 of Timothy grass pollen (*phleum pratense*; equivalent to a “very high” pollen count classification; concentration monitored using a Bukard continuous air sampler) in icreasing steps of 5 minute intervals in a computer controlled environmental chamber (Design Environmental, Ebbw Vale, UK) at a temperature of 20°C and 70% ambient humidity (average local conditions in June in the UK) until the concentration that caused ocular itching graded ≥3 (RQLQ grade) and a ≥0.5 unit change (Jenvis scale) in nasal and temporal bulbar conjunctival hyperaemia in both eyes was established . The concentration determined for each individual was used at all subsequent visits. The environmental chamber is a custom built compartmentalised walk-in chamber (width 5m X 5m, height 4m) where allergens can be introduced and distributed evenly within the air.

Once the concentration of pollen for each individual had been established, on separate occasions separated by at least one week, out of the allergy season, the subjects had baseline measurements taken and were then exposed to pollen at this concentration for 5 minutes and 5 minutes post exposure the same measurements were repeated. This was

followed by application bilaterally of either an AT applied to the temporal conjunctiva (Blink Refreshing Eye Drops 0.5ml single use vial, Abbot Medical Optics, USA), CC applied to the closed eye lid for 5 minutes (frozen gel-pack: Boots Pharmaceuticals, Nottingham, UK), AT combined with CC (for 5 minutes, 5 minutes after AT insillation) or no treatment (NT) to the eyes in random order at each visit (examiner masked). The same measures were then repeated every 10 minutes for 1 hour at each visit.

A subgroup of 11 randomly selected subjects (mean age of 29.1±12.9 years, range 20-65) attended for three further identical visits receiving 1 drop of Epinastine Hydrochloride (EH, Relestat 0.5mg/ml, Allergan, USA), 1 drop of EH combined with CC (for 5 minutes, 5 minutes after instillation of EH), or a single drop of saline (termed vehicle, equivalent to the same volume as the drug but without the active ingredients to determine how much of the effect was lubrication compared to pharmaceutical) in random order to assess the efficacy of non-pharmaceutical agents, against a dual action antihistamine/mast cell stabiliser licenced for seasonal allergic conjunctivitis (Abelson *et al*, 2004; Lanier *et al*, 2004; Monson *et al*, 2004; Whitcup *et al*, 2004).

Statistics

Statistical analysis was performed using SPSS for Microsoft Windows. As ocular surface temperature and conjunctival hyperaemia were found to be normally distributed (Kolmogorov-Smirnov Test >0.05), their changes over time were evaluated by repeated measures ANOVA, and post-hoc analysis was performed using paired t-tests. Changes in ocular symptomology were evaluated by the Freidman test and post-hoc analysis was performed using Wilcoxon signed-rank tests. Statistical significance was taken as $p<0.05$. Sample size for both the initial and subgroup of subjects met the requirements for sufficient replicates for a repeated measures experimental design (Armstrong *et al*, 2002). Using G-Power, effect size=0.5 (medium); $\alpha=0.05$; power=0.80; number of groups (treatments) =4; number of repeats=4; necessitating 8 subjects for a repeated measures within factor design

in the initial study; and 9 subjects for the subgroup (3 treatments, 3 repeats). We aimed to overrecruit owing the number of visits required and expected dropout.

7.4 Results

Non-Pharmaceutical Treatment Efficacy

Bulbar Conjunctival Hyperaemia

Hyperaemia was similar at baseline at each visit ($F=0.955$, $p=0.438$) as was the post exposure effect ($F=0.267$, $p=0.898$). There was no difference in conjunctival hyperaemia between the eyes ($F=0.112$, $p=0.742$), however, the nasal conjunctiva was more red than the temporal conjunctiva over the measurement period (1.71±0.62 versus 1.47±0.56 Jenvis units; $F=33.711$, $p<0.001$). There was a significant difference in conjunctival hyperaemia following each of the treatments ($F=68.211$, $p<0.001$; Figure 7.4.1), with a reduction in redness with time ($F=302.764$, $p<0.001$), although this recovery differed with treatment ($F=9.469$, $p<0.001$) and none of the treatments achieved complete recovery to baseline within 60 minutes ($p<0.001$). However, all treatments produced a significant improvement in hyperaemia over time compared to no treatment both nasally and temporally ($p<0.05$).

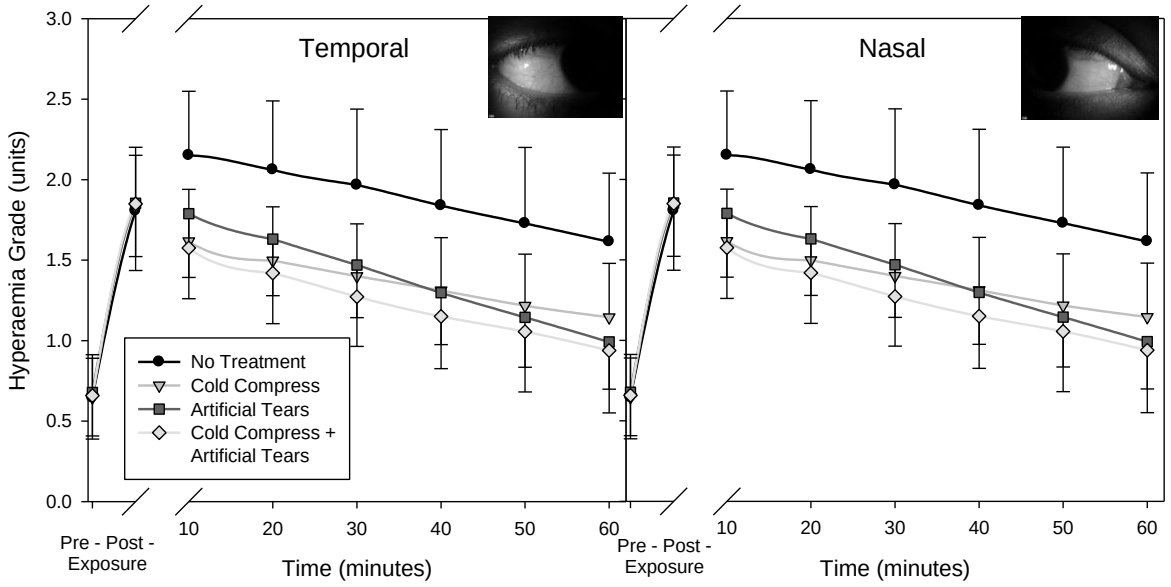

Figure 7.4.1: Hyperaemia grade pre and post pollen exposure and every 10 minutes thereafter up to 60 minutes for no treatment, cold compress, artificial tears and artificial tears combined with cold compress on the temporal and nasal bulbar conjunctiva. Data from right and left eyes was similar so were averaged (n=18 subjects, 36 eyes). Error bars represent ±1 standard deviation.

Ocular Surface Temperature

Ocular surface temperature was similar at baseline at each visit ($F=0.685$, $p=0.605$) as was the post exposure effect ($F=0.636$, $p=0.639$). There was no difference in temperature between the eyes ($F=0.017$, $p=0.897$), however there were significant differences in temperature between corneal, nasal and temporal locations ($F=97.899$, $p<0.001$). There was a significant difference in temperature following each of the treatments ($F=19.684$, $p<0.001$; Figure 7.4.2), with the temperature diverging toward baseline over time ($F=32.955$, $p<0.001$), although this recovery differed with treatment ($F=122.796$, $p<0.001$). Temporal bulbar conjunctival and corneal temperatures returned to baseline levels (was no longer significantly different; $p>0.05$) with the application of cold compress (within 50 minutes), artificial tears (within 40 minutes) and artificial tears combined with cold compress (within 40 minutes), whereas for the nasal bulbar conjunctiva the return to baseline temperature was

generally faster (40, 30 and 40 minutes respectively). Ocular surface temperature did not return to baseline levels without treatment at any location ($p<0.05$).

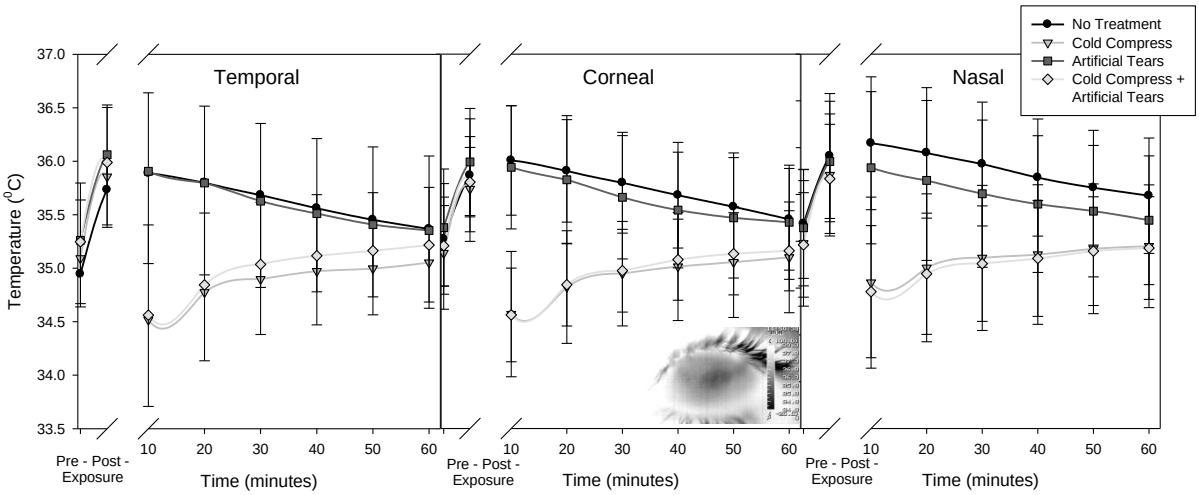

Figure 7.4.2: Ocular surface temperature pre and post pollen exposure and every 10 minutes thereafter up to 60 minutes for no treatment, cold compress, artificial tears and artificial tears combined with cold compress on the corneal and temporal and nasal bulbar conjunctival surfaces. Data from right and left eyes was similar so were averaged (n=18 subjects, 36 eyes). Error bars represent ±1 standard deviation.

Ocular Symptomology

Although the symptoms differed in overall magnitude, with itching rated as the severest symptom and swelling the least, the profile with time after treatment and recovery was similar for each of the symptoms so they were averaged for analysis. The global ocular symptom scores were similar at baseline at each visit ($X^2=6.091$, $p=0.107$) as was the post exposure effect ($X^2=2.729$, $p=0.435$). They decreased with time after treatment (CC: $X^2=88.489$, $p<0.001$; AT: $X^2=88.258$, $p<0.001$; AT+CC: $X^2=87.639$, $p<0.001$; Figure 7.4.3), with all treatments reducing symptoms more than no treatment ($p < 0.001$), but none of the treatments returned global ocular symptom scores to baseline levels within 1 hour after antigen exposure ($p<0.001$).

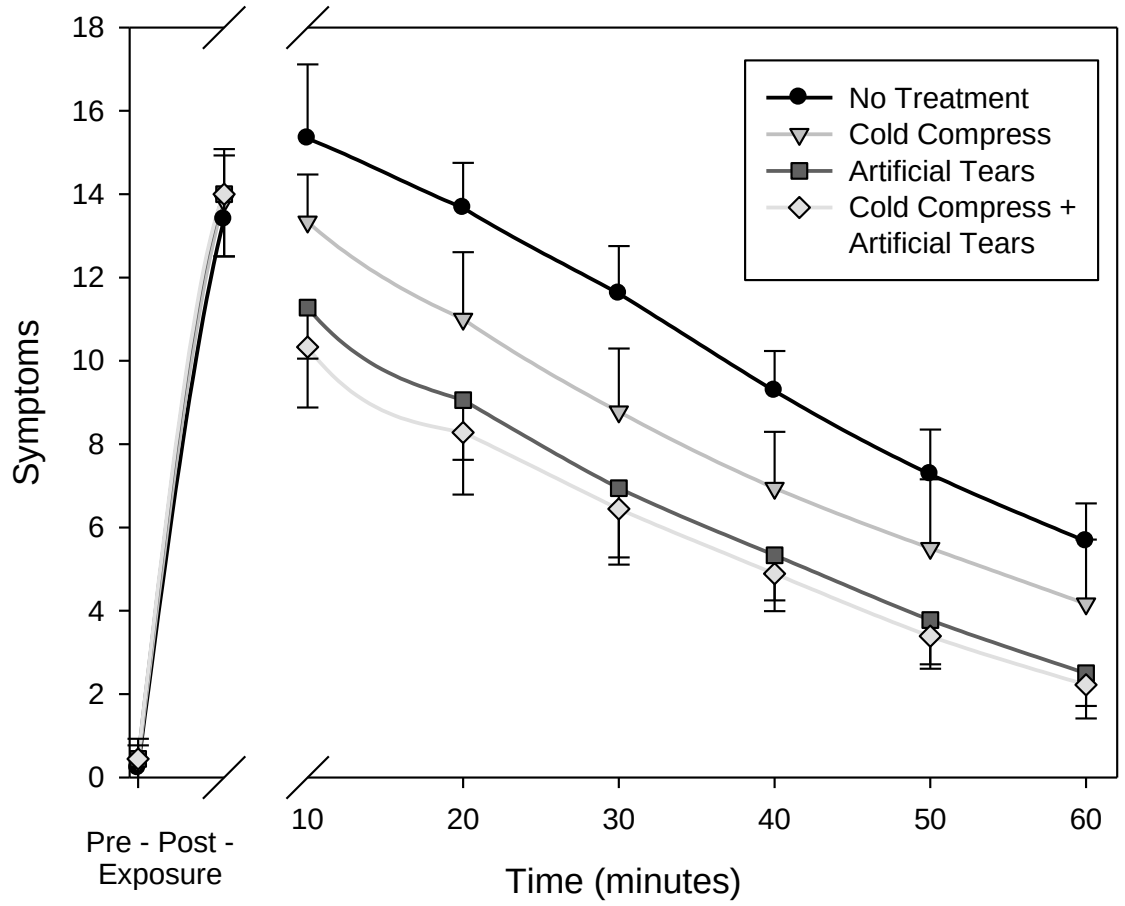

Figure 7.4.3: Ocular symptoms pre and post pollen exposure and every 10 minutes thereafter up to 60 minutes for no treatment, cold compress, artificial tears and artificial tears combined with cold compress. Although the symptoms differed in overall magnitude the profile with time after treatment and recovery was similar for each of the symptoms so they were averaged for analysis. N = 18. Error bars represent ±1 standard deviation.

Relative Efficacy of Non-Pharmaceuticals versus a Dual Action Pharmaceutical

Bulbar Conjunctival Hyperaemia

There was a significant difference in conjunctival hyperaemia between each of the treatments ($F=11.728$, $p<0.001$; Table 7.4.1), with a reduction in redness with time ($F=581.320$, $p<0.001$), although this recovery differed with treatment ($F=9.463$, $p<0.001$). AT

combined with CC outperformed AT, CC and EH alone and EH combined with CC nasally. The treatment effect of EH was enhanced by combining it with a CC. The saline volume control (vehicle) showed the action of EH was principally from the active pharmaceutical ingredients. AT instillation had similar effectiveness to a CC application used in isolation (Table 7.4.1).

		Significance (p)					
Treatment	Mean*	EH	EH+CC	CC	AT	AT+CC	Vehicle
EH	$1.46\pm0.43_n$	X	<0.001	0.378	0.045	0.042	<0.001
	$1.35\pm0.40_t$	X	<0.001	<0.001	<0.001	<0.001	<0.001
EH+CC	$1.33\pm0.41_n$		X	0.002	<0.001	0.559	<0.001
	$1.19\pm0.37_t$		X	0.929	0.220	0.014	<0.001
CC	$1.51\pm0.30_n$			X	0.349	<0.001	<0.001
	$1.19\pm0.29_t$			X	0.162	<0.001	<0.001
AT	$1.55\pm0.38_n$				X	<0.001	<0.001
	$1.24\pm0.35_t$				X	<0.001	<0.001
AT+CC	$1.36\pm0.31_n$					X	<0.001
	$1.08\pm0.37_t$					X	<0.001
Vehicle	$2.00\pm0.39_n$						X
	$1.65\pm0.38_t$						X

Table 7.4.1: Statistical comparison of nasal (n) and temporal (t) hyperaemia between epinastine hydrochloride (EH), epinastine hydrochloride combined with cold compress (EH+CC), cold compress (CC), artificial tear (AT), artificial tears combined with cold compress (AT+CC) and vehicle. Nasal and temporal regions significantly interacted with treatment and so have been presented separately. * = mean hyperaemia grade (Jenvis units) of right and left eyes averaged (n=11, 22 eyes) over 60 minutes.

Ocular Surface Temperature

There was a significant difference in ocular surface temperature between each of the treatments ($F=11.680$, $p<0.001$; Table 7.4.2), with a change in temperature toward baseline with time ($F=17.952$, $p<0.001$), although this recovery differed for each treatment ($F=144.816$, $p<0.001$). CC in combination with an AT or EH lowered the antigen raised ocular surface temperature below the pre-exposure baseline. AT instillation alone or in combination to a CC or EH significantly, but only slightly (<0.5ºC), reduced the temperature ($p < 0.05$; Table 2). CC combined with either a AT or EH had a similar cooling effect. The

saline vehicle volume control to EH had a similar cooling effect to an AT and no beneficial cooling effect over EH of the same volume but containing active pharmaceutical agents.

		Significance (p)					
Treatment	Mean*	EH	EH+CC	CC	AT	AT+CC	Vehicle
EH	35.31±0.48	X	<0.001	<0.001	<0.001	<0.001	0.057
EH+CC	34.72±0.63		X	0.228	<0.001	0.089	<0.001
CC	34.81±0.55			X	<0.001	<0.001	<0.001
AT	35.52±0.67				X	<0.001	0.319
AT+CC	34.57±0.34					X	<0.001
Vehicle	35.44±0.41						X

Table 7.4.2: Statistical comparison of ocular surface temperature between epinastine hydrochloride (EH), epinastine hydrochloride combined with cold compress (EH+CC), cold compress (CC), artificial tear (AT), artificial tears combined with cold compress (AT+CC) and vehicle. Ocular temperature was similar between eyes and did not interact with ocular surface region, so average data is presented. * = mean ocular surface temperature of right and left eyes and region combined (n=11, 22 eyes) over 60 minutes.

Ocular Symptomology

All ocular symptom changes with time were similar so they have been averaged for presentation and analysis. At all measurement time intervals, symptoms were reduced for both EH and EH in combination with a CC than a CC or AT alone or in combination ($p < 0.01$; Figure 7.4.4). Only EH alone and in combination with a CC reduced global ocular symptom scores to baseline levels within the post-antigen exposure hour over which subjects were monitored (after 60 minutes: $p=0.414$, $p=0.705$). A CC enhanced the pharmaceutical benefit of EH alone up to 20 minutes ($p<0.05$), where thereafter they were similarly efficacious ($p>0.05$). A CC also further reduced symptoms when combined with AT compared to AT use alone up to 20 minutes ($p < 0.05$). The drug effect was from the active ingredients rather than the saline vehicle control ($p < 0.001$).

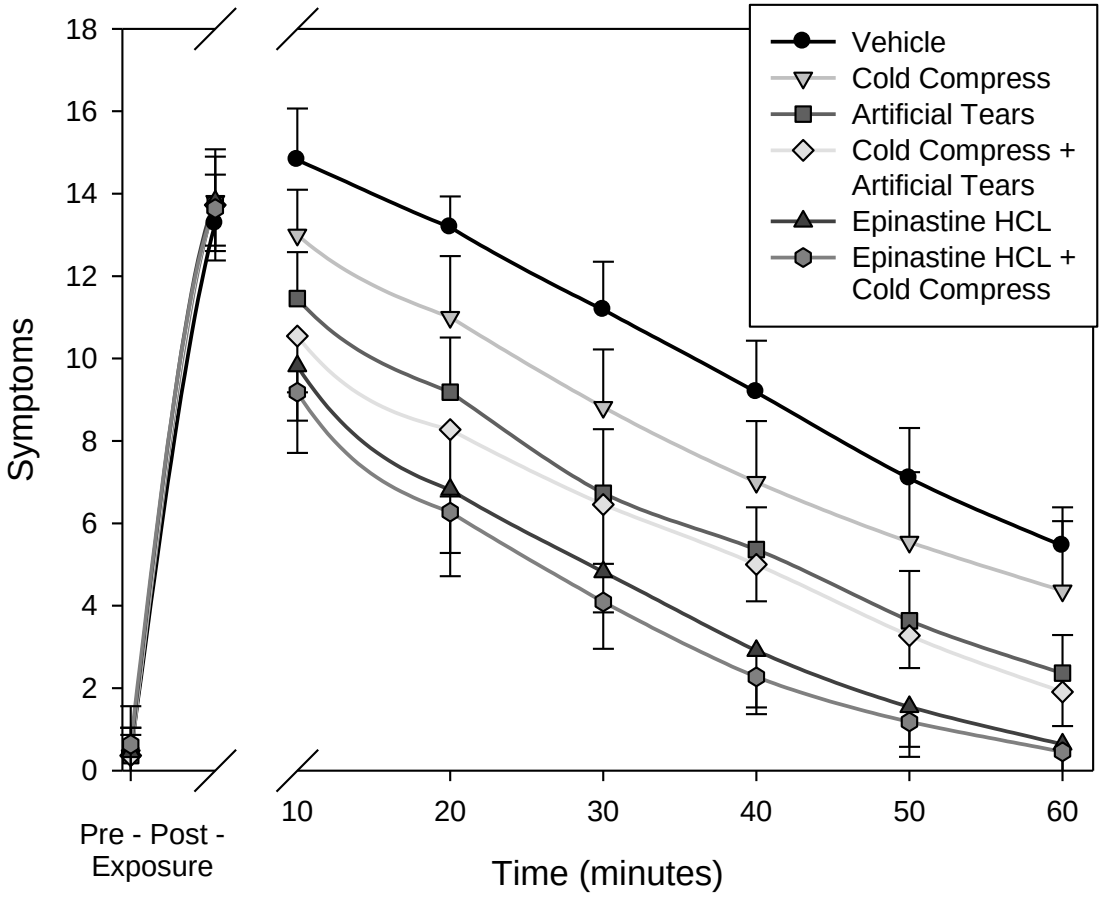

Figure 7.4.4: Ocular symptoms pre and post pollen exposure and every 10 minutes thereafter up to 60 minutes for the saline vehicle volume control, cold compress, artificial tears and artificial tears combined with cold compress, epinastine and epinastine combined with a cold compress. Although the symptoms differed in overall magnitude the profile with time after treatment and recovery was similar for each of the symptoms so they were averaged for analysis. N = 11. Error bars represent ±1 standard deviation.

7.5 Discussion

An environmental chamber model was used to simulate acute ocular seasonal allergic conjunctivitis (SAC) to grass pollen in an environment designed to reflect the local temperature and humidity conditions during the local pollen season. Subjects with a history of seasonal allergic conjunctivitis were skin prick tested to determine systemic sensitivity to

grass pollen and underwent conjunctival allergen challenge to confirm ocular sensitivity to grass pollen, thus ensuring only those with true SAC were enrolled into the study. As the experimental visits were conducted outside the local pollen season, the effect of environmental exposure to other allergens that the patient may be allergic to is limited (if not prevented) and combined with the exclusion of those presenting with signs and symptoms of SAC at each visit ensured that unintended exposure did not confound the results. At the first experimental visit, patients were exposed to increasing concentrations of grass pollen in the environmental chamber at five minute intervals until at least a mild ocular allergic reaction was achieved, and this concentration was used at all subsequent visits. As no statistically significant change in signs (conjunctival hyperaemia and ocular surface temperature) or symptoms (itching, swelling, watering, and soreness) was observed post exposure and between eyes at each visit, this environmental chamber model provides a reproducible, bilaterally homogenous baseline in a physiologically accurate manner from which a treatment effect may be detected. Further, the conditions within the environmental chamber simulated a natural, real world scenario of pollen exposure that patients may actually encounter. Thus, this model overcomes many of the shortcomings of the CAC and traditional environmental models and may serve as the most accurate replication of SAC in office that can be used to study the effect of treatment interventions and the pathophysiology of SAC itself. The detection of a marker of mast cell degranulation within the tear fluid would confirm the ocular allergic reaction induced by the environmental chamber model and validate the use of this model in future studies. One possible candidate is tryptase, considered as a very good marker of mast cell degranulation as it is an enzyme unique to mast cells (Butrus *et al*, 1990). Studies have found that tryptase levels are increased in symptomatic SAC patients and in the tears of patients after conjunctival challenge, the secretagogue compound 40/80, and after rubbing the eyelids (Butrus *et al*, 1990).

In the first phase of the study, the efficacy of artificial tears (AT), cold compress (CC) and in combination (AT+CC) were investigated by measuring conjunctival hyperaemia, ocular

surface temperature and ocular symptoms following exposure to grass pollen in the environmental chamber. Subjects were exposed over a 5 minute interval in the environmental chamber to a predetermined threshold of reactivity, to ensure that subjects had sufficient signs and symptoms in order to detect any treatment effect. The data shows that all treatments provided benefit in relieving hyperaemia, restoring physiological ocular temperature and reducing ocular symptoms during an acute episode of stimulated SAC compared to no treatment.

Although artificial tears (AT) are principally formulated to relieve ocular surface signs and symptoms in dry eye, they have been advocated to have a beneficial effect in SAC (Bielory, 2002; Chigbu, 2009b). The reduction in signs (conjunctival hyperaemia) and symptoms of SAC in this study are likely to have been principally caused by diluting and washing away the allergen from the eye (thus reducing the concentration of allergen on the ocular surface), and the AT acting as a barrier to further exposure by preventing the allergen from binding to the ocular surface (Friedlaender, 2001; Bielory, 2002; Chigbu, 2009b). This barrier effect to allergens has also been observed in contact lens wear, where patients wearing soft contact lenses exhibited reduced signs and symptoms of ocular allergy compared to non-contact lens wearing control visits following exposure in an allergen chamber, with a further benefit from using contact lenses with sustained release of a lubricating agent from within the material matrix (Wolffsohn & Emberlin, 2011). ATs are generally stored at room temperature, which could give them an additional soothing effect, but this study demonstrated that any benefit from the temperature change from AT is minor compared to its other properties such as lubrication, with the temperature reduction and consistency over time higher in the nasal region, compared to the cornea and lower still temporally, following the excretion pathway of the tear film.

In environmental studies of anti-allergy drug efficacy, the use of artificial tears as a control have been shown to have a drug effect of up 50-70% and this is considered to be a placebo effect (Lindsay-Miller, 1979; Abelson *et al*, 1990; Davies & Mullins, 1993; Abelson & Loeffler,

2003). However, as artificial tears may produce a real physical effect on the binding of allergens to the ocular surface, this mechanism cannot be considered purely as placebo and therefore should not be considered as an effective control in studies of acute SAC, whereas their use is warranted in investigating the prophylactic effect of an ocular anti-allergy drugs (Abelson & Loeffler, 2003).

The use of cold compresses (CC) has previously been recommended as supportive therapy in ocular allergy (Friedlaender 1995; Schmid & Schmid, 2000; Bielory, 2002) but no studies relating to the efficacy of cold compress treatment has been reported in the scientific literature. Therefore, this study has demonstrated the beneficial effects of cold compress therapy in ocular disease for the first time. The application of CC may reduce hyperaemia and relieve signs and symptoms by causing vasoconstriction of conjunctival blood vessels and subsequently prevent or minimise swelling and leakage of and inflammatory mediators involved in the allergic response (Hingorani & Lightman, 1995; Bhargava *et al*, 1998; Bielory, 2002). A potential limitation of the CC data was the ability to control the application to the closed eyelids, although the gel mask was held in place over the eyes with an attached elastic headband. This was, however, realistic to the clinical reality where the exact area and location of contact of the compress with the eyelid will vary between patients owing to differences in facial structure.

In the second phase of the study, the effectiveness of non-pharmaceutical treatments was compared to a dual action antihistamine / mast cell stabiliser pharmaceutical (EH), with or without the addition of a CC, in a randomly selected subgroup of subjects using the same acute induced SAC methodology. Comparison over the 60 minute observation period showed that the combination of artificial tears and cold compress was superior to all other treatments in reducing hyperaemia including over the pharmaceutical agent, although the antigen induced ocular redness could be improved to the equivalence effectiveness by combining EH with a CC. An AT or a CC used alone was more effective that the pharmaceutical used in isolation. The pharmaceutical agent effect, however, was confirmed

as being derived from the active ingredients rather than any ocular lubricating effect of its fluid vehicle and this was also the case for the pharmaceutical effect on ocular comfort.

A CC alone or in combination with an AT or EH pharmaceutical lowered the ocular surface temperature below baseline from the increased level caused by exposure to the antigen, whereas an AT alone had relatively little effect over ocular temperature, particularly over the temporal conjunctiva. As this treatment result differed from that of conjunctival hyperaemia and ocular symptoms, it could suggest that the inflammatory events causing increased ocular surface temperature following antigen exposure could differ from those driving other signs and symptoms or the results could be confounded by tear film thickness variations across the ocular surface and with time as this would have affected the radiated heat imaged by the thermal camera.

Ocular symptomology improved faster with EH compared to all other treatment modalities, reducing symptoms to baseline levels after 60 minutes, and the recovery profile was enhanced initially by the application of a CCs. Although none of the non-pharmaceutical treatments reduced symptoms to baseline levels, the mean scores were low, falling within the “hardly troubled at all” category. This data suggests that AT and CC, either alone or in combination, are effective methods of relieving the signs and symptoms of SAC during the active phase of the condition.

EH displays anti-histamine, anti-inflammatory and mast cell stabilising properties in animal and in-vitro studies (Trattler *et al*, 2006; Friedlaender, 2006). Conjunctival allergen challenge model clinical trials of EH have shown that it is significantly more effective in preventing the signs and symptoms of allergic conjunctivitis compared to its vehicle as confirmed in this study (Abelson *et al*, 2004; Lanier *et al*, 2004). The efficacy of EH has also been demonstrated to be effective in an environmental clinical trial (Whitcup *et al*, 2004), but these study designs are subject to variations in exposure and therefore limit their ability to detect drug effects. Only one study has previously utilised an environmental chamber, where EH

was found to be effective and in reducing the ocular signs and symptoms of cat-dander sensitive patients in an active control study (Monson *et al*, 2004). Thus, there has been a lack of studies investigating the efficacy of EH in acute SAC. In the present study, the combination of EH+CC was superior to EH alone in reducing ocular surface temperature ($p<0.001$), superior to EH in reducing hyperaemia both nasally ($p<0.001$) and temporally ($p<0.001$), and enhanced the symptom recovery profile within the first 20 minutes. This suggests that clinically, EH should be prescribed together with advice on applying cold compresses in acute episodes.

The results of the present study are applicable only on the ability of the treatments to relieve the signs and symptoms of simulated SAC during the acute phase of the ocular allergic response, thus it has no bearing on their ability to prevent signs and symptoms from developing through prophylactic treatment. It is not expected that the application of cold compress or artificial tears will have any effect before the ocular allergic response develops, unless they are applied frequently.

7.6 Conclusions

The data suggests that although EH resolves symptoms of SAC earlier, it appears to be less efficacious in resolving ocular signs of inflammation such as conjunctival hyperaemia and ocular surface temperature increases compared to an artificial tear or cold compress alone, or better in combination, during an acute episode of SAC. Therefore for occasional sufferers such self-management, with reduced risks of drug interactions and reduced patient expense, should be considered. For more frequent SAC sufferers, the benefits of cold a compress in addition to prophylactic pharmaceuticals should be considered as part of patient management when symptoms still occur. Further study is required to measure the immunologic response to ocular signs and symptoms induced by the environmental chamber and treatment strategies.

Chapter 8

General Discussion and Conclusions

The management of allergic conjunctivitis and dry eye, two common and related eye conditions that although relatively mild cause significant impact on the quality of life, has not been well studied, let alone in the UK. Further, a wide range of treatments for these conditions are available, yet non-pharmacological treatments for allergic conjunctivitis lack evidence to support their use, and evidence to support the use of eyelid warming therapy with commercially available devices for the treatment of the most common cause of dry eye is not present within the scientific literature. Many of these patients present to both pharmacies and optometrists in the UK where such treatments are readily available. Thus, the aim of this thesis was to determine the diagnostic and management capabilities of pharmacy staff, and provide an evidence base for non-pharmacological treatments for allergic conjunctivitis and dry eye so that clinicians may better understand these conditions, identify areas for professional improvement, and have an scientific support for the treatments that they prescribe as part of evidence based practice.

Using a mystery shopper design in Chapter 2, the history and symptoms questioning by UK pharmacists and pharmacy staff for the differential diagnosis of allergic conjunctivitis is generally poor, placing their confidence in diagnosing allergic conjunctivitis in only a few questions. Although treatments were recommended in most of consultations, just over half were based on evidence in the scientific literature, even though a wide range of topical anti-allergy medications are available over-the-counter or pharmacy-only on the premises. Interestingly, very few offered allergen avoidance advice despite being the most effective way of preventing an acute episode, but none of the pharmacy staff attempted to identify the potential allergens by elicting patient history. However, although pharmacy staff lacks the equipment and training to confirm sensitivity to a particular allergen via skin tests and conjunctival allergen challenge, a diagnosis of allergic conjunctivitis is often made upon

patient history and presenting symptoms and signs. Thus, not only does this study highlight the need for improved ophthalmology training for both pharmacists and pharmacy staff, there is a need to establish strong cross-referral links with optometrists. Indeed, this mystery shopper methodology may be applied to optometrists in order to determine a more accurate assessment of their behaviour, diagnostic capabilities, and management decisions not only for allergic conjunctivitis, but other common eye diseases, rather than relying on questionnaires or surveys which are prone to several forms of bias (Watson *et al*, 2006).

The mystery shopper design used in Chapter 2 was also applied to investigate the diagnostic capability and management decisions by UK pharmacy staff for dry eye. Again, the ability to diagnose dry eye was, with less than half giving the correct diagnosis. Interestingly, although the lack of distinct symptoms for dry eye may have contributed to this, none of the pharmacy staff in Chapter 2 gave a diagnosis of dry eye in the allergic conjunctivitis mystery shopper scenario, but 6% of pharmacy staff in Chapter 3 gave a diagnosis of allergic conjunctivitis as part of hay fever in the dry eye mystery shopper scenario. Thus, it may be that ocular itching, a pathognomic symptom volunteered by the mystery shopper, is a key differential between allergic conjunctivitis and dry eye. Of those that correctly identified dry eye, treatments advised were suitable and included artificial tears and lipid sprays, but it is currently unknown which treatment is more suitable, if at all, for different dry eye subtypes. In support of the findings in Chapter 2, there is clear need for improved education and training relating to the differential diagnosis and management of common eye disease which may present at community pharmacies, such as allergic conjunctivitis and dry eye which may coexist and indeed initiate one another (Fujishima *et al*, 1996a). Mystery shopper studies may also be used as an educational tool, where pharmacies and optometry practices are invited to take part in such a study to allow opportunites for direct constructive feedback after the mystery shop has been performed (Watson *et al*, 2006; Mesquita *et al*, 2010; Weiss *et al*, 2010). Although the participants would be aware that the mystery shopper may arrive any time during a specified period,

social desirability bias may actually improve their behaviour and subsequently improve patient care over this period and beyond due to the constructive feedback (Watson *et al*, 2006; Rhodes & Miller, 2012).

The classification of different dry eye subtypes has been complicated by the lack of correlation between dry eye tests and between symptoms, thus the decision to treat a particular subtype with a particular dry eye treatment in the presence of conflicting signs may be difficult. However, cluster analysis, studied in Chapter 4, appears to be a useful tool in identifying different clinically relevant dry eye subtypes based upon commonly used objective tests and is able to distinguish between those with and without symptoms, although some degree of overlap was expected in patients with minimal dry eye signs. Three subgroups were identified, one which may consist of those with subclinical dry eye characterised by low OSDI scores and relatively low menisus height (cluster 2), one with moderate OSDI scores and greater degree of corneal staining (cluster 2a), and one with high OSDI scores but lesser degree of corneal staining (cluster 1a). Based upon the clnical signs in each group, specific dry eye treatments were suggested that may alleviate the causative dry eye mechanism in that group. However, despite the wide range of dry eye treatments available, there appears to be no eveidence to suggest that a specific primary treatment strategy is suitable for particular dry eye subgroup. Future studies where patients in a particular cluster are randomly assigned a specific dry eye treatment in a cross-over design may help to clarify whether certain treatments are better suited to patients with particular dry eye signs, rather than current trial and error approaches used in clinical practice.

MGD is considered a major and most common form of dry eye, and is typically treated with eyelid warming therapy yet commercially available eyelid warming devices, such as the MGDRx EyeBag, appear to have no evidence supporting their efficacy. In Chapter 5, the MGDRx EyeBag has been demonstrated for the first time to produces an increase in eyelid temperature that causes an increase in tear film lipid layer thickness and tear film stability, validating its use as an eyelid warming device and suggesting that it may be an effective

treatment for MGD and MGD related evaporative dry eye. To support these findings, more accurate and objective tear film thickness measurements are required to quantify the actual increase such as with the DR-1 interferometer (Goto *et al*, 2003d) rather than relying on subjective interpretation of specular images, although the examiners were masked as to which eye received the heated eyebag. Similarly, measurement of tear film stability in an objective manner would help to minimise measurement bias. In Chapter 6, the efficacy and safety of the MGDRx EyeBag was evaluated in patients diagnosed with MGD related evaporative dry eye in a randomised, internally controlled, single masked, clinical trial. Compared to a non-heated control, the heated MGDRx EyeBag demonstrated statisitically significant improvements in conjunctival hyperaemia, tear film osmolarity, tear film stability; tear film lipid layer thickness, meibomian gland function, meibomian gland dropout, oculae surface staining and ocular symptoms after 2 weeks treatment. Further, no reduction in visual acuity was observed and no significant changes in corneal curvature occurred before and after treatment, and combined with very few adverse events (one report of transient stinging), the MGDRx EyeBag may be considered an effective eyelid warming device for MGD related evaporative dry eye that can be recommended by clinicians as a now evidence based treatment option. However, whether this treatment effect is sustained beyond the 2 week treatment period, and whether continuous treatment is required or contines to improve signs and symptoms remains unknown. Future parallel group studies where different treatment durations are used in patients and are followed up some time after treatment cessation may help to optimise treatment for a range of severities, and establish the long term safety of this eyelid warming device.

As part of Chapter 6, an automated NITBUT measurement using the Oculus Keratograph 5M was compared with both the Tearscope NITBUT and human video observation NITBUT to provide an objective measure of NITBUT and determine whether results from each method correlated. Although all three methods were strongly and significantly positively correlated, the automated Keratograph NITBUT was statistically significantly shorter than the

Tearscope NITBUT. As the Keratograph is sensitive to very small areas of break up within the tear film across the entire cornea, the shorter NITBUT values were not unexpected as a human observer, who must constantly gaze across the entire cornea and may have missed these early and possibly imperceptible areas of tear film break up. Nonetheless, these data suggest that automated NITBUT measurement is not only comparable to traditional methods but allows for objectivity, at least for patients with a form of dry eye. NITBUT measurement in normal healthy individuals or those with subclinical dry eye may vary considerably (Sullivan *et al*, 2010, 2012a, 2012b), and is it hypothesised that correlation between automated NIBUT and traditional methods of NIBUT measurement may be weak in these patients.

In the final experiemental Chapter, the efficacy of artificial tears and cold compresses, both non-pharmacological treatments long suggested to help relieve the signs and symptoms of seasonal allergic conjunctivitis (SAC), were evaluated using a novel model of ocular allergy. Both artificial tears and cold compresses were more effective than no treatment and the treatment effect, evaluated through measurement of signs of ocular inflammation (conjunctival hyperaemia and ocular surface temperature increases) was greater when then were combined. Further, the data suggests that during an acute episode of SAC, although epinastine hydrochloride, a topical ocular anti-allergic medication, resolves symptoms of SAC earlier, it appears to be less efficacious in resolving the ocular signs of inflammation compared to an artificial tear or cold compress alone, but was better in combination with cold compresses. Therefore, optometrists and pharmacists to whom SAC patients frequently present may recommend the use of these now evidence based non-pharmacological treatments that are readily available at these practices to occasional sufferers. This study not only lends support to the frequent recommendation of artificial tears for ocular allergy stated by optometrists (Wolffsohn, 2009), but allows those without specialist prescribing qualifications and pharmacists to recommend an evidence based treatment that reduces the risk of drug interactions and patient cost. For more frequent SAC sufferers, the benefits of cold a compress in addition to prophylactic pharmaceuticals such as epinatsine

hydrochloride should be considered when symptoms still occur, necessitating cross-referral between independent prescribers such as GPs and therapeutically qualified professionals.

The environmental chamber model used to elicit an ocular allergic response combines benefits from both the conjunctival allergen challenge and environmental models. The initial titration visits allow the concentration of pollen within the environmental chamber to be established which produces a physiologically accurate, repeatable and desired ocular allergic response level, thus allowing a sufficient homogenous baseline from which a treatment effect can be detected. Further, the administration of allergen to the eyes in the atmosphere within the environmental chamber simulates more natural exposure compared to direct application of relatively high allergen solution concentration to the eyes. Thus, this model of ocular allergy can be utilised for the investigation of other treatments, both non-pharmacological and pharmacological alike for SAC. Indeed, the pollen may be substituted for other allergens such as animal dander and dust mites that are implicated in PAC. However, further study is required to measure the immunologic response to ocular signs and symptoms induced by the environmental chamber to provide substantial support to the enivironmental chamber as an accurate model of ocular allergy, and what effect treatment, particularly non-pharmacological, has on this response.

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Appendix

A1: Patient Information Sheets and Consent Forms

A2: Peer Reviewed Scientific Journal Publications

(2013). Effectiveness of non-pharmacologic treatments for acute seasonal allergic conjunctivitis. *Ophthalmology*, IN PRESS

A2.4 Bilkhu PS, Wolffsohn JS, Naroo SA (2013). Effect of a commercially available warm compress on eyelid temperature and tear film in healthy eyes. *Optometry and Vision Science*, IN PRESS

Aston University
Life & Health Sciences

Investigating the Effect of the MGDRx EyeBag on Eyelid Temperature, Tear Film Lipid Layer Thickness and Tear Film Stability

(Version 1)

Investigators

Mr Paramdeep Bilkhu, Dr Shehzad Naroo and Prof James Wolffsohn

Background and Objectives

Meibomian gland dysfunction (MGD) is a common condition with a variety of causes. Obstructive MGD is the most common subtype where the meibomian glands along the upper and lower lid margin become blocked. The blockage is often caused by the abnormal meibomian gland lipid secretion (meibum). Normally the meibum is liquid, but the meibum in MGD has a higher melting temperature such that it does not melt at body temperature and becomes inspissated and stagnant. This can cause disturbances of the tear film lipid layer, which is normally replenished by the meibum and helps prevent evaporation of the tears. As the meibum is abnormal in MGD, the tear film lipid layer becomes functionally incompetent, resulting in increased evaporation and therefore irritative dry eye symptoms. MGD is therefore a major cause of evaporative dry eye.

Treatment of MGD therefore often involves eye lid warming therapies, where heat is applied to the eyelids to melt the abnormal meibum, increase tear film lipid layer thickness, improve tear film stability and relieve the irritative symptoms. The heat can come from variety of sources, such as warm moist air, infrared devices and warm compresses. The MGDRx EyeBag (The EyeBag Company Ltd, UK) is one such device used to perform warm compresses. It is an eye mask constructed from silk, suedex and filled with flaxseed (linseed) that can be heated in a microwave. However, there appears to be no scientific evidence in the literature demonstrating heat transfer to the eyelids or the effect on the tear film lipid layer and stability. The aim of the study is therefore to determine the temperature of the external and internal eyelids following warm compress and the effects on the tear film lipid layer and stability in normal subjects.

Inclusion and Exclusion Criteria

To take part in this study, you must be at least 18 years of age with no active eye disease, no ocular medications, and no systemic medications known to affect the eyes and no history of eye surgery in the last 3 months. You must remove your contact lenses at least 24 before any tests are carried out but you may re-insert them after testing. You must

not wear eye make-up or false eyelashes for the study visit, but you may apply after testing. If you are willing to take part in the study you will be asked to complete a consent form, but you may leave the study at any time without giving a reason.

Measurements

1. The temperature of the inner and outer upper and lower eyelids of each eye. This will be measured non-invasively using a thermal camera. The upper and lower eyelids will be gently folded with a cotton bud to measure the temperature of the inner eyelids. This does not cause any pain but may cause slight but very brief discomfort.

2. The thickness of the tear film lipid layer of each eye. This will be measured non-invasively with an instrument called the Tearscope in the eye under test. It involves a cold light source directed toward your eyes and the tear film will be examined through a slit lamp bio-microscope.

3. The stability of the tear film of each eye. This will be measured non-invasively by calculating the non-invasive tear film break up time with the Tearscope in the eye under test. You will be asked to blink, and then instructed to keep your eyes open as long as possible. This will be repeated 3 times.

4. An MGDRx EyeBag, after heating in the microwave to 45°C, will then be placed over one of your eyes (closed) selected at random for a period of 5 minutes. At the same time, a non-heated MGDRx EyeBag will be applied to the other eye. The heated eye mask will feel warm to the skin.

5. After 5 minutes have passed, the MGDRx EyeBags will be removed. The above measurements (1, 2 and 3) will then be repeated immediately and 10 minutes after removal.

Important Information

Following the application of the heated MGDRx EyeBag, you may experience temporary blurred vision (approximately 5 minutes). However, this is related to pressure applied to the eyes by the fingers on the warm compress and is therefore very unlikely to occur in this study.

Duration and Reimbursement

The study requires you to attend for one visit in the Vision Science Building at Aston University. The study visit will last up to 30 minutes.

You will be reimbursed £5 for the study visit to cover travel expenses and study time.

Study Information

The study has received ethical approval by Aston University Audiology and Optometry Research and Ethics Committee.

This study is being funded by Aston University. If you require any further information about the MGDRx EyeBag, registering or indeed any aspect of the study please contact Paramdeep Bilkhu (email: bilkhups@aston.ac.uk; telephone: 07535 633861).

YOU WILL BE GIVEN A COPY OF THIS PATIENT INFORMATION SHEET TO KEEP

Aston University
Life & Health Sciences

Personal Identification Number: _____

CONSENT FORM

Title of Project: **Investigating the Effect of the MGDRx EyeBag on Eyelid Temperature, Tear Film Lipid Layer Thickness and Tear Film Stability**

Research Venue: Vision Sciences Building, Aston University, Birmingham, B4 7ET

Name of Investigator(s): Mr Paramdeep Bilkhu, Dr Shehzad Naroo and Prof James Wolffsohn

Please initial box

1. I confirm that I have read and understand the information sheet (version .....) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐

3. I agree to take part in the above study. ☐

_____	_____	_____
Name of Research Participant	Date	Signature
_____	_____	_____
Name of Person taking Consent	Date	Signature

Aston University
Life & Health Sciences

Participant Information Sheet (Version 1)

Section A: The Research Project

1. Title of the study

   Investigating the efficacy of the MGDRx EyeBag in patients with meibomian gland dysfunction related dry eye.

2. Invitation to participate

   You are being invited to take part in a research study involving the application of an eyelid warming device to treat meibomian gland dysfunction. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask the investigator if there is anything that is not clear to you or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

3. Purpose of the study

   Meibomian gland dysfunction (MGD) is a common condition that causes the secretion from meibomian glands in the eyelids to become blocked. Normally, the secretion helps to maintain a healthy tear film. In MGD, the tear film becomes unstable and often causes dry eye symptoms. Treatments often involve gently warming the eyelids to melt this blockage which prevents tears from spreading over the eye. Although there has been some research on application of heat with warm moist flannels, the MGDRx EyeBag potentially offers a simpler and more effective method of applying heat to the eyelids. We would like to find out how effective an eyelid warming device is at melting the secretion and unblocking these glands.

4. Will it cost anything to be in this study?

   No, but the study does not replace the need for regular eye examinations.

5. What will happen to the results of the study?

   The results of the study may be published in a peer reviewed academic journal or report, but your name will not appear or be identifiable in these documents.

6. Who do I contact if I would like further information?

If you have any questions about the study please contact the investigator(s) listed on the consent form. If you have any questions regarding your rights as a research participant please contact the Aston University Research Ethics Committee.

Section B: Your participation in the Research Project

1. Why have I been invited to take part?

Your optometrist has noted that the meibomian glands which help to produce a healthy tear film are blocked. In order to determine whether or not the eyelid warming device is effective the study requires patients with a confirmed diagnosis of meibomian dysfunction and dry eye symptoms.

You will **not** be included in this study if:

- You are less than 18 years of age
- You are pregnant or breastfeeding or currently planning a pregnancy
- You have any other condition which affects the front of your eyes
- You have had surgery to the front of your eyes
- You have systemic conditions affecting the health of your eye
- You are taking ocular medications or systemic medications known to affect the health of your eyes
- You wear contact lenses

2. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this Participant Information Sheet and a copy of the Statement of Informed Consent Form. You will be asked to sign the attached Statement of Informed Consent Form once you have had the opportunity to read all the instructions and information provided and received satisfactory answers to any questions you may have. If you decide to take part you are still free to withdraw at any time and without giving a reason.

3. How do I withdraw from the study?

You are free to withdraw at any time and without giving a reason. Your decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive. To withdraw, please inform one of the investigators named on the Statement of Informed Consent Form.

4. What will happen to me if I take part?

Your optometrist will first determine your suitability to take part in the treatment phase of the study by performing a series of screening tests. This will take place at your eye care practice and includes:

- Examining your eyelids and tear film with a slit lamp microscope – this will involve gently moving and pressing the eyelids.
- Measuring the stability of your tear film with a slit lamp microscope – you will be asked to keep your eyes open after a blink for as long as you can. .

If you are found to be suitable you will be asked if you would like to take part in the treatment phase of the study, which will take place at Aston University. If you choose to take part, the following measurements will be taken at first visit:

- Completing a questionnaire about how your eyes feel
- Examining your eyes, eyelids and tear film with a slit lamp microscope – this will involve gently moving and pressing the eyelids.
- Examining your meibomian glands with an infra-red camera – this will involve gently moving the eyelids.
- Checking the health of the front of the eye using ophthalmic dyes – these dyes do not sting and have no effect on vision or driving. They will naturally wash out of the eye upon blinking after few minutes.

After these measurements, you will be asked to use an eyelid warming device for the treatment phase, which will last 2 weeks. The eyelid warming device is also known as the MGDRx EyeBag (eyebag), and is made of silk and suede like material filled with flaxseed, much like a bean bag. You will be given two eyebags, one for each eye, but you will be instructed to heat only one of them. The heated eyebag will be used for only one eye selected at random, and the non-heated eyebag for the other eye to be applied at the same time for 5 minutes – This is repeated twice a day for the two week treatment phase. Full instructions will also be given as how to heat and use them. We will also ask you to complete a treatment diary, so you can record when you use the eyebag.

After the 2 week treatment phase you will be asked to attend a second visit at Aston University, where the same measurements as the first visit will be taken (see above).

5. What are the possible risks of taking part?

Application of the ophthalmic dye may cause the eyes to become irritated, but this is very rare and there are no known reported cases. In the highly unlikely event this occurs, an experienced optometrist will be immediately available to manage this condition. Furthermore, these dyes are used routinely in professional eye care practice.

The eyebag may cause temporary blurred vision (approximately 5 minutes) after use. However, this is related to pressure applied to the eyes by the fingers is therefore very unlikely to occur in this study. Such an effect has not been reported in similar studies of eyelid warming therapy.

The heated eyebag will be warm to the touch, but is very unlikely to cause any injury to the skin and eyes as your eyes will be closed during treatment and applied for a relatively short period of time.

If any problems or questions arise during the study, you should contact the investigator of the study, on the contact details listed on the Statement of Informed Consent.

6. What if something goes wrong?

   Please feel free to contact Mr Paramdeep Singh Bilkhu (bilkhups@aston.ac.uk; 07535633861). If you have any concerns about the way in which the study has been conducted, then you should contact the Secretary of the Aston University Research Ethics Committee (j.g.walter@aston.ac.uk; 0121 204 4665).

7. What do I have to do while I am in the study?

   You will be required to attend the study visits as mutually agreed by yourself and the investigator, perform the treatment method as prescribed and keep a treatment diary as explained above.

8. What will happen to any information or data collected during the study?

   The data and information will be analyzed by the investigators and the results of the study may be published in a peer reviewed academic journal or report, but your name will not appear or be identifiable in these documents.

9. What are the possible benefits of taking part?

   The information obtained from this study will help to determine if the eyebag is an effective eyelid warming device for the treatment of meibomian gland dysfunction related dry eye.

10. Will my information be kept confidential?

    All information and data collected about you during the course of the study will be kept strictly confidential.

11. Who has reviewed the study?

    This research study has been reviewed and given ethical approval by the Aston University Research Ethics Committee.

12. What am I agreeing to by signing the Statement of Informed Consent Form?

By signing the Statement of Informed Consent Form you are confirming that you have read and understood all pages of the information presented above and that you are willing to participate in this study.

YOU WILL BE GIVEN A COPY OF THIS PATIENT INFORMATION SHEET TO KEEP

Aston University
Life & Health Sciences

Personal Identification Number: _____

CONSENT FORM

Title of Project: **Investigating the efficacy of the MGDRx EyeBag in patients with meibomian gland dysfunction related dry eye**

Research Venue: Vision Sciences Building, Aston University, Birmingham, B4 7ET

Name of Investigator(s): Mr Paramdeep Bilkhu, Dr Shehzad Naroo and Prof James Wolffsohn

Please initial box

1. I confirm that I have read and understand the information sheet (version .....) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐
3. I agree to take part in the above study. ☐

_____	_____	_____
Name of Research Participant	Date	Signature
_____	_____	_____
Name of Person taking Consent	Date	Signature

Aston University
Life & Health Sciences

National Pollen and Aerobiology Research Unit
at the University of Worcester

STUDY INFORMATION (Version 1)

Investigating the Efficacy of Artificial Tear Supplements and Cold Compresses for the treatment of Seasonal Allergic Conjunctivitis

Investigators
Mr Paramdeep Bilkhu, Dr Shehzad Naroo, Prof James Wolffsohn, Mrs Louise Robertson, and Prof Roy Kennedy.

Background and Objectives
Seasonal allergic conjunctivitis is an irritating eye condition that affects many people, caused by hypersensitivity to normally harmless substances such as pollen, and often accompanies seasonal hay fever. Treatments that can be used before initiating medical therapy include artificial tear supplements and cold compresses. However, there is no evidence in the scientific literature that demonstrates their efficacy compared to no treatment or their combined effect with anti-allergic medication. Therefore we aim to investigate the efficacy of artificial tear supplements and cold compresses alone, in comparison to anti-allergic medication, and cold compresses in combination with anti-allergic medication. In addition, we also aim to determine the time course of ocular allergic reactions. At the end of the study we will be able to see whether or not artificial tear supplements and cold compresses are effective in treating seasonal allergic conjunctivitis.

Inclusion and Exclusion Criteria
To take part in this study, you must be at least 18 years of age with a history of seasonal allergic conjunctivitis or seasonal allergic rhinoconjunctivitis (seasonal hay fever) that is not currently active. You must not suffer from asthma. You must also have had no eye surgery in the last 3 months, no other active eye condition, no history of an adverse reaction to ocular drugs or dyes and no history of anaphylaxis (severe systemic allergic reaction). You must not use any ocular medication or systemic medications (antihistamines, mast cell stabilisers, non-steroidal anti-inflammatory drugs or steroids) at least 14 days prior to the start and for the duration of the study but it will take place outside of the allergy season. You must remove your contact lenses before any tests are carried out but you may re-insert them 24 hours after testing. If you are willing to take part in the study you will be asked to complete a consent form, but you may leave the study at any time without giving a reason – your academic studies, welfare and healthcare will not be affected but any data that is already collected may be used in the study.

Screening

Before we take any measurements, we need to know whether or not you have allergic sensitivity to grass pollen. This is achieved by performing a skin prick test and conjunctival challenge test. Both the skin prick test and conjunctival challenge test are commonly performed tests in hospitals to confirm allergies and identify allergens. The skin prick test involves placing small drops of pollen solution on the forearm and gently pricking the underlying skin surface with a small, sterile needle – this is not an injection and does not typically cause bleeding but you may experience a sharp, pinching sensation. A positive response occurs when the small area of skin covered by the drop becomes itchy, red and raised. The conjunctival challenge test involves placing drops of pollen solution into the eye. A positive response occurs when the eye becomes itchy, red and swollen. The only risk involved with these tests is anaphylaxis (a severe systemic allergic reaction) but this is very rare. To minimise any risk these tests will be performed with medical cover present at all times with appropriate medical equipment. Only those who have a positive response to the conjunctival challenge test or conjunctival challenge test and skin prick test will be asked to continue in the study.

Measurements

There will be a total of 3 study visits, each lasting approximately 1 hour and separated by at least 2 days. At the beginning of each visit, a set of measurements will be taken by an experienced optometrist. These are:

 What symptoms you are feeling and how severe they are – you will be asked to complete a short questionnaire about how your eyes currently feel.
 The redness and temperature of your eyes – the front surface of both eyes will be photographed using digital cameras.

 The response of the immune system in your eyes – a tear sample is taken from each eye using a micro-sponge and is analysed for signs of an allergic reaction.

After these measurements we will ask you to stand inside a specially designed room where the environment can be controlled by a computer. Grass pollen will be introduced into the atmosphere of the room, so that the signs and symptoms of seasonal allergic conjunctivitis can be induced – this is intentional, but normally resolves within a few hours with no treatment. At each visit, you will be given one of no treatment, artificial tear supplement (preservative free ocular lubricant), cold compress (cooled gel eye mask) or anti-allergic medication (epinastine hydrochloride 500µg/mL). The measurements will then be repeated every 10 minutes for 1 hour.

After the final set of measurements, the front surface of your eyes will be assessed using a temporary dye with a blue light to highlight any changes. Fluorescein does not sting, lasts only a few minutes and has no effect on vision or driving. However it may cause a self limiting mild allergic reaction where the eyes become red, irritated and sore but this is highly unlikely as there are no known reported cases. In the unlikely event this does happen the experienced optometrist is immediately available to manage the condition. The micro-sponge

will be applied briefly to the eye to draw the tears and does not sting, but may cause a little temporary irritation which subsides quickly.

The anti-allergic drug epinastine hydrochloride (Relestat, Allergan) is a prescription only medicine indicated for the treatment of allergic conjunctivitis. As with all medications, there are potential side effects (1 in 10 to 1 in 100 people). There may be a slight burning sensation on application but this temporary and subsides quickly. The epinastine formulation also contains preservatives called benzalkonium chloride and disodium edetate. As with fluorescein sodium, these may cause a self limiting mild allergic reaction. In the unlikely event this does happen the experienced optometrist is immediately available to manage the condition.

Study Length and Reimbursement

The screening visit and the 3 measurement visits (total 4 visits) is separated at least 2 days therefore the minimum total study length for each participant is 10 days.
Each visit will last approximately 1 hour, for which you will be reimbursed £8 to cover travel expenses. The maximum reimbursement will be £32 (£8×4 visits).

Study Information

This trial is being funded by Aston University but all tests will be performed in NPARU (Charles Darwin Building) at the University of Worcester. If you require any further information about the treatments please contact Mr Paramdeep Bilkhu (email: bilkhups@aston.ac.uk)

If you require any information about appointments or registering for the trial please contact Dr Louise Robertson (email: l.robertson@worc.ac.uk or telephone 01905 855204) or visit our reception in the Charles Darwin Building.

The study has received ethical approval by Aston University Ethics Committee.

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Aston University
Life & Health Sciences

National Pollen and Aerobiology Research Unit
at the University of Worcester

Personal Identification Number: _____

CONSENT FORM

Title of Project: **Investigating the Efficacy of Artificial Tear Supplements and Cold Compresses for the treatment of Seasonal Allergic Conjunctivitis**

Research Venue: Vision Sciences Building, Aston University, Birmingham, B4 7ET

Name of Investigator(s): Mr Paramdeep Bilkhu, Dr Shehzad Naroo, Prof James Wolffsohn, Mrs Louise Robertson, and Prof Roy Kennedy

Please initial box

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Name of Research Participant	Date	Signature
_____	_____	_____
Name of Person taking Consent	Date	Signature