ENVIRONMENTAL RISK FACTORS FOR DRY EYE DISEASE*

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Doctor of Philosophy

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October 2019

* This thesis received funding by EU Horizon 2020 research & innovation programme under Marie Sklodowska-Curie grant agreement No 642760.

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SUMMARY

Dry eye disease (DED) is one of the most frequently encountered ocular conditions, which is clinically under-recognized mainly due to a poor consensus on its diagnosis. It is considered as a multifactorial disease of the ocular surface, where the homeostasis of the tear film is disrupted. In 2017, the Tear Film Ocular Surface Dry Eye Workshop II proposed a global consensus in the diagnosis of DED. For the present thesis, three different studies were performed, in which the recommended diagnostic criteria was used, to provide a wider insight into DED epidemiology. Prevalence rates and risk factors for DED and DED subtypes were estimated among a single population in the UK. DED subtypes included aqueous deficient (ADDE) and evaporative (EDE) forms of the disease, described by measurements of tear meniscus height (TMH), tear evaporation, tear lipid layer thickness (LLT) and meibomian gland dysfunction (MGD). Moreover, a self-administered DED diagnostic method, assessing DED symptoms and the Optrex^{TM} dry eye blink test, was proposed.

Accordingly, this thesis has determined:

- A prevalence of 19.0-56.3%, 6.2%, 64.2% and 11.1% for DED, ADDE, EDE, and both ADDE and EDE, respectively.
- Age, employment status, medication intake, female sex, the presence of any health conditions/problems, poor sleep quality and prolonged outdoor activity as significant risk factors for DED. The last four factors were the most significant.
- Age as a significant risk factor for EDE.
- Ocular surface staining as the most commonly observed DED sign.
- MGD as the most commonly observed EDE sign.
- DED diagnosis by symptoms and tear film stability as the most suitable diagnostic method for the disease.
- A diagnostic sensitivity of 100% and specificity of 54% of the proposed DED diagnostic method.

Keywords: diagnosis, epidemiology, prevalence, logistic regression, blink test

Meinen Eltern

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisors Prof. James S. Wolffsohn, Prof. Leon N. Davies and Dr. Alejandro Cerviño Expósito for their guidance throughout my PhD.

A special thanks goes to Prof. James S. Wolffsohn for his immense knowledge and encouragement. To the European Dry Eye Network (EDEN) group, particularly, to Dr. Francesco Menduni and Dr. Tugce Ipek. With their friendship, strength, faith and perseverance, they have made me complete my writing up.

A sincere thanks goes to the study participants - without them, none of this would have been possible. To Lisa-Marie Baker for pushing me forward in the last participant recruitment, to Dr. Richard Amstrong and Prof. Christof Backhaus for sharing their statistical expertise, and to Dr. Susie Jones for her remarkable support during my first PhD year.

Also, a big thanks goes to Dr. Tecla Bonci, Dr. Fiona Cruickshrank, Dr. Gurpreet Bhogal-Bhamra and Dr. Paramdeep Singh Bilkhu for their help and the fun times in and out of work environment.

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LIST OF ABBREVIATIONS

Abbreviation	Full name
ADDE	aqueous deficient dry eye
ARCHA	Aston Research Centre for Healthy Ageing
CI	confidence interval
CL	contact lens
CLD	contact lens discomfort
DED	dry eye disease
DEQ-5	5-item Dry Eye Questionnaire
DERFS	Dry Eye Risk Factor Survey
EDE	evaporative dry eye
EDEN	European Dry Eye Network
FBUT	fluorescein tear break-up time
FTMH	fluorescein tear meniscus height
IDEEL	Impact of Dry Eye on Everyday Life
K5M	Keratograph 5M
LFU	lacrimal functional unit
LLT	lipid layer thickness
LWE	lid-wiper epitheliopathy
MGD	meibomian gland dysfunction
MQ	McMonnie’s Questionnaire
n/a	not applicable
NEI	National Eye Institute
NIBUT	non-invasive tear break-up time
NIKBUT	non-invasive Keratograph tear break-up time

OR	odds ratio
OSDI	Ocular Surface Disease Index
PRT	phenol red thread
ROC	receiver operative characteristics
TFOS DEWS I	Tear Film Ocular Surface Dry Eye Workshop I
TFOS DEWS II	Tear Film Ocular Surface Dry Eye Workshop II
TMH	tear meniscus height
VDT	visual display terminal
WHS	Women’s Health Study

1. CHAPTER 1: LITERATURE REVIEW

1.1 Overview

The chapter compiles available literature on dry eye disease. Its purpose is to summarise current knowledge in the disease definition, classification and epidemiology, and to identify research problems in the literature that justify the rationale of the present thesis. Dry eye definitions and classification schemes were extracted from substantial reports about the disease (Lemp *et al.*, 2007; Craig *et al.*, 2017). Moreover, information about the disease epidemiology was taken from population-based cross-sectional studies that have been considered in (Stapleton *et al.*, 2017).

1.2 The lacrimal functional unit

Dry eye disease can result from any alteration occurring in the anatomy and physiology of the lacrimal functional unit (LFU) (Figure 1.1) (Lemp *et al.*, 2007).

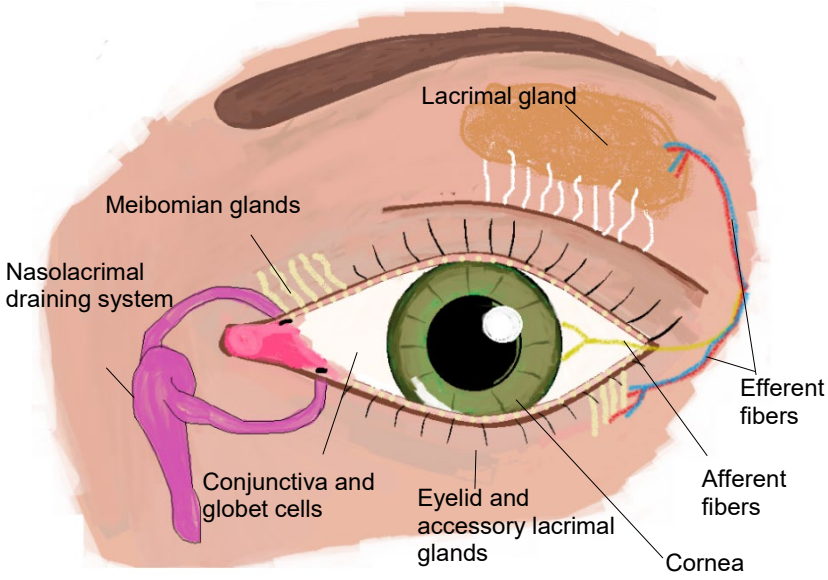


Figure 1.1 The lacrimal functional unit (LFU)

The LFU is an integrated ocular system that compromises the cornea, conjunctiva, lacrimal glands, meibomian glands, eyelids, nasolacrimal draining system and the involved afferent and efferent nerves (cranial nerves V and VII) (Lemp *et al.*, 2007). Its overall function is to maintain the integrity of the tear film (Lemp *et al.*, 2007).

1.3 The tear film

The tear film is a transparent fluid that covers the eye. As such, it preserves the health of the ocular surface. It is traditionally described at the cornea as a tri-laminar structure (Wolff, 1946; Holly and Lemp, 1977), consisting of:

- The lipid layer, which is the outermost layer of the tear film (Wolff, 1946; Holly and Lemp, 1977). It is composed of polar and non-polar lipids that are secreted primarily by the meibomian glands and, with a lesser amount, by the eyelid glands of Moll and Zeiss (Wolff, 1946; Holly and Lemp. 1977). The lipids are believed to retard tear evaporation from the ocular surface and hence to avoid ocular desiccation (Willcox *et al.*, 2017).
- The aqueous layer, which constitutes the bulk of the tear film (Wolff. 1946; Holly and Lemp. 1977). It contains mainly water and specific proteins and electrolytes that are protective and nutritive to the ocular surface (Willcox et al., 2017). The substances are secreted by the main lacrimal gland and the accessory lacrimal glands of Krause and Wolffring, with additional contributions arising from the conjunctival epithelial cells (Wolff, 1946; Holly and Lemp, 1977).
- The mucin layer, which is the innermost layer of the tear film (Wolff, 1946; Holly and Lemp, 1977). It consists of mucins that are secreted by the conjunctival goblet cells and distributed in decreasing gradient from the ocular surface towards the lipid layer (Wolff, 1946; Holly and Lemp, 1977). The mucins are

thought to prevent tear overspill by adhering the aqueous layer onto the ocular surface (Willcox et al., 2017).

A new consensus defines the tear film as a mixture of the aqueous and mucin layer with an overlying lipid phase (Doane, 1994). However, there is a continual return to the traditional model due to its explanatory simplicity (Willcox *et al.*, 2017).

1.4 Definition of dry eye disease

Efforts to define dry eye disease (DED) include the publication of three substantial reports (Lemp, 1995; Lemp *et al.*, 2007; Craig *et al.*, 2017). The National Eye Institute (NEI)/Industry Workshop on Clinical Trials in Dry Eyes was the first in defining DED (Lemp, 1995). Their definition was published in 1995 as follows:

"Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation, which causes damage to the interpalpebral ocular surface and is associated with symptoms of discomfort (Lemp, 1995)*."*

At this stage, DED was termed as a disorder of the tear film, where tear deficiency or excessive tear evaporation played causative roles (Lemp, 1995). The disorder was described as the presence of ocular signs that relate to symptoms of discomfort (Lemp, 1995).

In 2007, a better understanding of the pathogenesis of DED allowed the Tear Film Ocular Surface Dry Eye Workshop I (TFOS DEWS I) (Lemp *et al.*, 2007) to restructure the 1995 definition as follows:

"Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential

damage of the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface (Lemp *et al.*, 2007)*."*

For the first time, DED was defined as a disease with a multifactorial nature, where an alteration of the tear film and ocular surface was characterized by several underlying causes (Lemp *et al.*, 2007). Ocular discomfort, visual disturbance and tear film instability were considered as hallmarks of the disease (Lemp *et al.*, 2007). In addition, hyperosmolarity and both ocular surface damage and inflammation were recognized as further DED markers (Lemp *et al.*, 2007).

Finally, in 2017, a last definition by the Tear Film Ocular Surface Dry Eye Workshop II (TFOS DEWS II) (Craig *et al.*, 2017) described DED as follows:

"Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles (Craig *et al.*, 2017)*."*

Again, DED was characterized by the presence of ocular symptoms and signs. The concept of "loss of homeostasis of the tear film" was included to be considered as the pathophysiological core feature of the disease (Craig *et al.*, 2017). This would serve to acknowledge any changes occurring in the ocular surface and tear film, irrespective to which aetiological factor or combination of aetiological factors had initiated the disease process (Craig *et al.*, 2017).

1.5 Classification of dry eye disease

DED is classified into two main etiological entities (Figure 1.2), evaporative or aqueous deficient dry eye (Lemp *et al.*, 2007). Both forms may co-exist with

increasing disease severity, which can be easily assessed by scoring patients' symptomatology (Craig *et al.*. 2017; Wolffsohn *et al.*. 2017).

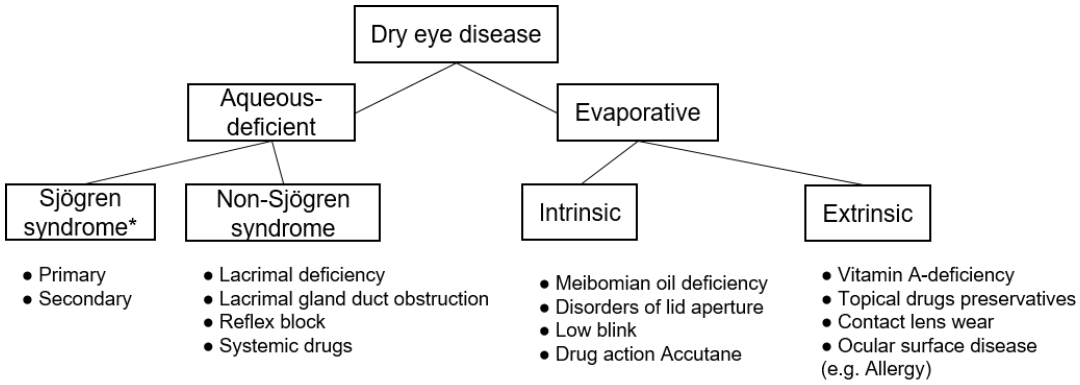


Figure 1.2 Sub-classification of DED

DED = dry eye disease. ***** Sjögren syndrome is an autoimmune disease that affects the body exocrine glands.

Aqueous deficient dry eye (ADDE) is characterized by a reduced secretion and volume of the aqueous component that arises from any damage, dysfunction or reduced innervation of the lacrimal gland and is divided into two major groupings, Sjögren syndrome and non-Sjögren syndrome (Lemp *et al.*, 2007; Craig *et al.*, 2017).

Evaporative dry eye (EDE) is described as an excessive tear film loss via evaporation from the ocular surface in the presence of normal lacrimal function (Lemp *et al.*, 2007; Craig *et al.*, 2017). This alteration is explained by an unstable lipid layer due to blink and eyelid abnormalities, which are classified as either intrinsic (i.e. meibomian oil deficiency) or extrinsic (i.e. contact lens wear) (Lemp *et al.*, 2007; Craig *et al.*, 2017).

1.6 Epidemiology of dry eye disease

One major challenge in the epidemiology of DED has been the lack of a standardized worldwide definition (Stapleton *et al.*, 2017). This has led to the use of diverse diagnostic criteria, which, in turn, complicates the comparison and interpretation of epidemiologic study results about the prevalence and risk factors of the disease (Stapleton *et al.*, 2017).

The most consistent diagnostic criteria in the literature appears to be that first adopted by the Women's Health Study (Stapleton *et al.*, 2017). Other epidemiological studies have diagnosed DED either by the presence of its symptoms, signs or both symptoms and signs (Stapleton *et al.*, 2017).

1.6.1 Dry eye prevalence by the Women's Health Study criteria

The diagnosis of DED by the Women's Health Study (WHS) criteria is based on the presence of self-reported symptoms of ocular dryness and irritation either often or constantly, or a previous disease diagnosis by a physician (Uchino *et al.*, 2008, 2011; Schaumberg *et al.*, 2009; Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014; Um *et al.*, 2014; Na *et al.*, 2015).

The WHS criteria has mostly been conducted in Asian populations and has shown a DED prevalence of 12.5-23.7%, with females more affected than males (Uchino *et al.*, 2011; Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014) (Table 1.1). The lowest prevalence rate of 4.3% has been reported in American males (Schaumberg *et al.*, 2009).

Studies describing DED by symptom self-report and clinical diagnosis separately have estimated a respective prevalence of 2.2-24.4% and 3.0-12.7% (Uchino et al.,

2008; Uchino et al., 2011; Schaumberg et al., 2009; Zhang, Chen and Wu, 2012; Ahn et al., 2014; Um et al., 2014; Na et al., 2015).

Table 1.1 DED prevalence by the WHS criteria*

Study	**Population**	**Prevalence (%[95%CI])**		
Authors, Year	Country Age (years) Sex (n)	By clinical diagnosis or symptom self-report	By clinical diagnosis	By symptom self-report
Schaum-berg et al., 2009	USA ≥ 50 25444 ♂	4.3[n/a]	3.0[n/a]	2.2[n/a]
Zhang et al., 2012	China n/a 927 ♀ 958 ♂	23.7[n/a]	1.3[n/a]	23.1[n/a]
Uchino et al., 2011	Japan ≥ 40 1423 ♀ 1221 ♂	♀ 21.6[19.5-23.9] ♂ 12.5[10.7-14.5]	♀ 7.9[6.6-9.5] ♂ 2.0[1.3-3.0]	♀ 18.7[16.7-20.8] ♂ 11.5[9.7-13.4]
Ahn et al., 2015	South Korea ≥ 19 6676 ♀ 4990 ♂	16.0[14.6-17.3]	8.0[7.3-8.7]	14.4[13.1-15.7]
Uchino et al., 2008	China 15-18 585 ♀ 2848 ♂	n/a	♀ 8.0[7.4-8.4] ♂ 4.3[3.9-4.6]	♀ 24.4[23.9-25.0] ♂ 21.0[20.1-21.8]
Um et al., 2014	South Korea ≥ 30 9398 ♀ 7033 ♂	n/a	All 10.4[9.9-10.9] ♀ 12.7[12.6-12.7] ♂ 4.6[4.6-4.6]	All 17.7[17.1-18.3] ♀ 19.4[19.4-19.5] ♂ 9.8[9.8-9.9]
Na et al., 2015	Korea ≥19 6655 ♀	n/a	12.3[n/a]	n/a

DED = dry eye disease. WHS = Women's Health Study. n/a = not applicable. ♀ = female. ♂ = male. CI = confidence interval.
* Included population-based cross-sectional studies that have been considered in (Stapleton *et al.*, 2017).

1.6.2 Dry eye prevalence by symptoms

The prevalence rates of DED diagnosed by symptoms (Table 1.2) are not as comparable as those diagnosed with the WHS criteria since they rely upon different definitions of symptomatic DED (Stapleton *et al.*, 2017). Nevertheless, it can be generally ascertained that symptomatic DED is more prevalent in females than in males (Lu *et al.*, 2008; Tongg *et al.*, 2009; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Han *et al.*, 2011; Hashemi *et al.*, 2014; Malet *et al.*, 2014; Paulsen *et al.*, 2014; Tan *et al.*, 2015).

Symptomatic DED has been described as either agreeing with a statement that defines the disease as having several symptoms (Vehof *et al.*, 2014; Na *et al.*, 2015), self-reporting to have at least one of several DED symptoms either often, sometimes, constantly or all of the time (Lu *et al.*, 2008; Jie *et al.*, 2009; Tongg *et al.*, 2009; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Han *et al.*, 2011; Paulsen *et al.*, 2014; Tan *et al.*, 2015), or showing a positive result to the Ocular Surface Disease Index (OSDI) questionnaire (Hashemi *et al.*, 2014; Malet *et al.*, 2014).

Studies defining symptomatic DED by symptom self-report were mostly conducted on Asian populations and showed different prevalence rates, ranging from 6.5% to 52.4% (Lu *et al.*, 2008; Jie *et al.*, 2009; Tongg *et al.*, 2009; Guo *et al.*, 2010; Han *et al.*, 2011; Tan *et al.*, 2015). In the USA (Paulsen *et al.*, 2014) and Spain (Viso, Rodriguez-Ares and Gude, 2009), the same method was used, reporting prevalence rates of 14.5% and 18.4%, respectively.

DED symptoms, which have been agreed to be either normally present or present for the past three months, were found similarly prevalent among Korean (20.0%) (Na *et al.*, 2015) and British females (20.8%) (Vehof *et al.*, 2014). On the other hand, an OSDI score of ≥ 23 estimated a disease prevalence of 18.3% in Iran (Hashemi *et al.*, 2014) and of 39.2% in France (Malet *et al.*, 2014).

Table 1.2 DED prevalence by symptoms*

Study	**Population**	**Prevalence (%[95%CI])**		
Authors, Year	Country Age (years) Sex (n)	By agreeing to a statement	By symptom self-report	By an OSDI ≥23
Na et al., 2015	Korea ≥19 6655 ♀	20.0[n/a][A]	n/a	n/a
Vehof et al., 2015	United Kingdom 20-83 1635 ♀	20.8[19.5-22.1][B]	n/a	n/a

Table 1.2 *(continued)*

Paulsen et al., 2014	USA 21-81 1789 ♀ 2271 ♂	n/a	All 14.5[n/a][C] ♀ 17.9[n/a][C] ♂ 10.5[n/a][C]	n/a
Han et al., 2011	Korea ≥ 65 340 ♀ 317 ♂	n/a	All 30.3[n/a][D] ♀ 34.7[n/a][D] ♂ 25.6[n/a][D]	n/a
Tan et al., 2015	Singapore 15-83 561 ♀ 443 ♂	n/a	All 12.3[10.3-14.4][E] ♀ 14.8[12.0-18.0][E] ♂ 9.0[6.5-12.][E]	n/a
Jie et al., 2009	China 40-84 1112 ♀ 3327 ♂	n/a	21.0[n/a][F]	n/a
Viso et al., 2009	Spain 40-96 411 ♀ 243 ♂	n/a	All 18.4[15.4-21.3][F] ♀ 21.8[17.9-25.8][F] ♂ 12.5[8.3-16.6][F]	n/a
Guo et al., 2010	China 40-91 837 ♀ 979 ♂	n/a	All 50.1[47.8-52.4][F] ♀ 50.2[46.8-53.6][F] ♂ 49.9[46.8-53.1][F]	n/a
Tong et al., 2009	Singapore 40-80 1704 ♀ 1576 ♂	n/a	All 6.5[5.7-7.4][F] ♀ 4.9[3.9-6.0][F] ♂ 8.2[6.9-9.7][F]	n/a
Lu et al., 2008	China ≥ 40 809 ♀ 1031 ♂	n/a	All 52.4[50.2-54.7][F] ♀ 52.9[49.5-56.3][F] ♂ 52.1[49.1-55.2][F]	n/a
Hashemi et al., 2013	Iran 40-64 595 ♀ 413 ♂	n/a	n/a	All 18.3[15.9-20.6] ♀ 20.0[16.9-23.1] ♂ 15.7[12.2-19.3]
Malet et al., 2014	France 73-94 561 ♀ 354 ♂	n/a	n/a	All 39.2[n/a] ♀ 44.7[n/a] ♂ 30.5[n/a]

DED = dry eye disease. n/a = not applicable. OSDI = Ocular Surface Disease Index. ♀ = female. ♂ = male. CI = confidence interval.

* Included population-based cross-sectional studies that have been considered in (Stapleton *et al.*, 2017).

A. Agreeing to have symptoms of dryness, foreign body sensation, itching burning or sandiness.

B. Agreeing to have symptoms of foreign body sensation, itching, burning or sandiness not related to allergy and for the past three months.

C. Self-reporting the use of artificial tears at least once per day or symptoms of dryness, grittiness or burning either moderately bothersome/greater or often/sometimes.

D. Self-reporting at least one of six symptoms (dryness, grittiness/sandiness, burning, stickiness of eyelids, watery eyes/tearing and redness) either often or all of the time and for the past two weeks.

E. Self-reporting at least one of five primary symptoms of the McMonnies questionnaire (soreness, scratchiness, dryness, grittiness and burning) either often or constantly.

F. Self-reporting at least one of six symptoms (dryness, grittiness/sandiness, burning, redness, crusting of eyelashes and morning stickiness of eyelids) either often or all of the time.

1.6.3 Dry eye prevalence by signs

The prevalence of DED by signs (Table 1.3) vary considerably. Following clinical tests were used to describe DED by signs:

- The fluorescein tear break-up time (FBUT) (Lu *et al.*, 2008; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Hashemi *et al.*, 2014; Malet *et al.*, 2014), which determines the stability of the tear film. It measures the number of seconds that elapse between a blink and the appearance of the first tear film disruption, which is easily seen following instillation of sodium fluorescein into the eye.
- The Schirmer test II (Lu *et al.*, 2008; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Hashemi *et al.*, 2014), which measures the aqueous production of the tear film. The test involves the insertion of a filter paper strip, after using ocular anaesthesia, over the one-third temporal lower eyelid margin. The length of the wet area (in millimeters) is read off after 5 minutes of application and gives an indication of the aqueous tear film volume.
- Fluorescein and rose bengal staining (Viso, Rodriguez-Ares and Gude, 2009; Hashemi *et al.*, 2014). Both sodium fluorescein and rose bengal are ophthalmic dyes used to visualize eventual ocular surface damage in DED. In DED epidemiology, sodium fluorescein has been exclusively used to evaluate corneal damage (Viso, Rodriguez-Ares and Gude, 2009; Hashemi et al., 2014). In contrast, rose bengal has been used to stain both cornea and conjunctiva (Viso, Rodriguez-Ares and Gude, 2009; Hashemi et al., 2014).

Table 1.3 DED prevalence by signs*

Study	**Population**	**Dry eye prevalence (%[95%CI])**			
Authors, Year	Country Age (years) Sex (n)	By FBUT ≤10s	By Schirmer test II <5mm/5min	By fluorescein staining ≥1†	By rose bengal staining ≥3‡
Malet et al., 2014	France 73-94 561 ♀ 354 ♂	All 44.9[n/a]**A** ♀ 43.7[n/a]**A** ♂ 46.6[n/a]**A**	n/a	n/a	n/a
Guo et al., 2010	China 40-91 837 ♀ 979 ♂	All 37.7[35.5-39.9] ♀ 35.1[31.9-38.4] ♂ 39.9[36.9-43.0]	All 19.9[18.4-22.1] ♀ 17.5[14.8-19.9] ♂ 22.2[19.6-24.8]	All 6.0[4.9-7.1] ♀ 6.1[4.5-7.7] ♂ 5.9[4.4-7.4]	n/a
Lu et al., 2008	China ≥ 40 809 ♀ 1031 ♂	All 35.3[33.1-37.5] ♀ 38.1[34.7-41.4] ♂ 33.1[30.2-36.0]	All 35.3[33.1-37.5] ♀ 38.1[34.7-41.4] ♂ 33.1[30.2-36.0]	All 5.8[4.7-6.9] ♀ 5.7[4.1-7.3] ♂ 5.9[4.5-7.4]	n/a
Viso et al., 2009	Spain 40-96 411 ♀ 243 ♂	All 15.6[12.7-18.5] ♀ 17.0[13.2-20.9] ♂ 12.8[8.5-17.2]	All 37.0[33.2-40.7] ♀ 37.1[32.4-41.9] ♂ 36.6[30.4.3-42.8]	All 7.0[4.9-8.9] ♀ 7.2[4.7-8.9] ♂ 6.4[3.3-9.6]	All 13.0[10.3-15.6] ♀ 11.8[8.5-15.0] ♂ 15.0[10.4-19.7]
Hashe-mi et al., 2014	Iran 40-64 595 ♀ 413 ♂	All 34.2[29.5-38.8] ♀ 37.6 [32.3-42.9] ♂ 29.1[23.7-34.5]	All 17.8[15.5-20.0] ♀ 17.1[14.2-20.1] ♂ 18.6[14.8-22.4]	All 11.3[8.5-14.1] ♀ 12.3[9.0-15.5] ♂ 9.9[6.5-13.3]	All 4.9[3.4-6.5]; ♀ 6.0[3.9-8.1] ♂ 3.5[1.5-5.5]

DED = dry eye disease. n/a = not applicable. FBUT= fluorescein tear break-up time. ♀ = female. ♂ = male. CI = confidence interval.
***** Included population-based cross-sectional studies that have been considered in (Stapleton *et al.*, 2017).
A. A FBUT ≤ 5s was used instead.
†. Corneal fluorescein staining was graded as 0 (no staining), 1 (mild staining with a few disseminated stains and limited to less than one third of the cornea), 2 (moderate staining with severity between grades 1 and 3) and 3 (severe staining with confluence stains and occupying half or more of the cornea).
‡. Corneal and conjunctival rose bengal staining was graded using the van Bijsterveld staining score system.

An FBUT of ≤10s (Lu *et al.*, 2008; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Hashemi *et al.*, 2014), Schirmer test II of <5mm/5min (Lu *et al.*, 2008; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Hashemi *et al.*, 2014), fluorescein score of ≥1 (Lu *et al.*, 2008; Viso, Rodriguez-Ares and Gude, 2009; Guo *et al.*, 2010; Hashemi *et al.*, 2014) and rose bengal score of ≥3 (Viso, Rodriguez-Ares and Gude, 2009; Hashemi *et al.*, 2014) have estimated a disease prevalence of 15.6-37.7%, 17.8-37.0%, 5.8-11.3% and 4.9-13.0%, respectively. However, when an FBUT value of ≤5s was used (Malet *et al.*, 2014), the prevalence of DED was reported to be up

to 44.9%. The discrepancy in the disease prevalence may be explained by poor standardisation and invasiveness of the tests (Stapleton *et al.*, 2017).

1.6.4 Dry eye prevalence by symptoms and signs

Four different population-based studies exist (Viso, Rodriguez-Ares and Gude, 2009; Hashemi *et al.*, 2014; Malet *et al.*, 2014; Vehof *et al.*, 2014), whereby the prevalence of DED has been estimated by a combination of symptoms and signs (Table 1.4). Unfortunately, due to the heterogeneity of used disease diagnoses, direct comparisons of the prevalence rates are not possible (Stapleton *et al.*, 2017).

Table 1.4 DED prevalence by symptoms and signs*

Study	**Population**	**Dry eye prevalence (%[95%CI])**			
Authors, Year	Country Age (years) Sex (n)	By clinical diagnosis and daily use of artificial tears	By an OSDI ≥23 or use of daily artificial tears	By an OSDI ≥23 and at least one sign (FBUT ≤10s, Schirmer test II <5mm/5min, fluorescein staining ≥1† or rose bengal staining ≥3‡)	By symptom self-report and at least one sign (FBUT ≤10s, Schirmer test II <5mm/5min, fluorescein staining ≥1† or rose bengal staining ≥3‡)
Vehof et al., 2015	United Kingdom 20-83 1635 ♀	9.6[8.7-10.6]	n/a	n/a	n/a
Malet et al., 2014	France 73-94 561 ♀ 354 ♂	n/a	All 21.9[n/a] ♀ 27.1[n/a] ♂ 13.6[n/a]	n/a	n/a
Hashemi et al., 2014	Iran 40-64 595 ♀ 413 ♂	n/a	n/a	All 8.7[6.9-10.6] ♀ 10.6[8.0-13.2] ♂ 6.1[3.8-8.3]	n/a
Viso et al., 2009	Spain 40-96 411 ♀ 243 ♂	n/a	n/a	n/a	All 11.0[8.6-13.3] ♀ 11.9[8.8-15.1] ♂ 9.0[5.3-12.6]

DED = dry eye disease. n/a = not applicable. OSDI = Ocular Surface Disease Index. FBUT= fluorescein tear break-up time. ♀ = female. ♂ = male. CI = confidence interval.
***** Included population-based cross-sectional studies that have been considered in (Stapleton *et al.*, 2017).
†. Corneal fluorescein staining was graded as 0 (no staining), 1 (mild staining with a few disseminated stains and limited to less than one-third of the cornea), 2 (moderate staining with severity between grades 1 and 3) and 3 (severe staining with confluence stains and occupying half or more of the cornea).
‡. Corneal and conjunctival rose bengal staining was graded using the van Bijsterveld staining score system.

1.6.5 Environmental risk factors for dry eye disease

Logistic regression analyses have been performed in epidemiological studies concerned with quantifying associations between environmental conditions and DED (Uchino *et al.*, 2008, 2011; Lu *et al.*, 2008; Tongg *et al.*, 2009; Jie *et al.*, 2009; Schaumberg *et al.*, 2009; Guo *et al.*, 2010; Han *et al.*, 2011; Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014; Um *et al.*, 2014; Vehof *et al.*, 2014; Hashemi *et al.*, 2014; Malet *et al.*, 2014; Paulsen *et al.*, 2014; Tan *et al.*, 2015; Na *et al.*, 2015). The term environment is broadly used to refer to any external and internal bodily states habitually experienced by an individual, such as demographic factors (i.e. age), lifestyle factors (i.e. contact lens wear), environmental factors (i.e. humidity) and physiological or genetic factors (i.e. health conditions) (Lemp *et al.*, 2007).

1.6.5.1 Assessing dry eye risk factors

In logistic regression, the odds ratio (OR) is widely used to quantify how much likely an environmental condition contributes to DED (Uchino *et al.*, 2008, 2011; Lu *et al.*, 2008; Tongg *et al.*, 2009; Jie *et al.*, 2009; Schaumberg *et al.*, 2009; Guo *et al.*, 2010; Han *et al.*, 2011; Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014; Um *et al.*, 2014; Vehof *et al.*, 2014; Hashemi *et al.*, 2014; Malet *et al.*, 2014; Paulsen *et al.*, 2014; Tan *et al.*, 2015; Na *et al.*, 2015) (Table 1.5).

Mathematically, the OR is calculated as follows: OR = odds of DED among individuals with the condition/ odds of DED among individuals without the condition, where “odds of DED” is defined as the probability of having the disease divided by the probability of not having the disease (Szumilas, 2010).

Table 1.5 DED risk factor assessment*

Study	DED diagnosis	Risk factor	Risk categories	OR [95%CI]	p-value
Schaum-berg et al., 2009	WHS criteria	♂ Age (years)	50-54	1.00	
			55-59	0.81 [0.64-1.04]	n/a
			60-64	0.72 [0.55-0.93]	n/a
			65-69	0.92 [0.71-1.20]	n/a
			70-74	1.18 [0.92-1.53]	n/a
			74-79	1.51 [1.15-1.97]	n/a
			≥80	1.76 [1.34-2.32]	n/a
		♂ Race/Ethnicity	White	1.00	
			African American	1.13 [0.76-1.68]	n/a
			Asian/Pacific Islander	1.36 [0.79-2.35]	n/a
			Hispanic	1.25 [0.93-1.67]	n/a
			Unknown/Other	0.93 [0.53-1.63]	n/a
		♂ Region of residence	South	1.00	
			West	0.93 [0.53-1.63]	n/a
			Midwest	1.01 [0.85-1.18]	n/a
			Northeast	0.96 [0.81-1.14]	n/a
			Other	1.61 [0.85-3.04]	n/a
		♂ Hypertension	No	1.00	
			Yes	1.28 [1.12-1.45]	n/a
		♂ Benign prostatic hyperplasia	No	1.00	
			Yes	1.26 [1.09-1.44]	n/a
		♂ Diabetes mellitus	No	1.00	
			Yes	0.97 [0.74-1.24]	n/a
Zhang et al., 2012	WHS criteria	Myopia	No	1.00	
			Yes	1.49 [0.99-2.23]	n/a
		Contact lens wear	No	1.00	
			Yes	1.22 [0.81-1.81]	n/a
		Inadequate refractive correction	No	1.00	
			Yes	1.98 [1.58-2.49]	n/a
		Frequent self-administered topical ophthalmic medication	No	1.00	
			Yes	1.84 [1.40-2.41]	n/a
		Poor sleep quality	No	1.00	
			Yes	1.34 [1.05-1.71]	n/a
Uchino et al., 2011	WHS criteria	♂ Age (years)	40-49	1.00	
			50-59	1.22 [0.67-2.20]	n/a
			60-69	1.03 [0.55-1.94]	n/a
			70-79	1.03 [0.54-1.99]	n/a
			≥80	0.95 [0.43-2.09]	n/a
		♂ Body mass index (kg/m^2)	18.5-24.9	1.00	
			<18.5	2.07 [0.98-4.39]	n/a
			>25.0	1.11 [0.72-1.70]	n/a
		♂ Visual terminal display use (hours)	No	1.00	
			0-2	0.71 [0.40-1.27]	n/a
			2-4	0.52 [0.21-1.26]	n/a
			≥4	1.10 [0.54-2.24]	n/a
		♂ Contact lens use	No	1.00	
			Yes	3.84 [1.46-10.10]	n/a
		♂ Stroke	No	1.00	
			Yes	1.33 [0.69-2.55]	n/a
		♂ Hypertension	No	1.00	
			Yes	1.39 [0.94-2.06]	n/a
		♀ Age (years)	40-49	1.00	
			50-59	1.08 [0.68-1.72]	n/a
			60-69	1.16 [0.71-1.89]	n/a
			70-79	1.52 [0.92-2.51]	n/a
			≥80	1.36 [0.79-2.34]	n/a

Table 1.5 *(continued)*

	♀ Body mass index (kg/m^2)	18.5-24.9	1.00	
		<18.5	1.17 [0.69-1.97]	n/a
		>25.0	0.69 [0.48-1.01]	n/a
	♀ Visual terminal display use (hours)	No	1.00	
		0-2	1.03 [0.62-1.70]	n/a
		2-4	2.33 [1.12-4.85]	n/a
		≥4	1.88 [0.95-3.73]	n/a
	♀ Contact lens use	No	1.00	
		Yes	3.61 [2.13-6.10]	n/a
	♀ Stroke	No	1.00	
		Yes	1.26 [0.70-2.28]	n/a
	♀ Myocardial infarction or angina	No	1.00	
		Yes	2.64 [1.51-4.62]	n/a
Clinical diagnosis	♂ Age (years)	40-49	1.00	
		50-59	1.92 [0.56-6.63]	n/a
		60-69	1.33 [0.33-5.43]	n/a
		70-79	0.96 [0.20-4.65]	n/a
		≥80	Omitted	n/a
	♂ Body mass index (kg/m^2)	18.5-24.9	1	
		<18.5	2.28 [0.49-10.59]	n/a
		>25.0	0.52 [0.15-1.79]	n/a
	♂ Visual terminal display use (hours)	No	1.00	
		0-2	0.90 [0.28-2.90]	n/a
		2-4	0.51 [0.06-4.14]	n/a
		≥4	1.24 [0.31-4.98]	n/a
	♂ Contact lens use	No	1.00	
		Yes	4.38 [0.87-22.04]	n/a
	♂ Stroke	No	1.00	
		Yes	0.94 [0.12-7.49]	n/a
	♂ Hypertension	No	1.00	
		Yes	0.68 [0.24-1.96]	n/a
	♀ Age (years)	40-49	1	
		50-59	1.78 [0.95-3.35]	n/a
		60-69	1.70 [0.86-3.37]	n/a
		70-79	0.45 [0.17-1.17]	n/a
		≥80	1.09 [0.47-2.50]	n/a
	♀ Body mass index (kg/m^2)	18.5-24.9	1.00	
		<18.5	0.98 [0.44-2.21]	n/a
		>25.0	0.72 [0.39-1.31]	n/a
	♀ Visual terminal display use (hours	No	1.00	
		0-2	0.97 [0.49-1.93]	n/a
		2-4	2.52 [1.04-6.13]	n/a
		≥4	1.40 [0.56-3.46]	n/a
	♀ Contact lens use	No	1.00	
		Yes	4.36 [2.33-8.17]	n/a
	♀ Stroke	No	1.00	
		Yes	1.24 [0.45-3.37]	n/a
	♀ Myocardial infarction or angina	No	1.00	
		Yes	0.97 [0.32-2.95]	n/a
Symptoms (WHS criteria)	♂ Age (years)	40-49	1.00	
		50-59	1.13 [0.61-2.11]	n/a
		60-69	1.00 [0.51-1.93]	n/a
		70-79	1.03 [0.52-2.03]	n/a
		≥80	1.02 [0.45-2.27]	n/a
	♂ Body mass index (kg/m^2)	18.5-24.9	1.00	
		<18.5	2.04 [0.93-4.48]	n/a
		>25.0	1.22 [0.78-1.88]	n/a
	♂ Visual terminal display use (hours)	No	1.00	
		0-2	0.76 [0.42-1.37]	n/a
		2-4	0.48 [0.18-1.25]	n/a
		≥4	1.00 [0.46-2.16]	n/a

Table 1.5 *(continued)*

		♂ Contact lens use	No	1.00	
			Yes	4.48 [1.69-11.90]	n/a
		♂ Stroke	No	1.00	
			Yes	1.42 [0.74-2.75]	n/a
		♂ Hypertension	No	1.00	
			Yes	1.56 [1.04-2.35]	n/a
		♀ Age (years)	40-49	1.00	
			50-59	1.00 [0.60-1.65]	n/a
			60-69	1.27 [0.75-2.14]	n/a
			70-79	1.90 [1.11-3.23]	n/a
			≥80	1.46 [0.82-2.60]	n/a
		♀ Body mass index (kg/m^2)	18.5-24.9	1.00	
			<18.5	1.34 [0.78-2.29]	n/a
			>25.0	0.74 [0.50-1.09]	n/a
		♀ Visual terminal display use (hours	No	1.00	
			0-2	1.10 [0.64-1.88]	n/a
			2-4	2.28 [1.05-4.96]	n/a
			≥4	2.44 [1.22-4.90]	n/a
		♀ Contact lens use	No	1.00	
			Yes	2.67 [1.54-4.65]	n/a
		♀ Stroke	No	1.00	
			Yes	1.12 [0.60-2.09]	n/a
		♀ Myocardial infarction or angina	No	1.00	
			Yes	2.71 [1.53-4.79]	n/a
Ahn et al., 2014	Clinical diagnosis	Age (years)	19-29	1.00	
			30-39	1.00 [0.7.-1.50]	0.84
			40-49	1.20 [0.90-1.70]	0.23
			50-59	1.80 [1.20-2.70]	<0.01
			60-69	1.70 [1.10-2.70]	0.02
			≥70	1.00 [0.60-1.70]	0.93
		Sex	Male	1.00	
			Female	2.80 [2.10-3.70]	<0.01
		Monthly household income	Lowest quintile	1.00	
			2nd-4th quintile	1.10 [0.80-1.60]	0.53
			Highest quintile	1.20 [0.80-1.80]	0.39
		Education	Elementary school	1.00	
			Middle school	1.30 [0.80-2.00]	0.24
			High school	1.50 [1.00-2.20]	0.06
			University/higher	1.60 [1.00-2.40]	0.05
		Residential area	Urban	1.00	
			Rural	1.00 [0.70-1.30]	0.90
		Occupation	Occupation Farming, fishing and forestry	1.00	
			Administrator, management, professional business and financial operations occupations	1.50 [0.80-2.90]	<0.17
			Sales and related occupations	1.30 [0.70-2.60]	<0.37
			Business and financial operations occupations	0.90 [0.50-1.70]	0.86
			Installation, maintenance and repair occupations or technicians	1.30 [0.70-2.50]	0.45
			Laborer	1.30 [0.70-2.40]	0.48
			Unemployed	1.50 [0.90-2.70]	0.14

Table 1.5 *(continued)*

		Hypertension	No	1.00	
			Prehypertension	0.90 [0.70-1.10]	0.30
			Hypertension	0.8 [0.60-1.00]	0.07
		Obesity (kg/m^2)	Underweight (<18.5)	1.00	
			Normal (18.5-24.9)	0.90 [0.60-1.40]	0.75
			Obesity (>25.0)	0.80 [0.60-1.30]	0.40
		Hypercholesterole-mia	No	1.00	
			Yes	1.20 [0.90-1.60]	0.13
		Hypertriglycemia	No	1.00	
			Yes	0.90 [0.70-1.30]	0.66
		Rheumatoid	No	1.00	
			Yes	1.30 [0.80-2.20]	0.29
		Thyroid disease	No	1.00	
			Yes	1.70 [1.20-2.40]	<0.01
		Lifetime smoker	No	1.00	
			Yes	0.70 [0.60-1.00]	0.09
		Sleep duration (hours)	6-8	1.00	
			<6	1.10 [0.90-1.50]	0.34
			>8	0.70 [0.50-1.10]	0.10
		Stress	Least stressful	1.00	
			Moderately stressful	1.30 [1.00-1.70]	0.07
			Extremely stressful	1.70 [1.10-2.60]	0.01
		Binge alcohol user	Never drink an alcohol	1.00	
			Not a binge alcohol user	0.80 [1.00-1.20]	0.82
			Yes	0.70 [1.00-1.30]	0.89
		History of eye surgery	No	1.00	
			Yes	2.60 [2.00-3.30]	<0.01
	Symptoms (WHS criteria)	Age (years)	19-29	1.00	
			30-39	1.10 [0.80-1.40]	0.62
			40-49	1.10 [0.90-1.50]	0.34
			50-59	1.50 [1.10-2.10]	0.01
			60-69	1.60 [1.10-2.30]	<0.01
			≥70	1.20 [0.80-1.90]	0.34
		Sex	Male	1.00	
			Female	1.90 [1.50-2.40]	<0.01
		Monthly household income	Lowest quintile	1.00	
			2nd-4th quintile	1.20 [0.90-1.60]	0.14
			Highest quintile	1.20 [0.90-1.60]	0.28
		Education	Elementary school	1.00	
			Middle school	1.10 [0.80-1.40]	0.65
			High school	1.00 [0.80-1.40]	0.93
			University/higher	1.50 [1.10-2.00]	0.02
		Residential area	Urban	1.00	
			Rural	1.10 [0.80-1.50]	0.63

Table 1.5 *(continued)*

		Occupation	Farming, fishing and forestry	1.00	
			Administrator, management, professional business and financial operations occupations	1.40 [0.80-2.30]	0.20
			Sales and related occupations	1.60 [0.90-2.60]	0.09
			Business and financial operations occupations	1.20 [0.70-1.90]	0.54
			Installation, maintenance and repair occupations or technicians	1.30 [0.80-2.20]	0.35
			Laborer	1.40 [0.80-2.20]	0.22
			Unemployed	1.50 [0.90-2.30]	0.09
		Hypertension	No	1.00	
			Prehypertension	1.00 [0.80-1.20]	0.92
			Hypertension	0.90 [0.70-1.10]	0.23
		Obesity (kg/m^2)	Underweight (<18.5)	1.00	
			Normal (18.5-24.9)	1.20 [0.80-1.70]	0.31
			Obesity (>25.0)	1.00 [0.70-1.40]	0.99
		Hypercholesterole-mia	No	1.00	
			Yes	1.40 [1.10-1.70]	<0.01
		Hypertriglycemia	No	1.00	
			Yes	0.90 [0.70-1.20]	0.50
		Rheumatoid	No	1.00	
			Yes	0.90 [0.50-1.50]	0.66
		Thyroid disease	No	1.00	
			Yes	1.50 [1.10-2.00]	0.01
		Lifetime smoker	No	1.00	
			Yes	0.90 [0.70-1.10]	0.30
		Binge alcohol user	Never drink an alcohol	1.00	
			Not a binge alcohol user	1.20 [1.0-1.40]	0.09
			Yes	1.10 [0.90-1.40]	0.30
		Sleep duration (hours)	6-8	1.00	
			<6	1.30 [1.00-1.60]	0.03
			>8	0.90 [0.60-1.20]	0.48
		Stress	Least stressful	1.00	
			Moderately stressful	1.30 [1.00-1.60]	0.03
			Extremely stressful	1.60 [1.10-2.30]	0.02
		History of eye surgery	No	1.00	
			Yes	2.20 [1.80-2.70]	<0.01
Uchino et al., 2008	Clinical diagnosis	♂ Contact lens use	No	1.00	
			Soft contact lenses	4.20 [2.80-6.20]	0.19
			Hard contact lenses	4.40 [1.30-15.40]	<0.001
		♀ Contact lens use	No	1.00	
			Soft contact lenses	4.90 [2.30-10.30]	<0.001
			Hard contact lenses	2.50 [0.50-12.20]	<0.001
	Symptoms (WHS criteria)	♂ Contact lens use	No	1.00	
			Soft contact lenses	4.60 [3.80-5.70]	<0.001
			Hard contact lenses	2.60 [1.10-5.90]	0.029

Table 1.5 *(continued)*

		♀ Contact lens use	No	1.00	
			Soft contact lenses	5.80 [3.60-9.30]	<0.001
			Hard contact lenses	5.50 [2.20-13.70]	0.003
Um et al., 2014	Clinical diagnosis	Sex	Male	1.00	
			Female	3.02 [2.61-3.50]	n/a
		Age (years)	30-30	1.00	
			40-49	0.91 [0.73-1.13]	n/a
			50-59	1.06 [0.85-1.31]	n/a
			60-69	1.37 [1.11-1.68]	n/a
			≥70	0.90 [0.71-1.14]	n/a
		City size	Rural	1.00	
			Metropolitan cities	1.68 [1.30-2.17]	n/a
			Other cities	1.58 [1.22-2.06]	n/a
	Symptoms (WHS criteria)	Sex	Male	1.00	
			Female	2.21 [1.96-2.48]	n/a
		Age (years)	30-30	1.00	
			40-49	0.97 [0.80-1.16]	n/a
			50-59	1.11 [0.93-1.33]	n/a
			60-69	1.26 [1.06-1.51]	n/a
			≥70	1.06 [0.87-1.29]	n/a
		City size	Rural	1	
			Metropolitan cities	1.39 [1.09-1.77]	n/a
			Other cities	1.27 [1.00-1.62]	n/a
Na et al., 2015	Clinical diagnosis	♀ Psychological stress perception	Low	1.00	
			Moderate	1.70 [1.20-2.40]	n/a
			Severe	2.00 [1.40-2.80]	n/a
			Very severe	2.70 [1.60-4.60]	n/a
		♀ Depressed mood	No	1.00	
			Yes	1.50 [1.10-2.00]	n/a
		♀ Suicidal thoughts	No	1.00	
			Yes	1.20 [0.90-1.50]	n/a
		♀ Psychological counseling	No	1.00	
			Yes	1.80 [1.00-3.10]	n/a
		♀ Depression diagnosis	No	1.00	
			Yes	1.40 [0.90-2.20]	n/a
		♀ Anxiety/Depression	None	1.00	
			Yes	1.50 [1.10-2.00]	n/a
	Symptoms (by agreeing to a statement)	♀ Psychological stress perception	Low	1.00	
			Moderate	1.70 [1.30-2.20]	n/a
			Severe	2.00 [1.40-2.70]	n/a
			Very severe	2.50 [1.60-4.00]	n/a
		♀ Depressed mood	No	1.00	
			Yes	1.30 [1.00-1.70]	n/a
		♀ Suicidal thoughts	No	1.00	
			Yes	1.30 [0.90-1.70]	n/a
		♀ Psychological counseling	No	1.00	
			Yes	2.00 [1.10-3.60]	n/a
		♀ Depression diagnosis	No	1.00	
			Yes	1.10 [0.70-1.60]	n/a
		♀ Anxiety/depression	No	1.00	
			Yes	1.50 [1.10-1.90]	n/a
Vehof et al., 2014	Symptoms (by agreeing to a statement)	♀ Age (years)		1.01 [1.01-1.02]	<0.0005
		♀ Use of contact lenses	No	1.00	
			Yes	1.78 [1.10-2.87]	0.018
		♀ Cataract surgery	No	1.00	
			Yes	1.70 [1.24-2.32]	0.001
		♀ Glaucoma	No	1.00	
			Yes	1.34 [0.89-2.02]	0.16
		♀ Age-related macular degeneration	No	1.00	
			Yes	1.52 [0.99-2.33]	0.054

Table 1.5 *(continued)*

	♀ Osteoporosis	No	1.00	
		Yes	1.20 [0.90-1.60]	0.23
	♀ Asthma	No	1.00	
		Yes	1.40 [1.14-1.71]	0.001
	♀ Eczema	No	1.00	
		Yes	1.70 [1.41-2.05]	<0.0005
	♀ Allergy (any)	No	1.00	
		Yes	1.42 [1.20-1.68]	<0.0005
	♀ Any thyroid	No	1.00	
	problems	Yes	1.38 [1.12-1.71]	0.003
	♀ Hypothyroidism	No	1.00	
		Yes	1.30 [0.93-1.82]	0.12
	♀ Hyperthyroidism	No	1.00	
		Yes	1.41 [0.80-2.49]	0.24
	♀ Rheumatoid	No	1.00	
	arthritis	Yes	1.34 [1.02-1.75]	0.034
	♀ Fertility problems	No	1.00	
		Yes	1.15 [0.88-1.52]	0.31
	♀			
	Hypercholesterole-	No	1.00	
	mia	Yes	1.31 [1.10-1.56]	0.002
	♀ Hypertension	No	1.00	
		Yes	1.12 [0.94-1.35]	0.20
	♀ Diabetes	No	1.00	
		Yes	1.22 [0.85-1.77]	0.28
	♀ Osteoarthritis	No	1.00	
		Yes	1.33 [1.11-1.59]	0.002
	♀ Cancer	No	1.00	
		Yes	1.17 [0.92-1.49]	0.21
	♀ Stroke	No	1.00	
		Yes	2.50 [1.55-4.02]	<0.0005
	♀ Migraine	No	1.00	
		Yes	1.24 [1.04-1.49]	0.018
	♀ Irritable bowel	No	1.00	
	syndrome	Yes	1.92 [1.60-2.30]	<0.0005
	♀ Chronic	No	1.00	
	widespread pain syndrome	Yes	2.61 [1.92-3.56]	<0.0005
	♀ Pelvic pain	No	1.00	
		Yes	1.64 [1.33-2.02]	<0.0005
	♀ Depression	No	1.00	
		Yes	1.67 [1.35-2.07]	<0.0005
Clinical	♀ Age (years)		1.05 [1.03-1.06]	<0.0005
diagnosis	♀ Use of contact	No	1.00	
and daily	lenses	Yes	1.01 [0.43-2.37]	0.99
use of	♀ Cataract surgery	No	1.00	
artificial		Yes	1.69 [1.16-2.47]	0.006
tears	♀ Glaucoma	No	1.00	
		Yes	1.56 [0.95-2.57]	0.077
	♀ Age-related	No	1.00	
	macular degeneration	Yes	1.56 [0.91-2.66]	0.11
	♀ Osteoporosis	No	1.00	
		Yes	1.22 [0.97-1.53]	0.08
	♀ Asthma	No	1.00	
		Yes	1.54 [1.17-2.04]	0.002
	♀ Eczema	No	1.00	
		Yes	1.48 [1.14-1.93]	0.004
	♀ Allergy (any)	No	1.00	
		Yes	1.42 [1.11-1.80]	0.005
	♀ Any thyroid	No	1.00	
	problems	Yes	1.61 [1.22-2.12]	0.001

Table 1.5 *(continued)*

		♀ Hypothyroidism	No	1.00	
			Yes	1.48 [0.95-2.29]	0.08
		♀ Hyperthyroidism	No	1.00	
			Yes	1.68 [0.83-3.40]	0.15
		♀ Rheumatoid arthritis	No	1.00	
			Yes	1.38 [1.02-1.87]	0.039
		♀ Fertility problems	No	1.00	
			Yes	1.45 [1.01-2.09]	0.04
		♀ Hypercholesterole-mia	No	1.00	
			Yes	1.14 [0.90-1.43]	0.28
		♀ Hypertension	No	1.00	
			Yes	0.98 [0.76-1.24]	0.84
		♀ Diabetes	No	1.00	
			Yes	1.53 [0.99-2.37]	0.06
		♀ Osteoarthritis	No	1.00	
			Yes	1.35 [1.08-1.68]	0.0007
		♀ Cancer	No	1.00	
			Yes	1.11 [0.80-1.53]	0.54
		♀ Stroke	No	1.00	
			Yes	1.64 [0.88-3.05]	0.12
		♀ Migraine	No	1.00	
			Yes	1.47 [1.15-1.88]	0.002
		♀ Irritable bowel syndrome	No	1.00	
			Yes	2.24 [1.76-2.85]	<0.0005
		♀ Chronic widespread pain syndrome	No	1.00	
			Yes	2.13 [1.42-3.18]	<0.0005
		♀ Pelvic pain	No	1.00	
			Yes	1.86 [1.41-2.46]	<0.0005
		♀ Depression	No	1.00	
			Yes	1.67 [1.27-2.19]	<0.0005
Paulsen et al., 2014	Symptoms (by self-report)	Age (years)		1.12 [1.00-1.27]	n/a
		Sex	Male	1.00	
			Female	1.45 [1.14-1.85]	n/a
		Contact lens use	Never	1.00	
			Past	1.09 [0.83-1.43]	n/a
			Current	2.09 [1.56-2.79]	n/a
		Arthritis	No	1.00	n/a
			Yes	1.41 [1.09-1.82]	
		Allergies	No	1.00	
			Yes	1.54 [1.18-2.01]	n/a
		Thyroid disease	No	1.00	
			Yes	1.40 [1.00-1.97]	n/a
		Migraine headache	No	1.00	
			Yes	1.44 [1.10-1.90]	n/a
		Antihistamines	No	1.00	
			Yes	1.41 [1.07-1.86]	n/a
		Steroids	No	1.00	n/a
			Yes	1.47 [1.10-1.97]	
Han et al., 2011	Symptoms (by self-report)	Sex	Male	1.00	
			Female	1.64 [1.15-2.33]	0.006
		Region	Rural	1.00	
			Urban	1.94 [1.35-2.80]	<0.001
		Age (years)	65-69	1.00	
			70-74	0.94 [0.61-1.44]	0.76
			75-79	1.32 [0.80-2.16]	0.28
			80-84	1.14 [0.58-2.25]	0.70
			≥85	1.93 [0.83-4.47]	0.12

Table 1.5 *(continued)*

Tan et al., 2015	Symptoms (by self-report)	Gender	Female	1.00	
			Male	0.82 [0.52-1.28]	0.38
		Age (years)	Young (<25)	1.00	
			Mid (25-45)	1.27 [0.72-2.24]	0.41
			Old (>45)	1.35 [0.71-2.56]	0.36
		Contact lens wear	No	1.00	
			Yes	2.96 [1.81-4.83]	<0.0005
		Alcohol use	No	1.00	
			Yes	1.49 [0.55-4.04]	0.43
			Sometimes	0.31 [0.04-2.37]	0.26
		Medication side effect	No	1.00	
			Yes	1.84 [0.99-3.44]	0.05
Jie et al., 2008	Symptoms (by self-report)	Age (years)		1.00	
				1.03 [1.02-1.05]	<0.001
		Gender	Male	1.00	
			Female	1.56 [1.23-1.98]	<0.001
		Region	Rural	1.00	
			Urban	1.89 [1.46-2.45]	<0.001
		Undercorrection of refractive error	No	1.00	
			Yes	1.42 [1.11-1.82]	0.005
		Low degree of nuclear cataract	No	1.00	
			Yes	0.81 [0.69-0.97]	0.02
Guo et al., 2010	Symptoms (by self-report)	Age-related cataract	No	1.00	
			Yes	4.05 [3.03-5.42]	<0.001
		Pterygium	No	1.00	
			Yes	3.35 [2.58-4.35]	<0.001
		Age (years)		1.00	
				3.42 [2.42-4.83]	<0.001
		Gender	Male	1.00	
			Female	1.01 [0.84-1.21]	>0.05
		Low education level (<3 years)	No	1.00	
			Yes	1.00 [0.76-1.33]	>0.05
		Low socioeconomic status	No	1.00	
			Yes	1.10 [0.92-1.33]	>0.05
		Smoking	No	1.00	
			Yes	1.06 [0.81-1.39]	>0.05
		Alcohol consumption	No	1.00	
			Yes	1.01 [0.75-1.36]	>0.05
		High altitude	No	1.00	
			Yes	1.26 [0.82-1.93]	>0.05
Tongg et al., 2009		Gender	Female	1.00	
			Male	1.16 [0.72-1.85]	n/a
		Age (years)	40-49	1.00	
			50-59	1.21 [0.83-1.75]	n/a
			60-69	0.88 [0.54-1.43]	n/a
			70-80	0.98 [0.58-1.67]	n/a
		Cigarette smoking	No	1.00	
			Yes	1.77 [1.17-2.66]	n/a
		Thyroid disease	No	1.00	
			Yes	2.58 [1.29-5.18]	n/a
		Income (S$)	<500	1.00	
			500-1000	1.00 [0.65-1.54]	n/a
			1000-2000	1.49 [0.83-2.61]	n/a
			2000-3000	1.88 [0.93-3.83]	n/a
			>3000	1.74 [1.13-2.68]	n/a
		Highest education attained	No formal education	1.00	
			Less than elementary	0.83 [0.42-1.66]	n/a
			Elementary school	1.14 [0.70-1.84]	n/a
			High school	1.26 [0.71-2.22]	n/a
			College/university	1.20 [0.59-2.44]	n/a

Table 1.5 *(continued)*

		Type of housing	1-2 room public flat	1.00	
			3-4 room public flat	0.95 [0.61-1.48]	n/a
			5 room public flat	1.48 [0.86-2.53]	n/a
			Private housing	1.07 [0.29-3.88]	n/a
		Outdoor work	No	1.00	
			Yes	1.23 [0.74-2.05]	n/a
		Currently driving vehicle	No	1.00	
			Yes	0.99 [0.67-1.46]	n/a
Lu et al., 2008		Pterygium	No	1.00	
			Yes	1.3 [1.00-1.70]	0.031
		Age (years)		3.29 [2.48-4.37]	<0.001
		Sex	No	1.00	
			Yes	1.03 [1.00-1.70]	>0.05
		Low education level (<3 years)	No	1.00	
			Yes	1.61 [1.22-2.12]	0.001
		Low socioeconomic status	No	1.00	
			Yes	2.39 [1.48-3.86]	<0.001
		Smoking	No	1.00	
			Yes	1.27 [0.97-1.60]	0.001
		Alcohol consumption	No	1.00	
			Yes	1.27 [0.97-1.60]	>0.05
		High altitude (≥4000 to 3300-3600)	No	1.00	
			Yes	1.85 [1.45-2.37]	<0.001
Hashe-mi et al., 2014	Symptoms (OSDI ≥ 23)	Pterygium	No	1.00	
			Yes	1.70 [n/a]	0.020
Malet et al., 2014	Symptoms (OSDI ≥ 23)	Education	No education or primary school	1.00	
			Short secondary school	0.75 [0.50-1.12]	0.16
			Long secondary school	0.49 [0.31-0.77]	0.002
			High school or University	0.62 [0.39-1.00]	0.05
		Body mass index (kg/m2)		0.98 [0.94-1.02]	0.41
		Smoking habits	Never	1.00	
			Former	0.82 [0.54-1.24]	0.35
			Current	0.80 [0.36-1.79]	0.59
		Daily time spent on screen (hours/day)	0-2	1.00	
			3-4	1.02 [0.60-1.74]	0.93
			5-10	1.16 [0.66-2.04]	0.60
		Hypothyroidism	No	1.00	
			Yes	1.18 [0.61-2.28]	0.62
		Best-corrected visual acuity of < 20/40	No	1.00	
			Yes	1.58 [0.75-3.33]	0.23
		Cataract extraction	No	1.00	
			Yes	1.22 [0.87-1.72]	0.24
		Late AMD	No	1.00	
			Yes	1.22 [0.61-2.47]	0.57
		Retinopathy	No	1.00	
			Yes	0.68 [0.36-1.30]	0.25
		Glaucoma	No	1.00	
			Yes	0.78 [0.36-1.73]	0.55
		Ocular hypertension	No	1.00	
			Yes	1.58 [1.00-2.50]	0.05

Table 1.5 *(continued)*

	Beta-blockers	No	1.00	
		Yes	0.90 [0.63-1.30]	0.58
	Diuretics	No	1.00	
		Yes	1.27 [0.86-1.87]	0.33
	Anxiolytics	No	1.00	
		Yes	1.53 [1.03-2.28]	0.04
	Antidepressant	No	1.00	
		Yes	1.33 [0.83-2.11]	0.23
	Antihistamines	No	1.00	
		Yes	1.48 [0.71-3.10]	0.25

DED = dry eye disease. OR = odds ratio. n/a = not applicable. ♀ = female. ♂ = male. CI = confidence interval.
*Included population-based cross-sectional studies that have been considered in (Stapleton *et al.*, 2017).

An OR of 1 is indicative of no effect relationship between the condition and the disease (Szumilas, 2010). ORs of <1 and >1 suggest that being exposed to the condition decreases and increases the occurrence of DED, respectively (Szumilas, 2010). In other words, an OR of >1 means that the condition is a risk factor of DED (Szumilas, 2010).

Importantly, similarly to DED prevalence rates, DED risk factors have been confounded by the disease diagnoses used and the population characteristics studied. A deeper description of identified DED associations is included further on (section 1.6.5.3.1 and section 1.6.5.3.2).

1.6.5.2 Reporting dry eye risk factors

The precision and statistical significance of ORs are often reported (Uchino *et al.*, 2008, 2011; Lu *et al.*, 2008; Tongg *et al.*, 2009; Jie *et al.*, 2009; Schaumberg *et al.*, 2009; Guo *et al.*, 2010; Han *et al.*, 2011; Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014; Um *et al.*, 2014; Vehof *et al.*, 2014; Hashemi *et al.*, 2014; Malet *et al.*, 2014; Paulsen *et al.*, 2014; Tan *et al.*, 2015; Na *et al.*, 2015). Both characteristics become important to understand how confident a researcher can be when generalizing the observed DED risk factors to the wider population.

1.6.5.2.1 The precision of dry eye risk factors

Generally, the 95% confidence interval (CI) is used to determine the precision of ORs (Browner and Newman, 1986). A large 95% CI refers to less precise ORs, whereas a small 95% CI refers to more precise ORs (Szumilas, 2010).

The upper and lower 95% CIs are calculated using the following formulas: Upper 95% CI = e ^ [ln(OR) + 1.96 √(1/a + 1/b + 1/c + 1/d)] and Lower 95% CI = e ^ [ln(OR) + 1.96 √(1/a + 1/b + 1/c + 1/d)], where “a” is the number of exposed individuals with DED, “b” the number of exposed individuals without DED, “c” number of unexposed individuals with DED and “d” the number of unexposed individuals without DED (Szumilas, 2010).

Importantly, the 95% confidence interval depends on the sample size and the standard deviation of the study groups (Szumilas, 2010). A large sample size gives narrower 95% CIs and hence more precise ORs. On the other hand, where the dispersity is high, the 95% CIs are wider and the ORs are consequently less certain (Szumilas, 2010).

1.6.5.2.2 The significance of dry eye risk factors

Often, an OR with a 95% CI that does not include the value of zero effect (OR =1) is interpreted as statistically significant (Szumilas, 2010). However, interpretation alone is not enough and hence the p-value is used (Szumilas, 2010).

The p-value is usually expressed as a proportion which can also easily interpreted as a percentage. Conventionally, an OR with a p-value less than 0.05 is considered statistically significant. A level of 0.05 means that only 5% of an association of this

size may arise in the sample by chance, so it is likely to represent a “real” association in the wider population (Szumilas, 2010).

Note that the statistical significance of an OR can never be absolutely certain as the p-value is a measure of probability. There is always the possibility of committing errors, including false positives (to conclude there is a relationship, but in fact there is not) and false negatives (to conclude there is no relationship, when in fact there is) (Szumilas, 2010).

1.6.5.3 Classifying dry eye risk factors

Environmental risk factors for DED can be classified into modifiable and non-modifiable (Stapleton *et al.*, 2017). Modifiable risk factors for DED are those that can be controlled in order to decrease the chance of developing or worsening the disease (Jones *et al.*, 2017). In contrast, non-modifiable cannot be changed, however, determining their presence may help in understanding a positive DED diagnosis (Wolffsohn *et al.*, 2017).

1.6.5.3.1 Non-modifiable dry eye risk factors

1.6.5.3.1.1 Sex

Females are often on major risk of DED symptoms and signs than males, suggesting that sex hormones may play an important role in the etiology of the disease (Sullivan *et al.*, 2017).

Sex hormones are synthesized by the gonads, by the adrenal glands or by conversion of steroid precursors in peripheral intracrine tissues (i.e skin or fat) to be then released into the blood circulation and regulate physiological functions of different structures of the LFU (Truong *et al.*, 2014).

Main classes of sex hormones include androgens, estrogens and progesterones. The three steroids are present in each sex at different levels, considering androgens the “male sex hormones” and both estrogens and progesterones the “female sex hormones” (Truong *et al.*, 2014; Sullivan *et al.*, 2017).

Androgens are believed to enhance the function of the meibomian glands by modulating the transport and synthesis of lipids, to regulate the secretion of the lacrimal gland and to stimulate the proliferation and immune response of corneal and conjunctival cells (Sullivan *et al.*, 2017). On the other hand, estrogens and progesterones are thought to antagonize the actions of androgens; however, further studies are needed to clarify the precise mechanism of these sex hormones (Sullivan *et al.*, 2017).

1.6.5.3.1.2 Ethnicity

The term ethnicity is used to classify any population study by their physical characteristics, such as skin colour, facial shape and hair type. In DED, Asians appear to be more affected by the disease, however, there are discrepancies in the literature (Stapleton *et al.*, 2017).

1.6.5.3.1.3 Age

Although DED can develop at any age, aging is recognized as a significant risk factor of the disease (Stapleton *et al.*, 2017). It encompasses inevitable structural and functional changes of the LFU, such as corneal irregularities accompanied with visual function degradation, atrophy of both lacrimal gland and meibomian glands, lid laxity or conjunctivochalasis (De Paiva, 2017).

The underlying mechanism of aging on the eye is often explained by increasing predisposition of older adults of systemic and topical medication use, hormonal

changes (menopause), inflammatory systemic conditions and oxidative stress (Sharma and Hindman, 2014). Nevertheless, the cause of aging itself remains largely elusive.

1.6.5.3.1.4 Health conditions

Whether health conditions precede from DED or not remains uncertain (Stapleton *et al.*, 2017). Health conditions that have been significantly associated with DED are hypertension (Ahn *et al.*, 2014), thyroid disease (Ahn *et al.*, 2014), hypercholesterolemia (Ahn *et al.*, 2014), stress (Ahn *et al.*, 2014), age-related macular degeneration (Vehof *et al.*, 2014), asthma (Vehof *et al.*, 2014), eczema (Vehof *et al.*, 2014), any type of allergy (Vehof *et al.*, 2014), rheumatoid arthritis (Vehof *et al.*, 2014), stroke (Vehof *et al.*, 2014), chronic wide pain syndrome (Vehof *et al.*, 2014), pelvic pain (Vehof *et al.*, 2014), depression (Vehof *et al.*, 2014), fertility problems (Vehof *et al.*, 2014). osteoarthritis (Vehof *et al.*, 2014), migraine (Vehof *et al.*, 2014), irritable bowel syndrome (Vehof *et al.*, 2014), cataract (Jie *et al.*, 2009; Guo *et al.*, 2010), pterygium (Lu *et al.*, 2008; Guo *et al.*, 2010; Hashemi *et al.*, 2014), anxiolytics (Malet *et al.*, 2014) and general intake of medication (Tan *et al.*, 2015). Further research is needed to clearly understand the nature of the association between these health conditions and DED (Stapleton *et al.*, 2017).

1.6.5.3.1.5 Ocular surgery

Ocular surgery has been recognised as a probable risk factor of DED (Stapleton *et al.*, 2017). The relationship between ocular surgery and the disease is explained by different surgical contributing factors.

The main hypothesised cause of DED due to ocular surgery relates to a neural-based mechanism (Belmonte, Acosta and Gallar, 2004). Most ocular surgeries, such as

cataract and refractive surgery, involve the disruption of corneal nerves by incisions that may potentially interrupt the neural feedback loop between the ocular surface and lacrimal gland (Belmonte, Acosta and Gallar, 2004). Consequently, the aqueous tear film secretion is impaired, inducing eventual ocular surface desiccation and inflammation (Labetoulle *et al.*, 2019).

Surgical changes in the ocular surface and/or palpebral fissure may also disturb the blinking pattern, which, in turn, alter the tear film flow and stability (Chen *et al.*, 2017). In refractive surgery, the correction of higher refractive errors implies deeper ablations depths and hence increases the risk of DED (Tuisku *et al.*, 2007; Nettune and Pflugfelder, 2010).

On the other hand, the use of a light microscope during surgery may be harmful to the ocular surface (Hwang and Kim, 2014). The continuous exposure to the strong light of the microscope on the ocular surface has shown to retard the incision closure (Ipek *et al.*, 2018). Similarly, ocular tissue wounding may be delayed by the use of surgical antiseptics drops (Thomas *et al.*, 2009) and post-surgical medications containing preservatives (Baudouin *et al.*, 2010).

1.6.5.3.1.6 Environmental conditions

The eye is directly exposed to the outside and therefore is endangered by a multitude of factors occurring in an individual's surrounding (Stapleton *et al.*, 2017).

At present, controlled adverse environment chambers have served to study closely the effect of the environment on DED (Calonge *et al.*, 2018). For instance, variations in temperature, airflow velocity and relative humidity, and passive cigarette smoking have demonstrated to alter the tear film homeostasis (by increasing tear film evaporation) and exacerbate DED symptoms (González-García *et al.*, 2007; Ward *et*

al., 2010; Tesón *et al.*, 2013; López-Miguel *et al.*, 2014; Martín-Montañez *et al.*, 2016).

Other studies have significantly associated high altitude (Lu *et al.*, 2008; Guo *et al.*, 2010), and higher ozone levels and lower humidity levels with DED symptoms (Hwang *et al.*, 2016). In addition, the chronic exposure to traffic derived air pollution can contribute to DED characterized by symptoms and signs of tear film instability (Novaes *et al.*, 2010).

1.6.5.3.2 Modifiable dry eye risk factors

1.6.5.3.2.1 Visual display terminals

DED symptoms may impact adversely an individual's ability to perform tasks requiring sustained visual concentration (Miljanović *et al.*, 2007) and may contribute to a lower quality of life (Tong *et al.*, 2010). Long-term use of visual display terminals (VDT), especially for more than four hours daily, has been associated with DED (Kojima *et al.*, 2011; Uchino and Schaumberg, 2013).

Vision problems related to VDT use have been designated as "computer vision syndrome (Gowrisankaran and Sheedy, 2015). The term computer vision syndrome includes symptoms of eyestrain, ocular fatigue, burning sensation, irritation, redness, blurred vision and dryness (Gowrisankaran and Sheedy, 2015).

DED symptoms due to VDT use has been suggested to occur due to both reduced blink rate and incomplete blinking (Wolkoff *et al.*, 2005; Portello, Rosenfield and Chu, 2013; Chu, Rosenfield and Portello, 2014; Argilés *et al.*, 2015). Changes in blinking pattern can further contribute to tear evaporation that leads to tear film instability and

mild epithelial damage (Wolkoff *et al.*, 2005; Portello, Rosenfield and Chu, 2013; Chu, Rosenfield and Portello, 2014; Argilés *et al.*, 2015).

Interestingly, the blink rate has been shown to decrease as font size and contrast are reduced (Gowrisankaran, Sheedy and Hayes, 2007) or cognitive demand of the task increased (Himebaugh, 2009; Jansen *et al.*, 2010).

1.6.5.3.2.2 Contact lens wear

Contact lenses are optical devices made from biocompatible polymers that are designed to be applied onto the eye to correct vision.

DED signs and symptoms that are exclusive to contact lens wear have extensively been studied in the literature as contact lens discomfort (CLD) (Nichols *et al.*, 2013). CLD is described as having either intermittent or persistent adverse ocular sensations during lens wear that are often interpreted as ocular dryness (Nichols *et al.*, 2013). Importantly, CLD has not clearly been distinguished from DED prior to contact lens wear and hence further research is needed in this area (Nichols *et al.*, 2013).

During lens wear, the tear film needs to lubricate and hydrate the contact lens for preserving the health of the ocular surface (Efron *et al.*, 2013). However, the physical presence of the contact lens in situ disrupts normal tear film function and stability dividing the tears into two compartments, the post-lens and pre-lens tear film (Holly 1981).

Both integrity and replenishment of the post-lens and pre-lens tear film are critical for cushioning the effect of blinking (Efron *et al.*, 2013). In the event of DED, contact lens wearers have reported low tear break-up times and ocular surface staining, characterized by the continuous friction between the eye and the contact lens (Efron

et al., 2013). Low tear film volume has also been associated with DED during contact lens wear (Efron *et al.*, 2013).

1.6.5.3.2.3 Poor sleep quality

The relationship between poor sleep quality and DED (Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014) has been scarcely studied. Poor sleep quality has been described either by short sleep duration (Ahn *et al.*, 2014) or having inadequate sleep (Zhang, Chen and Wu, 2012).

Sleep deprivation is thought to increase sympathetic and decrease parasympathetic tone (Tobaldini *et al.*, 2017). Whereas the lacrimal gland is innervated by both sympathetic and parasympathetic nervous systems, the latter is most extensive (Belmonte *et al.*, 2017) and hence any kind of sleep disturbance may considerably lessen tear secretion.

Accordingly, a small case-control study among healthy male sleepers has shown that staying awake for twenty-four hours reduces tear film volume and stability, as well as increases tear film osmolarity, leading to ocular discomfort (Lee et al., 2014).

Insomnia has also been related to DED by symptoms (Galor *et al.*, 2018) and both symptoms and signs (Ayaki *et al.*, 2016). The association is explained by the coexistence of mood disorders (Galor *et al.*, 2018) and ocular pain (Ayaki *et al.*, 2016) that possibly induces distress and exacerbates difficulties in falling asleep.

1.6.5.3.2.4 Nutrition

The association between certain conditions, such as vitamin A deficiency, anorexia, bulimia, malabsorption syndromes (usually associated to alcoholism) (Stapleton *et al.*, 2017), and DED allows nutrition to be identified as an important factor for the

homeostasis of the tear film. Hence, the involvement of nutritional and/or dietary components on tear composition and physiology has been studied.

Supplementary vitamins are believed to protect the ocular surface from oxidative stress (Seen and Tong, 2018). For example, vitamin C has been reported to play an important role in corneal wound healing after refractive surgery (Kasctsuwan *et al.*, 1999). Multivitamin-trace supplementations, including vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B9, vitamin E, vitamin C, calcium, iron, magnesium and/or zinc, have also shown to increase both tear film stability and volume of DED participants (Patel, Plaskow and Ferrier, 1993; Drouault-Holowacz *et al.*, 2009).

Moreover, DED participants have been benefited by a balanced intake of omega-3 and omega-6 (Roncone, Bartlett and Eperjesi, 2010; Rosenberg and Asbell, 2010; Oleñik, 2014; Bhargava *et al.*, 2015; Gatell-Tortajada, 2016). Both essential fatty acids are recommended as they display anti-inflammatory properties systematically and have shown to retard tear film evaporation and to enhance tear film secretion (Roncone, Bartlett and Eperjesi, 2010; Rosenberg and Asbell, 2010; Oleñik, 2014; Bhargava *et al.*, 2015; Gatell-Tortajada, 2016).

Importantly, more research is needed to understand which dose, composition and length of nutritional supplementation, either of vitamins (with or without trace elements) or essential fatty acids, are required to effectively treat DED (Stapleton *et al.*, 2017).

1.7 Thesis rationale

The prevalence and risk factors of DED are difficult to establish. Indeed, both have differed depending on the characteristics of the population studied and the definition

used for the diagnosis of the disease. Moreover, the knowledge about DED subtypes is limited. The goal of the present thesis is to perform research on DED; more specifically, to examine in isolation the impact of different diagnostic criteria on the disease prevalence (Chapter 3) and to evaluate the associated risk factors (Chapter 4), to understand if DED outcomes and risk factors may be more related to aqueous-deficient or evaporative components of the disease (Chapter 5), as well as to explore a cheap and feasible diagnostic method that may be useful for future population-based studies about DED (Chapter 6). To do so, data were collected from a single population in the UK and great care was taken in following a well-standardized study methodology (Chapter 2).

2. CHAPTER 2: STUDY METHODOLOGY

2.1 Overview

The chapter discusses the study methodology of the present thesis. It explains the rationale behind the chosen tests and the strengths and limitations of each method used.

2.2 Study design

A cross-sectional study was conducted at Aston University Eye Clinic. This research is observational in nature as it attempts to simultaneously explore the prevalence and risk factors of DED. The study was approved by the ethical committee of Aston University and conformed to the tenets of the Declaration of Helsinki.

2.2.1 Power calculation

Two hundred sixty-five participants were estimated to be an appropriate sample size for the present study. The sample size was calculated by considering a CI of 95% and using the following formula: $n = (((1.96^2)P(1-P))/d^2)$ (Arya, Antonisamy and Kumar, 2012), where *"n"* is the sample size, *"P"* the expected disease prevalence and *"d"* the allowable error. As *"P"*, the prevalence rate for DED of 22.1% from a British female cohort (Prevalence: 20.8% [95% CI, 19.5-22.1]) (Vehof *et al.*, 2014) was used. The rationale to set *"P"* at 22.1% is based on the fact that any value nearer to 50% leads to the largest *"n"* (Figure 2.1) and hence to more confident results (Arya, Antonisamy and Kumar, 2012). Moreover, because an allowable error of 0.05 has been generally recommended when *"P"* takes values between 10% and 90% (Arya, Antonisamy and Kumar, 2012), a *"d"* of 5% was applied.

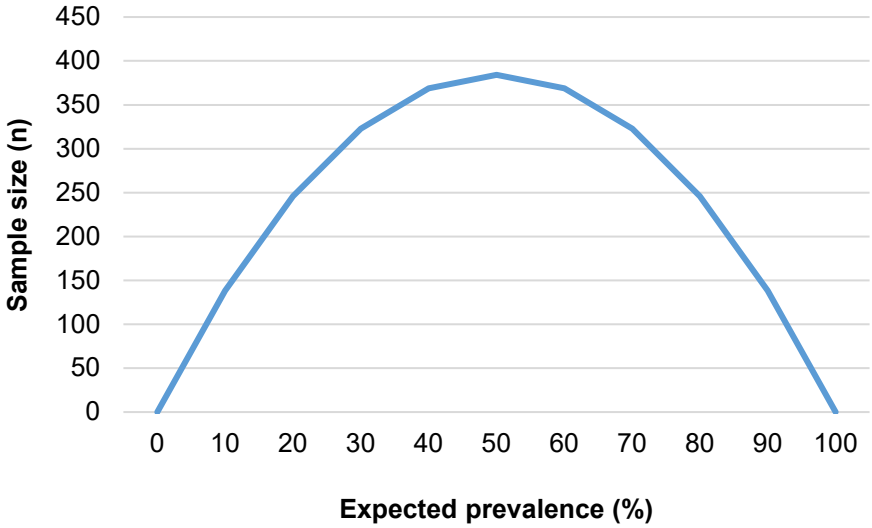


Figure 2.1 Relationship between sample size and expected prevalence

The sample size was given as a function of expected prevalence for z = 1.96 and d = 0.05.

2.2.2 Study population

The population about conclusions were drawn were female and male adult residents in Birmingham (UK), regardless of nationality. Estimates about Birmingham resident population for 2016 were obtained from the Birmingham City Council (www.birmingham.gov.uk/census) and considered together with the above power calculation to determine the required study participants (Table 2.1).

Table 2.1 Required study population (stratified by age and sex)

Age	(years)	18-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Sex	Males (n)	6	31	25	22	19	14	9	5
	Females (n)	6	31	25	22	20	14	10	7

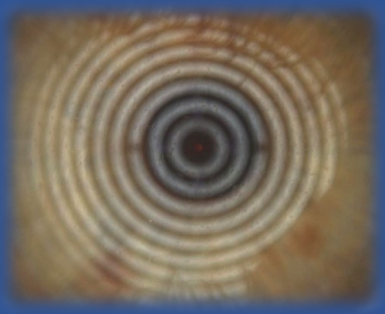
2.2.3 Recruitment

Great efforts were made to recruit as many eligible participants as possible. Recruitment mainly occurred through Aston University staff email advertising, via advertisement within the Aston Eye Clinic and Aston Research Centre for Healthy Ageing (ARCHA), and through posters pinned around the campus (Figure 2.2). Further recruitment methods included leaflet advertising in the Birmingham City Centre and posting an advert in the weekly news bulletin of the Birmingham City Council.

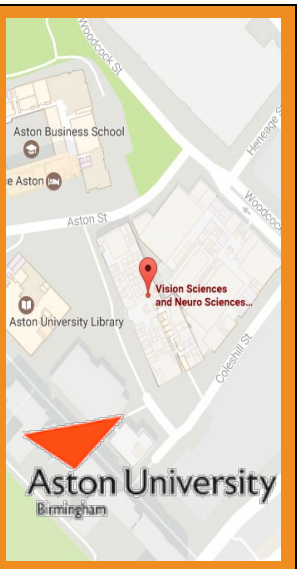
How good is the 'Tear Film' of your eyes?

We are currently looking for people aged ≥ 18 years-old to participate in our 'Tear Film Evaluation' study.

At our central Birmingham Eye Clinic, we will assess how healthy your tears are.
No discomfort to your eyes will be caused during the eye exam.



Phone: **0121 204 4400**
Email: **dryeye@aston.ac.uk**
Times and dates can be arranged at your convenience
Free parking provided on request

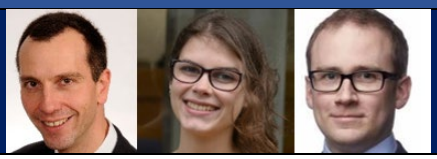


Aston University
Health Clinics

Vision Science
Building

Birmingham
B4 7EQ

Researchers
M Vidal-Rohr (middle)
Prof. J S Wolffsohn (left)
Prof. L N Davies (right)



Received funding by EU Horizon 2020 research & innovation programme under Marie Skłodowska-Curie grant agreement No 642760.

Figure 2.2 Study advertisement

2.2.4 Inclusion and exclusion criteria

To participate in the study, participants were required to be ≥18 years-old. They needed to be Birmingham residents who did not leave the country one month before the study to ensure that environmental differences between participants were minimised. The highest age limit was set at 90 years as the tests required considerable cooperation. Eligible participants were invited via email and advised to not wear contact lenses or use artificial tears twenty-four hours prior to the study.

2.2.5 Clinical assessment

In a single clinical session lasting approximately one hour, participants were first asked to complete a dry eye risk factor survey and two dry eye questionnaires, followed by a full ocular surface and tear film examination on their preferred eye.

Figure 2.3 Aston University Health Clinics

All participants were examined in an adjacent room of the Aston University Health Clinics (Figure 2.3) from September 2016 to March 2018. Throughout the study, the room temperature and humidity were kept by 22.4 ± 2.0°C and 49.3 ± 8.2%, respectively. Eye examinations only occurred after a minimum of 15 minutes of adaptation to the room conditions.

2.2.5.1 Diagnostic considerations

Minimal manipulation to the ocular surface may alter tear film physiology by inducing reflex tearing or adding foreign bodies into the tears. Invasive methods, such as the use of ophthalmic dyes to improve tear film visibility, are criticised due to disrupting the normal tear film state (Mooi *et al.*, 2017) and hence providing less reliable results (Nichols, Mitchell and Zadnik, 2004). In addition, automated methods are strongly recommended since they are less dependent on examiners´ clinical expertise (Nichols *et al.*, 2002).

The main instrument used for the present study was the Keratograph 5M (K5M; Oculus Optikgeräte GmbH, Wetzlar, Germany) (Figure 2.4), a topographer using a video analysis software to overcome inaccuracies made by invasive and subjective testing of important dry eye parameters. However, where non-invasive and objective methods were not possible, traditional methods were carefully adopted following substantial training. Finally, to minimise the impact of subsequent testing on tear film physiology, the involved diagnostic methods were performed in increasing order of invasiveness (Wolffsohn *et al.*, 2017), as listed below.

Figure 2.4 The Keratograph 5M (K5M)

2.2.5.2 Dry eye risk factor survey

Information about the exposure of DED risk factors was obtained through a self-administrated Dry Eye Risk Factor Survey (DERFS) (

Figure 2.5). The survey was developed aiming to evaluate all DED risk factors assessed in current cross-sectional studies (Chapter 1). It included questions about participants' demographics and life factors, including:

- Ethnicity. The term ethnicity has been used to classify populations into subgroups based on physical characteristics. Because Asians, including Korean, Chinese and Japanese populations, have been significantly associated with DED (Stapleton *et al.*, 2017), an ethnicity classification including this ethnic group was considered.
- Age. DED is known to increase with age (Stapleton *et al.*, 2017). Participants' age decade was recorded, as this is less intrusive than assessing their precise age. The approach has largely been used in DED epidemiological research (Schaumberg *et al.*, 2009; Tongg *et al.*, 2009; Uchino *et al.*, 2011; Ahn *et al.*, 2014; Um *et al.*, 2014).
- Sex. Sex hormones may play an important role in DED (Truong *et al.*, 2014; Sullivan *et al.*, 2017) and hence participants' sex was recorded.
- Living zone and outdoor activity. DED has been significantly related with urban areas (Jie *et al.*, 2009; Han *et al.*, 2011; Ahn *et al.*, 2014) and outdoor ozone air pollution (Hwang *et al.*, 2016). Therefore, both factors were assessed. The living zone was classified into rural and urban areas (Jie *et al.*, 2009; Han *et al.*, 2011; Ahn *et al.*, 2014). Because data collection on air pollution as in (Hwang *et al.*, 2016) was not possible, the exposure to outdoor air pollution was graded by participants' average hours spending outside on a regular leisure day.

- Education. DED has been significantly associated with higher educational level (Ahn *et al.*, 2014; Malet *et al.*, 2014), as well as office-based work (Ahn *et al.*, 2014). Education was categorized as elementary/primary school, middle/secondary school, high school/6th form, and university/higher (Ahn *et al.*, 2014). Work was described by participants' daily working hours. Importantly, classifying work as non-office-based and office-based might have been intercorrelated with computer use (Ahn *et al.*, 2014) and hence was not considered.
- Smoking. Smoking has been significantly associated with DED (Lu *et al.*, 2008; Ward *et al.*, 2010). Although smoking habits have been described as generally smoking (Lu *et al.*, 2008), the number of cigarettes smoked per day was asked to provide a more detailed analysis.
- Drinking. The relationship between alcohol consumption and DED is controversial (You, Qu and Yu, 2016). The risk factor was assessed by asking participants' weekly alcohol unit intake.
- Contact lens wear. Both rigid and soft contact lenses interrupt normal tear film function and physiology (Efron *et al.*, 2013; Nichols *et al.*, 2013), contributing most likely to DED (Uchino *et al.*, 2008, 2011; Zhang, Chen and Wu, 2012; Paulsen *et al.*, 2014; Vehof *et al.*, 2014; Tan *et al.*, 2015). The risk factor was assessed by recording participants' contact lens type (Uchino *et al.*, 2008) and wear frequency in days per week. The contact lens wear frequency was asked to provide a more detailed analysis.
- Computer use. Changes in blinking patterns due to computer use can lead to DED (Wolkoff *et al.*, 2005; Portello, Rosenfield and Chu, 2013; Chu, Rosenfield and Portello, 2014; Argilés *et al.*, 2015). As in (Uchino *et al.*, 2011; Malet *et al.*, 2014), computer use was determined by average hours per day.

- Current/past health conditions/problems, medication use and stress. DED has been significantly related to several systemic and mental health conditions/problems, as well as to the use of medication and stress (Lu *et al.*, 2008; Jie *et al.*, 2009; Guo *et al.*, 2010; Ahn *et al.*, 2014; Hashemi *et al.*, 2014; Malet *et al.*, 2014; Vehof *et al.*, 2014; Tan *et al.*, 2015). Hence these factors were asked. Moreover, participants' stress status was classified into least, moderate and extreme (Ahn *et al.*, 2014).
- Nutritional supplements intake. Recording of nutritional supplementation was considered since these are suggested as DED treatments (Jones *et al.*, 2017) and may undercover a possible DED diagnosis and/or risk factor.
- Sleep quality. Sleep deprivation has been significantly associated with DED (Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014). Participants' regular sleeping hours were recorded to assess their sleep quality (Ahn *et al.*, 2014).

THE DRY EYE RISK FACTOR SURVEY

To which group do you belong? *Underline if applicable*

Ethnicity	→	White/ Asian/ Black/ Others
Sex	→	Female/Male
Age	→	10s/ 20s/ 30s/ 40s/ 50s/ 60s/ 70s/ 80s
Residential area	→	Rural/Urban
Education	→	Elementary or primary school/ Middle or secondary school/ High school or 6th form/ University or higher

Do you … *Underline and/or complete if applicable*

Work?	No ↓	Yes	→	Hours/day		
Smoke?	No ↓	Yes	→	Cigarettes/day		
Drink alcohol?	No ↓	Yes	→	Units/week		*1 unit = 1 glass of beer, wine or spirit*
Wear contact lenses?	No ↓	Yes	→	Type	Days/week	
Use computer?	No ↓	Yes	→	Hours/day		
Have/ have had any health condition/problem?	No ↓	Yes	→	Describe		
Have had any eye surgery?	No ↓	Yes	→	Describe		
Take any medication regularly?	No ↓	Yes	→	Describe		
Take any nutritional supplement regularly?	No	Yes	→	Describe		

How … *Underline and complete if applicable*

Much sleep do you get?	→	Hours/night
Much do you spend outdoors on a regular leisure day?	→	Hours/leisure day
Stressful are your days?	→	Least stressful/ Moderately stressful/ Extremely stressful

Figure 2.5 The dry eye risk factor survey (DERFS)

2.2.5.3 Dry eye questionnaires

DED symptoms can be gathered either through non-scripted verbal interviews or self-administered questionnaires (Wolffsohn *et al.*, 2017). However, self-administered questionnaires are preferred given their enhanced standardization in recording the disease symptomatology (Wolffsohn *et al.*, 2017).

Currently validated symptom questionnaires with discriminative ability in DED include the Impact of Dry Eye on Everyday Life (IDEEL) (Guillemin *et al.*, 2012), the McMonnie's Questionnaire (MQ) (Gothwal *et al.*, 2010), the 5-item Dry Eye Questionnaire (DEQ-5) (Chalmers, Begley and Caffery, 2010) and the Ocular Surface Disease Index (OSDI) (Schiffman *et al.*, 2000).

The two questionnaires chosen for the present study were the DEQ-5 and OSDI. The questionnaires have been found to be concurrent with each other (Galor *et al.*, 2015) and are currently recommended to be used for the diagnosis of DED (Wolffsohn *et al.*, 2017). Whereas the DEQ-5 is found attractive due to its short length, the OSDI is recommended because of its strong establishment in the field of DED clinical trials (Wolffsohn *et al.*, 2017).

Symptomatic DED was determined using a DEQ-5 cut-off score of ≥6 (Chalmers, Begley and Caffery, 2010) and ODSI score of ≥13 (Schiffman *et al.*, 2000). The questionnaires were presented together on a single document page (Figure 2.6). Participants were also asked if they have had eye irritation, either rarely, sometimes, frequently or constantly, for the past month and a previous diagnosis of DED by a physician. The questions were added to further diagnose DED by the WHS criteria, which accounts as the most widely used previous diagnostic criteria in the epidemiology of DED (Stapleton *et al.*, 2017).

DRY EYE QUESTIONNAIRES

Instructions: Circle the number in the box that best represents each answer.

Have you experienced any of the following *during the last week*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Have problems with your eyes limited you in performing any of the following *during the last week*:

6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or a bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Have your eyes felt uncomfortable in any of the following situations *during the last week*:

10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air-conditioned?	4	3	2	1	0	N/A

1 Questions about EYE DISCOMFORT:					
a. During a typical day in the past month, **how often** did your eyes feel discomfort?	0 Never	1 Rarely	2 Sometimes	3 Frequently	4 Constantly
b. When your eyes feel discomfort, **how intense** was this feeling of discomfort at the end of the day, within two hours of going to bed?	Never have it 0	Not at all intense 1	2	3	4 Very intense 5
2 Questions about EYE DRYNESS:					
a. During a typical day in the past month, **how often** did your eyes feel dry?	0 Never	1 Rarely	2 Sometimes	3 Frequently	4 Constantly
b. When your eyes feel discomfort, **how intense** was this feeling of dryness at the end of the day, within two hours of going to bed?	Never have it 0	Not at all intense 1	2	3	4 Very intense 5
3 Questions about WATERY EYES:					
a. During a typical day in the past month, **how often** did your eyes feel watery?	0 Never	1 Rarely	2 Sometimes	3 Frequently	4 Constantly
4 Questions about IRRITATED EYES:					
a. During a typical day in the past month, **how often** did your eyes feel irritated?	0 Never	1 Rarely	2 Sometimes	3 Frequently	4 Constantly

Have you had a previous clinical diagnosis of dry eye? Yes ☐ No ☐

Figure 2.6 DED questionnaires used

DED = dry eye disease.

2.2.5.4 Tear film evaporation

Increased tear film evaporation is thought to result from tear film instability caused by an impaired lipid layer (Craig and Tomlinson, 1997). In the present study, tear film evaporation was evaluated, although its discriminative ability in DED has not been determined yet (Wolffsohn *et al.*, 2017).

Non-DED and DED individuals have shown tear film evaporation rates of 48.85 ± 23.47 g/m^2/h and 75.78 ± 50.26 g/m^2/h, respectively (Tomlinson, Doane and McFadyen, 2009). The tear film evaporation rates have been assessed using evaporimeters with open and closed chambers (Tomlinson, Doane and McFadyen, 2009). The evaporimeters contain sensors that detect changes occurring at the ocular surface, either of vapour pressure or relative humidity, from which the tear film evaporation is inferred (Tomlinson, Doane and McFadyen, 2009).

Measuring tear film evaporation is challenging. The tear film evaporation rate has shown to fluctuate highly with day time (Wojtowicz and McCulley, 2009), room humidity (Abusharha and Pearce, 2013) and temperature (Abusharha, Pearce and Fagehi, 2016). Moreover, tear film evaporation readings may be confounded by additional evaporation coming from the eyes' surrounding skin (Wolffsohn *et al.*, 2017).

The Delfin VapoMeter (Delfin Technologies Ltd, Kuopoi, Finland) (Figure 2.7) was used to assess the tear film evaporation rate. The core of the VapoMeter has a hygrometer sensor that monitors the increase of relative humidity, from which the tear film evaporation rate is deducted in units of g/m^2/h. The humidity is measured within a swimming google piece that is enclosed by the eye during measurement. Participants were asked to remain their eyes open during measurement, as specified

by the manufacturer. Three consecutive tear film evaporation readings were taken, and the mean was recorded.

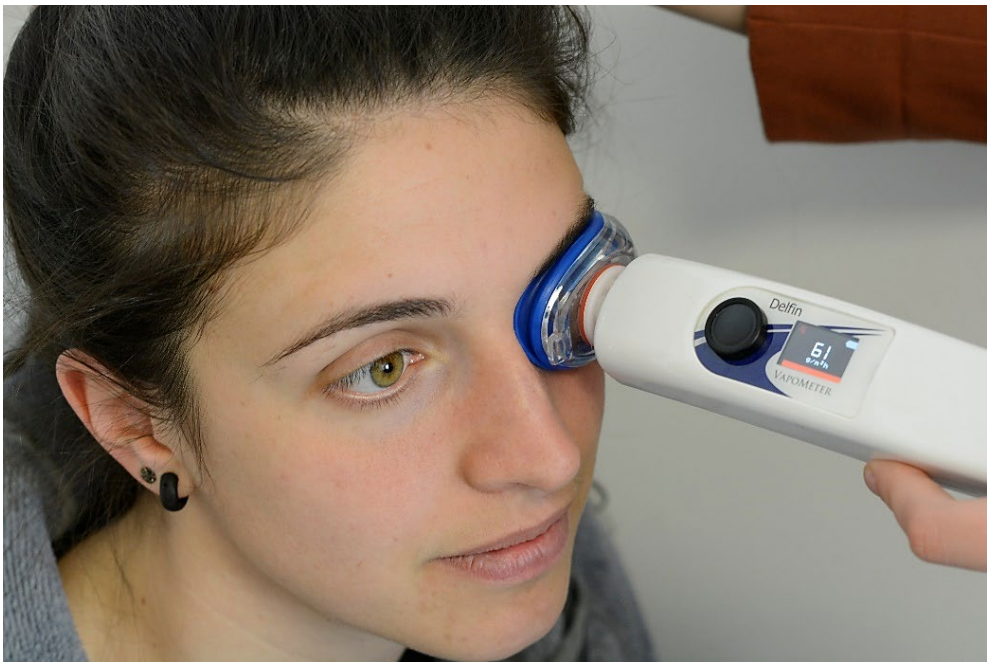


Figure 2.7 Tear evaporation measured with the Delfin VapoMeter

The standard adapter (swimming google piece with an opening diameter of 11mm) of the Delfin VapoMeter was applied on the participants' eye. The tear evaporation was deducted from the humidity increase measured by the hygrometer sensor of the adapter. Three consecutive tear film evaporation readings were taken, and the mean was recorded.

2.2.5.5 Tear film osmolarity

Higher than normal physiological solute concentration in the tear film is known as tear hyperosmolarity. Tear hyperosmolarity results either from excessive evaporation in the presence and/or absence of normal tear flow and constitutes the major trigger of other events leading to ocular surface damage and inflammation (Lemp *et al.*, 2007).

Among other clinical tests, tear film osmolarity has been demonstrated to be the best single monitoring marker for DED (Tomlinson *et al.*, 2006), as it has the strongest

correlation to the disease severity (Lemp *et al.*, 2011) and the lowest variability over time scales that are clinically relevant (Sullivan *et al.*, 2012).

Unlike non-DED individuals, DED individuals show unstable osmolarity values with greater intra- and interocular variability with increasing disease severity (Tomlinson *et al.*, 2006; Keech, Senchyna and Jones, 2013). Several cut-off scores have been suggested to distinguish both groups (Bron *et al.*, 2014). To date, a threshold of ≥308 mOsm/l of either eye or a difference of >8mOsm/l between eyes is globally recommended for the diagnosis of DED (Bron *et al.*, 2014).

Past measurements of tear film osmolarity are based on determining one of two colligative properties of the tear film: the freezing point or vapour pressure (Tomlinson, McCann and Pearce, 2010; Gokhale, Stahl and Jalbert, 2013). Both freezing point and vapour pressure techniques have been criticised for requiring considerable expertise as well as for being invasive (causing reflex tearing during tear collection) and time-consuming (allowing tear evaporation during tear analysis) (Tomlinson, McCann and Pearce, 2010; Gokhale, Stahl and Jalbert, 2013).

In the present study, the tear film osmolarity was measured temporally from the inferior tear meniscus with an electic impedance-based osmometer: the TearLab Osmolarity System (TearLab Corporation, California, USA) (Figure 2.8). During measurement, the participants were asked to look up and away from the instrument. Readings were collected from each eye, and both osmolarity values and the interocular difference were recorded.

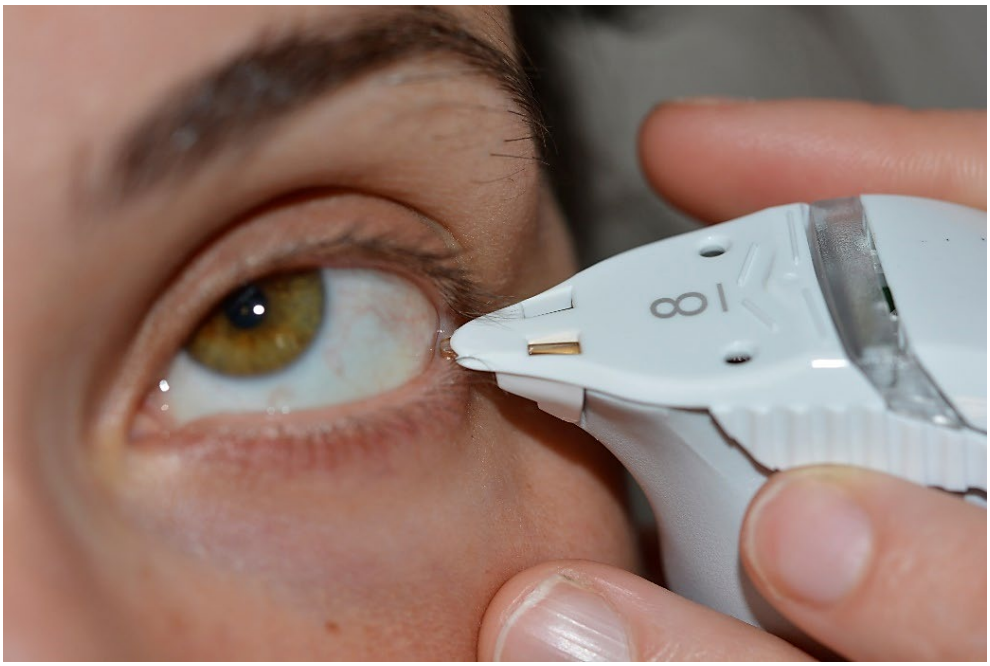


Figure 2.8 Tear osmolarity measured with the TearLab Osmolarity System

The osmolarity pen was gently lowered until the bottom of the tip touched the lower tear meniscus height of the temporal eyelid canthus. Osmolarity readings were obtained within 40 seconds of successful tear collection.

The TearLab Osmolarity System is considered minimally invasive, as it samples low tear film volume (less than 20nl) without direct contact with the ocular surface (Tomlinson, McCann and Pearce, 2010). However, it may be limited due to the fact that it measures the osmolarity of the tear film within the lower tear meniscus, which is hypothesised to be slightly more dilute than other parts of the tear film (Bron *et al.*, 2002).

2.2.5.6 Tear film volume

Evaluating tear film volume is essential for detecting aqueous-deficient components of DED (Wolffsohn *et al.*, 2017). In clinical settings, following diagnostic methods have been used to assess tear film volume:

- The Schirmer test. The Schirmer test involves the insertion of a filter paper strip, after using (Schirmer test II) or not using (Schirmer test I) ocular anesthesia, over the one-third temporal lower eyelid margin (Schirmer, 1903). The length of the wet area (in millimeters) is read off after 5 minutes of application and gives an indication of the aqueous tear film volume (Schirmer. 1903). A Schirmer score of ≤5 mm/5 min has been proposed to be abnormal, meaning the presence of aqueous tear deficiency (Bron *et al.*, 2007). Nevertheless, the clinical application of the Schirmer test is disputed, as it is highly variable, unreliable and poorly correlated to other DED signs and symptoms (Senchyna and Wax, 2008).

- The phenol red thread test. The phenol red thread (PRT) test consists of a phenol-red-impregnated cotton thread that is applied onto the eye, in a similar manner to the Schirmer test, but without needing topical anesthesia (Patel *et al.*, 1998). It has been developed to overcome the disadvantages of the Schirmer test, however, with no success (Senchyna and Wax, 2008). The PRT test has found to be poorly reliable as the Schirmer test (Moore *et al.*, 2009), falling in disuse for more than ten years ago (Wolffsohn *et al.*, 2017).

- The tear meniscus height. The tear meniscus is the collection of tears at the intersection of the bulbar conjunctiva and the eyelid margins. Its height represents 75-90% of the tear film volume (Holly, 1985) and is evaluated central inferior (in millimeters) with en-face slit lamp observation either using a reflective graticule (TMH) or sodium fluorescein (FTMH).

Among all tests, the TMH accounts the minimally invasive method to assess tear film volume (Wolffsohn *et al.*, 2017). A TMH of ≤0.2 mm is currently interpreted as a lack

of tear film homeostasis and has demonstrated good repeatability with low individual variability (Uchida *et al.*, 2007).

In the present study, TMH was assessed with the “Tear Meniscus Height” setting of the K5M and at magnification of 1.4x (Figure 2.9). Digital infrared-images were taken at the centre of the lower eyelid, with no eyelid manipulation. The TMH was centrally measured once, using the vertical alignment of the K5M reflective graticule as a reference.

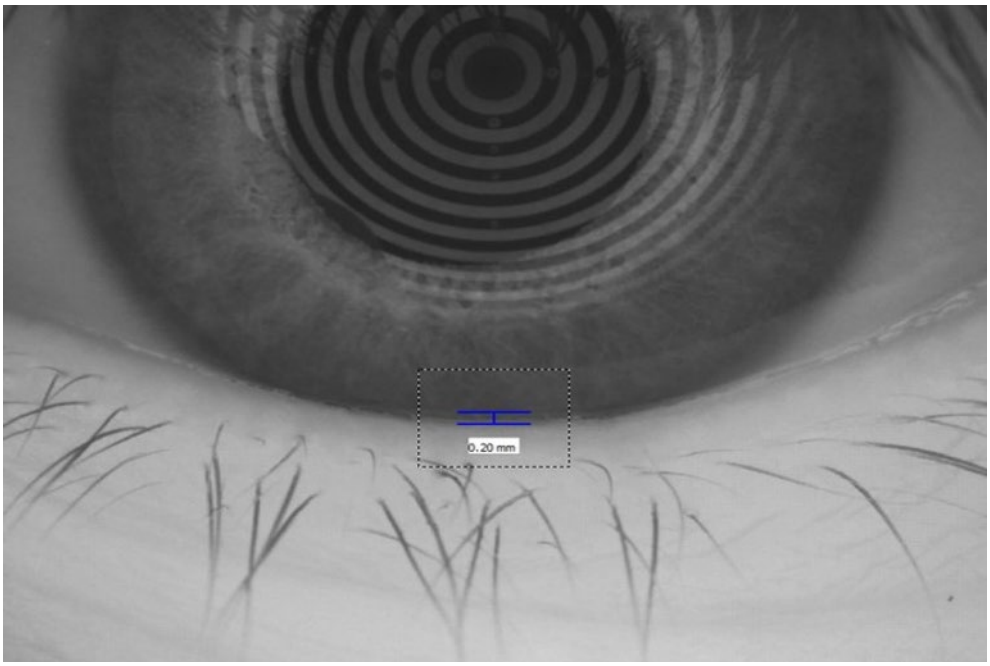


Figure 2.9 Lower TMH measured with the Keratograph 5M

TMH = tear meniscus height.

The central THM was analysed by the Tear Meniscus Height TF-Scan software of the Keratograph 5M. It was measured once, using the vertical alignment of the of the Keratograph 5M reflective graticule as a reference.

2.2.5.7 Lipid layer thickness

White light interferometry is a valuable technique for measuring the lipid layer thickness (LLT) of the tear film (Wolffsohn *et al.*, 2017). The LLT is inferred from

interferometric colour fringes observed by specular reflection at the tear lipid-aqueous interface (Guillon, 1982; Korb *et al.*, 1994).

Thickening of the lipid layer has shown to cause an interferometric colour change from grey to red, which is typically observed when the interpalpebral aperture is narrowed (McDonald, 1969) and may occur by forced blinking (Korb *et al.*, 1994).

Recently, an automated interferometer has been introduced (Goto *et al.*, 2003; Blackie *et al.*, 2009). Because we did not have access to the automated interferometer, the Guillon-Keeler grading scale (Craig and Tomlinson, 1997) was adopted for evaluating interferometric lipid videos of the K5M.

Table 2.2 The Guillon-Keeler grading scale

Grade	Description	LLT
1. Open meshwork	Indistinct, gray, marble-like pattern, frequently visible only by the post-blin movement.	≈ 15nm
2. Closed meshwork	Well defined, gray, marble-like pattern with a tight meshwork.	≈ 30nm
3. Wave pattern	Constantly changing, wave-like pattern.	≈ 30-80nm
4. Amorphous	Blue-whitish appearance with no discernible features.	≈ 80nm
5. Colour fringes	Appearance of coloured interference fringes.	≈ 80-300nm

LLT = lipid layer thickness.

The Guillon-Keeler grading scale is a validated grading scale with moderate inter- and intra-examiner agreement (Guillon, 1998; Nichols *et al.*, 2002). It classifies the lipid layer into five grades (by texture and colour of the observed lipid layer) (Table 2.2), where grade 1 (open meshwork) represents the lowest LLT and grade 5 (colour fringes) the highest (Craig and Tomlinson, 1997).

Videos of the lipid layer were recorded using the “Lipid Layer” software of the K5M at a modified magnification of 1.4x and for the duration of three non-forceful blinks (Figure 2.10). To ensure that the blinks were not forced, the participant was previously instructed to gaze forward comfortably while the instrument was set up for

the next measure. In case of observing a lipid layer with overlapping patterns, the most predominant pattern was considered for the analysis of LLT.

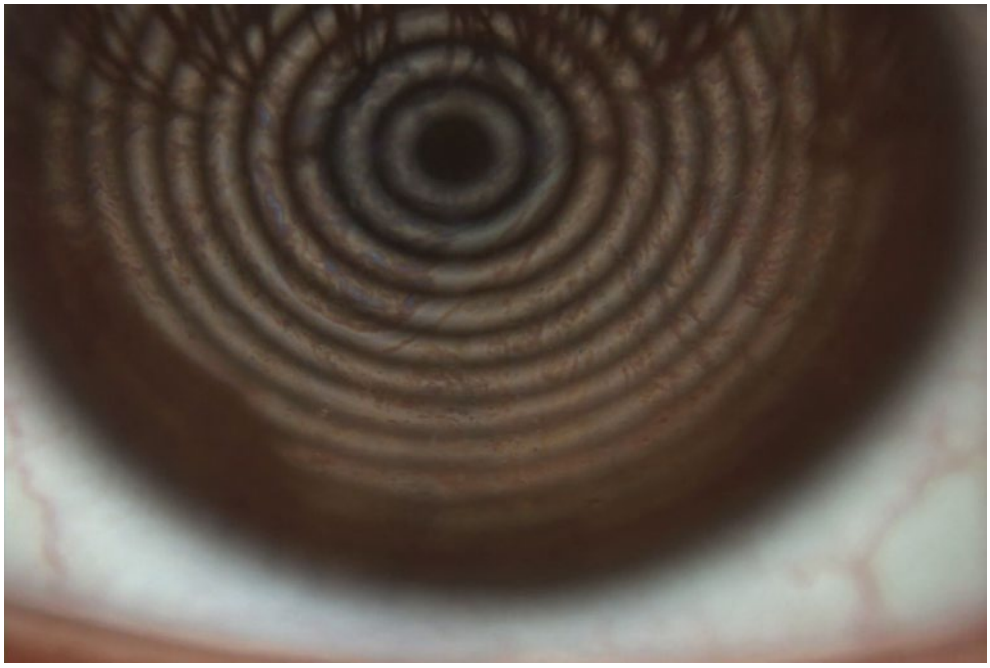


Figure 2.10 Colour fringe LLT observed with the Keratograph 5M

LLT = lipid layer thickness.

The LLT was evaluated using the Lipid Layer TF-Scan software of the Keratograph 5M. The Keratograph 5M reflective graticule was carefully focused on the tear lipid layer, centrally to the cornea. A lipid layer video was recorded for the duration of three non-forceful eyelid blinks.

2.2.5.8 Tear film stability

The number of seconds that elapse between the last blink and the appearance of the first tear film disruption is the so-called tear film break-up time (BUT) (Norn, 1969; Lemp *et al.*, 1970), which constitutes the most commonly employed clinical test to evaluate tear film stability (Wolffsohn *et al.*, 2017).

The BUT has been performed either invasively (FBUT) or noninvasively (NIBUT). Invasive methods involve instilling sodium fluorescein onto the eye and observing

tear film disruptions as areas of dye discontinuity. In contrast, non-invasive methods are based on the detection of any distortions of a reflected pattern from the tear film. Depending on the method used, BUT values of <5s (for FBUT) and <10s (for NIBUT) are associated with unstable tear films (Wolffsohn *et al.*, 2017).

By nature, the BUT is a highly variable measure (Sullivan *et al.*, 2012) and hence consistency in its procedure is important. NIBUT is preferred over FBUT (Wolffsohn *et al.*, 2017), as the use of sodium fluorescein alters tear dynamics and induces earlier tear disruptions than at a natural state (Mengher *et al.*, 1985; Mooi *et al.*, 2017). Moreover, the volume of instilled fluorescein is difficult to standardise (Nichols, Mitchell and Zadnik, 2004).

For the current study, the “NIKBUT” software of the K5M was used to evaluate the NIBUT (Figure 2.11). Infrared illuminated ring patterns were focused on the participant’s cornea. Recording of the reflected tear image occurred straight after having instructed the participant to deliver two natural blinks and keep their eyes open as long as possible. When the tear film was significantly broken or the participant had to blink again, the recording was automatically stopped and saved to be analysed.

The K5M software showed the analysis in an outcoming window, in which the quality of the reflections was mapped and two measures for NIBUT were given: the time at the first tear break-up occurred (NIKBUT-first) and the average of all tear break-up incidents (NIKBUT-average). Three consecutive NIKBUT-first readings were taken (separated by at least 60 seconds) and the mean was recorded.

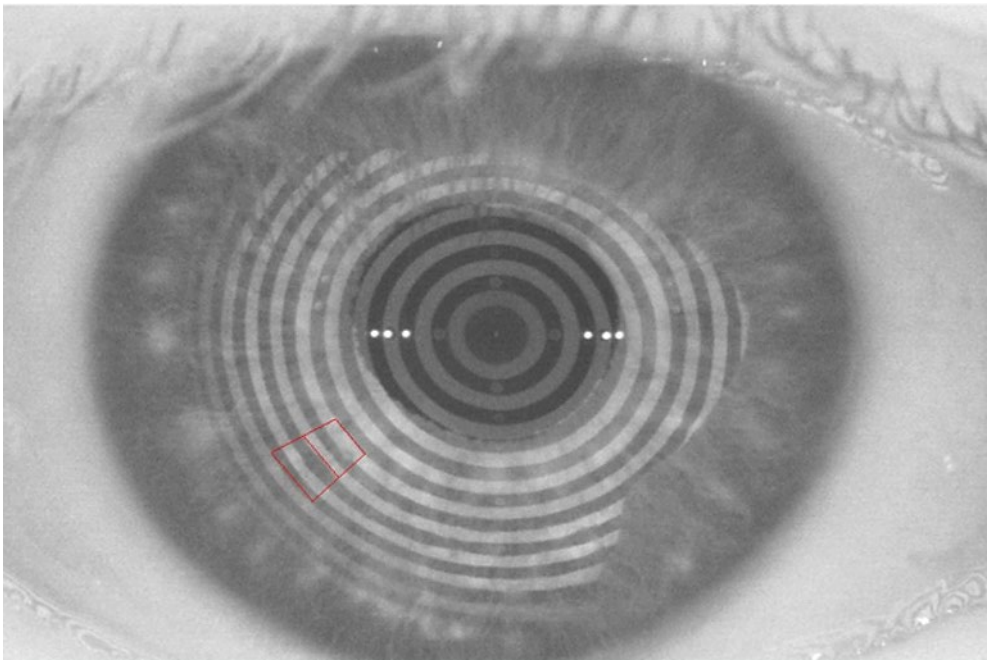


Figure 2.11 First NIKBUT observed with the Keratograph 5M

NIKBUT = non-invasive Keratograph tear break-up time.

The NIKBUT was analysed with the NIKBUT TF-Scan software of the Keratograph 5M. The Keratograph 5M reflective graticule was carefully focused on the tear film, centrally to the cornea. Three NIKBUT values were taken (separated by at least 60s), and the mean was recorded. Recording always stopped at participants' next blink.

2.2.5.9 Ocular staining

Ocular surface damage can be visualised following instillation of ophthalmic dyes. either in solution or via wetted filter paper strip. Ophthalmic dyes in current and past use are sodium fluorescein, rose bengal and lissamine green. Sodium fluorescein emits best fluorescence upon illumination through a blue excitation filter (peak wavelength of 495nm) with a yellow barrier filter (bandpass at 500nm) (Peterson, Wolffsohn and Fowler, 2006), staining epithelial cells with disrupted intercellular junctions (Feenstra and Tseng, 1992). Conversely, both rose bengal and lissamine green are believed to stain any epithelial cells, whose membrane is already

compromised/damaged or exposed due to lack of mucus cover (Kim and Foulks, 1999).

In DED, ocular surface damage is considered a relatively late stage of the disease (Wolffsohn *et al.*, 2017). At present, sodium fluorescein and lissamine green are recommended to be used simultaneously as part of the diagnosis of DED to assess corneal and conjunctival damage, respectively (Wolffsohn *et al.*, 2017). Lissamine green has largely replaced rose bengal, as it has similar staining patterns, but is far less toxic and irritating to the eye (Manning, Wehrly and Foulks, 1995; Machado, Castro and Fontes, 2009). Furthermore, the addition of 1% lissamine green to 2% sodium fluorescein does not significantly alter the fluorescence of the latter and provides optimal corneal and conjunctival staining with only slightly less efficacy than a non-well-tolerated mixture of 1% rose bengal and 2% sodium fluorescein (Korb *et al.*, 2008).

In the present study, 1mg sodium fluorescein (Bio Fluoro, Bio-Tech Vision Care Pvt Ltd, Gujarat, India) and 1.5 mg lissamine green (Green Glo, Hub Pharmaceuticals Llc, California, USA) were instilled via filter paper strips. The strips were wetted with saline (Sensitive Eyes^{TM} Plus Saline Solution, Baush & Lomb Incorporated, New York, USA) and applied near to the temporal canthus of the lower eyelid margin whilst the participant looked up and away (Wolffsohn et al., 2017). The strips were held in place few seconds until the dyes dropped (through surface tension) onto the eyelid margin (Figure 2.12).

Figure 2.12 Lissamine green instillation via wetted filter paper strip

The ophthalmic dye was applied via saline-wetted paper strip to the temporal eyelid canthus. The saline-wetted paper strip was held in place few seconds until the dye dropped (through surface tension) onto the eyelid margin.

Corneal and conjunctival staining were assessed using the “Fluo imaging” and “New Picture/Video” settings of the K5M. and within two (Peterson, Wolffsohn and Fowler, 2006) and four minutes (Hamrah *et al.*, 2011) of sodium fluorescein and lissamine green instillation, respectively. Fluorescein was instilled once (with the excess saline flicked off), whereas a whole drop of lissamine green (allowed to increase in concentration for 5s) was instilled twice, 5 minutes apart (Wolffsohn *et al.*, 2017).

Overall, ocular surface damage is clinically graded with subjective scoring systems, such as the Van Bijsterveld system, Oxford scheme, NEI/ Industry-recommended guidelines, Efron scale and Brien Hold Vision Institute Grading scale (Sook Chun and Park, 2014). However, for research purposes, corneal and conjunctival staining spots were objectively analysed with ImageJ version 1.51j8 (National Institutes of Health,

USA), following image processing (Figure 2.13 and Figure 2.14). The approach was based on a more continuous version of the Oxford grading scheme.

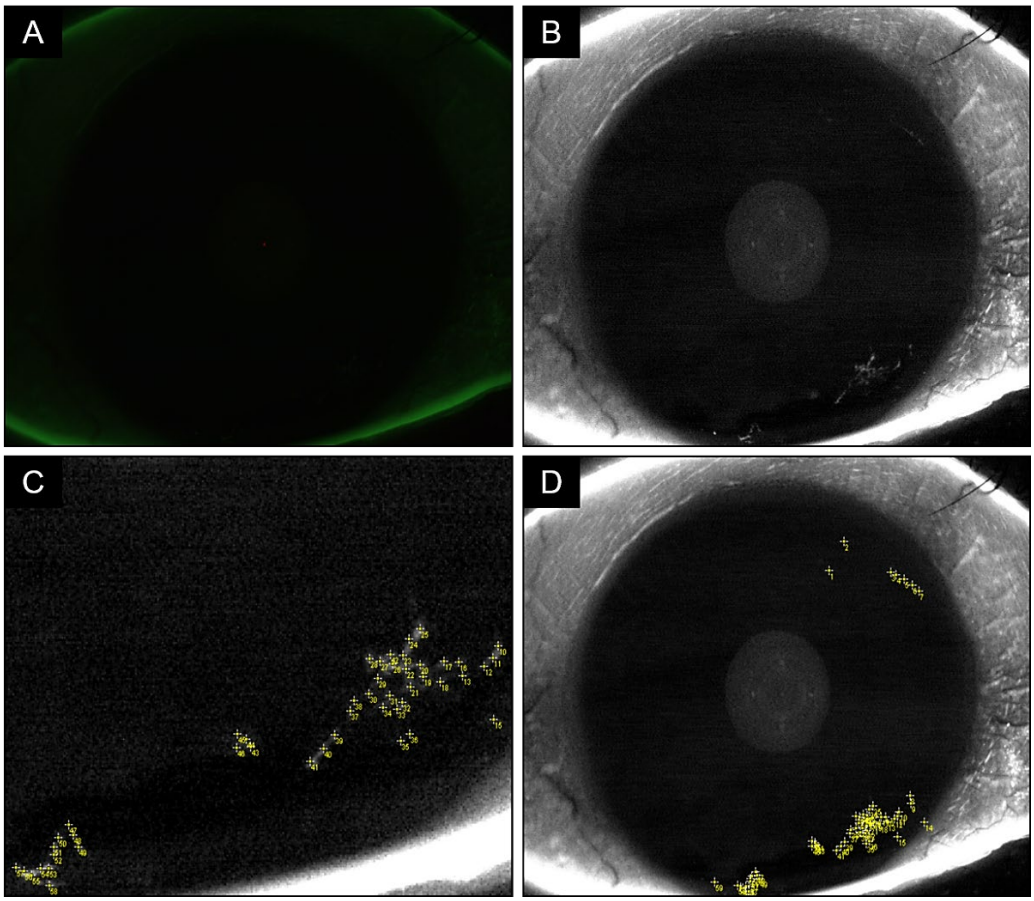


Figure 2.13 Fluorescein staining image analysis with ImageJ

A) Raw corneal staining image.

B) Processing the blue colour channel of the raw corneal staining image.

C) Zooming out the processed image by 75% and counting the observed staining spots.

D) Finalized fluorescein staining image analysis.

ImageJ was used to analyse fluorescein staining. The blue colour channel of the raw corneal staining image was processed to allow a better image contrast. Fifty-nine corneal staining spots were observed.

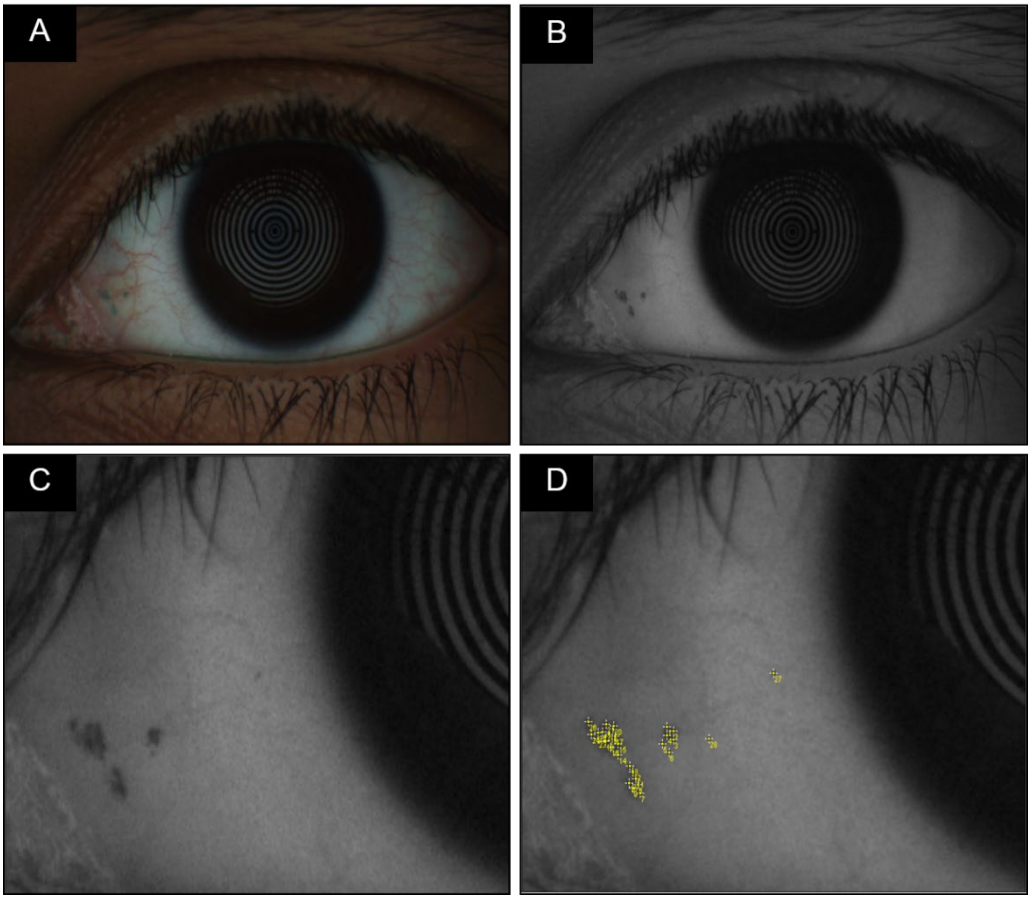


Figure 2.14 Lissamine green staining image analysis with ImageJ

A) Raw conjunctival staining image.

B) Processing the red colour channel of the raw conjunctival staining image.

C) Zooming out the processed image by 75%.

D) Counting the observed staining spots.

ImageJ was used to analyse lissamine green staining. The red colour channel of the raw conjunctival staining image was processed to allow a better image contrast. Twenty-eight conjunctival staining spots were observed.

2.2.5.10 Lid wiper epitheliopathy

Lid wiper epitheliopathy (LWE) was first described in the upper eyelid and subsequently in the lower eyelid. It is defined as an alteration of the marginal palpebral conjunctiva that comes in contact with the ocular surface (Korb *et al.*, 2002). It is diagnosed by vital staining and has been correlated to dry eye symptoms in contact lens wearers as well as non-contact lens wearer (Korb *et al.*, 2002, 2005, 2010).

LWE occurs because of tear film deficiency between the ocular surface and the eyelid wipers, contributing to continuous friction between the structures (Korb *et al.*, 2005) that may result in morphologically distinct staining patterns (Varikooty *et al.*, 2015). This friction effect is thought to be limited to just start of each blink cycle due to aqua-planning (Pult *et al.*, 2015).

LWE has recently been considered as a valuable diagnostic sign of DED (Efron *et al.*, 2016; Wolffsohn *et al.*, 2017). For the present study, LWE was evaluated by everting both eyelids and measuring the extent of lissamine green staining. Sufficient dye was instilled to ensure the visualization of the Marx line along the eyelid margin (Doughty *et al.*, 2004), and the “New Image/Video” setting of the K5M was selected.

The width and the length of the LWE staining were objectively analysed with ImageJ and classified according to the Korb four-point grading scale (For LWE width: score 0: 25% wide LWE staining; score 1: 25 – 49% wide LWE staining; score 2: 50 – 74 % wide LWE staining: score 3: ≥75% wide LWE staining) (For LWE length: score 0: <2 mm long LWE staining; score 1: 2 – 4 mm long LWE staining; score 2: 5 – 9 mm long LWE staining: score 3: >10 mm long LWE staining) (Korb *et al.*, 2005) (Figure 2.15). In non-contact lens wearers, an upper LWE cut-off value of 1 (based on this

scale) has shown a specificity of 96% and sensitivity of 48% of symptomatic DED (Shiraishi, Yamaguchi and Ohashi, 2014).

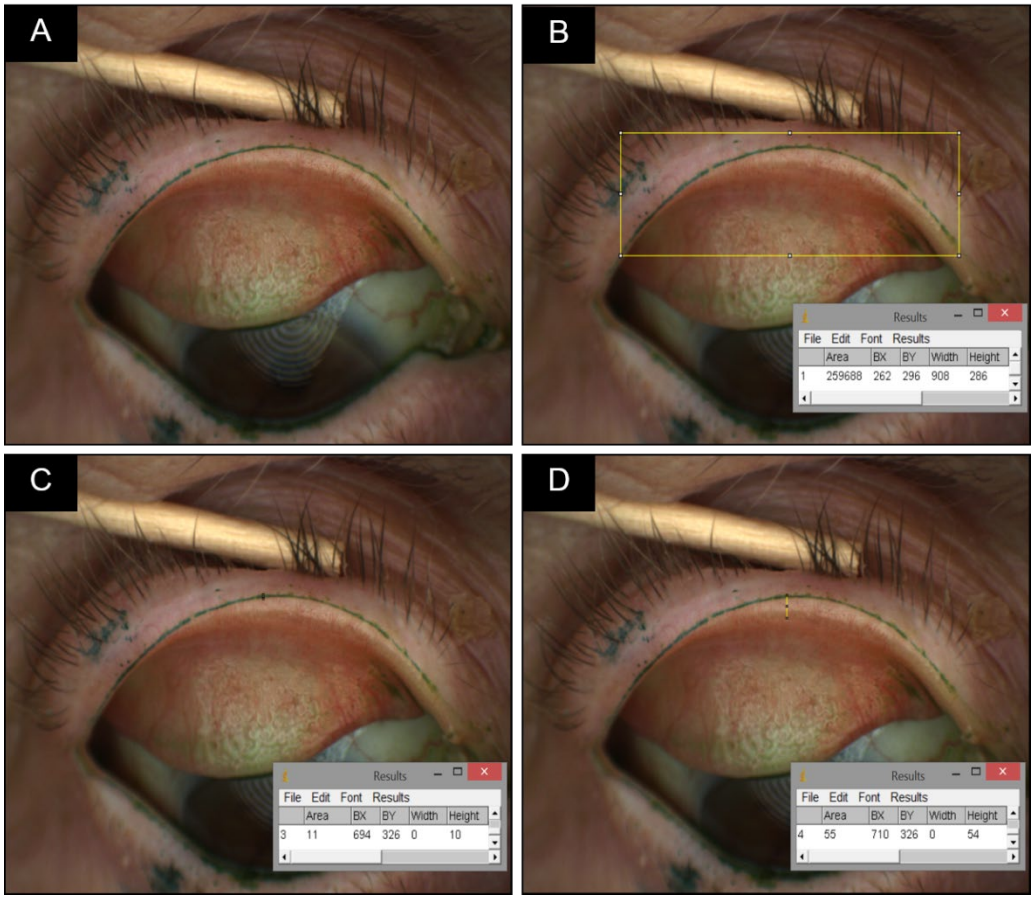


Figure 2.15 LWE staining image analysis with ImageJ

LWE = lid wiper epitheliopathy.

- **A)** Raw LWE staining image of the upper eyelid.
- **B)** Measuring the length of the observed lid wiper epitheliopathy (L = 908 pixels).
- **C)** Measuring the width of the observed lid wiper epitheliopathy (W_{LWE} = 10 pixels).
- **D)** Measuring the total width of the lid wiper (W_{LW} = 54 pixels).

An upper LWE length of 18.5mm and upper LWE width of 18.5% were observed. The LWE length was calculated using a scale convertor of 1 mm = 49 pixels (L /49). The LWE width percentage was obtained by dividing the width of the observed lid wiper epitheliopathy by the total width of the lid wiper (W_{LWE} /W_{LW}).

2.2.5.11 Meibomian gland dysfunction

Meibomian gland dysfunction (MGD) is defined as “chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/ quantitative changes in the glandular secretion” (Daniel Nelson *et al.*, 2011). It is considered a major cause of EDE, whereby the tear lipid layer loses subsequently its protective role in surface desiccation (Lemp *et al.*, 2007).

Meibomian glands are tubuloacinar, holocrine glands that are located within the upper and lower eyelid tarsal plates (Bron *et al.*, 2004). They are similar to sebaceous glands of the skin, but not related to hair follicles (Knop *et al.*, 2011). The duct orifices of the meibomian glands open just anterior to the mucocutaneous junction at the lid margins (Bron *et al.*, 2004).

Meibomian glands’ secretion is known as meibum (Bron *et al.*, 2004). The meibum is composed by polar and non-polar lipids (Green-Church *et al.*, 2011) and is believed to be regulated by hormonal and neural influences as well as the contraction of palpebral muscles (Knop *et al.*, 2011). Within a blink, the secreted lipids are released and spread onto the ocular surface to form the lipid layer of the tear film (Bron *et al.*, 2004).

In clinical practice, meibography consists of infrared-imaging of the morphological silhouettes of the meibomian glands of the everted eyelids (Arita *et al.*, 2008). For the present study, the technique was performed using the “Meibography Upper/Lower Lid” setting of the K5M. The absence of the meibomian gland (meibomian gland dropout) was evaluated in both eyelids and graded with a currently recommended (Wolffsohn *et al.*, 2017) and highly reproducible five-point meiboscale: the meiboscore (score 1: ≈0% loss of meibomian gland area; score 2: ≤25% loss of

meibomian gland area; score 3: 26 – 50% loss of meibomian gland area; score 4: 51 – 75% loss of meibomian gland area; score 5: >75% loss of meibomian gland area) (Pult and Riede-Pult, 2013). The relative areas of meibomian gland dropout were previously obtained with ImageJ, dividing the area with no visible glands by the total area of the tarsal conjunctiva (Pult and Riede-Pult, 2013) (Figure 2.16).

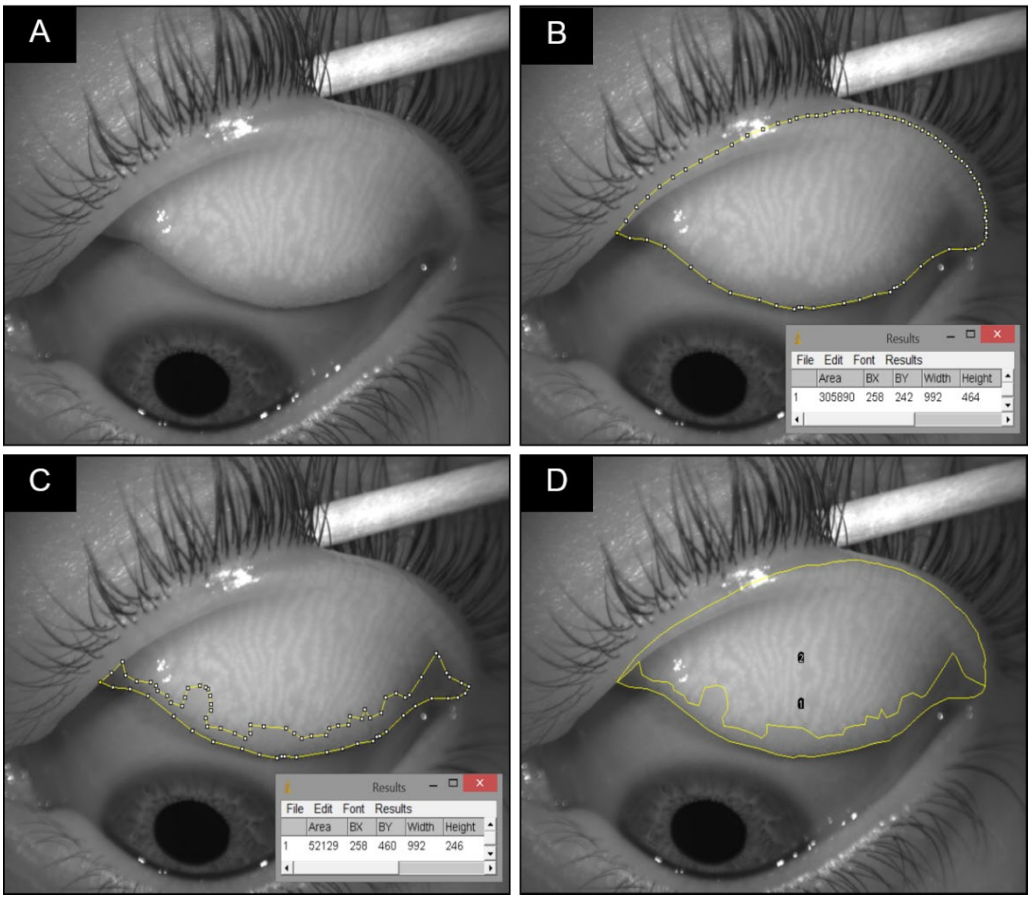


Figure 2.16 MGD analysis with ImageJ

MGD = meibomian gland dysfunction.

- **A)** Meibography of the upper eyelid.
- **B)** Measuring the total everted area of the upper tarsal conjunctiva (A_T = 305890 pixels).
- **C)** Meibomian the area of Meibomian dropouts of the upper tarsal conjunctiva (A_D = 52129 pixels).

A MGD of 17.0% was observed. The MGD severity was obtained by dividing the area of meibomian dropouts by the total everted area of the upper tarsal conjunctiva (A_D/A_T).

3. CHAPTER 3: THE PREVALENCE OF DRY EYE DISEASE IN THE UK

3.1 Overview

The chapter gives an overview of DED in Birmingham (UK). It includes data about the disease prevalence by different diagnostic criteria.

3.2 Introduction

The primary importance of prevalence rates is to gain an understanding of a disease burden to further plan and allocate health sources (Mann, 2003).

In DED, current cross-sectional studies have unfortunately relied on different diagnoses rendering incomparable conclusions about the disease prevalence (Stapleton *et al.*, 2017). Reported DED prevalence rates have widely ranged from 1.3% to 52.9% (Stapleton *et al.*, 2017).

The most consistent diagnostic criteria in the literature has been that first adopted by the WHS (Stapleton *et al.*, 2017). Other diagnostic methods have determined the disease either by the presence of its symptoms, signs or both symptoms and signs (Stapleton *et al.*, 2017). Details about all methods can be found in Chapter 1 (sections 1.6.1 - 1.6.4).

In view of standardisation, the TFOS DEWS II proposed a global diagnosis of DED (Wolffsohn *et al.*, 2017). This identifies an individual as having DED by the presence of one ocular sign (determined either by assessing the tear film stability, tear film osmolarity, or ocular surface staining) and a positive result from a validated questionnaire (either the DEQ-5 or OSDI test) (Wolffsohn *et al.*, 2017).

The present study is the first population-based study in the UK that estimates the prevalence of DED following the TFOS DEWS II diagnostic recommendations. Moreover, it determines the prevalence of DED by the WHS criteria to understand to which extent the TFOS DEWS II diagnostic criteria differs from past diagnostic techniques of the disease.

3.3 Methodology

The study methodology described in Chapter 2 was used to study the prevalence of DED by the WHS and TFOS DEWS II criteria. The WHS criteria defined DED by self-report of ocular dryness and irritation either often or constantly, or a previous clinical diagnosis of the disease (Schaumberg *et al.*, 2009; Uchino *et al.*, 2011; Zhang, Chen and Wu, 2012; Ahn *et al.*, 2014). The TFOS DEWS II criteria (Wolffsohn *et al.*, 2017) defined DED by an OSDI score of ≥13 or DEQ-5 score of ≥6 and either one of the following signs:

- Non-invasive tear breakup time of <10s;
- Tear film hyperosmolarity defined either by the highest osmolarity value of ≥308 mOsm/l among eyes or an interocular osmolarity difference of >8 mOsm/l;
- Ocular surface damage defined either by >5 corneal staining spots, >9 conjunctival staining spots, or a lower/upper LWE staining of ≥2 mm length and ≥25% width.

For the present study, the TFOS DEWS II criteria was adopted for a NIKBUT value of <8s. The rationale of considering this cut-off value is based on the fact that the K5M has shown to detect tear breakup times 2s earlier than subjective methods (for what a tear breakup time of <10s was originally set for) (Markoulli *et al.*, 2018). Also, the TFOS DEWS II strengthened the need for benchmarking techniques' cut-off

values when using objective measurements (Wolffsohn *et al.*, 2017). The report stated that tear break-up times "can be as low as 2.7s for automated algorithms, and up to 10s for subjective observation techniques" (Wolffsohn *et al.*, 2017).

3.3.1 Data processing

Collected scores of the DED questionnaires, tear film osmolarity, NIKBUT, and corneal, conjunctival and LWE staining were entered in a common Excel spreadsheet. Non-DED and DED outcomes were calculated by considering the above-mentioned diagnostic criteria and coded into values of 1 and 2, respectively.

3.3.2 Statistical analysis

Statistical analysis was performed with SPSS version 23 (IBM Corp. released in 2015. New York. US). DED prevalence rates were stratified by sex and age decades and presented with their 95% CIs. Where the sample size was ≤40, Jeffrey's interval corrections were made (DasGupta, Cai and Brown, 2001). Differences among prevalence rates were tested with McNemar's (for paired samples) and Chi-square tests (for unpaired samples). Correlations between DED symptoms and signs among DED participants (all previously confirmed to be not normally distributed with Kolmogorov-Smirnov normality tests) were evaluated with Spearman's rank correlation coefficients.

3.4 Results

Two-hundred and eighty-two Birmingham residents (43 ± 19 years, 56% females) participated in the study (Figure 3.1). Recruitment occurred by 60%, 30%, 6%, 3% and 1% in the Aston University campus, Birmingham City Centre, ARCHA,

Birmingham City Council and Aston Eye Clinic, respectively. Of all participants enrolled, thirty-one did not successfully complete the clinical assessment; either because they found the tests too time-consuming or too invasive.

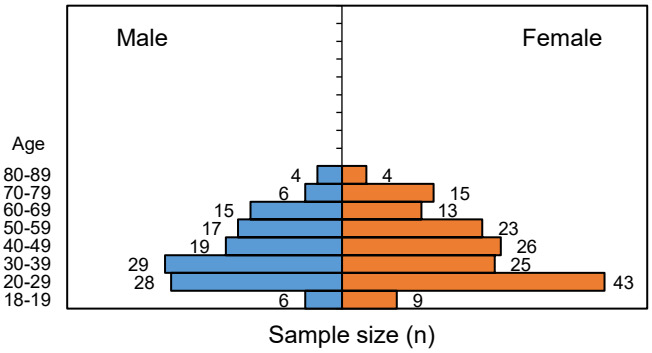


Figure 3.1 Study population distribution

The study population intended to map Birmingham's (UK) population census of 2016 (Figure 3.2).

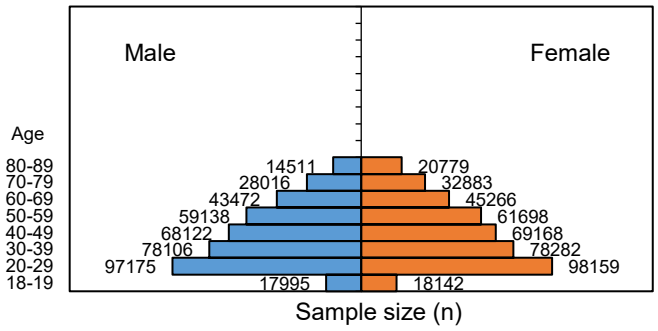


Figure 3.2 Birmingham's (UK) population census (2016)

3.4.1 Dry eye prevalence by the TFOS DEWS II criteria

The prevalence of DED by the TFOS DEWS II criteria varied with the diagnostic method used. DED diagnosis occurred significantly more often where ocular symptoms were assessed with the DEQ-5 than with the OSDI (Figure 3.3). The

highest prevalence rates were observed with those diagnostic methods involving ocular surface staining (Figure 3.3).

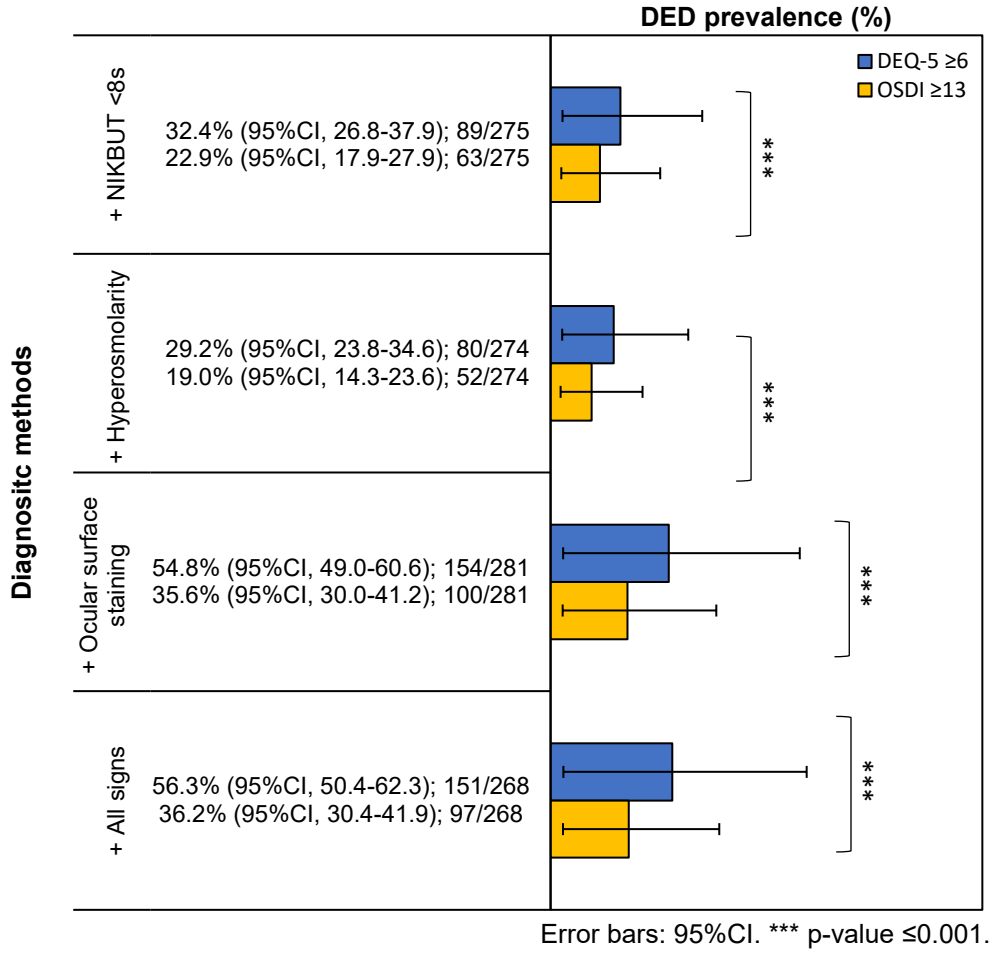


Figure 3.3 DED prevalence by the TFOS DEWS II criteria

DED = dry eye disease. NIKBUT = non-invasive Keratograph tear break-up time. DEQ-5 = 5-item Dry Eye Questionnaire. OSDI = Ocular Surface Disease Index.

The TFOS DEWS II criteria defined DED either by signs of tear film instability, hyperosmolarity or ocular surface staining, and a positive DEQ-5 or OSDI score.

DED by the TFOS DEWS II criteria was found significantly more prevalent in females than in males (Figure 3.4). The disease also differed with age, but without statistical significance (Figure 3.5).

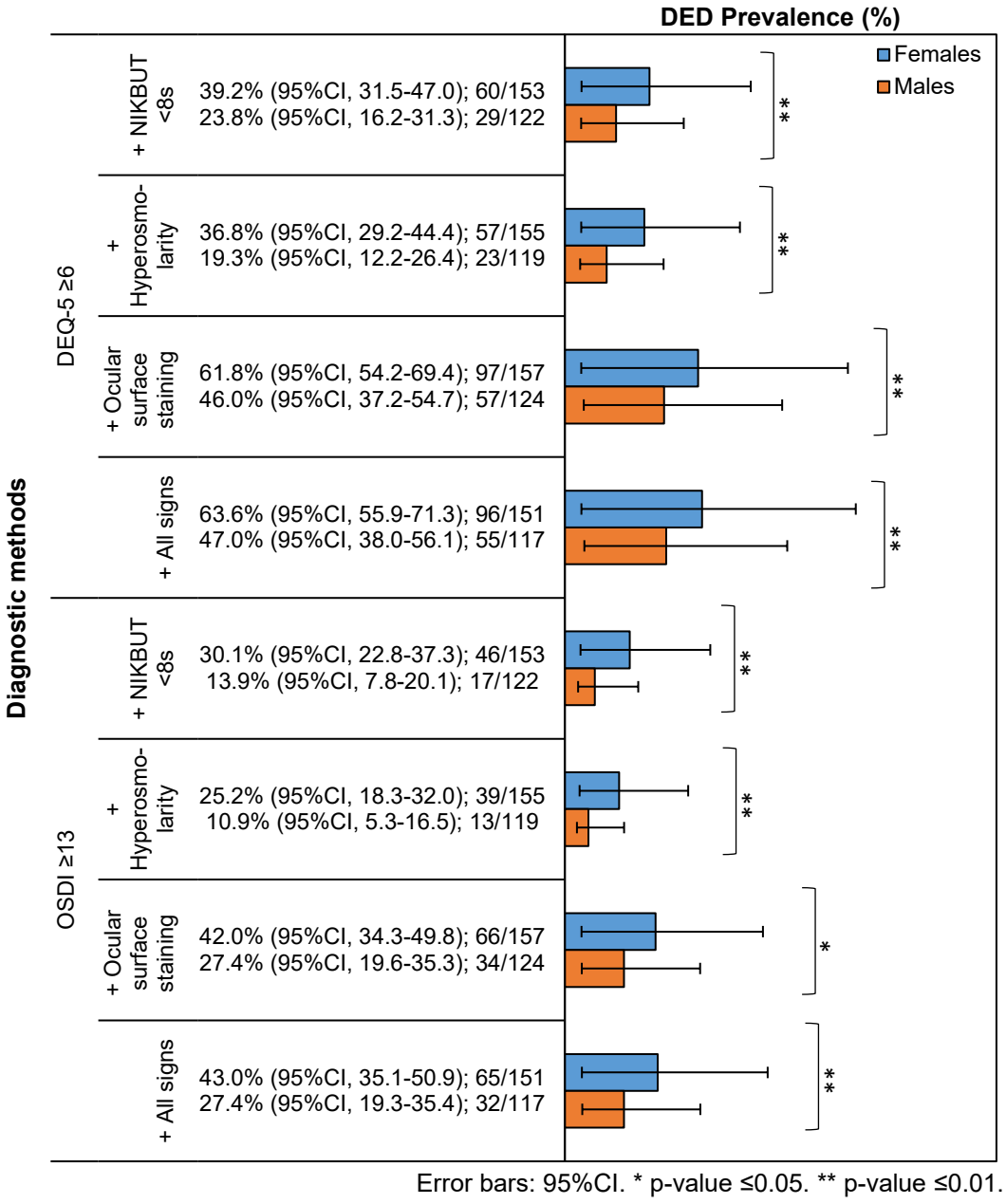


Figure 3.4 DED prevalence by the TFOS DEWS II criteria (stratified by sex)

DED = dry eye disease. NIKBUT = non-invasive Keratograph tear break-up time. DEQ-5 = 5-item Dry Eye Questionnaire. OSDI = Ocular Surface Disease Index.

The TFOS DEWS II criteria defined DED either by signs of tear film instability, hyperosmolarity or ocular surface staining, and a positive DEQ-5 or OSDI score.

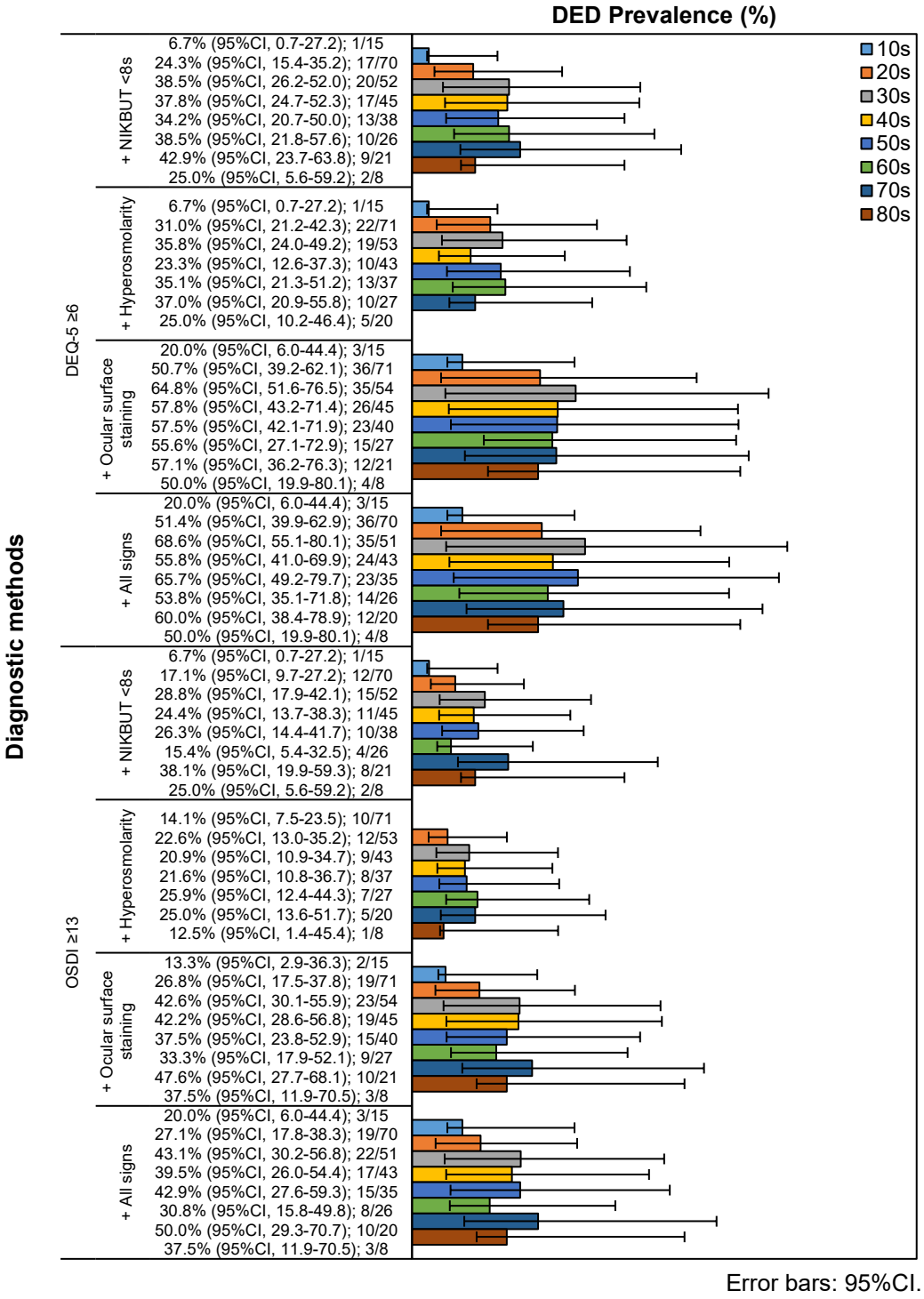


Figure 3.5 DED prevalence by the TFOS DEWS II criteria (stratified by age)

DED = dry eye disease. NIKBUT = non-invasive Keratograph tear break-up time. DEQ-5 = 5-item Dry Eye Questionnaire. OSDI = Ocular Surface Disease Index.

The TFOS DEWS II criteria defined DED either by signs of tear film instability, hyperosmolarity or ocular surface staining, and a positive DEQ-5 or OSDI score.

3.4.2 Dry eye prevalence by the WHS criteria

The WHS criteria estimated a DED prevalence of 29.5% (95%CI, 24.4-35.1). DED by the WHS criteria differed significantly with sex and age (Figure 3.6). Among all ages, participants of 70 to 79 years-old were the most affected by the disease.

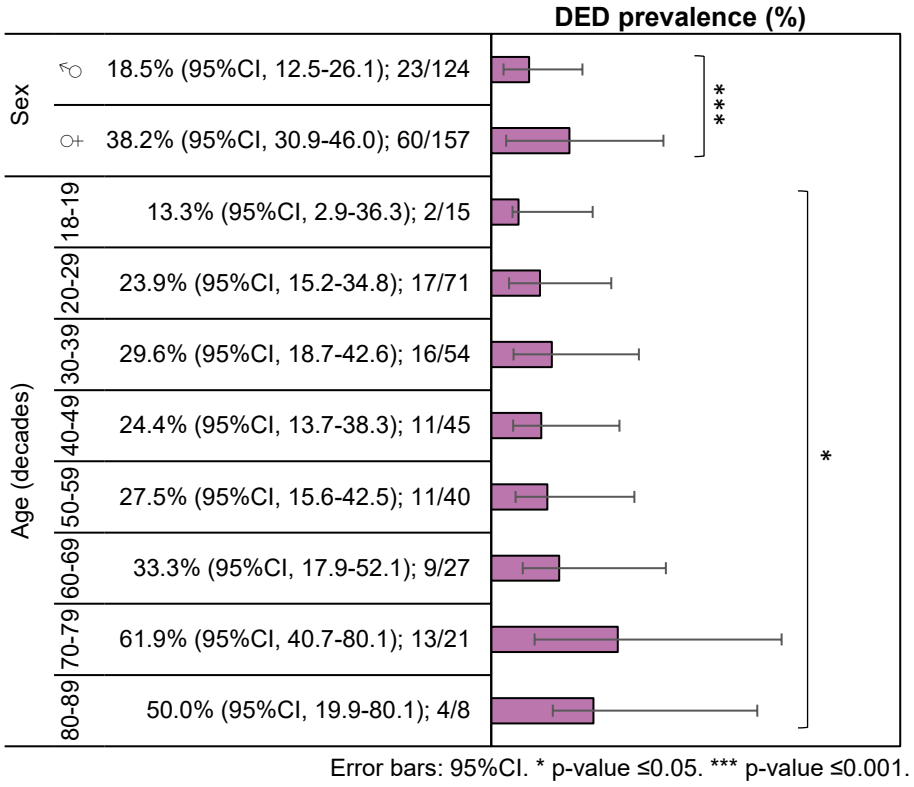


Figure 3.6 DED prevalence by the WHS criteria (stratified by sex and age)

WHS = Women's Health Study

The WHS criteria defined DED by self-report of ocular dryness and irritation either often or constantly, or a previous clinical diagnosis of the disease. Statistically significant differences between prevalence rates stratified by age resulted from significant differences between DED diagnoses of participants in their 10s and 70s (p-value ≤0.001).

3.4.3 Correlations between DED signs and symptoms in DED participants

NIKBUT values were significantly correlated to the DEQ-5 and OSDI among DED participants (Table 3.1). In addition, both symptom questionnaires were found to be significantly concurrent with each other (r_S, 0.518; p-value, ≤0.001).

Table 3.1 Correlations between DED signs and symptoms in DED participants

Ocular signs	Sample size	Correlation with DEQ-5 scores	Correlation with OSDI scores
		Spearman's rank coefficient	Spearman's rank coefficient
NIKBUT mean value (s)	162	-0.177*	-0.175*
Highest osmolarity value (mOsm/L)	161	-0.114	-0.107
Interocular osmolarity difference (mOsm/L)	161	-0.057	-0.034
Corneal staining spots	161	0.124	0.095
Conjunctival staining spots	160	0.058	0.091
Lower LWE length (mm)	162	-0.006	-0.026
Lower LWE width (%)	162	-0.040	-0.047
Upper LWE length (mm)	153	0.070	-0.046
Upper LWE width (%)	153	0.066	0.015

DED = dry eye disease. * p-value ≤0.05.

3.5 Discussion

The epidemiology of DED has been challenged by the failure of a standardised diagnostic method to rely on (Stapleton *et al.*, 2017). The present study is the first to determine the disease prevalence in the UK as conforming to the TFOS DEWS II diagnostic criteria (Wolffsohn *et al.*, 2017). The prevalence rates were stratified by sex and age and compared to those obtained with the WHS criteria.

The results showed that the prevalence of DED by the TFOS DEWS II varied considerably with the diagnostic method used, ranging from 19.0% to 56.3%. Prevalence rates were significantly higher where DED symptoms were assessed with the DEQ-5 than with the OSDI. Moreover, ocular surface staining was the most prevalent DED sign, followed by tear film instability and hyperosmolarity.

Lower prevalence rates of DED with the OSDI might be explained in the nature of the symptom questionnaire. As opposed to the DEQ-5, the OSDI assesses a smaller number of DED symptoms and hence may be less accurate in detecting symptomatic DED. Additionally, because the OSDI also measures the impact of environmental triggers on DED, it may be less simple to complete than the DEQ-5.

Previous evidence has noted that ocular surface staining might not only be an intrinsic feature of DED, but may also be presented in other conditions with eventual DED symptoms (Stapleton *et al.*, 2017). This might explain the higher prevalence rates of DED obtained when considering ocular surface staining compared to tear film instability or hyperosmolarity. Contrarily, the cut-off values used for corneal and conjunctival staining have been set somewhat subjectively (Whitcher *et al.*, 2010), probably misdiagnosing DED.

The WHS criteria determined a disease prevalence of 29.5%, with significant differences among sexes and age decades. The estimate was similar to those obtained with the TFOS DEWS II diagnostic methods based on the DEQ-5 and NIKBUT or tear film osmolarity. Notably, significant negative correlations between NIKBUT and DED symptoms highlighted the diagnostic suitability of tear film stability.

DED prevalence by symptoms and signs has also been reported in other European countries. Malet et al., describing DED by an OSDI score of ≥23 or the use of daily artificial tears, determined a disease prevalence of 21.97% in France; 27.1% in females and 13.6% in males (Malet *et al.*, 2014). A similar diagnostic method, involving symptom self-report and the assessment of ocular surface staining or tear film stability, was used in Spain and estimated a disease prevalence of 11.0%. with

females more affected than males (11.9% vs. 9.0%) (Viso, Rodriguez-Ares and Gude, 2009).

In agreement with these studies, DED by the TFOS DEWS II criteria differed significantly with sex, suggesting that sex hormones may play an important role in the disease predisposition. At present, male-specific sex hormones have shown to regulate both tear lipid and aqueous secretions, as well as the immune responses of corneal and conjunctival cells (Sullivan *et al.*, 2017). However, female-specific sex hormones appear to antagonise these functions (Sullivan *et al.*, 2017).

A meta-analysis on DED epidemiology has outlined that DED increases approximately linearly with age, with a steeper rise by decades in DED described by signs than by symptoms (Stapleton *et al.*, 2017). Interestingly, the prevalence of DED by the TFOS DEWS II criteria was not significantly different with age, nor presented an obvious linear relationship. The results might have been influenced by other DED risk factors.

In conclusion, differences in the DED diagnostic methods used resulted in variations in the disease prevalence. DED diagnosis involving the assessment of symptoms and tear film stability appears to be suitable and similar to the WHS criteria. The diagnostic method might be considered to diagnose DED on its own, reducing cost and time boundaries of future clinical and/or epidemiological DED studies (Savini *et al.*, 2008).

4. CHAPTER 4: THE RISK FACTORS OF DRY EYE DISEASE IN THE UK

4.1 Overview

The chapter gives an overview of DED in Birmingham (UK). It includes data about the risk factors of DED by the TFOS DEWS II diagnostic criteria.

4.2 Introduction

Epidemiological research on DED has focused on the assessment of risk factors to effectively prevent and control the disease (Stapleton *et al.*, 2017). A risk factor refers to any internal or external condition of an individual, which increases the likelihood of developing DED (Lemp *et al.*, 2007).

Assessing DED risk factors requires standardisation in obtaining information about individuals' health and lifestyle, as well as in differentiating between affected and unaffected eyes (Stapleton *et al.*, 2017). Yet, the use of different disease diagnoses has hindered to reach conclusive results on DED risk factors (Stapleton *et al.*, 2017).

The present study is the first population-based study in the UK that estimates the risk factors of DED following recent diagnostic recommendations of the TFOS DEWS II (Wolffsohn *et al.*, 2017).

4.3 Methodology

The study methodology described in Chapter 2 was used to study the risk factors of DED. The disease was defined as conforming to the TFOS DEWS II recommendations (Wolffsohn *et al.*, 2017), more specifically, by a DEQ-5 score of ≥6 or OSDI score of ≥13 and either one of the following:

- NIKBUT of <8s;
- Tear film hyperosmolarity defined either by the highest osmolarity value of ≥308 mOsm/l among eyes or an interocular osmolarity difference of >8 mOsm/l;
- Ocular surface staining defined by >5 corneal spots, >9 conjunctival spots or lower/upper LWE staining of ≥2 mm length and ≥25% width.

4.3.1 Data processing

Risk factors gathered by the DERFS questionnaire were treated as nominal or ordinal variables and coded into numerical scores (Table 4.1). Risk categories with a low frequency of endorsement (less than 5%) were collapsed (rather than being excluded from the start) for the statistical analysis.

Table 4.1 Recording of DED risk factors

DERFS question	Risk factor	Coding instructions
To which group do you belong?	Ethnicity	1= White, 2= Asian, 3= Black, 4= Others.
	Sex	1= Male, 2= Female.
	Age	1= 10s, 2= 20s, 3= 30s, 4= 40s, 5= 50s, 6= 60s, 7= 70s, 8= 80s.
	Residential area	1= Rural, 2= Urban.
	Education	1= Elementary or primary school, 2= Middle or secondary school, 3= High school or 6th form, 4= University o higher.
Do you work?	Employment status	1= Unemployed, 2= Employed.
Do you smoke?	Smoking	1= No, 2= Yes.
Do you drink alcohol?	Alcohol intake	1= No, 2= Yes.
Do you wear contact lenses?	Contact lens wear	1= No, 2= Yes.
Do you use computer?	Computer use	1= <3 hours/day, 2= 3-5 hours/day, 3= 6-8 hours/day, 4= >8 hours/day.
Do you have/have had any health condition/problem?	Health conditions/problems	1= No, 2= Yes. → Code 2 included any health condition/problem described by the participants.
Do you have had any eye surgery?	Ocular surgery	1= No, 2= Yes. → Code 2 included any past ocular surgery described by the participants.
Do you take any medication regularly?	Medication intake	1= No, 2= Yes. → Code 2 included any medication described by the participants.
Do you take any nutritional supplement regularly?	Nutritional supplement intake	1= No, 2= Yes. → Code 2 included any nutritional supplement described by the participants.

Table 4.2 *(continued)*

How much sleep do you get?	Sleep quality	1= >8 hours/night, 2= 6-8 hours/night, 3= <6 hours/night
How much do you spend outdoors on a regular leisure day?	Outdoor activity	1= <3 hours/leisure day, 2= 3-4 hours/leisure day, 3= >4 hours/leisure day.
How stressful are your days?	Stress level	1= Least stressful, 2= Moderately stressful, 3= Extremely stressful.

4.3.2 Statistical analysis

Statistical analysis was performed with SPSS version 23 (IBM Corp, released in 2015, New York, USA). Univariate analysis, including Chi-square tests, initially determined the significance of all self-reported risk factors. Risk factors with p-values of less than 0.10 were considered for further multivariate analysis using non-hierarchical enter binary logistic regression. Correlations between the selected risk factors were evaluated with point biserial correlation coefficients (between dichotomous and ordinal risk factors), Spearman's rank correlation coefficients (between two ordinal risk factors) and phi coefficients (between two dichotomous risk factors). Finally, the strength and precision of the DED risk factors were summarised using ORs and 95% CIs, respectively. ORs with p-values of ≤0.05 were considered statistically significant.

4.4 Results

Two-hundred and eighty-two Birmingham residents (43 ± 19 years, 56% females) participated in the study (Table 4.2). Recruitment occurred mostly at Aston University campus. Clinical assessments and DERFS questionnaires were successfully completed by 95% and 96% of all enrolled participants, respectively.

Table 4.2 Study population characteristics

Risk factor	Category	Frequency (n)	Percentage (%)
Ethnicity	White	166	58.9
	Asian	99	35.1
	Black	6	2.1
	Others	11	3.9
Sex	Male	124	44.0
	Female	158	56.0
Age (years)	18-19	15	5.3
	20-29	71	25.2
	30-39	54	19.1
	40-49	45	16.0
	50-59	40	14.2
	60-69	28	9.9
	70-79	21	7.4
	80-89	8	2.8
Residential area	Rural	40	14.8
	Urban	230	85.2
Education	Elementary or primary school	2	0.7
	Middle or secondary school	24	8.8
	High school or 6th form	49	17.9
	University or higher	199	72.6
Employment status	Unemployed	101	36.9
	Employed	173	63.1
Smoking	No	259	94.5
	Yes	15	5.5
Alcohol intake	No	111	40.5
	Yes	163	59.5
Contact lens wear	No	206	75.2
	Yes	68	24.8
Computer use (hours/day)	<3	61	22.3
	3-5	92	33.6
	6-8	95	34.7
	>8	26	9.5
Health conditions/problems*	No	86	31.4
	Yes	188	68.6
Ocular surgery**	No	232	85.0
	Yes	41	15.0
Medication intake***	No	142	51.8
	Yes	132	48.2
Nutritional supplement intake****	No	134	48.9
	Yes	140	51.1
Sleep quality (hours/night)	>8	14	5.1
	6-8	226	82.8
	<6	33	12.1
Outdoors activity (hours/leisure day)	<3	119	44.6
	3-4	84	31.5
	>4	64	24.0
Stress level	Least stressful	82	30.0
	Moderately stressful	173	63.4
	Extremely stressful	18	6.6

Table 4.2 *(continued)*

* Recorded health conditions/problems were migraine (n =31), asthma (n =31), eczema (n =23), acne (n =17), rosacea (n =9), psoriasis (n =3), dermatitis (n =1), morphea (n =1), vitiligo (n =1), vitamin D deficiency (n =28), iron deficiency (n =11), anxiety (n =25), depression (n =15), rheumatoid arthritis (n =28), hypertension (n =27), hypercholesterolemia (n =20), thyroid disease (n =14), cancer (n =13), polycystic ovary syndrome (n =4), bladder irritation (n =1), osteoporosis (n =5), irritable bowel syndrome (n =9), diabetes mellitus (n =11), lymphatic drainage problem (n =1), stroke (n =4), prostatitis (n =1), gout (n =1), keratoconus (n =1), pterygium (n =1), insomnia (n =2), Sjögren syndrome (n =1), tuberculosis (n =1), epilepsy (n =1), Ehlers-Danlos syndrome (n =1), sinusitis (n =2), familial dilated cardiomyopathy (n =1), Crown disease (n =1), Carpal tunnel syndrome (n =1), glaucoma (n =4), human immune deficiency virus (n =1), multiple sclerosis (n =1), thoracic outlet syndrome (n =1), audio sclerosis (n =1), diverticulosis (n =1), rhinitis (n =1), bronchiectasis (n =1), Best disease (n =1), age-related macular degeneration (n =1), Parkinson (n =1), traumatic glaucoma (n =1), ulcerative colitis (n =1), retinopathy (n =1), spinal stenosis (n =1). cataracts (n =1), pain in joints (n =1), back (n =8), pelvic (n =3) and hips (n =1), and allergy to pollen (n =44), grass (n =3), dust (n =12), penicillin (n =11), pets (n =7), nuts (n =3), feathers (n =2), flowers (n =1), wool (n =1), mould (n =1), mites (n =2), plasters (n =2), antibiotics (n =1), non-steroidal anti-inflammatory drugs (n =1), gluten (n =1), diary (n =1), soy (n =1), fish (n =1), eggs (n =1), zinc (n =1), statins (n =1), trimethoprim (n =1), efortil (n =1), morphine (n =1) inhaler (n =1) and opioids (n =1).
** Documented surgical ocular interventions were strabismus surgery (n =4), refractive surgery (n =13), dacryocystorhinostomy (n =2), cyst removal (n =7), corneal cross-linking (n =1), cataract surgery (n =11) and retinal surgery (n =2).
*** Medication intake included the use of oral contraceptives (n =18), antimigraine drugs (n =4), antihistamine drugs (n =22), pills for skin problems (n =7), antihistamine inhaler (n =11), anxiolytics (n =3), steroids (n =2), painkillers (n =7), blood pressure pills (n =19), anti-thyroid pills (n =11), pills for asthma (n =1), pills for digestive problems (n =2), pills for bladder control (n =4), cancer treatment (n =2), antidepressant (n =8), statins (n =19), diuretics (n =2), hormone therapy (n =3), pills for irritable bowel syndrome (n =1), diabetes treatment (n =6), aspirins (n =12), prostatitis treatment (n =2), heart treatment (n =1), pills for vertigo (n =1), sleeping tablets (n =5), dermatitis treatment (n =1), arthritis treatment (n =2), antibiotics (n =2), glaucoma drops (n =3), pills for palpitation (n =1), beta blockers (n =1), human immune deficiency virus treatment (n =1), osteoporosis treatment (n =1), stomach protector (n =3), antifungal pills (n =1), antihistamine nasal spray (n =1), antihistamine eyedrops (n =2), Parkinson treatment (n =1), morphine (n =1), epilepsy treatment (n =1), sinusitis nasal spray (n =1), gout treatment (n =1), and contraceptive implant (n =2).
**** Nutritional supplement intake include the use of vitamin D (n =42), cod liver oil (n =37), iron (n =22), proteins (n =4), multivitamins (n =44), vitamin C (n =16), vitamin B (n =9), calcium (n =5), zinc (n =2), vitamin E (n =1), weight gainer (n =1), folic acid (n =2), echinacea (n =1), glucosamine (n =11), hyaluronic acid (n =1), probiotics (n =1), herbal pills (n =1), magnesium (n =7), primrose oil (n =1), caffeine (n =1), essential amino acids (n =1), electrolytes (n =1), melatonin (n =1), collagen (n =1), lutein (n =2), yin yang (n =1), flaxseed oil (n =1), lysine (n =1), beetroot extract (n =1), turmeric (n =1) and Adalat (n =1).

4.4.1 Dry eye risk factors

Sex, age, employment status, health conditions/problems, medication intake, sleep quality and outdoor activity were considered for the multivariate analysis (Table 4.3). Risk factors which did not initially reach significance included ethnicity, residential area, education, smoking, alcohol intake, contact lens wear, computer use, ocular surgery, nutritional supplement intake and stress level (Table 4.3).

Table 4.3 Distribution of risk factors among non-DED and DED participants

Risk factor	Category	$N_{non-DED}$	N_{DED}	N_{Total}	X^2	p-value
Ethnicity	White	58	97	155	0.893	0.640
	Asian	42	55	97		
	Black and others	6	10	16		
Sex	Male	58	59	117	8.721	0.003*
	Female	48	103	151		
Age (decades)	18-19	11	4	15	12.201	0.058*
	20-29	33	37	70		
	30-39	15	36	51		
	40-49	16	27	43		
	50-59	12	23	35		
	60-69	10	16	26		
	70-79 and 80-89	9	19	28		
Residential area	Rural	14	25	39	0.439	0.508
	Urban	91	128	219		
Education	Elementary, primary, middle or secondary school	8	16	24	1.524	0.467
	High school or 6th form	16	30	46		
	University or higher	82	110	192		
Employment status	Unemployed	48	51	99	4.256	0.039*
	Employed	58	105	163		
Smoking habits	No	103	145	248	2.223	0.136
	Yes	3	11	14		
Drinking habits	No	40	67	107	0.710	0.399
	Yes	66	89	155		
Contact lens wear	No	83	116	199	0.537	0.464
	Yes	23	40	63		
Computer use (hours/day)	<3	23	36	59	2.438	0.487
	3-5	41	47	88		
	6-8	34	56	90		
	>8	8	17	25		
Health conditions/problems	No	46	38	84	10.501	0.001*
	Yes	60	118	178		
Ocular surgery	No	90	133	223	0.041	0.839
	Yes	16	22	38		
Medication intake	No	62	75	137	2.744	0.098*
	Yes	44	81	125		
Nutritional supplement intake	No	58	72	130	1.851	0.174
	Yes	48	84	132		
Sleep quality (hours/night)	>8	9	5	14	5.324	0.070*
	6-8	87	128	215		
	<6	9	23	32		
Outdoors activity (hours/leisure day)	<3	51	66	117	4.923	0.085*
	3-4	34	43	77		
	>4	17	44	61		
Stress level	Least stressful	29	52	81	4.284	0.117
	Moderately stressful	72	90	162		
	Extremely stressful	4	14	18		

DED = dry eye disease. n = sample size. X^2 = Chi-square test.
*Selected for logistic multivariate analysis.

DED associations that were identified statistically significant in the multivariate analysis were female sex, the presence of any health conditions/problems, poor sleep quality and prolonged outdoor activity (Table 4.4).

Table 4.4 Risk factors for DED

Risk factor		Univariate analysis			Multivariate analysis		
	Category	OR	95%CI	p-value	OR	95%CI	p-value
Ethnicity							
	White	1.000			n/a	n/a	n/a
	Asian	0.783	0.467-1.313	0.354			
	Black and others	0.997	0.344-2.885	0.995			
Sex							
	Male	1.000			1.000		
	Female	2.109	1.281-3.473	0.003	2.380	1.341-4.226	0.003
Age (years)							
	18-19	1.000			1.000		
	20-29	3.083	0.895-10.621	0.074	1.723	0.448-6.624	0.429
	30-39	6.600	1.811-24.053	0.004	3.185	0.707-14.342	0.131
	40-49	4.641	1.264-17.041	0.021	1.640	0.362-7.421	0.521
	50-59	5.271	1.380-20.138	0.015	1.658	0.336-8.191	0.535
	60-69	4.400	1.095-17.676	0.037	2.081	0.427-10.153	0.365
	70-79 and 80-89	5.806	1.443-23.363	0.013	2.880	0.615-13.491	0.179
Residential area							
	Rural	1.000			n/a	n/a	n/a
	Urban	0.788	0.388-1.598	0.082			
Education							
	Elementary, primary, middle or secondary school	1.000			n/a	n/a	n/a
	High school or 6th form	0.938	0.330-2.661	0.903			
	University or higher	0.671	0.274-1.642	0.382			
Employment status							
	Unemployed	1.000			1.000		
	Employed	1.704	1.025-2.832	0.040	1.826	0.850-3.923	0.123
Smoking							
	No	1.000			n/a	n/a	n/a
	Yes	2.605	0.709-9.570	0.149			
Alcohol intake							
	No	1.000			n/a	n/a	n/a
	Yes	0.805	0.486-1.334	0.400			
Contact lens wear							
	No	1.000			n/a	n/a	n/a
	Yes	1.244	0.693-2.234	0.464			
Computer use (hours/day)							
	<3	1.000			n/a	n/a	n/a
	3-5	0.731	0.375-1.432	0.362			
	6-8	1.052	0.536-2.066	0.882			
	>8	1.358	0.505-3.653	0.545			
Health conditions/problems							
	No	1.000			1.000		
	Yes	2.381	1.410-4.046	0.001	2.719	1.395-5.299	0.003
Ocular surgery							
	No	1.000			n/a	n/a	n/a
	Yes	0.930	0.463-1.869	0.839			
Medication intake							
	No	1.000			1.000		
	Yes	1.522	0.925-2.504	0.098	1.202	0.639-2.263	0.568
Nutritional supplement intake							
	No	1.000			n/a	n/a	n/a
	Yes	1.410	0.859-2.313	0.174			

Table 4.4 *(continued)*

Sleep quality (hours/night)						
>8	1.000			1.000		
6-8	2.648	0.858-8.171	0.090	2.471	0.660-9.256	0.179
<6	4.600	1.207-17.524	0.025	5.050	1.039-24.536	0.045
Outdoors activity (hours/leisure day)						
<3	1.000			1.000		
3-4	0.977	0.547-1.745	0.938	0.968	0.505-1.856	0.923
>4	2.000	1.025-3.902	0.042	2.369	1.108-5.066	0.026
Stress level						
Least stressful	1.000			n/a	n/a	n/a
Moderately stressful	0.697	0.402-1.208	0.198			
Extremely stressful	1.952	0.588-6.484	0.275			

DED = dry eye disease. OR = odds ratio. CI = confidence interval. n/a =not applicable.

4.4.2 Correlations between dry eye risk factors

Most of the selected DED risk factors were significantly correlated with age (Table 4.5). Health conditions/problems showed a significant positive and negative association with medication intake and outdoor activity, respectively (Table 4.5). A significant positive correlation was also observed between employment status and outdoor activity (Table 4.5).

Table 4.5 Correlations between DED risk factors

Correlations coefficient **Sample size**	Sex	Age	Employment status	Health conditions/ problems	Medication intake	Sleep quality	Outdoor activity
Sex		0.002 282	-0.070 274	0.080 274	0.063 274	0.006 273	-0.052 267
Age			-0.059 274	0.347* 274	0.341** 274	0.150* 273	-0.094 267
Employment status				-0.093 274	-0.051 274	0.093 273	0.192** 267
Health conditions/problems					0.385*** 274	0.038 273	-0.135* 267
Medication intake						0.050 273	-0.146 267
Sleep quality							0.025 266

DED = dry eye disease. * p-value ≤0.05. ** p-value ≤0.01. *** p-value ≤0.001.
*Included DED risk factors that have been selected for the logistic multivariate analysis.

4.5 Discussion

The present study is the first to identify DED risk factors as conforming to the TFOS DEWS II diagnostic criteria (Wolffsohn *et al.*, 2017). The criteria is evidence-based and currently recommended to be globally applied in DED research (Wolffsohn *et al.*, 2017).

The cross-sectional study design used allowed to evaluate different DED associations simultaneously (Mann, 2003). The associations were assessed in previous cross-sectional studies, whereby the disease was diagnosed either by the WHS criteria, symptoms, signs or both symptoms and signs (Stapleton *et al.*, 2017). Participants' characteristics were gathered through the DERFS in order to record more precisely the risk factors (Wolffsohn *et al.*, 2017).

The result showed that age, employment status, medication intake, female sex, the presence of any health conditions/problems, poor sleep quality and prolonged outdoors activity were potential risk factors for DED (p-values <0.20). The statistical significances of the last four factors were confirmed in the multivariate analysis (p-values ≤0.05).

In agreement with previous studies (Jie *et al.*, 2009; Han *et al.*, 2011; Ahn *et al.*, 2014), females were 2.317 times significantly more likely to present DED than males, reflecting the importance of sex hormones in the disease predisposition. Male-specific sex hormones are believed to regulate both tear lipid and aqueous secretions, as well as the immune responses of corneal and conjunctival cells (Sullivan *et al.*, 2017). In contrast, female-specific sex hormones appear to antagonise these functions (Sullivan *et al.*, 2017).

Health conditions/problems, including hypertension, hypercholesterolemia, thyroid disease, asthma, eczema, any allergy, rheumatoid arthritis, stroke, migraine, irritable bowel syndrome and pelvic pain, have been significantly related to DED (Ahn *et al.*, 2014; Vehof *et al.*, 2014). The same health conditions/problems were reported in the present study. Nevertheless, the rationale behind the relationship between each health condition/problem and DED is difficult to ascertain, as these were studied in conjunction.

Sleeping less than 6 hours/night contributed significantly to DED (OR = 5.050), as in (Ahn *et al.*, 2014). Short sleep duration is thought to decrease parasympathetic activity (Tobaldini *et al.*, 2017). The lacrimal gland is mostly innervated by the parasympathetic nervous system (Belmonte *et al.*, 2017), and hence any kind of sleep disturbance may considerably lessen tear secretion.

Participants engaging in out of doors for more than four hours on a regular leisure day were 2.369 times significantly more prone to DED. Outdoors activity can be related to environmental conditions, such as high altitude, sunlight exposure, temperature, humidity, wind, precipitation and air pollution, that have been associated with symptomatic and clinically diagnosed DED (Lu *et al.*, 2008; Guo *et al.*, 2010; Um *et al.*, 2014).

Although the statistical significance of age, employment and medication intake disappeared in the multivariate analysis, this does not mean that the risk factors have no clinical importance (Offord and Kraemer, 2000). Their contributions might have been influenced by inter-correlations observed with other risk factors that remained significant in the multivariate analysis.

Importantly, risk factors which did not initially reach significance in the univariate analysis, including ethnicity, residential area, education, contact lens wear, computer use, ocular surgery and stress level, might have been confounded by random sampling. Recruitment occurred mostly at Aston University, showing a strong University population profile. The participants were predominantly non-contact lens wearers, computer users and/o non-smokers, with moderate stress and/or no history of ocular surgery.

In conclusion, female sex, the presence of any health condition/problem, poor sleep quality and prolonged outdoor activity were significant risk factors for the DED. Future DED research, using the TFOS DEWS II diagnostic criteria in other study populations, would be of great value to better understand the disease burden worldwide and subsequently assist in the disease amelioration.

5. CHAPTER 5: SUBCLASSIFICATION OF DRY EYE DISEASE IN THE UK

5.1 Overview

The chapter gives an overview of DED in Birmingham (UK). It includes data about the prevalence and potential risk factors of DED subtypes.

5.2 Introduction

Prevalence studies assessing risk factors of ADDE and EDE may be useful to develop an effective treatment plan for both DED subtypes (Jones *et al.*, 2017). Both ADDE and EDE have similar ocular symptoms and general DED signs, however, they may be related to different risk factors and hence require a different therapeutical approach (Jones *et al.*, 2017).

Recent evidence on DED classification supports the hypothesis that ADDE and EDE may coexist with increasing disease severity and thus characteristics of each need to be considered in clinical practice (Craig *et al.*, 2017). Tests specific to ADDE and EDE evaluate the tear film volume (including the PRT test, Schirmer test and TMH) and tear film evaporation, lipid layer thickness and meibomian gland dysfunction, respectively (Wolffsohn *et al.*, 2017). The tests should not override a clinical diagnosis of DED but should assist in the disease amelioration (Wolffsohn *et al.*, 2017).

Unfortunately, although specific clinical tests have been assigned to diagnose ADDE and EDE, there is no apparent consistency in categorizing both DED subtypes (Jones *et al.*, 2017). For instance, in DED epidemiology, different sub-classification cut-off values were used, hindering direct comparisons of prevalence rates of ADDE and EDE (Albietz, 2000; Rege *et al.*, 2013; Asiedu, Dzasimatu and Kyei, 2018) (Table

5.1). Moreover, the disease diagnosis has been generally addressed somewhat subjectively (Wolffsohn *et al.*, 2017) (Table 5.1).

Table 5.1 Previous large-scale clinical-population-based studies on DED subtypes

Study	**Population characteristics**	**DED subtypes**			
		ADDE		**EDE**	
	Age (years) Sex (n)	Diagnosis	Prevalence (%[95%CI])	Diagnosis	Prevalence (%[95%CI])
Albietz 2000[A]	3-96 ♀ 912 ♂ 672	Lipid layer without colour fringes and meibomian glands without particulate, frothy or cloudy meibum, and PRT test of <10 mm/ 15s and TMH of <0.10 mm.	1.7 [n/a]	Lipid layer with colour fringes and meibomian glands with particulate, frothy or cloudy meibum, and PRT test of ≥10 mm/ 15s and TMH of ≥0.10 mm	4.0 [n/a]
Lemp et al. 2012[B]	46.3 ± 16.9 ♀ 218 ♂ 81	MGD score of ≤5 and Schirmer test II of <7 mm/5 min	10.3 [n/a]	MGD score of >5 and Schirmer test II 7 mm/5 min	35.3 [n/a]
Rege et al. 2013[C]	≥18 ♀ 2585 ♂ 2165	Meibomian glands without inspissated or toothpaste-like meibum, and Schirmer test II of <10 mm/ 5 min	13.36 [n/a]	Meibomian glands with inspissated or toothpaste-like meibum, and Schirmer test II of ≥10 mm/ 5 min	14.48 [n/a]
Asiedu. Dzasimatu and Kyei 2018[D]	17-35 ♀ 89 83 ♂	Meibomian glands without low expressibility and cloudy or toothpaste-like meibum, and Schirmer test I of ≤5 mm/5 min	5.2 [n/a]† 5.2 [n/a]‡	Meibomian glands with low expressibility and cloudy or toothpaste-like meibum, and Schirmer test I of >5 mm/5 min	11.6 [n/a]† 7.0 [n/a]‡

DED = dry eye disease. ADDE = aqueous deficient dry eye. EDE = evaporative dry eye. PRT = phenol red thread. TMH = tear meniscus height. n/a = not applicable.
A. DED was defined by at least one of five primary symptoms of the MQ questionnaire (soreness, scratchiness, dryness, grittiness and burning) either often or constantly, an FBUT of <10s and a rose bengal score of ≥1 (van Bjisterveld staining score) (Albietz, 2000).
B. DED was defined by an OSDI score of ≥5 and at least two of five signs: FBUT <7s, Schirmer test I <7 mm/5 min, corneal staining >0 (National Eye Institute/Industry Workshop scale), conjunctival staining >0 (National Eye Institute/Industry Workshop scale) and meiboscore of >5 (Bron/Foulks scoring system) (Sullivan *et al.*, 2010; Lemp *et al.*, 2012).
C. DED was defined by presenting a MQ score of ≥14.5 (Rege *et al.*, 2013).
D. DED was classified into symptomatic† and asymptomatic‡ DED. Symptomatic DED was defined by an OSDI score of ≥13 and fluorescein tear break-time of <10s or corneal and conjunctival fluorescein staining of ≥1 (Oxford grading scale). Asymptomatic DED was defined by an OSDI score of <13 and fluorescein tear break-time of <10s or corneal and conjunctival fluorescein staining of ≥1 (Oxford grading scale) (Asiedu, Dzasimatu and Kyei, 2018).

A standardized DED diagnosis is crucial to attempt towards an accurate disease classification. To this purpose, the TFOS DEWS II proposed an evidence-based DED

diagnostic criteria (Wolffsohn *et al.*, 2017) and recommended its use with additional sub-classification tests. The sub-classification tests included measurements of TMH, LLT, tear evaporation and MGD (Wolffsohn *et al.*, 2017), however, they had no established diagnostic cut-off values.

The present study is the first in proposing a sub-classification system (with established cut-off values) for DED that follows current diagnostic recommendations of the TFOS DEWS II. The sub-classification system was subsequently used to determine the prevalence and potential risk factors of ADDE and EDE among a single population of UK.

5.3 Methodology

The study methodology described in Chapter 2 was used to study the prevalence and potential risk factors of DED subtypes. DED was diagnosed by the TFOS DEWS II criteria (Wolffsohn *et al.*, 2017). The criteria defined the disease by an OSDI score of ≥13 or DEQ-5 score of ≥6 and either the presence of:

- Tear film instability (determined by a NIKBUT of <8s);
- Tear film hyperosmolarity (characterized either by the highest osmolarity value of ≥308 mOsm/l among eyes or an interocular osmolarity difference of >8 mOsm/l);
- Ocular surface damage (described either by >5 corneal staining spots, >9 conjunctival staining spots, or a lower/upper LWE staining of ≥2 mm length and ≥25% width).

Measurements of tear evaporation, TMH, LLT and lower/upper MGD were assessed to aim DED classification, as suggested by the TFOS DEWS II (Wolffsohn *et al.*, 2017). In line with current definitions of ADDE and EDE (Craig *et al.*, 2017), ADDE was described by a reduced TMH, whereas EDE was described by a reduced LLT or an increased tear evaporation or lower/upper MGD (Wolffsohn *et al.*, 2017).

MGD has been previously graded by the quality of the meibum, more specifically, by its expressibility and appearance (Albietz, 2000; Rege *et al.*, 2013; Asiedu, Dzasimatu and Kyei, 2018). In the present study, the condition was defined by meibomian gland dropouts. Meibomian gland dropouts have been previously correlated with altered meibum (Finis *et al.*, 2015) and hence explains the rationale behind the used approach. Besides, it was believed that the meibum might have been already confounded by previous tests involving eyelid eversion and the instillation of ocular dyes.

Sex, age, employment status, health conditions/problems, medication intake, sleep quality and outdoor activity were gathered by the DERFS questionnaire (section 2.2.5.2) and considered in the risk factor analysis of both DED subtypes. As resulted from Chapter 4, the factors have shown to be significant risk factors of DED and, amongst these, sex and health conditions/problems had the greatest statistical significance (p-value ≤0.01).

Sub-classification tests cut-off values were determined from tear evaporation, TMH, LLT and MGD readings of non-DED participants. The readings were initially stratified by sex and by the presence/absence of health conditions/problems to understand whether both factors might have confounded normal tear film characteristics. Because parallel testing of high specific tests gives greater confidence in the

differential diagnosis of DED (Wolffsohn *et al.*, 2017), the study aimed to adopt cut-off values that were as specific as possible.

5.3.1 Data processing

Collected scores of DED and DERFS questionnaires, tear evaporation, tear osmolarity, NIKBUT, TMH, LLT, ocular staining and MGD were entered in a common Excel spreadsheet. Initial and differential diagnoses of DED were performed in participants that have successfully completed the clinical assessment. Negative and positive diagnoses were coded as values of 1 and 2, respectively. A dichotomous variable encompassing purely positive EDE and ADDE outcomes was created to study the risk factors of both DED subtypes. Risk factors were identically categorized as in Chapter 4.

5.3.2 Statistical analysis

Statistical analysis was performed with SPSS version 23 (IBM Corp. released in 2015. New York. US). All ocular parameters were confirmed to be not normally distributed using Kolmogorov-Smirnov tests. Differences between ADDE and EDE signs of female and male non-DED participants with and without health conditions/problems were analysed with U-Mann Whitney tests. Prevalence rates of DED subtypes were presented with 95% CIs. Associations between ADDE and EDE signs and between the sub-classification signs and DED symptoms of DED participants were evaluated with Spearman's rank correlation coefficients. Finally, within DED participants, risk factors of pure ADDE and EDE were determined through phi (for dichotomous risk factors) and point biserial correlation coefficients (for ordinal risk factors).

5.4 Results

Two-hundred and eighty-two Birmingham residents (42.4 ± 18.7 years, 56% females) participated in the study. Recruitment occurred mostly at Aston University campus. DERFS questionnaires were successfully completed by 96% participants. One hundred and sixty-two positive and one hundred and six negative diagnoses of DED were concluded. Missing data resulted from any instrumentation failure or participants' poor collaboration in the clinical assessment.

5.4.1 Dry eye sub-classification signs of non-dry eye participants

Measurements of tear evaporation, TMH, LLT and lower/upper MGD of non-DED participants with health conditions/problems differed significantly among females and males (Table 5.2). However, this was not the case for non-DED participants without health conditions/problems.

Table 5.2 DED sub-classification signs of non-DED participants (stratified by sex and the absence/presence of health conditions/problems)

Subclassification signs (mean ± SD)	**Non-DED participants**								
	Without health conditions/problems				**With health conditions/problems**				
	♀	**n**	♂	**n**	♀	**n**	♂	**n**	
Tear evaporation ($g/m^2/h$)	43.34 ± 2.76	19	37.42 ± 1.86	26	56.48 ± 7.08	29	46.06 ± 5.42	31	
TMH (mm)	0.28 ± 0.02	19	0.28 ± 0.02	26	0.26 ± 0.02	29	0.38 ± 0.03	31	***
LLT score	3.68 ± 0.28	19	3.33 ± 0.23	27	3.83 ± 0.23	29	3.26 ± 0.19	31	*
Lower MGD (%)	19.32 ± 3.11	19	17.22 ± 2.49	27	21.17 ± 2.47	29	20.84 ± 2.33	31	
Upper MGD (%)	23.50 ± 3.77	18	20.93 ± 2.13	27	30.18 ± 3.30	28	26.39 ± 2.65	28	

DED = dry eye disease. * p-value ≤0.05. *** p-value ≤0.001. ♀ = female. ♂ = male. n = sample size. SD = standard deviation.

5.4.2 Cut-off values of dry eye sub-classification tests

The diagnostic cut-off values of the sub-classification tests were based on the distribution of tear evaporation rates, TMH, LLT and upper/lower MGD of non-DED participants without health conditions/problems. These were:

- Tear evaporation of >46 g/m^2/h (Figure 5.1);
- TMH of <0.2 mm (Figure 5.2);
- LLT of grade <3 (Figure 5.3);
- Upper/lower MGD of >28% (Figure 5.4);

Non-DED participants without health conditions/problems were selected to ensure that the cut-off values were applicable for both sexes (section 5.4.1).

5.4.3 Sub-classification of dry eye disease

DED participants with a TMH of <0.2 mm and a normal tear evaporation rate (≤46 g/m^2/h), LLT (a grade of ≥3) and lower/upper MGD (≤28%) were diagnosed with ADDE. In contrast, EDE was defined by a tear evaporation rate of >46 g/m^2/h, LLT of grade <3 and lower/upper MGD of >28%, but a normal TMH (≥0.2 mm).

Where the criteria of both ADDE and EDE was met, DED was classified into a third disease subtype representing an aqueous-deficient/evaporative DED. Conversely, where the criteria of both ADDE and EDE was not met, DED was classified into an unclassified DED.

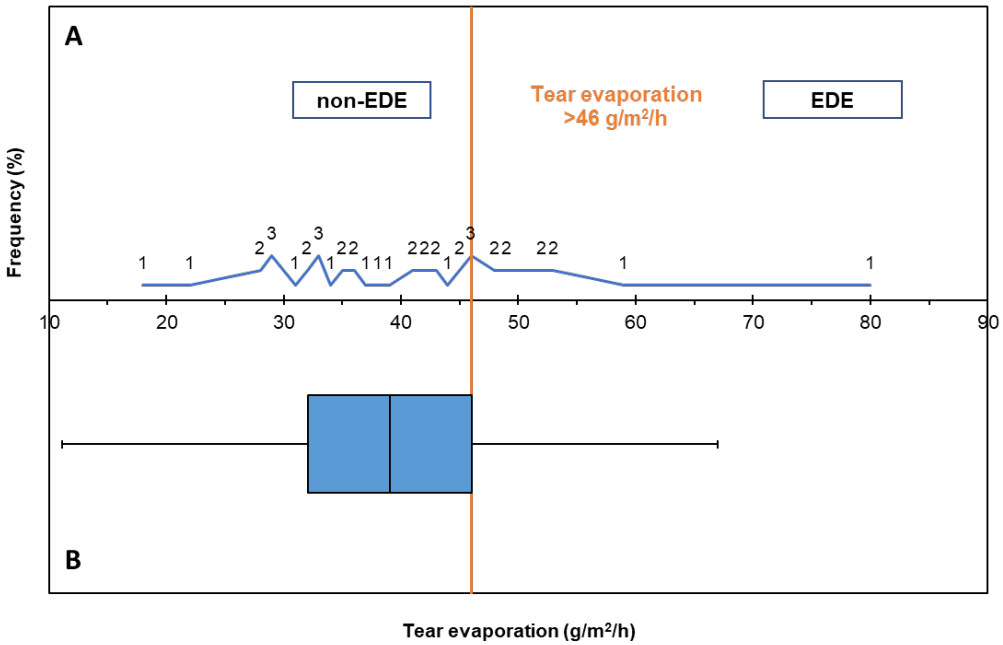


Figure 5.1 Tear evaporation distribution of healthy non-DED participants

EDE = evaporative dry eye; DED = dry eye disease.

(A) Frequency plot of tear evaporation measurements of healthy non-DED participants.

(B) Box plot of tear evaporation measurements of healthy non-DED participants.
First quartile = 32 g/m^2/h; Median = 39 g/m^2/h; Third quartile = 46 g/m^2/h; Minimum = 11 g/m^2/h; Maximum = 67 g/m^2/h.

The tear evaporation cut-off value was set at 46 g/m^2/h. The cut-off value was based on the third quartile of the tear evaporation distribution referring 35 of 45 non-DED participants as non-EDE. The approach used allowed to infer the highest possible test specificity of 77.8% (35/45).

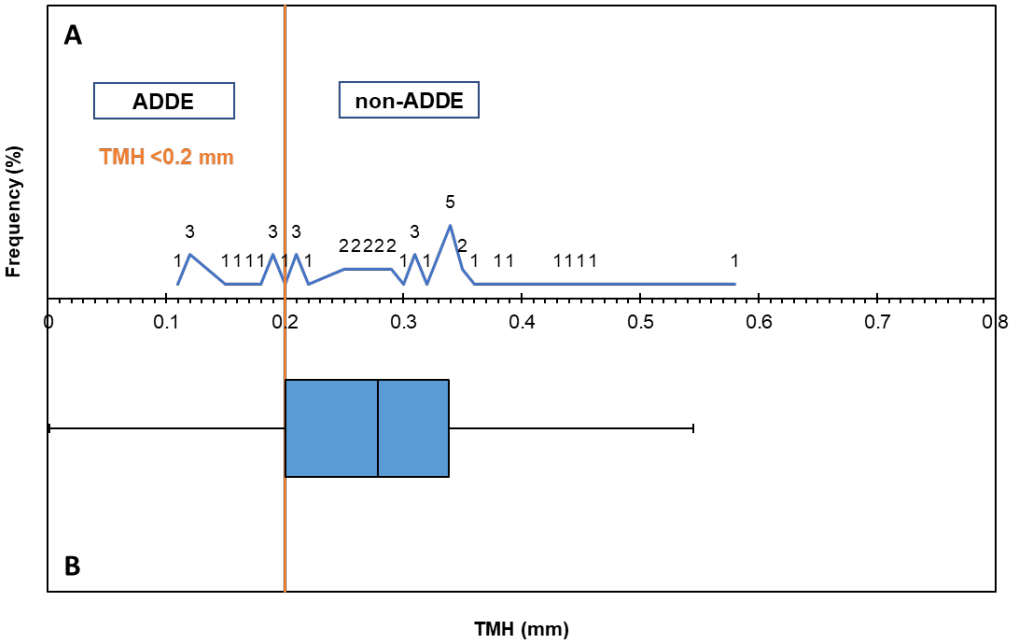


Figure 5.2 TMH distribution of healthy non-DED participants

TMH = tear meniscus height; ADDE = aqueous deficient dry eye; DED = dry eye disease.

(A) Frequency plot of TMH measurements of healthy non-DED participants.

(B) Box plot of TMH measurements of healthy non-DED participants.
First quartile = 0.203 mm; Median = 0.280 mm; Third quartile = 0.340 mm; Minimum = 0.004 mm; Maximum = 0.546 mm.

The TMH cut-off value was set at 0.2 mm. The cut-off value was based on the first quartile of the TMH distribution referring 35 of 46 non-DED participants as non-ADDE. The approach used allowed to infer the highest possible test specificity of 76.1% (35/46).

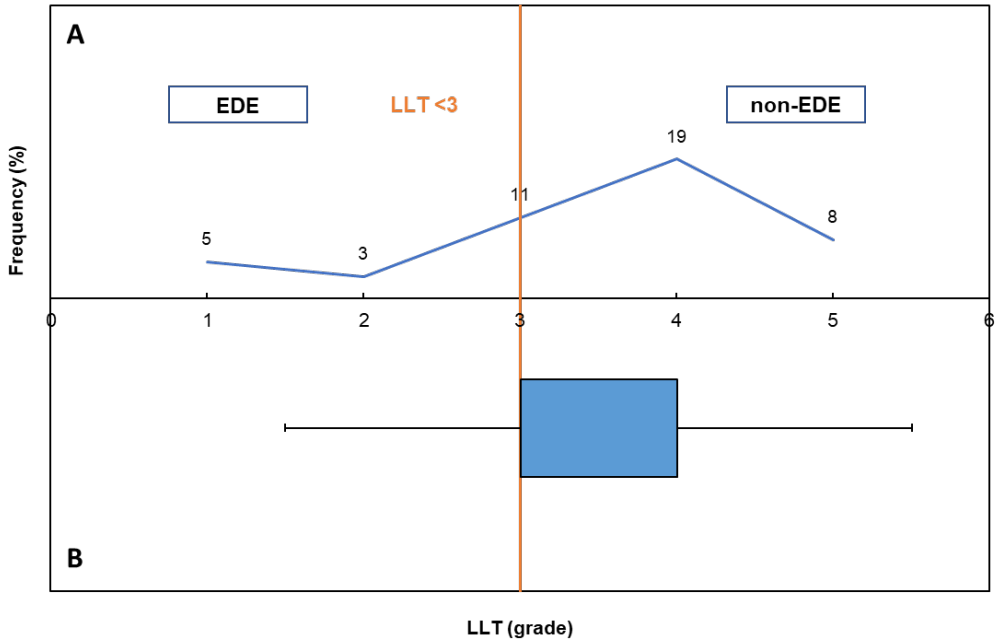


Figure 5.3 LLT distribution of healthy non-DED participants

LLT = lipid layer thickness; EDE = evaporative dry eye; DED = dry eye disease.

(A) Frequency plot of LLT measurements of healthy non-DED participants.

(B) Box plot of LLT measurements of healthy non-DED participants.
First quartile = 3.00; Median = 4.00; Third quartile = 4.00; Minimum = 1.50; Maximum = 5.50.

The LLT cut-off value was set at a grade of 3. The cut-off value was based on the first quartile of the LLT distribution referring 38 of 46 non-DED participants as non-EDE. The approach used allowed to infer the highest possible test specificity of 82.6% (38/46).

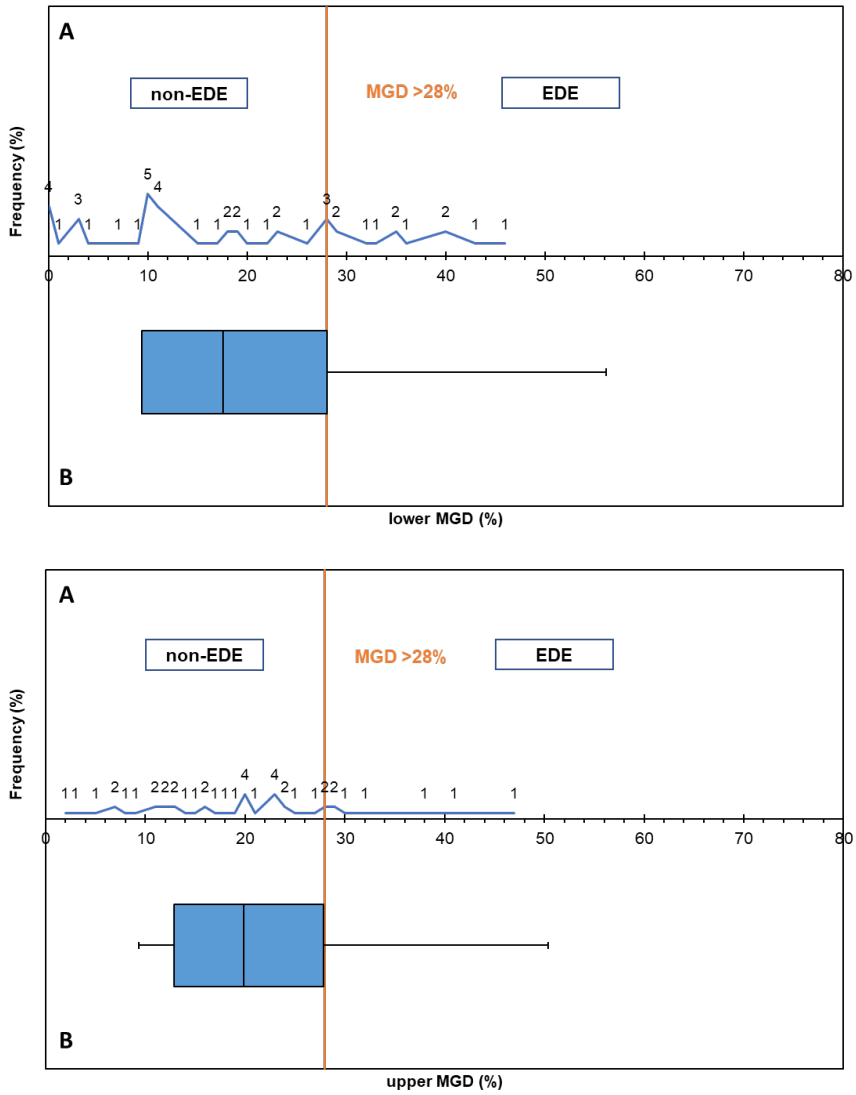


Figure 5.4 MGD distribution of healthy non-DED participants

MGD = meibomian gland dysfunction; EDE = evaporative dry eye; DED = dry eye disease.

(C) Frequency plot of lower/upper MGD measurements of healthy non-DED participants.

(D) Box plot of lower/upper MGD measurements of healthy non-DED participants.

For lower MGD: First quartile = 9.25%; Median = 17.50%; Third quartile = 28.00%; Minimum = 18.88%; Maximum = 56.13%. For upper MGD: First quartile = 13.00%; Median = 20.00%; Third quartile = 28.00%; Minimum = 9.50%; Maximum = 50.50%.

The MGD cut-off value was set at a grade of 28%. The cut-off value was based on the third quartile of the lower MGD and upper MGD distribution referring 35/46 and 35/45 of non-DED participants as non-EDE, respectively. The approach used allowed to infer the highest possible test specificity of 76.1% (35/46) for lower MGD and of 77.8% (35/45) for upper MGD.

5.4.4 Prevalence of dry eye subtypes

DED was classified either into unclassified DED (18.5%), EDE (64.2%), ADDE (6.2%) and both ADDE and EDE (11.1%) (Figure 5.5). Amongst all, EDE was the most prevalent DED subtype.

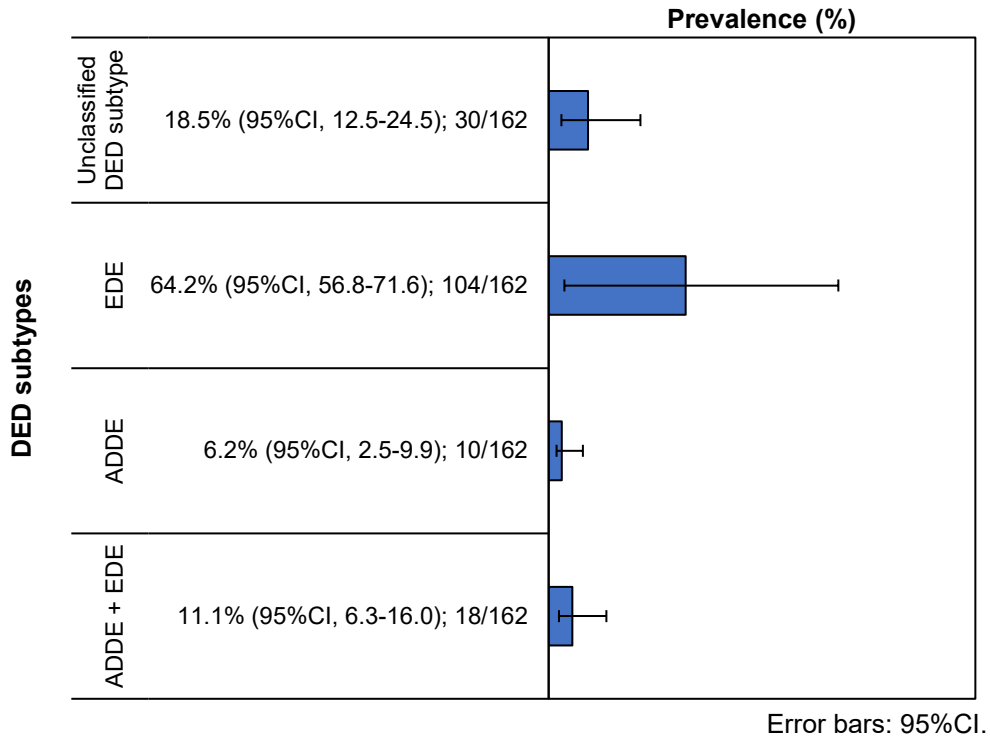


Figure 5.5 Prevalence of DED subtypes

DED = dry eye disease. EDE = evaporative dry eye. ADDE = aqueous deficient dry eye.

5.4.5 Frequency of evaporative dry eye signs in evaporative dry eye participants

MGD was the most commonly observed EDE sign, followed by increased tear evaporation and decreased LLT (Figure 5.6). Of all EDE participants, 45.2% (47/104) presented a combination of EDE signs (Figure 5.6).

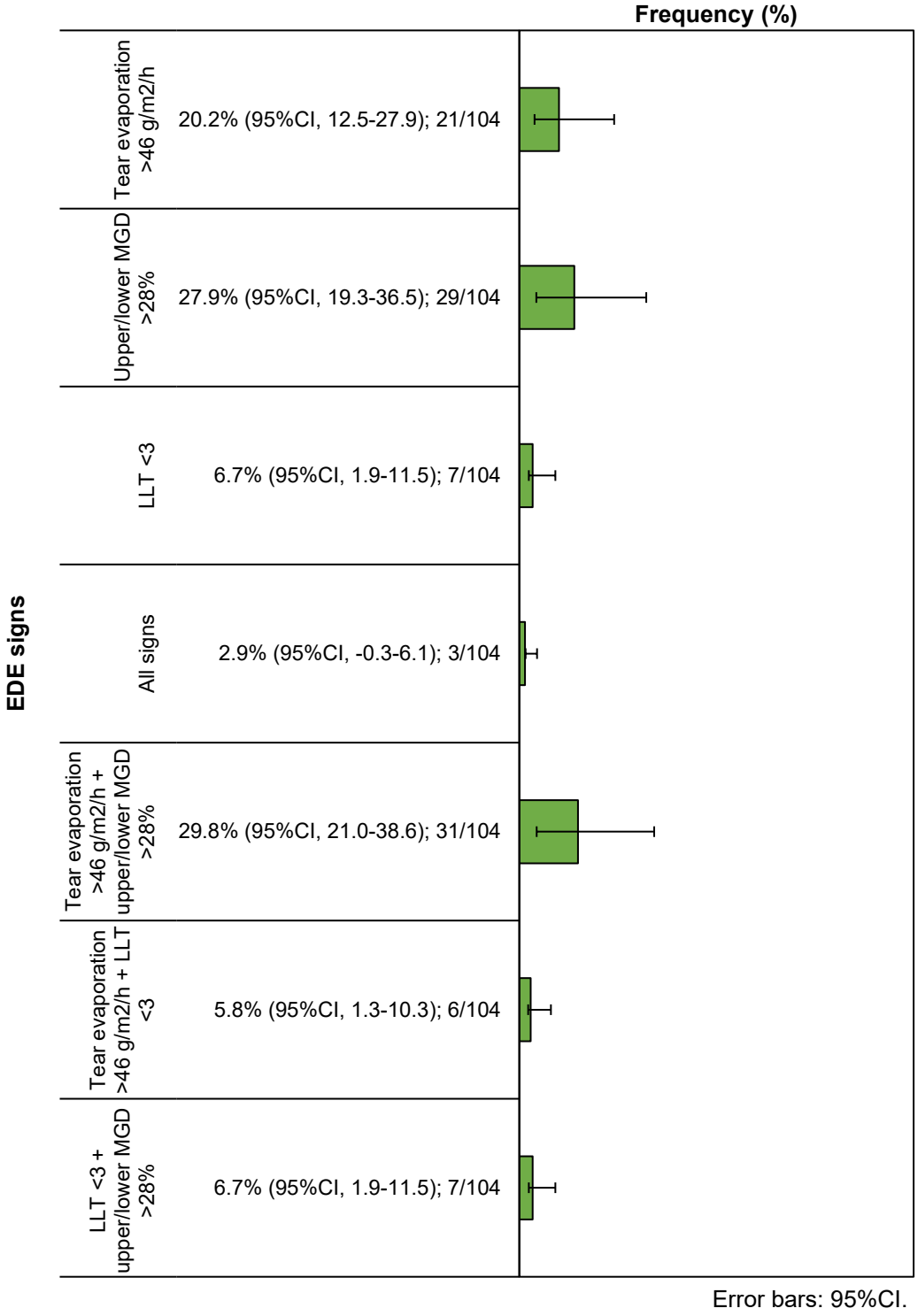


Figure 5.6 Frequency of EDE signs in EDE participants

EDE = evaporative dry eye. LLT = lipid layer thickness. MGD = meibomian gland dysfunction.

5.4.6 Relationship between dry eye sub-classification signs in dry eye participants

Tear evaporation and TMH were significantly positive correlated with each other, as well as with MGD (Table 5.3). LLT did not significantly correlate with any other signs specific to ADDE or EDE (Table 5.3).

Table 5.3 Correlations between DED sub-classification signs in DED participants

Correlation of sub-classification DED signs	Tear evaporation ($g/m^2/h$)		LLT grade		TMH (mm)		Lower MGD (%)		Upper MGD (%)	
	r_S	n	r_S	n	r_S	n	r_S	n	r_S	n
Tear evaporation ($g/m^2/h$)			-0.122	157	0.235**	157	0.171*	157	0.003	149
LLT grade					0.028	162	-0.017	162	0.021	153
TMH (mm)							0.065	162	0.197*	153
Lower MGD (%)									0.133	153

DED = dry eye disease. LLT = lipid layer thickness.TMH = tear meniscus height. MGD = meibomian gland dysfunction. * p-value ≤0.05. ** p-value ≤ 0.01. r_s = spearman rank correlation coefficient. n = sample size.

5.4.7 Relationship between dry eye sub-classification signs and symptoms in dry eye participants

Within dry eye participants, higher DEQ-5 and OSDI scores were significantly associated to increased tear evaporation (Table 5.4). However, no other ADDE and EDE sign was significantly related to DED symptoms (Table 5.4).

Table 5.4 Correlations between DED sub-classification signs and symptoms in DED participants

DED symptoms	Correlation with sub-classification DED signs									
	Tear evaporation ($g/m^2/h$)		LLT grade		TMH (mm)		Lower MGD (%)		Upper MGD (%)	
	r_S	n	r_S	n	r_S	n	r_S	n	r_S	N
DEQ-5 score	0.209**	157	-0.075	162	0.017	162	0.035	162	0.077	153
OSDI score	0.178*	157	0.020	162	-0.017	162	0.046	162	0.132	153

DED = dry eye disease. LLT = lipid layer thickness. TMH = tear meniscus height. MGD = meibomian gland dysfunction. DEQ-5 = 5-item Dry Eye Questionnaire. OSDI = Ocular Surface Disease Index. * p-value ≤0.05. ** p-value ≤0.01 r_s = spearman rank correlation coefficient. n = sample size.

5.4.8 Risk factors of dry eye subtypes

Age was significantly associated with EDE (Table 5.5). Sex, employment status, health conditions/problems, medication intake, sleep quality and outdoor activity did not result to be significant risk factors of either ADDE or EDE (Table 5.5).

Table 5.5 Correlations between pure DED subtypes† and potential risk factors of DED

Risk factors	**Correlation with DED subtypes**		
	$r_{pb/\varphi}$	**n**	
Sex	-0.162	114	
Age	-0.270	114	**
Employment status	-0.172	112	
Health conditions/problems	-0.058	112	
Medication intake	0.051	112	
Sleep quality	-0.062	112	
Outdoor activity	0.137	111	

DED = dry eye disease. ADDE = aqueous deficient dry eye. EDE = evaporative dry eye.
† EDE was coded as 1 and ADDE as 2.
* p-value ≤0.05; ** p-value ≤0.01.
r_{pb} = point biserial correlation coefficient (used for age, sleep quality and outdoor activity).
r_{φ} = phi correlation coefficient (used for sex, employment status, health conditions/problems and medication intake).
n = sample size.

5.5 Discussion

DED can be classified into ADDE and EDE, where the aqueous and lipid layer of the tear film are altered, respectively (Craig *et al.*, 2017). Both DED subtypes can be confused as they present similar symptoms and general DED signs, however, their differential diagnosis is of utmost importance for making right decisions when treating and managing the disease (Jones *et al.*, 2017).

At present, the TFOS DEWS II globally recommends an initial DED diagnosis involving the assessment of ocular symptoms (using either the DEQ-5 or OSDI questionnaire) and signs (including the assessment of NIKBUT, tear hyperosmolarity or ocular surface staining) (Wolffsohn *et al.*, 2017). The present study is the first to

adopt the recommended diagnostic criteria to further propose a sub-classification scheme of DED.

The proposed sub-classification scheme was used to determine the prevalence and risk factors of ADDE and EDE among a single population of UK. It included the evaluation of the tear evaporation, TMH, LLT and upper/lower MGD described by meibomian gland dropouts. The diagnostic tests were also formerly suggested by the TFOS DEWS II for attempting DED classification (Wolffsohn *et al.*, 2017); however, with no established diagnostic cut-off values.

In the present study, tear evaporation, TMH, LLT and upper/lower MGD cut-off values were determined from clinical data of non-DED participants without health conditions/problems. Health conditions/problems were excluded as these significantly confounded normal tear film functions. Cut-off values producing the highest possible diagnostic specificities of the sub-classification tests were selected to allow greater confidence in the differential diagnosis of the disease (Wolffsohn *et al.*, 2017). Accordingly, a TMH of <0.2 mm and LLT of grade ≥3, lower/upper MGD of ≤28% or tear evaporation rate of ≤46 g/m^2/h were used to diagnose DED participants with ADDE. In contrast, EDE was diagnosed by a TMH of ≥0.2 mm and tear evaporation rate of >46 g/m^2/h, LLT grade of <3, or lower/upper MGD of >29%.

Interestingly, the TMH cut-off value used was consistent with that of Uchida et al. (Uchida *et al.*, 2007). The cut-off values for tear evaporation, LLT and MGD were also in good agreement with previous studies associating tear evaporation rates of 48.85 ± 23.47 g/m^2/h (Tomlinson, Doane and McFadyen, 2009), LLT of ≥ 75 nm (Blackie *et al.*, 2009), and meibomian gland dropouts of 30.1 ± 17.4% (Pult, 2018) to non-DED individuals.

The study showed that EDE was the most common form of DED, with a prevalence rate of 64.2%. The findings were in accordance with previous research (Albietz, 2000; Rege *et al.*, 2013; Asiedu, Dzasimatu and Kyei, 2018) and suggest that, in a major DED cohort, the lipid layer may be more compromised compared to the aqueous layer. Indeed, MGD was the most frequently occurring sub-classification sign, followed by increased tear evaporation, impaired LLT and reduced TMH.

It should be noted that 17.3% DED participants had no obvious signs of ADDE and EDE. Unclassified DED has been observed in other studies attempting DED classification. Asiedu et al. reported 23.8% unclassified symptomatic DED participants and 25% unclassified asymptomatic DED participants (Asiedu, Dzasimatu and Kyei, 2018). Lemp et al. also were unable to categorise 29% of their study participants into evaporative, aqueous-deficient or mixed DED (Lemp *et al.*, 2012). Here, the disease might have been presented in the form of watery eyes (due to MGD) (Arita *et al.*, 2015), misleading ADDE diagnosis. Moreover, because the tear film is variable over time, it might be possible that the lack of evidence of the sub-classification signs was caused by stochastic or measurement noise.

Importantly, the DED sub-classification scheme used was composed by essential sub-classification tests. Significant associations between tear evaporation, MGD and TMH underline their combined diagnostic contributions. Moreover, TMH, as the only ADDE sign, and LLT, as the most independent EDE sign, were indispensable to aim DED classification.

Coexistence of both DED subtypes has been associated with increasing disease severity (Craig *et al.*, 2017). A severity matrix was proposed by Bron et al. (Bron *et al.*, 2007), however, due to the apparent severity differences in an individual in distinct

elements of the matrix, DED severity has been rather assessed from the participants' perspective by using symptom self-reports (Wolffsohn *et al.*, 2017). Sullivan et al. described the disease severity as a continuum rather than distinct grades (Sullivan *et al.*, 2010). This last approach is supported by poor associations between the sub-classification tests and DED symptoms, as only tear evaporation and DED symptoms were significantly related to each other.

Overall, the primary goal of DED treatment and management is to reconstruct the homeostasis of the tear film (Jones *et al.*, 2017). Artificial tears of different compositions account the mainstay of DED therapy (Jones *et al.*, 2017). Punctual plugging or tear stimulation, via topical medications, heating eyebags or essential fatty acid supplementation, are also of growing interest (Jones *et al.*, 2017). Other treatment options focus on lid hygiene or avoiding DED risk factors (Jones *et al.*, 2017). One study has shown that DED sub-classification can help to identify effectiveness of different artificial tears formulations in individuals with DED (Essaa, 2015). Future studies are needed to test other products using the new sub-classification scheme developed in this study.

Several large-scale population-based studies have associated DED to different risk factors (Stapleton *et al.*, 2017), but not specifically to DED subtypes. In the present study, EDE was found to be significantly related to age, whereas ADDE had no significant risk factors. The association between EDE and aging can be attributed by functional and structural changes of meibomian glands occurring with increasing age (Sharma and Hindman, 2014).

In conclusion, the study has demonstrated that EDE, as characterized by signs of LLT, tear evaporation and MGD, is far more common than ADDE in Birmingham

residents. Moreover, age is a significant risk factor of EDE. Further research, applying the proposed sub-classification scheme in different populations, is of interest for expanding the knowledge on DED subtypes.

6. CHAPTER 6: IMPROVING DRY EYE EPIDEMIOLOGICAL RESEARCH

6.1 Overview

The present chapter is a collaborative study between Aston University (Birmingham, UK) and the University of Auckland (Auckland, New Zealand) (Wolffsohn *et al.*, 2018). It discusses a cost-effective diagnostic method for DED by ocular symptoms and signs.

6.2 Introduction

Different epidemiological study designs exist to investigate the burden of DED and subsequently plan and allocate health sources (Mann, 2003; Stapleton *et al.*, 2017). The study designs are divided into experimental and observational and can be further sub-categorised as cross-sectional studies, case-control studies, and cohort studies (Mann, 2003).

Observational cross-sectional studies are the most commonly used approach in the epidemiology of DED, whereby the disease prevalence and risk factors are evaluated at one point in time (Stapleton *et al.*, 2017). However, common inferences are difficult to make since the studies have relied on different disease diagnoses (Stapleton *et al.*, 2017).

In view to standardization, the TFOS DEWS II recommended a global diagnosis of the disease (Wolffsohn *et al.*, 2017). This identifies an individual as having DED by a positive result to a validated questionnaire (either the DEQ-5 or OSDI test) and the presence of one ocular sign (determined either by assessing the NIKBUT, tear osmolarity or ocular surface staining) (Wolffsohn *et al.*, 2017).

The TFOS DEWS II criteria has unfortunately not been used in large-scale population studies yet. Cost and limited accessibility of the required clinical instrumentation might have been a barrier to its use. In fact, researchers' tendency is often to move away from high-cost diagnostic techniques towards economical diagnoses of DED that are commercially available in all parts of the world (Savini *et al.*, 2008).

Recently, the Optrex™ dry eye blink test has been advertised as a free rapid self-administered online test that indicates whether an individual might suffer from DED. Like the NIKBUT, it assesses the stability of the tear film, but based on the time that takes for the eyes to sense ocular discomfort when staring up to 15 seconds at a digital screen and without blinking.

The present study is the first to validate the diagnostic ability of the Optrex™ dry eye blink test. The study aim was to propose a cost-effective DED diagnostic method, involving the DEQ-5, OSDI and Optrex™ dry eye blink test, that conforms to the TFOS DEWS II criteria and is cost-effective for DED epidemiological research.

6.3 Methodology

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the institutional ethics committees of Aston University (Birmingham, UK) and University of Auckland (Auckland, New Zealand).

Study participants were recruited from both centres and enrolled after written consent inform. Participants were excluded if they had any active ocular diseases or were currently using ocular medications. The exclusion criteria did not include any further risk factors of DED as the study intended to involve participants of different DED severities in order to reduce spectrum bias (Wolffsohn *et al.*, 2017).

All investigators received substantial clinical training prior to the study. Ocular symptoms were gathered using both DEQ-5 and OSDI questionnaires. In the following order, tear osmolarity, NIKBUT, the Optrex^{TM} dry eye blink test and ocular surface staining (including the cornea, conjunctiva and upper/lower LWE) were assessed on participants' right eye.

Clinical assessment was conducted as described in Chapter 2. However, subjective methods, including two four-point grading scale (Korb *et al.*, 2005; Whitcher *et al.*, 2010) (Table 6.1), were used to score ocular surface staining. The rationale behind this amendment was to ease the clinical study performance between both sites.

Table 6.1 Ocular surface grading scales used

Ocular surface	**Grading scores**
Corneal/conjunctival (Whitcher et al., 2010)	score 0: 0-9 staining dots score 1: 10-32 staining dots score 2: 33-100 staining dots score 3: >100 staining dots
LWE staining (Korb et al., 2005)	score 0: <2 mm long and 25% wide staining score 1: 2 – 4 mm long and 25 – 49% wide staining score 2: 5 – 9 mm long and 50 – 74 % wide staining score 3: > 10 mm long and ≥ 75% wide staining

LWE = lid wiper epitheliopathy.

The Optrex^{TM} dry eye blink test was displayed on a 14-inch computer monitor (Lenovo^{TM} ThinkPad® T470p) at approximately 40 cm (Figure 6.1). The gaze angle varied depending on participants' height and sitting posture.

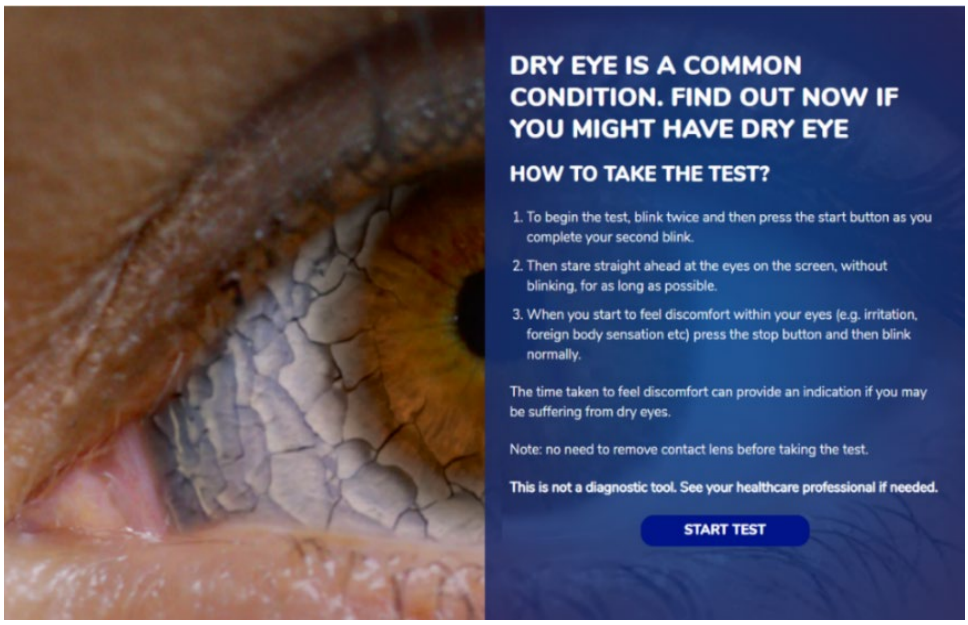


Figure 6.1 The Optrex™ dry eye blink test (reproduced with permission of Reckitt Benckiser)

Study participants were asked to deliver two non-force blinks and hold their blinking after having started the Optrex™ dry eye blink test. Testing with the Optrex™ dry eye blink test ended either after 15 seconds or when ocular discomfort was felt. Three consecutive measurements were taken, and the mean was recorded.

NIKBUT and Optrex™ dry eye blink test readings were compared to understand the level of interchangeability of both tear film stability measurements. Also, correlations of DED signs and symptoms with the Optrex™ dry eye blink test were examined.

DED was defined by the TFOS DEWS II criteria (Wolffsohn *et al.*, 2017), more specifically, by a DEQ-5 score of ≥6 or OSDI score of ≥13 and either one of the following:

- A NIKBUT of <10s,
- A tear film osmolarity of ≥308 mOsm/l or an intraocular osmolarity difference of >8 mOsm/l, or

- Ocular surface staining described by >5 corneal spots, >9 conjunctival spots or the presence of LWE at the inner eyelid margin (≥25% wide and ≥2mm long).

The diagnostic performance of the Optrex™ dry eye blink test was examined against the TFOS DEWS II criteria. An Optrex™ dry eye blink test cut-off score was subsequently determined. The cut-off value was adopted together with a DEQ-5 score of ≥6 or OSDI score of ≥13 to further define DED by ocular symptoms and signs following TFOS DEWS II diagnostic recommendations.

6.3.1 Power calculation

The required sample size of eighty-five participants was calculated using following formula: $n = [(Z_\alpha + Z_\beta)/(0.5x\ln[(1+r)/(1-r)]]^2 +3$, where n was the sample size, α the rate of false positives of DED, Z_α the standard deviation of α, β the rate of false negatives of DED, Z_β the standard deviation of β and r the correlation coefficient (Hulley *et al.*, 2013). The sample size was determined to seek a sizeable correlation coefficient of at least 0.30 between the Optrex™ dry eye blink test and NIKBUT (Jacob Cohen, 1992). Conventional values of α and β were used (α, 0.05; β, 0.20) (Hulley *et al.*, 2013).

6.3.2 Data processing

Because of the Optrex™ dry eye blink test having a maximum duration of 15s, NIKBUT readings of >15s were capped into values of 15s. Moreover, for statistical purposes, both Optrex™ dry eye blink test and NIKBUT readings underwent logarithmic (log) transformation.

6.3.3 Statistical analysis

Statistical analysis was performed using SPSS version 23 (IBM Corp. 2015). Except for tear film stability measurements, DED symptoms and signs were found to be not normally distributed using Kolmogorov-Smirnov tests.

Measurements of Optrex™ dry eye blink test and NIKBUT were compared using paired t and F tests. Correlations of DED signs and symptoms with the Optrex™ dry eye blink test were evaluated with Spearman correlation coefficients. For NIKBUT though, Pearson correlation coefficients were used.

A receiver operative characteristics (ROC) curve of the Optrex™ dry eye blink test was illustrated. The ROC curve was constructed by plotting the rate of true positives against the rate of false positives of DED by the TFOS DEWS II criteria for every possible Optrex™ dry eye blink test value. The area under the ROC curve determined the diagnostic ability of the Optrex™ dry eye blink test. Younden's J indexes (computed as $J = \frac{true\ positives}{true\ positives + false\ negatives} + \frac{true\ negatives}{true\ negatives + false\ positives} - 1 = sensitivity + specificity - 1$) were calculated for all Optrex™ dry eye blink test values. The Optrex™ dry eye blink test value with the greatest Younden's J index (and hence with maximal diagnostic sensitivity and specificity) was defined as the cut-off score.

Finally, both diagnostic sensitivity and specificity of the proposed DED diagnostic method involving the Optrex™ dry eye blink test and DEQ-5 or OSDI questionnaire were evaluated.

6.4 Results

Eighty-seven participants (38 ± 17 years, 44 females) were included in the present study (Table 6.2). Of these, 71% fulfilled the TFOS DEWS II diagnostic criteria of DED.

Table 6.2 Tear film and ocular surface characteristics of the study participants

Characteristics	n	mean ± SD/ median (range)
DEQ-5 score	87	8.72 ± 4.46
OSDI score	87	19.19 ± 15.85
Highest tear film osmolarity value (mOsm/l)	87	305.08 ± 13.57
Interocular osmolarity difference (mOsm/l)	87	8.71 ± 7.40
NIKBUT (s)	87	9.52 ± 7.33
Optrex™ dry eye blink test (s)	87	9.83 ± 3.95
Corneal staining score	87	0 (0-1)
Conjunctival staining score	87	0 (0-1)
Upper LWE score	87	0 (0-0)
Lower LWE score	87	0 (0-2)

DEQ-5 = 5-item Dry Eye Questionnaire, OSDI = Ocular Surface Disease Index. NIKBUT = non-invasive Keratograph tear break-up time. LWE = lid wiper epitheliopathy. n = sample size. SD = standard deviation.

Measurements of the Optrex™ dry eye blink test and NIKBUT were not significantly different (p-value, 0.150). Nevertheless, the Optrex™ dry eye blink test showed a significantly narrower distribution compared to that of the NIKBUT (p-value, <0.001).

Among all DED parameters, significant correlations with the Optrex™ dry eye blink test were observed with OSDI, DEQ-5, NIKBUT, conjunctival staining and lower LWE (Table 6.3).

Table 6.3 Correlations of DED symptoms and signs with the Optrex™ dry eye blink test

	Correlation with the Optrex™ dry eye blink test		
Characteristics	**n**	**Correlation coefficient**	**p-value**
DEQ-5 score	87	-0.364	0.004**
OSDI score	87	-0.290	0.006**
Highest tear film osmolarity value (mOsm/L)	87	-0.066	0.55
Interocular osmolarity difference (mOsm/L)	87	-0.010	0.93
NIKBUT (s)	87	0.470	0.001***
Corneal staining score	87	-0.163	0.13
Conjunctival staining score	87	-0.237	0.03*
Upper LWE score	87	0.018	0.87
Lower LWE score	87	-0.251	0.02*

DEQ-5 = 5-item Dry Eye Questionnaire. OSDI = Ocular Surface Disease Index. NIKBUT = non-invasive Keratograph tear break-up time. LWE = lid wiper epitheliopathy. n = sample size. SD = standard deviation. * p-value ≤0.05. ** p-value ≤0.01. *** p-value ≤0.001.

The diagnostic ability of the Optrex™ dry eye blink test was significant moderately strong (p-value, <0.001), showing an area under the ROC curve of 0.77 (Figure 6.2).

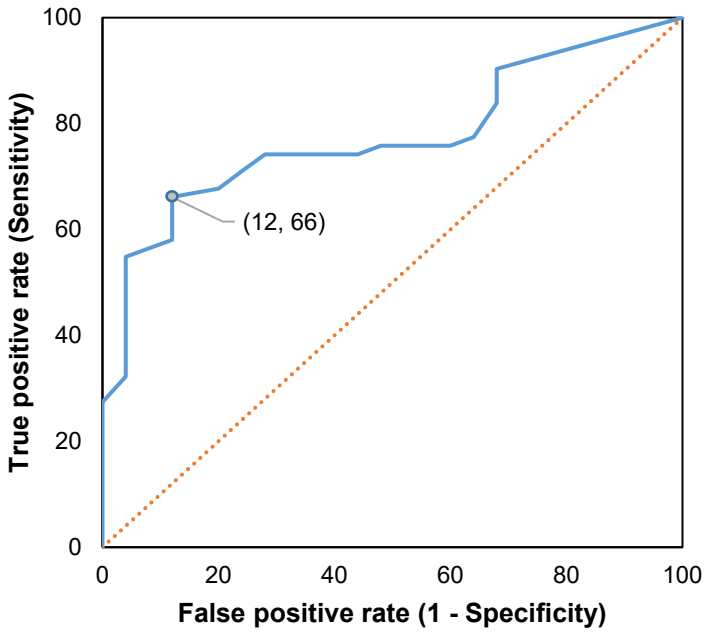


Figure 6.2 ROC curve assessing the diagnostic ability of the Optrex™ dry eye blink test

The greatest Younden's J index of 0.54 was found with an Optrex™ dry eye blink test value of ≤10s. The Optrex™ dry eye blink test value presented a sensitivity of 66% and specificity of 88%.

DED diagnosis by an Optrex™ dry eye blink test of ≤10 s and either by DEQ-5 score of ≥6 or OSDI score of ≥13 presented a diagnostic sensitivity and specificity of 100% and 54%, respectively (Table 6.4).

Table 6.4 DED outcomes by the proposed DED diagnostic method and the TFOS DEWS II criteria

DED outcomes of the proposed diagnostic method	**DED outcomes of the TFOS DEWS II criteria**		
	Negative	Positive	Total
Negative	25	21	46
Positive	0	41	41
Total	25	62	87

6.5 Discussion

Researchers take specially care in balancing the costs and diagnostic accuracy of clinical tests for DED (Savini *et al.*, 2008). In the present study, a rapid online test assessing tear film stability (the Optrex™ dry eye blink) was validated to further propose a DED diagnostic method by ocular symptoms and signs. The proposed method involved the use of the DEQ-5, OSDI and Optrex™ dry eye blink test. It should serve as a simple self-administered DED diagnostic method that conforms to the TFOS DEWS II criteria (Wolffsohn *et al.*, 2017) and is cost-effective for DED epidemiological research.

The results showed that NIKBUT and Optrex™ dry eye blink test readings were similar, enforcing the idea that the Optrex™ dry eye blink test constitutes a feasible alternative method for assessing tear film stability. It is important to note, however, that the Optrex™ dry eye blink test had a significantly narrower distribution than the NIKBUT, even when NIKBUT values were capped at 15s (Wolffsohn *et al.*, 2018). Hence, beyond this point, the disagreement between both tests would probably increase (Wolffsohn *et al.*, 2018).

As expected, a significant positive correlation between the NIKBUT and Optrex's dry eye blink test was observed. It is believed that any tear disruption results in a transient and localised tear osmolarity increase that stimulates nociceptors responsible for driving the blink reflex to replenish the tear film (Varikooty and Simpson, 2009). If tear disruptions account as well as a direct trigger to ocular discomfort, it would be expected that the correlation between the NIKBUT and Optrex™ dry eye blink test would have been greater than moderate (Wolffsohn *et al.*, 2018). Accordingly, the results suggest that symptom self-report might have been influenced by patients' high tolerance to pain (Wolffsohn *et al.*, 2018).

Higher Optrex™ dry eye blink test readings were also significantly associated with decreasing ocular symptoms, either assessed by the DEQ-5 or OSDI, conjunctival staining and lower LWE staining. The associations are supported by current literature (Yeniad, Beginoglu and Bilgin, 2010; Zhang *et al.*, 2017). Other DED signs showed similarly negative trends with higher Optrex™ dry eye blink test readings but without statistical significance.

Generally, the diagnostic ability of a test can be described by its sensitivity and specificity (Lalkhen and McCluskey, 2008). The sensitivity and specificity are measures that describe how well a test correctly identified those with and without a disease, respectively (Lalkhen and McCluskey, 2008). A trade-off typically exists between the two measures with the precise values for each selected cut-off value for a positive diagnosis (Lalkhen and McCluskey, 2008). In the present study, the Optrex™ dry eye blink test showed a significant moderately strong diagnostic ability when performed against the TFOS DEWS II criteria. The diagnostic ability was given graphically by the area under the ROC curve of 0.77, showing a maximal sensitivity of 66% and specificity of 88% for an Optrex™ dry eye blink test cut-off value of ≤10s. The cut-off value was similar to the NIBUT cut-off value reported by the TFOS DEWS II (Wolffsohn *et al.*, 2018).

Because the last updated definition of DED describes the disease by a combination of symptoms and signs (Craig *et al.*, 2017), the Optrex™ dry eye blink test of ≤10s on its own can only be considered as an indicative diagnostic method for DED. Interestingly, DED diagnosis by an OSDI score of ≥13 or DEQ-5 score of ≥6 and Optrex™ dry eye blink test of ≤10s was 100% sensitive and 54% specific to the TFOS DEWS II. The approach may be used as a screening tool as it is rapid and simple to administer.

In conclusion, the Optrex™ dry eye blink test combined with symptoms self-reports could work as a cost-effective diagnostic method for DED, especially for future epidemiological DED research. The Optrex™ dry eye blink test cut-off value used aligned closely with that reported by the TFOS DEWS II, for NIBUT (Wolffsohn *et al.*, 2018).

7. CHAPTER 7: DISCUSSION AND CONCLUSIONS

The epidemiology of DED aims to answer basic research questions – How many people are affected by the disease? Which are the risk factors for the disease? Which are the care health sources needed? These questions, however, encompass enormous methodological and interpretive complexity.

Researchers have assessed DED prevalence and risk factors differently, using a range of disease diagnoses and risk factor assessments. The inconsistencies across published cross-sectional studies have created barriers to interpreting the results. Moreover, the heterogeneity in the characteristics of the population studied has further complicated the research.

A thorough evaluation of existing differences in epidemiological research on DED is essential. In providing such information, researchers would gain a better understanding on DED epidemiology and identify research gaps that need to be filled for future research improvement.

This thesis initially includes a literature review of current DED prevalence rates and risk factors (Chapter 1). It extracted information of cross-sectional studies that have been published since the last decade, emphasizing on the DED diagnostic methods used, the characteristics of the population studied and logistic regression analyses of DED risk factors.

Given the limitations of the current state of epidemiological literature on DED, the overarching goal of this thesis was to perform a well-standardized DED cross-sectional study. Accordingly, a study methodology following global Tear Film Ocular Surface Dry Eye Workshops II (TFOS DEWS II) diagnostic recommendations of the

disease published in 2017 was considered (Chapter 2). This should ensure comparability with future reports.

Prevalence rates of DED (Chapter 3) and DED subtypes (Chapter 5) were estimated among a single population in the UK. Potential risk factors of DED (Chapter 4) and DED subtypes (Chapter 5) were also assessed using a self-administered evidence-based dry eye risk factor survey (DERFS). Lastly, a simple, cost-effective and self-administered DED diagnostic criteria by the TFOS DEWS II diagnostic criteria was developed (Chapter 2).

A summary of the main findings of this thesis by chapter is detailed below:

- **Chapter 1.** This chapter reviewed prevalence rates and risk factors reported in recent epidemiological studies of DED. The prevalence of DED was found to range from 1.3% to 52.9%. Identified risk factors for DED were either modifiable or non-modifiable and included age, sex, health conditions/problems, ambient conditions, contact lens wear, VDT use, diet and sleep duration. The chapter highlighted that the reported prevalence rates and risk factors of the disease varied with the diagnostic methods used and the characteristics of the population studied. DED diagnosis was based either on the WHS criteria, symptoms, signs or both symptoms and signs. Asians were the most commonly studied population in the epidemiology of the disease.

- **Chapter 2.** Having noted the influence of the diagnostic methodology on the disease prevalence and risk factors (Chapter 1), the next key aspect was to optimise this based on the current consensus around diagnosis, but also to consolidate previously identified risk factors into a simple to complete

questionnaire as no suitable 'validated' form currently existed. Hence Chapter 2 included a broad summary of the study methodology used for this thesis. DED questionnaires, including the OSDI and DEQ-5, and measurements of tear osmolarity, tear evaporation, LLT, TMH, NIKBUT, ocular surface staining and MGD were considered. The use of these tests for the initial and differential diagnosis of DED is supported by previous clinical research. According to the TFOS DEWS II, they account together the most efficient battery to diagnose DED as per the last updated disease definition and classification. Moreover, the DERFS questionnaire was used to assess potential risk factors for DED. The survey was developed based on current evidence on DED risk factors discussed in Chapter 1.

- **Chapter 3.** Having clarified the appropriate methodology for studying DED epidemiological estimates and developed the DERFS (Chapter 2), an ethical opinion and governance procedure for the study was sought and granted allowing a cross-sectional study to commence. As such, Chapter 3 determined the prevalence of DED at a single point in time. The diagnostic criteria used was the TFOS DEWS II criteria. The study population included female and male Birmingham (UK) residents aged 18 to 88 years-old. These were stratified in recruitments so that they were representative to the Birmingham population census of 2016. The prevalence of DED varied with the diagnostic method used, ranging from to 19.0% to 56.3%, and was significantly higher where ocular surface staining was assessed. Moreover, ocular symptoms were significantly more prevalent with the DEQ-5 than with the OSDI test. Notably, the study was the first in estimating the prevalence of DED by the TFOS DEWS II criteria. The estimates obtained ranged similar to those discussed in Chapter 1. The DED

diagnostic method based on DED symptoms and tear film instability appeared the most suitable method for the disease.

- **Chapter 4.** Having assessed the prevalence of DED population by the TFOS DEWS II criteria, DED and non-DED participants were identified to allow risk factors to be assessed from the DERFS. Female sex, age, employment status, the presence of any health conditions/problems, medication intake, poor sleep quality and prolonged outdoor activity were found to be significant risk factors of DED. Amongst these, female sex, the presence of any health conditions/problems, poor sleep quality and prolonged outdoor activity had the greatest statistical significance. The results were in accordance with other studies reporting risk factors of DED by either the WHS criteria, symptoms, signs or symptoms and signs.

- **Chapter 5.** DED is sub-classified as having ADDE and EDE forms. The forms are intended to inform management decisions and hence are important to be clinically distinguished. Unfortunately, a review of the literature acknowledged no apparent consistency in the sub-classification of DED. To this purpose, Chapter 5 proposed a differential diagnosis of ADDE and EDE. The differential diagnosis was in line with current TFOS DEWS II diagnostic recommendations. DED and non-DED participants were identified in Chapter 3. ADDE was characterized by a reduction in TMH; conversely, EDE was defined by signs of LLT, tear evaporation and MGD. Diagnostic cut-off values of the sub-classification tests, that were as specific as possible in discriminating ADDE and EDE, were determined from clinical data of healthy non-DED participants. Whereas older DED participants were at major risk of EDE, there was no significant risk factor for ADDE.

Prevalence rates of 6.2% for ADDE, 64.2% for EDE and 11.1% for both ADDE and EDE were estimated. This was in accordance with previous research reporting EDE as the more common form of DED. The approach taken will allow clinicians to make a more informed choice for an initial DED management for individual patients, although further research is needed to warrant advocating this.

- **Chapter 6.** So far, the chapters involved complex clinical testing which is not available to many eye care practitioners and other health care professionals, such as pharmacists and general medical practitioners, who are often approached by patients about DED symptoms and need to be able to make an informed differential diagnosis. Chapter 6 proposed a self-administered diagnostic method for DED that conformed to the TFOS DEWS II criteria. DED was diagnosed by an OSDI score of ≥13 or DEQ-5 score of ≥6 and Optrex^{TM} dry eye blink test of ≤10s. This last test assessed the tear film stability based on the time that takes for the eyes to sense ocular discomfort. The proposed DED diagnosis was found to be 100% sensitive and 54% specific to the TFOS DEWS II diagnostic criteria. The diagnostic method should help patients in empowering their self-management of symptoms with the caveat that, if these persist, a full examination with a suitably equipped and skilled eye-care professional should be sought. Moreover, the diagnostic method could be used for DED epidemiological research, as it is rapid and cost-effective and hence reduces both cost and time boundaries that are usually faced in the disease epidemiology.

As the first of its kind, this thesis serves as an insight into prevalence and risk factors of DED and DED subtypes of a single population in the UK, following current global diagnostic recommendations of the TFOS DEW II.

Clinicians should be aware that ocular surface staining is a common sign that does not necessarily translate into DED. DED by symptoms and ocular surface staining should be carefully distinguished with other ocular conditions involving symptoms and ocular hyperaemia. On the other hand, in the absence of a complete test battery for DED (as recommended by the TFOS DEWS II), the assessment of tear film stability and symptoms may be suitable on its own to diagnose the disease. The Optrex™ dry eye blink test may be used for this purpose.

The main limitations of this thesis are intrinsic to the study design. The association and certainty of the obtained cross-sectional risk factors of DED and DED subtypes would be stronger in a longitudinal study. The sample size calculation might be limited as it was based on a British female cohort; however, this cohort was the only available UK data that could be referred to at the time of the thesis. The prevalence and risk factors of DED and DED subtypes obtained were also specific to the Birmingham, UK population.

In view to future directions in DED research, the author has collected equivalent data in Valencia, Spain. The developed protocol was also facilitated to other colleagues of the European Dry Eye Network (EDEN) and researchers based in New Zealand, China and Mexico to further allow similar data collection and hence future reliable and comparable research data on the epidemiology and sub-classification of DED.

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