

Ocular *Demodex folliculorum*: prevalence and associated symptoms in an Irish population

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Abstract

Purpose To investigate the prevalence of ocular *Demodex folliculorum* (DF) in an Irish population. To validate a modified Ocular Surface Disease Index questionnaire and employ it to evaluate the relationship between dry eye symptoms and the presence of DF.

Methods One hundred and fifty-six subjects were enrolled in an epidemiological cross-sectional prevalence study. Each subject completed a novel questionnaire on ocular symptoms and was assessed for the presence of DF. Data was analysed to assess prevalence and to search for significant links between each symptom and DF.

Results An overall prevalence of 68% was found. Total mean number of DF found on microscopic examination was 3.83 mites per subject (range 0–25). The presence of symptoms was higher among individuals with DF ($P = 0.04$). Itch was found to be the symptom most significantly associated with the presence and number of DF ($P = 0.025$ and $P = 0.035$, respectively). The questionnaire showed good internal

consistency (Cronbach's alpha; $\alpha > 0.7$) and good reliability (Intra-class Correlation Co-efficient; ICC > 0.7). The sensitivity and specificity of a positive symptom result using the questionnaire were 70.8% and 46.9%, respectively.

Conclusion There is a strong association between the prevalence of DF and symptoms, in particular itchy eyes. However, not all patients with DF will be symptomatic. The newly developed questionnaire is a reliable instrument for measuring change in symptoms over a period of time and suitable for observing patient reported outcomes in interventional treatment studies.

Keywords *Demodex folliculorum* · Prevalence · Dry eye symptoms · Blepharitis

Introduction

Demodex folliculorum (DF) and *Demodex brevis* (DB) are eight legged ectoparasites that reside in the pilosebaceous units of human skin [1–5]. The mites feed on epidermal skin cells and sebum and are, therefore, most commonly found in areas rich in sebaceous glands—cheeks, nose, chin and the periocular area [2, 4–6]. DF inhabits the eyelash follicles. It uses its claws to scrape at the internal walls of the lash follicles resulting in follicular distention, epithelial hyperplasia and reactive hyperkeratinisation

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[3, 5, 7, 8]. It is suggested that this forms a translucent cuff at the base of the lash [9]: cylindrical dandruff (CD). It has also been proposed that CD is the result of an inflammatory cicatrix formed from dead DF within the follicle [10]. CD is considered a pathognomonic sign for DF infestation of the eyelash follicles [11]. Studies suggest DB resides in the sebaceous glands and contributes to meibomian gland dysfunction (MGD) by causing granulomatous changes to the glandular cells and also physically blocking the gland resulting in an accumulation of meibum within the duct [3, 5, 8]. Given the location of the mites on the eyelids; DF appears to be associated with anterior blepharitis and DB with MGD. The resulting eyelid inflammation causes inflammatory proteins to be released into the tear film causing ocular surface inflammation and irritation [12, 13].

Various ocular abnormalities such as anterior blepharitis, MGD, aqueous deficient and evaporative dry eye share similar symptoms involving the ocular surface; itch, irritation, redness, burning sensations [7, 14, 15]. This creates a challenge for practitioners to distinguish between each condition when screening patients based on symptoms alone.

Another dilemma is that dry eye is a multifactorial disease, and the symptoms of dry eye and ocular surface disease fluctuate and often do not correlate well with the degree of ocular signs present [16–19]. Symptoms and severity of symptoms may vary according to the condition and time of day [20]. MGD and blepharitis are more commonly associated with a foreign body sensation and sticky eyes in the morning, while aqueous deficient dry eye appears to get worse throughout the day [20]. DF and DB can produce an inflammatory reaction on the ocular surface [21]. Itching and crusting at the eyelid margin are among the most common symptoms associated with DF infestation [7, 15, 22, 23]. This is most likely due to the presence of CD on the eyelash margin and the movement of the mites across the surface of the skin. As mites are most active at night [24], it could be hypothesised that patients with DF infestation would be more symptomatic at night or in the morning after the mites have been most active. To try to better understand the relationship between DF and symptoms, it is important for subjects to report on the frequency and severity of them. This also applies to functional vision such as reading, using a computer, and environmental conditions known to exacerbate

symptoms in certain ocular surface diseases, for example, wind and air conditioning [20, 25].

Increasingly, patient reported outcomes (PROs) are becoming an integral part of clinical trials [26, 27]. Monitoring PROs after treatment is a good way of investigating the effect of treatment on the patient [27]. In 2011, the International Workshop on Meibomian Gland Dysfunction discussed the increasing importance of PROs in clinical trials. A recommendation was also made to attempt to identify specific symptoms for specific conditions, as the difficulty in distinguishing between symptoms of different anterior abnormalities is an ever present challenge [28]. The present questionnaire was developed and analysed to assess the relationship between DF and dry eye symptoms. The capacity for the questionnaire to be used as a diagnostic screener for DF blepharitis and its ability to assess change in symptoms over the course of treatment was also evaluated.

Materials and methods

Ethics statement

This study was conducted under the Tenets of Helsinki Declaration of Human Studies [29] after approval by the Dublin Institute of Technology Research Ethics Committee.

Examination

Subjects from the National Optometry Centre, local catchment area, and staff and students of Dublin Institute of Technology were invited to part-take in a cross-sectional prevalence study for ocular DF. Inclusion criteria: ≥ 18 years of age. Exclusion criteria: subjects currently being treated for blepharitis or who had used treatment in the past 6 months, active ocular infection (excluding blepharitis) or ocular surgery within the past 6 months. Each subject completed the novel questionnaire and was assessed for the presence of DF. Presence of DF was defined as: positive sighting of DF on lash rotation and/or one or more DF counted on microscopic examination. One eyelash from each eyelid was first manipulated in clockwise and counter-clockwise directions using a sterile forceps, to stimulate DF tails, if present, to emerge from the lash follicle. Subsequently, the lash was epilated

for microscopic examination. Currently, rotation of the eyelash prior to epilation is not a standardised technique. However, its use in visualising DF prior to epilation has been utilised in previous studies [5, 30]. Furthermore, it has been highlighted that lash manipulation has the potential to provide an alternative method to lash epilation for visualising DF in a clinical setting, although further research is required at present [31, 32]. Thus, epilation and microscopic examination remain the recommended method for DF diagnosis [7, 32]. Adult DF count was recorded using the modified Coston method [11]. Data was analysed to assess the overall prevalence of DF found, and to search for significant links between each symptom and the presence and quantity of DF.

Questionnaire development

The symptom questionnaire was styled on the validated OSDI symptom questionnaire (Attachment 1). It has been suggested that altering an existing questionnaire may help increase sensitivity of the symptom questionnaire for the diagnosis of MGD or blepharitis [33]. The OSDI format was chosen, as it has shown good repeatability and validity for effectively measuring the severity of dry eye [34]. It is one of the most commonly used symptom questionnaires administered to subjects in DF related clinical trials [15, 35, 36], and the scoring system utilised by OSDI is scored on scale of 0–100 with higher scores indicating increased severity of symptoms. Each item in the questionnaire is graded on a 4-point Likert scale indicating frequency of the symptom in question; where 0 equals none of the time, 1—some of the time, 2—half of the time, 3—most of the time, and 4—all of the time. The total OSDI score is calculated using the following formula: [total symptom number (A) \times 25/number of questions answered (B)] [34, 37]. As the formula takes into consideration the number of questions answered, it is possible to use the formula to get OSDI values for each of the subscales [34]. Previous studies have also combined questions from different subscales to produce separate subscores using the OSDI formula [38]. Lee et al. [36] modified the OSDI questionnaire by adding questions relating to blepharitis (itchy eyes and matter along the eyelid margin), to increase the questionnaires' sensitivity to detect DF. Similar to Lee et al. [36], questions relating to itchy eyes and particulate matter along the eyelid margin were added

to the current questionnaire. Questions on blurred vision and poor vision were removed from the symptom subscale and replaced with symptom questions from other validated dry eye questionnaires; McMonnies (dryness, burning), 5-item Dry Eye Questionnaire (DEQ-5) (dryness, watery), Standard Patient Evaluation of Eye Dryness (SPEED) (dryness, burning sensation, watery eyes). Novel questions (itchy eyes and red eyes) not found in previous dry eye questionnaires were included due to the high reports of such symptoms found in the literature [7, 15]. Symptoms were analysed in three different ways: the presence of symptoms, the total modified OSDI score, and the severity of symptoms. The presence of symptoms was defined as asymptomatic or symptomatic (regardless of severity). The severity of symptoms was graded from the total modified OSDI symptom score as shown in Table 1 [34, 37, 39].

Questionnaire validation

Suitability for use is dependent on the questionnaire being reliable and reproducible: that any change detected by the instrument is real and not as a result of poor repeatability [40]. The reliability and reproducibility of the questionnaire was measured in two ways. First, the internal consistency of the questionnaire was assessed using Cronbach's α . In keeping with the literature, alpha value > 0.7 was accepted [41, 42]. Second, the intra-rater reliability was assessed using intra-class correlation (ICC) [43] and the test–retest method; $P < 0.4$ signifies poor reliability, $0.4 \leq P \leq 0.75$ signifies fair to good reliability and $P \geq 0.75$ signifies excellent reliability [44].

It is necessary that the questionnaire is also responsive and sensitive to change as a result of treatment [40]. This was measured by conducting a test–retest post-treatment for both a non-treatment

Table 1 Severity of symptoms in accordance with total modified OSDI score

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

group and a treatment group and comparing the mean using a 2-tailed *t* test ($P < 0.05$ significance).

To allow for comparison with previous methods of validation of dry eye questionnaires, a factor analysis was applied to the symptom section of the questionnaire to determine if subscales similar to the OSDI dry eye questionnaire existed. Cronbach's α was applied to each subscale.

A receiver operating characteristics curve was generated to determine the sensitivity of the symptom questionnaire for the diagnosis of DF infestation at each symptom grade.

Statistical analysis

Statistical analysis was performed using the mathematical programme IBM SPSS (ver. 24.0). All summarised continuous data was expressed as mean and range. The data between categorical variables was assessed using Chi square analysis (X^2). Between-group data was assessed using the Student's *t* test, Mann–Whitney *U* test (MWU) and Kruskal–Wallis H (K–W) test where appropriate. Spearman's correlation co-efficient (r_s) was used to assess scaled and continuous variables.

Results

Questionnaire validation

One hundred and fifty-six subjects completed the questionnaire once. Suitable subjects were invited to enrol in a separate interventional DF study. Data analysis is ongoing and will be discussed in a separate paper upon completion. Fifty separate subjects completed the questionnaire twice for the test–retest assessments.

Factor analysis was applied to results from the 156 participants (age: 45.18 ± 18.36 years, gender: male $n = 70$; 45%, female $n = 86$; 55%) who filled out the questionnaire at least once. Extraction method 'principal axis factoring' was chosen, as the data was non-parametrically distributed [45]. Factor analysis showed three sub-scales for the questionnaire similar to the OSDI questionnaire: ocular symptoms, vision related function and environmental triggers [34] (Table 2).

Cronbach's α was applied to each subscale and to the questionnaire as a whole. Cronbach's α for the overall symptom questionnaire was good at 0.824, each of the subscales had a slightly lower α value but were still > 0.7 , see Table 3 [41, 42].

Fifty separate subjects completed the questionnaire for the test–retest method to examine the reliability of the questionnaire. The questionnaire was administered twice, two weeks apart, at the same time of day, with no change to their daily routines or general circumstances between testing. The test–retest reliability of the questionnaire was determined by calculating the ICC. Results can be seen in Table 3. All scores exceeded 0.7 which is the desired criteria to be met [44].

An assumption of the test–retest reliability assessment is that a subject's condition remains stable between the first test and the retest, as no intervention took place. This is evident from the strong ICC value seen for symptom score of 0.893. Taking this into consideration, a post hoc ICC was performed to compare the repeatability of the symptom score after two weeks of treatment in the ongoing interventional treatment study. It was expected that the correlation would be much weaker, as subjects' symptoms should have changed after receiving treatment. This hypothesis was confirmed with an $ICC = 0.655 < 0.893$. A two-tailed *t*-test was applied to both sets of data. There was no significant difference in total symptom score in the test–retest group ($P = 0.536$) in comparison to a highly significant difference in retest total symptom score in the group that received treatment ($P = 0.000$). Treatments included were; Dr. Organic Tea Tree Face Wash, OcuSoft Plus lid scrubs and a once off treatment with BlephEx followed by nightly lid scrubs using OcuSoft Plus. The placebo effect of receiving treatment must be taken into consideration when assessing how effective treatments are at improving symptoms. However, for the purpose of assessing the questionnaires ability to measure change in subjective symptoms, the placebo effect is considered extraneous.

The receiver operator characteristic (ROC) curve was plotted to evaluate the diagnostic ability of the symptom questionnaire to assess for the presence of DF. A relatively flat ROC curve was formed, with an area under the curve (AUC) of 0.614 (Fig. 1). A positive symptom result $\geq G1$ gives a sensitivity of 70.8% and a specificity of 46.9% for the questionnaire.

Table 2 Factor analysis was applied to the modified OSDI questionnaire

	Factor		
	Ocular symptoms	Vision related function	Environmental triggers
<i>Factor analysis</i>			
Dryness	0.534		
Gritty/irritated	0.696		
Itchy	0.636		
Red eyes	0.546		
Burning	0.403	0.373	– 0.233
Photophobia		0.342	
Watery			0.614
Lids stuck together	0.209		
Reading		0.722	
Night driving		0.518	
Computer		0.745	
Television		0.499	0.244
Wind			0.886
Cold air	0.230		0.610
Air conditioning	0.220	0.246	0.277

Extraction method: principal axis factoring

Rotation method: oblimin with Kaiser normalization

Kaiser–Meyer–Olkin measure of sampling adequacy: 0.784

Results show 3 subscales similar to OSDI; Ocular Symptoms, Vision Related Function, Environmental Triggers. Burning sensation, discomfort in cold air and discomfort in air conditioned environments loaded on more than one factor. This is likely due to the multifactorial nature of dry eye and common crossover between symptoms and causes

Table 3 Reliability analysis: Cronbach's α measuring internal consistency, and ICC measuring repeatability for the questionnaire

	Internal consistency: Cronbach's α (95% confidence interval) ($n = 156$)	Test–retest: Intra-class Correlation Co-efficient (95% confidence interval) ($n = 50$)
Reliability of the symptom questionnaire		
Ocular symptoms	0.741	0.824
Vision related function	0.795	0.725
Environmental triggers	0.825	0.887
Symptom questionnaire as a whole	0.839	0.897

Results are shown for each of the subscales and for the overall questionnaire as a whole. All values were > 0.7

As expected, as severity of symptoms increase (G3 severe symptoms), the sensitivity drops to 33%, but the specificity increases to 85.7%.

Questionnaire application

One hundred and fifty-six subjects completed the questionnaire and were assessed for presence and

quantity of DF. The overall prevalence of DF found was 68%. The total mean number of DF found per subject on microscopic examination was 3.83 mites (range 0–25). Subjects included 70 males and 86 females with a mean age of 45.98 ± 18.36 years (range 19–82 years). The presence and quantity of DF was not significantly different between genders ($P = 0.061$ and $P = 0.695$, respectively). Females,

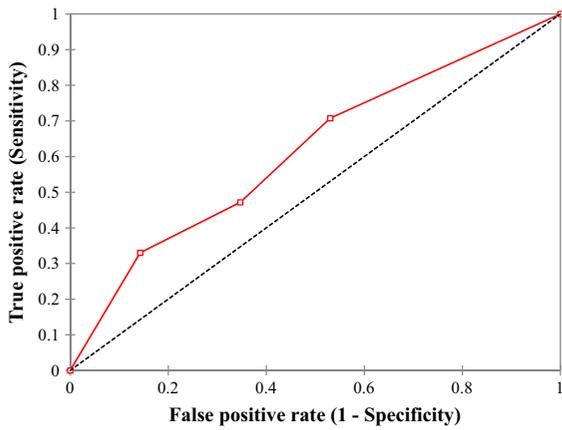


Fig. 1 ROC curve demonstrates the ability of the novel questionnaire to assess for presence of DF using symptom grade (Normal—Severe: 0–3). AUC = 0.614

however, were more symptomatic ($P = 0.026$) (Table 4). The presence of DF was significantly associated with increasing age (t -test; $P < 0.001$). Likewise, the quantity of DF was significantly associated with increasing age also (r_s 0.386; $P < 0.001$).

Presence of *Demodex folliculorum* versus symptoms

Subjects with DF were more symptomatic than those without DF (X^2 ; $P = 0.04$) (Fig. 2). As such, the total modified symptom score was significantly higher in subjects with DF (25.01 ± 18.86) in comparison to subjects without DF (18.81 ± 15.55) (MWU; $P = 0.05$). Likewise, the severity of symptoms increased in subjects with DF in comparison to subjects without DF (X^2 ; $P = 0.028$) (Fig. 3). The greatest difference was found in the severe symptom group. Very few ($n = 7$) subjects without DF had severe symptoms (Fig. 3). The majority of subjects without DF were asymptomatic. By comparison, the majority of subjects with DF had symptoms; the

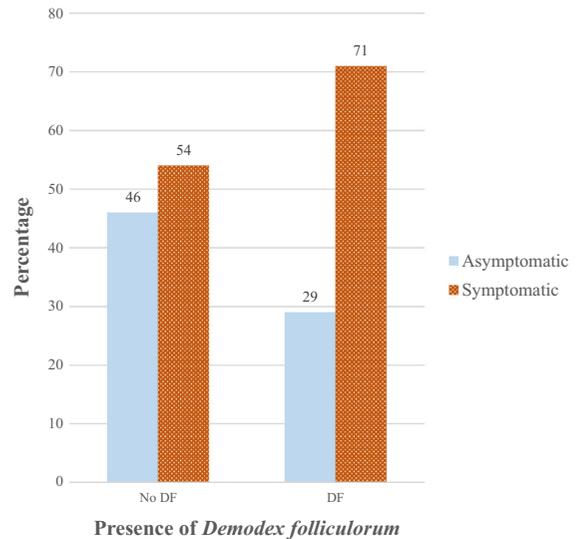


Fig. 2 Percentage frequency distribution of symptomatic and asymptomatic subjects, with and without DF. Subjects with DF were significantly more symptomatic (X^2 ; $P = 0.04$)

majority of which were severely symptomatic ($n = 35$) (Fig. 3).

The prevalence of each symptom reported by subjects with and without DF is shown in Table 5. The presence of the symptom itch was significantly associated with the presence of DF. A symptom of ‘itchy eyes’ was more commonly reported by subjects with DF than those without (69 vs. 52%) (X^2 ; $P = 0.025$). The severity of itch for the presence of DF was not found to be significant (X^2 ; $P = 0.131$). Overall subjects with ‘itchy eyes’ were not significantly older than those without ‘itchy eyes’ (MWU; $P = 0.826$). Subject’s with ‘itchy eyes’ and DF were significantly older than those with ‘itchy eyes’ and no DF (MWU; $P < 0.001$). Asymptomatic individuals with DF were also older, but not significantly (MWU; $P = 0.323$).

A functional vision symptom of ‘discomfort watching television’ was also significantly associated with

Table 4 Comparison of age, presence and quantity of DF, and OSDI scores for male and female study participants*

Sex	N	Age (years)	Prevalence (%)	Demodex count	OSDI score
Male	70	44.83 ± 18.03	74.29	4.02 (range 0–22)	19.54 ± 15.69
Female	86	46.93 ± 18.69	62.79	3.69 (range 0–25)	25.87 ± 19.39
	$P = 0.477$ (A)	$P = 0.126$ (B)	$P = 0.695$ (A)	$P = 0.026$ (A)	

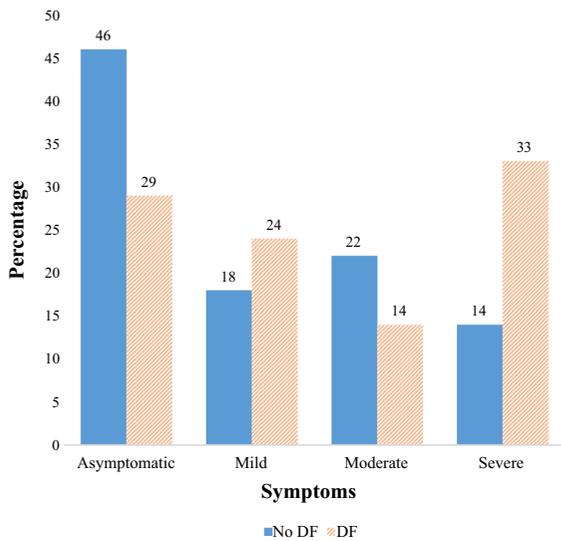


Fig. 3 Percentage frequency distribution of grade of severity of symptoms among subjects with and without DF (X^2 ; $P = 0.028$). 46% of subjects without DF were asymptomatic and only 14% had severe symptoms. Only 29% of subjects with DF were asymptomatic, however, 33% had severe symptoms. It is also evident that the majority of symptomatic subjects with DF were severely symptomatic

the presence of DF (42 vs. 12%) (X^2 ; $P = 0.000$). However, overall subjects reporting ‘discomfort when watching television’ were significantly older than those without any discomfort (MWU; $P = 0.010$). As the symptomatic group is older, they will be

predisposed to an increase in presence of DF given the significant risk factor that age is for DF [15, 46].

Quantity of *Demodex folliculorum* versus symptoms

The total mean number of DF found per subject on microscopic examination was 3.83 mites (range 0–25). Not all subjects with DF were symptomatic. The mean number of mites found was significantly higher among symptomatic subjects (4.53 mites: range 0–25) in comparison to asymptomatic subjects (2.51 mites: range 0–19) (MWU; $P = 0.021$). Spearman’s correlation showed no significant relationship between quantity of DF and the modified OSDI score ($r_s = 0.122$; $P = 0.128$). However, a low positive correlation was established between the number of DF mites and increasing grade of severity of symptoms ($r_s = 0.162$; $P = 0.0439$). This correlation is expressed in the equation: $Y = 0.0267 X$ (number of DF) + 1.2565, and suggests that on average 1 DF mite = G1.28 symptoms. An increase of 28 more mites is required to produce moderate symptoms, and a further 38 extra (minimum 66 mites) required to produce severe symptoms. Spearman’s correlation demonstrated an equation similar to above, $Y = 0.0264 X$ (number of DF) + 1.0552, that an increase in severity of ‘itchy eyes’ was also positively associated with an increase in the quantity of DF

Table 5 Prevalence of symptoms reported by subjects with and without DF; given as percentage of subjects in each group

Symptom	Subjects with <i>Demodex folliculorum</i> (%)	Subjects without <i>Demodex folliculorum</i> (%)	P value (X^2)
Gritty/irritated	72	70	0.685
Itchy	69	52.0	0.025
Dryness	68	68	0.880
Wind	57	62	0.459
Air conditioning	55	44	0.251
Watery	53	60	0.347
Photophobia	45	46	0.959
Red eyes	45	40	0.378
Computer	43	40	0.656
Cold air	43	30	0.167
Problems reading	42	36	0.300
Television	42	12	0.000
Burning sensation	28	18	0.137
Lids stuck together	21	12	0.184
Night driving	19	8	0.063

($r_s = 0.172$; $P = 0.035$). Likewise, this equation suggests that 1 DF = G1.08 ('itchy eyes' some of the time), but on average, an increase of a further 36 DF mites, respectively, are required to produce respective increases in severity of symptoms. Thus, the above equations for severity of symptoms and severity of itch show that the presence of just 1 DF can produce mild symptoms, but symptoms can also be present without DF, as seen previously.

An increase in severity of 'discomfort when watching television' was found to be significantly associated with an increase in quantity of DF ($r_s = 0.164$; $P = 0.044$). Further analysis showed that this increase was directly parallel to an increasing age for the same symptom ($r_s = 0.24$; $P = 0.003$). Given the low overall prevalence (32%) of 'discomfort when watching television', even among subjects with DF, and the significant increase in age of those symptomatic, it is possible that age-related dry eye changes may be contributing to this symptom. However, presence and quantity of DF should still be considered as it is an age-related change, and very few control subjects reported discomfort.

Time of day did not appear to affect symptoms with respect to DF. No other individual symptom was found to be significantly associated with the presence or quantity of DF.

Pathogenic infestation

Earlier studies have suggested not all incidence of DF is pathogenic, and it appears to be an increase in quantity of DF that causes "pathogenic" infestation [47, 48]. Using the severity scale suggested by Randon et al. [48], ≥ 3 mites per follicle, data was analysed to look at the prevalence of "pathogenic" DF in the study population, and any associations between "pathogenic" DF infestation and age and symptoms (Table 6).

Subjects with "pathogenic" DF infestation were significantly older than subjects with "non-pathogenic" DF infestation (MWU; $P = 0.014$) and subjects with no DF (MWU; $P < 0.001$). Subjects with "non-pathogenic" DF infestation were older, but not significantly, than subjects with no DF (MWU; $P = 0.067$).

Subjects with "pathogenic" and "non-pathogenic" DF were more symptomatic than subjects with no DF, although this difference was not found to be significant

for either group (MWU; $P = 0.076$ and $P = 0.118$, respectively). The highest prevalence of the symptom 'itchy eyes' was among subjects with "pathogenic" DF, however, when compared to subjects with no DF, this was not significant (X^2 ; $P = 0.077$).

Discussion

Demodex folliculorum is commonly associated with ocular surface disease [7]. The prevalence of DF reported in the literature varies from 20 to 100%, increasing with age and existing presence of ocular surface disease. Kemal et al. [49] found a prevalence of 28.8% in blepharitis subjects and 26.7% among normal individuals. Gao et al. [11] reported a prevalence of 100% among patients with CD, and 22% among patients without CD. Kabataş et al. [22] discovered a prevalence of 67.2% in blepharitis patients and 54.9% in control patients. Roth [46] reported an overall prevalence of 84% that increased to 100% in subjects over 70 years of age. Lee et al. [36] found an overall prevalence of 70% in their demographic epidemiology study. The present study, in good agreement with previous studies, found an overall prevalence of 68% which was higher in older and in symptomatic subjects.

The current study discovered a relatively high prevalence of DF, even among normal population. However, there is an ongoing debate over the pathogenicity of the ectoparasites [7, 50–52]. The findings in this study are in agreement with previous authors [53, 54], that DF can be found among asymptomatic individuals. The present study found an overall mean number of 2.51 mites (range 0–19) among asymptomatic individuals. Lacey et al. [52] suggested that in many cases DF is a mere commensal organism with possible beneficial roles—ingesting bacteria and other micro-organisms in the lash follicle. Forton et al. [55] demonstrated in dermatology that < 5 mites/cm² is considered normal, and that higher densities are seen in the presence of disease or immunodeficiency. Baima et al. [50] postulated that the mite has the potential to become pathogenic to the host where numbers of mites increase beyond a critical level. Randon et al. [48] suggest this level as ≥ 3 mites per follicle. The increase in symptoms, as seen in previous studies [15, 22, 23, 36] and in the current study also, would suggest that DF does have

Table 6 Comparison of quantity of DF, age, presence of symptoms, modified OSDI score, and presence of itch for subjects with; no DF, mild “non-pathogenic” infestation, and “pathogenic” infestation of DF. A = Kruskal–Wallis P value; B = χ^2 P value

	Frequency (n) (Prevalence %)	Quantity of mites (mean \pm SD)	Age (yrs) (mean \pm SD)	Presence of Symptoms (%)	Modified OSDI (mean \pm SD)	Presence of Itch (%)
No <i>Demodex folliculorum</i>	50 (32%)	0.00 \pm 0.00	37.76 \pm 17.02	54.00	18.81 \pm 15.55	52.00
Mild/Normal infestation (< 3 mites per follicle)	39 (25%)	1.54 \pm 0.68	44.62 \pm 19.07	64.10	25.79 \pm 19.49	66.67
Pathogenic infestation (\geq 3 mites per follicle)	60 (38.5%)	8.97 \pm 5.20	53.60 \pm 16.51	75.00	24.02 \pm 17.33	71.67
Mites visible on lash manipulation but not on microscope	7 (4.5%)	0.00 \pm 0.00	47.14 \pm 14.36	71.43	29.27 \pm 28.79	57.14
			< 0.001 (A)	0.141 (B)	0.274 (A)	0.161 (B)

pathogenic potential as the numbers of DF present an increase. Furthermore, in an ongoing study, these symptoms were shown to be improving following treatment, thus strengthening the argument that the DF mites do have pathogenic potential. Sędzikowska et al. [23] recently published results of a large-scale study investigating the association between DF and presence of symptoms reported by patients without the use of a questionnaire. The results propose that a minimum of 7 DF mites per 8 epilated eyelashes is required to produce 1 symptom, and that a further 18 mites are required to produce a second symptom. In the study conducted by Sędzikowska et al. [23], they did not measure severity of these symptoms reported by patients: intermittent vs constant, sometimes vs all of the time, merely the presence of the symptom. The current study investigated the severity of symptoms reported by patients using a modified version of the OSDI questionnaire. Applying a similar statistical analysis as that applied by Sędzikowska et al. [23], the current study found a much higher number (33) of DF was required to produce moderate symptoms. While the results of both studies show good agreement, they are not directly comparable, as the current study counted DF mites on 4 epilated eyelashes, whereas Sędzikowska et al. [23] counted DF mites on 8 epilated eyelashes.

Symptoms can be present in the absence of DF, and similarly DF can be present in the absence of symptoms. A prevalence of 68% DF was found in the current study. Of these affected individuals, only 71% (75/106) had symptoms, (χ^2 ; $P = 0.028$)

(Fig. 2). A potential reason for this is that chronic inflammation of the anterior ocular surface can lead to changes in corneal morphology [56], leading to corneal hypoesthesia, thus resulting in reduced symptoms in the presence of severe infestation and inflammation [57–59]. As a result, patient reported symptoms do not always correlate well with clinical signs of ocular surface disease, as was demonstrated in the current study. While it appears that the majority of individuals with DF have symptoms; nonetheless, asymptomatic individuals can also have DF. As such, this study found that among those 106 individuals with DF, the quantity of DF seen on microscopic examination was similar between asymptomatic and symptomatic subjects (4.39 ± 4.37 mites vs 6.16 ± 5.82 mites, respectively; MWU; $P = 0.185$). A potential cause for this could be that chronic infestation and inflammation has resulted in hypoesthesia at the ocular surface resulting in an asymptomatic subject with large numbers of DF. However, as DF reside in the follicle, the crawling, scrapping movement of the mites would be expected to create an itching sensation, which should not be affected by corneal hypoesthesia. A more likely possibility is that not all DF was removed during lash epilation. During the study, it became apparent that especially in cases where lashes were loose in damaged lash follicles due to the presence of high numbers of DF, the lash would slip out leaving the DF still inside the lash follicle. This further enhances the need to combine symptoms and a thorough clinical work-up when screening and treating patients for DF. It should be taken into consideration

when assessing for the presence of DF that as the severity of infestation increases, the looser the lashes become. This increases the possibility of DF mites remaining in the lash follicles. The authors recommend using lash manipulation in combination with epilation to accurately assess the severity of DF infestation. Further research into the benefit of lash manipulation is currently taking place and will be described in more detail in a future paper.

The literature reports certain symptoms commonly associated with DF; itch, burning sensation, foreign body sensation, redness and crusts along the lid margins, blurred vision and misdirection of eyelashes [7, 15, 22, 23, 60–62]. Ocular dryness (74.7%), itching (42.78%), and irritation (39.1%) were reported in a study by Koo et al. [15]. Kabataş et al. [22] stated prevalence values of symptoms of redness (80%), itching (63.6%) and foreign body sensation (55.6%) in DF positive blepharitis patients. Sędzikowska et al. [23] reported similar symptoms but lower values: itching (28%), redness (21%), watery eyes (15%), and dryness (6%) among others. The use of specific questionnaires was not discussed by Kabataş et al. [22] or Sędzikowska et al. [23]. The lower prevalence values reported in the study by Sędzikowska et al. [23] are likely as a result of symptom reporting not being prompted by the use of a questionnaire; but depended on each subject complaining of a symptom of their own accord. The most commonly reported symptoms by subjects with DF in the current study were gritty irritated eyes (72%) followed by; itch (69%), dryness (68%), watery (57%), photophobia (45%), red eyes (45%), burning sensation (28%), and lids stuck together (21%). Many of these symptoms were also commonly reported by subjects that did not have any DF, and were not found to be significantly associated with DF. However, in agreement with previous studies [22, 23, 62], the current study found that the symptom ‘itchy eyes’ was associated with an increased presence of DF. Furthermore, the current study demonstrated that the severity of ‘itchy eyes’ increased as the number of DF increased. This further strengthens the basis for ‘itchy eyes’ as a significant symptom of DF infestation.

It should be noted that the symptom ‘itchy eyes’ was also reported by approximately 50% of the control subjects, this was not unexpected. Dry eye is multifactorial and its symptoms are subjective, and therefore can be interpreted differently by each individual.

For example, itch is one of the hallmark symptoms of allergy. Data collection for the current study took place over 2 years, therefore, it is possible that a history of allergy influenced the severity of the symptom itch. However, Chi square analysis showed no significant correlation between the presence of allergy and the presence of general symptoms, or symptoms of itch among subjects that did not have DF (X^2 ; $P = 0.79$ and $P = 0.085$, respectively). The authors are not suggesting ‘itchy eyes’ as a diagnostic symptom of DF; merely that it appears to be a more prevalent and frequently reported symptom among individuals affected with DF infestation. Potentially, the presence of the symptom itch is as an allergic response to the presence of DF which has been hypothesised previously [7, 63].

The OSDI questionnaire is one of the most commonly used symptom questionnaires administered to subjects in DF related clinical trials [15, 35, 36]. Results from the present study and previous studies outlined above have demonstrated that itch is amongst the most common complaint in subjects with DF. However, no question on the original OSDI exists to ask about ‘itchy eyes’. The modified questionnaire developed by Lee et al. [36] included a question on ‘itchy eyes’, and demonstrated that the overall OSDI score was significantly associated with increased numbers of DF. Nonetheless, the authors of the current study were unable to infer if the questionnaire modified and administered by Lee et al. [36] had been validated and therefore would suggest the utilisation of the current questionnaire, or similar validated questionnaire (that includes questions regarding itchy eyes) in future studies relating to DF.

Conclusion

The novel questionnaire demonstrated good internal consistency (Cronbach’s α was > 0.7 for both the total questionnaire and each of the subscales) and good to very good repeatability (> 0.75) for both the total questionnaire and each of the subscales in the test–retest ICC. The strong repeatability component of the questionnaire shows that it can be used as a valid means of monitoring patient’s subjective symptoms as a result of treatment over time in a clinical setting. This is becoming increasingly more important as

PROs become a critical part of patient-centred management in the health sector [26].

The questionnaire displayed a reasonable sensitivity value of 70.8% for correctly identifying subjects found positive for DF infestation. However, for verifying the presence of DF infestation and knowing who requires further intervention, this would not be sufficient and indicates that a thorough clinical work-up would also be required to confirm diagnosis.

To the best of the authors' knowledge, this is the first study that has looked at the prevalence of ocular DF and its associated symptoms in an Irish population. There was a strong link between the presence and quantity of DF and severity of symptoms, using the modified OSDI questionnaire. Itch was significantly associated with the presence of DF above any other symptom reported by patients. In clinical practice, it is important to remember the presence of DF in patients complaining of itchy eyes. Based on the findings of the current study, it would be advisable to utilise the current questionnaire or a questionnaire that includes questions about itch when managing and treating anterior ocular disorders such as blepharitis. However, a thorough clinical work-up is still required for differential diagnosis between various anterior ocular disorders.

Not all patients with DF will be symptomatic, even when infestation is seemingly severe. As with many anterior ocular disorders, symptoms can be similar and may not always be present. The relationship between DF infestation and corneal hypoesthesia needs to be investigated further and additional research into the triggers that cause a patient to become symptomatic is still required.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. This research was conducted as part of a self-funded postgraduate research degree.

Human and animal rights All procedures performed in this study involving human participants were in accordance with the ethical standards of Dublin Institute of Technology and with the *Tenets of Helsinki Declaration of Human Studies* [29].

Informed consent Informed consent was obtained from all individual participants included in the study.

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