• Clinical Research •

Demodex-infected qualitative index based on meibomian gland dropout in *Demodex* blepharitis

Lin Fu[1], *Xiao-Yu Chen*[1], *Sen Wang*[1], *Sheng-Lu Yao*[1], *Heng-Li Lian*[1], *Ya-Na Fu*[1], *Xin-Xin Yu*[1], *Zu-Hui Zhang*[1], *Qi Dai*[1,2]

[1]National Clinical Research Center for Ocular Diseases, Eye Hospital, Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China

[2]College of Mathematical Medicine, Zhejiang Normal University, Jinhua 321004, Zhejiang Province, China

Co-first Authors: Lin Fu and Xiao-Yu Chen

Correspondence to: Qi Dai. National Clinical Research Center for Ocular Diseases, Eye Hospital, Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China. dq@mail.eye.ac.cn

Received: 2026-04-14 Accepted: 2026-06-24

Abstract

● **AIM:** To explore a novel morphological feature of the meibomian gland (MG) and evaluate its prevalence and diagnostic utility in *Demodex* blepharitis.

● **METHODS:** This study included an initial cohort examined for *Demodex* using *in vivo* confocal microscopy and an additional validation cohort examined using conventional light microscopy. The new *Demodex*-infected qualitative (DQ) dropout was defined as the appearance of one or more fully atrophied glands among relatively healthy MG. Diagnostic efficiency was assessed using a receiver operating characteristic (ROC) curve, and diagnostic consistency was tested using Cohen's kappa. The association between ocular surface parameters and DQ dropout was assessed.

● **RESULTS:** The initial cohort included 105 participants, comprising 70 *Demodex*-positive (DP; 18 males and 52 females; median age, 38y; range, 21–51y) and 35 *Demodex*-negative (DN; 11 males and 24 females; median age, 33y; range, 24–55y) individuals. The DQ dropout feature was 64.3% and 2.8% in the DP and DN groups, respectively (P<0.001). ROC analysis yielded an area under the curve (AUC) of 0.807. Substantial inter-rater agreement was achieved in confirming DQ dropout, with a Cohen's kappa of 0.617. In the validation cohort of 887 patients, DQ dropout prevalence remained higher in the DP group than in the DN group (59.9% *vs* 28.5%, P<0.001), with strong expert agreement (kappa=0.642, P<0.001), and an AUC of 0.671. A multivariable logistic regression model incorporating tear meniscus height, MG area, and energy curve achieved an AUC of 0.969.

● **CONCLUSION:** This novel morphological feature of DQ dropout is common in *Demodex* infection, demonstrating good diagnostic ability and substantial inter-observer agreement.

● **KEYWORDS:** *Demodex* blepharitis; meibomian gland dysfunction; *Demodex*-infected qualitative dropout

DOI:10.18240/ijo.2026.11.06

Citation: Fu L, Chen XY, Wang S, Yao SL, Lian HL, Fu YN, Yu XX, Zhang ZH, Dai Q. *Demodex*-infected qualitative index based on meibomian gland dropout in *Demodex* blepharitis. *Int J Ophthalmol* 2026;19(11):2148-2155

INTRODUCTION

Demodex blepharitis is a meibomian gland dysfunction (MGD) caused by *Demodex* infestation, a common ectoparasite in humans first described in 1841[1]. Eyelid inflammation due to *Demodex* infestation is usually characterized by redness and encrustation of the lid margins, viscous yellowish discharge, eyelash loss, sleeve formation, and increased pigmentation around the roots of the remaining lashes[2]. *Demodex* disturbs the ocular surface microenvironment and causes microstructural changes in the meibomian gland (MG), which can induce or aggravate MGD; greater mite burden correlates with more severe structural damage. However, *Demodex* blepharitis remains underdiagnosed and undertreated worldwide[3].

Despite the obvious clinical manifestations of *Demodex* blepharitis, 58.7% of patients are never diagnosed with blepharitis[4], which often leads to misdiagnosis and inappropriate treatment. A positive correlation exists between advancing age and susceptibility to *Demodex* infestation[5], suggesting a higher incidence among older individuals. However, this demographic distribution may also reflect historical deficiencies in training, education, and public awareness, which have impeded clinicians' understanding of *Demodex* blepharitis. These gaps have likely resulted

in fewer targeted screenings and under-reporting of cases. With enhanced diagnostic acumen and adoption of advanced examination techniques such as confocal microscopy, the true prevalence of ocular *Demodex* infestations is expected to be higher than previously anticipated.

The low diagnostic rate of *Demodex* blepharitis stems from the absence of specific symptoms or signs of isolated infections. Patients with MGD, especially those with *Demodex* blepharitis MGD, have a characteristic MG dropout or more severe total dropout, with unevenly distributed MG morphology[6-7]. Previously, we employed a deep learning-based artificial intelligence system to analyze the morphological changes in MG and developed digital biomarkers, such as the uneven index and energy curve[8-9], to detect and diagnose *Demodex* blepharitis. Indeed, recent advancements in ocular imaging have increasingly utilized deep learning algorithms to automatically quantify MG loss and analyze microstructural alterations with high precision[10-12]. However, these indices are quantitative parameters that require complex calculations, making them more suitable for research purposes and challenging to implement in clinical settings.

To enhance clinical applicability, this study defines a novel qualitative morphological index of *Demodex* blepharitis and investigates its relationship with ocular surface health, thereby improving the clinical management of *Demodex* blepharitis.

PARTICIPANTS AND METHODS

Ethical Approval The study protocol was approved by the Institutional Review Board of Wenzhou Medical University (approval number: H2023-022-K-20) and was conducted in accordance with the Declaration of Helsinki.

Participants This two-stage retrospective cohort study enrolled patients with MGD who presented to the Dry Eye Department of the Eye Hospital, Wenzhou Medical University, between February 2019 and August 2024. Stage 1 (diagnostic-transition cohort) aimed to compare the clinical characteristics between *Demodex*-positive (DP) and *Demodex*-negative (DN) patients with MGD following the introduction of *in vivo* confocal microscopy (IVCM). Stage 2 (historical-validation cohort) involved evaluating the predictive value of MG dropout for *Demodex* infestation when the conventional eyelash epilation method was routinely used. Based on our pilot data (n=50), the area under the curve (AUC) for *Demodex*-infected qualitative (DQ) dropout was estimated to be 0.80. Post-hoc power analysis indicated that a minimum of 94 participants (assuming a 2:1 ratio of DP to DN cases) would be required to achieve 80% statistical power at α=0.05. Beginning in September 2022, *Demodex* diagnosis was transitioned from light microscopy. Due to the relatively low false-negative rate of IVCM and to minimize verification bias, all patients who underwent IVCM after this date and tested negative for *Demodex* were automatically enrolled in the DN group (n=35). Each DN patient was matched with two DP patients of the same sex and age (±5y), yielding a 1:2 individually matched DP group (n=70). Prior to this transition, diagnosis relied on traditional eyelash epilation followed by light microscopic mite detection. To validate the predictive performance of MG dropout, we retrieved the records of 887 consecutive patients with MGD who had undergone standard eyelash epilation examination before IVCM became available. Exclusion criteria included history of ocular trauma or surgery, current acute ocular surface inflammation, other ocular or systemic diseases affecting the MG and tear film function, corneal contact lens wear within 2wk, use of medications or eye drops affecting the MG and tear film function within 2wk, and MG density<0.05 (to reduce the error caused by extreme MG dropout).

Diagnosis of *Demodex* Blepharitis The diagnostic criteria for blepharitis were based on the patient's history and positive slit-lamp findings, according to the Blepharitis Preferred Practice Pattern of the American Academy of Ophthalmology[13]. The diagnosis of DP blepharitis is primarily based on ocular surface-related symptoms, including dry eye, itchy eye, foreign body sensation, lid margin congestion, and cylindrical dandruff on the eyelashes, as well as positive microscopy findings[13]. For the conventional *Demodex* examination, three eyelashes were taken from each eyelid under slit-lamp microscopy, placed parallel to each other on a glass slide, covered with a coverslip, and examined under light microscopy for *Demodex* mite identification and counting. With recent advances in IVCM, direct visualization and counting of *Demodex* mites under a confocal microscope have gradually replaced eyelash epilation. Owing to its simplicity, non-invasiveness, and superior reliability, IVCM has become the mainstream diagnostic modality[14]. Using light microscopy, DP infection was defined according to the Chinese diagnostic criteria: 1) observation of all life stages of *Demodex*, such as mites, larvae, or eggs; 2) observation of more than three mites in any eyelid; 3) findings below these criteria classified as suspicious positive, requiring clinical correlation for diagnosis[15]. During standard IVCM examination, the presence of more than one mite per eyelash was considered consistent with DP[14]. DN was diagnosed in patients without *Demodex* infestation or suspected *Demodex* infestation. Both standards were used in this study: IVCM for the diagnostic cohort, and conventional eyelash epilation with light microscopy for the validation cohort. Since these two methods differ in detection principle, sensitivity, and classification schema, the diagnostic certainty of negative status was not fully equivalent between the two cohorts. But this allowed us to evaluate the performance of DQ dropout across real-world clinical scenarios.

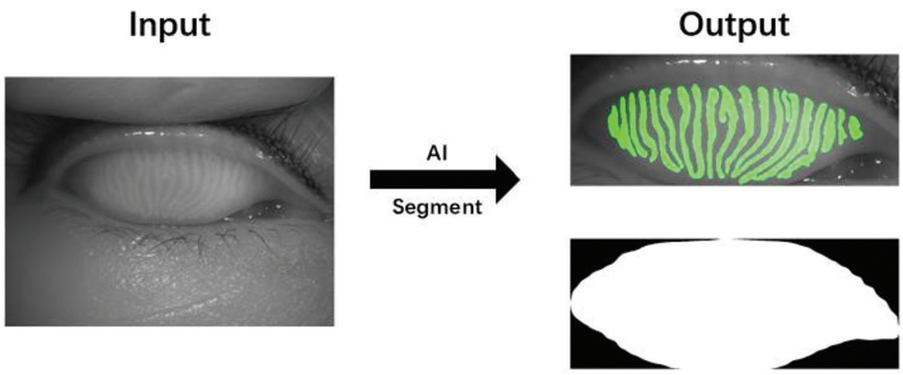


Figure 1 Segmentation of the meibomian gland AI: Artificial intelligence.

Ocular Surface Examination All patients underwent slit-lamp examination and completed ocular surface evaluations, including tear meniscus height (TMH) and the first and average non-invasive tear film break-up times (NIBUT). Both examinations were performed by experienced examiners using the Keratograph 5M (Oculus, Wetzlar, Germany).

Acquisition and Analysis of Meibomian Gland Images Meibography images were acquired using the Keratograph 5M (Oculus, Wetzlar, Germany) to scan the upper eyelids[16]. After the images were exported, they were processed using our previously developed MG segmentation artificial intelligence system[17] to delineate the glands from the tarsal plate (Figure 1), enabling the subsequent calculation of additional metrics such as the energy curve.

Morphological Indices

Characteristic DQ dropout DQ dropout is a newly identified morphological sign in the present study, which is defined as the coexistence of one or more completely atrophied glands alongside relatively intact glands with preserved lipid content and no full atrophy. It is characterized by the total loss of the gland or only remnants of the gland opening (Figure 2 A1–A2).

Prior to the formal grading session, two experts received a 1h standardized training module. During the formal reading phase, a double-blind, randomized sequence was strictly followed; the two physicians independently completed two rounds of scoring within 2wk, using separate workstations and without time constraints.

To evaluate the predictive efficiency and consistency of DQ dropout for *Demodex* blepharitis, the two experts examined the meibography images from 105 participants who underwent IVCM. Subsequently, both experts evaluated the meibography images of patients whose eyelashes had previously been epilated for mite detection. This validation cohort comprised 887 patients, encompassing 968 DP eyes and 766 DN eyes, and was used to assess the predictive performance of DQ dropout as a biomarker for *Demodex* infestation.

Statistical Analysis All statistical analyses were performed using SPSS version 27.0 (SPSS for Windows, Chicago, IL, USA). Data are presented as mean±standard deviation, range, or median (interquartile range). An independent samples *t*-test or the Mann-Whitney *U* test was used to compare differences between groups. Cohen's kappa was used to evaluate the concordance in the diagnosis of *Demodex* blepharitis between the two experts. A P-value<0.05 was considered statistically significant. The diagnostic efficacy of each parameter was evaluated using the receiver operating characteristic (ROC) curve analysis.

RESULTS

Among the patients who underwent IVCM, 70 were DP, and 35 were DN. The baseline characteristics and ocular surface examination results are presented in Table 1. No significant differences were observed between the two groups for sex or age. The TMH was significantly lower in the DP group than in the DN group (P=0.029). Regarding NIBUT, neither the first measurement nor the average NIBUT differed significantly between the groups (both P>0.05). As shown in Table 2, the MG quantitative parameters in the DP group, including the MG area and density, were both reduced compared with those in the DN group (both P<0.05). No significant difference in the vagueness values was observed between the groups (P=0.13). As shown in Table 3, the energy curves[9] between the two groups differed significantly (P<0.001).

Based on these quantitative MG parameters, the characteristic DQ dropout was first defined in this study as one or more fully atrophied MG or remnant MG openings among relatively healthy MGs (Figure 2). Two experts evaluated the images according to the DQ dropout definition. In the IVCM cohort, the proportion of DQ dropout was significantly higher in the DP group than in the DN group (64.3% *vs* 2.8%, P<0.001). Inter-expert agreement was substantial, with a kappa coefficient of 0.617 (P<0.001). To validate and compare the diagnostic performance of DQ dropout and the energy curve, ROC curves were generated and the AUCs were calculated. As shown in Figure 3, the DQ dropout achieved an AUC of 0.807 [95% confidence interval (CI): 0.725–0.889], with a sensitivity

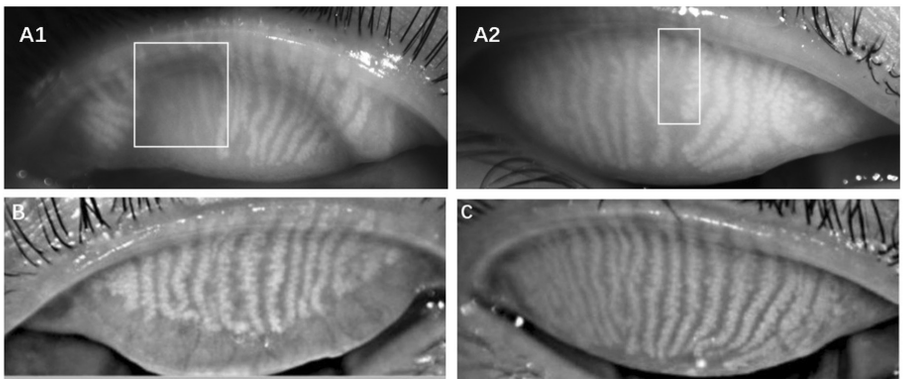


Figure 2 The feature of *Demodex*-infected qualitative (DQ) dropout (A1–A2) compared to even meibomian gland (MG) dropout (B) and normal MG (C).

Table 1 Participant characteristics and ocular surface parameters

Parameters	*Demodex* negative group (*n*=35)	*Demodex* positive group (*n*=70)	*P*
Sex	M=11 (31.4%); F=24 (68.6%)	M=18 (25.7%); F=52 (74.3%)	0.537
Age (y)	33 (28, 39)	38 (29.75, 42)	0.139
Range	24–55	21–51	
Tear meniscus height (mm)	0.274±0.082	0.230 (0.190, 0.260)	0.029
Range	0.150–0.460	0.100–0.400	
First NIBUT (s)	4.210 (3.060, 6.500)	4.685 (2.870, 7.520)	0.547
Range	1.720–10.520	1.340–12.810	
Average NIBUT (s)	6.989±2.534	6.785 (4.963, 9.898)	0.708
Range	1.720–12.270	2.290–14.480	

M: Male; F: Female; NIBUT: Non-invasive tear film break-up time.

Table 2 Upper lid MGs quantitative compare from the meibography examination

Parameters	*Demodex* negative group (*n*=35)	*Demodex* positive group (*n*=70)	*P*
MG area	95340.540±15396.426	66051.790±19705.534	<0.001
MG density	0.302±0.033	0.228±0.063	<0.001
Vagueness value	16.776±3.674	18.140±4.601	0.130

MG: Meibomian gland.

Table 3 Upper lid energy curves

Parameters	*Demodex* negative group (*n*=35)	*Demodex* positive group (*n*=70)	*P*
Energy curve	622.892 (573.975, 677.609)	686.223 (646.867, 710.950)	<0.001

of 0.643 and specificity of 0.971, outperforming the energy curve (AUC=0.771, 95%CI: 0.680–0.862).

In the multivariable logistic regression model (incorporating TMH, MG area, and energy curve), DQ dropout remained an independent predictor of *Demodex* blepharitis [adjusted odds ratio (OR)=207.652; 95%CI: 8.683–4966.208, *P*<0.001]. The new model achieved an AUC of 0.969 (95%CI: 0.942–0.997), significantly superior to DQ dropout alone (ΔAUC=0.162), indicating a marked improvement in predictive performance after incorporating clinical parameters.

Given that eyelash epilation followed by mite counting has long been the prevailing diagnostic method, we further analyzed 887 patients who underwent this procedure. The prevalence of DQ dropout was markedly higher in the DP group than in the DN group (59.9% *vs* 28.5%, *P*<0.001), and the inter-rater agreement was substantial (kappa 0.642, *P*<0.001). Consistent with the preceding analyses, ROC curves were constructed, and the AUCs were calculated to evaluate the diagnostic performance of DQ dropout for *Demodex* blepharitis. As shown in Figure 4, DQ dropout achieved an AUC of 0.671 (95%CI: 0.635–0.707).

DISCUSSION

This study defined DQ dropout, a novel MG feature characterized by the presence of one or more fully atrophied glands among healthy glands. Although DQ dropout demonstrated moderate diagnostic ability for *Demodex* blepharitis, it is a morphological sign that does not require quantitative evaluation and has promising diagnostic consistency among experts. Moreover, it correlated with ocular surface parameters, which indicate the severity of blepharitis.

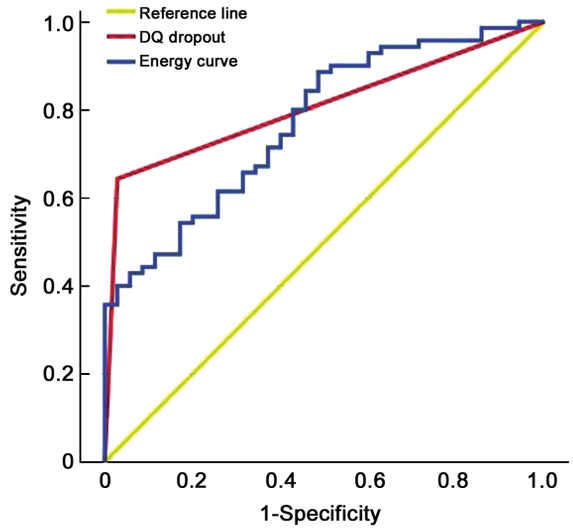


Figure 3 ROC curves of DQ dropout and energy curve ROC: Receiver operating characteristic curve; DQ: *Demodex*-infected qualitative.

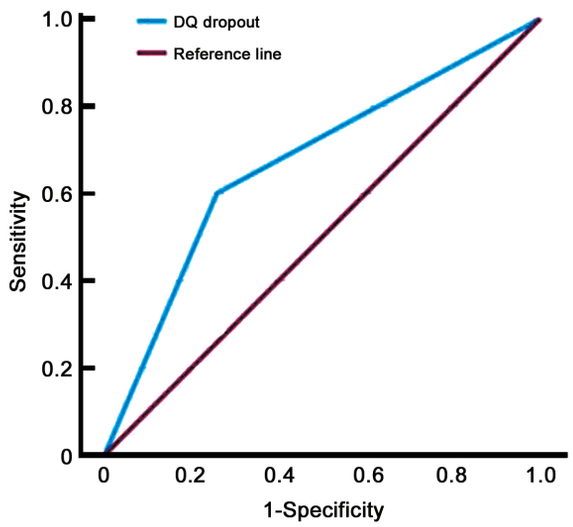


Figure 4 ROC curves of DQ dropout for assessing *Demodex* blepharitis ROC: Receiver operating characteristic curve; DQ: *Demodex*-infected qualitative.

The prevalence of *Demodex* infestation in blepharitis is high, ranging from 29% to 90%[1]. However, the condition remains underdiagnosed. Various studies have been conducted to improve diagnostic efficacy using IVCM and light microscopy[18]. However, these examinations are complicated and time-consuming and are not indicated for every patient with blepharitis. Moreover, ophthalmologists who are not specialized in ocular surface diseases may overlook the characteristics of *Demodex* infestation or the need for mite-related examinations, which may explain why some patients with ocular *Demodex* infestation are missed. Our previous study focused on the morphological changes in MGs in *Demodex* infestation and revealed uneven MG dropout in both manual and artificial intelligence methods[8-9,19-20]. Furthermore, we found that the complete dropout of glands between two normal glands can lead to abnormally high uneven indices. Of note, this dropout is distinctly different from the typical morphological changes of isolated MGD, patients with simple MGD generally exhibit progressive, uniform, and diffuse gland atrophy.

The DQ dropout showed good diagnostic ability with an AUC of 0.807. Inter-rater reliability was substantial, with kappa coefficients of 0.617 and 0.642 in the IVCM and light microscopy groups, respectively. This indicates that DQ dropout is easily identifiable by clinicians. The AUC (0.671) for the validation cohort using eyelash microscopy was lower than that of the initial cohort (0.807). The lower AUC of DQ dropout in the validation cohort may be largely explained by inherent differences in diagnostic performance between the two reference standards: IVCM demonstrates higher sensitivity and lower false-negative rate[21-22], whereas conventional light microscopy carries a certain risk of missed diagnosis. Although this difference aligns with the well-recognized clinical characteristics of the two methods and reflects the real-world performance of the index, such an explanation remains inferential. Because DQ dropout was evaluated against diagnostic benchmarks with differing sensitivity and false-negative profiles, direct cross-cohort comparison and interpretation of its universal generalizability should be made with greater caution.

Missed *Demodex* infestation by DQ dropout suggest that this sign appeared only in moderate-to-severe blepharitis and remained absent during the early stages of infestation. In our cases, a new DQ feature was introduced 1y after the case occurred. DQ dropout on meibography can alert ophthalmologists to the possibility of *Demodex* infestation and prompt further biological examination. This novel sign of DQ dropout may help improve diagnoses of *Demodex* infestation.

In the multivariable logistic regression model incorporating clinical parameters (TMH, MG area, and energy curve), the combined model achieved an AUC of 0.969 (95%CI: 0.942–0.997), which was significantly superior to the diagnostic performance of DQ dropout alone (ΔAUC=0.162). This indicates a strong correlation between DQ dropout and *Demodex* blepharitis, further validating its independence and reliability as a specific morphological sign of the disease. Notably, the adjusted OR of DQ dropout was extremely high with a very wide confidence interval, suggesting some instability in effect size estimation that may be related to data sparsity in this relatively small cohort; thus, the result should be interpreted with caution. The excellent performance of the combined model suggests a potential synergistic association among TMH, MG area, energy curve, and DQ dropout. Although our retrospective diagnostic dataset does not directly demonstrate causal pathways, we hypothesize that this synergy is biologically plausible: *Demodex* infestation may directly

cause local atrophy of MG to form DQ dropout, and induces a decrease in the overall secretory function of MG (manifested by reduced MG area), abnormalities in the tear film lipid layer[23] (associated with the energy curve[9]), and further impacts tear retention (reflected by decreased TMH) through mechanical obstruction by mites, stimulation by metabolic products, and inflammatory responses[24-25]. Therefore, combining multiple parameters can more comprehensively capture the pathophysiological essence of the disease and reduce the potential diagnostic blind spots of a single indicator. However, as this was an exploratory model developed without internal or external validation, potential overfitting may exist given the limited sample size. Variables were selected a priori based on clinical relevance, and collinearity diagnostics confirmed no significant collinearity. The robustness and generalizability of this model need to be confirmed in future larger-scale and externally validated studies.

To our knowledge, this is the first study to define the characteristic dropout of MG in patients with blepharitis and *Demodex* infestation. The finding of DQ dropout is independent of microscopic examination and reduces misdiagnoses. DQ dropout may serve as a more precise indicator for clinicians to determine the need for treatment. Many patients with a positive IVCM test for *Demodex* exhibit no symptoms or eyelid margin abnormalities. It was previously believed that such patients could not be diagnosed with *Demodex* blepharitis and did not require treatment. However, DQ dropout suggests that the MG microstructure may already be altered, even in the absence of explicit symptoms[26]. While our current cross-sectional data cannot dictate therapeutic decisions, this morphological sign highlights the need for close clinical monitoring. Future longitudinal and treatment-response studies[27-28] are required to determine whether early intervention in these subclinical cases can prevent further deterioration of MG function and the onset of related complications[29].

We speculated that DQ dropout may be related to the quantity and activity of *Demodex*. Currently, there are three main mechanisms of *Demodex* blepharitis pathogenesis. First, microabrasions of the MG caused by direct injury from mite claws, blockage of the MG orifice, and epithelial cell depletion. For instance, *Demodex folliculorum* mites can physically damage the hair follicle-sebaceous gland unit, subsequently stimulating abnormal tissue repair. This leads to epithelial hyperplasia and reactive hyperkeratosis, impairing eyelash growth[30]. Additionally, *Demodex folliculorum* mites and their metabolic byproducts mechanically obstruct MG ducts and orifices. This increases resistance to lipid transport within the ducts, blocks gland openings, and disrupts MG function, eventually leading to abnormalities in the lipid layer of the tear film[31]. Second, *Demodex* mites serve as carriers for various pathogens, including *Bacillus vegetativus*, *Propionibacterium acnes*, and *Staphylococcus spp.*[32-33]. Their presence may disrupt the homeostasis of the ocular surface microbiome, leading to dysbiosis and potentially secondary microbial infections[34]. Meibum is crucial in maintaining ocular surface health by preventing tear evaporation and enhancing tear film stability[35]. Both *Demodex* mites and their associated microorganisms secrete lipases that degrade meibum, leading to alterations in the tear film lipid layer, reduced tear film stability, and possible ocular surface damage. Third, hypersensitivity reactions triggered by *Demodex* mite debris or metabolic byproducts may damage MGs[36-37]. Georgala *et al*[38] found that the epidermis of patients who tested positive for *Demodex* mites contained a large number of Langerhans cells and macrophages, whereas the immune cell distribution in patients who tested negative was normal. This indicates that *Demodex folliculorum* may induce delayed hypersensitivity reactions. These pathological changes can greatly affect MG morphology. Moreover, an overpopulation of *Demodex* mites may also alter MG morphology, with greater mite density associated with more severe gland damage[6,39]. Therefore, the DQ dropout of MG may be due to differences in the quantity and activity of *Demodex* in the gland ducts in different regions. Different degrees of gland damage, in turn, lead to uneven MG dropout, ultimately resulting in the characteristic DQ dropout feature.

Our study has some limitations. First, it only provided qualitative confirmation of *Demodex* infestation; the exact mite load and its spatial distribution were not quantified, precluding a precise assessment of the relationship between parasite burden and DQ dropout. Future studies should correlate DQ dropout with specific mite counts and their eyelid distribution through detailed *in vivo* localization and quantitative analyses. Besides, the multivariable model yielded a high OR with a wide CI for DQ dropout, which may be affected by data sparsity and limited sample scale, warranting cautious interpretation of relevant statistical results. Second, this was a retrospective study, and the IVCM-DN subgroup was relatively small, potentially introducing information bias; however, it was sufficient to compare the statistical differences. The combined diagnostic model was exploratory without internal or external validation, and its robustness needs further confirmation; potential overfitting should also be noted in result explanation. Third, the two cohorts employed different clinical reference standards for *Demodex* diagnosis, this design was intended to reflect real-world clinical practice and evaluate the generalizability of DQ dropout across common diagnostic settings. Nevertheless, inherent differences in sensitivity and false-negative rates between the two methods complicate direct cross-cohort comparison of diagnostic performance

and warrant cautious interpretation of universal applicability. Lastly, as a retrospective analysis, we did not differentiate infections caused by *Demodex folliculorum* and *Demodex brevis*. Species identification and comparative analysis of the two *Demodex* species will be performed in our future prospective studies.

In conclusion, the newly defined DQ dropout of MGs may aid in the diagnosis of *Demodex* blepharitis and demonstrates high consistency among clinicians. Its association with ocular surface parameters suggests that it may serve as a valuable adjunctive morphological clue for clinicians evaluating *Demodex* infection and MGD severity.

ACKNOWLEDGEMENTS

The authors would like to thank the experts for their contribution: Dr Shuangqing Wu, Dandan Wang, Yinying Zhao.

Authors' Contributions: Fu L and Chen XY contributed equally. Data analysis, Dai Q, Chen XY, Wang S, and Lian HL; Methodology, Chen XY, Zhang ZH, Fu YN, Yu XX, Yao SL and Wang S; Project administration, Dai Q and Fu L; Software, Chen XY and Lian HL; Writing–original draft, Fu L and Chen XY; Writing–review and editing, Chen XY, Wang S, Yao SL, and Dai Q. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI-Generated Content Disclosure: No AI-generated content was used in this manuscript.

Foundation: Supported by the Joint TCM Science & Technology Projects of National Demonstration Zones for Comprehensive TCM Reform (No.GZY-ZJ-KJ-23086).

Conflicts of Interest: Fu L, None; **Chen XY,** None; **Wang S,** None; **Yao SL,** None; **Lian HL,** None; **Fu YN,** None; **Yu XX,** None; **Zhang ZH,** None; **Dai Q,** None.

REFERENCES

1 Zhang AC, Muntz A, Wang MTM, *et al*. Ocular Demodex: a systematic review of the clinical literature. *Ophthalmic Physiol Opt* 2020;40(4):389-432.

2 Rabensteiner DF, Aminfar H, Boldin I, *et al*. Demodex mite infestation and its associations with tear film and ocular surface parameters in patients with ocular discomfort. *Am J Ophthalmol* 2019;204:7-12.

3 Shah PP, Stein RL, Perry HD. Update on the management of demodex blepharitis. *Cornea* 2022;41(8):934-939.

4 Barnett M, Simmons B, Vollmer P, *et al*. The impact of *Demodex* blepharitis on patient symptoms and daily life. *Optom Vis Sci* 2024;101(3):151-156.

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

6 Cheng SN, Zhang MC, Chen H, *et al*. The correlation between the microstructure of meibomian glands and ocular Demodex infestation: a retrospective case-control study in a Chinese population. *Medicine (Baltimore)* 2019;98(19):e15595.

7 Yin Y, Gong L. Uneven meibomian gland dropout over the tarsal plate and its correlation with meibomian gland dysfunction. *Cornea* 2015;34(10):1200-1205.

8 Liu XY, Fu YN, Wang DD, *et al*. Uneven index: a digital biomarker to prompt *Demodex* blepharitis based on deep learning. *Front Physiol* 2022;13:934821.

9 Wang MJ, Chen XY, Wang KS, *et al*. A new digital biomarker of Demodex blepharitis: energy curve of the meibomian edge. *Front Cell Dev Biol* 2025;13:1627327.

10 Swiderska K, Blackie CA, Murakami D, *et al*. Advancements in artificial intelligence for meibography: clinical utility and contributions to meibomian gland dysfunction management. *Clin Exp Optom* 2026;109(3):398-414.

11 Liu SH, Shah M, Leslie L, *et al*. Artificial intelligence for diagnosing meibomian gland dysfunction: a systematic review and meta-analysis of diagnostic test accuracy studies. *Cornea* 2026;45(10): 1318-1331.

12 Expert Workgroup of Expert consensus on classification and annotation methods processes and quality control for dry eye imaging in artificial intelligence applications (2025), Intelligent Ophthalmology Branch of China Medical Education Association. Expert consensus on classification and annotation methods, processes and quality control for dry eye imaging in artificial intelligence applications (2025). *Chinese Journal of Experimental Ophthalmology* 2025;43(4):289-300.

13 Amescua G, Akpek EK, Farid M, *et al*. Blepharitis preferred practice pattern®. *Ophthalmology* 2019;126(1):P56-P93.

14 Yan TT, Dai Q, Yu GS. *In vivo* confocal microscopy: a standard operating procedure for the detection of *Demodex* mites at the eyelid margin. *J Vis Exp* 2025(221).

15 Asian Dry Eye Association China Branch (2018), Ocular Surface and Tear Disease Study Group, Ophthalmology Professional Committee, Cross-Strait Medical Exchange Association. Expert Consensus on Diagnosis and Treatment of Demodex Blepharitis in China. *Zhonghua Yan Ke Za Zhi* 2018;54(7):491-495.

16 Markoulli M, Duong TB, Lin M, *et al*. Imaging the tear film: a comparison between the subjective keeler tearscope-plus™ and the objective oculus® keratograph 5M and LipiView® interferometer. *Curr Eye Res* 2018;43(2):155-162.

17 Dai Q, Liu XY, Lin XL, *et al*. A novel meibomian gland morphology analytic system based on a convolutional neural network. *IEEE Access* 2021;9:23083-23094.

18 Arici C, Mergen B, Bahar Tokman H, *et al*. Investigation of the *Demodex* lid infestation with *in vivo* confocal microscopy versus light microscopy in patients with seborrheic blepharitis. *Ocul Immunol Inflamm* 2022;30(4):973-977.

19 Yu XX, Fu YN, Lian HL, *et al*. Uneven meibomian gland dropout in patients with meibomian gland dysfunction and *Demodex* infestation. *J Clin Med* 2022;11(17):5085.

20 Wang KS, Chen XY, He CL, *et al*. Information entropy-based framework for quantifying curve tortuosity in meibomian glands uneven atrophy. *Transl Vis Sci Technol* 2026;15(5):25.

21 Wang YJ, Ke M, Chen XM. Prospective study of the diagnostic accuracy of the *in vivo* laser scanning confocal microscopy for ocular demodicosis. *Am J Ophthalmol* 2019;203:46-52.

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

23 Kim WJ, Ahn YJ, Kim MH, *et al*. Lipid layer thickness decrease due to meibomian gland dysfunction leads to tear film instability and reflex tear secretion. *Ann Med* 2022;54(1):893-899.

24 Rhee MK, Yeu E, Barnett M, *et al*. Demodex blepharitis: a comprehensive review of the disease, current management, and emerging therapies. *Eye Contact Lens* 2023;49(8):311-318.

25 Li JF, Cui HH, Sun X, *et al*. Topical cyclodextrin minocycline inclusion complex inhibited the inflammation in blepharitis-related dry eye disease. *Int Immunopharmacol* 2025;165:115509.

26 Sapir S, Mimouni M, Sela T, *et al*. Asymptomatic meibomian gland dysfunction in refractive surgery candidates. *J Refract Surg* 2026;42(4):e299-e306.

27 Chen KY, Chan HC, Chan CM. Which treatment works better for Meibomian Gland Dysfunction: LipiFlow or intense pulsed light A systematic review and network meta-analysis. *Photodiagn Photodyn Ther* 2025;53:104630.

28 Chen KY, Chan HC, Chan CM. How effective and safe are punctal plugs in treating dry eye disease? A systematic review and meta-analysis. *Contact Lens Anterior Eye* 2025;48(5):102438.

29 Cooper JM, Mahmoud R, Jennings CJ, *et al*. Pharmacological, natural and emerging therapies for meibomian gland dysfunction: a review. *Drugs* 2026;86(5):675-718.

30 Murphy O, O' Dwyer V, Lloyd-McKernan A. The clinical use of eyelash manipulation in the diagnosis of Demodex folliculorum blepharitis. *Eye Contact Lens* 2020;46 Suppl 1:S33-S38.

31 Sabeti S, Kheirkhah A, Yin J, *et al*. Management of meibomian gland dysfunction: a review. *Surv Ophthalmol* 2020;65(2):205-217.

32 Zhu MY, Cheng C, Yi HS, *et al*. Quantitative analysis of the bacteria in blepharitis with demodex infestation. *Front Microbiol* 2018;9:1719.

33 Szkaradkiewicz A, Chudzicka-Strugała I, Karpiński TM, *et al*. Bacillus oleronius and Demodex mite infestation in patients with chronic blepharitis. *Clin Microbiol Infect* 2012;18(10):1020-1025.

34 Liang XT, Li YL, Xiong K, *et al*. Demodex infection changes ocular surface microbial communities, in which meibomian gland dysfunction may play a role. *Ophthalmol Ther* 2021;10(3):601-617.

35 Muntz A, Sandford E, Claassen M, *et al*. Randomized trial of topical periocular castor oil treatment for blepharitis. *Ocul Surf* 2021;19:145-150.

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

37 Martínez-Pulgarín DF, Ávila MY, Rodríguez-Morales AJ. Interventions for Demodex blepharitis and their effectiveness: a systematic review and meta-analysis. *Contact Lens Anterior Eye* 2021;44(6):101453.

38 Georgala S, Katoulis AC, Kylafis GD, *et al*. Increased density of *Demodex folliculorum* and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. *J Eur Acad Dermatol Venereol* 2001;15(5):441-444.

39 Hao YR, Zhang XY, Bao JY, *et al*. *Demodex folliculorum* infestation in meibomian gland dysfunction related dry eye patients. *Front Med (Lausanne)* 2022;9:833778.