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Article

Influence of *Demodex* Infection in the Occurrence and Prognosis of Keratitis in Patients With Meibomian Gland Dysfunction Related Dry Eye

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Abstract

Aims/Background: Meibomian gland dysfunction (MGD)-related dry eye results in reduced tears, unstable tear film, ocular surface damage, and even causes keratitis. *Demodex* infection causes inflammatory diseases of the ocular surface. This study aimed to explore the impact of *Demodex* infection on the occurrence and prognosis of keratitis in patients with MGD-related dry eye. **Methods**: A total of 122 MGD patients who visited the Department of Ophthalmology, The First People's Hospital of Chun'an County from June 2022 to June 2023 were selected for retrospective study. Patients were divided into keratitis group (n = 65) and non-keratitis group (n = 57) according to the presence of keratitis. MGD patients with keratitis were followed up for one year, and divided into good prognosis group (n = 36) and poor prognosis group (n = 29) based on their prognosis. The *Demodex* infection status and ocular surface parameters were detected in these two groups of patients. Logistic regression was adopted to analyze the influencing factors of keratitis complicated with MGD. **Results**: The positive rate of *Demodex* infection in the keratitis group was 81.5%, which was significantly higher than that in the non-keratitis group (35.1%, $p < 0.05$). Compared with non-keratitis group, keratitis group had reduced tear film break-up time (BUT), and increased corneal fluorescein staining (CFS), meibomian gland (MG) dropout, and plugging of MG orifices ($p < 0.05$). Logistic multivariate regression analysis showed that *Demodex* infection (odds ratio [OR]: 6.209, 95% confidence interval [CI]: 2.101–18.348), CFS (OR: 2.627, 95% CI: 1.562–4.416) and plugging of MG orifices (OR: 3.174, 95% CI: 1.616–6.235) were independent risk factors for keratitis in patients with MGD-related dry eye ($p < 0.05$), and BUT (OR: 0.768, 95% CI: 0.606–0.972) was a protective factor ($p < 0.05$). The age and MG expression in the good prognosis group were lower than those in the poor prognosis group ($p < 0.05$). No significant difference was observed between the good prognosis and poor prognosis groups in patients with *Demodex* infection ($p > 0.05$). **Conclusion**: The positive rate of *Demodex* infection is higher in patients with MGD combined with keratitis. *Demodex* infection, CFS and plugging of MG orifices are independent risk factors for keratitis, while the tear film BUT is a protective factor in MGD-related dry eye patients. *Demodex* infection does not affect the prognosis of keratitis.

Keywords: mite; keratitis; meibomian gland dysfunction; dry eye

1. Introduction

Meibomian gland (MG) is a special type of sebaceous gland located in the eyelid meibomian plate. The fat secreted by MG is the main component of the lipid layer on the surface of the tear film, which can promote the stability of the tear film and maintain the health of eye surface [1]. Meibomian gland dysfunction (MGD) is the most common ocular surface disease in clinical setting, giving rise to symptoms such as redness, itching, irritation, dryness, decreased vision, and tearing. It is usually characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion [2]. MGD can cause abnormal tear film and ocular surface inflammation [3]. It was reported that MGD was also the main cause of various types of ocular surface diseases, such as dry eye disease, eyelid-associated keratoconjunctivitis, and keratitis [1,4,5]. MGD-related dry eye could experience reduced tears, unstable tear film, ocular surface damage, and even cause keratitis [1]. Keratitis is the main cause of blindness, and most vision loss caused by keratitis can be avoided through early detection and treatment [6,7]. However, the pathological mechanism and influencing factors of keratitis are complex and remain to be elucidated.

Demodex is widely prevalent parasites that primarily feed on secreted oils. Researches have shown that *Demodex* infection of the eyelids was significantly associated with MGD, ocular surface inflammation, and blepharokeratoconjunctivitis [8,9]. Previous study reported that *Demodex* infection was a risk factor of MGD [10]. In addition to causing direct damage to epithelial cells of the hair follicle, *Demodex* infection also could cause marginal corneal infiltration, phlyctenule-like lesions, and superficial corneal opacity [11–13]. Meanwhile, *Demodex* can carry concomitant bacteria (such as *Streptococci* and *Staphylococci*), which are capable of producing antigens and triggering immune response [14]. In addition, the metabolic byproducts of *Demodex* can trigger an inflammatory cascade by activat-

ing Toll-like receptor 2 (TLR2) innate immune pathway, leading to up-regulation of inflammatory cells and pro-inflammatory factors [15]. Overall, *Demodex* infection is associated with the inflammatory diseases of the ocular surface. Exploring the correlation between *Demodex* infection and MGD, a previous study found that the infection was an influencing factor for MGD, particularly prominent in elderly patients aged 41 to 70 years [16]. As studies that explore the impact of *Demodex* infection on the development and prognosis of keratitis remain scarce, it is still unclear whether *Demodex* contributed to the occurrence of keratitis in MGD patients. Thus, this study was designed to retrospectively analyze clinical data of patients with MGD-related dry eye, aiming to clarify the relationship between *Demodex* infection and the occurrence, clinical characteristics, and prognosis of keratitis. This study will provide a basis for the clinical diagnosis and treatment of MGD combined with keratitis.

2. Methods

2.1 Patients

A total of 122 patients with MGD-related dry eye who visited the Department of Ophthalmology, The First People's Hospital of Chun'an County from June 2022 to June 2023 were selected for this retrospective study. This study was approved by The First People's Hospital of Chun'an County Ethics Committee (Approval No. 2024-04-12-06) and was conducted in compliance with the Declaration of Helsinki. Informed consent was obtained from all patients. MGD patients were divided into keratitis group (n = 65) and non-keratitis group (n = 57) according to whether or not they developed keratitis. A keratitis diagnosis was verified based on a combination of diagnostic tests, including assessment of clinical symptoms, slit lamp examination, and laboratory findings [17]. Patients who satisfied the following criteria were included in the study: (1) meeting the diagnostic criteria for MGD-related dry eye [18]; (2) having complete clinical data; and (3) aged >18 years. Patients who met any of the following criteria were excluded: (1) having undergone eye surgery within 3 months, such as cataract removal surgery and laser/pulse therapy; (2) suffering an eye injury within 3 months; (3) having received treatment for *Demodex* infection within 3 months; (4) having received eye treatment with systemic antibiotics or eye drops within 3 months; (5) suffering from other ophthalmic diseases, including acute glaucoma, dacryocystitis, cataract and retinopathy; (6) having other diseases affecting the eyes, including diabetes, hypertension, hyperthyroidism, and Sjogren's syndrome; and (7) having a loss of follow-up. The flowchart for patient selection is displayed in Fig. 1.

2.2 Demodex Infestation

Three eyelashes were obtained from the upper and lower eyelids of patients, respectively. The samples were observed under a microscope (10× and 40×; BM1000, Jiangnan Yongxin Optical Co., Ltd., Nanjing, China) after glycerol drops were given. The number of *Demodex* (including dead or alive mites, larvae, and eggs) observed on each eyelash was recorded and counted. According to the diagnostic criteria [19], positive infection is defined as there are ≥3 *Demodex* on the three eyelashes from the same eyelid. The status of *Demodex* infection in all subjects was tested through standardized examination by two experienced ophthalmologists, who had received training about *Demodex* infection.

2.3 Ophthalmic Examination

The clinical information of patients, including gender, age, course of disease, history of eye trauma/burns, eye make-up, usage of contact lenses, and admission time were collected. All patients underwent ophthalmic examinations, which covered ocular surface disease index (OSDI), tear film break-up time (BUT), corneal fluorescein staining (CFS), Schirmer I test (SIT) and MG examination. The ophthalmic examinations of all patients were conducted under blinded conditions by an experienced ophthalmologist (7 years of clinical experience in ophthalmology) following standardized protocols.

The OSDI was used to diagnose patients with dry eye and evaluate the severity of the condition [20]. With a score range of 0–100, a higher score represents greater disability.

The measurement of BUT requires dropping 0.5% fluorescein sodium into the conjunctival sac of patients, who were then asked to blink several times. The appearance time of the first dry spot was recorded. The measurement was repeated three times and the average value was calculated [21].

CFS test was carried out to evaluate cornea damage [22]. The fluorescein sodium was dropped into the conjunctival sac, and the cornea were observed using a slit-lamp. The cornea was separated into four quadrants, with each quadrant scored from a range of 0–3 points according to National Eye Institute (NEI) scoring system (0 = no staining, 3 = severe). The test had a maximum of 12 points [23].

SIT was performed using a 5 mm × 35 mm tear filter strip. It was inserted between the middle and lateral third of each lower lid margin. After closing eyes for 5 minutes, the length of the wetting strip was read [21]. According to the Tear Film and Ocular Surface Dry Eye Workshop II (TFOS DEWS II) criteria, SIT <10 mm/5 min was the threshold value for dry eye [24].

MG examination encompasses MG dropout, plugging of MG orifice, and MG expression. Keratograph 5M (Oculus, Wetzlar, Germany) was used for the assessment of MG. (1) MG dropout: 0–3 points for each eyelid: 0 = no loss; 1 = MG loss of less than 1/3 area; 2 = MG loss between 1/3 and 2/3 area; 3 = MG loss of more than 2/3 area. The average score of the upper and lower eyelids was calculated [22]. (2) The plugging of MG orifices: 0–3 points for each eyelid, 0

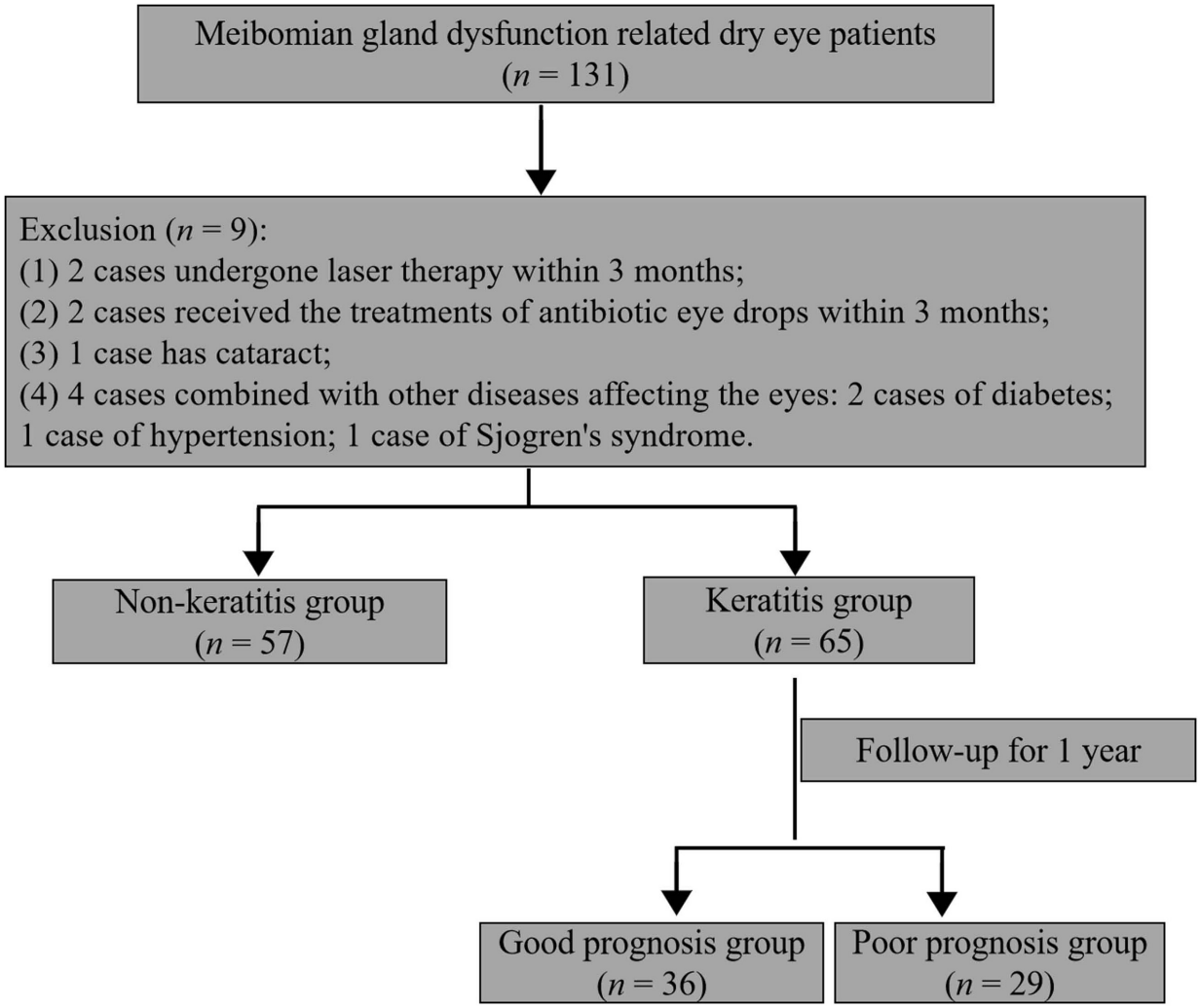

Fig. 1. Flowchart depicting patient inclusion and exclusion.

= no plugging; 1 = plugging <3 orifices; 2 = plugging ≥3 orifices, with a distribution of less than half of the full length of the lid; 3 = plugging ≥3 orifices, with a distribution of half or more of the full length of the lid. The average score of the upper and lower eyelids was calculated [25]. (3) MG expression: five glands of the upper eyelid and five glands of the lower eyelid were evaluated; grade 0: all glands expressible; grade 1: 3–4 glands expressible; grade 2: 1–2 glands expressible; grade 3, no glands expressible. The average score of upper and lower eyelids was calculated [22].

2.4 Treatment Procedure

For dry eye, the patients were treated with sodium hyaluronate eye drops (H201 73249, Santen Pharmaceutical, Osaka, Japan) 4 times daily for a consecutive 4 weeks. Meanwhile, they received fumigation and MG massage once a week for 4 weeks. Specifically, an atomizer was used for fumigation for 15–20 min. After fumigation, MG massage was performed on the meibomian gland to unblock MG. For individuals with *Demodex* infection, Tea Tree Oil Professional Mite Removal Wipes (OcuSOFT, Beijing, China) were used to clean the roots of the eyelashes and eyelids of patients. Specifically, after cleaning the eyelids, patients apply a wet compress for 10–15 minutes, twice daily for 4 weeks. Keratitis was treated with medications to ameliorate inflammation. Among the drugs for keratitis treatment are levofloxacin eye drop (H20203122, Wanhan Pharmaceutical, Zhongshan, China), pranoprofen eye drop (H20130682, Senju Pharmaceutical, Osaka, Japan), and tobramycin dexamethasone eye drop (H20150119, Alcon NV, Puurs-Sint-Amands, Belgium), which should be chosen based on the patient's condition.

2.5 Follow-up

MGD patients with keratitis (n = 65) discharged from the hospital were followed up for 1 year, with a follow-up every 3 months. The follow-up entailed recording the clinical symptoms of the patient's eyes and examining the corneal condition. The patients were categorized into a good prognosis group and a poor prognosis group according to the results of follow-up. Patients in the good prognosis group (n = 36) showed no significant eye discomfort,

Table 1. Clinical data of MGD patients.

Clinical data	MGD patients (n = 122)	Keratitis group (n = 65)	Non-keratitis group (n = 57)	t/χ^2	p
Age (years)	36.48 ± 7.79	37.38 ± 7.15	35.44 ± 8.40	1.378	0.171
Gender (male/female)	63/59	34/31	29/28	0.025	0.875
Course of disease (months)	13.19 ± 6.58	12.46 ± 6.25	14.02 ± 6.90	1.310	0.193
Eye make-up (n, %)				0.151	0.698
Yes	47 (38.5%)	24 (36.9%)	23 (40.4%)		
No	75 (61.5%)	41 (63.1%)	34 (59.6%)		
Contact lens usage (n, %)				3.650	0.056
Wearing	54 (44.3%)	34 (52.3%)	20 (35.1%)		
Not wearing	68 (55.7%)	31 (47.7%)	37 (64.9%)		
History of eye trauma/burns (n, %)				1.623	0.203
Yes	23 (18.9%)	15 (23.1%)	8 (14.0%)		
No	99 (81.1%)	50 (76.9%)	49 (86.0%)		
Admission time (n, %)				1.745	0.627
March to May	25 (20.5%)	15 (23.1%)	10 (17.5%)		
June to August	38 (31.1%)	17 (26.2%)	21 (36.8%)		
September to November	39 (32.0%)	22 (33.8%)	17 (29.8%)		
December to February	20 (16.4%)	11 (16.9%)	9 (15.8%)		
Demodex infection (n, %)				27.266	<0.001
Positive	73 (59.8%)	53 (81.5%)	20 (35.1%)		
Negative	49 (40.2%)	12 (18.5%)	37 (64.9%)		

Table 2. Comparison of ocular surface parameters between the keratitis group and the non-keratitis group.

Ocular surface parameters	Keratitis group (n = 65)	Non-keratitis group (n = 57)	t/Z	p
OSDI	32.32 ± 4.61	30.91 ± 5.82	1.491	0.138
SIT (mm)	5.34 ± 2.73	6.11 ± 2.09	1.730	0.086
BUT (s)	4 (3, 6)	6 (4, 8)	3.634	<0.001
CFS	4 (3, 5)	3 (1.5, 3)	5.541	<0.001
MG dropout	2.0 (1.5, 2.5)	1.5 (1.0, 2.3)	2.719	0.007
Plugging of MG orifices	2.0 (1.5, 2.5)	1.5 (1.0, 2.0)	4.332	<0.001
MG expression	2.0 (1.5, 2.5)	2.0 (1.0, 2.0)	1.561	0.119

Note: BUT, break-up time; CFS, corneal fluorescein staining; MG, meibomian gland; OSDI, ocular surface disease index; SIT, Schirmer I test.

controlled corneal inflammation, regression of lesions, neovascularization regression, and no recurrence of keratitis, whereas for those in the poor prognosis group (n = 29), corneal inflammation was not effectively controlled or recurrence occurred.

2.6 Statistical Analysis

SPSS version 21.0 software (IBM Corp., Armonk, NY, USA) was adopted for performing statistical analysis of raw data. The normal distribution of data ware tested using the Shapiro-Wilk test. The data with normal distribution are presented as mean ± standard deviation (SD), and t test was employed for the comparison between two groups. The non-normal data are expressed as median (quartiles), and Mann-Whitney U test was utilized for comparison. Categorical data are expressed as frequency (%), and chi-square test was adopted for comparison. Logistic regression was adopted to analyze the influencing factors of keratitis complicated with MGD. The factors with $p < 0.05$ in the univariate logistic analysis were tested in the multivariate analysis. Multicollinearity was examined in the multivariate logistic regression analysis, and a variance inflation factor (VIF) <5 indicates no multicollinearity. The Hosmer-Lemeshow test was used to evaluate the goodness of fit. Additionally, $p < 0.05$ was considered a criterion for judging significant differences.

3. Results

3.1 Clinical Data of MGD Patients

Among all MGD patients, the incidence rate of keratitis was 53.3% (65/122), and the positive rate of *Demodex* infection was 59.8% (73/122). The MGD patients were divided into the keratitis group (n = 65) and the non-keratitis group (n = 57) based on whether they have keratitis or not. Among them, the positive rate of *Demodex* infection in the

Table 3. Logistic regression analysis of influencing factors of MGD complicated with keratitis.

	Univariate analysis					Multivariate analysis[&]				
	β	SE	Wald	OR (95% CI)	p	β	SE	Wald	OR (95% CI)	p
Age	0.033	0.024	1.886	1.033 (0.986–1.083)	0.170					
Male	0.057	0.363	0.025	1.059 (0.520–2.158)	0.875					
Course of disease	–0.037	0.028	1.693	0.964 (0.912–1.019)	0.193					
Eye make-up	–0.145	0.373	0.151	0.865 (0.417–1.797)	0.698					
Contact lens usage	0.708	0.372	3.610	2.029 (0.978–4.210)	0.057					
History of eye trauma/burns	0.608	0.482	1.595	1.837 (0.715–4.724)	0.207					
Admission time										
March to May	/	/	/	Reference	/					
June to August	–0.617	0.523	1.393	0.540 (0.194–1.503)	0.238					
September to November	–0.148	0.521	0.080	0.863 (0.311–2.393)	0.777					
December to February	–0.205	0.607	0.114	0.815 (0.248–2.679)	0.736					
Demodex infection	2.101	0.423	24.619	8.171 (3.564–18.734)	<0.001	1.826	0.553	10.909	6.209 (2.101–18.348)	<0.001
OSDI	0.053	0.036	2.182	1.054 (0.983–1.131)	0.140					
SIT	–0.129	0.076	2.868	0.879 (0.758–1.020)	0.090					
BUT	–0.312	0.084	13.753	0.732 (0.621–0.863)	<0.001	–0.265	0.121	4.804	0.768 (0.606–0.972)	0.028
CFS	0.968	0.201	23.301	2.633 (1.777–3.901)	<0.001	0.966	0.265	13.275	2.627 (1.562–4.416)	<0.001
MG dropout	0.678	0.237	8.175	1.969 (1.238–3.133)	0.004	0.369	0.344	1.150	1.446 (0.737–2.840)	0.284
Plugging of MG orifices	1.103	0.274	16.149	3.013 (1.760–5.160)	<0.001	1.155	0.344	11.244	3.174 (1.616–6.235)	<0.001
MG expression	0.327	0.222	2.166	1.387 (0.897–2.144)	0.141					

Note: [&]$p = 0.376$ (Hosmer-Lemeshow test). BUT, break-up time; CI, confidence interval; CFS, corneal fluorescein staining; MG, meibomian gland; OR, odds ratio; OSDI, ocular surface disease index; SIT, Schirmer I test.

keratitis group was 81.5% (53/65), significantly higher than that in the non-keratitis group (35.1%, 20/57) ($p < 0.05$). Moreover, no significant differences in age, gender, course of disease, history of eye trauma/burns, admission time, eye make-up, and contact lens usage were observed between the two groups of patients (both $p > 0.05$) (Table 1).

3.2 Comparison of Ocular Surface Functions Between Two Groups

Compared with the non-keratitis group, the keratitis group exhibited reduced BUT value, increased CFS, MG dropout, and plugging of MG orifices ($p < 0.05$). Moreover, there were no significant differences between OSDI, SIT, and MG expression ($p > 0.05$) (Table 2).

3.3 Logistic Regression Analysis of Influencing Factors Influencing MGD Complicated With Keratitis

The results of univariate logistic analysis showed that the *Demodex* infection (odds ratio [OR]: 8.171, 95% confidence interval [CI]: 3.564–18.734, $p < 0.001$), BUT (OR: 0.732, 95% CI: 0.621–0.863, $p < 0.001$), CFS (OR: 2.633, 95% CI: 1.777–3.901, $p < 0.001$), MG dropout (OR: 1.969, 95% CI: 1.238–3.133, $p = 0.004$), and plugging of MG orifices (OR: 3.013, 95% CI: 1.760–5.160, $p < 0.001$) were correlated with keratitis ($p < 0.05$, Table 3).

The above associated risk factors were included in the multivariate logistic regression analysis. The analysis of multicollinearity indicated that the VIF values of all variables were less than 5. *Demodex* infection (OR: 6.209, 95% CI: 2.101–18.348, $p < 0.001$), CFS (OR: 2.627, 95% CI: 1.562–4.416, $p < 0.001$) and plugging of MG orifices (OR: 3.174, 95% CI: 1.616–6.235, $p < 0.001$) were the independent risk factors for keratitis in MGD-related dry eye patients ($p < 0.05$), and BUT (OR: 0.768, 95% CI: 0.606–0.972, $p = 0.028$) was the protective factor (Table 3).

3.4 Prognosis of Patients With MGD-related Dry Eye Complicated With Keratitis

Patients with keratitis ($n = 65$) were followed up for one year, and divided into a good prognosis group ($n = 36$) and a poor prognosis group ($n = 29$) based on their prognosis. The age and MG expression in the good prognosis group were lower than that in the poor prognosis group ($p < 0.05$). No significant differences were observed between the good prognosis and poor prognosis groups in gender, history of eye trauma/burns, admission time, eye make-up, contact lens usage, *Demodex* infection, OSDI, SIT, BUT, CFS, MG dropout, and plugging of MG orifices ($p > 0.05$) (Table 4).

4. Discussion

MG is a lipid-secreting sebaceous gland located in the eyelid. MGD is a chronic, diffuse inflammatory disease caused by excessive keratinization of multiple MG terminal ductal epithelium and dysfunction of their glandular secretions [26]. Dry eye caused by MGD can lead to various forms of eye discomforts and complications, affecting the stability of the tear film and the quality of tears [27]. Furthermore, MGD also causes continuous stimulation and damage to conjunctival cells and corneal epithelial cells through inflammation and immune response, leading to a variety of ocular surface inflammatory diseases, such as eyelid-associated keratoconjunctivitis and keratitis [1,4,28]. In this study, a total of 122 MGD-related dry eye patients were included, of whom 65 patients had keratitis, with a prevalence rate of 53.3%.

Demodex mites are parasites that feed on abnormally secreted oils. Eyelids are particularly vulnerable to *Demodex* infection due to the high level of secreted oils [12]. It has been confirmed that the overgrowth and colonization of *Demodex* mites, as well as their derived metabolites, are the foundation of MGD pathogenesis. Meanwhile, these metabolites further induce epithelial hyperkeratosis by stimulating capillary dilation and inducing inflammation. In addition, the accumulation of metabolites exacerbates glandular obstruction and terminal duct hyperkeratosis [15]. In addition, *Demodex* mites can serve as carriers of pathogens and pathogenic microorganisms, such as *Staphylococcus* and *Streptococcus*, which can trigger the host immune reaction [14]. Therefore, *Demodex* infection is also an important factor contributing to ocular surface inflammation. In the current study, the positive rate of *Demodex* infection in MGD patients was 59.8%. Moreover, the positive rate of *Demodex* infection in patients with keratitis was higher than that in the non-keratitis group, reaching 81.5%. Previous literature reported that the infection rate of *Demodex* in patients with refractory keratitis was as high as 100% [29], further validating the high prevalence of *Demodex* infection in patients with keratitis.

Our study analyzed the influencing factors of keratitis in MGD patients, revealing that *Demodex* infection was the independent risk factor for keratitis in MGD patients. Previous study reported that among patients with *Demodex* infection, the incidence of keratitis reaches 65%; in these *Demodex* infections, and the severity of MG loss was correlated with the severity of keratitis [30]. This also lends supportive evidence for our conclusion. It should be noted that we did not detect the influence of *Demodex* infection on the prognosis of MGD patients with keratitis. This indicates that *Demodex* infection was related to the occurrence of keratitis in MGD patients, but did not affect the prognosis of keratitis patients, probably related to the complex pathological mechanism of keratitis. Multiple factors including bacteria, viral infections, trauma, allergies, and immunity are involved in the pathogenesis of keratitis [31]. The recurrence of keratitis may be influenced by other factors. In addition, this study included a small sample of only 65 patients with keratitis for prognosis analysis, which had limited ability in examining the impact of *Demodex* infection on the prognosis of keratitis. Meanwhile, due to the small

Table 4. Comparison of clinical data between good prognosis group and poor prognosis group.

	Good prognosis group (n = 36)	Poor prognosis group (n = 29)	t/χ^2/Z	p
Age (year)	34.61 ± 7.08	40.83 ± 5.65	3.845	<0.001
Gender (male/female)	16/20	18/11	2.000	0.157
Eye make-up (n, %)			0.023	0.880
Yes	13 (36.1%)	11 (37.9%)		
No	23 (63.9%)	18 (62.1%)		
Contact lens usage (n, %)			0.837	0.360
Wearing	17 (47.2%)	17 (58.6%)		
Not wearing	19 (52.8%)	12 (41.4%)		
History of eye trauma/burns (n, %)			0.600	0.439
Yes	7 (19.4%)	8 (27.6%)		
No	29 (80.6%)	21 (72.4%)		
Admission time (n, %)			2.128	0.546
March to May	9 (25.0%)	6 (20.7%)		
June to August	7 (19.4%)	10 (34.5%)		
September to November	14 (38.9%)	8 (27.6%)		
December to February	6 (16.7%)	5 (17.2%)		
Demodex infection			2.292	0.130
Positive	27 (75.0%)	26 (89.7%)		
Negative	9 (25.0%)	3 (10.3%)		
OSDI	32.56 ± 4.32	32.03 ± 5.01	0.458	0.649
SIT (mm)	5.77 ± 2.84	4.82 ± 2.53	1.407	0.164
BUT (s)	4.69 ± 1.98	4.10 ± 2.02	1.184	0.241
CFS	4 (3, 5)	4 (3, 5)	0.088	0.930
MG dropout	2.0 (2.0, 2.5)	2.0 (1.5, 2.5)	0.519	0.604
Plugging of MG orifices	2.0 (1.6, 2.5)	2.0 (1.3, 3.0)	0.020	0.984
MG expression	2.0 (1.0, 2.4)	2.0 (1.8, 3.0)	2.254	0.024

Note: BUT, break-up time; CFS, corneal fluorescein staining; MG, meibomian gland; OSDI, ocular surface disease index; SIT, Schirmer I test.

sample size, we did not conduct further regression analysis to explore the factors affecting the prognosis of keratitis. The influence and potential mechanisms of *Demodex* infections in the prognosis of keratitis should be further verified and explored using a larger cohort in the future.

In the current study to measured ocular surface parameters, we detected a decrease of BUT value and increases of CFS, MG dropout, and plugging of MG orifices in keratitis patients compared with non-keratitis patients, which are suggestive of ocular surface function weakening in MGD patients with keratitis. Nevertheless, OSDI score was not significantly different between the keratitis and non-keratitis groups. Despite the pathological changes on the ocular surface, subjective perception of MGD patients with keratitis did not experience significant enhancement. This may be due to the fact that the initial symptoms of keratitis are not obvious. Another reason may be the similarity and overlap in clinical symptoms between keratitis and MGD-related dry eye [1,17], leading to nonsignificant difference in subjective perception between the cohorts separately affected by the two diseases. In addition, OSDI was usually applied to evaluate the symptoms of dry eye, but may not be suitable for assessing the eye symptoms in keratitis. Therefore, it is recommended to conduct detailed ophthalmic examinations for MGD-related dry eye patients to identify overlooked pathological changes.

In this study, multivariate logistic regression analysis showed that *Demodex* infestation, CFS and plugging of MG orifices were the independent risk factors for keratitis in MGD-related dry eye patients, while BUT was the protective factor. CFS can be used to evaluate the integrity of the cornea and its pathological damage. It was reported that the CFS in patients with severe keratitis complicated with dry eye were significantly reduced after treatment [32]. BUT is utilized to evaluate the stability of the tear film. Dry eye patients are unable to keep the tear film intact due to decreased tear production, exhibiting a decrease of BUT. Containing proteins, peptides, lipids, mucins, and electrolytes, the tear film plays a role in maintaining the health of the ocular surface [33]. Instability of the tear film causes damages of the corneal epithelium, where pathogens can easily colonize the corneal surface and induce keratitis. In addition, the decrease of protective tear film proteins in the patients with dry eye has been identified as a contributor to keratitis [34]. MG plugging can increase the risk of keratitis by reducing tear film lipids and tear film stability, and altering the mi-

crobial environment on the ocular surface [35,36]. Meanwhile, the inflammatory response caused by MG plugging may also serve as a driver of keratitis [37].

Based on previous literature, contact lenses were considered a risk factor of keratitis [31]. However, in this study, we did not observe significant differences in contact lens usage between the keratitis group and the non-keratitis group. Such discrepancy is probably caused by the inclusion of different samples. Interestingly, the nonsignificant difference in age between the keratitis group and the non-keratitis group indicates that age is not a factor affecting the occurrence of keratitis in MGD patients. However, we found that subjects in the poor prognosis group were older than those in the good prognosis group, suggesting that age is related to the prognosis of MGD patients with keratitis. Previous study reported that age was a risk factor for MGD and keratitis, and older patients were at higher risk of MGD and keratitis [16,31]. Harbiyeli *et al*. [38] reported that old age was associated with delayed recovery and poor visual prognosis in polymicrobial keratitis, consistent with our finding.

Several limitations of this study should be acknowledged. The sample size of this study is relatively small, especially in the keratitis patient group intended for prognosis analysis. The retrospective nature of the study does not allow for strict randomization procedures and control of confounding factors. Prospective large-scale cohort studies should be performed to validate relevant conclusions in the future.

5. Conclusion

Overall, the positive rate of *Demodex* infection is higher in patients with MGD complicated with keratitis than those without keratitis. *Demodex* infection, CFS and plugging of MG orifices are the independent risk factors for keratitis, whereas BUT is the protective factor for MGD-related dry eye patients. Additionally, *Demodex* infection does not significantly impact the prognosis of keratitis. In summary, this study sheds light on factors influencing the clinical diagnosis and treatment of MGD and keratitis.

Key Points

- Among the patients with meibomian gland dysfunction (MGD)-related dry eye, the incidence rate of keratitis was 53.3%, and the positive rate of *Demodex* infection was 59.8%.
- The positive rate of *Demodex* infection in the keratitis patients is higher than that in the non-keratitis patients.
- *Demodex* infection, corneal fluorescein staining (CFS) and plugging of meibomian gland orifices are the independent risk factors for keratitis, whereas tear film break-up time (BUT) is the protective factor for patients with MGD-related dry eye.
- *Demodex* infection does not significantly impact the prognosis of keratitis.

Availability of Data and Materials

The data required during the current study are available upon reasonable request from the corresponding author.

Author Contributions

HL designed the research study. JHX and LYW collected and analyzed the data. HL wrote the first draft. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The research was approved by the Ethics Committee of The First People's Hospital of Chun'an County (Approval No: 2024-04-12-06) and conducted in accordance with the Declaration of Helsinki. All patients signed the informed consent form.

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Conflict of Interest

The authors declare no conflict of interest.

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