

Питання клінічної офтальмології

Optimization of therapeutic approaches for meibomian gland dysfunction in women of reproductive age with dry eye disease

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Оптимізація терапевтичних підходів до лікування дисфункції мейбомієвих залоз у жінок репродуктивного віку із синдромом сухого ока

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Abstract

Objective. To evaluate the clinical effectiveness of oral vitamin D supplementation as an adjunct to conventional therapy in reproductive-aged women with meibomian gland dysfunction (MGD)-associated dry eye disease (DED) and vitamin D deficiency by assessing changes in serum 25-hydroxyvitamin D levels, tear film stability, lipid layer thickness, tear meniscus height, meibomian gland morphology, and ocular surface function.

Materials and Methods. This prospective comparative study included 60 women (120 eyes) of reproductive age diagnosed with MGD-associated DED. Forty patients with confirmed vitamin D deficiency were allocated into two treatment groups: Group I received oral vitamin D supplementa-

tion combined with preservative-free artificial tears, whereas Group II received preservative-free artificial tears alone. Twenty women with normal vitamin D levels comprised Group III (control group) and received preservative-free artificial tears. Ocular surface evaluation includes non-invasive tear breakup time (NIBUT), lipid layer thickness (LLT), tear meniscus height (TMH), and meibography using the LacryD-iag® ocular surface analyzer (Quantel Medical, France). Serum 25-hydroxyvitamin D concentrations were measured at baseline and after three months of treatment.

Results. Combination therapy with oral vitamin D supplementation resulted in significantly greater improvement in tear film stability, LLT, TMH, and meibomian gland morphology compared with artificial tears alone ($p < 0.05$). The greatest clinical improvement was observed in patients with mild-to-moderate MGD. Patients receiving vitamin D supplementation also demonstrated a marked reduction in dry eye symptoms and better restoration of ocular surface function than those treated with artificial tears alone.

Conclusion. Personalized correction of vitamin D deficiency based on serum 25-hydroxyvitamin D levels represents an important pathogenetic component in the treatment of MGD-associated DED in women of reproductive age. The addition of oral vitamin D to preservative-free artificial tears significantly improves tear film stability, meibomian gland function, and clinical symptoms, particularly in mild-to-moderate disease. This approach may provide an effective strategy for optimizing DED management.

Keywords: dry eye disease, meibomian gland dysfunction, vitamin D deficiency, tear film stability, fertile-aged women, LacryDiag, oral vitamin D supplementation.

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Резюме

Мета. Оцінити клінічну ефективність перорального прийому вітаміну D як доповнення до звичайної терапії у жінок репродуктивного віку з синдромом сухого ока (ССО), пов'язаним з дисфункцією мейбомієвих залоз (ДМЗ) та дефіцитом вітаміну D, шляхом оцінки змін рівня 25-гідроксिवітаміну D у сироватці крові, стабільності слізної плівки, товщини ліпідного шару, висоти слізного меніска, морфології мейбомієвих залоз та функції поверхні ока.

Матеріали та методи. Це проспективне порівняльне дослідження охопило 60 жінок (120 очей) репродуктивного віку з ССО, пов'язаного з ДМЗ. 40 пацієнток з підтвердженим дефіцитом вітаміну D були розділені на дві групи лікування: група I отримувала пероральні добавки вітаміну D у поєднанні зі штучними сльозами без консервантів; група II отримувала лише штучні сльози без консервантів. 20 жінок з нормальним рівнем вітаміну D увійшли до групи III (контрольна група) та отримували штучні сльози без консервантів. Оцінка поверхні ока включає неінвазивний час розливу слізної плівки (NIBUT), товщину ліпідного шару (LLT), висоту слізного меніска (BCM) та мейбографію за допомогою аналізатора поверхні ока LacryDiag® (Quantel Medical, Франція). Концентрації 25-гідроксिवітаміну D у сироватці крові вимірювалися на початку дослідження та через три місяці лікування.

Introduction

Dry eye disease (DED) is a multifactorial disorder of the ocular surface characterized by a loss of tear film homeostasis and accompanied by ocular symptoms. Tear film instability, tear hyperosmolality, ocular surface inflammation and damage, and neurosensory abnormalities are recognized as the principal etiological mechanisms of the disease [1,2]. According to the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II), MGD is the leading cause of evaporative DED and contributes to excessive tear evaporation, tear film instability, and chronic ocular surface inflammation [3,4]. DED is one of the most common ocular surface disorders, with epidemiological studies reporting a higher prevalence among women and demonstrating associations with sex, hormonal status, aging, and environmental factors [5–7]. In particular, hormonal factors may play an important role in the development and progression of ocular surface disorders in women [8]. Recent studies have demonstrated an association between vitamin D deficiency and DED. Systematic reviews and meta-analyses have shown that lower serum 25-hydroxyvitamin D [25(OH)D] concentrations are associated with an increased risk and greater severity of DED [9,10]. Furthermore, clinical studies have reported improvement in dry eye symptoms and ocular surface parameters following vitamin D supplementation in patients with vitamin D deficiency [11]. These findings suggest that vitamin D deficiency may contribute to ocular surface dysfunction and should be considered during the evaluation of patients with DED. Assessment of tear film stability and ocular surface func-

Результати. Комбінована терапія з пероральним прийомом вітаміну D призвела до значно більшого покращення стабільності слізної плівки, LLT, BCM та морфології мейбомієвих залоз порівняно з використанням лише штучних сліз ($p < 0,05$). Найбільше клінічне покращення спостерігалось у пацієнтів з легкою та помірною формою ДМЗ. Пацієнти, які отримували добавки вітаміну D, також продемонстрували помітне зменшення симптомів сухого ока та краще відновлення функції поверхні ока, ніж ті, хто отримував лише штучні сльози.

Висновок. Персоналізована корекція дефіциту вітаміну D на основі рівня 25-гідроксिवітаміну D у сироватці крові є важливим патогенетичним компонентом у лікуванні ССО, пов'язаної з ДМЗ, у жінок репродуктивного віку. Додавання перорального вітаміну D до штучних сліз без консервантів значно покращує стабільність слізної плівки, функцію мейбомієвих залоз та клінічні симптоми, особливо при легкому та помірному перебігу захворювання. Цей підхід може забезпечити ефективну стратегію для оптимізації лікування ССО.

Ключові слова: синдром сухого ока, дисфункція мейбомієвих залоз, дефіцит вітаміну D, стабільність слізної плівки, жінки фертильного віку, LacryDiag, пероральний прийом вітаміну D.

tion is essential for the diagnosis and monitoring of patients with MGD-associated DED. The TFOS DEWS II Diagnostic Methodology Report recommends objective evaluation of tear film parameters and meibomian gland morphology to improve the accuracy and reproducibility of diagnosis and treatment monitoring [12]. Despite increasing evidence linking vitamin D deficiency with DED, previous studies have mainly focused on the association between vitamin D status and ocular surface abnormalities or have evaluated vitamin D supplementation in heterogeneous patient populations. Limited data are available regarding the effectiveness of individualized oral vitamin D supplementation as an adjunct to conventional therapy, specifically in reproductive-aged women with MGD-associated DED and vitamin D deficiency. Moreover, few studies have simultaneously evaluated biochemical vitamin D status together with comprehensive objective non-invasive ocular surface parameters, NIBUT, LLT, TMH and meibomian gland morphology. Therefore, the present prospective study aimed to evaluate the clinical efficacy of individualized oral vitamin D supplementation in combination with preservative-free artificial tears in reproductive-aged women with MGD-associated DED and confirmed vitamin D deficiency, using both biochemical assessment of vitamin D status and objective non-invasive ocular surface imaging.

Scientific novelty and clinical significance. Unlike previous studies that primarily investigated the association between vitamin D deficiency and DED or evaluated vitamin D supplementation in heterogeneous patient populations,

the present study specifically focused on reproductive-aged women with MGD-associated DED and confirmed vitamin D deficiency. The scientific novelty of the study lies in the comprehensive evaluation of individualized oral vitamin D supplementation using biochemical assessment of vitamin D status together with objective non-invasive ocular surface parameters. The findings may provide clinically relevant evidence supporting a personalized approach to the correction of vitamin D deficiency as an adjunct to conventional therapy in this specific patient population.

Objective of the study. To evaluate the clinical effectiveness of oral vitamin D supplementation as an adjunct to conventional therapy in reproductive-aged women with MGD associated with DED and vitamin D deficiency by assessing changes in serum 25-hydroxyvitamin D levels, tear film stability, LLT, TMH, meibomian gland morphology, and ocular surface function.

Materials and Methods

This prospective, comparative, non-randomized study was conducted at the Republican Specialized Scientific and Practical Medical Center of Eye Microsurgery (Tashkent, Uzbekistan) between January 2024 and March 2026. A total of 60 women (120 eyes) of reproductive age diagnosed with DED associated with MGD were enrolled. The mean age of the participants was 28.2 ± 4.2 years. The diagnosis of DED was established according to the recommendations of TFOS DEWS II, based on patients' symptoms and objective ocular surface examinations [1,12]. Women with vitamin D deficiency were assigned to two treatment groups according to the predefined non-randomized treatment protocol. Group I (20 patients, 40 eyes) received individualized oral vitamin D supplementation according to 25-hydroxyvitamin D levels in combination with preservative-free artificial tears (Ocutears[®], one drop four times daily for 3 months). Group II (20 patients, 40 eyes) received preservative-free artificial tears alone. Group III (20 patients, 40 eyes) consisted of patients with normal serum vitamin D concentrations and received preservative-free artificial tears, serving as the control group. All participants underwent ophthalmological examination at baseline and three months after treatment. The examination includes best-corrected visual acuity assessment, slit-lamp biomicroscopy, NIBUT, LLT, TMH, and non-contact meibography using the LacryDiag[®] ocular surface analyzer (Quantel Medical, Cournon-d'Auvergne, France). Serum 25(OH)D concentrations were determined using the iFlash 1800 Chemiluminescence Immunoassay Analyzer (Shenzhen YHLO Biotech Co., Ltd., Shenzhen, China). Vitamin D status was classified according to the predefined categories used in this study as severe deficiency (<10 ng/mL), deficiency (10–20 ng/mL), insufficiency (20–30 ng/mL), and sufficient status (≥ 30 ng/mL) [11].

Vitamin D status was categorized using the same predefined categories throughout the study: severe deficiency (<10 ng/mL), deficiency (10–20 ng/mL), insufficiency

Table 1. Classification of serum 25-hydroxyvitamin D [25(OH)D] concentrations according to the Endocrine Society Clinical Practice Guideline (2011) [11].

Level	Classification
<10 ng/mL	Severe deficiency
10–20 ng/mL	Deficiency
20–30 ng/mL	Insufficiency
≥ 30 ng/mL	Sufficient

(20–30 ng/mL), and sufficient status (≥ 30 ng/mL) [11] (Table 1).

A comprehensive non-invasive assessment of the ocular surface and tear film was performed using the LacryDiag[®] ocular surface analyzer (Quantel Medical, Cournon-d'Auvergne, France). The examination protocol included the following parameters:

1. NIBUT was automatically measured to assess tear film stability and indirectly evaluate the mucin layer; the normal reference range was considered 8–20 seconds.
2. Lipid layer thickness was determined by interferometry, with normal values ranging from 50 to 80 nm.
3. Non-contact meibography was carried out to examine the structure and function of the meibomian glands in both upper and lower eyelids; physiological gland loss was defined as 0–20%.
4. Tear meniscus height was measured to estimate the aqueous component of the tear film, with normal values between 0.25 and 1.0 mm. (Figure 1 – see cover page 2).

Vitamin D Supplementation Protocol Oral vitamin D dosing was individualized according to tear and serum 25(OH)D levels, based on Endocrine Society (2011) recommendations (Table 2) [11].

Ethical considerations. At the time the study was conducted, the Republican Specialized Scientific and Practical Medical Center of Eye Microsurgery did not have a separate institutional ethics committee. Therefore, the study protocol was reviewed and approved by the Scientific Council of the Center (Extract from Minutes No. 2, dated 27 February 2025), whose membership includes a representative of the National Ethics Committee of Uzbekistan (NECU). Written informed consent was obtained from all participants prior to enrollment. All signed informed consent forms are securely retained at the institution and are available to the editorial office upon reasonable request in accordance with patient confidentiality requirements. All study procedures were performed in accordance with the ethical principles of the Declaration of Helsinki (2013 revision).

Inclusion criteria. Women aged 18–45 years diagnosed with dry eye disease associated with meibomian gland dysfunction, availability of serum and tear fluid D [25(OH)D] measurements, and willingness to participate in the study with completion of all scheduled follow-up examinations.

Exclusion criteria. Patients with previous ocular surgery within the preceding six months, contact lens wear, active ocular infection or allergy, autoimmune diseases

Table 2. Oral vitamin D supplementation regimen according to baseline 25-hydroxyvitamin D [25(OH)D] concentrations and the Endocrine Society Clinical Practice Guideline (2011) [11]. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ng/mL, nanograms per milliliter

Serum 25(OH)D level	Vitamin D status	Initial treatment (8–12 weeks)	Alternative regimen	Maintenance dose
<10 ng/mL	Severe deficiency	50,000 IU/week for 8 weeks	7,000 IU/day	2,000–4,000 IU/day
10–20 ng/mL	Deficiency	50,000 IU/week for 6 weeks	4,000 IU/day	1,500–2,000 IU/day
20–30 ng/mL	Insufficiency	1,000–2,000 IU/day for 8–12 weeks	–	800–1,000 IU/day
≥30 ng/mL	Sufficient	Treatment not required	–	800–1,000 IU/day (maintenance, if indicated)

affecting the ocular surface, pregnancy or breastfeeding, systemic diseases known to affect tear production (e.g., Sjögren syndrome), use of topical anti-inflammatory medications within one month before enrollment, current systemic vitamin D supplementation, or refusal to provide written informed consent were excluded from the study.

Statistical analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean $\pm$ standard deviation (SD). The normality of data distribution was assessed using the Shapiro–Wilk test. Comparisons between baseline and post-treatment values within each study group were performed using the paired Student's *t*-test for normally distributed variables or the Wilcoxon signed-rank test for non-normally distributed data. Treatment effects over time among the three study groups were evaluated using two-way repeated-measures analysis of variance (ANOVA), with Time (baseline and 3-month follow-up) as the within-subject factor and Treatment Group as the between-subject factor. Bonferroni post hoc correction was applied for multiple comparisons when appropriate. A two-sided *p*-value < 0.05 was considered statistically significant.

Results

At baseline, 60 women with MGD-associated DED were examined. A reduced or absent tear meniscus was observed in 46 patients (76.7%), conjunctival mucus fila-

ments in 13 (21.7%), localized bulbar conjunctival prolapse in 50 (83.3%), mild conjunctival hyperemia in 36 (60.0%), and tear film debris in 28 patients (46.7%), indicating marked ocular surface instability before treatment.

Changes in NIBUT are presented in Table 3. Baseline NIBUT values were comparable among the three treatment groups across all grades of MGD. Following treatment, Group I (oral vitamin D plus preservative-free artificial tears) demonstrated significant improvement in NIBUT at all MGD severity grades compared with baseline (paired comparison, $p < 0.001$). Group III (normal vitamin D status treated with preservative-free artificial tears) also showed significant improvement ($p < 0.001$). In contrast, Group II (preservative-free artificial tears alone in vitamin D-deficient patients) demonstrated only modest increases that did not reach statistical significance (all $p > 0.05$). Repeated-measures ANOVA demonstrated a significant Group $\times$ Time interaction ($p < 0.001$), indicating that the magnitude of improvement differed significantly among treatment groups and that adjunctive vitamin D supplementation produced greater restoration of tear film stability than artificial tears alone.

Changes in TMH are summarized in Table 4. A significant increase in TMH was observed in Group I across all MGD severity grades after treatment compared with baseline (paired comparison, $p < 0.001$). Similar significant improvement was observed in Group III ($p < 0.001$), whereas Group II showed only limited changes without statistical

Table 3. Changes in non-invasive tear breakup time (NIBUT) before and after treatment (mean $\pm$ SD)

Variable	Time	Group I (Vitamin D + AT) (n=20)	Group II (AT only) (n=20)	Group III (Normal vitamin D + AT) (n=20)	Between-group <i>p</i>
NIBUT (s)	Baseline	6.1 $\pm$ 2.4	5.6 $\pm$ 2.3	6.2 $\pm$ 2.5	0.62
	3 months	10.2 $\pm$ 2.7*	6.7 $\pm$ 2.4	10.3 $\pm$ 2.6**	0.003

Notes: Data are presented as mean $\pm$ SD. $p < 0.05$ was considered statistically significant. * Statistically significant difference compared with Group II at 3 months. ** Statistically significant difference compared with Group II at 3 months; no statistically significant difference between Groups I and III. Within-group comparisons between baseline and 3 months: Group I, $p < 0.001$; Group II, $p = 0.08$; Group III, $p < 0.001$. Between-group treatment effects were evaluated using two-way repeated-measures ANOVA (Group $\times$ Time interaction).

Abbreviations: NIBUT, non-invasive tear breakup time; AT, preservative-free artificial tears; SD, standard deviation. Within-group comparisons were performed using the paired Student's *t*-test (or Wilcoxon signed-rank test where appropriate). Between-group treatment effects were analyzed using two-way repeated-measures ANOVA (Group $\times$ Time interaction).

significance ($p > 0.05$). Repeated-measures ANOVA confirmed a significant Group $\times$ Time interaction ($p < 0.001$), demonstrating that oral vitamin D supplementation resulted in significantly greater recovery of tear meniscus height than artificial tears alone.

The interferometric evaluation of lipid layer thickness is presented in Table 5. After treatment, LLT increased significantly in Group I at all grades of MGD compared with baseline (paired comparison, $p < 0.001$). Significant improvement was also observed in Group III ($p < 0.001$), while Group II exhibited only small, statistically non-significant changes ($p > 0.05$). Repeated-measures ANOVA revealed a significant Group $\times$ Time interaction ($p < 0.001$), indicating that adjunctive vitamin D supplementation provided significantly greater restoration of the tear film lipid layer.

Group I demonstrated a significant reduction in meibomian gland loss after treatment across all MGD severity grades compared with baseline (paired comparison, $p < 0.001$). Group III also showed significant improvement ($p < 0.001$), whereas only minimal, non-significant changes were observed in Group II ($p > 0.05$). To account for baseline differences in disease severity, analysis of covariance (ANCOVA) was additionally performed using baseline MGD grade as a covariate. The treatment effect remained statistically significant ($p < 0.001$), confirming that the observed improvement was independent of the initial severity of MGD (Table 6).

Overall, patients receiving oral vitamin D supplementation in combination with preservative-free artificial tears (Group I) demonstrated the greatest improvement in tear film stability, tear meniscus height, lipid layer thick-

Table 4. Changes in tear meniscus height (TMH) before and after treatment (mean $\pm$ SD)

Variable	Time	Group I (Vitamin D + AT) (n=20)	Group II (AT only) (n=20)	Group III (Normal vitamin D + AT) (n=20)	Between-group p
TMH (mm)	Baseline	0.18 $\pm$ 0.08	0.17 $\pm$ 0.07	0.20 $\pm$ 0.08	0.58
	3 months	0.37 $\pm$ 0.07*	0.26 $\pm$ 0.06	0.36 $\pm$ 0.07**	0.004

Notes: Data are presented as mean $\pm$ SD. * $p < 0.05$ compared with Group II at 3 months. ** $p < 0.05$ compared with Group II at 3 months; no statistically significant difference compared with Group I. Within-group comparisons (baseline vs. 3 months): Group I, $p < 0.001$; Group II, $p = 0.07$; Group III, $p < 0.001$. Between-group treatment effects were analyzed using two-way repeated-measures analysis of variance (ANOVA) to evaluate the Group $\times$ Time interaction. A two-sided p-value < 0.05 was considered statistically significant. Abbreviations: TMH, tear meniscus height; AT, preservative-free artificial tears; SD, standard deviation.

Table 5. Changes in lipid layer thickness (LLT) before and after treatment (mean $\pm$ SD)

Variable	Time	Group I (Vitamin D + AT) (n=20)	Group II (AT only) (n=20)	Group III (Normal vitamin D + AT) (n=20)	Between-group p
LLT (nm)	Baseline	22.8 $\pm$ 8.9	23.4 $\pm$ 8.2	22.4 $\pm$ 8.8	0.74
	3 months	39.8 $\pm$ 6.5*	29.7 $\pm$ 6.2	39.4 $\pm$ 6.3**	0.002

Notes: Data are presented as mean $\pm$ SD. * $p < 0.05$ compared with Group II at 3 months. ** $p < 0.05$ compared with Group II at 3 months; no statistically significant difference compared with Group I. Within-group comparisons (baseline vs. 3 months): Group I, $p < 0.001$; Group II, $p = 0.06$; Group III, $p < 0.001$. Between-group treatment effects were analyzed using two-way repeated-measures analysis of variance (ANOVA) to evaluate the Group $\times$ Time interaction. A two-sided p-value < 0.05 was considered statistically significant. Abbreviations: LLT, lipid layer thickness; AT, preservative-free artificial tears; SD, standard deviation; nm, nanometers.

Table 6. Changes in meibomian gland loss before and after treatment (mean $\pm$ SD)

Variable	Time	Group I (Vitamin D + AT) (n = 20)	Group II (AT only) (n = 20)	Group III (Normal vitamin D + AT) (n = 20)	Between-group p
Meibomian gland loss (%)	Baseline	45.1 $\pm$ 15.6	45.7 $\pm$ 14.8	44.8 $\pm$ 15.3	0.91
	3 months	26.4 $\pm$ 12.1*	39.2 $\pm$ 13.4	25.8 $\pm$ 11.9**	0.001

Notes: Data are presented as mean $\pm$ SD. * $p < 0.05$ compared with Group II at 3 months. ** $p < 0.05$ compared with Group II at 3 months; no statistically significant difference compared with Group I. Within-group comparisons (baseline vs. 3 months): Group I, $p < 0.001$; Group II, $p = 0.09$; Group III, $p < 0.001$. Between-group treatment effects were analyzed using two-way repeated-measures analysis of variance (ANOVA) to evaluate the Group $\times$ Time interaction. A two-sided p-value < 0.05 was considered statistically significant. Abbreviations: AT, preservative-free artificial tears; SD, standard deviation.

ness, and meibomian gland morphology. Improvements in Group III were also significant but were generally comparable to those observed in Group I because these patients had normal baseline vitamin D levels. In contrast, patients treated with preservative-free artificial tears alone despite vitamin D deficiency (Group II) exhibited only modest functional improvement. Collectively, these findings indicate that correction of vitamin D deficiency enhances the therapeutic response in women with MGD-associated DED by improving both tear film function and ocular surface morphology.

Discussion

DED is currently recognized as a multifactorial disorder characterized by the loss of tear film homeostasis, tear film instability, hyperosmolarity, ocular surface inflammation, epithelial damage, and neurosensory abnormalities [1,2]. According to the TFOS DEWS II reports, MGD is the leading cause of evaporative DED, resulting in reduced lipid secretion, excessive tear evaporation, and chronic ocular surface inflammation [3–5]. The contribution of MGD to tear film instability and ocular surface damage has long been established, making restoration of meibomian gland function one of the principal therapeutic goals in patients with evaporative DED [13]. The present study evaluated the effectiveness of individualized oral vitamin D supplementation combined with preservative-free artificial tears in women of reproductive age with MGD-associated DED and vitamin D deficiency. The results demonstrated that combined therapy produced significantly greater improvement in objective ocular surface parameters than artificial tears alone. Patients receiving vitamin D supplementation exhibited significant increases in NIBUT, TMH, and LLT, together with a significant reduction in meibomian gland loss assessed by meibography. In contrast, patients with vitamin D deficiency treated only with preservative-free artificial tears demonstrated modest improvements that did not reach statistical significance for most outcome measures. One of the major strengths of the present study is the statistical approach used to evaluate treatment efficacy. Unlike studies relying solely on comparisons of post-treatment values, treatment response was assessed using paired comparisons between baseline and the three-month follow-up within each study group. Furthermore, repeated-measures analysis of variance demonstrated significant Group $\times$ Time interactions, confirming that improvements differed significantly among the treatment groups over time and were attributable to the therapeutic intervention rather than to baseline differences. This longitudinal analytical approach provides stronger evidence for treatment efficacy and directly addresses an important methodological limitation of studies based solely on post-treatment comparisons. The superiority of combined therapy observed in Group I suggests that correction of vitamin D deficiency enhances the therapeutic effect of conventional lubrication therapy. Although preservative-free artificial tears improve ocular surface lubrication and

reduce patient discomfort, they do not directly modify the inflammatory mechanisms or MGD responsible for evaporative DED. In contrast, restoration of vitamin D status may improve tear film homeostasis through several biological mechanisms, thereby potentiating the response to conventional treatment. Vitamin D regulates both innate and adaptive immune responses, suppresses the production of pro-inflammatory cytokines, promotes epithelial barrier integrity, and contributes to the maintenance of lacrimal functional unit homeostasis [11]. Vitamin D deficiency has been associated with increased ocular surface inflammation, epithelial dysfunction, goblet cell loss, and impaired tear secretion, all of which contribute to tear film instability and the progression of DED. Restoration of adequate vitamin D levels may therefore reduce ocular surface inflammation, improve epithelial regeneration, normalize tear film composition, and enhance meibomian gland function, thereby explaining the significant improvements observed in the present study. The increase in NIBUT following vitamin D supplementation reflects improved tear film stability, which represents one of the principal therapeutic targets in evaporative DED. Similarly, the significant increase in TMH indicates improved tear volume and enhanced maintenance of the precocular tear film. Improvement in LLT suggests recovery of meibomian gland secretory function and a reduction in excessive tear evaporation. These functional improvements were accompanied by a significant reduction in meibomian gland loss detected by meibography, indicating that combined therapy may contribute not only to functional recovery but also to the preservation of gland morphology. Structural assessment of meibomian glands has become an essential component of modern DED evaluation because gland loss correlates closely with disease severity and tear film dysfunction [14]. Our findings are consistent with previous clinical investigations. Askari et al. [9], in a systematic review and meta-analysis, demonstrated that vitamin D deficiency is significantly associated with shorter tear breakup time, lower Schirmer test values, increased ocular surface staining, and more severe dry eye symptoms. Similarly, Liu et al. [10] reported that vitamin D deficiency significantly increases the risk of DED and suggested that correction of vitamin D deficiency may improve both subjective symptoms and objective clinical findings. Bae et al. [8] also demonstrated significant improvement in clinical dry eye parameters following oral vitamin D supplementation in patients refractory to conventional treatment. Previous studies have also shown that therapeutic interventions directly targeting MGD, including intense pulsed light (IPL), can significantly improve tear film stability, lipid layer quality, and meibomian gland function [15]. Although IPL therapy was not included in the present study, the improvement observed after vitamin D supplementation suggests that modulation of systemic inflammatory pathways may provide additional therapeutic benefits in patients with MGD-associated DED, particularly in those with confirmed vitamin D deficiency. The present study further ex-

tends previous evidence by simultaneously evaluating serum vitamin D status together with multiple objective non-invasive ocular surface parameters, including NIBUT, TMH, LLT, and meibography. While many earlier studies primarily relied on symptom questionnaires or conventional clinical tests, the comprehensive imaging-based assessment used in the present investigation provides additional evidence regarding the effects of vitamin D supplementation on both functional and structural components of the ocular surface. The results of the present study are also consistent with the TFOS DEWS II Epidemiology Report, which identified female sex, hormonal status, aging, and MGD as major risk factors for DED [6]. Since all participants in the present study were women of reproductive age, correction of vitamin D deficiency may represent an important additional therapeutic strategy in this specific patient population. Another important observation is that treatment efficacy remained significant after adjustment for baseline disease severity. Because disease severity was comparable among the study groups at baseline, the observed improvements cannot be explained by differences in the initial stage of MGD. This finding supports the conclusion that vitamin D supplementation may exert an independent beneficial effect on tear film stability and meibomian gland function regardless of baseline disease severity. From a clinical perspective, these findings suggest that evaluation of serum 25-hydroxyvitamin D concentrations should be considered in women presenting with MGD-associated DED, particularly in patients demonstrating an insufficient response to conventional lubricant therapy. Early identification and correction of vitamin D deficiency may improve treatment outcomes, restore tear film homeostasis, and reduce the progression of MGD. In addition to tear film instability and ocular surface inflammation, persistent ocular discomfort in some patients may also be influenced by neurosensory abnormalities and neuropathic ocular pain, which are increasingly recognized as important contributors to DED [16]. Although neurosensory function was not evaluated in the present study, future investigations incorporating ocular pain assessment and inflammatory biomarkers may provide a more comprehensive understanding of the mechanisms underlying the beneficial effects of vitamin D supplementation. The present study has several limitations. First, the sample size was relatively small and included only women of reproductive age, which may limit the generalizability of the findings. Second, the follow-up period was limited to three months, preventing assessment of long-term therapeutic outcomes. Third, inflammatory cytokines, oxidative stress markers, and neurosensory biomarkers were not evaluated. Finally, although disease severity was considered during statistical analysis, larger multicenter randomized controlled trials with longer follow-up periods are required to confirm these findings and further clarify the role of vitamin D supplementation in the management of MGD-associated DED.

In conclusion, individualized oral vitamin D supplementation combined with preservative-free artificial tears

produced significantly greater improvement in tear film stability, tear volume, lipid layer thickness, and meibomian gland morphology than preservative-free artificial tears alone in women of reproductive age with MGD-associated dry eye disease and vitamin D deficiency. The use of paired within-group comparisons together with repeated-measures ANOVA confirmed that these improvements were attributable to treatment rather than baseline differences among study groups. These findings support the incorporation of serum vitamin D assessment into the comprehensive evaluation of patients with MGD-associated DED and suggest that correction of vitamin D deficiency may represent an effective adjunctive therapeutic strategy for improving both functional and structural ocular surface outcomes.

Author Contributions

K.M.Kh.: Conceptualization, Analysis, Project Administration, Conceptualization, Writing – review & editing; V.M.SH.: Data collection and analysis, K.M.Kh. and Kh.Z.A.: Writing – original draft; V.M.SH.: Writing – review & editing, Formal Analysis, Data collection and analysis, Formal Analysis. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no conflicts of interest that could influence their opinion on the subject matter or materials described and discussed in this manuscript.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

DED – dry eye disease, MGD – meibomian gland dysfunction, NIBUT – non-invasive tear breakup time, TFOS DEWS II – Tear Film & Ocular Surface Society Dry Eye Workshop II.

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