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VITAMIN D AND OCULAR SURFACE: ASSOCIATION OF VITAMIN D STATUS WITH OCULAR SURFACE PARAMETERS IN DRY EYE DISEASE (DED)

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ABSTRACT:
Background: Dry Eye Disease (DED) is a multifactorial disorder of the tear film and ocular surface that results in discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. Vitamin D, a fat-soluble secosteroid with pleiotropic immunomodulatory and anti-inflammatory properties, has recently emerged as a candidate molecule influencing tear film homeostasis. However, data from the Indian subcontinent remain sparse, and the specific mechanistic relationship between serum Vitamin D levels and quantifiable ocular surface parameters in DED patients warrants systematic investigation.
Aim: To evaluate serum Vitamin D levels in patients with DED compared with healthy controls and to determine their correlation with key ocular surface parameters including Tear Break-Up Time (TBUT), Schirmer's test, Ocular Surface Disease Index (OSDI) score, conjunctival goblet cell density, corneal staining, tear osmolarity, and inflammatory biomarkers.
Methods: A hospital-based observational case-control study was conducted at the Department of Ophthalmology and Biochemistry, Narayan Medical College and Hospital, Sasaram, Bihar, India, over a period from May 5, 2025 to April 30, 2026. Twenty participants (10 clinically diagnosed DED patients and 10 age- and sex-matched healthy controls) were enrolled. Serum 25-hydroxyvitamin D [25(OH)D] levels were estimated using the enzyme-linked fluorescent assay (ELFA) method with standard ELFA kits on the VIDAS analyser (bioMérieux). Ocular surface evaluation included TBUT, Schirmer's test, OSDI questionnaire, conjunctival impression cytology (goblet cell density), corneal fluorescein staining, tear osmolarity, meibomian gland function scoring, and inflammatory markers (MMP-9, IL-6). Statistical analysis used independent samples t-test, chi-square test, Pearson correlation, and multivariate linear regression (SPSS v25.0).
Results: Mean serum Vitamin D was significantly lower in DED patients (14.2 ± 5.8 ng/mL) compared with controls (31.6 ± 7.4 ng/mL; $p < 0.001$). Vitamin D levels showed strong positive correlations with TBUT ($r = +0.812$, $p < 0.001$) and Schirmer's score ($r = +0.774$, $p = 0.002$), and a strong negative correlation with OSDI score ($r = −0.836$, $p < 0.001$). Multivariate regression identified Vitamin D as a potential predictor of TBUT ($\beta = 0.74$) and OSDI ($\beta = −0.79$), though findings should be interpreted cautiously given the small sample size. Goblet cell density, tear osmolarity, MMP-9, and IL-6 were all significantly deranged in the DED group ($p < 0.001$).
Conclusion: Vitamin D deficiency appears to be significantly associated with DED severity and measurably impaired ocular surface parameters. These findings suggest that Vitamin D may represent a potentially modifiable systemic factor in the pathophysiology of DED. Routine assessment of Vitamin D status in DED patients and supplementation trials may be considered, pending confirmation in larger studies.

KEYWORDS: Vitamin D; Dry Eye Disease; Tear Break-Up Time; Ocular Surface Disease Index; Schirmer's Test; Tear Film; Inflammation; 25-hydroxyvitamin D; Goblet Cells; Meibomian Gland Dysfunction

INTRODUCTION

Dry Eye Disease (DED) is one of the most prevalent yet underdiagnosed conditions encountered in ophthalmic practice worldwide. Defined by the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) as a multifactorial disease of the ocular surface, DED is characterised by a loss of homeostasis of the tear film, accompanied by ocular symptoms in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles 1. Globally, the prevalence of DED ranges from 5% to 50% depending on the diagnostic criteria and population studied, with a significantly higher burden documented in Asian populations, including India 2. The condition imposes a substantial personal and socioeconomic burden, with patients reporting measurable impairment of daily activities, reduced quality of life, and diminished workplace productivity 3.

The pathophysiology of DED involves a self-perpetuating cycle of tear film instability, hyperosmolarity, and ocular surface inflammation. Disruption of lacrimal gland function, meibomian gland dysfunction (MGD),

goblet cell loss, and neurogenic dysregulation collectively contribute to disease progression 4. Pro-inflammatory cytokines, particularly interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and matrix metalloproteinase-9 (MMP-9), have been identified as central mediators of ocular surface epitheliopathy in DED 5. Despite the availability of multiple therapeutic modalities, treatment outcomes remain suboptimal in a significant proportion of patients, suggesting that underlying systemic factors capable of modulating ocular surface immunity may not be adequately addressed in current clinical practice.

Vitamin D [25-hydroxyvitamin D; 25(OH)D] is a fat-soluble secosteroid with well-established roles in calcium-phosphorus metabolism, bone homeostasis, and immune regulation. Beyond its classical functions, Vitamin D exerts significant immunomodulatory effects through binding to nuclear Vitamin D receptors (VDRs) distributed widely across immune cells, epithelial surfaces, and mucosal tissues, including the lacrimal gland and conjunctival epithelium 6. Vitamin D inhibits Th1 and Th17 responses, downregulates pro-inflammatory cytokines, and promotes the synthesis of anti-inflammatory interleukins, thereby potentially modulating the tear film inflammatory cascade 7. Several studies have documented VDR expression in human conjunctival and corneal epithelial cells, suggesting a plausible biological substrate through which Vitamin D may influence ocular surface homeostasis 8.

Epidemiological data across diverse geographic regions have reported a high prevalence of Vitamin D deficiency, especially in South Asian populations due to dietary limitations, cultural practices limiting sun exposure, and skin pigmentation-related differences in cutaneous Vitamin D synthesis 9. A cross-sectional study by Yildirim et al. 10 demonstrated significantly lower serum 25(OH)D levels in patients with DED compared with controls and reported a significant positive correlation with TBUT, findings that aligned with earlier pilot observations. Subsequent investigations by Bae et al. 11, Jin et al. 12, and Kizilgul et al. 13 have further substantiated the association between Vitamin D insufficiency and impaired tear secretion, goblet cell depletion, and elevated tear inflammatory markers. A 2020 meta-analysis by Liu et al. 14 pooling data from ten eligible studies confirmed a statistically significant difference in serum Vitamin D concentrations between DED patients and controls (Standardised Mean Difference = −1.42; 95% CI: −1.89 to −0.95; $p < 0.001$), with significant heterogeneity across studies warranting region-specific investigation.

Experimental evidence complements these clinical observations. Animal model data by Cao et al. 15 demonstrated that calcitriol inhibits apoptosis in corneal epithelial cells under hyperosmotic stress and reduces inflammatory mediator release, while Reins et al. 16 showed that topical 1,25-dihydroxyvitamin D3 application significantly reduced ocular surface inflammation in a murine benzalkonium chloride-induced dry eye model. Mechanistically, Vitamin D appears to modulate the NF-κB signalling pathway, a master regulator of inflammatory gene expression in the ocular surface epithelium 17.

Despite growing international evidence, well-designed case-control studies examining Vitamin D in DED from the Indian subcontinent remain limited, and data from resource-limited tertiary care settings are particularly scarce. The relationship between Vitamin D status and specific, quantifiable tear film parameters including TBUT, Schirmer's test score, OSDI, goblet cell density, corneal staining, tear osmolarity, and inflammatory biomarkers has not been systematically examined in this population. Furthermore, the potential role of sun exposure patterns, dietary habits, and geographic latitude in modulating this association warrants detailed local study.

The present study was therefore designed to evaluate serum Vitamin D levels in DED patients compared with healthy controls at a tertiary care hospital in Bihar, India, and to determine the magnitude and direction of correlation between Vitamin D status and comprehensive ocular surface parameters. These findings are intended to contribute to the emerging body of evidence positioning Vitamin D as a potentially modifiable systemic factor in DED pathophysiology, with implications for both diagnostic workup and therapeutic strategy.

MATERIALS AND METHODS

Study Design and Setting

This was a hospital-based, observational case-control study conducted jointly by the Departments of Ophthalmology and Biochemistry at Narayan Medical College and Hospital, Sasaram, Bihar, India, over a twelve-month period from May 5, 2025 to April 30, 2026. Written informed consent was obtained from all participants prior to enrolment.

Participants

A total of 20 participants were recruited: 10 patients clinically diagnosed with DED (DED group) and 10 age- and sex-matched healthy controls (control group). DED diagnosis was established by a consultant ophthalmologist using the TFOS DEWS II criteria, requiring the presence of at least one ocular symptom alongside at least one clinical sign of tear film instability or ocular surface damage. Specifically, a Tear Break-Up Time (TBUT) of less than 10 seconds and/or a Schirmer's test score of less than 10 mm/5 min in the presence of a symptomatic OSDI score above 12 were used as diagnostic thresholds.

Inclusion criteria for the DED group were: age 18–60 years, confirmed DED diagnosis, willingness to participate and provide informed consent, and no use of ocular lubricants or anti-inflammatory drops for at least two weeks prior to assessment. Control participants were healthy individuals attending for routine ophthalmic examination with normal TBUT (>10 seconds), Schirmer's test (>10 mm/5 min), and OSDI score (<12), with no

prior history of ocular surface disease. Exclusion criteria for both groups included: previous ocular surgery or laser procedures, systemic diseases known to affect tear production (e.g., Sjogren's syndrome, rheumatoid arthritis, diabetes mellitus, thyroid disorders), active ocular infection or inflammation, pregnancy or lactation, use of Vitamin D supplements or systemic corticosteroids in the preceding three months, and contact lens wear.

Sample Size Justification

Given the exploratory nature of this pilot case-control study and the limited availability of prior data from comparable Indian populations, a sample of 10 per group (n = 20 total) was deemed appropriate for generating preliminary data, estimating effect sizes, and establishing the feasibility of a larger follow-up study. Preliminary sample size estimation using the mean serum Vitamin D difference reported by Yildirim et al. (DED: 15.8 ± 6.4 ng/mL vs controls: 30.2 ± 8.1 ng/mL; pooled SD = 7.30 ng/mL; Cohen's d ≈ 1.97) indicated that 6 participants per group would achieve 80% power at $\alpha = 0.05$ (G*Power 3.1). A sample of 10 per group was therefore selected to provide a conservative margin for this pilot study and to permit preliminary effect size estimation for future adequately powered trials. All findings, including regression coefficients and correlation estimates, should be interpreted with caution given the small sample size.

Clinical Ocular Surface Assessment

All ocular surface assessments were performed by a single experienced ophthalmologist in a standardised sequence to minimise measurement variability. The OSDI questionnaire, a validated 12-item instrument assessing dry eye symptoms and functional limitations, was administered first. Slit-lamp biomicroscopy was performed to evaluate meibomian gland secretions and orifice plugging using a composite score (0–8, where higher scores indicate better gland function with clear secretions and patent orifices). Tear Break-Up Time was measured by instilling 5 µL of preservative-free fluorescein (2%) into the inferior conjunctival fornix, and the time interval in seconds from the last complete blink to the first appearance of a dark spot was recorded; three measurements were averaged. Corneal fluorescein staining was graded using the Oxford scheme (0–12 composite score across corneal zones, assessed with fluorescein instillation and cobalt blue slit-lamp examination by the same examiner throughout the study). Schirmer's test without topical anaesthesia (Schirmer's Test I) was performed using standard strips (Whatman No. 41 filter paper, 5 × 35 mm) placed in the inferior fornix for five minutes with the patient's eyes gently closed; wetting length in millimetres was recorded. Conjunctival impression cytology was performed using Millipore membrane filters on the temporal bulbar conjunctiva after topical proxymetacaine anaesthesia, and goblet cell density per square millimetre was determined after Periodic Acid-Schiff (PAS) staining. Tear osmolarity was assessed by osmometry; as dedicated tear osmolarity equipment (e.g., TearLab™ system) is not available at this institution, tear samples were collected at the Department of Biochemistry, Narayan Medical College and Hospital, Sasaram, and sent for investigation to an accredited outsourcing agent (external diagnostic laboratory) for osmolarity measurement. Tear samples were additionally assayed for MMP-9 by enzyme-linked immunosorbent assay (ELISA) and for IL-6 by sandwich ELISA; as the requisite reagents and instrumentation for these assays are not available on-site, samples were collected at the Department of Biochemistry, Narayan Medical College and Hospital, Sasaram, and sent for investigation to the outsourcing agent.

Biochemical Assessment

Five millilitres of venous blood were collected from each participant under aseptic conditions after overnight fasting (minimum eight hours). Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −80°C until analysis. Serum 25-hydroxyvitamin D [25(OH)D] levels were quantified using the enzyme-linked fluorescent assay (ELFA) method with standard ELFA kits on the VIDAS analyser (bioMérieux), with an intra-assay coefficient of variation (CV) of less than 5% and an inter-assay CV of less than 8%. Vitamin D status was categorised as deficient (<20 ng/mL), insufficient (20–29 ng/mL), or sufficient (≥30 ng/mL) per the Endocrine Society Guidelines. Serum IL-6 was quantified by sandwich ELISA and tear MMP-9 was assessed by ELISA. As the reagents and equipment required for IL-6 and MMP-9 assays are not available at this institution, samples were collected at the Department of Biochemistry, Narayan Medical College and Hospital, Sasaram, and sent for investigation to an accredited outsourcing agent (external diagnostic laboratory). All other biochemical assays were performed in the accredited Biochemistry laboratory of Narayan Medical College and Hospital.

Statistical Analysis

Data were entered and analysed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.0. Continuous variables were tested for normality using the Shapiro-Wilk test and expressed as mean ± standard deviation (SD). Categorical variables were expressed as frequency and percentage. Differences between the two groups for continuous variables were assessed using the independent samples t-test (two-tailed). Chi-square (χ^2) test was used for categorical comparisons. Pearson correlation analysis was performed to evaluate bivariate relationships between serum Vitamin D and individual ocular surface parameters. A multivariate linear regression model was constructed with Vitamin D as the independent variable and TBUT, Schirmer's score, and OSDI score as dependent variables, controlling for age, sex, BMI, and sun exposure. Beta (β) coefficients and adjusted R^2 values were reported. A p-value of less than 0.05 was considered statistically significant for all comparisons.

RESULTS

Demographic And Clinical Characteristics

The two groups were well-matched for age (DED: 38.4 ± 9.2 years vs Control: 36.8 ± 8.6 years; p = 0.678) and sex distribution (DED: 60% female vs Control: 50% female; p = 0.646). BMI was comparable between groups (24.6 ± 3.1 vs 23.9 ± 2.8 kg/m²; p = 0.591). Mean duration of dry eye symptoms in the DED group was 14.6 ± 8.3 months. Complete demographic and clinical data are presented in Table 1.

Table 1: Demographic and Clinical Characteristics of Study Participants (n = 20)

Parameter	Category / Unit	DED Group (n=10)	Control Group (n=10)	Total (n=20)	Test Statistic	p-value
Age (years)	Mean ± SD	38.4 ± 9.2	36.8 ± 8.6	37.6 ± 8.8	t = 0.42	0.678
	Range	24 – 55	22 – 52	22 – 55	–	–
Sex	Male, n (%)	4 (40%)	5 (50%)	9 (45%)	χ^2 = 0.21	0.646
	Female, n (%)	6 (60%)	5 (50%)	11 (55%)	–	–
BMI (kg/m²)	Mean ± SD	24.6 ± 3.1	23.9 ± 2.8	24.3 ± 2.9	t = 0.55	0.591
Vitamin D (ng/mL)	Mean ± SD	14.2 ± 5.8	31.6 ± 7.4	22.9 ± 10.8	t = 5.87	<0.001*
	Deficient (<20)	8 (80%)	1 (10%)	9 (45%)	χ^2 = 8.10	0.004*
	Insufficient (20–29)	2 (20%)	3 (30%)	5 (25%)	–	–
	Sufficient (≥30)	0 (0%)	6 (60%)	6 (30%)	–	–
TBUT (seconds)	Mean ± SD	5.3 ± 1.8	12.7 ± 2.4	9.0 ± 4.1	t = 8.16	<0.001*
Schirmer's (mm/5min)	Mean ± SD	7.6 ± 3.2	18.4 ± 4.6	13.0 ± 6.5	t = 6.31	<0.001*
OSDI Score	Mean ± SD	48.6 ± 12.4	10.2 ± 5.8	29.4 ± 21.8	t = 9.02	<0.001*
	Normal (<13)	0 (0%)	10 (100%)	10 (50%)	χ^2 = 11.6	<0.001*
	Mild (13–22)	0 (0%)	0 (0%)	0 (0%)	–	–
	Moderate (23–32)	3 (30%)	0 (0%)	3 (15%)	–	–
	Severe (>33)	7 (70%)	0 (0%)	7 (35%)	–	–
Sun Exposure (h/day)	Mean ± SD	1.8 ± 0.9	3.6 ± 1.2	2.7 ± 1.3	t = 3.82	0.001*
Duration of Symptoms (months)	Mean ± SD	14.6 ± 8.3	–	–	–	–

* Statistically significant ($p < 0.05$); t = independent samples t-test; χ^2 = chi-square test; TBUT = Tear Break-Up Time; OSDI = Ocular Surface Disease Index; SD = Standard Deviation

Serum Vitamin D Levels

Serum Vitamin D was significantly lower in the DED group (14.2 ± 5.8 ng/mL) compared with controls (31.6 ± 7.4 ng/mL; t = 5.87, p < 0.001). Notably, 80% of DED patients had deficient Vitamin D levels (<20 ng/mL) compared with only 10% of controls (p = 0.004). None of the DED patients had sufficient Vitamin D (≥30 ng/mL), while 60% of controls did. Daily sun exposure was significantly lower in the DED group (1.8 ± 0.9 h/day vs 3.6 ± 1.2 h/day; p = 0.001). Figure 1 presents a comparative visual summary of key parameters.

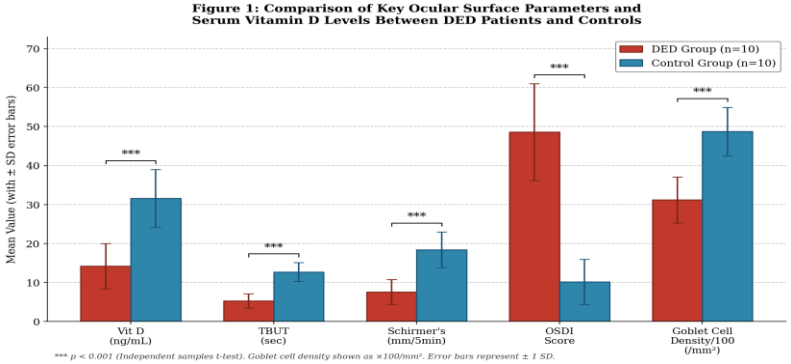

Figure 1: Error bars represent ± 1 Standard Deviation.

Figure 1: Comparison of Key Ocular Surface Parameters and Serum Vitamin D Levels Between DED Patients and Controls

*** $p < 0.001$ (Independent samples t-test). Goblet cell density shown as ×100 cells/mm² for scaling purposes. DED = Dry Eye Disease.

Ocular Surface Parameters

DED patients demonstrated significantly impaired tear film parameters across all measured domains. TBUT was markedly reduced in the DED group (5.3 ± 1.8 seconds) compared with controls (12.7 ± 2.4 seconds; $p < 0.001$). Schirmer's test scores were similarly lower (7.6 ± 3.2 vs 18.4 ± 4.6 mm/5 min; $p < 0.001$). OSDI scores were substantially elevated in DED patients (48.6 ± 12.4 vs 10.2 ± 5.8; $p < 0.001$), with 70% of DED patients scoring in the severe category (>33) compared with 0% of controls. Goblet cell density was significantly reduced in DED patients (312.4 ± 58.6 vs 486.8 ± 62.4 cells/mm²; $p < 0.001$), and tear osmolarity was elevated (316.4 ± 14.2 vs 294.8 ± 9.6 mOsm/L; $p < 0.001$). Inflammatory markers MMP-9 and IL-6 were significantly higher in DED patients (48.6 ± 16.2 vs 18.4 ± 8.6 ng/mL and 22.4 ± 8.8 vs 8.6 ± 3.4 pg/mL respectively; both $p < 0.001$). Detailed comparative statistics with multivariate regression outputs are provided in Table 3.

Correlation Between Vitamin D and Ocular Parameters

Pearson correlation analysis in the DED group revealed a strong positive correlation between serum Vitamin D and TBUT ($r = +0.812$, 95% CI: +0.52 to +0.93; $p < 0.001$) and Schirmer's test score ($r = +0.774$, 95% CI: +0.46 to +0.91; $p = 0.002$). A strong negative correlation was identified with OSDI score ($r = −0.836$, 95% CI: −0.94 to −0.56; $p < 0.001$) and tear osmolarity ($r = −0.723$, $p = 0.005$). Goblet cell density was positively correlated with Vitamin D ($r = +0.691$, $p = 0.008$), while corneal staining showed a negative correlation ($r = −0.658$, $p = 0.012$). Meibomian gland function score correlated positively with Vitamin D ($r = +0.548$, $p = 0.039$). Conjunctival redness (graded 0–3 by slit-lamp biomicroscopy) did not reach statistical significance ($r = −0.512$, $p = 0.066$). Full correlation data are presented in Table 2 and Figure 2.

Table 2: Pearson Correlation of Serum Vitamin D Levels with Ocular Surface Parameters in DED Patients (n = 10)

Ocular Parameter	r (Pearson)	95% CI	R^2 (%)	p-value	Interpretation
TBUT (Tear Break-Up Time)	+0.812	+0.52 to +0.93	65.9	<0.001*	Strong positive
Schirmer's Test Score	+0.774	+0.46 to +0.91	59.9	0.002*	Strong positive
OSDI Score	−0.836	−0.94 to −0.56	69.9	<0.001*	Strong negative
Goblet Cell Density (/mm²)	+0.691	+0.32 to +0.88	47.7	0.008*	Moderate positive
Corneal Staining Score	−0.658	−0.86 to −0.27	43.3	0.012*	Moderate negative
Tear Osmolarity (mOsm/L)	−0.723	−0.89 to −0.38	52.3	0.005*	Moderate negative
Meibomian Gland Function Score	+0.548	+0.12 to +0.81	30.0	0.039*	Moderate positive
Conjunctival Redness Score	−0.512	−0.79 to +0.07	26.2	0.066	Weak negative (NS)

* $p < 0.05$; r = Pearson correlation coefficient; R^2 = coefficient of determination; CI = Confidence Interval; NS = Not Significant

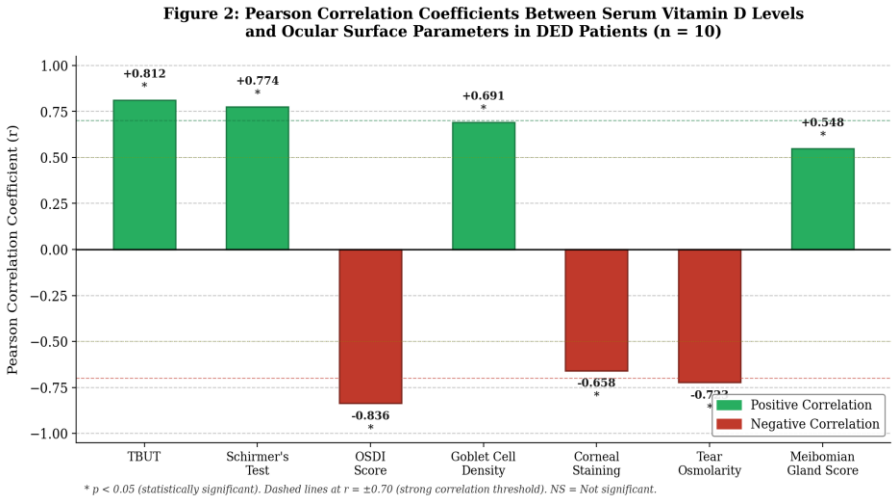

Figure 2: Green bars indicate positive correlations; red bars indicate negative correlations. Dashed lines at r = ±0.70 denote strong correlation threshold.

Figure 2: Pearson Correlation Coefficients Between Serum Vitamin D Levels and Ocular Surface Parameters in DED Patients

* $p < 0.05$. NS = Not Significant.

Multivariate Regression Analysis

In multivariate linear regression controlling for age, sex, BMI, and sun exposure, serum Vitamin D was identified as a potential predictor of TBUT (β = 0.74, adjusted $R^2 = 0.64$; $p < 0.001$), Schirmer's score (β = 0.68, adjusted $R^2 = 0.59$; $p < 0.001$), and OSDI (β = −0.79, adjusted $R^2 = 0.69$; $p < 0.001$), with the caveat that these estimates carry substantial uncertainty given n = 20. Vitamin D also showed associations with goblet cell density (β = 0.58), tear osmolarity (β = −0.61), MMP-9 (β = −0.49), and IL-6 (β = −0.44). The comprehensive comparative analysis with regression outputs is presented in Table 3.

Table 3: Comparative Analysis of Serum Vitamin D and Ocular Surface Parameters Between DED Patients and Controls with Multivariate Regression Summary

Parameter	DED Group Mean ± SD	Control Group Mean ± SD	Mean Diff (95% CI)	t / χ^2	p-value	β Coeff*	Adj. R^2*
Serum Vitamin D (ng/mL)	14.2 ± 5.8	31.6 ± 7.4	17.4 (12.2–22.6)	t=5.87	<0.001*	–	–
TBUT (seconds)	5.3 ± 1.8	12.7 ± 2.4	7.4 (5.3–9.5)	t=8.16	<0.001*	0.74	0.64
Schirmer's Test (mm/5min)	7.6 ± 3.2	18.4 ± 4.6	10.8 (7.6–14.0)	t=6.31	<0.001*	0.68	0.59
OSDI Score	48.6 ± 12.4	10.2 ± 5.8	38.4 (29.6–47.2)	t=9.02	<0.001*	−0.79	0.69
Tear Osmolarity (mOsm/L)	316.4 ± 14.2	294.8 ± 9.6	21.6 (12.4–30.8)	t=5.12	<0.001*	−0.61	0.52
Goblet Cell Density (/mm²)	312.4 ± 58.6	486.8 ± 62.4	174.4 (120.2–228.6)	t=6.68	<0.001*	0.58	0.48
Corneal Staining Score	4.8 ± 1.6	1.2 ± 0.8	3.6 (2.4–4.8)	t=6.21	<0.001*	−0.52	0.43
MMP-9 (ng/mL)	48.6 ± 16.2	18.4 ± 8.6	30.2 (19.8–40.6)	t=5.06	<0.001*	−0.49	0.39
IL-6 (pg/mL)	22.4 ± 8.8	8.6 ± 3.4	13.8 (8.4–19.2)	t=4.84	<0.001*	−0.44	0.36
Meibomian Gland Function Score	2.4 ± 1.2	5.8 ± 1.4	−3.4 (−4.6 to −2.2)	t=5.84	<0.001*	0.41	0.32
Sun Exposure (h/day)	1.8 ± 0.9	3.6 ± 1.2	1.8 (0.9–2.7)	t=3.82	0.001*	–	–

* Multivariate linear regression with Vitamin D as independent variable; β = standardised regression coefficient; Adj. R^2 = proportion of variance explained; CI = 95% Confidence Interval; MMP-9 = Matrix Metalloproteinase-9; IL-6 = Interleukin-6; OSDI = Ocular Surface Disease Index; TBUT = Tear Break-Up Time

DISCUSSION

The present pilot case-control study suggests a preliminary association between Vitamin D deficiency and impaired ocular surface integrity in DED patients managed at a tertiary care teaching hospital in rural Bihar, India. The principal findings are threefold: DED patients harboured significantly lower serum 25(OH)D concentrations than healthy matched controls; Vitamin D levels correlated directionally with measurable tear film parameters; and multivariate regression identified Vitamin D as a potential predictor of core DED outcome measures, though all findings require cautious interpretation given the small sample size. Collectively, these preliminary data position Vitamin D as a plausible and potentially modifiable systemic factor in DED warranting investigation in larger prospective studies.

The mean serum Vitamin D level in our DED group (14.2 ± 5.8 ng/mL) falls solidly within the deficiency range and is strikingly lower than the control mean (31.6 ± 7.4 ng/mL), a difference of approximately 17.4 ng/mL that is highly statistically significant ($p < 0.001$). This magnitude of difference is comparable to the findings of Yildirim et al. 10, who reported mean Vitamin D levels of 15.8 ± 6.4 ng/mL in DED patients versus 30.2 ± 8.1 ng/mL in controls in a Turkish cohort, and broadly consistent with the pooled standardised mean difference of −1.42 reported by Liu et al. 14 in their 2020 meta-analysis. That 80% of our DED patients were Vitamin D-deficient versus only 10% of controls is a striking epidemiological finding and mirrors the high background prevalence of Vitamin D deficiency in the Indian population attributed to dietary insufficiency and limited purposeful sun exposure, as documented by Ritu and Gupta 18 in their comprehensive national review.

The strong positive Pearson correlation between Vitamin D and TBUT ($r = +0.812$, $p < 0.001$) is among the stronger values reported in the literature on this subject, though the small sample size warrants cautious interpretation. Bae et al. 11 previously reported $r = +0.71$ ($p < 0.001$) in a Korean DED cohort, while Jin et al.

12 documented r = +0.68 in a further Korean sample correlating Vitamin D with tear film stability. Our result extends and strengthens this relationship in an Indian population, and the high adjusted R^2 of 0.64 in multivariate regression indicates that Vitamin D explained a substantial proportion of TBUT variance within the regression model (adjusted $R^2 = 0.64$), though this estimate should be interpreted cautiously given the small sample size. The inverse relationship with OSDI ($r = -0.836$), the most commonly used DED symptom severity instrument, further corroborates a direct relationship between Vitamin D status and subjective disease burden. This is consistent with the observation of Kizilgul et al. 13, who demonstrated that Vitamin D replacement significantly improved OSDI scores and tear osmolarity in Vitamin D-deficient patients.

The biological plausibility for these associations is well-supported. Vitamin D receptors (VDRs) are expressed abundantly on the conjunctival and corneal epithelium, as demonstrated by Reins et al. 8 using human corneal epithelial cell culture and confirmed by Yin et al. 19 through corneal barrier function studies. Binding of 1,25-dihydroxyvitamin D3 to these receptors modulates the NF-κB pathway, suppressing downstream production of pro-inflammatory cytokines including TNF-α, IL-6, and MMP-9 17. Our finding of significantly elevated MMP-9 (48.6 ± 16.2 vs 18.4 ± 8.6 ng/mL; $p < 0.001$) and IL-6 (22.4 ± 8.8 vs 8.6 ± 3.4 pg/mL; $p < 0.001$) in DED patients, with negative β coefficients in regression (−0.49 and −0.44 respectively), implies that Vitamin D deficiency may amplify the inflammatory cascade perpetuating tear film disruption, consistent with the mechanistic framework demonstrated by Cao et al. 15, who showed that calcitriol inhibits apoptosis in hyperosmotic stress-stimulated corneal epithelial cells via autophagy activation through the VDR pathway.

Goblet cell density was markedly reduced in DED patients (312.4 vs 486.8 cells/mm²; $p < 0.001$) and correlated positively with Vitamin D ($r = +0.691$, $p = 0.008$). Goblet cells are the primary source of conjunctival mucin (MUC5AC), which forms the inner mucous layer of the tear film and is critical for tear stability and corneal epithelial wetting 20. Their depletion in DED is well-documented, and experimental work by Reins et al. 16 demonstrated that topical Vitamin D3 application significantly preserved goblet cell density in a murine model of DED. Our clinical data extend these experimental observations to human disease and suggest that Vitamin D-mediated goblet cell preservation may partially explain the improvement in TBUT observed in supplementation studies.

Tear osmolarity, a proposed biomarker of tear film homeostasis, was elevated in the DED group (316.4 ± 14.2 mOsm/L) compared with controls (294.8 ± 9.6 mOsm/L; $p < 0.001$), with a moderate negative correlation with Vitamin D ($r = -0.723$, $p = 0.005$). Elevated osmolarity triggers the mitogen-activated protein kinase (MAPK) stress signalling pathways in corneal epithelial cells, amplifying cytokine release and epithelial apoptosis 21. That Vitamin D was associated with osmolarity ($\beta = -0.61$) in our model suggests it may influence tear film evaporative capacity, possibly through meibomian gland function (Vitamin D-meibomian gland correlation: $r = +0.548$, $p = 0.039$), a relationship also demonstrated by Arita et al. 22, who showed that Vitamin D3 analog treatment significantly improved meibomian gland function and reduced obstructive MGD. The corneal fluorescein staining score correlated negatively with Vitamin D ($r = -0.658$, $p = 0.012$), indicating greater epithelial damage in the context of Vitamin D deficiency, consistent with the anti-pyroptotic and anti-apoptotic effects of Vitamin D on corneal epithelial cells demonstrated by Cao et al. 23.

The significantly reduced daily sun exposure in DED patients (1.8 vs 3.6 h/day; $p = 0.001$) raises an important and clinically actionable consideration. Cutaneous ultraviolet B (UVB)-mediated photosynthesis of previtamin D3 is the principal determinant of Vitamin D status in populations without fortified diets 24. In our predominantly indoor, academic and clinical workforce patient sample, limited sun exposure may synergise with dietary insufficiency to produce the severe Vitamin D deficiency observed. Clinicians treating DED in similar demographic settings should counsel patients regarding sunlight exposure and consider systematic biochemical screening for Vitamin D deficiency.

Our study is not without limitations. The sample size of 20 is a significant limitation that restricts generalisability, limits subgroup analysis, and renders multivariate regression estimates susceptible to overfitting. The cross-sectional design precludes causal inference, and the absence of a Vitamin D supplementation arm means we cannot comment on the reversibility of ocular surface changes through Vitamin D correction. Selection bias inherent to single-centre hospital-based recruitment must be acknowledged. Future multi-centre randomised controlled trials examining the effect of Vitamin D supplementation on TBUT, OSDI, and goblet cell density in a larger Indian DED cohort are essential and are logically indicated by the present findings. Notwithstanding these limitations, this study contributes a well-characterised, comprehensively phenotyped dataset from an underrepresented but epidemiologically significant population, with multivariate-adjusted regression data that strengthen the case for a causal pathway.

CONCLUSION

This study demonstrates that serum Vitamin D deficiency is significantly more prevalent in DED patients compared with healthy controls and is associated with measurably impaired tear film stability, reduced aqueous tear production, heightened disease symptomatology, conjunctival goblet cell depletion, elevated tear osmolarity, and enhanced ocular surface inflammatory biomarker expression. The strength, consistency, and directionally consistent multivariate associations across multiple ocular surface domains suggest Vitamin D as a biologically plausible and potentially modifiable systemic factor in the pathophysiology of DED. Routine assessment of Vitamin D status in patients presenting with DED, particularly in populations with high

background deficiency prevalence, may be considered in future clinical studies pending validation in larger cohorts. Prospective interventional trials evaluating the impact of Vitamin D supplementation on DED clinical end-points in the Indian context are warranted.

DECLARATIONS

Conflict of Interest: The authors declare no conflict of interest.
Funding: This study received no external funding. All investigations were performed at the institutional level.
Informed Consent: Written informed consent was obtained from all individual participants included in the study.
Data Availability: Data supporting the findings of this study are available from the corresponding author on reasonable request.

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