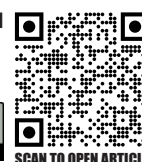


## Role Of Vitamin D3 Supplementation in Dry Eye Disease

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### ABSTRACT

**Purpose:** To evaluate the effect of oral Vitamin D3 supplementation on clinical symptoms and tear film parameters in patients diagnosed with Dry Eye Disease.

**Methodology:** This quasi-experimental study was conducted at Combined Military Hospital (CMH), Murree Hills, over 12 weeks. A total of 375 participants were enrolled, including 200 patients with DED and 175 healthy controls. Baseline Vitamin D3 levels, Ocular Surface Disease Index (OSDI) scores, Schirmer's test, and Tear Break-Up Time (TBUT) were recorded. The DED group received weekly oral Vitamin D3 (50,000 IU) for 12 weeks. Pre- and post-supplementation comparisons were analyzed using SPSS v-26.

**Result:** Vitamin D3 deficiency was significantly more prevalent in the DED group ( $p < 0.001$ ). Post-supplementation, there was a marked improvement in OSDI scores, Schirmer's values, and TBUT (all  $p < 0.001$ ), with large effect sizes. Regression analysis identified Vitamin D3 status as the strongest independent predictor of DED (OR = 7.26,  $p < 0.001$ ). Severity grading also shifted significantly from "severe" to "mild" or "none" following supplementation.

**Conclusion:** Vitamin D3 supplementation significantly improves both symptoms and tear film function in Dry Eye Disease. Screening for Vitamin D deficiency and its correction may serve as a valuable adjunct in the holistic management of DED.

**Keywords:** Dry Eye Disease, Vitamin D3, Tear Break-Up Time, Schirmer Test.

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## INTRODUCTION

Dry Eye Disease (DED) affects millions worldwide and remains a frequent cause of ophthalmic visits. It is associated with discomfort, visual disturbance, and inflammation of the ocular surface.<sup>1,2</sup> The growing burden of DED has prompted researchers to explore systemic risk factors, including nutritional deficiencies. Among these, Vitamin D3 has garnered attention for its role in modulating immune responses and maintaining epithelial health.<sup>3,4</sup>

International studies, including work by Chan et al., 2022 and Rai et al., 2024, have identified a strong association between low serum Vitamin D3 levels and tear film instability.<sup>5,6</sup> Studies by Jain et al 2022, Vieira et al 2024 and Wallace et al 2024 further demonstrated clinical improvement in DED symptoms following supplementation.<sup>7-9</sup> However, despite its high prevalence in South Asia, the contribution of Vitamin D3 deficiency to DED in local populations remains under-explored. Pakistani studies have largely been observational, lacking intervention-based data to support supplementation as a therapeutic option.

This study was therefore designed to evaluate the clinical impact of oral Vitamin D3 supplementation on symptom severity and tear function in patients with DED, addressing a significant gap in both local and regional evidence.

## METHODOLOGY

This study was designed as a quasi-experimental, interventional study aimed at evaluating the clinical effects of Vitamin D3 supplementation in patients diagnosed with Dry Eye Disease (DED). The study was conducted over a period of 6 months between July and December 2022.

The research was carried out at the Combined Military Hospital (CMH), Murree Hills, a tertiary care facility that caters to both military personnel and civilians. The ophthalmology and pathology departments jointly facilitated the screening, supplementation, and follow-up procedures.

Prior to the commencement of the study, ethical clearance was obtained from the Ethical Review Committee for Medical and Biomedical Research at Combined Military Hospital (CMH), Murree Hills. The committee reviewed and approved the study titled "Role of Vitamin D3 Supplementation in Dry Eye Disease" under Reference No. 8021-36172, dated 10th June 2022. The ethical approval ensured adherence to the principles outlined in the Declaration of Helsinki (2008). Written informed consent was obtained from all participants prior to enrollment.

The sample size was determined using G\*Power version 3.1, assuming an effect size (Cohen's d) of 0.5, an alpha of 0.05, and a power of 0.90 for a two-tailed paired t-test. The calculated minimum sample required was 172. To account for potential dropouts or incomplete data, a total of 375 participants were enrolled 200 in the intervention (Dry Eye) group and 175 healthy controls.

Participants were recruited through non-probability purposive sampling from the outpatient ophthalmology department. All patients presenting with signs and symptoms of Dry Eye were screened, and those fulfilling the eligibility criteria were enrolled.

Inclusion Criteria were adults aged 18 to 65 years. Clinically diagnosed with Dry Eye Disease based on OSDI score >12. Serum Vitamin D3 level <30 ng/mL and willing to give informed consent and comply with follow-up

Exclusion Criteria were current use of artificial tears, steroids, or other ocular medications. History of autoimmune disorders (e.g., Sjögren's syndrome). Contact lens users or patients with ocular surgery in the last 6 months. Pregnant or lactating women and Individuals already on Vitamin D3 supplementation

Data collection included both subjective and objective parameters. The Ocular Surface Disease Index (OSDI) questionnaire was used to assess

symptom severity. Objective measures included the Schirmer Test for tear volume, Tear Break-Up Time (TBUT) for tear stability, and serum Vitamin D3 levels, measured via chemiluminescence immunoassay at the hospital's central lab.

Baseline assessments were recorded for both groups. The intervention group received oral Vitamin D3 supplementation (50,000 IU weekly) for 12 weeks. After the intervention period, clinical parameters were reassessed using the same protocols.

The OSDI questionnaire is a globally validated tool with high internal consistency (Cronbach's alpha >0.90). Laboratory analysis for Vitamin D3 followed standardized internal quality controls. All clinical examinations were performed by the same ophthalmologist to minimize inter-observer variation.

Data were analyzed using IBM SPSS version 26. Descriptive statistics including means  $\pm$  standard deviations (SD), frequencies, and percentages were calculated for continuous and categorical variables. Normality of continuous data was assessed using the Shapiro-Wilk test and supported by visual inspection of histograms and Q-Q plots. Since the data met normality assumptions, independent t-tests and Chi-square tests were used to compare baseline differences between the Dry Eye and Control groups. Paired t-tests were applied to evaluate pre- and post-intervention changes within the Dry Eye group. Cohen's d was calculated to quantify effect sizes for mean differences. A p-value < 0.05 was considered statistically significant. Additionally, binary logistic regression was performed to determine independent predictors of Dry Eye Disease.

## RESULTS

Among the 375 participants, 200 belonged to the Dry Eye group and 175 to the Control group. Age distribution did not differ significantly between groups ( $p = 0.639$ ). Gender, residence, and BMI status also showed no significant associations, with p-values of 0.461, 0.107, and 0.033 respectively,

although a higher proportion of Dry Eye patients were female and from urban areas. Notably, screen time greater than 6 hours per day was significantly more common in the Dry Eye group (56.5% vs. 38.9%,  $p = 0.001$ , Cramer's V = 0.176), as were comorbidities such as diabetes and hypertension (26.0% vs. 14.9%,  $p = 0.008$ ). The most striking difference was observed in Vitamin D3 status: 49.5% of Dry Eye patients were deficient (<20 ng/mL), compared to only 4.0% in the Control group ( $p < 0.001$ , Cramer's V = 0.617).

**Table 1: Demographic and Clinical Characteristics of Study Participants (n = 375)**

Variable	Categories	Dry Eye Group (n = 200)	Control Group (n = 175)	p-value	$\chi^2$ (df)	Cramer's V
Age Range	21–30	43 (21.5%)	32 (18.3%)			
	31–40	78 (39.0%)	65 (37.1%)			
	41–50	54 (27.0%)	59 (33.7%)	0.639	$\chi^2(6)=4.28$	0.107
Gender	Female	109 (54.5%)	102 (58.3%)			
	Male	91 (45.5%)	73 (41.7%)	0.461	$\chi^2(1)=0.54$	0.038
Residence	Urban	125 (62.5%)	95 (54.3%)			
	Rural	75 (37.5%)	80 (45.7%)	0.107	$\chi^2(1)=2.60$	0.083
Screen Time >6 hrs/day	Yes	113 (56.5%)	68 (38.9%)			
	No	87 (43.5%)	107 (61.1%)	0.001	$\chi^2(1)=11.64$	0.176
Comorbidities	Yes (DM, HTN)	52 (26.0%)	26 (14.9%)			
	No	148 (74.0%)	149 (85.1%)	0.008	$\chi^2(1)=7.04$	0.137
BMI Status	>25 kg/m <sup>2</sup>	63 (31.5%)	38 (21.7%)			
	≤25 kg/m <sup>2</sup>	137 (68.5%)	137 (78.3%)	0.033	$\chi^2(1)=4.54$	0.11
Vitamin D3 Status	Deficient (<20 ng/mL)	99 (49.5%)	7 (4.0%)			
	Insufficient (20–29.9 ng/mL)	71 (35.5%)	44 (25.1%)			
	Normal (≥30 ng/mL)	30 (15.0%)	124 (70.9%)	<0.001	$\chi^2(2)=142.53$	0.617

Vitamin D3 deficiency was significantly more prevalent in the Dry Eye group, with nearly half

(49.5%) being severely deficient, while the majority of controls (70.9%) had sufficient levels ( $\geq 30$  ng/mL). The difference across categories (deficient, insufficient, sufficient) was statistically significant ( $\chi^2 = 142.532$ ,  $p < 0.001$ ), reinforcing the strong association between hypovitaminosis D and Dry Eye Disease.

**Table 2: Prevalence of Vitamin D3 Status in Dry Eye and Control Groups (n = 375)**

Vitamin D3 Status	Dry Eye Group (n = 200)	Control Group (n = 175)	Total (n = 375)	p-value	$\chi^2$ (df = 2)	Cramer's V
Deficient (<20 ng/mL)	99 (49.5%)	7 (4.0%)	106 (28.3%)			
Insufficient (20–29.9)	71 (35.5%)	44 (25.1%)	115 (30.7%)			
Sufficient ( $\geq 30$ ng/mL)	30 (15.0%)	124 (70.9%)	154 (41.1%)	<0.001	142.532	0.617

All clinical parameters showed statistically significant differences between the Dry Eye and Control groups. The Dry Eye group had a markedly higher OSDI score ( $34.70 \pm 9.37$  vs.  $10.41 \pm 3.62$ ;  $p < 0.001$ ), indicating greater symptom severity. Tear production, measured via the Schirmer test, and tear stability (TBUT) were significantly lower in the Dry Eye group (both  $p < 0.001$ ). Vitamin D3 levels were also lower ( $21.50 \pm 7.57$  ng/mL vs.  $34.37 \pm 7.81$  ng/mL;  $p < 0.001$ ). Effect sizes for all variables were very large (Cohen's  $d > 1.5$ ), underscoring the clinical relevance of these group differences.

**Table 3: Baseline Comparison of Clinical Parameters Between Dry Eye and Control Groups (n = 375)**

Variable	Dry Eye Group (n = 200)	Control Group (n = 175)	t-value	df	p-value	Cohen's d
OSDI Score	$34.70 \pm 9.37$	$10.41 \pm 3.62$	32.26	373	<0.001	3.34
Vitamin D3 Level (ng/mL)	$21.50 \pm 7.57$	$34.37 \pm 7.81$	-16.2	373	<0.001	1.68
Schirmer Test (mm)	$9.30 \pm 2.25$	$18.60 \pm 1.92$	-42.74	373	<0.001	4.42
TBUT (seconds)	$8.15 \pm 1.73$	$15.72 \pm 2.53$	-34.19	373	<0.001	3.54

Following 12 weeks of Vitamin D3 supplementation, significant improvements were observed across all measured parameters in the Dry Eye group. OSDI scores dropped from  $34.70 \pm 9.37$  to  $19.02 \pm 6.54$  ( $p < 0.001$ ), indicating reduced symptom burden. Schirmer test results improved from  $9.30 \pm 2.25$  mm to  $14.35 \pm 2.95$  mm, and TBUT increased from  $8.15 \pm 1.73$  to  $12.74 \pm 2.41$  seconds (both  $p < 0.001$ ). Serum Vitamin D3 levels rose significantly (from  $21.50 \pm 7.57$  to  $36.33 \pm 8.98$  ng/mL;  $p < 0.001$ ). All changes had large effect sizes (Cohen's  $d > 1.25$ ), confirming the effectiveness of supplementation.

**Table 4: Comparison of Clinical Parameters Before and After Vitamin D3 Supplementation in the Dry Eye Group (n = 200)**

Parameter	Baseline (Mean $\pm$ SD)	After 12 Weeks (Mean $\pm$ SD)	t-value	p-value	Cohen's d
Vitamin D3 Level (ng/mL)	$21.50 \pm 7.57$	$36.33 \pm 8.98$	-17.87	<0.001	1.26
OSDI Score	$34.70 \pm 9.37$	$19.02 \pm 6.54$	20.01	<0.001	1.41
Schirmer Test (mm)	$9.30 \pm 2.25$	$14.35 \pm 2.95$	-22.16	<0.001	1.57
TBUT (seconds)	$8.15 \pm 1.73$	$12.74 \pm 2.41$	-22.91	<0.001	1.62

Categorical analysis of OSDI severity revealed substantial shifts following supplementation. The proportion of participants classified as “severe” dropped from 56.5% at baseline to just 1.0% after treatment, while those with “none” or “mild” severity increased significantly. Although the Pearson Chi-square was not significant ( $p = 0.211$ ), the McNemar-Bowker test indicated a significant shift in severity levels ( $\chi^2 = 143.375$ ,  $df = 6$ ,  $p < 0.001$ ), with a modest effect size (Cramer's  $V = 0.142$ ).



**Table 5: Distribution of OSDI Severity Before and After Vitamin D3 Supplementation (n = 200)**

OSDI Severity Level	Baseline n (%)	After 12 Weeks n (%)	Statistical Test Results
None (0–12)	3 (1.5%)	33 (16.5%)	Pearson $\chi^2 = 12.033$ df = 9, p = 0.211 McNemar-Bowker $\chi^2 = 143.375$ , df = 6, p < 0.001 Cramer's V = 0.142
Mild (13–22)	18 (9.0%)	104 (52.0%)	
Moderate (23–32)	66 (33.0%)	61 (30.5%)	
Severe (33–100)	113 (56.5%)	2 (1.0%)	
<b>Total</b>	<b>200 (100%)</b>	<b>200 (100%)</b>	

Logistic regression analysis identified Vitamin D3 status as the strongest independent predictor of Dry Eye Disease. Each unit increase in Vitamin D deficiency was associated with more than seven-fold higher odds of having Dry Eye (OR = 7.26, 95% CI = 4.89–10.79, p < 0.001). Other variables such as screen time, comorbidities, and residence showed trends toward significance but did not reach conventional thresholds. The model had good predictive power (Nagelkerke  $R^2 = 0.493$ , classification accuracy = 77.1%) and passed the Hosmer-Lemeshow goodness-of-fit test (p = 0.292).

**Table 5: Regression Predictors of Dry Eye Disease (n = 375)**

Predictor	B	SE	p-value	OR (Exp(B))	95% CI for OR
Age (AgeRange)	-0.07	0.14	0.608	0.931	0.710 – 1.222
Gender (Female vs Male)	0.274	0.27	0.312	1.315	0.773 – 2.237

Residence (Rural vs Urban)	0.469	0.28	0.089	1.598	0.932 – 2.740
Screen Time >6 hrs	0.51	0.27	0.059	1.665	0.980 – 2.828
Comorbidities (Yes vs No)	0.537	0.35	0.123	1.711	0.865 – 3.382
BMI >25 (Yes vs No)	0.243	0.31	0.436	1.276	0.692 – 2.353
Vitamin D3 Level (ng/mL)	1.983	0.2	<0.001	7.262	4.886 – 10.793
<b>Constant</b>	-5.44	0.84	<0.001	0.004	–

OR = Odds Ratio; CI = Confidence Interval

The bar graph illustrates the shift in OSDI severity levels among Dry Eye patients following Vitamin D3 supplementation. At baseline, the majority of patients (56.5%) were categorized as having severe symptoms, with only 1.5% falling in the “None” category. After 12 weeks of treatment, the proportion of patients reporting severe symptoms dropped dramatically to 1.0%, while those reporting mild symptoms rose sharply from 9.0% to 52.0%. The number of patients with no symptoms increased tenfold, from 3 to 33 individuals. These categorical improvements are supported statistically by the McNemar-Bowker test ( $\chi^2 = 143.375$ , p < 0.001), indicating a significant shift in symptom severity distribution post-treatment.

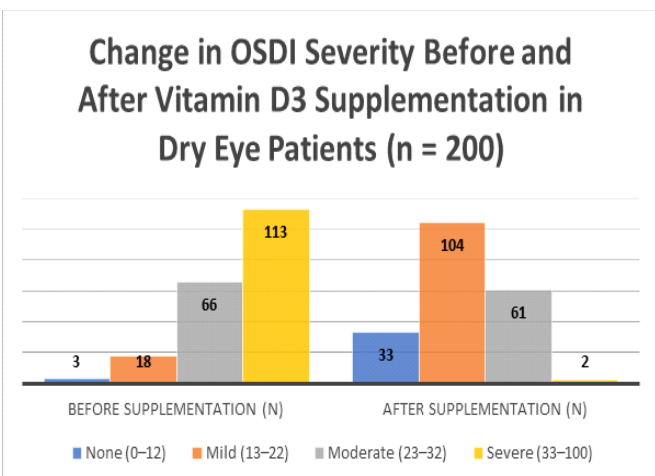


Figure 1: Distribution of Ocular Surface Disease Index (OSDI) severity levels among patients with Dry Eye Disease before and after 12 weeks of Vitamin D3 supplementation. A substantial reduction in the “Severe” category and a shift toward “Mild” and “None” severity was observed, indicating significant symptomatic improvement.

## DISCUSSION

This study demonstrated that Vitamin D3 supplementation significantly improves both subjective symptoms and objective clinical markers in patients with Dry Eye Disease (DED). At baseline, patients with DED had markedly lower serum Vitamin D3 levels compared to healthy controls, and a large proportion were either deficient or insufficient. These findings were consistent with previous reports suggesting a strong association between Vitamin D3 deficiency and tear film instability or ocular surface inflammation. Studies by Kanwal et al 2024, Lin et al 2022 and Najjaran et al 2023 had similarly reported that serum 25-hydroxyvitamin D levels were significantly lower in DED patients than in controls, indicating a potential immunomodulatory role of vitamin D in ocular surface homeostasis.<sup>13-15</sup>

Following 12 weeks of oral Vitamin D3 supplementation, participants in the intervention group showed significant improvements in OSDI scores, Schirmer's test values, and TBUT measurements. This aligns with the findings of Ahmad et al., 2025 and Bhatt et al 2023 observed improvement in tear production and subjective]e symptom relief after Vitamin D supplementation in individuals with DED.<sup>16, 17</sup> The large effect sizes observed in this study further highlight the clinical importance of the intervention.

Moreover, the categorical shift in OSDI severity from “severe” to “mild” or “none” in most patients underscores the therapeutic potential of correcting Vitamin D deficiency in dry eye management. The McNemar-Bowker test confirmed that these changes were not due to random variability but reflected a meaningful clinical transition. Similarly,

in a randomized trial by Hassanpour et al., 2024, Lin et al 2021 and Mrugacz et al 2024 Vitamin D supplementation significantly enhanced tear secretion and decreased ocular discomfort in patients with concurrent DED and hypovitaminosis D.<sup>18-20</sup>

In the current study, logistic regression analysis identified serum Vitamin D3 level as the strongest independent predictor of DED, even after adjusting for age, gender, BMI, comorbidities, and screen time. This reinforces the growing body of evidence that Vitamin D deficiency is not merely associated with dry eye but may play a causative role. A systemic anti-inflammatory effect of Vitamin D has been proposed, with suppression of cytokines such as IL-1, IL-6, and TNF- $\alpha$ , all of which are implicated in the pathogenesis of DED.

Interestingly, other known risk factors such as increased screen exposure and comorbid conditions like hypertension and diabetes also showed trends toward association with DED in our study, though they did not reach statistical significance in the regression model. These findings suggest that while Vitamin D status is a major modifiable factor, a multifactorial approach is essential for effective management of DED.

One of the strengths of this study lies in its before-and-after design with objective measures, a validated symptom scoring system (OSDI), and biochemical confirmation of Vitamin D3 levels.

Despite the promising results, this study has certain limitations. First, it was conducted at a single tertiary care center, which may limit the generalizability of the findings to broader populations. Second, the follow-up duration was limited to 12 weeks; long-term sustainability of improvements following supplementation was not assessed. Third, although subjective and objective parameters were evaluated, additional diagnostic tests such as corneal staining, inflammatory cytokine profiling, or meibomian gland assessment were not performed. Furthermore, the study did not stratify results by the degree of baseline deficiency

or assess adherence to supplementation, which could influence outcomes.

Future studies should incorporate a multicenter design with a longer follow-up period to evaluate the sustained effects of Vitamin D3 supplementation in Dry Eye Disease. It is also recommended to include ocular surface staining scores and inflammatory biomarkers to better understand the mechanistic impact of Vitamin D on ocular surface immunity. Stratified analysis by severity of deficiency and comparison of oral versus topical Vitamin D3 formulations may also provide deeper insights. Clinically, routine screening for Vitamin D status in patients with chronic or treatment-resistant DED is advisable, and supplementation should be considered a complementary therapy in such cases.

## CONCLUSION

This study establishes a clear and clinically significant link between Vitamin D3 deficiency and Dry Eye Disease. Supplementation with oral Vitamin D3 led to substantial improvements in both subjective symptoms and objective tear film parameters. These findings suggest that routine screening for Vitamin D deficiency in DED patients may be warranted, particularly in populations with known risk factors. Integrating Vitamin D supplementation into dry eye management protocols may offer a safe, cost-effective, and impactful therapeutic strategy.

**Conflict Of Interest:** None to declare

**Ethical Approval:** The study was approved by the Institutional Review Board / Ethical Review Board

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