

Effect of Short-Term Oral Vitamin D₃ Supplementation on Tear Film in Dry Eye Subjects

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Abstract: Vitamin D deficiency is common worldwide, particularly in the Middle East and North Africa. The prevalence of vitamin D deficiency (<50 nmol/L) is very high in Saudi Arabia and is associated with several chronic illnesses. The aim of the study is to investigate the effect of short-term oral vitamin D₃ supplementation on tear film stability in dry eye subjects. Thirty male dry eye subjects aged 19–37 years (mean \pm standard deviation (SD) = 26.2 \pm 5.0 years) completed the study. An age-matched control group of 30 normal eye male subjects (25.6 \pm 4.3 years) was also recruited. Each subject received an oral vitamin D₃ supplement (400 IU daily) for 3 consecutive days. The tear ferning (TF), phenol red thread (PRT), and tear break-up time (TBUT) tests were performed before and 24 hours after the third dose of vitamin D₃ supplement. TF grades were significantly (Wilcoxon test, $p < 0.001$) decreased (median (IQR) = 1.8 (0.8) in dry eye subjects after vitamin D₃ supplementation compared to those obtained before supplementation (2.3 (0.6)). TF grades were improved in 86.7% of dry eye subjects ($n=26$). Although PRT and TBUT tests scores were higher post-vitamin D₃ supplementation, the differences were not significant (Wilcoxon test, $p > 0.05$). No significant differences in TF grades and PRT and TBUT scores were observed between pre- and post-vitamin D₃ supplementation in healthy controls. For the study group, there were strong correlations between measurements of PRT (OD) and PRT (OS) and between TBUT (OD) and TBUT (OS) pre- and post-vitamin D₃ supplementation. No correlation was found between TF (OD) and TF (OS) grades pre- and post-vitamin D₃ supplementation. The TF test suggests that short-term oral vitamin D₃ supplementation can improve the quality of tears in dry eye subjects. Oral vitamin D₃ supplementation can be potentially used as an effective treatment for subjects with dry eye symptoms. Our study offers important insights into how vitamin D₃ supplementation can improve tear film in dry eye patients and in turn improving patients' quality of life. Future studies are needed to investigate the long-term effect of vitamin D₃ supplementation on the stability of the tear film.

Keywords: Vitamin D₃ Supplement, Dry Eye, Tear Film, Tear Ferning Test, Phenol Red Thread Test, Correlation

1. Introduction

Excessive evaporation or low secretion of tears leads to a disorder within the tear film, known as dry eye [1]. Dry eye can cause visual disturbance as a result of the damage that occurs within the ocular surface [2]. It is associated with discomfort symptoms, itchiness, redness, high tear osmolality, inflammation, and light sensitivity [3, 4], and

because of these symptoms, dry eye negatively affects patient quality of life. Dry is common in elderly and in particular women [5], and various dry eye tests and questionnaires are used to assess the instability within tear film, and thus ultimately diagnose dry eye [6, 7]. Smoking, diabetes, thyroid gland disorders, vitamin deficiency, high temperature and humidity, wearing contact lenses, and eye surgeries are the most common risk factors for dry eye [3, 8-10].

Vitamin D₃ (cholecalciferol; Figure 1) is the most important member of the vitamin D chemical group. It is a steroid-type, fat-soluble, large molecule that is derived from 7-dehydrocholesterol [11]. It originates from a reaction of cholesterol within the skin in the presence of ultraviolet light. It is also naturally present in some foods. Vitamin D enhances the absorption of calcium, magnesium, iron, zinc, and phosphate in the intestine [12], and has anti-inflammatory properties that play a role in controlling localized autoimmune disease [13].

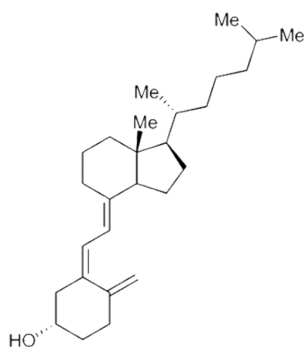


Figure 1. Chemical structure of vitamin D₃ (cholecalciferol).

Vitamin D deficiency is common worldwide, particularly in the Middle East and North Africa, and is more prevalent in women than in men [14, 15]. The prevalence of vitamin D deficiency (<50 nmol/L) is very high (81%) in Saudi Arabia [16]. Vitamin D deficiency is associated with several chronic illnesses including cancer, diabetes mellitus, multiple sclerosis, osteoporosis, hypertension, stroke, cardiovascular disease, inflammatory bowel disease, and mental illness [17-19]. Vitamin D deficiency can lead to genetic changes that might cause ocular diseases such as diabetic retinopathy, nearsightedness, uveitis, and dry eye [20, 21]. Lacrimal and accessory gland fluids have high levels of vitamin D [22], and corneal epithelium, retinal pigmentary epithelium, and endothelium contain vitamin D receptors, evidencing that vitamin D is involved in proper eye function [23]. Vitamin D enhances the barrier function in the corneal epithelial layer and regulates the fluid and ion transport in tear secretion [24-26]. Because Vitamin D acts as an immunoregulatory agent and its deficiency could lead to dry eye symptoms [27, 28]. Consequently, vitamin D supplement may also function as a treatment for dry eye symptoms.

This study is the first report to assess the short-term effects of vitamin D₃ supplements on tear film in dry eye subjects using the tear ferning (TF) test. This test has been recently employed as an efficient tool to assess the quality of tears [29-32]. Therefore, it serves as an important tool to measure how dry eye symptoms respond to vitamin D₃ supplements.

2 Materials and Methods

2.1. Subjects

Thirty male dry eye subjects aged 19–37 years (mean \pm standard deviation (SD) =26.2 \pm 5.0 years) completed the

study. An age-matched control group of 30 normal eye male subjects (25.6 \pm 4.3 years) was also recruited. Exclusion criteria for study participation included diabetes, smoking history, recent ocular surgeries, ocular diseases, abnormalities of the eyelids or lashes, consuming medications, wearing contact lenses, and thyroid gland disorders. The ocular surface disease index (OSDI) and a slit-lamp were used for classification of subjects as having normal or dry eyes. Each subject received an oral vitamin D₃ supplement (400 IU equivalent to 10 μ g daily) for 3 consecutive days, at dosages equivalent to the estimated average requirement of vitamin D₃ for an adult [33]. Vitamin D₃ capsules were supplied by Jamieson Laboratories Ltd. (Toronto, Canada). The TF, phenol red thread (PRT), and tear break-up time (TBUT) tests were performed before the treatment and 24 hours after the third dose of the vitamin D₃ supplementation. A 10-minute interval was allowed between the tests. The measurements were performed on the right eye, followed by the left eye, with a 10-minute gap between the two. The Helsinki declaration [34] was followed, and written informed consent was obtained from each participant following an explanation of the study nature. This study was approved by the College of Applied Medical Sciences Ethics Committee of King Saud University. The study was conducted at the Optometry and Visual Science Clinic, College of Applied Medical Sciences, King Saud University, Riyadh. All measurements were carried out between 11 am and 4 pm under control conditions of temperature and humidity, by the same examiner.

2.2. The OSDI

The OSDI was completed by each participant and scores greater than 12 were considered as dry eyes [35].

2.3. The TF Test

A glass capillary tube (10 μ l) purchased from Merck (Gillingham, UK) was used to collect a tear sample (1 μ l) from the lower meniscus of the right eye from each subject. The tears were dried for 10 minutes over a glass slide at room temperature and at a humidity of less than 40%. The TF patterns produced were observed using an Olympus DP72 microscope (Tokyo, Japan) with 10 \times magnification. The five-point numeric TF grading scale was used to grade the TF patterns using 0.1 increments [36]. TF grades of greater than or equal to two were defined as dry eye [36].

2.4. The PRT Test

A Zone-Quick PRT obtained from Showa Yakuhin Kako Co, Ltd (Tokyo, Japan) was used to measure tear volume. The cotton thread changes color when moisten with tears. A 3-mm length of the thread was folded and one-third of the length was inserted into the temporal canthus of the lower eyelid. After 15 seconds, the thread was removed, and the red-colored portion of the thread was measured in millimeters. A measurement of less than 10 mm was considered as dry eye [37].

2.5. The TBUT Test

The TBUT test was performed using Pro Glo Fluorescein Strips (Eye Care & Cure, Tucson, USA). The participant was asked to look down, and a strip was gently touched against the superior conjunctiva. The participant was asked to blink several times and to keep his eyes open, fixating on a distant target. The time period between the last blink and the first appearance of dark spots on the pre-corneal tear film was recorded in seconds. A measurement of less than 10 seconds was defined as dry eye [38].

2.6. Statistical Analysis

The data were analyzed using SPSS software (version 22; IBM, Armonk, NY, USA). The Pearson correlation coefficient (r) was used to describe the correlation between different parameters. The correlation was defined as weak ($r=0.10-0.29$), medium ($r=0.30-0.49$), or strong ($r=0.50-1.00$) [39]. Data obtained from the OSDI and TF, PRT, and TBUT tests in the study and control groups were not normally distributed (Wilcoxon test; $p<0.05$), and were presented using medians and interquartile ranges (IQRs).

3. Results

The average OSDI scores before treatment were 14.3 (5.3) and 6.1 (2.5) for the study and control groups, respectively, demonstrating that the study group had dry eyes before treatment. The TF grades were significantly (Wilcoxon test, $p<0.001$) improved (median (IQR) = 1.8 (0.8)) in dry eye subjects post-vitamin D₃ supplementation compared with those obtained pre-supplementation (2.3 (0.6)). The TF grades were improved in 86.7% of dry eye subjects ($n=26$). Representative images of TF obtained pre- and post-vitamin D₃ supplementation from a dry eye and a healthy eye subject are shown in Figures 2 and 3, respectively. Although PRT and TBUT test scores were slightly higher post vitamin D₃ supplementation than those before supplementation, the differences were not significant (Wilcoxon test, $p>0.05$). In the control group, there were no significant differences in the TF grades and PRT and TBUT scores pre- and post-vitamin D₃ supplementation. The averages for TF grades and PRT and TBUT scores are presented in Table 1 (study group) and Table 2 (control group). Side-by-side boxplots for TF grades, PRT (OD), PRT (OS), TBUT (OD), and TBUT (OS), pre- and post-vitamin D₃ supplementation within the study group are shown in Figures 4–8.

Table 1. The median TF grades, PRT, and TBUT measurements in the study group ($n=30$) pre- and post-vitamin D₃ supplementation.

Test	Pre-vitamin D ₃	Post-vitamin D ₃
TF*	2.3 (0.6)	1.8 (0.8)
PRT (OD, mm)	28.0 (6.0)	29.0 (5.0)
PRT (OS, mm)	27.0 (5.5)	27.2 (6.0)
TBUT (OD, s)	8.0 (3.5)	9.0 (5.0)
TBUT (OS, s)	8.0 (5.0)	9.0 (4.0)

* $p<0.05$.

Table 2. The median TF grades, PRT, and TBUT measurements in the control group ($n=30$) pre- and post-vitamin D₃ supplementation.

Test	Pre-vitamin D ₃	Post-vitamin D ₃
TF	1.2 (0.3)	1.0 (0.5)
PRT (OD, mm)	29.7 (6.5)	30.2 (6.8)
PRT (OS, mm)	30.3 (7.1)	30.6 (8.0)
TBUT (OD, s)	12.3 (7.0)	13.0 (7.2)
TBUT (OS, s)	13.0 (6.5)	13.8 (6.8)

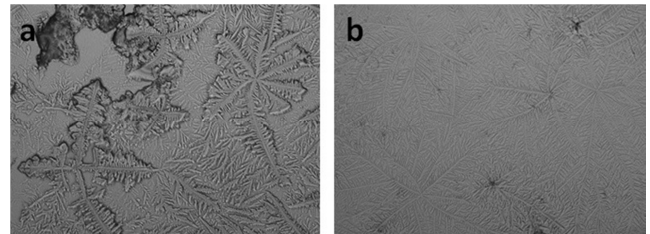


Figure 2. TF images obtained (a) before and (b) 24 hours after 3-day vitamin D₃ supplementation for a dry eye subject.

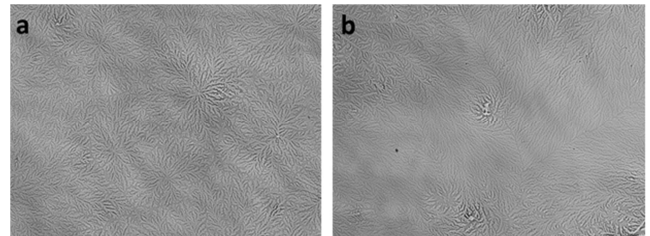


Figure 3. TF images obtained (a) before and (b) 24 hours after 3-day vitamin D₃ supplementation for a normal eye subject.

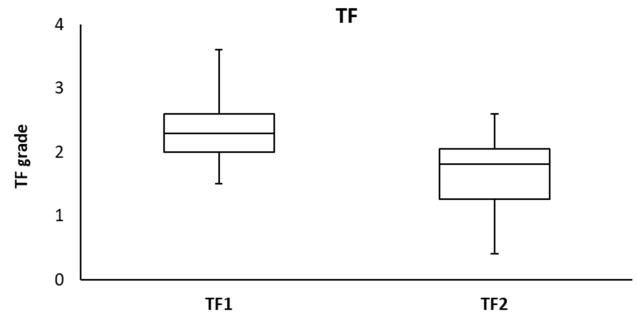


Figure 4. Side-by-side boxplots for TF grades before (TF1) and 24 hours after (TF2) 3-day vitamin D₃ supplementation in the study group ($p<0.05$).

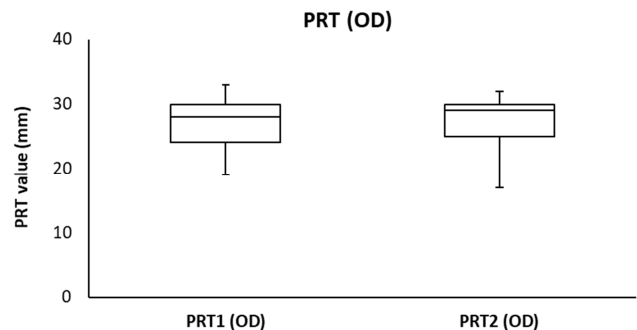


Figure 5. Side-by-side boxplots for PRT measurements from the right eyes before [PRT1 (OD)] and after 24 hours [PRT2 (OD)] 3-day vitamin D₃ supplementation in the study group.

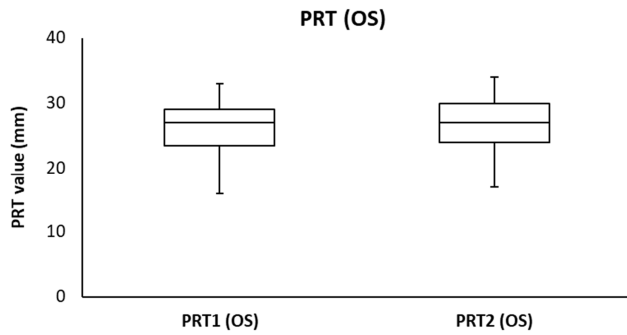


Figure 6. Side-by-side boxplots for PRT measurements from the left eyes before [PRT1 (OS)] and 24 hours after [PRT2 (OS)] 3-day vitamin D₃ supplementation in the study group.

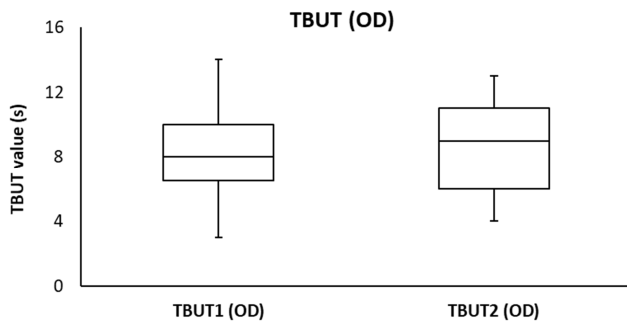


Figure 7. Side-by-side boxplots for TBUT measurements from the right eyes before [TBUT1 (OD)] and 24 hours after [TBUT2 (OD)] 3-day vitamin D₃ supplementation in the study group.

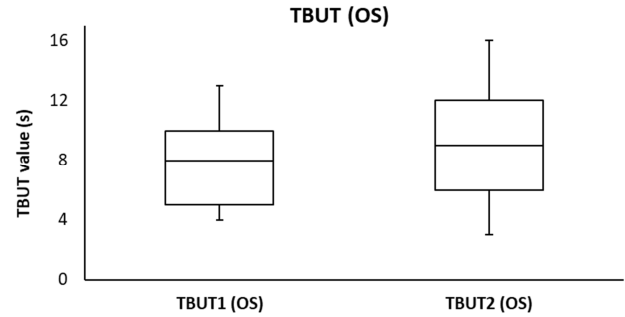


Figure 8. Side-by-side boxplots for TBUT measurements from the left eyes before [TBUT1 (OS)] and 24 hours after [TBUT2 (OS)] 3-day vitamin D₃ supplementation in the study group.

Correlations between TF, PRT, and TBUT scores obtained in the study group are shown in Table 3. For the study group, there were strong correlations between PRT (OD) and PRT (OS) measurements (Wilcoxon test; $r=0.564$; $p=0.001$ for pre-supplementation, and $r=0.785$; $p=0.001$ for post-supplementation) and between the TBUT (OD) and TBUT (OS) measurements (Wilcoxon test; $r=0.859$; $p=0.001$ for pre-supplementation and $r=0.895$; $p=0.001$ for post-supplementation). No correlation was found between the TF (OD) and TF (OS) grades pre- and post-vitamin D₃ supplementation for the study group.

Table 3. Correlation of TF, PRT, and TBUT scores obtained within the study group ($n=30$).

Test	TF		PRT (OD)		PRT (OS)		TBUT (OD)		TBUT (OS)	
	TF1	TF2	PRT1	PRT2	PRT1	PRT2	TBUT1	TBUT2	TBUT1	TBUT2
TF1										
r	1	0.001	0.003	0.057	0.063	0.177	0.012	-0.266	0.044	-0.167
p	—	0.994	0.988	0.752	0.726	0.325	0.947	0.134	0.806	0.352
TF2										
r	0.001	1	-0.056	-0.318	-0.138	-0.318	-0.155	-0.011	-0.082	-0.068
p	0.994	—	0.756	0.071	0.443	0.071	0.523	0.950	0.651	0.708
PRT1 (OD)										
r	0.003	-0.056	1	0.416	0.564	0.436	-0.019	0.061	-0.104	-0.012
p	0.988	0.756	—	0.106	0.001	0.011	0.915	0.738	0.565	0.949
PRT2 (OD)										
r	0.057	-0.318	0.416	1	0.458	0.785	0.458	0.208	0.256	0.220
p	0.752	0.071	0.016	—	0.007	0.001	0.007	0.245	0.151	0.218
PRT1 (OS)										
r	0.063	-0.138	0.564	0.496	1	0.496	0.131	-0.060	-0.107	-0.029
p	0.726	0.433	0.001	0.003	—	0.003	0.469	0.742	0.552	0.874
PRT2 (OS)										
r	0.177	-0.241	0.406	0.785	0.496	1	0.426	0.284	0.363	0.284
p	0.325	0.176	0.011	0	0.003	—	0.013	0.110	0.038	0.110
TBUT1 (OD)										
r	0.012	-0.115	-0.160	0.245	0.131	-0.426	1	0.654	0.859	0.721
p	0.947	0.523	0.915	0.170	0.467	0.013	—	0.001	0.001	0.001
TBUT2 (OD)										
r	-0.266	-0.011	0.061	0.208	-0.060	0.284	0.654	1	0.668	0.895
p	0.134	0.950	0.738	0.245	0.742	0.114	0.001	—	0.001	0.001
TBUT1 (OS)										
r	0.044	-0.082	-0.107	0.256	-0.107	0.363	0.859	0.667	1	0.0667
p	0.806	0.651	0.565	0.151	0.565	0.038	0.001	0.001	—	0.001
TBUT2 (OS)										
r	-0.167	-0.068	-0.120	0.220	-0.029	0.280	0.721	0.895	0.667	1
p	0.352	0.708	0.949	0.218	0.874	0.114	0.001	0.001	0.001	—

4. Discussion

Dry eye syndrome is caused by various illnesses and environmental factors. Vitamin D deficiency is associated with dry eye symptoms [10, 26, 40]. In our study, the TF grades decreased significantly (Wilcoxon test, $p < 0.05$) in dry eye subjects after consumption of vitamin D₃ supplementation for a short duration (3 consecutive days), suggesting that consumption of vitamin D₃ supplements improves tear quality. These findings are consistent with those of a previous study, which found that dry eye symptoms were improved after vitamin D treatment with a high injection dose [41]. However, the PRT and TBUT tests in our study showed no significant ($p > 0.05$) differences for the study and control groups pre- and post-supplementation. It seems that the duration of vitamin D₃ supplementation were short to cause an effect on tear secretion.

The effects of an intramuscular injection of a high dose (200,000 IU) of vitamin D₃ on the tear film parameters have been tested in older subjects (58.2±12.9 years) who suffer from vitamin D deficiency [41]. Tear secretion and TBUT time were found to increase post- vitamin D₃ treatment relative to pre-treatment. For example, the Schirmer test score was 6.7±3.9 mm ($n=105$) pre-treatment, 8.6±6.3 mm ($n=78$; $p=0.006$) two weeks post-treatment, 8.9±7.6 mm ($n=54$; $p=0.015$) six weeks post-treatment, and 8.4±7.2 mm ($n=49$; $p=0.140$) ten weeks post-treatment [41]. For the TBUT measurements, the average score was 3.2±2.3 seconds pre-treatment, 5.6±2.4 seconds ($p < 0.001$) two weeks post-treatment, 5.2±2.3 seconds ($p < 0.001$) six weeks post-treatment, and 4.5±2.6 seconds ($p=0.066$) ten weeks post-treatment [41]. Clearly, the highest tear secretion and TBUT were observed after six and two weeks post-vitamin D₃ treatment, respectively [41]. Similarly, the OSDI scores were lowest (21.1±16.5, $p=0.004$) ten weeks post-treatment compared with the scores recorded pre-treatment (34.4±24.9) [41]. However, another study conducted among dry eye subjects ($n=29$) that consumed oral vitamin D supplementation (1000 IU daily) for a long duration (2 months) suggested no correlation between the level of vitamin D and dry eye signs and symptoms [42].

Premenopausal women ($n=50$) with vitamin D deficiency are known to develop dry eye symptoms [43]. For example, significantly ($p=0.001$) higher OSDI scores (35.8±21.4) were obtained from premenopausal women compared with those from the control group (18.7±17.2) [43]. In addition, they showed significantly ($p=0.001$) lower scores in Schirmer (12.7±8.0 mm) and TBUT (7.9±4.0 seconds) tests than the control group (24.7±5.9 mm and 13.3±5.1 seconds, respectively) [43]. Another study conducted on subjects with vitamin D deficiency ($n=34$) showed similar results in which the average scores from Schirmer ($p=0.007$) and TBUT ($P=0.01$) tests were significantly lower compared with the scores obtained from the control group [44]. Subjects with vitamin D deficiency ($n=30$) had significantly ($p < 0.001$) higher tear osmolality (309±9 mOsm/L) and OSDI scores (35.8±21.4) compared to the control group (295±10 mOsm/L

and 18.7±17.2, respectively) [18]. In addition, they showed significantly ($p < 0.001$) lower scores for Schirmer (8.5±3.7 mm) and TBUT (8.7±0.6 seconds) measurements compared with those found in the control group (16.6±2.4 mm and 18.1±0.5 seconds, respectively) [18]. The tear secretion ($r=0.428$, $p < 0.001$) and TBUT ($r=0.389$, $p=0.001$) were found to be correlated with vitamin D deficiency [26]. The tear secretion ($p=0.004$) and TBUT ($p=0.022$) were significantly shorter relative to the control group [26]. Based on this evidence, vitamin D deficiency is a risk factor for dry eye, but vitamin D supplementation, especially when taken long term, can improve tear film stability. Indeed, the prevalence of dry eye is known to decrease when the vitamin D concentration in serum is increased [45, 46].

The current study has some limitations. First, the sample size was relatively low. Second, all subjects were male. Third, the duration of vitamin D supplementation was for a very short, meaning that long term effects could not be measured. A future study that involves a larger number of dry eye subjects, both male and female, and a longer duration of vitamin D₃ supplementation is required to confirm the association between the supplement and improvement in tear film stability for more people with dry eye.

5. Conclusion

The tear ferning test suggests that short-term oral vitamin D₃ supplementation can improve the quality of tears in dry eye subjects. Oral vitamin D₃ supplementation has the potential to be an effective treatment for subjects with dry eye symptoms. Our study offers important insights into how vitamin D₃ supplementation can improve tear film in dry eye patients and in turn improving patients' quality of life. Future studies are needed to investigate the long-term effects of vitamin D₃ supplements on the stability of tear film.

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Conflict of Interest

All the authors do not have any possible conflicts of interest.

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