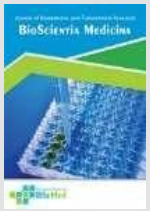
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Vitamin D Supplementation is Associated with Attenuated Ocular Surface Oxidative Stress in Mild Thyroid Eye Disease: A Preliminary Interventional Study

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ARTICLE INFO

Keywords:
Graves' orbitopathy
Malondialdehyde
Oxidative stress
Thyroid eye disease
Vitamin D

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i12.1451

A B S T R A C T

Background: Thyroid eye disease (TED) is an autoimmune orbitopathy where inflammation drives significant oxidative stress, contributing to patient morbidity. Malondialdehyde (MDA), a lipid peroxidation product, is a key biomarker of this oxidative damage. While vitamin D has known systemic immunomodulatory effects, its capacity to mitigate local oxidative stress on the ocular surface in TED is poorly understood. This study aimed to investigate the association between oral vitamin D supplementation and tear film MDA levels in patients with mild TED. **Methods:** A prospective, single-center, quasi-experimental pre-post study without a control group was conducted on 15 patients diagnosed with mild, active TED (Clinical Activity Score ≤3). Participants received 1000 IU of oral cholecalciferol (Vitamin D3) daily for 21 consecutive days. The primary outcome was the change in tear film MDA concentration, measured by ELISA. Secondary outcomes included serum 25-hydroxyvitamin D [25(OH)D] levels and clinical ocular surface parameters (Ocular Surface Disease Index [OSDI], Tear Break-Up Time [TBUT], Schirmer's I test). **Results:** A statistically significant reduction in mean tear film MDA levels was observed, decreasing from a baseline of 8.69 ± 4.15 ng/L to 5.70 ± 1.56 ng/L post-intervention ($p<0.001$). This was accompanied by a significant increase in mean serum 25(OH)D levels from 18.2 ± 5.9 ng/mL to 29.8 ± 6.4 ng/mL ($p<0.001$). Significant improvements were also noted in OSDI scores ($p=0.002$) and TBUT ($p=0.005$). The reduction in tear film MDA showed a significant negative correlation with the increase in serum 25(OH)D ($r = -0.68$, $p=0.005$). **Conclusion:** In this preliminary, uncontrolled study, short-term oral vitamin D supplementation was associated with a significant attenuation of ocular surface oxidative stress in patients with mild TED. These findings provide initial biochemical evidence for the potential localized benefits of vitamin D and support the need for larger, placebo-controlled randomized trials to validate its role as a safe adjunctive therapy for managing the ocular surface component of this disease.

1. Introduction

Autoimmune diseases constitute a diverse and challenging group of disorders arising from a breach in immunological tolerance, leading to a dysregulated immune response against self-antigens.[1] These conditions affect a significant portion of the global population and can manifest as either organ-specific or systemic pathologies. Among them, Graves' disease is the most common cause of hyperthyroidism worldwide, representing the archetypal organ-specific systemic autoimmune disorder. The central pathogenic event in Graves' disease is the generation of autoantibodies directed against the thyroid-stimulating hormone receptor (TSHR), resulting in constitutive activation of the thyroid gland and excessive production of thyroid hormones.[2]

While the systemic effects of thyrotoxicosis are profound, the most common and often most debilitating extrathyroidal manifestation of Graves' disease is thyroid eye disease (TED), also known as

Graves' Orbitopathy.[3] TED is a complex, disfiguring, and potentially sight-threatening autoimmune condition that targets the orbital and periorbital tissues. The clinical course is classically biphasic, characterized by an initial "active" inflammatory phase of variable duration (typically 12-24 months), which may subsequently evolve into a quiescent, fibrotic, or "inactive" phase. The clinical spectrum of TED is remarkably heterogeneous, ranging from mild ocular surface irritation, dryness, and conjunctival injection to severe complications, including significant proptosis, exposure keratopathy, restrictive myopathy causing diplopia, and, in its most severe form, compressive optic neuropathy leading to irreversible vision loss.[4]

The pathophysiology of TED is an intricate interplay of cellular and humoral immunity occurring within the unique anatomical confines of the orbit.[5] The primary autoantigen is the TSHR, which is aberrantly expressed on the surface of orbital fibroblasts. In genetically predisposed individuals, autoreactive T-lymphocytes infiltrate the orbit, recognize these TSHR-expressing fibroblasts, and, along with stimulating TSHR autoantibodies (TRAb), initiate a self-perpetuating inflammatory cascade. This activation drives the proliferation of orbital fibroblasts and their differentiation into two key pathological cell types: mature adipocytes (leading to de novo adipogenesis) and myofibroblasts. These activated fibroblasts become potent secretory cells, releasing a barrage of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-8 (IL-8), and chemokines that recruit further immune cells. Furthermore, they synthesize excessive quantities of hydrophilic glycosaminoglycans (GAGs), predominantly hyaluronic acid. The massive accumulation of GAGs, subsequent osmotic imbibition of water, and expansion of the orbital fat volume are the primary drivers of the characteristic volume expansion of orbital tissues, which clinically manifests as proptosis, periorbital edema, and venous congestion.[6]

A critical, and increasingly appreciated, component of this inflammatory milieu is the induction of profound local oxidative stress.[7] The intense metabolic activity of infiltrating immune cells (such as macrophages and neutrophils) and activated orbital fibroblasts results in a massive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This surge of free radicals overwhelms the endogenous antioxidant defense systems, creating a state of severe oxidative imbalance. These highly reactive molecules inflict widespread damage on essential biomolecules. Of particular importance is lipid peroxidation, a chain reaction in which ROS attack polyunsaturated fatty acids within cell membranes, disrupting membrane integrity, fluidity, and function. Malondialdehyde (MDA) is a stable and measurable end-product of this lipid peroxidation process, and it has been established as a reliable biomarker for quantifying the extent of local and systemic oxidative damage in numerous inflammatory and autoimmune diseases.[8] In TED, this oxidative stress is not merely an epiphenomenon but an active participant in the disease process, contributing to direct tissue injury and perpetuating the inflammatory cycle.

The management of TED is stratified by disease activity and severity. For patients with moderate-to-severe, active disease, high-dose systemic corticosteroids remain the first-line therapy, with novel biologic agents such as teprotumumab, an insulin-like growth factor-1 receptor inhibitor, revolutionizing treatment for this cohort. However, these potent therapies carry a significant risk of systemic side effects.[9] A large proportion of TED patients present with mild disease, for which the standard of care is often conservative, involving "watchful waiting" and symptomatic management of ocular surface discomfort with lubricants. While this approach avoids the risks of systemic immunosuppression, it fails to address the underlying low-grade inflammation and oxidative stress that cause the significant patient-reported morbidity—dry eye symptoms, foreign body sensation, redness, and

tearing—that severely impacts quality of life. This creates a "therapeutic limbo" for patients with mild TED, highlighting an unmet need for safe and effective therapies that can modulate the underlying disease process.

In this context, Vitamin D, a secosteroid hormone primarily known for its role in calcium homeostasis, has emerged as a potent modulator of the immune system. The active form of vitamin D, 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$], exerts its effects by binding to the Vitamin D Receptor (VDR), which is expressed on a wide array of immune cells, including T-cells, B-cells, macrophages, and dendritic cells. Mechanistically, vitamin D can suppress key inflammatory pathways in TED by inhibiting the production of pro-inflammatory cytokines like IL-6 and TNF-α, and by modulating T-cell differentiation, skewing the response from a pro-inflammatory Th1/Th17 phenotype towards an anti-inflammatory Th2/Treg phenotype. Importantly, the VDR and the enzymatic machinery required for local vitamin D metabolism (including 1-α-hydroxylase) are expressed in ocular surface tissues, including the cornea and conjunctiva, suggesting a plausible direct protective role.

Despite this strong biological rationale, a direct link between systemic vitamin D supplementation and the quantitative mitigation of local oxidative stress at the ocular surface in TED patients has not been clinically established. The tear film is a unique and accessible biological fluid that directly mirrors the biochemical environment of the ocular surface, making it an ideal medium for assessing local pathology.[10] Therefore, the primary aim of this preliminary investigation was to quantitatively determine the effect of short-term oral vitamin D3 supplementation on the concentration of MDA in the tear film of patients with mild, active TED. We hypothesized that systemic vitamin D supplementation would be associated with a statistically significant reduction in tear film MDA levels. The novelty of this study lies in its use of a specific, localized biomarker to obtain direct biochemical data on vitamin D's potential antioxidant effects at the primary site of patient discomfort, potentially establishing a rationale for its use as a safe, targeted adjunctive therapy in the management of mild TED.

2. Methods

This study was conducted as a single-center, prospective, quasi-experimental pre-test/post-test investigation. The choice of this preliminary design was to establish proof-of-concept and gather initial data on the biochemical efficacy of the intervention before embarking on a larger controlled trial. The research protocol was rigorously reviewed and received formal approval from the Research Ethics Committee of Dr. M. Djamil General Hospital, Padang, Indonesia (Approval No. DP.04.03/D.XVI.XI/564/2024). The study was performed in strict accordance with the ethical principles for medical research involving human subjects as outlined in the Declaration of Helsinki. After a comprehensive verbal and written explanation of the study's objectives, procedures, and potential risks and benefits, written informed consent was voluntarily obtained from every participant prior to enrollment.

Participants were recruited via purposive sampling from the outpatient ophthalmology clinic at Dr. M. Djamil General Hospital, a tertiary referral center, between December 2024 and February 2025. Inclusion criteria were; (1) Adult patients (age ≥ 18 years) with a confirmed diagnosis of Graves' disease; (2) A clinical diagnosis of active, mild Thyroid Eye Disease, defined by a Clinical Activity Score (CAS) of 3 or less on the 7-point scale; (3) Willingness and ability to provide informed consent and adhere to the 21-day study protocol; (4) No active treatment with systemic or retrobulbar corticosteroids, radiation, or other immunosuppressive agents for at least 3 months prior to enrollment. Exclusion criteria included; (1) Presence of other ocular surface diseases that could independently influence tear film composition (such as severe meibomian gland dysfunction, Stevens-

Johnson syndrome, chemical burns, or active infectious keratitis); (2) Co-existing systemic autoimmune diseases; (3) History of ocular or orbital surgery within the preceding 6 months; (4) Current use of vitamin D supplements exceeding 400 IU/day or topical ophthalmic medications with known anti-inflammatory effects (such as cyclosporine or steroids); and (5) Pregnancy or lactation.

The intervention consisted of oral supplementation with cholecalciferol (Vitamin D3) at a dose of 1000 International Units (IU) per day. Participants were provided with a 21-day supply of the supplement and instructed to take one capsule daily. Adherence was monitored through a patient-completed study diary and a pill count at the final study visit.

All assessments were performed at two time points: at baseline (Day 0, pre-intervention) and at the conclusion of the study (Day 22, post-intervention). At the initial visit, a structured case report form was used to document demographic data (age, gender) and relevant medical history. All participants underwent a comprehensive ophthalmic examination, including Best-Corrected Visual Acuity (BCVA), intraocular pressure (IOP) measurement, slit-lamp biomicroscopy, and dilated fundus examination. TED-specific evaluation included: (1) Clinical Activity Score (CAS): Assessed on the 7-point scale (spontaneous retrobulbar pain, pain on eye movement, redness of eyelids, redness of conjunctiva, swelling of caruncle/plica, chemosis, swelling of eyelids); and (3) Proptosis: Measured in millimeters using a Hertel exophthalmometer. The following clinical tests were performed in a standardized sequence to assess the ocular surface: (1) Ocular Surface Disease Index (OSDI): Participants completed this validated 12-item questionnaire to quantify the severity of dry eye symptoms. Scores range from 0 to 100, with higher scores indicating greater disability; (2) Tear Film Break-Up Time (TBUT): A fluorescein strip was applied to the inferior palpebral conjunctiva. The interval between the last complete blink and the appearance of the first dry spot on the cornea was measured in seconds. The average of three consecutive measurements was recorded; (3) Schirmer's I Test: Performed without topical anesthesia, a standardized filter paper strip was placed in the inferior conjunctival fornix for 5 minutes. The length of the wetted portion was measured in millimeters.

To avoid reflex tearing, no topical anesthetic was administered. Pooled tears from the inferior conjunctival fornix were carefully collected using a 10-microliter glass capillary micropipette without touching the ocular surface. The collected sample (5-10 µL per eye) was immediately transferred to a sterile, pre-chilled microcentrifuge tube, placed on ice, and transported to the laboratory. Samples were centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatant was then aliquoted and stored at -80°C until analysis.

Tear film MDA concentration was quantified at the Biomedical Laboratory, Faculty of Medicine, Universitas Andalas, using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (CAS Registry Number: 542-78-9), following the manufacturer's protocol. All samples and standards were assayed in duplicate. Absorbance was read at 450 nm, and MDA concentrations were calculated from a standard curve and expressed in nanograms per liter (ng/L).

Venous blood samples were collected at both study visits. Serum was separated and stored at -80°C. Total serum 25(OH)D concentration, the primary indicator of vitamin D status, was measured using a competitive chemiluminescence immunoassay on an automated analyzer. Results were expressed in nanograms per milliliter (ng/mL).

Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0. The normality of continuous variables was assessed using the Shapiro-Wilk test. The primary outcome (change in tear film MDA) and secondary continuous outcomes (serum 25(OH)D, OSDI, TBUT, Schirmer's test) before and after the intervention were analyzed using a paired-samples t-test. Pearson's correlation coefficient (r) was calculated to assess the relationship between the change in serum 25(OH)D (Δ25(OH)D) and the change

in tear film MDA (ΔMDA). Subgroup analyses based on gender (independent-samples t-test) and age group (one-way ANOVA) were performed. A two-tailed p-value of < 0.05 was considered statistically significant. A formal a priori power calculation was not performed, as this was considered a preliminary, hypothesis-generating study. Data are presented as mean ± standard deviation (SD).

3. Results

A total of 18 patients were screened for eligibility; 3 were excluded (one was taking high-dose vitamin D, one had co-existing severe blepharitis, and one declined participation). The remaining 15 patients were enrolled and completed the 21-day study protocol with excellent reported adherence.

The study cohort was predominantly female (n=11, 73.4%), with a mean age of 37.5 ± 10.8 years. All patients had mild, active TED. A detailed breakdown of the baseline CAS revealed that 5 patients (33.3%) had a CAS of 1, 9 patients (60.0%) had a CAS of 2, and 1 patient (6.7%) had a CAS of 3. The mean baseline serum 25(OH)D level was 18.2 ± 5.9 ng/mL, indicating a high prevalence of vitamin D insufficiency (<30 ng/mL) in this population. Baseline demographic and clinical characteristics are detailed in Table 1.

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants (n=15)

Characteristic	Value (Mean ± SD or N (%))
Age (years)	37.5 ± 10.8
20-29	5 (33.3%)
30-39	3 (20%)
40-49	6 (40%)
50-59	1 (6.7%)
Gender	
Male	4 (26.6%)
Female	11 (73.4%)
Clinical Activity Score (CAS)	
CAS = 1	5 (33.3%)
CAS = 2	9 (60.0%)
CAS = 3	1 (6.7%)
Proptosis (mm)	19.8 ± 2.1
OSDI Score	35.6 ± 12.4
TBUT (seconds)	6.1 ± 1.9
Schirmer's I (mm)	8.9 ± 3.5
Serum 25(OH)D (ng/mL)	18.2 ± 5.9

OSDI: Ocular Surface Disease Index; TBUT: Tear Break-Up Time; 25(OH)D: 25-hydroxyvitamin D.

The central finding of this study was a highly statistically significant reduction in tear film MDA concentration following the 21-day vitamin D supplementation period. As shown in Table 2 and Figure 1, the mean tear film MDA level decreased from a baseline of 8.69 ± 4.15 ng/L to 5.70 ± 1.56 ng/L post-intervention. This mean reduction of 2.99 ng/L was highly significant ($t(14)=4.98$, $p < 0.001$). Notably, every one of the 15 participants in the study demonstrated a decrease in their tear film MDA level from baseline.

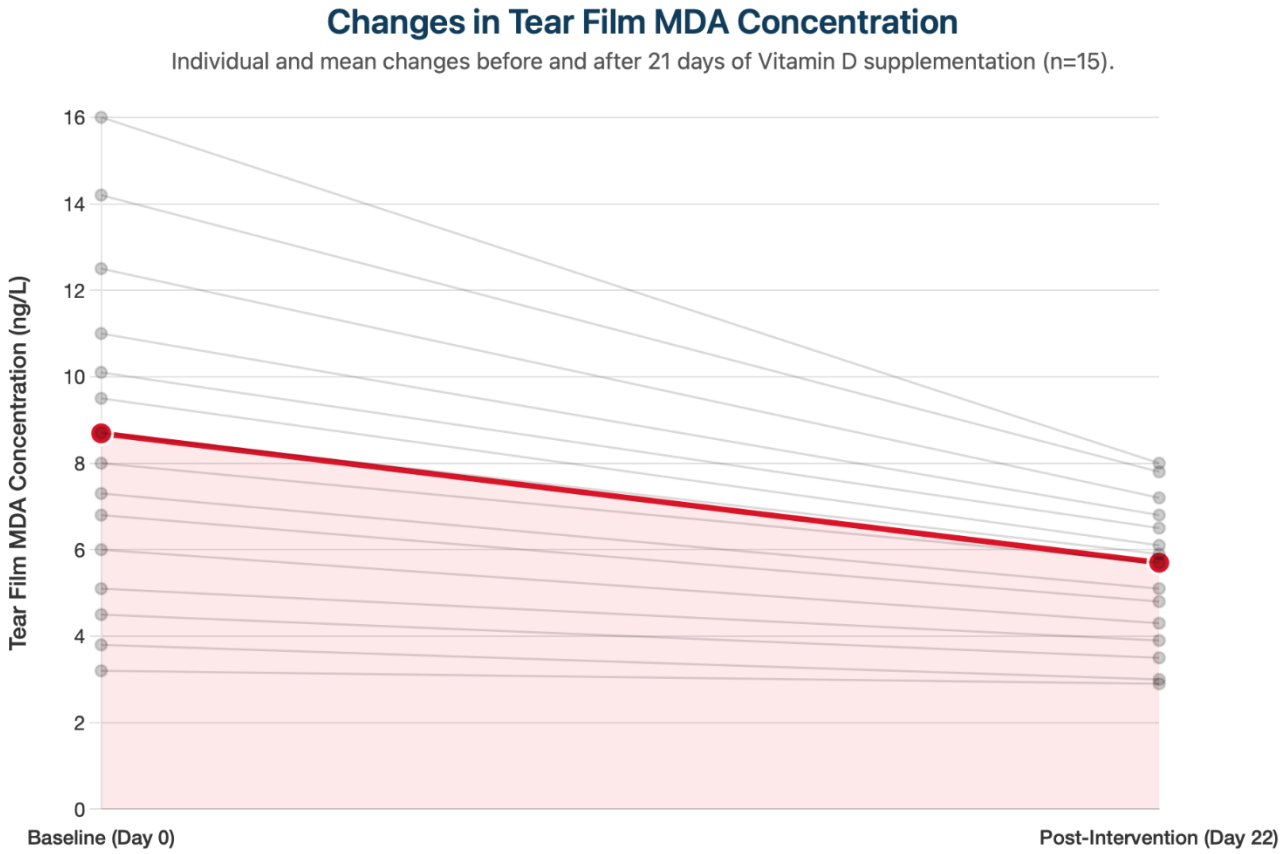

Figure 1. Individual changes in tear film MDA concentration before and after 21 days of vitamin D supplementation. Each line represents an individual participant (n=15), demonstrating a consistent reduction in MDA levels post-intervention. The bold lines represent the mean values before and after.

The oral supplementation was effective in improving systemic vitamin D status. The mean serum 25(OH)D concentration significantly increased from 18.2 ± 5.9 ng/mL to 29.8 ± 6.4 ng/mL post-intervention ($p<0.001$). Consistent with the reduction in the oxidative stress biomarker, significant improvements were also observed in clinical parameters of ocular surface disease (Table 2). The mean OSDI score, reflecting subjective symptoms, decreased significantly from 35.6 ± 12.4 to 26.1 ± 10.9 ($p=0.002$). Furthermore, the mean TBUT, an objective measure of tear film stability, significantly increased from 6.1 ± 1.9 seconds to 8.5 ± 2.3 seconds ($p=0.005$). The change in Schirmer's I test values, a measure of aqueous tear production, was not statistically significant ($p=0.081$).

Table 2. Comparison of Primary and Secondary Outcomes Before and After Vitamin D3 Supplementation (n=15)

Variable	Baseline (Mean ± SD)	Post-Intervention (Mean ± SD)	Mean Difference (95% CI)	*p*-value
Tear Film MDA (ng/L)	8.69 ± 4.15	5.70 ± 1.56	● -2.99 (-4.28, -1.70)	<0.001
Serum 25(OH)D (ng/mL)	18.2 ± 5.9	29.8 ± 6.4	● +11.6 (9.31, 13.89)	<0.001
OSDI Score	35.6 ± 12.4	26.1 ± 10.9	● -9.5 (-15.21, -3.79)	0.002
TBUT (seconds)	6.1 ± 1.9	8.5 ± 2.3	● +2.4 (0.91, 3.89)	0.005
Schirmer's I (mm)	8.9 ± 3.5	10.1 ± 3.8	● +1.2 (-0.19, 2.59)	0.081

Notes: Data are presented as Mean ± Standard Deviation. *p*-values were calculated using a paired-samples t-test.

Legend: ● Favorable Clinical Change ● Non-Significant Change

Notes: Data are presented as Mean ± Standard Deviation. p-values were calculated using a paired-samples t-test. CI: Confidence Interval.

To investigate the association between systemic vitamin D improvement and the local reduction in oxidative stress, a Pearson correlation analysis was performed. The analysis revealed a strong, statistically significant negative correlation between the increase in serum 25(OH)D levels and the decrease in tear film MDA levels ($r = -0.68$, $p=0.005$). This relationship, depicted in Figure 2, indicates that participants who experienced a greater increase in their systemic vitamin D levels also tended to have a more pronounced reduction in their ocular surface oxidative stress marker.

The reduction in tear film MDA did not differ significantly between male (mean reduction: 2.96 ± 1.5 ng/L) and female participants (mean reduction: 3.01 ± 2.1 ng/L) ($p=0.361$). Similarly, there was no statistically significant difference in the mean MDA reduction across different age groups ($p=0.482$), suggesting the observed association was consistent across these demographic strata in our cohort.

4. Discussion

Within the limitations of a preliminary, uncontrolled study design, the primary finding of this investigation is that short-term oral vitamin D supplementation was associated with a profound and statistically significant reduction in tear film MDA levels in patients with mild, active TED.[11] This result provides initial, quantitative biochemical data suggesting that a systemic nutritional intervention may effectively attenuate local oxidative stress on the ocular surface in this patient population. The concurrent and significant increase in systemic vitamin D status, alongside the strong negative correlation between the rise in serum 25(OH)D and the fall in tear film MDA, strengthens the link between the intervention and the observed local biochemical effect.[12] This study moves beyond theoretical association to provide clinical evidence that warrants further investigation into the therapeutic potential of vitamin D in the multifaceted management of TED.

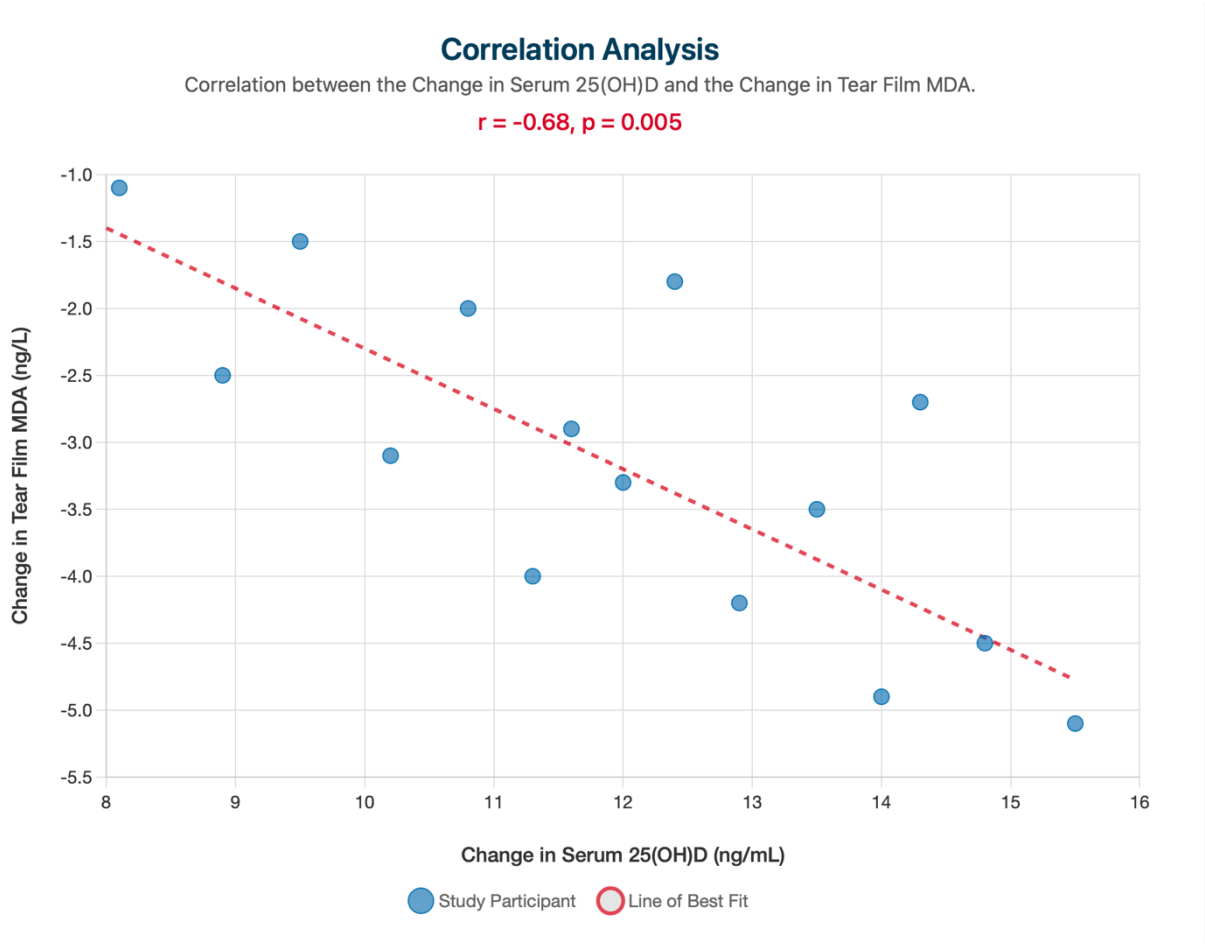

Figure 2. Correlation between the change in serum 25(OH)D and the change in tear film MDA. The scatter plot demonstrates a significant negative linear relationship, where a greater increase in serum vitamin D is associated with a greater decrease in tear film MDA (r = -0.68, p = 0.005).

To appreciate the significance of these findings, they must be interpreted within the complex pathophysiological landscape of TED. The observed reduction in MDA is likely not an isolated event but rather a downstream consequence of vitamin D's potential influence on the core drivers of the disease: autoimmunity, inflammation, and the resultant oxidative cascade.[13]

The orbit in active TED is a site of intense immunological activity.[14] The process, driven by autoreactive T-lymphocytes and autoantibodies, triggers a vicious cycle wherein activated orbital fibroblasts release a barrage of pro-inflammatory cytokines (such as IFN-γ, TNF-α, and IL-6), leading to GAG production and tissue expansion. This inflammatory storm is inextricably linked to overwhelming oxidative stress from two primary sources: 1) the "respiratory burst" from activated immune cells releasing massive amounts of ROS, and 2) cytokine-induced mitochondrial dysfunction in orbital fibroblasts and muscle cells, promoting endogenous ROS generation. This flood of free radicals initiates a self-propagating chain reaction of lipid peroxidation in cell membranes, of which MDA is a stable byproduct. The accumulation of MDA in the tear film serves as an integrated footprint of this entire upstream cascade of inflammation-driven oxidative injury.[15]

Our results suggest that vitamin D may disrupt this pathological cycle via a sophisticated, two-pronged attack: (1) Indirect antioxidant effect via potent immunomodulation: This is believed to be the dominant mechanism. The active form of vitamin D, $1,25(OH)_2D_3$, functions as a powerful brake on the immune system. In the context of TED, its actions are critically relevant. Vitamin D has been shown to suppress the proliferation of pro-inflammatory T-helper 1 (Th1) and Th17 cells, the key drivers of autoimmunity in TED, while promoting anti-inflammatory T-helper 2 (Th2) and T-regulatory (Treg) cells. It also directly inhibits the production of key pro-inflammatory cytokines like IL-6 and TNF-α. By dampening the overall T-cell-mediated inflammation and cytokine "noise" in the orbit, vitamin D may indirectly reduce the primary stimulus for ROS production by downstream effector cells, leading to a measurable decrease in lipid peroxidation and, consequently, lower MDA levels; (2) Direct cellular and antioxidant effects: There is emerging evidence that vitamin D may also exert more direct protective effects at the cellular level. The VDR is expressed on corneal and conjunctival epithelial cells.[16] In vitro studies have shown that vitamin D can enhance epithelial barrier function and directly suppress local inflammatory responses. Furthermore, some studies suggest vitamin D can bolster cellular antioxidant defenses by upregulating the expression of enzymes like glutathione peroxidase. Therefore, the reduction in tear film MDA may reflect not only a decrease in ROS production from infiltrating immune cells but also an enhanced capacity of the ocular surface epithelium itself to withstand oxidative insults (Figure 3).

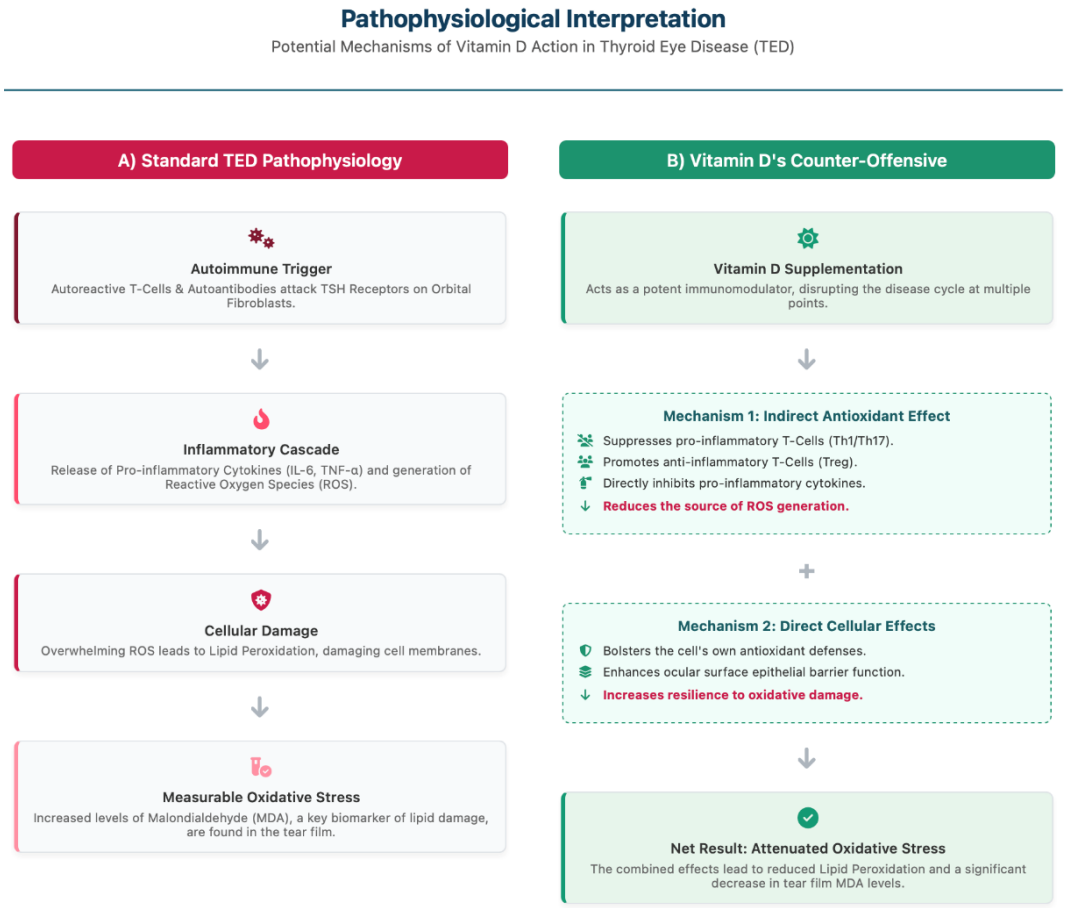

Figure 3. Pathophysiological interpretation.

A critical question is how an oral supplement can exert such a specific effect on the tear film. The answer likely lies in the local metabolism of vitamin D. The precursor we ingest and measure in the blood is the relatively inactive 25(OH)D. However, ocular tissues, including the corneal and conjunctival epithelium, express the enzyme 1-α-hydroxylase, which locally converts 25(OH)D into the biologically potent hormone $1,25(OH)_2D_3$. By significantly raising the systemic levels of the 25(OH)D substrate (from 18.2 to 29.8 ng/mL), our intervention effectively provided more raw material for the ocular surface tissues to generate their own supply of active vitamin D. This facilitates a localized, high-concentration immunomodulatory and antioxidant effect at the disease interface. The strong negative correlation we found between the rise in serum 25(OH)D and the fall in tear MDA ($r=-0.68$) provides powerful support for this "systemic-to-local" pathway, demonstrating a clear exposure-response relationship.

The clinical relevance of our findings is substantial, particularly for the large cohort of TED patients with mild disease who exist in a therapeutic limbo.[17] These patients suffer from chronic and bothersome symptoms of ocular surface disease that significantly impair their quality of life, yet their condition is not severe enough to warrant high-risk systemic immunosuppression. Our study shows a concurrent significant improvement in subjective OSDI scores and objective TBUT measurements alongside the reduction in MDA. This suggests that targeting a fundamental pathological mechanism—oxidative stress—may lead to clinically meaningful improvements in the patient experience.

Interestingly, we did not observe a significant change in Schirmer's I test values. This important negative finding suggests that the beneficial effect of vitamin D in this context is likely related to improving tear film quality and stability by reducing inflammation-mediated lipid peroxidation, rather than increasing aqueous tear quantity from the lacrimal gland. This aligns perfectly with the primary biochemical outcome and strengthens the hypothesis that vitamin D is acting on the inflammatory component of ocular surface disease in TED.[18]

Vitamin D supplementation represents an ideal candidate for an adjunctive therapy in this population. It is safe, widely available, inexpensive, and corrects a nutritional deficiency that is common in patients with autoimmune diseases.[19] By potentially reducing local inflammation and oxidative stress, vitamin D could help mitigate symptoms and form an integral part of a more holistic management strategy for mild TED. It is imperative to interpret these promising findings within the context of the study's significant limitations. The most critical weakness is the quasi-experimental, pre-post design, which lacked a placebo control group. This design is susceptible to several biases. The observed improvements in clinical outcomes, particularly the subjective OSDI score, could be influenced by a placebo effect. Furthermore, because patients often seek treatment when their symptoms are at their worst, the improvements could be partially attributable to regression to the mean. Finally, the natural history of mild TED can be variable, and the 21-day study period may have coincided with a spontaneous fluctuation in disease activity.

Second, the small sample size (n=15) limits the external validity and generalizability of our findings. While the primary outcome reached a high level of statistical significance, the study was likely underpowered to detect smaller effects or to perform robust subgroup analyses. Third, the short follow-up duration (21 days), while adequate for assessing a dynamic biochemical marker, is insufficient to evaluate long-term effects on the clinical course of TED, such as changes in CAS, proptosis, or eyelid retraction.[20] Given these limitations, this study should be viewed as preliminary and hypothesis-generating. The results provide a strong rationale for future research. A definitive assessment of vitamin D's efficacy in TED requires a large-scale, double-masked, placebo-controlled randomized clinical trial. Such a trial should incorporate a longer follow-up period to assess both biochemical and anatomical outcomes, and potentially explore other inflammatory

biomarkers in the tear film (such as cytokines) to further elucidate the mechanism of action.

5. Conclusion

In this preliminary, uncontrolled study, short-term oral supplementation with 1000 IU of vitamin D3 per day was associated with a significant attenuation of oxidative stress on the ocular surface in patients with mild, active Thyroid Eye Disease. The marked reduction in tear film malondialdehyde, a key biomarker of lipid peroxidation, establishes a clear link between a safe systemic intervention and a measurable local biochemical effect. This finding, supported by a strong correlation with the improvement in systemic vitamin D status and concurrent improvements in clinical signs and symptoms of ocular surface disease, highlights the potential immunomodulatory and protective roles of vitamin D. While validation in larger, placebo-controlled trials is essential to establish causality and define its therapeutic role, our results strongly support the consideration of vitamin D supplementation as a promising, non-invasive adjunctive strategy aimed at mitigating the inflammatory and oxidative components of TED.

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