Vitamin D Enhances the Efficacy of Topical Artificial Tears in Patients With Dry Eye Disease

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Purpose: To investigate the efficacy of topical carbomer-based lipid-containing artificial tears (CLAT) and hyaluronate (HU) in patients with dry eye disease (DED) based on serum 25-hydroxyvitamin D (25HD) levels and cholecalciferol (vitamin D) supplementation.

Methods: A total of 116 patients with DED from June 2015 to June 2016 were included. The participants were divided into the vitamin D deficiency (VDD) group and the non-VDD group according to their serum 25HD levels. The patients determined the ways of cholecalciferol supplementation. Ocular Surface Disease Index (OSDI) score, visual analog pain scale score, lid hyperemia, tear breakup time (TBUT), corneal fluorescein staining score, and Schirmer test were compared between baseline and 2 weeks posttreatment after topical applications and between before and after cholecalciferol supplementation.

Results: The OSDI and visual analog pain scale scores of both VDD and non-VDD groups decreased after application of topical CLAT and HU compared with baseline values ($P < 0.05$ for all, paired t test). TBUT, corneal fluorescein staining score, and lid hyperemia in the VDD group remained unaffected by topical CLAT and HU, whereas those in the non-VDD group were improved (3.2 ± 1.7 vs. 4.1 ± 2.2, 0.5 ± 0.7 vs. 0.4 ± 0.6, and 2.2 ± 0.8 vs. 1.9 ± 0.7 in the non-VDD group, $P = 0.001$, 0.030, and 0.012, respectively). OSDI score, TBUT, and lid margin hyperemia were improved in the intramuscular group after cholecalciferol supplementation compared with pretreatment (33.2 ± 23.2 vs. 28.5 ± 21.9, 3.5 ± 1.9 vs. 6.0 ± 2.5, and 2.2 ± 0.7 vs. 1.2 ± 0.8, $P < 0.05$, Wilcoxon rank test).

Conclusions: The effect of topical CLAT and HU was dependent on serum 25HD levels. Cholecalciferol supplementation enhanced the efficacy of topical treatment and may be a useful adjuvant therapy for patients with DED refractory to topical lubricants.

Key Words: carbomer-based lipid-containing artificial tears, vitamin D deficiency, dry eye disease, hyaluronate

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The tear film is a thin fluid layer providing a smooth surface over the cornea.[1] It is composed of 3 layers: the innermost mucin layer, an aqueous layer, and the outermost lipid layer.[2] Furthermore, the tear film is a complex mix of electrolytes, proteins (lipocalins, lactoferrin, transferrin, defensin, and lysozyme), phospholipids, oligopeptides, glycopeptides, and immunoglobulins.[3] The tear film also includes a variety of surfactants, including surfactant proteins A and D.[4] It is essential for the health of the eye that tear film components work in harmony.[3] Tear film stability is, therefore, the result of the harmonious balance between tear components.[5] A disturbance in one or more of the tear layers leads to the occurrence of dry eye disease (DED).[4]

DED is a disease of the tear film and ocular surface, leading to ocular discomforts and pain.[4] The pathophysiology of DED includes tear film instability and ocular surface inflammation.[3] DED has been treated with artificial tears such as sodium hyaluronate (HU), carbomer-based lipid-containing artificial tears (CLAT), and sodium carboxymethylcellulose, anti-inflammatory agents, autologous serum, and the procedure of punctal occlusion.[6] However, the treatment outcomes of DED cannot be predicted,[7] and the factors associated with treatment-refractory DED remain unclear.

Vitamin D is well known to have a function in calcium absorption, bone growth, and bone remodeling.[8] Vitamin D affects the synthesis of surfactants and the ability of calcium to bind to proteins and repression of inflammation.[9] Thus, vitamin D supplementation can be used as potential adjuvant therapy to conventional treatments of DED. In this study, we investigated the efficacy of topical lubricants on the tear film and ocular surface of patients with DED based on serum 25-hydroxyvitamin D (25HD) levels and cholecalciferol supplementation. We suggested that vitamin D supplementation can be used as adjuvant therapy for patients with DED.

METHODS

This study was conducted in accordance with the tenets of the Declaration of Helsinki for research involving human subjects. This retrospective, observational study was approved by the Institutional Review Board of Hallym University Medical Center. The medical charts of a total of 116 patients with DED who visited the Hallym University

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Kangnam Sacred Heart Hospital from June 2015 to June 2016 were retrospectively reviewed. The patients' charts were randomly selected.

DED was diagnosed as previously described based on tear instability, Schirmer tear secretion, and ocular discomforts.[10] Patients with DED who were treated with a carbomer-based lipid-containing artificial tear formulation (CLAT; Liposic EDO, Bausch & Lomb, Berlin) and 0.15% sodium HU (HU; New hyaluni, Taejoon, Seoul) were included in this study. The serum 25HD concentration was measured for each patient. The individuals were assigned into 2 groups according to the serum 25HD levels: the vitamin D deficiency (VDD) group (<12 ng/mL) and the non-VDD group (≥12 ng/mL). The exclusion criteria included history of autoimmune diseases, corneal surgery, corneal diseases, and the presence of corneal opacity. Data were obtained at baseline and at 2 weeks after topical application of lubricants, CLAT, and HU.

Then, after the patients were provided with results regarding their serum 25HD levels, related information, and suggestion of vitamin D supplementation, the patients chose their source of vitamin D supplementation: none, injection of 200,000 IU cholecalciferol [intramuscularly; intramuscular (IM) group], or oral supplementation of cholecalciferol (2000 IU daily: oral group). In the IM group of patients, 200,000 IU cholecalciferol was injected intramuscularly into the buttocks. Patients who preferred oral administration (oral group) were asked to take 2000 IU of cholecalciferol every day and were checked at the outpatient clinic. Patients who did not want to take any supplements were observed only with eye drops and without supplementation. The effects of cholecalciferol supplementation on the tear film and ocular surface were evaluated 2 weeks later.

DED symptoms were quantified using the Ocular Surface Disease Index (OSDI) questionnaire and visual analog pain scale (VAPS) score.[11] Individuals were asked to complete questionnaires about the DED symptoms during a 1-week recall period. The scale for each answer ranged from 0 (no discomfort) to 4 (significant ocular discomfort).[11] The OSDI scores were calculated and ranged from 0 to 100. The symptoms of DED were also graded numerically, using the VAPS (0–10). The individuals were encouraged to describe ocular pain using the VAPS system.

Tear breakup time (TBUT), corneal fluorescein staining score (CFSS), severity of lid hyperemia, and Schirmer tear secretion test were assessed. TBUT and CFSS evaluation was performed as previously described.[12,13] A fluorescein strip (Haag-Streit, Köniz, Switzerland) was used to measure the TBUT and CFSS. Eyelid margin hyperemia was graded as follows: 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Tear secretion was assessed by the Schirmer test without topical anesthesia.[14]

Statistics

Paired *t* tests were used to compare the TBUT, CFSS, lid hyperemia scores, Schirmer tear secretion test values, OSDI scores, and VAPS scores between baseline and 2 weeks after topical application of CLAT and HU. The Wilcoxon rank test was used to compare the parameters in each group between before and 2 weeks after cholecalciferol supplementation. SPSS Statistics 23.0 (IBM Corp, Chicago, IL) was used. $P < 0.05$ was considered statistically significant.

RESULTS

In total, 116 patients were enrolled in this study (Table 1 and Fig. 1). There were 34 men and 82 women. Mean age was 55.1 ± 12.4 years (range: 23–84 years). The mean serum 25HD level was 15.46 ± 8.32 ng/mL. Fifty-two patients were in the VDD group and 64 patients in the non-VDD group. In the VDD group, 46 patients were in the IM group and 5 patients in the oral group. In the non-VDD group, 8 patients were not supplemented (none), 48 patients received IM injections of 200,000 IU vitamin D (IM group), and 4 patients determined oral supplementation of 2,000 IU vitamin D (oral group). The mean serum 25HD level was 13.87 ± 7.06 ng/mL in the IM group and 16.70 ± 15.33 ng/mL in the oral group.

Response to Topical CLAT and HU

The mean OSDI score was 39.2 ± 23.0 at baseline and decreased to 33.8 ± 23.2 at 2 weeks after topical application of CLAT and HU ($P = 0.002$, paired *t* test). The mean VAPS score was 3.0 ± 2.5 at baseline and decreased to 2.1 ± 2.3 at 2 weeks after topical application of CLAT and HU ($P = 0.001$, paired *t* test). The mean TBUT was 3.2 ± 1.7 seconds at baseline and increased to 3.7 ± 2.0 seconds at 2 weeks after topical application of CLAT and HU ($P = 0.012$, paired *t* test). The results of the Schirmer tear secretion test were 9.8 ± 8.4 mm at baseline and 8.9 ± 7.7 mm at 2 weeks after topical application of CLAT and HU ($P = 0.207$). The mean fluorescein staining score was 0.5 ± 0.7 at baseline and 0.4 ± 0.6 at 2 weeks after topical application of CLAT and HU ($P = 0.061$, paired *t* test). The mean lid hyperemia score was 2.2 ± 0.8 at baseline and 2.1 ± 0.8 at 2 weeks after topical application of CLAT and HU ($P = 0.294$, paired *t* test).

Response to Topical CLAT and HU Is Dependent on VDD

The individuals were assigned to 2 groups according to the serum 25HD levels: the VDD group (<12 ng/mL) and the

TABLE 1. Changes in the Tear Film and Ocular Surface After Topical Application of Carbomer-Based Lipid-Containing Artificial Tear and Hyaluronate

	Baseline	After 2 wk	*P*
OSDI score	39.2 ± 23.0	33.8 ± 23.2	0.002*
VAPS score	3.0 ± 2.5	2.1 ± 2.3	0.001*
TBUT (s)	3.2 ± 1.7	3.7 ± 2.0	0.012*
CFSS	0.5 ± 0.7	0.4 ± 0.6	0.061
Lid hyperemia	2.2 ± 0.8	2.1 ± 0.8	0.294
Tear secretion test (mm)	9.8 ± 8.4	8.9 ± 7.7	0.207

*$P < 0.05$ by the paired *t* test.

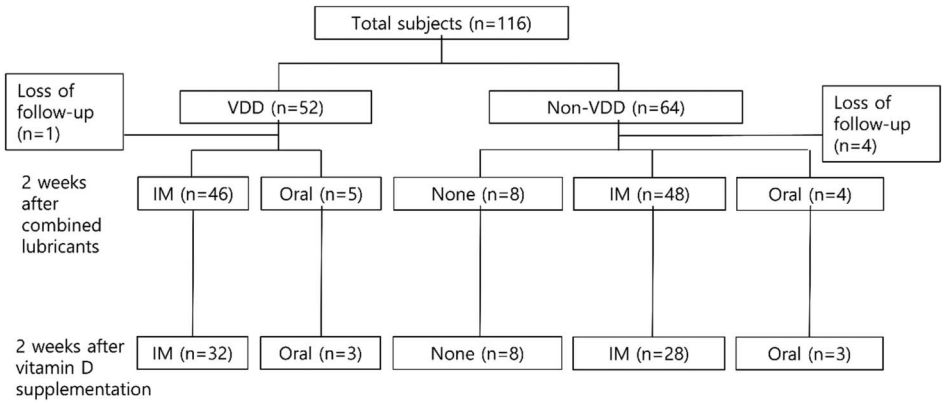

FIGURE 1. Flowchart of the patients included in the study.

non-VDD group (≥12 ng/mL) (Table 2). The measurements of the OSDI and VAPS for both groups decreased after topical application of CLAT and HU ($P = 0.027$ and 0.034 for the VDD and non-VDD groups, respectively, for the OSDI, paired t test; and $P = 0.028$ and 0.016, respectively, for the VAS, paired t test) (Figs. 2A, B). However, the OSDI and VAS scores were not different between the groups at 2 separate time points [$P = 0.401$ and 0.794, respectively, repeated-measure analysis of variance (ANOVA)]. The TBUT in the VDD group remained unaffected after topical application of CLAT and HU, whereas the TBUT of the non-VDD group was reported to have increased from baseline values ($P = 0.001$, paired t test; Fig. 2C). The TBUT was different between the groups at 2 different time points ($P = 0.011$, repeated-measure ANOVA). The tear secretion values of the Schirmer test in the VDD group were reduced after topical application of CLAT and HU ($P = 0.043$, paired t test; Fig. 2D), whereas those of the non-VDD group were unaffected. However, the values of the Schirmer tear secretion test were not different between the groups at 2 separate time points ($P = 0.185$, repeated-measure ANOVA). The CFSS of the VDD group was also unaffected by topical application of CLAT and HU, whereas the FSS of the non-VDD group decreased compared with the baseline values ($P = 0.030$, paired t test; Fig. 2E). The scores of the lid margin hyperemia parameter in the VDD group were not affected by topical application of CLAT and HU; whereas a decrease in the severity of lid margin hyperemia was reported in the non-VDD group after topical application of CLAT and HU ($P = 0.012$, paired t test; Fig. 2F). The severity of lid margin hyperemia was different between the groups at 2 separate time points ($P = 0.010$, repeated-measure ANOVA).

Response to Topical CLAT and HU Is Better After Cholecalciferol Supplementation

The OSDI score was decreased in the IM group after cholecalciferol supplementation compared with pretreatment ($P = 0.009$, Wilcoxon rank test), whereas that in the none group and oral group was not different between after cholecalciferol supplementation and pretreatment (Fig. 3A and Table 3). The VAS score was not different in all groups between after cholecalciferol supplementation and pretreatment (Fig. 3B). The TBUT was increased in the IM group after cholecalciferol supplementation compared with pretreatment ($P < 0.001$, Wilcoxon rank test), whereas that in the none group and oral group was not different between after cholecalciferol supplementation and pretreatment (Fig. 3C). Tear secretion and CFSS were not different in all groups between after cholecalciferol supplementation and pretreatment (Figs. 3D, E). Lid margin hyperemia was decreased in the IM group after cholecalciferol supplementation compared with pretreatment ($P < 0.001$, Wilcoxon rank test), whereas that in the none group and oral group was not different between after cholecalciferol supplementation and pretreatment (Fig. 3F).

DISCUSSION

Treatment of DED is to reduce ocular discomfort and ocular damage and to restore tear film stability. However, the

TABLE 2. Changes in the Tear Film and Ocular Surface After Topical Application of Carbomer-Based Lipid-Containing Artificial Tear and Hyaluronate Depending on VDD

	VDD Group			Non-VDD Group		
	Baseline	**After 2 wk**	***P***	**Baseline**	**After 2 wk**	***P***
OSDI score	42.4 ± 23.3	35.3 ± 23.5	0.027*	36.8 ± 22.6	32.6 ± 23.2	0.034*
VAPS score	2.9 ± 2.5	2.1 ± 2.3	0.028*	3.0 ± 2.9	2.3 ± 2.5	0.016*
TBUT (s)	3.2 ± 1.8	3.2 ± 1.7	0.741	3.2 ± 1.7	4.1 ± 2.2	0.001*
CFSS	0.5 ± 0.6	0.5 ± 0.7	0.799	0.5 ± 0.7	0.4 ± 0.6	0.030*
Lid hyperemia	2.2 ± 0.7	2.3 ± 0.7	0.212	2.2 ± 0.8	1.9 ± 0.7	0.012*
Tear secretion test (mm)	10.1 ± 8.0	8.2 ± 6.2	0.043*	9.5 ± 8.7	9.4 ± 8.7	0.948

*$P < 0.05$ by the paired t test.

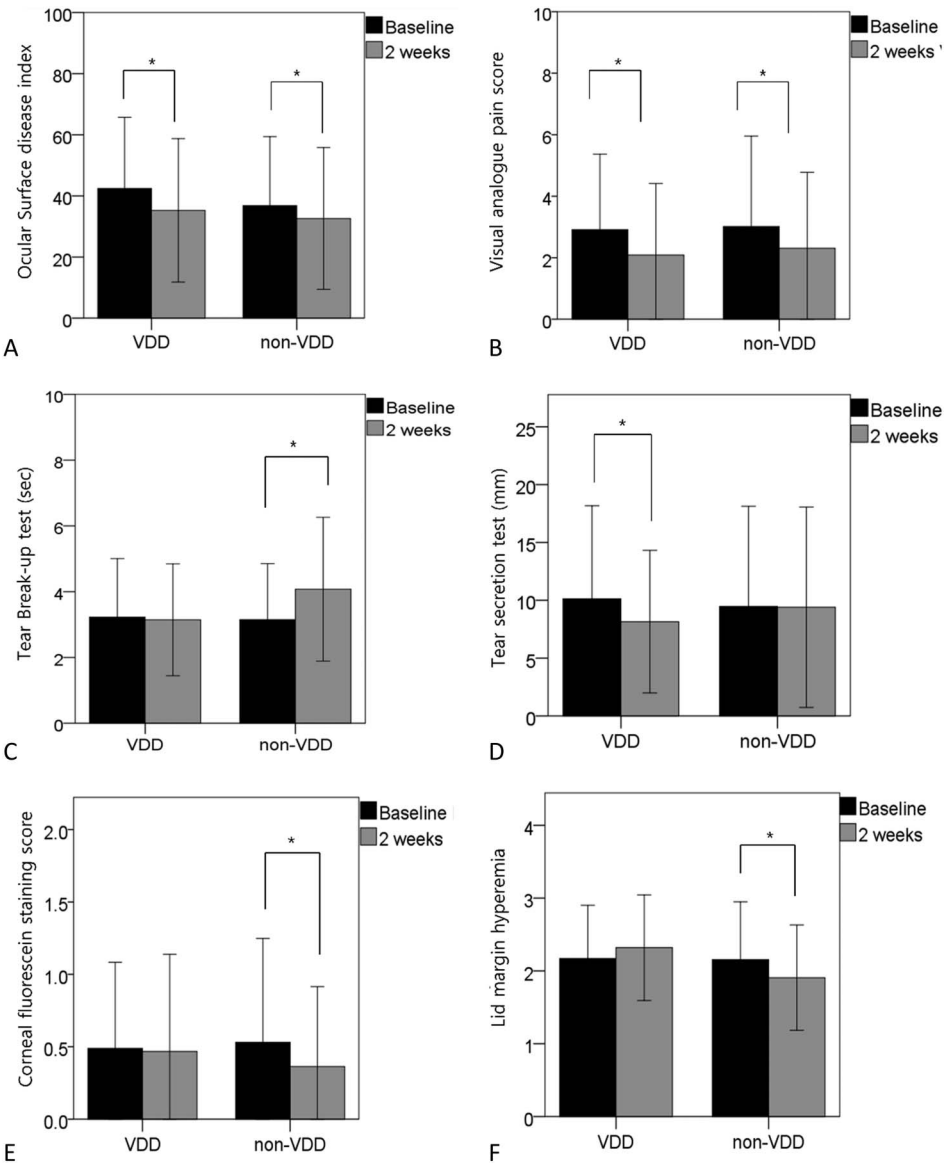

FIGURE 2. The effects of CLAT and HU on the symptoms of dry eye syndrome are influenced by VDD. A, The OSDI score of both groups decreased after topical application of lubricants compared with the baseline values (P = 0.027 and 0.034, respectively, paired t test). B, The VAPS of both groups decreased after topical application of lubricants compared with the baseline values (P = 0.028 and 0.016, respectively, paired t test). C, After topical application of CLAT and HU, the TBUT of the VDD group was unaffected compared with the baseline values, whereas the TBUT in the non-VDD group had increased (P = 0.001, paired t test). D, The values of the Schirmer tear secretion test in the VDD group were reduced after topical application of CLAT and HU compared with the baseline values (P = 0.043, paired t test), whereas those of the non-VDD group were unaffected after topical application of lubricants compared with the baseline values. E, After application of topical CLAT and HU, the CFSS of the VDD group was unaffected compared with the baseline values, whereas the CFSS of the non-VDD group had increased (P = 0.030, paired t test). F, Topical application of CLAT and HU had no effect on the severity of lid margin hyperemia in the VDD group compared with the baseline values but showed an increase in the non-VDD group (P = 0.012, paired t test). *Statistical significance compared by the paired t test.

factors underlying the variable treatment responses remain unexplained. In this study, combination of CLAT and HU alleviated the ocular symptoms in both VDD and non-VDD groups. Lubricants, such as CLAT, have been commonly used for symptomatic alleviation of DED. Furthermore, HU is involved in the tissue repair process and also contributes to the tissue hydrodynamics, the process of cell migration, and the interactions between the cell surface receptors. CLAT, an artificial tear formulation that provides the 3 layers of the tear film,[15,16] has been reported to reduce the symptoms of DED. However, in this study, TBUT, CFSS, and lid margin hyperemia improved only in the VDD group but not in the non-VDD group. The TBUT is an indicator of tear film stability.[17] CFSS and lid margin hyperemia are related to inflammation of the ocular surface and the eyelid.[17] It is notable that the change in the clinical indices related to DED, including TBUT, CFSS, and lid margin hyperemia, in response to combination of CLAT and HU was dependent on the serum 25HD levels. The values of the Schirmer test in the non-VDD group were increased after application of lubricants compared with the baseline values. We used the Schirmer test I, without anesthesia, to measure the extent of total tear secretion (basal and reflexive).[18] Reflex tearing might be induced in the non-VDD group before topical application of lubricants. In patients with DED, this can occur as a hypersensitive response of the ocular surface.[19] In addition, the values of the Schirmer test were not different between the groups at 2 separate time points using repeated-measure ANOVA. In this study, we measured tear secretion using the Schirmer test without topical anesthesia to evaluate basal tear secretion and the function of the main lacrimal gland. Reduction in Schirmer test values in the VDD group may be due to the high variability of the Schirmer test without anesthesia[18] or due to the decreased reflex tear flow in response to the decreased ocular surface symptoms.[20]

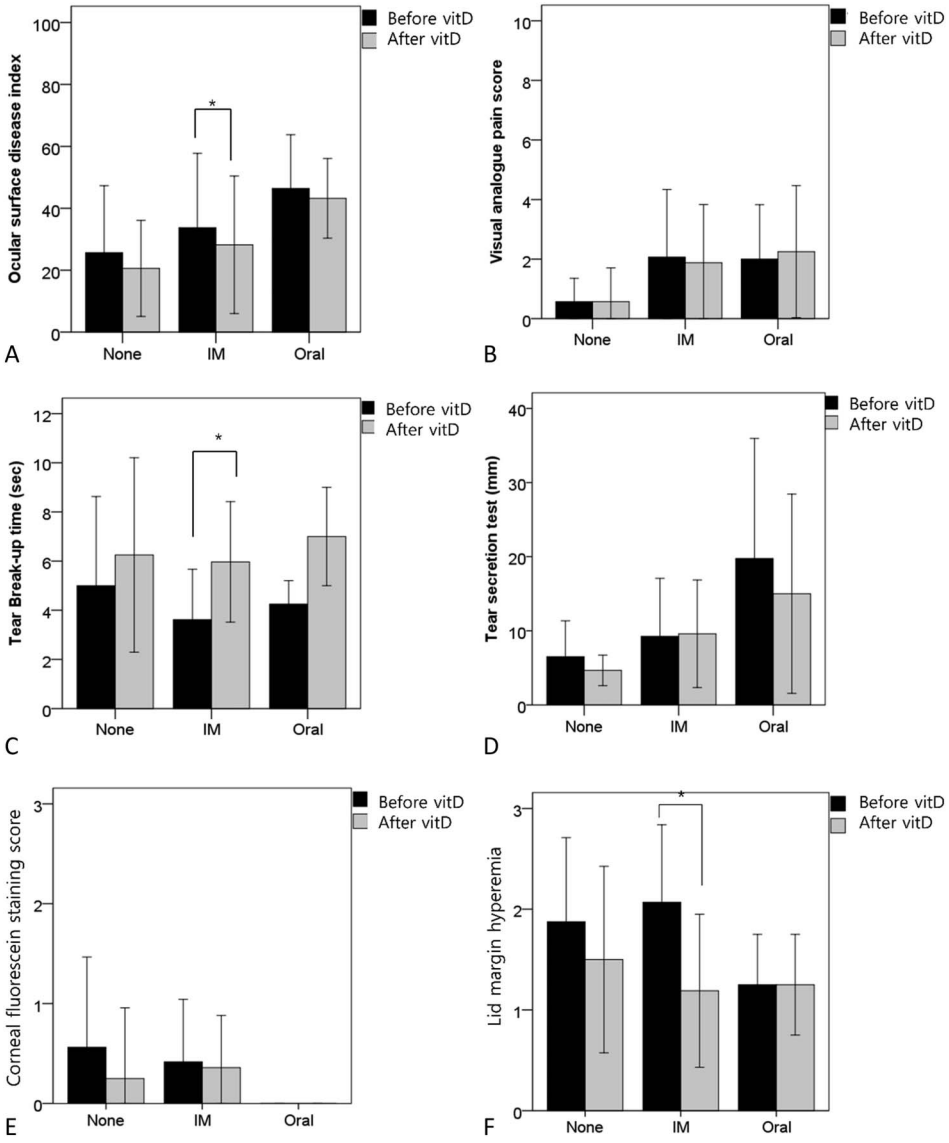

FIGURE 3. Response to topical CLAT and HU after cholecalciferol (vitamin D) supplementation. A, The OSDI score was decreased in the IM group after cholecalciferol supplementation compared with pretreatment ($P = 0.009$, Wilcoxon rank test), whereas that in the none group and oral group was not different between after cholecalciferol supplementation and pretreatment. B, The VAPS was not different in all groups between after cholecalciferol supplementation and pretreatment. C, The tear breakup test was increased in the IM group after cholecalciferol supplementation compared with pretreatment ($P < 0.001$, Wilcoxon rank test), whereas that in the none group and oral group was not different between after cholecalciferol supplementation and pretreatment. D and E, Tear secretion and CFSS were not different in all groups between after cholecalciferol supplementation and pretreatment. F, Lid margin hyperemia was decreased in the IM group after cholecalciferol supplementation compared with pretreatment ($P < 0.001$, Wilcoxon rank test), whereas that in the none group and oral group was not different between after cholecalciferol supplementation and pretreatment. *Statistical significant compared by the Wilcoxon rank test.

TABLE 3. Response to Topical Application of Carbomer-Based Lipid-Containing Artificial Tear and Hyaluronate After Vitamin D Supplementation

	None Group			IM Group			Oral Group		
	Before	After 2 wk	*P*	Before Cholecalciferol Supplementation	After Cholecalciferol Supplementation	*P*	Before Cholecalciferol Supplementation	After Cholecalciferol Supplementation	*P*
OSDI score	26.1 ± 20.8	19.4 ± 14.7	0.225	33.2 ± 23.2	28.5 ± 21.9	0.009*	50.5 ± 15.9	43.2 ± 12.9	0.715
VAPS score	1.6 ± 2.3	0.5 ± 1.1	1.000	2.2 ± 2.3	1.9 ± 1.9	0.356	2.5 ± 2.3	2.25 ± 2.2	1.000
TBUT (s)	5.2 ± 3.1	6.3 ± 4.0	0.066	3.5 ± 1.9	6.0 ± 2.5	<0.001*	3.7 ± 1.5	7.0 ± 2.0	0.068
CFSS	0.5 ± 0.8	0.3 ± 0.7	0.180	0.4 ± 0.6	0.4 ± 0.5	0.385	0.3 ± 0.8	0.0 ± 0.0	1.000
Lid hyperemia	1.9 ± 0.8	1.5 ± 0.9	0.257	2.2 ± 0.7	1.2 ± 0.8	<0.001*	1.3 ± 1.0	1.3 ± 0.5	1.000
Tear secretion test (mm)	6.6 ± 5.1	5.0 ± 2.1	0.336	8.7 ± 7.1	9.9 ± 7.4	0.332	14.7 ± 14.8	15.0 ± 13.4	0.715

*$P < 0.05$ by the Wilcoxon rank test.

In this study, OSDI score, TBUT, and lid margin hyperemia in response to topical CLAT and HU were improved only in the IM group after cholecalciferol supplementation. High-dose IM cholecalciferol has been reported to be more effective to increase serum 25HD levels compared with oral supplementation.[21] IM injection of 200,000 IU cholecalciferol elevates significantly the serum 25HD levels by around 15 ng/mL,[22] whereas oral supplementation of 2000 IU cholecalciferol may be insufficient to elevate the serum 25HD levels.[23] In addition, the patients in the oral group might determine the oral supplementation because their ocular symptoms were not severe to endure.

Although the interaction between HU or CLAT application and vitamin D has not been reported, there are several indications that vitamin D may act in synergy with HU or CLAT application. Tear osmolarity and instability is a main mechanism of DED.[24] Vitamin D decreases tear osmolarity and increased tear stability.[25,26] Vitamin D stimulates phospholipid synthesis and surfactant release.[9,27] The inner, polar phospholipids are bound to protein lipocalins within an aqueous layer that interacts with hydrophobic molecules and contributes to tear viscosity.[28] Surfactants decrease surface tension, contribute to the host defense mechanism, and regulate innate immunity and inflammation.[13] Surfactant proteins are involved in the incidence of several ocular surface diseases.[4,29] Furthermore, the tear film components of healthy individuals are in equilibrium; therefore, the influence of vitamin D on the ocular surface may alleviate the symptoms and signs of DED. Vitamin D enhances the corneal epithelial barrier functions[30] and promotes keratinocyte differentiation and proliferation.[31] In human cells, vitamin D has been reported to repress the responses of both Th_1- and Th_2-type cells[32]; this is significant as DED is characterized by inflammation of the ocular surface.[33,34] Thus, vitamin D supplementation may be able to harmonize the components of the tear film, through production of surfactants, and to reduce ocular surface inflammation. Vitamin D promotes production of surfactants, which is a mixture of lipids and proteins and enough ambiphilic to incorporate the lipid component of CLAT to the aqueous layer of tears. In addition, vitamin D contributes to absorption of calcium in intestine,[8] and calcium plays an essential role in fluid secretion in salivary glands and lacrimal glands.[35] Calcium ointment has been reported to improve DED symptoms.[36] The influence of Vitamin D on the ocular surface may relieve the symptoms and signs of patients with DED. Vitamin D may contribute to keep the tear film and ocular surface smooth and healthy, as well as in reducing inflammation.

This study is a retrospective cohort study, which is the same methodology as for the prospective study except for backward. The limitation of this study is that the number of oral vitamin D supplementation groups is small. Another limitation of this study is that the vitamin D levels were not measured after vitamin D supplementation. In this study, the patients were self-assigned to the treatment group. However, patients' charts were randomly selected in the retrospective study for generalization.[37] The further study is necessary to evaluate the vitamin D levels before and after supplementation.

In conclusion, the effect of topical lubricants depends on the serum 25HD levels. Vitamin D supplementation enhanced the efficacy of topical treatment. Vitamin D supplementation can be used as potential adjuvant therapy for patients with DED.

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