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Original Article

The correlation between vitamin D deficiency with tear film break-up time in patients with dry eye syndrome

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Abstract

Dry eye syndrome (DES) is a chronic inflammatory disorder of the ocular surface. Vitamin D (V-D) is a multifunctional hormone, and its roles are calcium metabolism and bone health, it is anti-inflammatory, has immune-regulatory properties and has been suggested to be a contributory factor in DES. The study aimed to determine the correlation between vitamin D deficiency and the magnitude of dry eye syndrome. A prospective comparison study was conducted from October 2022 to May 2023, enrolling 100 (200 eyes) cases of dry eye symptoms. Patients were subjected to the following examinations: slit-lamp examination, non-contact tonometry, fundus examination with 90D as a routine examination, and TBUT. Blood collected as five ml of venous blood drawn from them. The kit used is Elecsys Vitamin D kit. Tear film break-up time (TBUT) values (7 seconds) and (8 seconds) were more prevalent at 35% and 37%, respectively. V-D (ng/ml) concentration was deficient in 32%. V-D deficient was found to be more in TBUT value (5 seconds) as (21%), with a high statistically significant difference ($p<0.0001$). To the best of our knowledge, this is the first study to determine the association of V-D levels in patients with DES. Female gender, greater BMI, and elderly are the strongest risk factors for DES. Patients with dry eye symptoms have shorter TBUT values. The higher the V-D deficiency, the shorter the TBUT values, and the more severe the DES. V-D deficiency is a strong predictor for the development of DES. The V-D has a proportional relationship with TBUT values.

Keywords: dry eye syndrome, TBUT, Vitamin D deficiency, Vitamin D kit.

Introduction

Dry eye syndrome is a chronic inflammatory disorder of the ocular surface brought about by hyper-osmolarity of tear [1–4]. A recent international workshop (DEWS 2007a) provided the following definition: it is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with damage to the ocular surface. Another, dry eye syndrome (DES) or keratoconjunctivitis sicca (KCS) defined as a group of disorders that affect various components of LFU, resulting in the dysfunction of the ocular tear film and/or the integrity of the ocular surface [5, 6].

Internationally, DES is a common and chronic disease. Individuals with DE are 2–3 times more likely to report problems with reading, performing professional work, computer use, watching TV and daytime or nighttime driving and those with refractive errors are unsuitable for refractive surgery and are limited in their ability to wear contact lenses or use cosmetics [7, 8]. It is a significant problem for up to 35% of the population, commonly in women, 66%. Regarding population-based studies, the estimated data showed that the prevalence varied widely from 3.5% to 68.3% [9]. The prevalence data revealed a higher DE rate in studies of Asian populations compared with reports of Caucasian studies [9, 10].

Chan *et al.* suggested the order of tests in 2015 as symptom questionnaire, clinical history, tear film break-up time (TFBUT) with fluorescein, corneal staining with fluorescein, Schirmer 1, lid margin examination and meibomian gland expression for detection of DES [11].

Knowledge about the pathophysiology of DE has advanced from the simple concepts of aqueous deficiency or lipid contents to include concepts of tear hyper-osmolarity, ocular surface inflammation and neurosensory abnormalities [12, 13]. Recently, different studies concluded that DE is a chronic inflammatory disease with many properties of autoimmune disease [14–16].

Vitamin D (V-D) is a multifunctional hormone, and its roles are calcium metabolism and bone health, as well as anti-inflammatory and immune-regulatory properties [17].

Authors have determined that vitamin D receptor (VDR) expression is in the majority of immune cells [18], which regulate several genes involved in inflammation, immunity, cellular proliferation, differentiation, and apoptosis [19, 20]. Previously, an association of vitamin D deficiency (VDD) with chronic illness, autoimmune disease and cancer estimated well [21].

Recently, VDD has been suggested to be a contributory factor in DE [22]. Researchers presumed that cases with VDD are more prone to DE [15, 23–27]. Other studies were unable to demonstrate such association between DE and a low V-D level [28, 29].

Shetty *et al.* [29], Askari *et al.* [30], Jeon *et al.* [27], Meng *et al.* [31], and Khamar *et al.*, [32] showed that patients had a mean serum V-D level significantly lower than that in control groups (p=0.002).

By systematic review and meta-analysis, Liu and colleagues demonstrated an association between the serum V-D level and DE syndrome. They found the serum V-D level was lower in patients with DES than in healthy controls and VDD was associated with an elevating in symptoms of DE and a decline in reflex tear production [15], indicating that VDD is a potential risk factor for developed DES. Others concluded that V-D has anti-inflammatory and immune-regulatory features, and its deficiency resulted in inflammatory or immune-mediated dryness of the eyes [17, 33].

VDD is prescribed for various health problems. Recently, it has been linked to musculoskeletal disorders, mental health [34], cancers [35–37], diabetes mellitus and metabolic syndrome [38], cardiovascular diseases [39, 40], and autoimmune disorders [41], and eye diseases [42, 43].

The study aims to determine the correlation of vitamin D deficiency with the magnitude of dry eye syndrome.

Material and methods

Study design and setting

A prospective comparison study was carried out from October 2022 to May 2023. This study enrolled 100 (200 eyes) cases of DE symptoms. The study was conducted at the Ophthalmic Consultant Clinic in Al-Basrah Teaching Hospital and the Ophthalmic outpatient clinic in Al-Sadi Private Hospital.

Inclusion criteria

a. Both genders suffer from DE symptoms;
b. Cooperative patients;
c. Aged 16 to 60 years.

Exclusion criteria

a. The age under 16-year-old and older than 60-year-old;
b. History of corneal surgery, corneal ulcer and corneal infections;
c. Ocular diseases (glaucoma or uveitis, ocular allergy, pterygium or blepharitis);
d. Hormonal replacement therapy and Postmenopausal women;
e. DM, HTN, malignancies, endocrine and metabolic disorders and thyroid disease;
f. Laser vision correction;
g. Smoker;
h. Current contact lens user;
i. Treatment cause DE (antihistamines, antidepressants, birth control pills, decongestants, gabapentin, sildenafil citrate, anticholinergic drugs, blood pressure medications, postmenopausal estrogen therapy, beta-blockers, antispasmodics and diuretics);
j. Eyelids disease;
k. Nasolacrimal pathway disorders;
l. Pregnancy;
m. General malnutrition;
n. Patients who were unable to comprehend the questionnaire;
o. Patients who were refusing to give consent for the study.

Data collection

All patient demographic data included age, gender, body height, body weight and BMI [44].

Ophthalmologic examination

Patients were subjected to the following examinations: slit-lamp examination, non-contact tonometry to measure intraocular pressure, fundus examination with 90D as a routine examination, and TBUT.

Examination of dry eye

Approximately 5µL (a drop) of normal saline was instilled into the strip, which was then shaken to remove extra liquid to minimize the volume of fluorescein fluid. Afterward, the strip was gently touched with the inferior temporal bulbar conjunctiva for 1–2 seconds. Participants were asked to blink three times naturally to facilitate the uniform distribution of fluorescein on the ocular surface. The time from the last blink of the eye to the first dry spot on the tear film was measured under a cobalt-blue filter. Three consecutive measurements were recorded with a time interval of 30 seconds. Two eyes were observed separately.

Tear film break-up time (TBUT) is a method for determining the stability of the tear film and checking EDE. In testing for TBUT, sodium fluorescein dye is added to the eye and the tear film is observed under the slit lamp while the patient avoids blinking until tiny dry spots develop. Generally, >10 seconds is considered normal, 5 to 10 seconds, marginal, and <5 seconds is considered low [45].

Blood sample

Blood patients had five ml of venous blood drawn from them. The blood is collected in a gel tube, and the serum is extracted quickly. For 10 minutes, the blood samples were centrifuged at 3000 rpm. The serum samples were then transferred into a new clean disposable plain tube to be utilized later.

Kits

The kit used in this study is the Elecsys Vitamin D kit (Roche).

Vitamin D measurement

Principle (Competition principle)

- 1st incubation: By incubating the sample (20 µL) with pretreatment reagent 1 (V-D binding protein) and 2(25-hydroxyvitamin D), bound 25-hydroxy-vitamin D is released from the Vitamin D Binding Protein (VDBP).
- 2nd incubation: The processed sample is incubated with the ruthenium labeled VDBP, which forms a complex between the 25 hydroxyvitamin D and the ruthenylated VDBP. Cross-reactivity to the V-D metabolite 24,25 dihydroxyvitamin D is inhibited by a specific unlabeled antibody that binds to it in the sample.
- 3rd incubation: After adding streptavidin-coated microparticles and 25 biotin-labeled hydroxyvitamin D, unbound ruthenylated VDBP are busy. Combining biotin and streptavidin, a complex of vitamin D-binding protein ruthenylated and 25-hydroxyvitamin D biotinylated binds to the solid phase.
- Microparticles are magnetically caught on the electrode surface after the reaction mixture is drawn into the measurement cell. Then, ProCell/ProCell M is used to remove unbound components. The photochemical emission resulting from the application of a voltage to the electrode is detected by a photomultiplier.
- Results are obtained by a calibration curve, which is a 2-point calibration method and a master curve that is provided through the bar-code or bar-code of the reagent [46].

Statistical analysis

Statistical analysis was performed using SPSS v24 (IBM Inc., Chicago, IL, USA). Descriptive statistics consist of numbers, and percentages were measured. Mean, median and SD for categorical data were calculated. Pearson's Chi-square test measured an association between DES clinical and biochemical parameters. Two–way ANOVA analysis was used to describe the association between groups. A two-sided P value of less than 0.05 was considered statistically significant.

Results

Basic characteristics, vitamin D of patients

Table 1 shows the basic characteristics and V-D of patients. Males were (30%) whereas females were (70%). The higher percentage of age distribution belonged to the group (51–60 years) as (56%). A greater percentage of patients were included in the BMI group (25–29 kg/m^2) as (57%).

Patient percentages according to Tear film break-up time (TBUT) values (7 seconds) and (8 seconds) were

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Table 1: Basic characteristics, Vitamin D and immune-anti-genes of patients in the study (N=100).

Parameters	No.	%	P-value
Gender			
Male	30	30.0	0.99
Female	70	70.0	
Age groups (years)			
11–20	6	6.0	
21–30	1	1.0	
31–40	9	9.0	<0.0001
41–50	23	23.0	
51–60	56	56.0	
>60	5	5.0	
Mean±SD		**49.65±11.834**	
Median		**54**	
Range		**14–65**	
BMI (kg/m²)			
18–21	3	3.0	
21–25	33	33.0	<0.0001
25–29	57	57.0	
>29	7	7.0	
Mean±SD		**25.46±2.575**	
Median		**26.1**	
Range		**19.4-31.0**	
Patient percentage according to Tear film break-up time (TBUT)			
5 seconds	23	21.0	
6 seconds	5	5.0	<0.0001
7 seconds	35	35.0	
8 seconds	37	37.0	
Mean±SD		**7.04±0.89**	
Median		**7**	
Mode		**8**	
Patient percentage according to Questionnaire score			
1	65	65.0	
2	23	23.0	0.037
3	12	12.0	
Mode		**2**	
Patient percentage according to Vitamin D (ng/ml)			
Deficiency (<20)	32	32.0	
Insufficient (20–30)	45	45.0	0.011
Normal (>30)	23	23.0	
Mean±SD		**26.99±15.72**	
Median		**23.45**	
Range		**5.2–70**	

Note: BMI – body mass index; SD – standard deviation.

reported to be more prevalent at 35% and 37%, respectively. In addition, TBUT value (5 seconds) was recorded in 23% of patients. The last one was (6 seconds) in 5% (p<0.0001).

Patient percentages according to V-D (ng/ml) concentration were deficient in 32%, insufficient in 45% and normal in 23%, with a high significant difference (p=0.011).

The comparison of Vitamin D levels in dry eye patients concerning TBUT

Among DE patients, the V-D deficient was found to be more in TBUT value (5 seconds) in 21%, followed by value (7 seconds) in 7%, value (6 seconds) in 3% and value (8 seconds) in 1%, with a high statistical significant difference (p<0.0001) (Table 2 and Figure 1).

The comparison of clinical and biochemical parameters of dry eye patients according to the severity of the disease

Table 3 shows the comparison of DE patients' clinical and biochemical parameters according to the disease's severity.

In the severe group of DE syndrome, the mean age was younger (35.75±20.79 years) than in the mild group (50.48±9.72 years) and moderate group (51.92±8.252 years), with a high statistical significant difference (p<0.0001).

Regarding gender distribution and disease severity, there was no significant difference among males and females (p=0.44). Among BMI and severity, there was no significant difference in females (p=0.842).

Regarding TBUT groups, there was a high statistically significant difference among groups (p<0.0001). Severe disease contributed to only TBUT (5 seconds) and (6 seconds), while mild disease contributed to only TBUT (7 seconds) and (8 seconds).

In the severe form of the disease, there was a high statistically significant difference among the mean V-D concentration (p<0.0001). The concentration of V-D for the severe group was very lower than in mild and moderate groups (10.7±5.05 ng/ml), (43.89±18.66 ng/ml) and (24.03±9.84 ng/ml), respectively.

Discussion

Dry eye syndrome (DES) is a multifactorial illness of the tears and ocular surface of the eye, causing discomfort, visual disturbances and tear film instability [2–5, 47–58].

Vitamin D deficiency (VDD) is a cause of DE symptoms that lead to rising ocular surface inflammation and decreased TBUT, rising eyelid margin hyperemia, and dropping tear secretion [56]. The mechanisms or the roles beyond it make the epithelium of the corneal cell barrier function better via gap and tight junction regulation [54]. Also, it induces IL10 production and the dropping of inflammatory cytokines/factors like IL1-6, C-reactive protein and TNF alpha [48]. It plays a role in suppressing the Th1 and Th2 cell proliferation by raising antioxidant cytokines in the tear system to reduce the inflammatory process [58, 59]. Furthermore, V-D presents as a cathelicidin production indicator, conjunctival and corneal wound healing promoter, tear osmolarity reducer, and tear film stability improver [58–63].

In this study, males were (30%) whereas females were (70%). This was contested by Nanda *et al.* [47], who studied patients with DES with a male-to-female ratio of (1:1) and also contested by Watts *et al.* [48], who studied 90 patients, 52 were females and 38 were males. This could be because DES differs according to latitude, gender, ethnicity and culture [49]. As a result, DES is more commonly present in females than in males. This is in accordance with a report from TFOS DEWS II by Craig *et al.* [50], which also states that DES is more common in females.

Banik *et al.* [51] reported a greater prevalence of females with DE than males, similar to the data observed in the study of Sahai and Malik [52]. These differences in sex distribution could be attributed to the effects of hypothalamic-pituitary hormones, sex steroids, glucocorticoids, insulin-like growth factor-1, insulin and thyroid hormones [48, 49].

Table 2: The percentage of patients by the comparison of Vitamin D level concerning TBUT (N=100).

Vitamin D (ng/ml)	TBUT (seconds) / No. (%)				P-value*
	5	6	7	8	
Deficiency	21 (21.0)	3 (3.0)	7 (7.0)	1 (1.0)	**<0.0001**

Note: * – ANOVA (two-sided).

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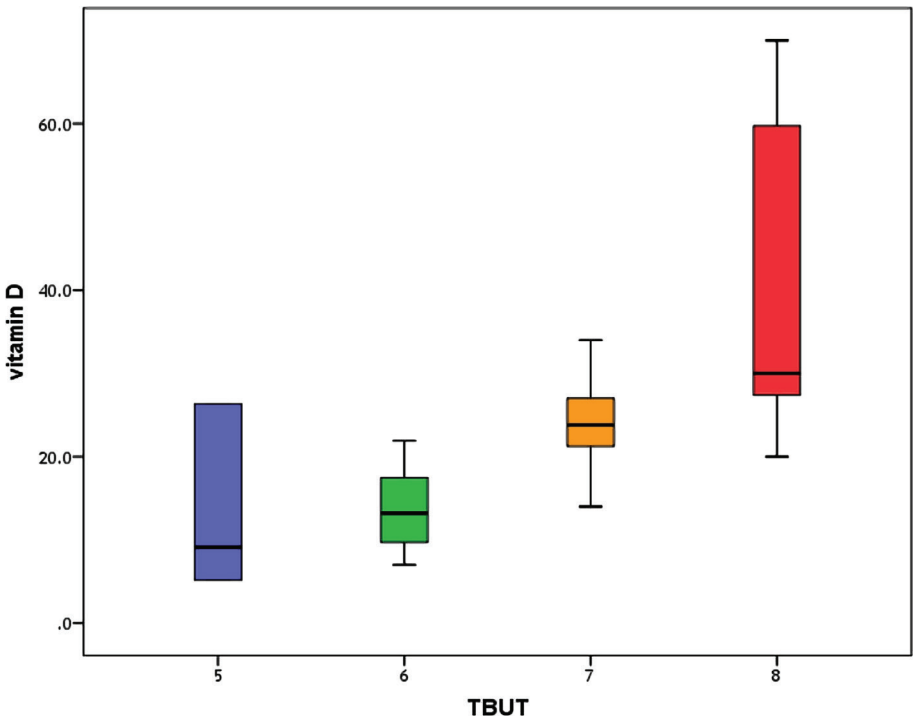

Figure 1: The comparison of Vitamin D level in dry eye patients in relation to TBUT.

In terms of the DE severity, there was no significant difference among males and females (p=0.44) in this study, while Watts *et al.* [48] concluded that female gender represented a strong risk factor for DES.

The higher percentage of age distribution belonged to the group (51-60 years) at (56%), with a mean age of 49.65. This was slightly higher than the mean age of the study by Nanda *et al.* [47]. Watts *et al.* [48] reported that the mean age of (44.87±4.77 years) and the maximum number of participants was in the age group (31–50 years) which was younger than our patients.

Banik *et al.* [51] reported an age group peak of (31–40 years) was the same as the age after >70 years age group of DE cases. Also, Sahai and Malik [52] reported the same findings.

In the severe group of DES in the present study, the mean age was younger (35.75±20.79 years) than in mild and moderate groups, with a high statistical significant difference (p<0.0001). This is consistent with many studies in India by Nanda *et al.* [47] and Jain *et al.* [58], in Türkiye by Yildirim *et al.* [62] and Kurtul *et al.* [63] and in Iran by Hashemi *et al.* [64] while disagreeing

Table 3: The comparison of dry eye patients' clinical and biochemical parameters according to the disease's severity.

Parameters		Dry eye severity (mean±SD)			P-value*
		Mild	Moderate	Severe	
Age		50.48±9.72	51.92±8.252	35.75±20.79	**<0.0001**
Gender	Male	17 (73.9%)	43 (66.2%)	10 (83.3%)	0.44
	Female	6 (26.1%)	22 (33.8%)	2 (16.7%)	
BMI		25.81±1.96	25.17±2.63	26.3±3.17	0.842
TBUT	5	-	2 (3.1%)	3 (25%)	**<0.0001**
	6	-	14 (21.5%)	9 (75%)	
	7	4 (17.4%)	31 (47.7%)	-	
	8	19 (82.6%)	18 (27.7%)	-	
Vitamin D (ng/ml)		43.89±18.66	24.03±9.84	10.7±5.05	**<0.0001**

Note: BMI – body mass index; TBUT – Tear film break-up time; SD – standard deviation.

with other studies such as Shah and Jani [65], Jin *et al.* [66] and Demirci *et al.* [67].

The greater percentage of patients belonged to the BMI group (25–29 kg/m^2) (57%), with a higher statistically significant difference than those with normal weight (33%) and obese (7%) (p<0.0001). There was no significant difference among BMI and severity (p=0.842). In the literature of our previous studies, we found no relation between BMI and DES [47–52].

In the present study, the TBUT values (7 seconds) and (8 seconds) were reported as more prevalent, with a mean of 7.04 seconds. Meanwhile, the mean TBUT in the Nanda *et al.* [47] study was 8.7 seconds.

Regarding TBUT groups, there was a high statistically significant difference among groups (p<0.0001). Severe disease contributed to only TBUT (5 seconds) and (6 seconds). These findings agree with Nanda *et al.* [47], Yildirim *et al.* [62] and Kurtul *et al.* [63] and disagree with Shah and Jani [65] and Demirci *et al.* [67].

In 2020, Watts *et al.* [48] calculated TBUT by group-wise comparison of mean TBUT before treatment with V-D. They found no statistically significant between any pair of groups.

Among DE patients, the V-D deficiency was found to be more in TBUT value (5 seconds) in 21%, with a high statistically significant difference (p<0.0001). Nanda *et al.* found that mean TBUT was grossly decreased in patients with V-D deficiency [47].

Galor et al, found that there must be an increase in V-D supplements to decrease DES symptoms. Moreover, showed that the TBUT, eyelid margins hyperemia, DE severity and tear secretion test improved after V-D supplementation compared to pretreatment status [53].

Jin *et al.* stated that improved TBUT and better tear secretion correlated with serum V-D levels [66]. They conducted a retrospective observational study by measuring TBUT. The mean age was (53.38±13.69 years). Mean serum 25-(OH) D level was (14.41±5.98 ng/ml). TBUT positively correlated with serum 25-(OH) D levels (P<0.001). TBUT was shorter in the V-D deficient group compared to the sufficient group (P=0.022), which resamples our study findings.

Kurtul *et al.* [63] concluded that TBUT was lower in the V-D deficient group (p=0.01 and 0.007), but Jee *et al.* [17] did not find an association between serum V-D levels and TBUT of DES (p>0.05).

Yildirim and colleagues found that low TBUT scores were mostly documented in VDD [62]. They reported that DE and tear function impairment in VDD, thus indicating a protective role of 25-(OH) D in the DES by enhancing tear film parameters improvement and declining the inflammatory process of the ocular surface. Furthermore, they recommended that every case of VDD should be evaluated for DES status [62].

Rolando and Barabino, in 2023, concluded that possibly because of V-D's positive effect in modulating the inflammatory and immune responses, systemic V-D supplementation should be considered as a potentially effective therapeutic strategy, especially but not only for cases affected by DES. They have been considering the V-D hormonal roles and suggested pharmacological doses of V-D (>10,000 IU) that might be required to restore the severe V-D deficiency status correlated with eye diseases [61].

In the current study, patients' percentages according to V-D (ng/ml) concentration were deficient in 32%, insufficient in 45% and normal in 23%, with a mean of (26.99±15.72 ng/ml). Our findings were much greater than the findings of Nanda *et al.* [47] study in Cuttack City, India. They found that the mean 25-(OH) D level in DE cases was 14.14±5.98 ng/ml, and regarding the incidence of VDD, they reported that 40% of DE cases had deficiency [47]. In addition, Watts *et al.* [48] found no statistical difference in the mean serum V-D level before treatment in all groups (P=0.258).

Furthermore, Yoon *et al.* [54] found that the mean serum 25-(OH) D levels of subjects with DES were (16.90±6.0 ng/ml). They suggested that inadequate sunlight exposure time and low serum 25-(OH) D level were found to be the main etiological factors for the syndrome.

In 2018, a study by Yang *et al.* [56] found that V-D supplement increased the V-D levels, thus improving DES, tear quality and ocular surface conditions.

In fact, we found a high statistically significant difference among the mean V-D concentration in the severe form of the DE disease (p<0.0001). The concentration of V-D for the severe group was much lower than in mild and moderate groups at (10.7±5.05 ng/ml), (43.89±18.66 ng/ml) and (24.03±9.84 ng/ml), respectively. Almost every study discussed this problem showed the same results [47–57, 59, 61, 67–70]. Thus, the V-D has a proportional relationship with DE TBUT values.

VDD has been recorded to be associated with immune disorders, and vitamin derivatives and analogs have been used to manage topical or systemic treatment of immune-mediated diseases [71]. In addition, it has been reported to play a role in wound healing response, which is important for host protection and the healing process of ocular surface damage [72].

In 2023, a randomized controlled trial by Najjaran *et al.* [73] concluded that V-D supplementation as an

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adjuvant to routine DE disease treatment improves ocular surface hemostasis parameters (i.e., TBUT), results in better tear stability and a more improved tear osmolarity in cases with vitamin deficiency. In contrast, Jee *et al.* [68] studied more than 16000 subjects and found no correlation between DE and V-D levels, plus vitamin levels were the same in the diseased and non-diseased eye groups.

Conclusion

To the best of our knowledge, this is the first time study to determine the association of V-D levels within patients with DES. Patients with DE symptoms have shorter Tear film break-up time (TBUT) values. The higher the V-D deficiency, the shorter the TBUT values, and the more severe the DES. V-D deficiency is a strong predictor for the development of DES. The V-D has a proportional relationship with TBUT values.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The approval for this study was obtained from the College of Pharmacy, University of Basrah (approval ID: #351 on 23/01/2023).

Consent to participate

Written informed consent was obtained from all participants in this study.

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