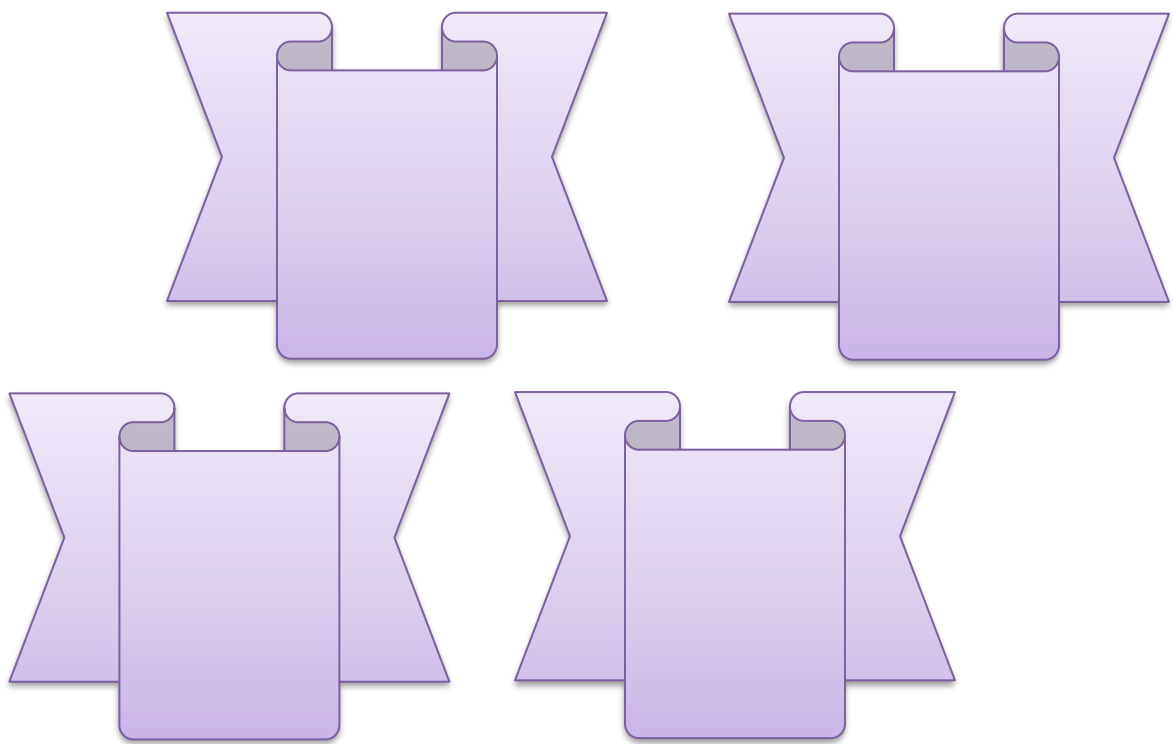


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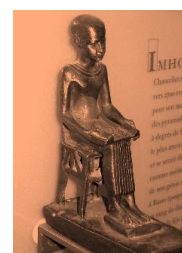


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Original Article

Effect of Vitamin D Deficiency and Its Supplementation on Both Conjunctival Impression Cytology and Tear-Film Changes

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ABSTRACT

Article information

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Background: Deficiency of vitamin-D led to dry eye disease and its supplementations improves this condition.

Aim of the work: This study aims to assess conjunctival impression cytology [CIC] and tear-film parameters in patients of vitamin-D deficiency [VDD], as well as how vitamin-D supplementation affects them.

Patients and Methods: This prospective study included 20 participants [40 eyes] with serum vitamin D levels below normal, 20 control subjects [40 eyes] with normal serum vitamin D levels, and 20 participants [40 eyes] after treatment with supplementation of vitamin D. A thorough ophthalmological examination, tear break-up time [TBUT], Schirmer II test, and CIC tests were performed on each subject.

Results: Regarding mean serum vitamin D levels [$p < 0.001$], TBUT scores [$p < 0.001$], and Schirmer II values [$p < 0.001$], there was a statistically significant difference among the groups. 65% of patients in the deficient group showed grades 2 or 3 impression cytology, compared to 30% in the control group and 25% in the post-treatment group, according to the Nelson staging system [$p < 0.001$].

Conclusion: Our findings show that VDD causes dry eye, resulting in decreased tear film parameters, conjunctival squamous metaplasia, and loss of goblet cell, whereas vitamin D supplementation improves parameters of the tear film and impression cytology.

Keywords: Dry eye; Conjunctival impression cytology; Tear break-up time; Vitamin D deficiency; Schirmer II test.



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INTRODUCTION

A prohormone that is fat-soluble is vitamin-D [VD]. It plays a significant part in a number of biological processes in addition to its classical role in regulating calcium levels. It is produced in the body following sun exposure and has an anti-inflammatory, immunomodulatory, anti-neoplastic, and antioxidant effects ^[1].

It has been suggested that VDD contributes to dry eye disease [DED]. Since main cause of DED is inflammation, vitamin D works by reducing pro-inflammatory cytokines and raising anti-inflammatory cytokines in tears, which improves DED signs and symptoms ^[2].

DED, a multifunctional ocular surface disease marked by a loss of homeostasis of the tear film, is characterized by inflammation and injury to the ocular surface, abnormalities of neurosensory system, and tear film instability and hyperosmolarity ^[3]. Symptoms include pain, a feeling of a foreign body, itching, burning, or light sensitivity while signs include unstable tear films, and ocular surface inflammation ^[4]. Dry eye symptoms are caused upon with vitamin D deficiency ^[5]. Schirmer test [ST] can help to evaluate tear volume, whereas TBUT can evaluate tear film stability ^[6,7].

For evaluating the ocular surface, CIC is a very helpful, generally non-invasive as well as simple method. Cells are extracted from the surface of the conjunctiva by adhering to a substrate, often a filter made of a microporous synthetic substance. These cells are processed for the purpose of cytological examination. The amount of squamous metaplasia on the conjunctival surface can be used to determine the severity of the disease ^[8].

The most important factor for evaluating the ocular surface is that the number of goblet cells of the conjunctiva is reduced, so using therapeutic methods to enhance the goblet cell count and control inflammation on the ocular surface ^[9]. Treatment for DED in VDD patients that improves tear production, reduces instability of the tear, and reduces ocular surface inflammation and eyelid edge inflammation is vitamin D supplementation ^[10].

This study's purpose is to evaluate CIC and tear-film parameters in VDD patients, as well as how vitamin D treatment affects them.

PATIENTS AND METHODS

A prospective non-randomized comparative case-control study was carried out between November 2022 and February 2023 in the Histo-pathology and Ophthalmology Departments of the AL-Zahraa University Hospital in Egypt. In this study, 60 volunteers between the ages of 20 and 52 contributed 120 eyeballs. Twenty participants in group [A] had normal serum levels of vitamin-D; twenty participants in group [B] had VDD; and twenty participants in group [C] had supplementation for VDD.

Inclusion Criteria: Age over 18 years and patients with vitamin D serum level below normal [< 20 ng/mL].

Exclusion Criteria: Ocular diseases such glaucoma, uveitis, ocular allergies, ocular infections, and problems of the cornea were excluded. Antidepressants, birth control pills, blood pressure drugs, beta-blockers, and diuretics were among the concomitant medications that were excluded from being administered to patients because they could result in dry eyes. illnesses of the nasolacrimal pathway or disorders of the lid. wearers of lenses. dryness following ocular surgery. A patient who is uncooperative or mentally unstable. Patients who rejected providing their consent for the study.

Ethical consideration: Each patient who participated in the trial was given an explanation of the study before providing their written informed consent. The study complies with Al-Azhar Medical Research Ethics Committee's ethical code.

Using the ELISA method, the serum 25 [OH] VD levels were measured, and levels below 20 ng/mL were considered deficient.

All subjects underwent a thorough ophthalmological examination, which included measuring visual acuity [VA] using a Landolt C chart that was transformed to decimal form for statistical analysis, an anterior segment slit-lamp examination, and a posterior segment fundus inspection using a Volk 90D lens.

The same ophthalmologist handled the collection of samples for CIC, all Schirmer II tests, and TBUT tests. The same pathologist assessed each impression cytology specimen.

Schirmer II test: measures the amount of wetness of a sterile paper strip by inserting it for five minutes into the lower eyelid where it connects with the ocular surface following local anesthetic administration. An abnormal value is ≤ 5 mm.

Tear break-up time [TBUT] test: The lower conjunctival sac is loaded with fluorescein dye [one drop]. The time it takes for the first dry spot to form on the corneal surface after last blinking is then timed in seconds. Cobalt blue light is used to study the film of the tear under a slit lamp biomicroscope. Pathological values are those with values less than 10s.

Conjunctival impression cytology [CIC]: A cellulose acetate filter sheet [3x5 mm, pore size: 0.2 μ m] is applied to the temporally bulbar conjunctiva for 10 seconds after topical anesthetic with proparacaine has been applied. The strip is then placed on a clear glass plate and fixed for two hours in a 95% ethyl alcohol solution before being stained with hematoxylin and eosin [H&E]. According to Nelson's procedure, evaluations were carried out at the pathology department using a light microscope. The technique can identify four phases [0–3], with stages 0 and 1 [nucleus/cytoplasm ratios of 1:2 and 1:3 respectively] being normal and stages 2 and 3 [ratios of 1:4 and 1:6] being changed.

Statistical analysis: Utilizing SPSS version 24 [Statistical Program for Social Science], the data were evaluated. Quantitative data was presented as mean \pm SD. To express qualitative data, frequency and percentage were utilized. By dividing the sum of values by the total number of values, the mean [average] of a set of discrete numbers is determined. The standard deviation serves as a measurement for the dispersion of a set of numbers [SD]. A low SD indicates that the values are close to the established mean, compared to a high SD, which indicates that the values are distributed over a wider range. When comparing more than two means, use a one-way analysis of variance [ANOVA] [for normally distributed data] [F]. The Kruskal-Willis test [KW], which is used when comparing more than two means [for abnormally distributed data]. It was decided to compare non-parametric data using the Chi-square test [X^2]. For multiple comparisons between distinct variables, the post-hoc test was used. Pearson correlation coefficient [r] was used to correlated different variables. P-values < 0.05 , was regarded as significant.

RESULTS

Regarding visual acuity, no statistically significant difference was present [p-value = 0.874] among the groups under study in groups A, B, and C, it was 0.78 ± 0.27 , 0.82 ± 0.21 , and 0.82 ± 0.21 respectively.

In table [1], there was no gender or age difference among the studied groups [p = 1.00 and p = 0.087, respectively], however, vitamin D levels in group B were considerably less than in groups A and C [p-value 0.001].

Table [2] demonstrates ocular surface tests and shows that there was a statistically significant [p-value = 0.003] rise in the percentage of abnormal Schirmer II test results between group B and groups A and C and a statistically significant [p-value < 0.001] decline in the mean of the Schirmer II test in group B when group B was in comparison to groups A and C, the percentage of abnormal TBUT increased with a statistically significant [p-value < 0.001] increase, while group B's mean TBUT score declined with a statistically significant [p-value < 0.001]. When compared with group A [12 eyes, 30%] and group C [10 eyes, 25%], there was a statistically significant [p-value = 0.002] higher percentage of late squamous metaplasia in group B [26 eyes, 65%].

Table [3] shows that in group A, a statistically [p-value < 0.001] significant negative [r = -0.65] correlation was found between vitamin D and CIC but no statistically significant [p-value = 0.531, 0.058 respectively] correlations between vitamin D and ST or TBUT.

While there was no statistically significant link between vitamin D and CIC [p-value = 0.109], there was a positive correlation between vitamin D and ST and TBUT in group B [r = 0.33 and 0.35, respectively], with statistical significance [p-values = 0.036 and 0.023, respectively].

In group C, there was a statistically significant, positive correlation between vitamin D and ST and TBUT [p-value = 0.011, p-value < 0.001] [r = 0.39, 0.57, respectively]. Vitamin D and CIC have a statistically significant [p-value < 0.001] negative correlation [r = - 0.83].

Histological grades of CIC are show in figures [1-4].

Table [1]: Comparisons between the studied groups as regards age, gender, and vitamin D level

		Groups						X ²	P-value
		Group A [20 pt, 40 eyes]		Group B [20 pt, 40 eyes]		Group C [20 pt, 40 eyes]			
Gender	Male	6	30%	6	30%	6	30%	0.1	1.0 NS
	Female	14	70%	14	70%	14	70%		
Age [years]	Mean	37.2		31.8		31.9		2.5	0.087 NS
	±SD	9.2		8.2		8.4			
Vitamin D level[ng/ml]	Mean	39.8		14.2		44.5		67.9	< 0.001 S
	±SD	10.3		3.7		10.7			

Table [2]: Ocular surface tests rates in the groups under study

		Groups						Test	P-value
		Group A [20 pt, 40 eyes]		Group B [20 pt, 40 eyes]		Group C [20 pt, 40 eyes]			
Schirmer II test	Normal	31	77.5%	20	50%	33	82.5%	X ² = 11.7	0.003 S
	Abnormal	9	22.5%	20	50%	7	17.5%		
Schirmer II test	Mean	9.35		5.4		9.5		F = 17.3	< 0.001 S
	±SD	4.04		2.7		3.8			
TBUT	Normal	35	87.5%	6	15%	28	70%	X ² = 46.8	< 0.001 S
	Abnormal	5	12.5%	34	85%	12	30%		
TBUT	Mean	12.3		7.9		11.3		KW = 17.3	< 0.001 S
	±SD	1.89		1.78		2.6			
CIC	Normal cytology	10	25%	4	10%	8	20%	X ² = 16.5	0.002 S
	Early squamous metaplasia	18	45%	10	25%	22	55%		
	Late squamous metaplasia	12	30%	26	65%	10	25%		

Table [3]: Vitamin D and other analyzed parameters were correlated across all studied groups

Vitamin D	Group A		Group B		Group C	
	r	p-value	r	p-value	r	p-value
Schirmer test	0.1	0.531 NS	0.33	0.036 S	0.39	0.011 S
TBUT	0.3	0.058 NS	0.35	0.023 S	0.57	< 0.001 S
CIC	- 0.65	< 0.001 S	- 0.25	0.109 NS	- 0.83	< 0.001 S

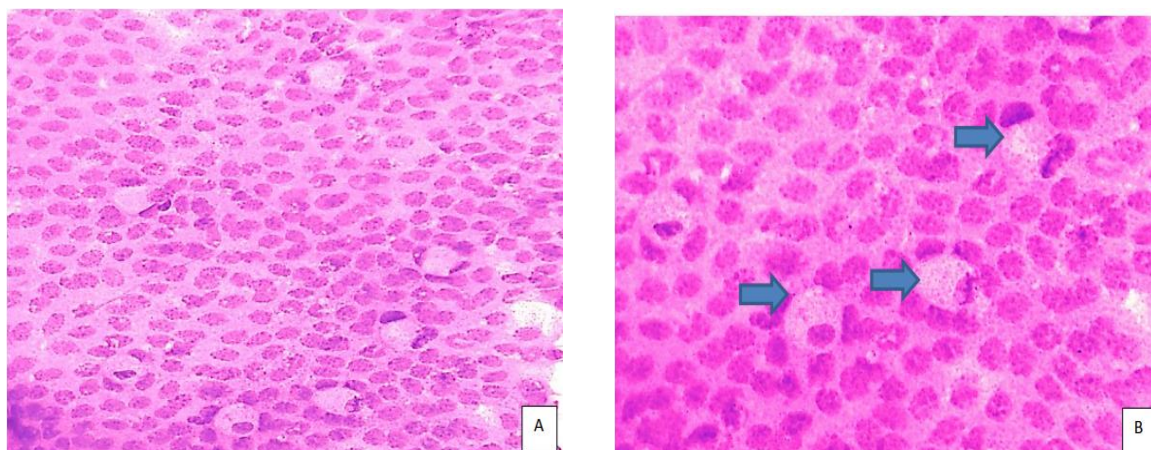


Figure [1]: Grade 0: sheets made up of small cuboidal cells with nucleocytoplasmic ratio 1:2 and abundant goblet cells [arrows]. Original magnification [A: 100, B: 200].

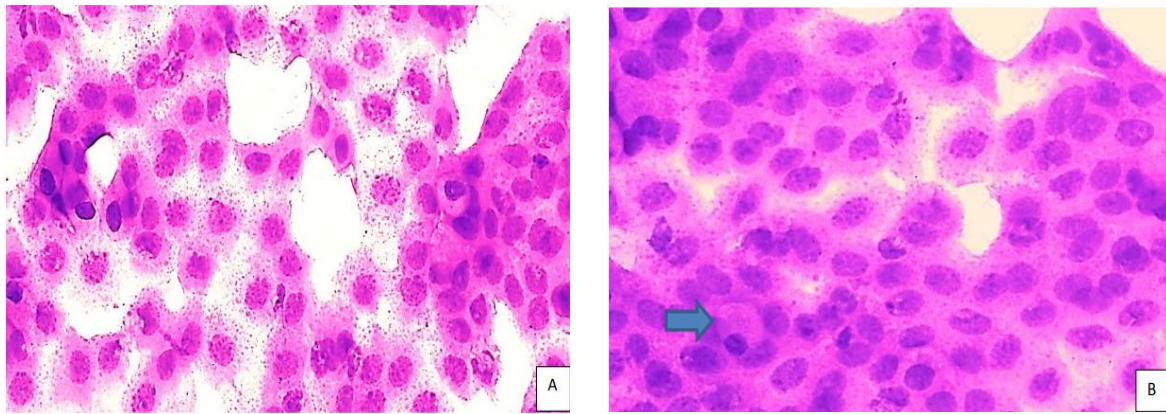


Figure [2]: Grade 1: sheets of larger polygonal cells with slightly decreased nucleocytoplasmic ratio 1:3 and decreased goblet cells. Original magnification [A: 100, B: 200]

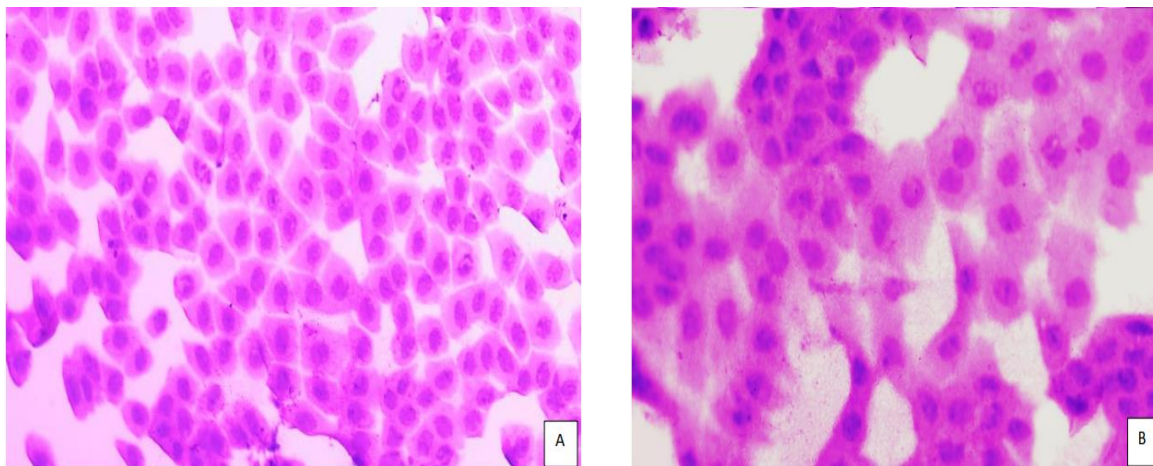


Figure [3]: Grade 2: Discohesive large polygonal cells with moderate decrease of nucleocytoplasmic ratio 1:4 and marked decrease of goblet cells. Original magnification [A: 100, B: 200].

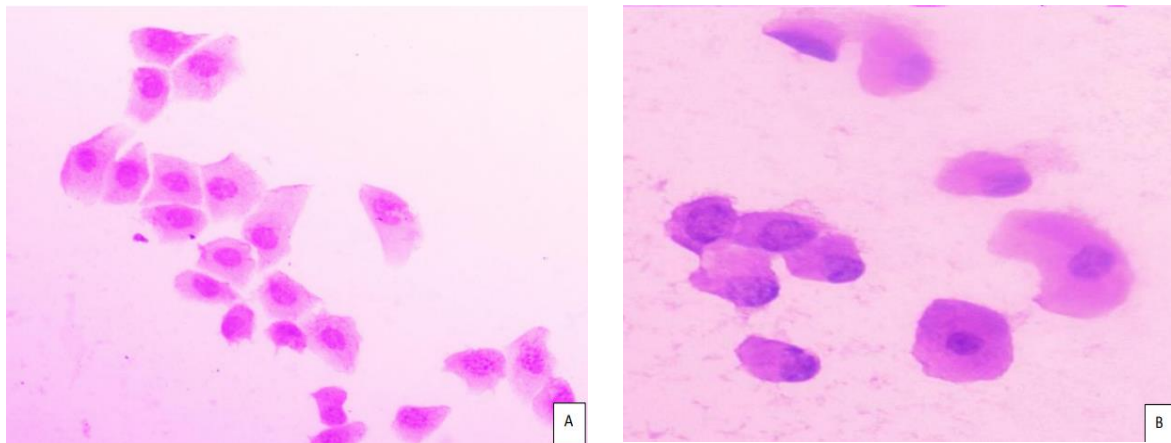


Figure [4]: Grade 3: large polygonal cells showing marked discohesion & marked decrease of nucleocytoplasmic ratio 1:6. Original magnification [A: 100, B: 200].

DISCUSSION

VDD can result in ocular surface inflammation and, eventually, DED. Vitamin D, on the other hand, may alleviate DED by inhibiting interleukin-6 [IL-6], the primary source of localized inflammation. Furthermore,

vitamin D can inhibit inflammatory cytokine production while encouraging the elimination of antioxidant cytokines in tears. The functions of the corneal epithelial barrier can be improved by vitamin D, which may improve eye conditions [11].

The "gold standard" method for identifying morphology of cells in human ocular surface cells is impression cytology [IC], which is a minimally invasive procedure [12].

Age and gender were not significantly different amongst the studied groups in our study [$p=0.087$ and $p=1.00$, respectively]. This result is supported by the study of Kurtul *et al.* [13].

Visual acuity measurements in all participant's eyes do not differ significantly from one another [p -value = 0.874]. This is supported by the outcomes of the study of Dikci *et al.* [9], which demonstrates that, the best corrected decimal visual acuity scores in all participants' eyes varied from 0.8 to 1.0 on the Snellen chart.

With a statistically significant [p -value < 0.001] difference between the mean vitamin D levels in the deficiency group [14.2 ± 3.7 ng/ml] and the control group [39.8 ± 10.3 ng/ml], respectively. There is agreement according to a study by Kurtul *et al.* [13], that found that the mean levels of vitamin D were [32.8 ± 8.72 ng/ml] in the control group and [11.50 ± 1.8 ng/ml] in the study group [$P=0.001$].

The outcomes of our research revealed that the study group's TBUT scores and Schirmer II test results were significantly lower than those of the control group.

According to ST, the mean score for subjects in the deficiency group was [5.4 ± 2.7 mm], the mean score for the subjects in the post-treatment group was [9.5 ± 3.8 mm], and in the control group the mean score for the subjects was [9.35 ± 4.04 mm]. This difference was statistically significant with a P -value of <0.001. This is parallel with the research by Jain *et al.* [14], which demonstrated a significant difference in the mean ST values between the deficient group [4.65 ± 2.069 mm] and the control group [9.34 ± 5.522 mm] with a P -value of < 0.001.

Furthermore, the mean TBUT score among patients in the deficiency group was [7.9 ± 1.78 s], whereas the mean score for patients in the post-treatment group was [11.3 ± 2.6 s], and the mean value for participants in the control group was [12.3 ± 1.89 s]. This difference was statistically significant with a P -value of <0.001. This is parallel to the findings of Karaca *et al.* [15], which shown that TBUT increased from [5.53 ± 3.12 s] in the deficient group to [9.13 ± 3.01 s] in the post treatment group with a

statistically significant [$p < 0.001$] improvement. This is supported by the results of the study by Yildirim *et al.* [16] that showed that the mean TBUT of the deficient group is lower [7.85 ± 4.02 s] than that of the control group [13.27 ± 5.12 s].

90% of the CIC findings for the deficit group patients exhibited grades 1 and 2&3 in 25% and 65% of the eyes, respectively. In contrast, 80% of the patients in the post-treatment group had CIC findings with grades 1 and 2 and 3 [55% and 25%, respectively]. In addition, 75% of the eyes with CIC results had grades 1 and 2&3 [45% and 30% respectively], which was statistically different from the control patients [P -value = 0.002].

In accordance with the staging by Nelson system, 65% of subjects in the study group had impression cytology that was grade 2 or 3, as opposed to 30% of those in the control group [$p = 0.007$]. This is consistent with the research by Dikci *et al.* [9] findings which showed that 69.4% of participants in the study group had impression cytology that was grade 2 or 3, compared to 18.5% of individuals in the control group [$p < 0.001$].

It is assessed how serum vitamin D levels correlate to results from the TBUT, the Schirmer II test, and the CIC. Serum vitamin D levels significantly correlate negatively [$r = - 0.65$, $p < 0.001$] and negatively [$r = - 0.83$, $p 0.001$] with CIC findings in groups A and C, respectively. While there is no correlation to group B's CIC findings.

In group B, positive correlation [$r= 0.35$, $p= 0.023$] was found with TBUT scores, while in group C, there is a very significant correlation [$r= 0.57$, $p < 0.001$].

In group A, there is no correlation [$r= 0.1$, $p= 0.531$] between serum vitamin D levels and the Schirmer II test. This is in line with a study by Dikci *et al.* [9] that discovered no relationship between serum vitamin D levels and the Schirmer II test [$r = 0.169$, $p = 0.185$], a weakly positive relationship between serum vitamin D levels and TBUT scores [$r = 0.384$, $p = 0.002$], and a moderately negative relationship between serum vitamin D levels and CIC results [$r = - 0.595$, $p < 0.001$]. Groups B and C, however, show a positive correlation [$r=0.33$, $p=0.36$, and $r=0.39$, $p=0.011$, respectively].

In group B, there is no correlation with CIC, although in group A there is no correlation with TBUT. Similar to what was seen in the **Dikci *et al.***^[9] research, serum levels of vitamin D were demonstrated to have slightly positive relation with TBUT results [$r = 0.384$, $p = 0.002$] and a moderately negative [$r = - 0.595$, $p < 0.001$] correlation with CIC outcomes.

Conclusion: Patients who are deficient in vitamin-D have a severe form of eye dryness. Dry eye symptoms, tear film parameters and impression cytology improve after taking vitamin-D supplementation.

Recommendations: Impression cytology can be performed to diagnose DED in people who are vitamin D deficient and to assess the degree of dryness of the eye and how vitamin D treatment would affect it.

Conflict of Interest and Financial Disclosure: None.

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