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Research Article

The Effects of Vitamin D Deficiency on Corneal Thickness and Endothelial Parameters

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Abstract

Purpose: To evaluate the effect of vitamin D deficiency on central corneal thickness and endothelial cell indices.

Method: A prospective cross-sectional study was performed on 39 healthy adults between 22 and 58 years of age recruited from King Saud Medical City in Riyadh, Saudi Arabia. Enrolled participants were classified into three groups based on vitamin D level: Group 1 was composed of 10 individuals (20 eyes) as a control group with serum vitamin D levels of 30-100 ng/ml, and Group 2 was formed of 9 age-matched participants (18 eyes) with vitamin D insufficiency (serum vitamin D level 20-30 ng/ml), and Group 3 was included 20 age-matched participants (40 eyes) with vitamin D deficiency were serum vitamin D level < 20 ng/ml. Corneal endothelial cell indices: CD, CV, HEX, and central corneal thickness CCT were measured using specular microscopy and pentacam. The obtained results were compared among the three different groups.

Results: no significant difference was obtained between the normal, insufficiency, and deficiency groups on all measured study outcomes. The mean CD values were 2626 ± 163, 2725 ± 265, and 2598 ± 261 (p = 0.406) in the control, insufficiency, and deficiency groups, respectively. The mean CV values were 28.9 ± 3.83, 26.9 ± 3.73, and 28.7 ± 4.11 (p = 0.228). The mean HEX values were 66.9 ± 7.88, 64.5 ± 12.5, and 67.3 ± 6.27 (p = 0.850), respectively.

Conclusion: The results indicate no statistically significant difference in any of the corneal endothelial cell indices in relation to different vitamin D levels among participants in our study.

Keywords: Vitamin D Deficiency; Corneal Endothelium; Corneal Thickness; Specular Microscopy

Introduction

Vitamin D is a collective term that describes a group of vitamins that could be produced endogenously in suitable biological conditions and are relatively soluble in fat. The various biologically available forms of Vitamin D comprise Vitamin D3, also called Cholecalciferol, and Vitamin D2, known as Ergocalciferol. Vitamin D3 is generated through its predecessor, 7-dehydrocholesterol, in the presence of ultraviolet radiation (UV Rays) in the human skin [1]. Dietary foodstuffs provide minimal quantities of Vitamin D3. On the other hand, Vitamin D2 is produced by its precursor ergo sterol (human sterol) in the presence of UV radiation in human membranes [1].

Vitamin D3 is transformed to calcidiol or calcifediol 25-hydroxyvitamin D3 (25OH D3), and Vitamin D2 is transformed to 25-hydroxy-ergocalciferol through the 25-hydroxylase in the human liver. Furthermore, in the kidneys and various tissues, 25OH D3 is converted to 1, 25-dihydroxy-cholecalciferol or 1, 25[OH]-2D3-calcitriol by the hydroxylase enzyme. This is the final active form of Vitamin D3 [1,2]. To calculate and investigate the levels of Vitamin D in the blood of human beings, these two Vitamin D metabolites are estimated. As per the regulations presented by Kidney Dialysis Outcomes Quality Initiative guidelines, if the flowing 25OH D3 status is lower than 5 ng/ml, then the patient suffers from severe deficiency of Vitamin D. If the status of Vitamin D is between 5 to 15 ng/ml then the person is suffering from a slight Vitamin D shortage. Whereas, if the levels are between 15 and 29 ng/ml, it is considered Vitamin D ineffectuality, and levels above 30 ng/ml are accepted as normal. But the average range of Vitamin D in human beings should be between 40 to 60 ng/ml [1,3]. Vitamin D provides many benefits to the body in the form of inflammation depressing cytokines production, and diminishing the generation of inflammation-causing cytokines. Vitamin D plays a significant role in immune regulation, cell propagation, differentiation, apoptosis, and angiogenesis [1,4].

Many research studies of vitamin D have been conducted in different areas of ocular disorders such as ocular inflammation, age-related macular degeneration, the optic nerve in multiple sclerosis, myopia, glaucoma, diabetic retinopathy, dry eye syndrome, and ocular vasculature [1,5,6]. Based on the literature, it is obvious that vitamin D deficiency has been related to several ocular diseases. Thus, vitamin D significantly maintains ocular health from the anterior to the posterior segment [2]. However, studies regarding corneal endothelial involvement during vitamin D deficiency are limited. Because of accumulated multiple toxic products and inflammatory cytokines in the aqueous humor of the patients with vitamin D deficiency and because of direct contact between the corneal endothelium and aqueous humor, we hypothesized that these processes in aqueous humor of patients with vitamin D deficiency affected corneal endothelial health.

Corneal endothelial cells are a group of sensitive cells with high metabolic activities that line the inner part of the cornea in a single layer [7]. They play an essential role in maintaining corneal clarity through tight regulation of the corneal hydration level facilitated by different ion channels [8]. The health of corneal endothelial cells can be assessed by analyzing a group of parameters on specular microscopy, such as coefficient of variation (CV), hexagonal cell ratio (HEX), Corneal Endothelial cell density (CD), and central corneal thickness (CCT) [9].

In this research, we aimed to investigate the effect of different Vitamin D levels on the health and function of corneal endothelial cells and CCT demonstrated on specular microscopy and pentacam, respectively. To the best of our knowledge, this is the first research in the literature to compare the effect of vitamin D deficiency and vitamin D insufficiency on the corneal endothelium.

Material and Methods

This prospective cross-sectional study was performed on 39 healthy adults between 22 and 58 years of age recruited from patients and employees at the Ophthalmology and Optometry Department, King Saud Medical City in Riyadh, Saudi Arabia, from November 2020 to May 2022. All enrolled subjects provided written informed consent, and the Institutional Review Board (IRB) Committee approved the study at King Saud Medical City. All participants underwent a comprehensive eye examination, including visual acuity assessment using Snellen E letter acuity chart, refraction using an auto-refractometer, intraocular pressure measurement, slit lamp biomicroscopic examination, and fundus examination. Subjects who had a systemic disease such as diabetes mellitus and hypertension, previous intraocular surgery or laser therapy, history of any corneal disorders or opacity, ocular inflammation or infection, trauma history, contact lens wear, cataract, glaucoma,

presence of high myopia, and pregnancy were excluded from this study. Moreover, patients older than 60 years were not included in this study since the possibility of age-related corneal endothelial changes.

The enrolled participants were divided into three groups based on vitamin D level: Group 1 was composed of 10 individuals (20 eyes) as a control group with serum vitamin D levels of 30-100 ng/ml, and Group 2 was formed of 9 age-matched participants (18 eyes) with vitamin D insufficiency (serum vitamin D level 20-30 ng/ml). Group 3 included 20 age-matched participants (40 eyes) with vitamin D deficiency with serum vitamin D level < 20 ng/ml. These categories were classified according to the laboratory of the King Saud Medical City reference values.

The individuals whose serum vitamin D levels were detected within the categories mentioned above (levels of serum vitamin D) were referred to the Ophthalmology and Optometry Department and included in the study as Group 1,2 or 3. Pentacam and Specular microscopy imaging were performed on the eyes of all enrolled participants to measure the central corneal thickness (CCT), and corneal endothelial parameters, respectively. The OCULUS Pentacam is a rotating Scheimpflug camera that generates three-dimensional images of the anterior eye segments in 2 seconds. It provides topography and elevation measurements of the anterior and posterior surfaces of the cornea in addition to the corneal thickness and others [10]. Specular Microscopy is a non-invasive technique to evaluate the integrity of corneal endothelium; the non-contact type is the clinical standard. It is used to determine cell loss or changes in the shapes of cells (pleomorphism) or cell size (polymegethism) [11]. We compared central corneal thickness and corneal endothelial parameters (corneal endothelial cell density CD (cells/mm^2), coefficient of variation CV, and hexagonal cell ratio HEX) between the groups.

Statistical analysis

Descriptive data were expressed as means with standard deviations (SD) if normally distributed; otherwise, a median with interquartile range (IQR) was used. Normality was determined using Kolmogorov–Smirnov test. The categorical variables were summarized as frequencies with percentages.

Measured study outcomes included: visual acuity with correction (VA C.C), spherical equivalent (SE), central corneal thickness (CCT), cell density (CD), coefficient of variation (CV), and hexagonal cell ratio (HEX). We reported study outcomes for the overall cohort and by groups: normal versus insufficient versus deficiency group. Between groups, analysis was carried out using one-way ANOVA. Comparative analyses were done using a chi-square test for the categorical variables and a t-test for continuous variables. Correlation analysis between variables was carried out using Spearman's correlation. *P*-values < 0.05 were considered statistically significant.

SPSS software version 23 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis.

Results

A total cohort of 39 subjects with an average age of 35 ± 8 years who met the inclusion criteria were enrolled in the present study. The demographic characteristics of the subjects are demonstrated in Table 1.

Characteristic		Overall (n = 39)	Normal (n = 10)	Insufficient (n = 9)	Deficiency group (n = 20)	p-value*
Age, mean (± SD)		35 (8)	37 (7)	33 (4)	35 (11)	0.241
Gender, n (%)	Male	9 (23)	1 (10)	1 (11)	7 (35)	0.037
	Female	30 (77)	9 (90)	8 (89)	13 (65)	
Vitamin D (ng/ml), median (IQR)		21.2 (14.3)	40.9 (8.87)	25.9 (2.79)	9.12 (1.99)	0.001

Table 1: Demographic data for participants.

*Comparing the normal group to the deficiency group using the non-parametric test for groups.

From the included 39 individuals, 10 (26%) subjects (20 eyes) had normal vitamin D levels (normal group; mean [SD]: 40.9 [± 8.87]) with an average age of 37 ± 7 while 9 (23%) (18 eyes) had vitamin D insufficiency (mean [SD]: 25.9 [± 2.79]), with an average age of 33 ± 4. On the other hand, the vitamin D deficiency group included 20 (51%) participants (40 eyes) (deficiency group; mean [SD]: 9.12 [[± 1.99]) aged 35 ± 11 (Table 1). From a total of 78 eyes, there was no significant difference between the normal, insufficiency, and deficiency groups on all measured study outcomes (Table 2). Even while comparing the normal to deficiency group, no significant difference was calculated for the study outcomes (Table 3).

Parameter	Normal (n = 20)	Insufficient (n = 18)	Deficiency group (n = 40)	p-value*
VA C.C	0.05 (0.16)	0.02 (0.05)	0.04 (0.08)	0.488
SE	-0.80 (1.26)	-0.17 (0.77)	-0.49 (1.26)	0.395
CCT	541 (38.3)	527 (34.8)	551 (39.6)	0.106
CD	2626 (163)	2725 (265)	2598 (261)	0.406
CV	28.9 (3.83)	26.9 (3.73)	28.7 (4.11)	0.228
HEX	66.9 (7.88)	64.5 (12.5)	67.3 (6.27)	0.850

Table 2: Study Parameters (Total eyes).

*Comparing between groups using the non-parametric test for group.

Parameter	Normal (n = 20)	Deficiency group (n = 40)	p-value*
VA C.C	0.05 (0.16)	0.04 (0.08)	0.313
SE	-0.80 (1.26)	-0.49 (1.26)	0.228
CCT	541 (38.3)	551 (39.6)	0.500
CD	2626 (163)	2598 (261)	0.931
CV	28.9 (3.83)	28.7 (4.11)	0.493
HEX	66.9 (7.88)	67.3 (6.27)	0.509

Table 3: Study Parameters (Total eyes).

*Comparing between groups using non-parametric test for group.

Correlation analysis revealed no significant correlation between the vitamin D levels and corneal endothelial parameters CD, CV, and HEX, as shown in the following graphs.

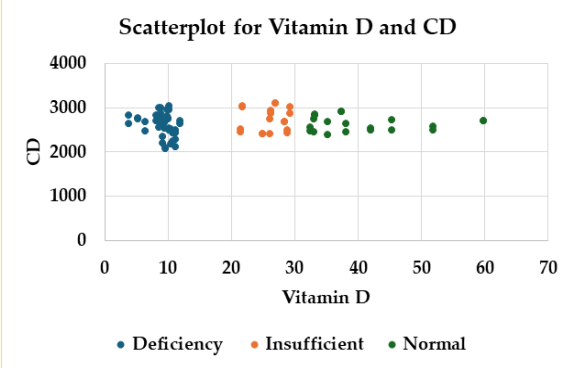

Figure 1: Correlations between vitamin D levels and endothelial cell density.

Discussion

Recently, it has been discovered that cell cultures of ciliary body non-pigmented epithelium, corneal epithelial and endothelial cells, scleral fibroblasts, and retinal pigment epithelium all exhibit vitamin D hydroxylase function. It has also been demonstrated that the majority of these cells can transform 25OH D3 into 1, 25 OH 2D3, which is the dynamic system of vitamin D. It's interesting to note that cultures of corneal limbal epithelial cells can make vitamin D from scratch, much like skin cells can after being exposed to UV-B light. Suggesting that vitamin D is a crucial biological media-

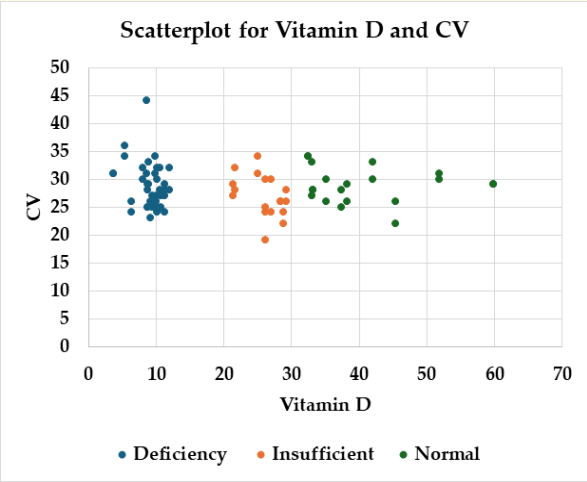

Figure 2: Correlations between vitamin D levels and coefficient of variation.

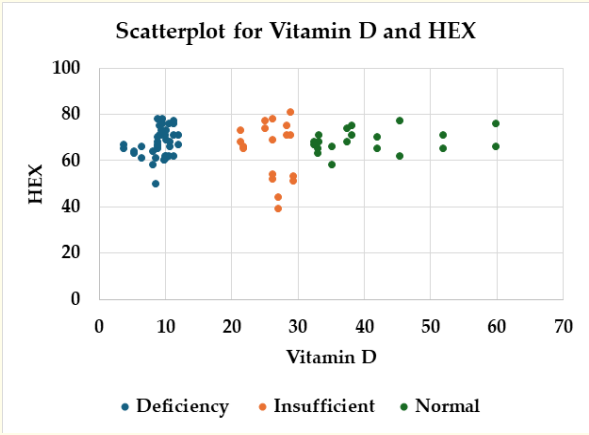

Figure 3: Correlations between vitamin D levels and hexagonal cell ratio.

tor in the eye; the cornea and other ocular tissues can regulate and activate vitamin D metabolism independently [4,12,13].

Vitamin D has been shown to prevent oxidative damage to human retinal pigment epithelium and endothelial cells. So, Vitamin D shortage can lead to greater oxidation and inflammation [14]. This inflammation and oxidation could impact corneal endothelial functioning. According to research by Ghiță., *et al.* [15] in 2019, intraocular inflammation impacts how corneal endothelial cells operate, increasing the thickness of the cornea and resulting in changes in corneal endothelial parameters such as CD, CV, and HEX. Moreover, one of the fundamental apparatuses that may cause damage to corneal endothelial cells, similar to the contrivance found in uveitis, is the provocative cytokines that may arise in the aqueous humor of people with vitamin D shortage. Hence, this study aimed to evaluate if vitamin D deficiency and insufficiency would affect corneal endothelial function, and we also wanted to compare the various effects of different vitamin D levels on corneal endothelial cells indices, including CV, HEX, corneal endothelial CD, and CCT by specular microscopy and OCULUS pentacam, respectively.

Cankaya., *et al.* [1] have found lower HEX, CD values, and higher CV values, indicating polymegethism and pleomorphism in the corneal endothelial cell layer in patients with vitamin D deficiency. On the contrary, we have found no statistically significant difference in any of the corneal endothelial cell indices in relation to different vitamin D levels among participants in our study. This can be attributed to various factors, including the difference in the mean vitamin D level between our deficiency group and theirs, 9.12 ± 1.99 and 4.93 ± 2.91 ng/ml, respectively. Our study population included 39 subjects with an average age of 35 ± 8 years compared to Cankaya., *et al*. where they had 98 subjects with a mean age of 44.4 ± 10.89 and 41.5 ± 8.17 in the deficiency group and control group, respectively. Our sample included a younger study population in comparison to other studies, and we saw no association between vitamin D levels and corneal endothelial parameters. We elected to study a younger age population where endothelial cells are at a better health capacity and are not yet exposed to the effect of accumulative oxidative stress. Moreover, the present study reported no statistically significant difference in CCT among deficiency, insufficiency, and control groups which agreed with the study conducted by Cankaya., *et al.* [1].

Even though the country's three largest cities, including Riyadh, Jeddah, and Dammam, are located in the tropics, printed statistics show that the Saudi Arabian inhabitants suffer from a vitamin D deficit that may be as high as 100%. Other than nutrition and sunlight, various things affect vitamin D levels. Saudis had a high incidence of hypovitaminosis D than non-Saudis who resided in the same areas and likely had similar diets. Compared to the inhabitants with standard points, Saudi persons who possess the GG allele of the three SNPs (Single Nucleotide Polymorphisms) had considerably lower amounts of 25OHD [16]. However, despite the higher

prevalence of vitamin D deficiency and the underlying genetic basis in our Saudi population, we still could not demonstrate the effect of different vitamin D levels on endothelial function. However, one of the limitations of the present study is the smaller sample size compared to other studies in the literature, which would only allow the generalization of our results if further larger studies were carried out in our Saudi population.

Conclusion

This study indicates no statistically significant difference in any of the corneal endothelial cell indices in relation to different vitamin D levels among participants in our research.

Conflict of Interest

The authors declared that there is no conflict of interest.

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