

## Investigation of Vitamin D Levels in Patients with Vitiligo Vulgaris

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**SUMMARY** The aim of the study was to investigate serum 25-hydroxyvitamin D (25(OH) D<sub>3</sub>) levels in patients with vitiligo vulgaris in terms of causal relation and extension of the disorder.

This study is a clinical cross-sectional study carried out in order to determine 25-hydroxyvitamin D levels among 25 patients with vitiligo vulgaris and in 41 controls. Fitzpatrick skin phototypes, history of autoimmune disease, family history of vitiligo, and duration of the disease were also evaluated.

The mean levels of vitamin D in patient and the control group were 15.2±5.2 ng/dL and 14.4±6.2 ng/dL respectively ( $P>0.05$ ). In our study, 48% of the patients had insufficient (<30 ng/mL) and 52% had very low (<15 ng/mL) levels of vitamin D. There was no correlation between age, duration of the disease, and body surface area affected with vitamin D levels. There was no significant difference in vitamin D levels between patients who had family history of vitiligo (5 patients, 20%) and those that did not.

Vitamin D levels were found to be insufficient (<30 ng/mL) or very low (<15 ng/mL) in most of the patients with vitiligo vulgaris, but not statistically significantly different as a group when compared to the controls. More studies are needed to differentiate between the effects of low vitamin D levels on pathogenesis of vitiligo vulgaris and lower vitamin D levels as a result of the disease.

**KEY WORDS:** vitamin D, vitiligo, autoimmunity, hypopigmentation

### INTRODUCTION

Vitiligo is an acquired disorder characterized by depigmentation of skin that affects 1-2% of the population worldwide (1). It is the most common disorder that is equally likely in both genders and can occur at any age. Although the real cause of vitiligo

is unknown, the autoimmune theory is the one that is best supported. Vitiligo can occur along with other autoimmune diseases including pernicious anemia, hyperthyroidism, Hashimoto's thyroiditis, alopecia areata, and adrenocortical failure (2,3). The disease

is inherited multifactorially, with a polygenic pattern and incomplete penetrance (1).

Histological studies have shown the absence of melanocytes in the affected skin (4). Evidence supports that both humoral and cellular immunity play a role in vitiligo (5-7). The importance of the T regulatory CD4<sup>+</sup> cell subset (Treg) in the pathogenesis of many autoimmune diseases is already known. At the site of depigmentation, T cell infiltrates are invariably seen in patients with active vitiligo, along with a high frequency of cytotoxic T lymphocytes specific for melanocytic antigens, suggesting a direct melanocyte specific T cell attack (8).

Vitamin D deficiency is a global health problem. Although the effects of vitamin D on bone and mineral metabolism are well established, its implications on the immune system are still under investigation. Vitamin D receptors have been found in all immune-system cells, including activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, B cells, neutrophils, and antigen presenting cells (macrophage and dendritic cells) (9). The association between vitamin D and autoimmune diseases, such as type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, asthma, and inflammatory bowel disease, has been established (10). Whether this association indicates a causal relation is a matter of debate.

Currently, topical vitamin D3 analogues such as calcipotriene are used to treat vitiligo, but the blood levels of vitamin D in patients with vitiligo are not clinically well established.

In this study our aim was to investigate the serum 25(OH) D<sub>3</sub> levels in patients with vitiligo vulgaris (VV) in terms of causal relation and extension of the disorder.

## PATIENTS AND METHODS

The present study is a clinical cross-sectional study carried out among the patients with VV in the Department of Dermatology at the Mustafa Kemal University Hospital between 2010 and 2011. The study was approved by the local Ethical Committee of the Mustafa Kemal University (Hatay, Turkey). The study was conducted in accordance with the Declaration of Helsinki. Informed consent of the all participants was obtained. The VV is diagnosed by an investigator (G.S). Patients taking oral vitamin D supplements or topical vitamin D, with a history of phototherapy in the last year, history of systemic disease, or with the presence of chronic inflammatory disease and incomplete documentation were excluded from the study.

During the interview in the clinic, Fitzpatrick skin phototypes, history of autoimmune disease, family

**Table 1.** Characteristics of the vitiligo group (yr: years; n: numbers; SD: standard deviation; BSA: Body surface area)

Age (yr; mean ± SD)	33.9 ± 19.4
Sex	
Male, n	13
Female, n	12
Duration of disease (yr) mean ± SD	5.36 ± 6.41
Family history, n	4
BSA involved, n	
0-9 %	15
10-100%	10
Fitzpatrick skin type	
II, n	6
III, n	19

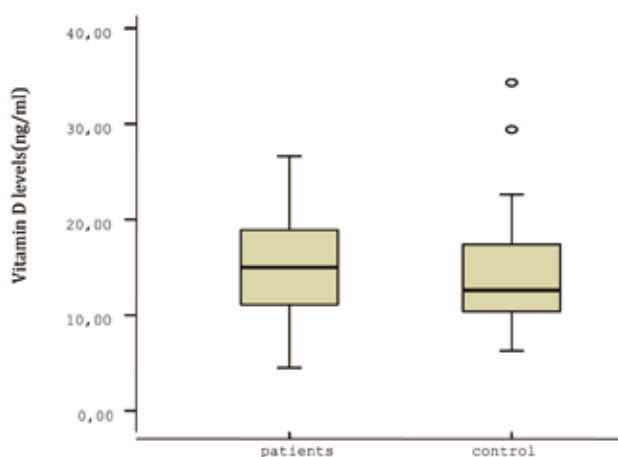
history of vitiligo, and duration of the disease were recorded for patients included in the study.

Twenty five of patients with VV and 41 from control group had sera drawn to determine 25(OH)-D<sub>3</sub> levels. To avoid seasonal variations, blood samples from the control group were taken at the same time as the patient group. All the subjects were from the metropolitan area of Hatay, in order to avoid geographic differences in sun exposure and vitamin D levels. Laboratory testing was performed in the biochemistry laboratory of Mustafa Kemal University, Turkey. Blood samples were drawn from the antecubital vein by careful vein puncture in a 21 G sterile syringe without stasis at 08.00–10.00 AM after a fasting period of 12 h and then centrifuged at 4000 g for 5 min to separate plasma for 25(OH)-D. The blood samples were also analyzed for data on complete blood counts, creatinine, alanine aminotransferase, aspartate aminotransferase, and thyroid-stimulating hormone (TSH).

Serum 25-hydroxyvitamin D levels were determined via chemiluminescence by ARCHITECT i2000 system. Vitamin D deficiency is defined as a 25(OH)D<sub>3</sub> level below 20 ng/mL, very low levels as 15 ng/mL, insufficiency as 21–29 ng/mL, and sufficiency as a 25(OH)D<sub>3</sub> level of 30–100 ng/mL (11,12).

## STATISTICAL ANALYSIS

Statistical analysis was performed with the SPSS software version 15. The variables were investigated using visual and analytical methods (Kolmogorov-Siminov/Shapiro-Wilk test) to find out whether or not they are normally distributed. Descriptive analysis was presented using medians for the non-normally



**Figure 1.** Vitamin D levels of the patient and the control groups.

distributed variables. The Mann-Whitney U test was used to compare ages and vitamin D levels between the patient and control groups. P-value of less than 0.05 was considered statistically significant.

## RESULTS

A total of 25 patients and 41 controls were included in the study. The patient population consisted of 13 men and 12 women, with an overall mean ( $\pm$  standard deviation) age of  $33.9 \pm 19.4$  years. The control group consisted of 20 men and 21 women, with an overall mean age of  $34.7 \pm 15.9$ . There was no significant difference in mean age between the patient and the control group ( $P > 0.05$ ). The mean levels of vitamin D in the patient and control group were  $15.2 \pm 5.2$  ng/dL and  $14.4 \pm 6.2$  ng/dL respectively ( $P > 0.05$ ). Twenty patients and 34 control group had vitamin D deficiency. Four patients had a family history of vitiligo. Only one patient had a history of another autoimmune disease: Graves disease. There was no correlation between the vitamin D levels and percentage of the affected body surface area ( $P > 0.05$ ,  $r = 0.002$ ). There was also no correlation between vitamin D levels and duration of the vitiligo ( $P > 0.05$ ,  $r = 0.209$ ).

Six patients had Fitzpatrick skin phototype 2 while the 19 had phototype 3. Control and the patient groups did not significantly differ in Fitzpatrick skin phototype.

## DISCUSSION

Our study investigated vitamin 25(OH) $D_3$  levels of patients with VV. A case control study in a Chinese population showed no correlation between vitamin d

levels and onset of vitiligo (13). In a case control study from Egypt, Saleh *et al.* found 25(OH) $D_3$  deficiency in vitiligo patients with and without autoimmune diseases (14). Another study by Silverberg *et al.* has limitations because there was no control group, but is worth considering (12). In our study, there was no difference between the patients and the control group with respect to 25(OH)  $D_3$  levels. In Silverberg *et al.*, 55.6% of the subjects had insufficient ( $< 30$  ng/mL) and 13.3% had very low ( $< 15$  ng/mL) vitamin D levels (12). In comparison, in our study 48% had insufficient ( $< 30$  ng/mL) and 52% of the patients had very low ( $< 15$  ng/mL) levels of vitamin D. None of the patients had sufficient ( $> 30$  ng/mL) levels. On the other hand, in the control group 31.7% had insufficient, 65.9% had very low, and only one participant had sufficient vitamin D levels (Figure 1). None of the subjects in control group had severe deficiency. In our study, the low levels of vitamin D may be related to the time the blood samples were collected, since all of the patients participated in the study during winter and autumn.

Regarding the Fitzpatrick skin phototype, there was no statistical difference in vitamin D levels between the skin type II and III. Silverberg *et al.* found a correlation between vitamin D insufficiency and Fitzpatrick skin phototype, but the correlation did not exist with very low levels when using the 15 ng/mL cut-off point. There are some studies that show correlation between age and vitamin D, whereas others found no correlation (15-17). In our study, there was no correlation between age and vitamin D levels.

Silverberg *et al.* found that patients with comorbid autoimmunity were more likely to have very low vitamin D levels (12). We had only one patient who had Graves' disease, with a vitamin D level of 19 ng/mL which is considered to be deficient but not very low ( $< 15$  ng/mL). There is a study by Yamashita *et al.* that shows increased prevalence of vitamin D deficiency in Graves' disease (18).

There was no significant difference with respect to vitamin D levels between patients who had family history of vitiligo ( $n = 5$ , 20%) and those that did not. There was also no correlation between the vitamin D and duration of the disease and body surface area affected. Six (24%) of the patients had relapsing VV, but there was no significant difference in vitamin D levels compared with the non relapsing group.

Vitamin D receptors play a central role in the biological actions of vitamin D, and these receptors are found in almost all immune-system cells including T cells, B cells, neutrophils, and antigen presenting cells (9,19). There is large number of studies that implicates that 1.25 (OH) $_2D_3$  influences the immune

system, but whether this relation is a cause or result of the autoimmune diseases is not clear. Vitamin D levels can vary significantly in populations due to geographical, economic, and social factors. The levels that may cause bone disease (<8 ng/mL) or elevate parathyroid hormone levels (<30 ng/mL) have been established, but the levels that triggers autoimmune diseases should be studied (20).

## CONCLUSION

More studies are needed to establish the effect of low vitamin D levels as part of the pathogenesis of immune diseases rather than a result of the diseases.

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