

Serum vitamin D level in patients with newly diagnosed pemphigus vulgaris

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Background: Pemphigus vulgaris (PV) is a rare autoimmune blistering disease of the skin and mucous membranes. Because vitamin D deficiency is associated with many immune disorders, we compared the levels of vitamin D between PV patients and healthy controls.

Methods: Vitamin D level of 20 patients with PV was compared with 20 healthy individuals matched for age, gender, hours spent in the sun, body mass index (BMI) and daily vitamin D intake at the same time interval. The severity of the disease was estimated according to ABSIS score. The serum level of 25-hydroxyvitamin D (25OHD) was measured by a commercial ELISA test.

Results: Vitamin D deficiency in the pemphigus vulgaris group (75%) was higher than healthy controls (45%) ($P = 0.053$). Mean level of vitamin D in PV patients was significantly lower than healthy controls, and was in the range of vitamin deficiency ($P \leq 0.05$). No significant relationship was observed between the severity of the PV disease and vitamin D levels ($P > 0.05$).

Conclusions: Based on the results of the present study, the mean level of vitamin D in patients with PV was significantly lower than healthy people. Based on our findings and given the important role of vitamin D in preventing inflammatory and autoimmune diseases, vitamin D deficiency can be considered as a factor triggering the onset of PV.

Keywords: pemphigus vulgaris, vitamin D, autoimmune disease

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INTRODUCTION

Pemphigus Vulgaris is a rare and life-threatening autoimmune blistering disease caused by antibodies produced against desmosomal connective proteins, especially desmoglein (Dsg) 1 and 3, which are responsible for maintaining the integrity of the epidermis ¹. Vitamin D may play a role in the development of PV due to its regulatory effects on the immune system. This vitamin plays a major regulatory role in the immune system through affecting the function and proliferation of B and T cells, the differentiation of T helper (Th) cells, and the regulation of regulatory T cells (Tregs) ²⁻⁴. In

vitro studies have shown that vitamin D suppresses the function of Th1 cells, while inducing Th2 activity and, consequently, increasing cytokines associated with it ^{5,6}. It has been further shown that IL-17 has a positive effect on the development of allergic and autoimmune diseases ^{7,8}. Suppressing the production of IL-17 ⁹, vitamin D can increase the population of Tregs cells ¹⁰, hence having a repressive role in the immune system and precluding autoimmune diseases ¹¹.

It was previously shown that vitamin D deficiency can affect the proper functioning of the immune system and may be the cause of the onset or exacerbation of autoimmune diseases ¹²⁻¹⁵. So far,

research has shown different results in terms of the role of vitamin D deficiency in the development of pemphigus¹⁶⁻²⁰. Therefore, further studies are required to investigate the association between vitamin D deficiency and the disease.

According to a 10-year study in Iran, PV is the most abundant form of autoimmune bullous diseases (81.2%)²¹, necessitating further studies to find the underlying causes of the disease and to provide preventive and therapeutic strategies in Iran. A systematic review of vitamin D status in populations worldwide shows the prevalence of vitamin D deficiency in Iranian population²². Given the relationship between vitamin D deficiency and the occurrence of autoimmune diseases, this study was conducted to investigate the association between PV and vitamin D deficiency.

MATERIALS & METHODS

Participants and study design

In this case-control study, 20 patients referring to the dermatology clinic, Imam Reza Hospital (Mashhad, Iran) from September to December of 2015 were confirmed for PV according to the pathologic and immunofluorescence results. The same number of healthy individuals that matched the patients' age, daily hours spent in the sun, BMI, and daily intakes of vitamin D were included in the study. Subjects, all of whom were selected from the northeastern regions of Iran, were studied in one season to exclude the effect of seasonal variations in vitamin D levels. Exclusion criteria were topical treatment with vitamin D derivatives or systemic therapy (phototherapy) in the past six months, residents of other regions of Iran, use of vitamin D supplement and the presence of other chronic inflammatory diseases. The severity of the disease in patients was recorded in a questionnaire and calculated according to Autoimmune Bullous Skin Disorder Intensity Score (ABSIS)¹. The advantage of this system is that it includes both qualitative and quantitative factors. Patients were selected according to the criteria associated with entering and excluding from the study and blood sampling (5 ml blood from brachial vein). The questionnaires and informed consents were completed and signed by all participants, and the samples were delivered to the laboratory. This study was approved by

the research ethical committee of the Faculty of Medicine, Mashhad University of Medical Sciences.

The serum level of 25-hydroxyvitamin D (25OHD) was measured by ELISA method (25(OH) Vitamin D ELISA Kit, Biosource, Los Angeles, CA, USA) in a laboratory. Samples with a serum level of more than 30 ng/ml were regarded as normal. Samples with a serum level of less than 10 ng/ml were considered deficient, and those between 10-30 ng/ml were deemed insufficient.

Statistical methods

Data were described using mean, standard deviation and frequency indices. Data analysis was performed using Chi-square, T-student test and Pearson correlation. In order to control the interfering factors, general linear models were used. In all tests, a p-value of less than 0.05 was considered to be statistically significant. SPSS software (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows was employed for analysis.

Ethical considerations

The written informed consent was obtained from all participants.

RESULTS

In this study, 20 patients with pemphigus vulgaris and 20 healthy controls were enrolled. Gender distribution was the same in both groups, with each group comprised of 9 (45%) males and 11 (55%) females. The mean (SD) age of participants was 50.42 (15.7) years old. The mean (SD) ABSIS of the patients was 31.93 (12.4).

Comparing the age of the two groups, T-test did not show any significant difference in terms of the mean age between the two groups ($P = 0.56$).

Of the 40 participants in the study, 16 (40%) people had normal levels of vitamin D (> 30 ng/ml) and 24 (60%) had abnormal levels of vitamin D (< 30 ng/ml); moreover, there was a significant difference between PV patients and healthy controls as concerns this distribution, because subjects with abnormal levels of vitamin D were more in the case group ($P_v = 0.48$). Of these 24 cases, 6 had vitamin D deficiency (serum levels of 0-10 ng/ml), while

Table 1. Comparison of vitamin D levels in pemphigus patients and controls

Subjects	Deficiency (0-10ng/ml)	Insufficiency (10-30ng/ml)	Sufficiency (30-150ng/ml)	Total
HCS, n (%)	2 (10)	7 (35)	11 (55)	20 (100)
Patients, n (%)	4 (20)	11 (55)	5 (25)	20 (100)
Total	6 (15)	18 (45)	16 (40)	40 (100)

HCS: healthy controls; n: number

18 had insufficient vitamin D levels (serum levels of 10-30 ng/ml), (Table 1).

Comparing the mean level of vitamin D between patients (20.97 ng/ml) and healthy controls (32.64 ng/ml), the average level of vitamin D for the healthy controls was 11.67 ng/ml higher than that for the patients. The mean level of vitamin D for the healthy subjects was within the normal range, while regarding the patients, the mean level was in an abnormal range. To evaluate the significance of the difference, independent T-test was used to compare the means. Based on this test, the difference between the mean level of vitamin D was significant and the level of vitamin D in the patient group was less than the control ($P = 0.016$).

According to the results of Pearson correlation test, there was no significant relationship between vitamin D level and age and ABSIS ($P = 0.7$, $r = -0.18$).

T-test did not show any significant differences concerning vitamin D levels and ABSIS scores between the two genders in PV patients ($P_v = 0.54$, 0.98 , respectively).

DISCUSSION

Our main goal in this study was to investigate the association between vitamin D deficiency and PV. We found that PV was associated with abnormal levels of vitamin D compared with healthy controls ($P \leq 0.05$). Based on this finding, vitamin D deficiency can be considered as a predisposing factor for the onset of PV disease. In the previous studies, there were different results regarding the association between vitamin D deficiency and PV. Most studies have found lower levels of vitamin D in PV patients compared to healthy controls¹⁶⁻²⁰. In a study by EL-Komy *et al.*, which investigated the status of vitamin D in 34 patients with pemphigus, the level of vitamin D was significantly lower in patients¹⁶. In another study, a severe vitamin D deficiency was observed in patients with PV²³. Zarei *et al.* found that vitamin D was significantly

lower in PV patients, and there was a negative correlation between vitamin D levels and the oral severity of the disease¹⁸.

Contrary results have also been reported in studies that have so far been conducted in different countries regarding the relationship between vitamin D deficiency and PV¹⁶⁻²⁰. In a study conducted by Joshi *et al.* in North India, a region where hypovitaminosis is common, deficient levels of vitamin D were detected in both PV patients and healthy controls¹⁷. A notable finding of that study was a dramatic reduction in TGF- β /IL-17 ratio in PV patients, revealing the dysregulation of T cells functions¹⁷. Contrary to the results observed by Marzano *et al.*²³, Tukaj *et al.* found no difference between vitamin D serum levels of bullous pemphigoid patients and healthy controls²⁰.

Despite the similarity of the factors affecting the level of vitamin D such as race, skin type and climate²⁴, there are contradictory results concerning the association of PV with vitamin D deficiency in studies conducted in Iran^{18,19}. In a study performed by Zarei *et al.*, PV patients had significantly lower serum levels of vitamin D¹⁸. On the other hand, Moravvej *et al.* observed that Vitamin D deficiency is common both in PV patients and healthy controls¹⁹. Adding the results of this study to previous studies may indicate the potential role of vitamin D deficiency in the development of the disease in Iran.

We further examined the correlation between vitamin D deficiency and PV severity based on ABSIS, and found no significant correlations. This is in contrast with previous studies, where a negative correlation was found between vitamin D levels and the severity of disease according to Harman's scores¹⁸ and Pemphigus Area and Activity Score (PAAS)¹⁹.

In this study, the average level of vitamin D between the two genders in the patient group showed that vitamin D level was lower in women (19.25 ng/ml) compared with men (23.07 ng/ml). Women are less exposed to sunlight than men due

to the differences in clothing in Iran, and this must be taken into consideration for the prevention and treatment of the disease.

The results of this study indicated that the average level of vitamin D in the control group was within the normal range, while the cases had an abnormal range of Vitamin D. These low levels were not related to the patient's age, climate, daily hours spent in the sun, BMI, and daily intakes of vitamin D3. The results of this study and the previous ones highlight the potential role of vitamin D deficiency as a cause for the onset or exacerbation of PV. Accordingly, in future studies, the effects of using vitamin D in preventing the onset or treatment of the disease should be investigated.

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Conflict of Interest: None declared.

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