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Original Article

Evaluation of Vitamin D Status in Newly Diagnosed Pemphigus Vulgaris Patients

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Abstract

Background: Pemphigus vulgaris (PV) is an autoimmune blistering disorder of the skin or mucosa. Since low vitamin D status has been linked to many immune disorders, we designed this study to compare the vitamin D status in PV patients with healthy controls.

Methods: In this case-control study, vitamin D status of 32 newly diagnosed PV patients was compared with 36 healthy control subjects. All patients were selected from the specialized dermatology departments of Razi Hospital, Tehran University of Medical Sciences in a 2-year period (2009-2010). The severity of the disease was estimated according to Harman's scores. Serum concentration of 25(OH)D was measured by Roche Elecsys System. Data were analyzed by independent t-test.

Results: Both groups were similar based on sex, age and body mass index. The mean duration of disease was 5.57±0.93 months. The mean oral and skin severities were 1.81±0.20 and 2.31±0.17 respectively, based on Harman's scores. Serum 25(OH)D was significantly lower in PV patients compared to controls (-8.90; 95% CI, 2.29-15.51 and $P = 0.009$). There was a negative correlation between vitamin D level and the oral severity of disease ($r = -0.39$ and $P = 0.02$).

Conclusion: PV patients had significantly lower serum level of 25(OH)D compared to healthy subjects which might contribute to worsen the disease. These data indicate the importance of improving vitamin D level in pemphigus patients.

Keywords: Pemphigus vulgaris, Vitamin D, Calcitriol

Introduction

Pemphigus is a group of chronic, life-threatening, autoimmune bullous diseases resulting from autoantibodies aimed to desmosomal proteins. This severe skin disorder is typically characterized by damaging epithelial adhesion molecules, especially desmogleins 1 and 3, which lead to loss of cell to cell contact (acantholysis) (1). Pemphigus vulgaris (PV) is the most severe and prevalent type (2). The incidence of PV varies from 0.5 to 4 cases per 100000 person-years however; it depends on geographical location and is nearly 1 per 100000 populations in Iran and it is more common in middle-aged patients and especially in the Mediterranean region, Middle East and India (3, 4). The underlying causes of PV are not yet well recognized. However, it seems that genetic factors play a piv-

otal role and environmental stimulants influence the disease incidence as well.

Low vitamin D status, as an environmental factor, has been linked to many autoimmune disorders such as type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis and etc. (5, 6) Immune cells can synthesize the active form of vitamin D3 [1,25 $(OH)_2$ D] and express the vitamin d receptor (VDR) which mediates the genomic function of the vitamin (7). Vitamin D is known as an important immunomodulator agent, since it regulates both innate and adaptive immune systems by affecting monocyte/macrophages and dendritic cells, as well as T and B lymphocytes. Totally, it can down-regulate the adaptive immune system to lower the incidence of autoimmune disease while boost the innate immune system to fight infections (8, 9). In addition, it has been investigated that cutaneous immunity is controlled by ultraviolet (UV) irradiation, which affects keratinocytes, antigen-presenting cells, and T lymphocytes. Epidermal expression of 1,25 $(OH)_2$ D connects the environment to the immune system via expansion of CD4+ CD25+ regulatory T cells and down-regulating cutaneous immune responses (10). Moreover, Calcitriol acts directly on suppressing autoimmune responses by inhibiting Th1 cytokines and promoting Th2 cytokines (11). The diverse immune-modulatory potentials of 1,25 $(OH)_2$ D, the high prevalence of vitamin deficiency in Iran and insufficient data about the vitamin status in PV patients, intrigued us to design and perform this case-control study to compare the vitamin D status in PV patients with healthy controls.

Materials and Methods

Subjects

In this case-control research, we studied 32 patients with PV and 36 healthy people. The sample size was estimated with an assumption of α=0.05 and power of 80%, to detect the minimum meaningful differences of 5 ng/ml between groups. All patients were selected from the specialized dermatology departments of Razi Hospital, Tehran University of Medical Sciences during a 2-year period (2009-2010), so the effect of vitamin D seasonal fluctuation was omitted. We used simple sampling so every patient admitted to the hospital and met our criteria entered the study. PV was diagnosed based on clinical, histological (suprabasal cleft and acantholysis) and immunological (intercellular deposits of IgG and/or C3) criteria of the disease (12). Patients were newly diagnosed and not receiving corticosteroids or any other systemic treatment for pemphigus. They were taking no supplements or contraceptive pills. Patients with any hepatic or renal disorders were not enrolled. Healthy people were recruited from volunteers. They should have had no history of autoimmune or inflammatory disorders. Ones taking supplements, corticosteroids and contraceptives, and ones affected with cardiovascular diseases and diabetes were excluded (Fig. 1). Frequency matching resulted in equal distribution of age, sex and body mass index (BMI) in case and control groups.

Data collecting

The severity of disease was assessed according to Harman's scores (13). Patients were examined by one expert physician to minimize observer bias resulting from inter-individual variations. The extent of involvements of skin and oral mucosa were measured and recorded separately and scored as a number from 0 to 3. The patients and controls were interviewed about their medical history and administration of drugs and supplements. Their body weight (with minimal clothing) and height (without shoes and in upright position) were measured using a measuring scale (Seca GmbH & co KG, Hamburg, Germany) with an accuracy of 0.1 kg and 0.5 cm respectively. Body mass index (BMI) was calculated using the following formula: weight (kg) / height $(cm)^2$. After taking written consent from all subjects, blood samples were collected in the morning, after 12-14 hours fasting from antecubital vein and were centrifuged at 3000 rpm for 10 min at 4°C. The separated plasma was immediately stored at -80°C until biochemical analysis. Serum concentration of 25(OH)D was measured by Roche Elecsys System with intra- and inter-assay coefficients of variation (CV) of 5.4% and 6.1%, respectively.

Ethical points

All subjects were deeply informed about the purposes and procedures of the study and signed written consent form before entering the study. In case of being under 18 years old, one of the parents signed the consent form. Research protocol was approved by the Ethics Committee of Tehran University of Medical Sciences.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Normality of data distribution was checked by Kolmogorov-Smirnov test. Groups were compared with independent sample t test or chi square test for continuous and categorical data, respectively. Correlation between vitamin D level and severity of disease were measured using Spearman's rho. A *P* value <0.05 was considered to be statistically significant. All the statistical analysis was done by the Statistical Package for the Social Sciences (Version11.5, SPSS Inc, Chicago, IL, USA).

Results

A total of 32 PV patients fulfilled the inclusion criteria and enrolled in this study (15 males and 17 females, aged 16-69 years) and all of them finished the study. We did not have missing data and all measurements were used in statistical analysis. The mean duration of disease in patient group was 5.57±0.93 months. The mean severity score of skin and oral involvement were 2.31±0.17 and 1.81±0.20, respectively, based on Harman's scores. Thirty-six healthy persons were served as control group (consisted of 9 males and 27 females, aged 20-60 years). Some confounding factors such as sex distribution, age, weight, height, and BMI were checked at the baseline and no significant difference was observed between groups (Table 1). The serum vitamin D level was significantly lower in PV patients compared to healthy controls (11.79 ± 1.55 vs. 20.69 ± 2.79 and *P* = 0.009, Table 2). Since the distribution of the data was not normal, we transformed them into vitamin D logarithm. Comparing vitamin D logarithm between two groups showed statistically significant difference too (*P* = 0.004). Interestingly, there was a negative correlation between vitamin D level and the total severity of disease (r = -0.35 and *P* = 0.05). However, the negative correlation between vitamin D and oral severity was more conspicuous (r = -0.39 and *P* = 0.02, Table 3).

Table 1: Sex distribution, age and anthropometric indices of patients and healthy subjects

	Patients (n=32)	Controls (n=36)	*P* value
Sex (number of male/female)	15/17	9/27	0.08*
Age (yr)	43.75±11.58	44.08±10.63	0.90#
Weight (kg)	65.12±14.11	66.75±11.86	0.60#
Height (cm)	161.87±8.17	160.57±7.38	0.45#
BMI (kg/m²)	24.92±4.98	26.26±3.83	0.23#

Data are presented as Mean±SD./ * Chi square test/# Independent sample *t* test

Table 2: Vitamin D status in pemphigus vulgaris patients and healthy subjects

	Group	Number	Mean	Std. Deviation	Mean difference with 95% CI	*P* value*
Serum vitamin D (ng/ml)	Patients	32	11.7966	8.82	-8.90 (-15.51, -2.29)	0.009
	Controls	36	20.6975	16.77		

* Independent sample *t* test

Table 3: Correlations of vitamin D with duration and severity of disease

		Duration of disease*	Total severity#	Skin severity#	Oral severity#
Vitamin D	*r*	-.182	-.349	-.078	-.394
	P value	.319	.051	.673	.026

* Pearson's correlation coefficient/# Spearman's rho

Discussion

Serum vitamin D in pemphigus patients

According to our findings, serum vitamin D was significantly lower in newly diagnosed PV patients compared to healthy control subjects. These data is in consistence with the study of El-Komy et al., who reported a lower serum 25(OH)D and a higher incidence of suboptimal vitamin D levels in PV patients. They conclude that the associated vitamin D insufficiency in patients may exacerbate their disease through various immune related mechanisms (14). In addition, Marzano et al. found the same data and hypothesized that vitamin D deficiency may play a key role in the pathophysiology of pemphigus vulgaris (15). Despite the importance of the potential relationship between vitamin D and PV, there are a few published studies on this subject and all of them showed the same results as ours. However, the exact underlying mechanisms are not well recognized yet. The strength of the present study is to include only newly diagnosed PV patients who did not use any medication until then. However, in other studies patients used to take drugs such as steroids in addition to either azathioprine or mycophenolate mofetil. Administration of steroids might affect the calcium and vitamin D levels. There was a potential for selection bias, which was minimized by sampling from the greatest referral center of dermatology in Iran, and patients were referred from all over the country. Therefore, we think it would be possible to generalize these data to other patients.

Low vitamin D because of disease

The low vitamin D status in PV patients might be due to several reasons. One possible suggestion is the decrease in skin capacity to produce vitamin D as it happens in burned patients (16). In addition, these patients might have a reduced sun exposure to protect their damaged skin. Some studies reported the production of anti-vitamin D antibodies in autoimmune skin diseases such as systemic lupus erythematosus (17). However, more studies are needed to find out if there are such antibodies in PV patients.

Low vitamin D as a cause of disease

On the other hand, the insufficient vitamin D level can be considered as an environmental factor that contributes to the pathophysiology of disease. The key immune-modulatory role of vitamin D is well established. Low serum vitamin D level has been observed in several autoimmune diseases such as systemic lupus erythematosus (SLE) (18), insulin-dependent diabetes mellitus (IDDM) (19), rheumatoid arthritis (RA) (20) and multiple sclerosis (MS) (21).

Possible underlying mechanisms

Calcitriol can prevent autoimmune diseases by inhibiting T helper 1 (Th1) cytokines (Interleukin-2, tumor necrosis factor alpha and interferon gamma) (22), promoting Th2 cytokines (IL-4 and IL-10) (23), suppressing Th-17 activity and IL-17 production (24, 25) and most importantly inducing regulatory T cells (Tregs) to modulates/down-regulates *immune responses (26). Since r*ecent studies suggest that control of Desmoglein-3-reactive lymphocyte by Tregs can prevent the development of pemphigus vulgaris, vitamin D can be considered as a potential candidate to facilitate this process (27). In addition, there is a tight relationship between vitamin D and skin. Vitamin D precursor is produced by skin cells and plays a pivotal role in skin health. It can stimulate keratinocyte differentiation and induce toll like receptor 2 (TLR2) that initiate the innate immune response to invading infections and help wound

healing and tissue repair (28). Therefore, an optimal level of calcitriol is necessary for preventing the exacerbation of skin diseases like PV.

Conclusion

This study shows that serum vitamin D level is significantly lower in newly diagnosed pemphigus vulgaris patients who did not use any medication compared to healthy controls. There was a negative correlation between the vitamin D level and the severity of disease, specially the oral severity. An optimal level of calcitriol might be important in preventing both incidence and exacerbation of pemphigus vulgaris and policy makers should pay more attention to solve the problem of common vitamin D deficiency/insufficiency in populations. However, no causality can be concluded from this case-control study. To determine the exact effect of vitamin D, some well-designed clinical trials and cohort studies are recommended.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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