British Journal of Medicine & Medical Research
5(1): 50-56, 2015, Article no.BJMMR.2015.005
ISSN: 2231-0614

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Vitamin D Status in Moroccan Patients with Pemphigus

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/BJMMR/2015/5596
Editor(s):
(1) Divya Kesanakurti, Dept. of Cancer Biology and Pharmacology, University of Illinois College of Medicine, USA.
(2) Anonymous
Reviewers:
(1) Anonymous, Boston University School of Medicine, USA.
(2) Anonymous, Pasteur institute of Morocco, Morocco.
(3) Anonymous, University of Milan, Italy.
(4) Anonymous, Aix Marseille University, France.
Peer review History: http://www.sciencedomain.org/review-history.php?iid=644&id=12&aid=5943

Original Research Article

Received 27th June 2013
Accepted 25th September 2013
Published 5th September 2014

ABSTRACT

Objective: The aim of this study was to assess the vitamin D status and the factors that may influence this vitamin in Moroccan patients with pemphigus.
Study Design: Case-control study.
Place and Duration of Study: Department of Dermatology at the University Hospital of Rabat-Salé between 2012 and 2013.
Methods: In this case-control study, 30 patients with pemphigus were included. Sixty healthy persons were recruited to serve as controls. The patients underwent anthropometric assessment and clinical evaluation. Serum 25 hydroxyvitamin [25(OH) D] D2 and D3 were measured using radioimmunoassay. Hypovitaminosis D was defined as serum 25(OH) D <30 ng/ml.
Results: The average age of participants was 56 years ± 11.4. Hypovitaminosis D was observed in

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100% of patients. The prevalence of hypovitaminosis D was higher ($p < 0.001$) in patients than in controls. Serum 25(OH) D levels were associated with disease duration ($r=0.34$, $p=0.05$), ESR ($r=-0.31$, $p=0.05$) and PTH ($r=-0.66$, $p<0.001$). No relationship was found between serum 25(OH) D levels and the other characteristics related to patients or pemphigus.

Conclusions: Our study suggested that serum levels of vitamin D were significantly lower in Moroccan patients with pemphigus. Future studies with a larger population are needed to confirm our results.

Keywords: Pemphigus; 25-hydroxyvitamin D.

1. INTRODUCTION

Pemphigus is a rare group of autoimmune bullous diseases characterized by widespread blistering and erosions of the skin and mucous membranes [1]. Its incidence has been estimated at between 1 to 16 new cases per million people per year [2]. Pemphigus follows a potentially life-threatening course; immunosuppressive agents, particularly corticosteroids, represent the treatment of choice for this disease [3]. It is caused by circulating autoantibodies targeting epidermal cell surfaces, which results in disruption of epidermal cell adhesions and formation of intraepidermal blisters [4].

In recent years, vitamin D has attracted a significant amount of attention from researchers. Epidemiologic studies suggest that the prevalence of vitamin D insufficiency is increasing globally [5]. In addition to its important metabolic activities, vitamin D also contributes to the regulation of the immune system [6]. Epidemiological evidence indicates a significant association between vitamin D deficiency and an increased incidence of autoimmune diseases [7]. Vitamin D has been recognized as a factor that may trigger or exacerbate autoimmunity and whose deficiency is associated with the incidence and/or severity of different autoimmune disorders [8]. Major immune system-mediated rheumatic diseases like rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis or overlap syndromes, such as undifferentiated connective tissue disease and others, are characterized by low-serum levels of vitamin D that often correlated to the severity of the disease [9]. Previous studies have shown that 25-hydroxyvitamin D_3 ($1,25(OH)_2D3$) represses mRNA and transcription of several proinflammatory cytokines, including IL-2, IL-17 and granulocyte macrophage colony-stimulating factor [10]. It expands circulating Treg populations and directly affects B-cell homoeostasis and inhibits proliferation and increases apoptosis of B cell [11]. As we know, In Pemphigus vulgaris, the loss of keratinocyte intraepidermal cell-cell adhesion and acantholysis is a result of pathogenic Dsg3-antibodies production by B cells [12-13]. Vitamin D3 inhibits proliferation and induces differentiation of murine and human keratinocytes in culture [14]. And with its synthetic analogs, they inhibit excessive proliferation of keratinocytes in psoriasis [15]. All these data suggest the important role of the vitamin in pemphigus. However, data is limited regarding the status of vitamin D in patients with pemphigus. The aim of our study was to examine the vitamin D status and the factors that may influence it in Moroccan patients with pemphigus.

2. MATERIALS AND METHODS

This case-control study recruited 30 patients with pemphigus. They were admitted to the Department of Dermatology at the University Hospital of Rabat-Salé between 2012 and 2013. Patients were excluded if they had either an additional chronic disease or were receiving treatment (except corticosteroids) that could influence vitamin D status. No patient was taking vitamin D supplementation.

A detailed questionnaire was completed based on the information obtained from the patient's medical records and by interviewing all of the participants. Collected data included age, sex, wearing a veil, skin phototype, Body Mass Index (BMI), subtype of pemphigus, disease duration, and medication (corticosteroid, immunosuppressive drugs).

The diagnosis of pemphigus was based on typical clinical findings as well as histopathological and immunopathological (indirect immunofluorescence assay) criteria. Pemphigus has been classified superficial and deep. The disease severity was evaluated using the pemphigus disease area index (PDAI) and the autoimmune bullous skin disorder intensity score (ABSIS) [16]. Erythrocyte sedimentation

rates (ESR), C-reactive protein (CRP), indirect immunofluorescence (IFI) were collected. Indirect immunofluorescence detects circulating IgG antibodies directed against the epithelial cell surface.

2.1 Serum 25-hydroxyvitamin D

Serum concentrations of albumin-adjusted calcium, parathyroid hormone (PTH) and 25-hydroxyvitamin D_2 and D_3 [25(OH) D] were measured. The measurements were done in the same laboratory. The PTH was measured by immunometric assay and serum 25(OH) D was measured in nanograms per milliliter using radioimmunoassay (RIA). The RIA method is based on an antibody with specificity to 25-OH-D. We defined levels <20 ng/ml (50 nmol/L) as vitamin D deficiency; levels from 20–29 ng/ml (50–72 nmol/L) as vitamin D insufficiency; levels ≥30 ng/ml (75 nmol/L) as adequate [17, 18] and hypovitaminosis D was defined as 25-OH vitamin D levels < 30 ng/ml. Daily sun exposure over the previous week was assessed using a reported questionnaire [19].

2.2 Controls

The control group consisted of 60 patients with similar age and gender to the study group with a comparable sun exposure score. Patients selected for the study were recruited from the city of Rabat through advertisements in local hospitals. They underwent physical examination and demographic characteristics were noted. Details concerning vitamin D were also collected. Individuals with chronic diseases were excluded from the control group.

2.3 Statistical Analysis

Analyses were performed using the software program SPSS (Windows Version 13.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to assess the demographic variables and characteristics of disease. The association between levels of vitamin D and other variables was studied using Student's t-test for qualitative variables and correlation for quantitative. The Chi Square and Student's t-test were used to compare levels of vitamin D in both patients and healthy controls. P values ≤0.05 were considered significant.

3. RESULTS

Thirty patients, 22 females (73%), with pemphigus were included in this case-control study. The average age of participants was 56 years ±11.4. The median disease duration was 2 years [0-7.25]. The most common subtype was profound pemphigus, 21 patients (70%). The median ABSIS, PDAI, and indirect immunofluorescence were respectively 20.5 [4.5-35.56], 22.5 [13-37.25] and 50 [20-160]. Patients' characteristics are presented in Table 1.

Table 1. Socio-demographic and disease characteristics of the patients

Females[1]	22 (73)
Age (years)[2]	56 ± 11.4
Wearing a veil [1]	21 (70)
Menopausal patients [1]	18 (60)
BMI (kg/m^2) [2]	26±5.6
Sun exposure[1]	1(3.3)
Skin phototype[1]	
1	0
2	0
3	3 (10)
4	24 (83)
5	3 (10)
Pemphigus type[1]	
superficial pemphigus	9 (30)
profound pemphigus	21 (70)
Disease duration (month)[3]	2 [0-7.25]
PDAI activity score[3]	22.5 [13-37.25]
PDAI sequel score[3]	0.5 [0-4]
ABSIS[3]	42.5 [29.12-114.25]
Skin ABSIS[3]	36.6 [4.5-97.2]
Mucosal ABSIS[3]	3 [0-5.25] / 3.75[0-30.3]
Medications used[3]	
Cumulative dose of corticosteroid (mg/day)	2030 [1072.5-8122.5]
Immunosuppressive (mg/day)	3000 [750-7500]
Corrected serum calcium (mg/l)[2]	99.4±6.63
Parathormone serum level (ng/l)[2]	72±24
ESR(mm/h [3]	22 [14-41]
CRP (mg/l)[3]	8 [3-52.5]
IFI(UI/L)[3]	50 [20-160]

[1]Number and percentage N (%); [2]mean and standard deviation; [3]median and IQR interquartile range; BMI: Body Mass Index; ABSIS: Autoimmune Bullous Skin Disorder Intensity Score; PDAI: pemphigus disease area index; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; IFI: indirect immunofluorescence

Table 2. Association between 25(OH)D levels and participant characteristics

	r or mean of vitamin D	p
Gender		
Male	17,9±6,5	0,1
Females	14,58±5,58	
Age (years)	0,02	0,99
Wearing a veil	14,5±5,7	0,2
Menopausal patients	14±5,6	0,1
Pemphigus type		
Superficial pemphigus	15±6,4	0,5
Profound pemphigus	16±4,7	
Disease duration (months)	0,34	**0,05**
BMI (kg/m^2)	-0.08	0.67
Cumulative dose of corticosteroid (mg/day)	0,20	0,26
Cumulative dose of immunosuppressive (mg/day)	-0,18	0,5
PDAI activity score	-0,11	0,5
PDAI sequel score	0,10	0,57
ABSIS	-0.02	0.16
IFI (UI/l)	-0,10	0,6
ESR (mm/h)	-0,31	**0,05**
CRP (mg/l)	-0,27	0,13
PTH (ng/ml)	-0,66	**<0,001**

BMI: Body Mass Index; ABSIS: Skin Disorder Intensity Score; PDAI: pemphigus disease area index *ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; IFI: indirect immunofluorescence, PTH: Parathormone serum level*

All patients had hypovitaminosis D [25(OH) D <30 ng/ml] (Fig. 1). The mean serum 25(OH) D level was 15.45ng/ml ±5.91.

The average levels of corrected serum calcium and parathormone serum were respectively 99.4 ±6.63 mg/l and 72 ±24 ng/l.

Serum 25(OH) D levels were associated with disease duration (r=0.34, P=.05), ESR (r=-0.31, P=.05) and PTH (r=-0.66, P<.001) (Table 2). We didn't find any significant association between serum 25(OH) D levels and other characteristics related to patients or pemphigus (Table 2).

The median age of control patients was 56 years ±10. Their characteristics are presented in Table 3. Seventy percent of them had hypovitaminosis D (Fig. 1).

The mean serum 25(OH) D level was 27.6 ng/ml ±10. The prevalence of hypovitaminosis D was higher (P<.001) and the serum 25(OH) D levels lower (P<.001) in patients than in controls (Table 4).

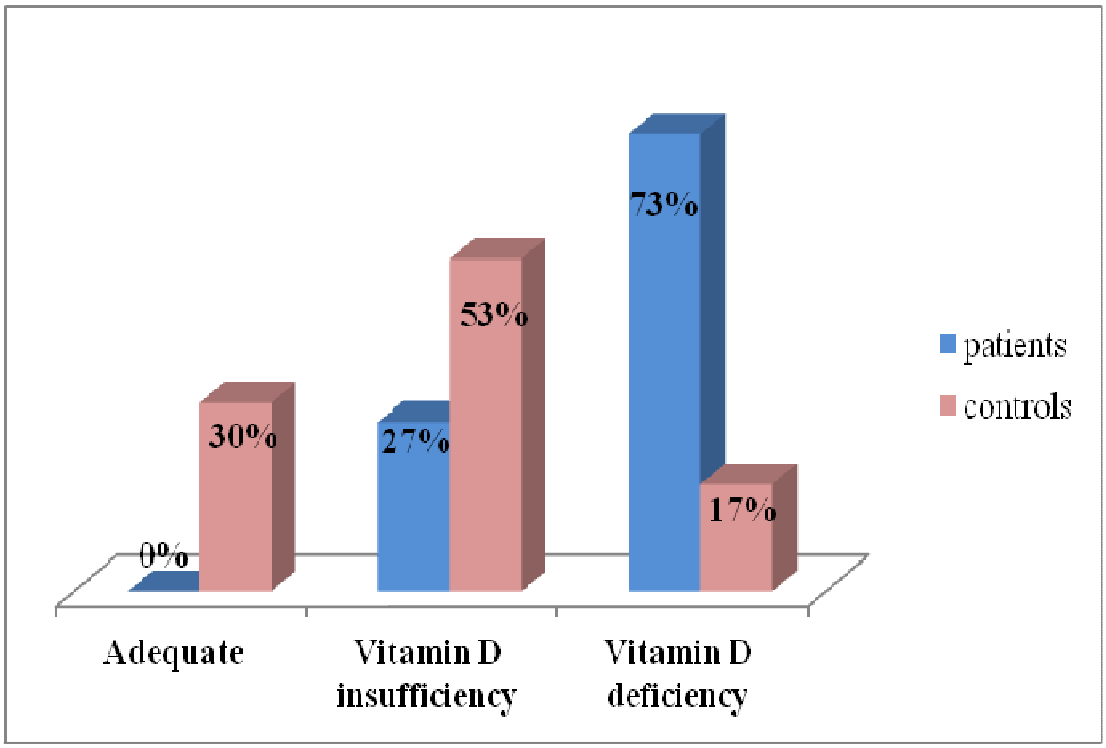

Fig. 1. Characteristics of 25-hydroxyvitamin D in patients and controls

73% of patients had vitamin D deficiency, while 17% of controls had. 53% of healthy controls had vitamin D insufficiency against 27% in patients. no patient had normal levels of vitamin D

Table 3. Characteristics of controls

Female[1]	44(73)
Age (years) [2]	56±10
Wearing a veil [1]	24 (54.5)
Menopausal patients [1]	31 (70.5)
BMI (kg/m^2) [2]	28±5
Parathormone serum level (ng/l) [2]	65±27.6
serum 25(OH) D level (ng/ml) [2]	27.6±10

[1]Number and percentage N (%); [2]mean and standard deviation; BMI: Body Mass Index; *25(OH) D: 25 hydroxyvitamin D*

Table 4. Comparison between patients and healthy controls

	Patients (n = 30)	Controls (n = 60)	P
BMI (kg/m^2), mean ± SD	26±5.64	28±4.9	0.07
25-Hydroxyvitamin D (ng/mL), mean±SD	15.4±6	27.6±9.7	<0.001
Subjects with hypovitaminosis D, n (%)	30(100%)	42(70%)	<0.001
Parathormone serum level (ng/l), mean ± SD	72±24	65±27.6	0.2

BMI: Body Mass Index

4. DISCUSSION

To the best of our knowledge, this is the first study in Morocco to evaluate vitamin D status in patients with autoimmune bullous skin disorders, especially pemphigus.

The main objective of this study was to determine the vitamin D status of Moroccan patients with pemphigus and the factors that may influence it in them. Our study showed a high level of hypovitaminosis D (100%) in patients with pemphigus. It found also that the percentage of patients with lower serum 25-OHD was high compared to the healthy controls. Our results agreed with those found in a two recent studies made by Marzano et al. [8] and EL-Komy and his team [11]. They found lower serum 25(OH) D levels in patients with pemphigus vulgaris or bullous pemphigoid and a higher prevalence of severe hypovitaminosis D than in controls. In fact, the high prevalence of vitamin D insufficiency is defined as a global health problem [20] and in recent years, many genetic and epidemiological studies have attempted to link the defects in the vitamin D system to increased prevalence of autoimmune diseases [21]. The fact that vitamin D has been implicated as a factor in several different autoimmune diseases suggests that vitamin D might be an environmental factor that normally participates in the control of self-tolerance [22]. Pelajo et al. [23] compared two groups, one with and one without autoimmune disorders. They found that of those individuals with autoimmune disorders, 23% had vitamin D deficiency [serum 25(OH) D <20 ng/ml], and of those in the non autoimmune group, 14% were vitamin D deficient. In a German population, vitamin D status was shown to strongly correlate with multiple sclerosis lesion frequency given a 2-month lag time [24]. Our findings also support those results since we found an association between serum 25(OH) D levels and disease duration.

Our results join with those of Marzano et al. [8], a study that showed no association existed between vitamin D levels and disease severity. This can be due to the limited number of patients. However our study showed an association between pemphigus and ESR. On the other hand, not all studies showed an association between vitamin D and disease activity. In Copenhagen, systemic lupus erythematosus patients had lower levels of 25(OH) D than healthy or osteoarthritis controls, yet vitamin D levels did not correlate with disease activity [25]. The same result was shown in a study on patients with cutaneous lupus erythematosus in whom no significant correlation could be found between vitamin D levels and disease activity parameters [26].

Intact PTH was significantly associated with hypovitaminosis D. Like us, Pepe et al. [27] found that in subjects with vitamin D insufficiency (n=53) [25(OH) D <30 nmol/l], mean serum levels of PTH were significantly higher (P<0.001) than those in subjects of similar age with normal vitamin status (n=209) with all the assays employed. Some authors reported that this association was best described by an exponential curve with an inflexion when serum 25(OH) D levels fell below a certain limit [28-29]. Steingrimsdottir et al. [30] reported that serum PTH was lowest in the group with a serum 25-hydroxyvitamin D level of more than 18 ng/mL, but highest in the group with a serum 25-hydroxyvitamin D level of less than 10 ng/ml. This association is not always present. EL-Komy and his team didn't find statistically significant correlation between serum 25OHD and parathormone [11]. Although a cross-sectional study such as our study is not sufficient to demonstrate the association between vitamin D

status and serum PTH levels. However, intervention studies are needed to further address this issue.

We also investigated the association of hypovitaminosis D with other factors related to patients or to disease including the wearing of a veil, menopause, BMI and cumulative dose of corticosteroid, but no association was found. Such a lack of association can be due to the small number of patients in the present study.

Despite the methodological limitations of this study (small sample size, cross-sectional, single-center study), our results could support the others studies reported in literature concerning the association between serum 25(OH) D levels and pemphigus disease.

5. CONCLUSION

This study adds evidence to the growing knowledge regarding vitamin D and autoimmunity. It showed that all of the patients had hypovitaminosis D and that they had more vitamin D deficiency than controls. Further large studies aimed at evaluating the vitamin D status and role of factors potentially affecting this vitamin in patients with autoimmune bullous skin diseases are necessary.

CONSENT

All authors declare that oral consent was obtained from the patients.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=644&id=12&aid=5943