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A case-control study: evaluation of vitamin D metabolism in patients with vitiligo

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Background/aim: Reduced vitamin D is considered as one of the environmental factors that can increase the prevalence of certain autoimmune diseases. This study aimed to assess vitamin D metabolism in patients with vitiligo.

Materials and methods: A prospective case-control study was conducted on 44 consecutive patients with vitiligo vulgaris and 43 healthy controls. Their plasma 25-hydroxyvitamin D (25(OH)D), parathormone (PTH), calcium, magnesium, and phosphate levels were measured.

Results: There was no significant difference in the mean age, sex and Fitzpatrick skin phototype between the patient and control groups ($P > 0.05$). The plasma levels of 25(OH)D and calcium were significantly decreased ($P = 0.002$, $P < 0.0001$, respectively) and PTH and magnesium levels were significantly increased in patients with vitiligo ($P < 0.0001$, $P < 0.0001$, respectively). The advancement of age ($P = 0.03$, $R = -0.18$) and comorbid autoimmune illnesses ($P = 0.04$) were found to be significantly associated with lower 25(OH)D levels.

Conclusion: There is a universal lack of 25(OH)D in the Turkish population. Screening for vitamin D may be a tool for the presence of comorbid autoimmune diseases. Further studies are needed to understand the role of vitamin D metabolism in the pathogenesis of vitiligo.

Key words: Vitiligo, 25-hydroxyvitamin D, vitamin D metabolism

1. Introduction

Vitiligo is a common acquired depigmenting condition of an unknown cause. Its immunopathogenesis is not completely understood but autoimmune etiology appears to be the most plausible (1). The relationship of vitiligo with other autoimmune disorders, and especially its coexistence with autoimmune thyroid diseases, has previously been reported (2,3). Recently, many autoimmune diseases including vitiligo have been found to be associated with reduced vitamin D levels (4,5).

Vitamin D has been implicated as one of the environmental factors that may trigger or exacerbate some autoimmune disorders including inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, type 1 diabetes mellitus, and rheumatoid arthritis (4,6). Vitamin D is a fat-soluble steroid hormone that is obtained through diet, supplements, or synthesis in the skin upon exposure to ultraviolet (UV) B radiation. 25-Hydroxyvitamin D (25(OH)D), with a half-life of about 2 weeks, is the major circulating vitamin D metabolite, and it is the routinely assessed as the most appropriate indicator of vitamin D levels in humans (7–9). Vitamin D classically regulates bone metabolism and calcium homeostasis. The nonclassic actions of vitamin D include regulation of cellular proliferation and differentiation, hormone secretion, and the immune system in both innate and adaptive immunity (4,10).

Vitiligo is an autoimmune depigmentation disorder and topical vitamin D analogs are effective for repigmentation (11,12). Furthermore, vitamin D is also considered to be associated with autoimmune disorders, and therefore the present study was designed to determine whether there is a relationship between vitiligo and vitamin D metabolism in comparison to healthy controls.

2. Materials and methods

This prospective case-control study consisted of patients who had been admitted to the outpatient clinic of

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dermatology at the Keçiören Research and Training Hospital (Ankara, Turkey) during the period of November 2011 to February 2012. In order to minimize the effect of seasonal changes on vitamin D levels, the study was conducted in the same winter period. All the patients enrolled in the study were living in Ankara, located in central Anatolia, where these months are considered to be winter and there is a lower level of exposure to UV light. This study was approved by the local ethics committee and written informed consent was obtained from each patient. A total of 44 patients with vitiligo (20 females, 24 males, mean age: 34.5 ± 16.1 years (range: 16–60 years)) and 43 control subjects (33 females, 10 males, mean age: 33.0 ± 12.6 years (range: 17 - 60 years)) were included in the study. Vitiligo was diagnosed on the basis of clinical findings. Healthy control subjects were recruited from among patients who were referred to our clinic for the treatment of nevus and had no systemic disease. In order to minimize the differences due to dietary intake of vitamin D, the patient and control groups were asked to estimate the amount of dairy products eaten per day and take notice of similar food habituation. Exclusion from the study was applied to the patients and controls who used any vitamin D supplements, topical vitamin D, or calcium-phosphate modifying drugs, or having any of the following problems: sensitivity or dairy allergy, and recent history of phototherapy, sunscreen usage, malignancy, hypercalcemia, hypocalcemia, hyperparathyroidism, or functional renal or liver abnormalities. Participants in either group with any history of smoking (current and former smokers) were also excluded from the study. Demographic data, duration of vitiligo and family history of vitiligo, and personal and family history of comorbid autoimmune disease were acquired from patient interviews. The Fitzpatrick skin phototype, clinical type, and affected body surface area (BSA) were assessed through a physical examination. The clinical appearance of vitiligo is classified into generalized (multiple scattered lesions in a symmetrical distribution pattern) and localized occurrence (13). Individuals with segmental vitiligo were not included the study. The BSA of depigmentation was divided into 2 groups: 1%–19% BSA and ≥20% BSA. Fasting blood samples were obtained by venipuncture of the large antecubital veins of the patients without stasis and after 12 h of fasting. The samples were immediately centrifuged; the plasma was separated and stored at –80 °C. In order to avoid variation, all samples were analyzed on the same day and using the same kit. The serum 25(OH) D level was measured by the RIA kit (25OH-VIT.D3 RIA-CT kit (Catalog No: KIP1961)). The serum calcium, phosphate, parathormone (PTH), and magnesium levels were measured using standard laboratory techniques. Vitamin D deficiency is defined as 25(OH)D below 20 ng/mL, and >30 ng / mL was considered normal (14).

Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). All the numerical variables are expressed as the mean ± standard deviation (SD). The normality of data was analyzed using a Kolmogorov–Smirnov test. A paired sample t-test was performed for the initial and final values of hormonal and biochemical data with homogeneous variability. A Wilcoxon signed-ranks test was used to analyze data with nonhomogeneous variability. For the comparison of categorical variables or percentages, we used Fisher's exact and chi-square tests. Differences between numeric variables were tested with Student's t-test or the Mann–Whitney U test and correlation was tested with Spearman's rank order or the Pearson correlation coefficient. $P < 0.05$ was considered to be statistically significant.

3. Results

The demographic data, characteristics of vitiligo, and comorbid autoimmune illnesses are given in Table 1. There was no significant difference in mean age, sex, and Fitzpatrick skin phototype between the patient and the control groups ($P > 0.05$). Of the patients, 6.8% had localized vitiligo and 93.2% had the generalized form of the disease. Six patients had a history of comorbid autoimmune illnesses, 2 patients had Hashimoto thyroiditis, 1 patient had Graves disease, and 3 patients had type 2 diabetes mellitus. A family history of autoimmune disorders was reported in 9.1% of the patients. The hormonal markers and biochemical parameters are given in Table 2. The analyses revealed that the 25(OH)D and serum calcium levels were significantly lower ($P = 0.002$, $P < 0.0001$, respectively) while PTH and serum magnesium levels were significantly higher ($P < 0.0001$, $P < 0.0001$, respectively) in the patient group than the control group. The serum 25(OH)D concentrations were deficient (<20 ng/mL) in 32 (72.7%) of the patient group, while in the control group 13 (30.23%) of the subjects had 25(OH)D deficiency ($P = 0.001$).

We found that advancement of age ($P = 0.03$, $R = –0.18$) and comorbid autoimmune illnesses ($P = 0.041$) were significantly associated with lower 25(OH)D levels. We did not observe a statistically significant correlation between vitamin D levels and sex, the clinical type of the vitiligo, percentage of the affected BSA, duration of the vitiligo, family history of vitiligo, or family history of autoimmune diseases in the patient group ($P > 0.05$).

4. Discussion

To the best of our knowledge, this is the first study investigating vitamin D metabolism including not only the vitamin D level but also the PTH, calcium, magnesium, and phosphate levels of patients with vitiligo in comparison with healthy controls. We found that calcium and 25(OH)

Table 1. Demographics of study subjects, characteristics of vitiligo, and comorbid autoimmune illnesses.

	Number of subjects	%
Mean age, years	37.5 ± 16.1	
Sex Male	24	54.5
Female	20	45.5
Fitzpatrick skin type		
II	16	36.3
III	28	63.7
Duration of disease (months)		
Mean ± SD	38.8 ± 59.1	
Type		
Localized	3	6.8
Generalized	41	93.2
Body surface area		
1%–19%	35	79.6
≥20%	9	20.4
Family history	4	9.1
Comorbid autoimmune illness		
Negative	39	88.6
Positive	6	13.6
Hashimoto disease	2	4.5
Graves	1	2.4
Diabetes mellitus	3	6.8
Family autoimmune history		
Negative	40	90.9
Positive	4	9.1

Table 2. Comparison of hormonal and biochemical parameters between patients and control group.

	Patients	Controls	P
25(OH)D (ng/mL)	16.35 ± 7.41	23.98 ± 13.5	0.002
PTH (pg/mL)	98.69 ± 41.23	58.65 ± 24.17	0.0001
Calcium (mg/dL)	9.36 ± 0.37	9.84 ± 0.39	0.0001
Phosphate (mg/dL)	3.15 ± 0.69	3.36 ± 0.48	0.10
Magnesium (mmol/L)	2.1 ± 0.13	0.80 ± 0.1	0.0001

25(OH)D, 25 hydroxyvitamin D; PTH, parathormone.

D levels decreased significantly while the PTH and magnesium levels were significantly increased in patients with vitiligo. We also observed that advancement of age and comorbid autoimmune illnesses were significantly associated with lower 25(OH)D levels in patients with vitiligo.

Vitamin D deficiency was found in both the patients and the controls. Hekimsoy et al. measured serum levels of 25(OH)D of adults in the Aegean region of Turkey and found a high prevalence of vitamin D deficiency (15). Van der Meer et al. demonstrated that the vitamin D status in the Turkish population varied widely in Turkey, according

to darker skin colors, sunscreen usage, insufficient intake of vitamin D in the diet, and the habit of using clothing to cover most of the body (16). In addition to these factors, in the present study, the low levels of vitamin D may be related to the time at which blood samples were collected, since all of the subjects were enrolled in the study in winter.

Accumulating data give rise to a link with vitamin D in the etiopathogenesis of vitiligo. Vitamin D exerts its effects through the nuclear vitamin D receptor (VDR). There are studies showing an association between VDR gene polymorphisms and vitiligo that avoids the increased risk for developing vitiligo (17,18). Saleh et al. detected highly significant 25(OH)D deficiency in patients with vitiligo when compared to healthy controls (19). However, they did not observe any significant correlation between associated autoimmune diseases and the serum 25(OH)D levels of patients. Xu et al. investigated the serum 25(OH) D deficiency in Chinese patients with vitiligo (20). Their data did not reveal a correlation between vitamin D levels and onset of vitiligo. On the other hand, they demonstrated that patients with vitamin D deficiency were at an increased risk of developing autoimmune diseases. In the study by Silverberg et al., 55.6% of the patients with vitiligo had insufficient (<30 ng/mL) and 13.3% had very low (<15 ng/mL) vitamin D levels (5). They found that comorbid autoimmune illness in subjects was associated with very low 25(OH)D levels, suggesting that vitamin D levels in patients with vitiligo may help to determine if additional testing for other autoimmune diseases is warranted. In the present study, serum 25(OH)D concentrations were significantly deficient (72.7% of the patient group) in vitiligo patients when compared to healthy controls and comorbid autoimmune illnesses were significantly associated with lower 25(OH)D levels in patients with vitiligo, supporting the findings of Silverberg et al.

In this study, we found significantly higher magnesium levels in the vitiligo group. This finding may be a mere stimulation of magnesium reabsorption in the renal tubule, absorption in the gut, and release of the ion from bone by the hyperproduction of parathormone due to vitamin D deficiency (21).

The exact cause of melanocyte loss in vitiligo is still debatable. It is considered to be the result of an autoimmune-mediated process, including both humoral (circulating antibodies targeting melanocyte antigens) and cellular immune aberrations leading to the destruction of melanocytes. Vitiligo is associated with overproduction of proinflammatory cytokines such as TNF-α, IL-6, and IL-2 (22). A less traditional function of vitamin D has been demonstrated as immunoregulation, including the regulation and differentiation of the cells of both the innate and adaptive immune systems (23,24). Active vitamin D has multiple immunosuppressant properties that inhibit the adaptive immune response by suppressing the proinflammatory processes (25). It has been shown to inhibit the differentiation and maturation of myeloid dendritic cells, and to suppress the antigen-presenting capacity of macrophages and dendritic cells. It also has antiproliferative effects on B and T cells and inhibits production of several proinflammatory cytokines such as IL-2, interferon-γ, and TNF-α of Th1 cells, known to be associated with vitiligo (4,10,26–28). Vitamin D compounds might limit the melanocyte loss in vitiligo by counteracting the local immune process in the skin, oxidative stress, programmed cell death, and aberrant calcium fluxes and by photoprotecting the epidermal melanin unit (28). In addition, vitamin D may stimulate the differentiation of immature melanocyte precursors and regulate melanocyte development and melanogenesis (29).

Vitamin D deficiency has been shown to facilitate the progression of existing autoimmune disease (30). The exact mechanism by which vitamin D affects autoimmunity is still unknown. It is a challenging question as to whether low 25(OH)D levels are the consequence or the cause of autoimmune disease. A further question is whether the low 25(OH)D levels in patients with vitiligo make subjects more susceptible to secondary autoimmunity, or whether vitamin D deficiency is a contributory cause of autoimmune inflammation.

Given the importance of vitamin D for a functional immune system and the profound deficiency observed in autoimmune diseases, as well as the correlation of vitamin D deficiency with the disease activity, the question arises as to whether the cells' immunity in autoimmune diseases are capable of responding appropriately to vitamin D. Prospective epidemiologic data on vitamin D intake and the risks of developing autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, and multiple sclerosis have been studied (31–33). However, given the small number of studies, no conclusions can be drawn.

However, as the pathogenesis of autoimmune diseases per se is still unclear, many of the molecular pathways suggest a potential role of vitamin D insufficiency in the progression of vitiligo. Furthermore, the mechanisms by which the deficiency of vitamin D triggers the autoimmune process are not fully elucidated. In addition to the previously reported data in the literature, our study revealed an altered vitamin D metabolism (significant decrease of 25(OH)D and calcium levels and significant elevation of PTH and magnesium levels) in patients with vitiligo. In this study, comorbid autoimmune illnesses were also significantly associated with lower 25(OH)D levels in patients with vitiligo. Further interventional studies are needed to understand the role of vitamin D metabolism in the pathogenesis of vitiligo and autoimmunity.

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