

# 25-hydroxyvitamin D status in patients with alopecia areata

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## Abstract

**Introduction:** Alopecia areata (AA) is a T cell-mediated autoimmune disease that causes inflammation around anagen-phase hair follicles. Insufficient levels of vitamin D have been implicated in a variety of autoimmune diseases.

**Aim:** To investigate the status of serum 25-hydroxyvitamin D (25(OH)D) in patients with AA, serum 25(OH)D concentrations were compared between AA patients and healthy controls and thus determine if a possible association exists between serum 25(OH)D levels and AA.

**Material and methods:** The study comprising 41 patients diagnosed with AA and 32 healthy controls was conducted between October 2010 and March 2011. The serum vitamin D levels of the study group were determined by high performance liquid chromatography. Serum levels of calcium, phosphorus, alkaline phosphatase, and parathyroid hormone were also evaluated.

**Results:** The study was based on 41 patients aged between 20 and 50 (mean: 32.8 ±7.5). The control group included 32 healthy persons aged between 20 and 51 (mean: 32.7 ±7.5). Serum 25(OH)D levels in patients with AA ranged from 5.0 to 38.6 ng/ml with a mean of 8.1 ng/ml. Serum 25(OH)D levels in healthy controls ranged from 3.6 to 38.5 ng/ml with a mean of 9.8 ng/ml. There was no statistically significant difference in the serum vitamin D level between AA patients and healthy controls ( $p > 0.05$ ).

**Conclusions:** Deficient serum 25(OH)D levels are present in patients with AA. However, considering the high prevalence of vitamin D deficiency in Turkey, no difference was noted between AA patients and controls.

**Key words:** alopecia areata, autoimmunity, vitamin D.

## Introduction

Alopecia areata (AA) is a tissue-specific autoimmune T cell-mediated disease. The exact pathogenesis of AA is not fully understood. However, recent available evidence supports an autoimmune targeting of hair follicles. The histological feature of AA is lymphocyte infiltration around and within affected hair follicles. Also macrophages and Langerhans cells around and within the hair follicles have been observed [1, 2]. Alopecia areata is an autoimmune disease and many autoimmune conditions are associated with reduced vitamin D levels, including rheumatoid arthritis, diabetes mellitus and multiple sclerosis [3].

Vitamin D is a fat soluble steroid synthesized in the skin from 7-dehydrocholesterol (as a hormone) or ingested with food (as a vitamin). It has a role in mediating the normal function of both the innate and adaptive immune systems and initiates biological responses via binding to

the vitamin D receptor (VDR) that is widely distributed in most tissues. Vitamin D has been implicated in processes that may trigger or exacerbate autoimmunity [4, 5]. Various studies report that vitamin D levels are associated with the incidence and/or severity of some autoimmune diseases including multiple sclerosis, lupus erythematosus, type 1 diabetes mellitus, and rheumatoid arthritis [6–9]. Also vitamin D analogues are effective topical therapies for cutaneous autoimmune conditions including psoriasis and vitiligo [10].

## Aim

The aim of this study was to evaluate the status of vitamin D in patients with AA. Because of the high prevalence of vitamin D deficiency in our geographic area, we also evaluated the levels in a healthy control group and compared the two groups between October 2010 and

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March 2011. In order to minimize the effect of seasonal changes on vitamin D levels, the study was conducted during the fall and winter months.

## Material and methods

This study was carried out in the Department of Dermatology with the approval of the Hospital Ethics Committee. Patients and controls had to sign informed consent, according to the Declaration of Helsinki.

### Patients

This study was conducted in the Department of Dermatology of the Turgut Ozal University Hospital, Ankara, Turkey from October 2010 to March 2011. Forty-one patients with AA (26 males and 15 females) and 32 healthy control subject (18 males and 14 females) were studied. Exclusion from analysis was based upon oral vitamin D supplementation; major cardiovascular, liver, kidney or digestive disease; treatment for AA 1 month before testing; or refusal to have laboratory testing.

Patient demographics including gender, age, history of AA onset, main site of involvement, duration and progression of the disease, Fitzpatrick skin phenotype, and personal and family history of comorbid autoimmune diseases were acquired during patient interviews in the department.

### Methods

We evaluated the levels of 25-hydroxyvitamin D (25(OH)D), calcium, phosphorus, alkaline phosphatase (ALP), and parathyroid hormone (PTH). Vitamin D was assessed as deficient if 25(OH)D levels were  $< 20$  ng/ml, insufficient if between 20 and 30 ng/ml, and sufficient if  $> 30$  ng/ml.

### Assays

Serum calcium, phosphorus, and ALP levels were measured with a spectrophotometric device using a commercial kit (Roche Integra 800). Vitamin D was quantified by high performance liquid chromatography (HPLC) (Vertical Mark of Column device by UFLC-SHIMADZU, with features by Verti Sep™ GES C18HPLC Column, ImmuChrom GmbH lot number VD-130218F). Serum levels of PTH were measured by a chemiluminescence immunoassay device (Siemens Centaur XP).

### Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, Illinois, United States). Normal or non-normal distributions of continuous variables were determined by the Shapiro Wilk test. The mean differences between groups were compared by using the Student's *t*-test; otherwise, the Mann-Whitney *U* or Kruskal-Wallis tests were used according to the

number of independent groups for the comparisons of median values. When the *p*-value from the Kruskal-Wallis test statistics is statistically significant, the Conover's non-parametric multiple comparison test was used to determine which group differs from the others.

Categorical data were analysed by the Pearson's  $\chi^2$  or Fisher's exact test (where applicable). The degree of association between the duration of symptoms and vitamin D levels was evaluated by Spearman's correlation analysis. Multiple Logistic Regression analysis was applied for determining the best predictor(s) to discriminate between case and control groups. Odds ratio and 95% confidence intervals for each independent variable were also calculated. A value of *p* less than 0.05 was considered statistically significant.

## Results

The study included 41 patients (26 males, 15 females) aged between 20 and 50 (mean:  $32.8 \pm 7.5$ ). The control group included 32 healthy people (18 males, 14 females) aged between 20 and 51 (mean:  $32.7 \pm 7.5$ ). There was no statistically significant difference between patient and control groups with respect to the mean age. Fifteen patients had a single patch and 26 patients had multiple patches; all lesions were on the scalp (Table 1).

Among the 41 patients with AA, a family history of AA was present in 12 (29.3%) patients; Hashimoto thyroiditis was found in 4 (9.8%) patients; type I diabetes mellitus occurred in 6 (14.6%) patients, and rheumatoid arthritis was found in 2 (4.9%) patients. An autoimmune disease was present in 4 (9.8%) patients (Table 2).

Serum 25(OH)D levels in patients with AA ranged from 5.0 to 38.6 ng/ml with a mean of 8.1 ng/ml. Overall, 93.8% had a vitamin D deficiency, 3.1% had a vitamin D insufficiency, and 3.1% had sufficient levels of vitamin D. Serum 25(OH)D levels in the healthy control group ranged from 3.6 to 38.5 ng/ml with a mean of 9.8 ng/ml. Overall, 85.3% had a deficiency, 9.8% had an insufficiency, and 4.9% had sufficient levels of vitamin D. There was no statistically significant difference in the serum vitamin D level between AA patients and healthy controls ( $p > 0.05$ ) (Figures 1 and 2). When the proportion of men and women in the study groups was investigated, there was no statistically significant difference in 25(OH)D levels ( $p > 0.05$ ).

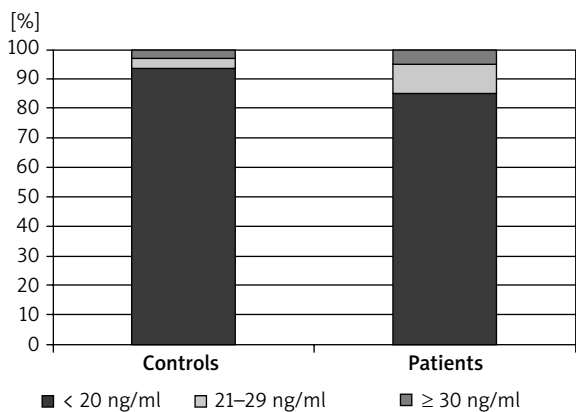
The serum 25(OH)D levels under 10 ng/ml were observed in 53.7% of patients and 59.4% of the control group. There was no statistically significant difference in the serum vitamin D levels under 10 ng/ml between AA patients and controls ( $p > 0.05$ ).

There was no statistically significant difference between the patient and control group with the respect to the levels of calcium, phosphorus, ALP, and PTH ( $p > 0.05$ ).

**Table 1.** Demographic data of the patient and control groups

Variables	Controls (n = 32)	Patients (n = 41)	P-value
Age [years]	32.7 ±7.5	32.8 ±7.5	0.961 <sup>†</sup>
Gender:			0.535 <sup>‡</sup>
Male	18 (56.3%)	26 (63.4%)	
Female	14 (43.7%)	15 (36.6%)	
Fitzpatrick skin phenotype:			0.587 <sup>‡</sup>
Type II	4 (12.5%)	8 (19.5%)	
Type III	20 (62.5%)	21 (51.2%)	
Type IV	8 (25.0%)	12 (29.3%)	
Vitamin D [ng/ml]	8.1 (5.0–38.6)	9.8 (3.6–38.5)	0.508 <sup>†</sup>
Vitamin D [ng/ml]:			
≥ 30	1 (3.1%)	2 (4.9%)	1.000 <sup>#</sup>
21–29	1 (3.1%)	4 (9.8%)	0.377 <sup>#</sup>
< 20	30 (93.8%)	35 (85.3%)	0.453 <sup>#</sup>
< 10	19 (59.4%)	22 (53.7%)	0.479 <sup>#</sup>
Calcium	9.4 ±0.44	9.4 ±0.47	0.954 <sup>†</sup>
Phosphorus	3.4 ±0.43	3.4 ±0.56	0.678 <sup>†</sup>
ALP	71.3 ±20.0	78.9 ±18.8	0.101 <sup>†</sup>
PTH	57.1 (19.8–247.0)	64.5 (22.7–143.5)	0.415 <sup>†</sup>

<sup>†</sup>Student's t test, <sup>‡</sup>Pearson's  $\chi^2$ , <sup>#</sup>Mann-Whitney U test, <sup>\*</sup>Fisher's exact test.



**Figure 1.** Comparison of serum 25(OH)D levels between alopecia areata patients and controls

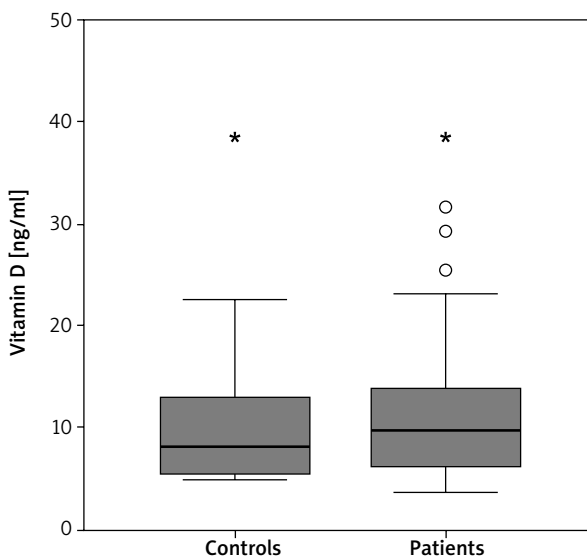
Results of the logistic regression models are presented in Table 3. The logistic regression analysis showed no significant effect of age, gender, or skin type on vitamin D status.

**Discussion**

The hair follicle is a highly hormone-sensitive organ [2]. Vitamin D is a hormone that plays an important role

**Table 2.** Clinical characteristics of patients with AA (n = 41)

Variables	Result
Duration of alopecia areata [months]:	
< 1	13 (31.7%)
1–3	10 (24.4%)
4–6	5 (12.2%)
> 6	13 (31.7%)
Involvement:	
Single	15 (36.6%)
Multiple	26 (63.4%)
Comorbid autoimmune disease:	4 (9.8%)
Hashimoto thyroiditis	4 (9.8%)
Family history:	12 (29.3%)
Hashimoto thyroiditis	4 (9.8%)
Type I diabetes mellitus	6 (14.6%)
Rheumatoid arthritis	2 (4.9%)



**Figure 2.** Comparison of vitamin D levels between controls and patients. The horizontal lines in the middle of each box indicates the median, while the top and bottom borders of the box mark the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively. The whiskers above and below the box mark the maximum and minimum vitamin D levels

in regulation of calcium homeostasis, both in cell growth and differentiation, as well as immune system regulation [4, 5]. Based on biological effects, a normal 25(OH)D level is ≥ 30 ng/dl. Vitamin D deficiency is being increasingly recognised worldwide due to differences in the dietary intake of vitamin D, varying durations of exposure to sunlight or the use of supplements, the prevalence of vitamin D deficiency shows different patterns across various populations [11]. Various studies report that vitamin

D levels are associated with the incidence and/or severity of some autoimmune disorders including type I diabetes mellitus, systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease [6]. Recent studies have indicated that vitamin D deficiency can be a significant risk factor for AA occurrence [12–15].

A study by Yilmaz *et al.* revealed low serum 25(OH)D levels in patients with AA compared with healthy controls [12]. Mahamid *et al.* found a strong correlation between AA and vitamin D deficiency in a study of 23 patients [13]. In another study, Aksu *et al.* showed that serum 25(OH)D levels were inversely correlated with disease severity of AA [14]. In a study based on 156 patients with AA and 148 controls, d'Ovidio *et al.* found that the insufficiency or deficiency of 25(OH)D was not significantly different between patients with AA and controls. However, a deficiency in 25(OH)D was present in 42.4% of patients, which was significantly higher than the 29.5% observed in healthy controls. In addition, the decreased level of 25(OH)D was not correlated with a pattern or extent of hair loss [15].

Our results do not agree with previous reports demonstrating the association between AA and vitamin D deficiency. In our study, we found that patients had a deficiency of 25(OH)D, but there was no statistically significant difference in the serum vitamin D levels between AA patients and healthy controls ( $p > 0.05$ ). This may be due to the universal tendency toward lower values of 25(OH)D in our geographical area. Hekimsoy *et al.* found a high prevalence of vitamin D deficiency (74.9%) and an insufficiency (13.8%) in a population-based sample in the Aegean region of Turkey [16]. In a study on 1010 paediatric patients in Turkey, Orun *et al.* found that the deficiency (24.3%) and insufficiency (16.5%) of 25(OH)D were frequent in childhood, especially in the adolescent period [17]. Van der Meer *et al.* demonstrated that vitamin D status in the Turkish population varied widely in Turkey, according to sunscreen usage, insufficient intake of vitamin D in the diet, darker skin colours, and the habit of using clothing to cover most of the body [18].

Vitamin D is recognized as the sunlight vitamin. The major source of this vitamin is skin synthesis of vitamin D. More than 90% of the vitamin D requirement for most people comes from casual exposure to sunlight. Natural dietary sources of vitamin D are limited [19]. In Middle Eastern populations who live in sunny climates, very low vitamin D levels have been reported, particularly populations from Lebanon, Iran, Jordan and Turkey [20–22]. This may be due to common environmental factors such as latitude, seasonality, pollution, customs or cultural issues, diet, or fortified-food policies. In addition, individual sociocultural and behavioural factors such as clothing, use of sunscreens with high sun protection factor, sunbathing habits, skin pigmentations, time spent outdoors, and insufficient playgrounds may affect the status of serum vitamin D levels.

**Table 3.** The results of multivariate analysis

Variables	Odds ratio	95% CI	P-value
Age [years]	0.986	0.922–1.054	0.675
Gender:			
Female	1.000	–	–
Male	0.819	0.254–2.636	0.737
Fitzpatrick skin phenotype:			
Type II	1.000	–	–
Type III	0.568	0.138–2.347	0.435
Type IV	0.627	0.132–2.979	0.557
Vitamin D	1.036	0.961–1.116	0.359
Calcium	0.823	0.259–2.619	0.741
Phosphorus	1.410	0.497–3.995	0.518
ALP	1.024	0.994–1.054	0.117
PTH	0.999	0.984–1.014	0.901

Our study has a few limitations. The study sample of 41 healthy individuals was small and the fact that blood samples were collected only once during the late fall and winter months between October and March. It would be useful to evaluate patients at different times of the year to study seasonal variations. Multicentre studies from different geographic areas around Turkey are needed.

## Conclusions

We found decreased serum 25(OH)D levels in patients with AA, but there was no statistically significant difference in the serum vitamin D level between AA patients and healthy controls. Further studies are needed to clarify the association between a deficiency of 25(OH)D and AA. But still in our opinion, we recommend screening blood vitamin D levels in AA patients and if deficient, adding oral vitamin D to the AA treatment protocol.

## Conflict of interest

The authors declare no conflict of interest.

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