Association of vitamin D level with alopecia areata

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Background: Alopecia areata (AA) is an autoimmune disorder of hair follicles. We aimed to find the association between Vitamin D level and AA.

Method: Eligible AA patients and controls were enrolled in this case-control study and serum samples were assessed for 25-hydroxy vitamin D (25-(OH)-D3). The levels of 25-(OH)-D3 were categorized as deficient (<10ng/ml), insufficient (10 to <30 mg/ml) and sufficient (>30ng/dl) and the SALT (Severity of Alopecia Tool) score was used to assess the severity of the disease. The data was analysed and the association between vitamin D levels and AA, disease distribution, and the pattern of hair loss was investigated.

Result: Twenty eight patients (19 males, 9 females) and 44 healthy controls (16 males, 28 females) were assessed. There was no statistically significant difference between patients and controls with regard to the level of $25(OH)D_3$ when the data was adjusted for gender (Ordinal odds ratio: 0.49 (0.18-1.34 and 95% CI, p-value=0.16). The level of $25(OH)D_3$ was lower in patients with nail involvement in contrast to those without it (P=0.02); moreover, no significant difference was found between patients with different patterns of hair loss.

Conclusion: After adjustment for gender, there was no association between AA and the level of vitamin D.

Keywords: 25-hydroxyvitamin D3, alopecia areata, severity of alopecia tool score, vitamin D

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INTRODUCTION

Alopecia areata (AA) is a common inflammation-induced hair loss disorder that is associated with increased risk of other autoimmune disorders [1,2]. Although 0.1 to 0.2% of the general population are affected [3], the lifetime risk of disease is estimated around 2% [4]. Patients in all ages may be affected; however, the majority of the cases are younger than 30 years of age. Moreover, the disease is shown to be more severe in males and those who are affected in early childhood [5]. AA is a common comorbidity in patients with rickets; on the other hand, mutations in vitamin D receptor (VDR), which is proposed as the underlying cause of AA in some rickets patients, has highlighted the importance of vitamin D in the pathogeneses of this disorder [6].

Vitamin D is a steroid hormone that primarily regulates calcium metabolism; it is synthesized from cholesterol under the effect of ultraviolet on keratinocytes of the skin and after modification in the kidney and liver, changes to an activated form. Calcitriol or $1,25(OH)_2D_3$ is the active form of vitamin D which bounds to VDR, a member of the steroid-thyroid-retinoid receptor gene superfamily of nuclear transcription factors, and regulates the expression of several genes [6].

Although the effect of vitamin D on keratinocytes and its effect on the pathogenesis of different dermatologic disorders is studied and reviewed [7,8], there is not enough data regarding vitamin D effects on AA. The aim of this study was to find the association between AA and level of vitamin D.

PATIENTS AND METHODS

We conducted this case-control study to evaluate the association of the serum level of vitamin D and AA in patients attending the dermatology clinics of Loghman-e-Hakim and Shohada-e-Tajrish University Hospitals from September 2010 through March 2012. The study protocol was designed in accordance with the Declaration of Helsinki and was implemented after obtaining the approval of the Ethics Board of Shahid Beheshti University of Medical Sciences.

Eligible patients were those with at least 10 years of age in whom the diagnosis of AA of the scalp was made by a dermatologist. As for the control group, we recruited healthy volunteers who accompanied patients with different dermatology problems at their attendance in our clinics.

In both groups, participants with the following conditions were excluded from the study: taking vitamin D or calcium supplements, frequent ingestion of alcohol, gastrointestinal problems, bone or renal disease, sarcoidosis, or any other metabolic and systemic disorder that could affect $25(OH)D_3$ absorption, metabolism, or serum level, treatment by systemic or topical steroids, barbiturates, bisphosphonates, sulfasalazine, or receiving PUVA or NBUVB during the previous three months.

A problem in measuring $25(OH)D_3$ was the seasonal changes in its levels; the highest and the lowest levels are seen at the end of the summer and at the end of the winter, respectively; Therefore, the number of controls who were enrolled in each season was proportionate to the number of patients enrolled in the same season.

The active form of vitamin D (1,25 (OH)2D3) is not a good indicator of serum vitamin D levels due to its short half-life and low serum levels; instead, the circulating form of vitamin D or 25(OH) D_3 has a longer half-life and higher serum levels and is more convenient to measure. Therefore, we measured 25(OH) D_3 in our study [9].

Baseline characteristics of the participants of both groups were documented. In order to assess the severity of alopecia in patients, our dermatologists used the SALT (Severity of Alopecia Tool) score, which is devised by National AA Foundation working committee [10]. In this scoring system, the scalp is divided to four areas of vertex, the right profile of the scalp, the left profile of the scalp, and the posterior aspect of the scalp which as account for 40%, 18%, 18%, and 24% of the scalp surface area, respectively. The SALT score is calculated by summation of hair loss percentage in these four areas.

In order to measure $25(OH)D_3$, 3.5 to 5 ml venous blood was obtained from the antecubital area by the hospital laboratory technicians while the participant was in the prone position for 10 minutes. The blood samples were centrifuged at 3000 rpm and the plasma was collected and stored at -80°C in the lab refrigerator for later measurement. The samples were sent to a reference laboratory in a container and after thawing, the level of $25(OH)D_3$ was measured by LIAISON kits (Diasorin LIAISON, Stillwater, Mn, USA) and via automated chemiluminescence immunoassay (CLIA). According to the levels of $25(OH)D_3$, the status of Vitamin D in the participants was graded as deficient (<10 ng/ml), insufficient (10 through <30 ng/ml), and sufficient ($\geq$30 ng/ml).

All statistical analyses were performed using the statistical software SPSS 16.0.0. (SPSS Inc. Chicago, IL, USA). Two-sided P values less than 0.05 were considered statistically significant. Continuous variables were expressed as mean±SD or as median with total and interquartile (25^{th}-75^{th} percentiles) ranges. In this study, multivariable ordinal logistic regression (which considers cut-off values for 25 (OH) D) was applied to determine the characteristics associated with the vitamin D status. The participants were categorized into 3 ordinal categories of vitamin D status: 0 to <10, 10 to <30, and $\geq$30 ng/mL. First, unadjusted ordinal logistic regression models were fitted to examine the association of vitamin D status with the participants' sex and group status (patients or healthy controls). Factors for which the unadjusted odds ratios (ORs) had a P value less than 0.20 were included in the multivariable ordinal logistic regression model.

The serum concentration of 25 (OH) D was

compared in patients with nail involvement versus patients with no involvement of nails using the Mann-Whitney-U test. The Kruskal-Wallis test was applied to compare the concentration of 25 (OH) D among the patients with different patterns of hair loss.

RESULTS

This study included 28 patients with AA (9 women and 19 men) and 44 healthy controls (28 women and 16 men). Baseline demographics and clinical characteristics of the patients with AA and healthy controls are summarized in Table 1. The distribution of participants across the ranges of 25-hydroxy vitamin D is illustrated in Table 2.

In the unadjusted ordinal logistic regression models, low 25 (OH) D concentrations were less likely to be observed in participants who were male and had AA (P=0.001). In our multivariable ordinal logistic regression model, the group status adjusted for participants' sex was not statistically associated with the 25 (OH) D status (p=0.16).

In this study, the serum concentration of 25(OH) D was significantly lower in patients with nail involvement when compared to patients with no involvement of the nails (p=0.02, Mann-Whitney-U-test). No significant differences were observed among the patients with different patterns of hair loss according to their serum concentrations of 25(OH) D (p=0.75, Kruskal-Wallis test). According to our findings, the median serum concentration of 25(OH) D was lower in patients with active hair loss although the difference was not statistically significant (p=0.07).

DISCUSSION

The levels of vitamin D were lower in controls in comparison with the patients in this study; however, no significant difference was detected

Table 1. Baseline demographics and clinical characteristics of patients with alopecia areata and healthy controls

	Patients with alopecia areata (n=28)	Healthy controls (n=44)
Age, years		
Mean±SD	27.75±7.97	33.16±12.52
Median (range)	27.5 (18-48)	30.5 (10-83)
Gender, no. (%)		
Female	9 (32.14%)	28 (63.63%)
Age at onset of disease, years		
Mean±SD	19.90±9.65	-
Median (range)	18.5 (4-42)	-
Duration of disease, years		
Median (range)	6 (0.5-20)	-
Subgroups of hair loss duration		
<3 months	0	-
3–12 months	1 (3.57%)	-
12–24 months	1 (3.57%)	-
>2–5 years	5 (17.86%)	-
>5 years	21 (75.00%)	-
SALT† score		
0-24	6 (21.40%)	-
25-49	4 (14.30%)	-
50-74	1 (3.60%)	-
75-99	0	-
100	17 (60.70%)	-
Pattern of hair loss		
Patchy	10 (35.70%)	-
Totalis	8 (28.60%)	-
Universalis	9 (32.10%)	-
Ophiasis pattern	1 (3.60%)	-
Body hair loss		
None	12 (42.86%)	-
Some	10 (35.71%)	-
100%	6 (21.43%)	-
Nail involvement, no. (%)	10 (35.71%)	-
Activity of hair loss	14 (50.00%)	-
History of PTSD before onset of disease	10 (35.71%)	-
History of thyroid disease	3 (10.71%)	-
Family history of alopecia areata	1 (3.57%)	0
History of atopic	8 (28.57%)	-

* Values are mean±SD unless otherwise noted
† SALT: Severity of Alopecia Tool Score ranges from 0 to 100, with 0 indicating no alopecia areata and 100 indicating severe disease

Table 2. Distribution of 25-hydroxy vitamin D

		25-hydroxy vitamin D			
Groups of study	**Sex**	**<10 ng/mL**	**10 to <30 ng/mL**	**≥ 30 ng/mL**	**Total**
Patients	Male	5	10	4	19
	Female	4	4	1	9
	Total	9 (31.1%)	14 (50.0%)	5 (17.9%)	28
Healthy controls	Male	4	11	1	16
	Female	19	9	0	28
	Total	23 (52.3%)	20 (45.4%)	1 (2.3%)	44

between patients with AA and healthy controls after adjustment for sex. The proportion of female participants was 63% and 32% of the patients and controls, respectively. Lower levels of $25(OH)D_3$ were more frequently found in female patients rather than males in both groups, which is most likely due to the women's limited exposure to UV because of religious and regional dress codes.

The role of vitamin D in the pathogenesis of AA has been a matter of interest for many years; the evidence has arisen from the reports of AA in patients with hereditary vitamin D resistant rickets (HVDRR) [11]; in fact, the mutation of VDR is demonstrated to be the cause of hair loss in these patients. On the other hand, the effect of vitamin D on protecting hair follicles from chemotherapy-induced alopecia [12,13] provides further evidence for the role of vitamin D in hair growth and possibly hair loss; however, this effect also depends on the applied chemotrapeutic agent [8]. While most of the studies have investigated the role of vitamin D in AA in HVDRR, there is limited clinical or experimental research focusing on the role of vitamin D in the pathogenesis of AA in the general population. In contrast to HVDRR, the prevalence of AA in the general population, the autoimmune nature of disease, and its reversibility cannot be explained by a single receptor mutation; that is to say, there are differences in genotype with regard to ethnicity [14]. In one study in Turkey, no relationship was found between AA and VDR gene polymorphism [15]. Therefore, the mechanism of the pathogenesis in AA might be the effect of vitamin D on immune cells rather than changes in VDR.

The association of vitamin D levels with autoimmune diseases including diabetes mellitus [16], systemic lupus erythematosus [17], and rheumatoid arthritis [16] has been already investigated. The serum level of vitamin D has also been assessed in another autoimmune disease, i.e. vitiligo. In a cohort study, 25(OH) D3 demonstrated bimodal distribution in vitiligo vulgaris patients; there were some patients with normal levels while low levels were detected in others. In addition, they demonstrated incremental changes in vitamin D levels as the skin complexion became lighter according to Fitzpatrick skin type classification [18]. Evidence suggests a relationship between autoimmune diseases and UV radiation and therefore vitamin D, with respect to latitude. For example, the course of multiple sclerosis fluctuates with changes in the levels of vitamin D along with seasonal changes [19]. In addition, the prevalence of psoriasis is higher toward the poles and decreases in the areas around the equator [20] and a case-control study demonstrated lower levels of vitamin D in these patients in contrast to healthy controls [21].

AA is a $CD8^+$ T lymphocyte-dependent autoimmune disease; in rat models, depleting $CD8^+$ T-cells leads to hair restoration [22]. Transferring T-cells from an affected mouse to a normal model can lead to AA [23]. The collapse of constitute immune privilege is hypothesized to be the underlying cause of AA [24]. This hypothesis states that in the presence of immune signals, other immune system components, preexisting auto-activated $CD8^+$ lymphocytes can attack hair follicles [25]. On the other hand, vitamin D implies its immunomodulatory effects via its nuclear receptors expressed in antigen-presenting cells (APCs) and T-lymphocytes [26]. The rate limiting enzyme in converting 25(OH) D3 to the active form of vitamin D, namely 1-α-hydroxylase, is expressed by macrophages and unlike its renal form, not only is this enzyme under regulation of immune system signals but also no negative feedback is implied by the end product. This enzyme is also expressed by dendritic cells, another important APC [27,28]. In dendritic cells, calcitriol inhibits IL-12 production and hence, suppresses T-cells activation. In addition, vitamin D can promote helper T-cells type 2 (Th_2) proliferation and increase the production of IL-4, IL-5, IL-10, interferon-gamma (IF-γ) in antigen-stimulated $CD4^+$ T-cells [29]. Therefore, if any association exists between AA and vitamin D, it would be from the effects on the immune system and modulation of T-cells functions rather than the VDR polymorphism.

Our study could neither link the vitamin D levels to AA nor reject such an association; it might be to some extent due to the small sample size and not matching patients for gender. The levels of exposure to the UV, seasonal changes and altitude, level of activity, and body mass index (BMI) should be considered to justify the level of vitamin D in patients in further studies; moreover, the gender was a confounding factor in our study and participants must be matched for gender. This pilot study was the first study that examined the association of vitamin D levels and AA. Further investigation with focus on the

immunomodulatory effects of vitamin D in the pathogenesis or treatment of this disease would disclose the role of vitamin D in AA.

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