

Association between Vitamin D Levels and Alopecia Areata

Mahmud Mahamid MD^{1,2,3}, Omar Abu-Elhija MD^{2,3}, Mosab Samamra MD¹, Ammad Mahamid MD³ and William Nseir MD^{2,3}

¹Digestive Disease Institute, Shaare Zedek Medical Center, Jerusalem, Israel

²Department of Internal Medicine, Holy Family Hospital, Nazareth, Israel

³Metabolic Diseases Unit, Holy Family Hospital, Nazareth, Israel

ABSTRACT: **Background:** Alopecia areata (AA) is an autoimmune disease, based on the response to local and/or systemic corticosteroid treatment. The role of vitamin D in the pathogenesis of immune/autoimmune mediated diseases has been widely studied. **Objectives:** To investigate a possible association between serum 25-hydroxyvitamin D levels and alopecia areata. **Methods:** The study included 23 patients diagnosed with AA followed at our outpatient clinic during the period March 2010 to May 2011, as well as a control group matched for age and gender. All subjects underwent a complete work-up and medical examination, anthropometric measurements and laboratory tests. Laboratory tests included complete blood count, C-reactive protein (CRP), and vitamin D levels. **Results:** Mean CRP values were significantly higher in the AA group than the control group (1.1 ± 0.7 mg/dl vs. 0.4 ± 0.8 mg/dl, $P < 0.05$). Vitamin D levels were significantly decreased in the AA group (11.32 ± 10.18 ng/ml vs. 21.55 ± 13.62 ng/ml in the control group, $P < 0.05$). Multivariate analysis showed that CRP (odds ratio 3.1, 95% confidence interval 2.6–4.2, $P = 0.04$) and serum vitamin D levels < 30 ng/ml (OR 2.3, 95%CI 2.2–3.1, $P = 0.02$) were associated with AA. **Conclusions:** We found a significant correlation between AA and vitamin D deficiency. Vitamin D deficiency can be a significant risk factor for AA occurrence.

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KEY WORDS: alopecia areata (AA), vitamin D, immune disease, vitamin D receptor (VDR)

Alopecia areata is the most common cause of inflammation-induced hair loss (non-scarring hair loss) that can involve any hair-bearing area [1]. Clinically, AA can present with different clinical manifestations varying from reversible patchy hair loss to complete baldness or complete body hair loss [2]. The prevalence of AA is 0.1 to 0.2%, with a calculated lifetime risk of 2%. AA affects both children and adults, and hair of all colors. There is no gender predilection, but according to the literature more men are affected [3].

AA is considered an organ-specific autoimmune disease associated with an increased overall risk of autoimmune disorder (16%) such as systemic lupus erythematosus, vitiligo

and autoimmune thyroiditis [4]. Studies from the last decade have clearly demonstrated that AA is an autoimmune disease mediated by T lymphocytes in which autoantigens are necessary to activate T cells that generate the disease [5]. Animal experimental models of AA have shown complex immunological changes, such as inflammatory infiltrate consisting of CD4⁺CD8⁺ lymphocytes, macrophages and B cells, and a local increase in the cytokines interleukins-2 and 6 [6,7].

While the treatment for patchy AA is effective, treatment for widespread disease is unsatisfactory. The available options are topical steroids, topical minoxidil, intralesional steroids and, for extensive disease, oral or pulse steroid therapy [8,9].

More than 1 billion people worldwide have been identified as vitamin D-deficient [10]. Vitamin D deficiency has also been widely reported in all age groups from sun-rich countries such as Israel [11]. The key role played by vitamin D together with calcium in bone health is well known, and other non-classical actions of vitamin D are recognized. Interaction with the immune system is one of the most well-established non-classical effects of vitamin D [12]. Vitamin D deficiency has also been associated with increased risk of respiratory disease including infections (influenza and *Mycobacterium tuberculosis*) and chronic respiratory diseases such as cystic fibrosis [13].

The active form of vitamin D, 1,25-dihydroxyvitamin D, mediates its action by binding to specific vitamin D receptors located in the nucleus of target cells. It has been demonstrated that VDR is strongly expressed in the key structure of human hair follicles [14]. The role of vitamin D deficiency in the development of immune mediated disease in the last two to three decades sparked considerable interest. Studies suggest a link between vitamin D deficiency and autoimmune diseases, such as rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus, as well as cancer [15,16].

In the present study we sought a possible association between serum 25-hydroxyvitamin D levels and alopecia areata.

PATIENTS AND METHODS

This was a prospective study in which 23 consecutive patients diagnosed with AA were enrolled and followed at the outpatient clinics of the Holy Family Hospital, a 150-bed primary care hospital in Nazareth, Israel, and Maccabi Healthcare Services (one of four health funds in the country) between March 2010 and

AA = alopecia areata

May 2011. The diagnosis of AA was made by two senior dermatologists according to the clinical evaluation. If the diagnosis was not clear after clinical evaluation, a skin biopsy was performed to confirm the diagnosis. Demographic, clinical and laboratory data of the patients were recorded. Excluded from the study were patients with other skin disorders and/or another cause of alopecia (tinea capitis, trichotillomania, androgenic alopecia, scarring alopecia, traction alopecia, secondary syphilis, telogen effluvium, female androgenic alopecia), as well as patients with autoimmune or systemic diseases (SLE, rheumatic arthritis, scleroderma, thyroiditis, hyperthyroidism, hypothyroidism).

The control group included 20 individuals without a history of AA who were enrolled randomly from our clinics. Demographic, clinical, anthropometric measurements (height, weight, body mass index) and laboratory data of the control group were also collected. All subjects underwent a complete medical examination and laboratory tests. Laboratory tests were performed within 30 days of enrollment in the study and included complete blood count, C-reactive protein and vitamin D levels. In all patients diagnosed with AA, serum 25 (OH) vitamin D levels were measured twice a year (in the winter and summer seasons due to previous data that showed a seasonal variability) using a commercial enzyme immunoassay. The normal cutoff for CRP was 0–0.5 mg/L, while the normal range of vitamin D levels was 30–50 ng/ml. We then defined vitamin D insufficiency as vitamin D < 30 ng/ml and vitamin D deficiency as < 20 ng/ml.

STATISTICAL ANALYSIS

Data were analyzed using SPSS version 19 (IBM SPSS, Chicago, IL, USA). Continuous variables were expressed as the mean \pm standard deviation. The chi-square test was used to test differences in categorical variables between the two groups. A multivariate analysis was performed to determine the association between the occurrence of AA and the variables. A *P* value < 0.05 was considered significant.

RESULTS

A total of 43 patients were enrolled in our study, 23 patients with AA and 20 who served as controls. The AA group comprised 14 males and 9 females with a mean age of 24.2 ± 12.3 years and mean duration of diagnosis 1.3 ± 1.4 years. The mean BMI was 22.3 ± 4.7 . Eighteen patients had patchy disease and 5 patients had extensive disease; 5 patients were treated with local topical steroids, one patient with intralesional steroids and 17 were without current therapy. Of the 20 patients in the control group, 13 were males and 7 were females with a mean age of 27 ± 11.26 years and mean BMI 23.1 ± 3.2 (clinical and laboratory data are presented in Table 1).

SLE = systemic lupus erythematosus
CRP = C-reactive protein
BMI = body mass index

Table 1. Demographic and laboratory data comparing AA and control group

	AA group (n=23)	Control group (n=20)	P value
Gender (male)	14	13	NS
Age (yr)	24.2 ± 12.3	27 ± 11.26	NS
Body mass index	22.3 ± 4.7	23.1 ± 3.2	NS
White blood count	$9.8 \pm 2.3 \times 10^3 / \text{mm}^2$	$7.4 \pm 3.4 \times 10^3 / \text{m}$	NS
C-reactive protein	1.1 ± 0.7	0.4 ± 0.8	< 0.05
Serum vitamin D	11.32 ± 10.18 ng/ml	21.55 ± 13.62 ng/ml	< 0.05
Extensive AA	5	–	–
Patchy AA	18	–	–
Systemic steroid therapy	None		
Local topical steroid therapy	6	–	–
No current or recent therapy (within 6 months)	17	–	–
No. of patients with vitamin D deficiency (%)	16/23 (69.5%)	5/20 (25%)	< 0.05

The results are presented as mean \pm standard deviation

AA = alopecia areata, NS = not significant

Table 2. The results of multivariate analysis

	OR (95%CI)	P value
Age (yr)	1.1 (0.25–1.8)	0.75
Male gender	1.17 (0.32–5.75)	0.37
BMI	0.97 (0.92–1.03)	0.35
CRP > 1	3.1 (2.6–4.2)	0.04
Serum 25(OH)D < 30 ng/ml	2.3 (2.2–3.1)	0.02

Multivariate analysis showed that CRP > 1 and serum vitamin D levels < 30 ng/ml were associated with AA

CI = confidence interval, OR = odds ratio

The laboratory tests showed a white blood count of $9.8 \pm 2.3 \times 10^3 / \text{mm}^2$ in the AA group and $7.4 \pm 3.4 \times 10^3 / \text{mm}^2$ in the control group (*P* = 0.72). The mean CRP level was 1.1 ± 0.7 mg/dl in the AA group and 0.4 ± 0.8 mg/dl in the control group (*P* < 0.05).

Vitamin D levels were significantly decreased in the AA group as compared with the control group (11.32 ± 10.18 vs. 21.55 ± 13.62 ng/ml, *P* < 0.05); 16 patients in the AA group (69.5%) versus only 5 patients (25%) in the control group had low vitamin D levels [Table 1]. There was no significant difference between the levels of vitamin D during the summer and winter in the whole study population (*P* = 0.95). Multivariate analysis showed that vitamin D levels < 30 ng/ml (odds ratio 2.3, 95% confidence interval 2.2–3.1, *P* = 0.02) and high CRP levels > 1 (OR 3.1, 95%CI 2.6–4.2, *P* = 0.04) were associated with AA occurrence (the results of multivariate analysis are shown in Table 2).

OR = odds ratio
CI = confidence interval

DISCUSSION

To the best of our knowledge this is the first study to examine the association between vitamin D and AA. In this prospective study a significant association was found between serum levels of 25(OH) D below 30 ng/ml and the occurrence of AA.

The mechanism(s) that link vitamin D deficiency with AA syndrome are unknown. The hair follicle is a highly hormone-sensitive organ [17]. Vitamin D is a hormone that plays an important role, in addition to calcium homeostasis, in immune regulation, cell growth and differentiation. The active form of vitamin D, 1,25-dihydroxyvitamin D₃, mediates its action by binding to specific vitamin D receptors located in the nucleus of target cells [18]. VDR is a member of the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor regulating vitamin D-responsive genes [18]. It has been demonstrated that VDR is strongly expressed in key structures of human and murine hair follicles [18]. A lack of VDR is associated with reduced epidermal differentiation and hair follicle growth [19]. Expression of VDR in keratinocytes is necessary for the maintenance of the normal hair cycle [20]. In addition, patients with hereditary 1,25-dihydroxyvitamin D₃-resistant rickets type II and VDR knockout mice exhibit a phenotype that includes alopecia [21]. AA is a tissue-specific autoimmune disease. VDR gene polymorphisms and levels of vitamin D influence susceptibility to autoimmune diseases, such as Graves' disease and psoriasis [22]. In view of the above we wondered whether a relationship exists between serum vitamin D levels and AA, another autoimmune disease. To the best of our knowledge this clinical relationship has never been investigated.

Vitamin D deficiency has been associated with several adverse health consequences that include autoimmune diseases, cardiovascular diseases, and infections. 1,25- dihydroxyvitamin D₃ acts as an immunomodulator targeting various immune cells, such as monocytes, macrophages, dendritic cells, as well as T lymphocytes and B lymphocytes, thus modulating both innate and adaptive immune responses [23]. Prospective studies in the involvement of vitamin D in immune/autoimmune disorders are understandably limited, but most cross-sectional studies have shown an inverse relationship between concentration of vitamin D and disease activity [24]. In another study on patients with rheumatoid arthritis, the authors concluded that the serum concentrations of vitamin D were inversely related to disease activity [25]. In vitro studies suggest that when vitamin D is added, many immunological characteristics of SLE are resolved, suggesting that vitamin D deficiency shifts the immunological response towards the loss of tolerance [25].

The pathogenesis of AA is likely to be autoimmune and inflammatory. The ability of corticosteroids to abort AA suggests that the symptoms may be caused by inflammatory cytokines

rather than another etiology. Another finding of our study was the elevation in CRP levels in subjects with AA (albeit a mild and non-statistically significant increase). This finding suggests inflammatory mechanisms apart from the proposed immune mechanism.

There are some limitations to our study. Firstly, the study group was relatively small. Secondly, we did not exclude obese and/or overweight patients who may have low levels of vitamin D levels. Thirdly, we did not have a subgroup with vitamin D supplementation to assess the implication on disease severity and extension. Fourthly, we did not exclude patients who were treated with vitamin D supplements or a high vitamin D diet. Finally, vitamin D levels were measured once, regardless of ethnicity, skin color or sun exposure, which may bias the results.

In summary, we found a strong correlation between AA and vitamin D deficiency, suggesting that vitamin D deficiency can be a significant risk factor for AA occurrence. More studies with a large number of patients are needed to confirm this hypothesis.

Correspondence

Dr. M. Mahamid

Digestive Disease Institute, Shaare Zedek Medical Center, Jerusalem 94342, Israel
email: mahmudmahamid@yahoo.com

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VDR = vitamin D receptor

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