Abstract

**Background:** Alopecia areata (AA) is an immune-mediated disease in which autoantigens play an important part in activating T-lymphocytes. Vitamin D has been associated with various autoimmune diseases, and Vitamin D receptors are strongly expressed in hair follicles and their expression in keratinocytes is necessary for the maintenance of the normal hair cycle.

**Aim:** The aim of this study was to find the association between Vitamin D level and AA.

**Materials and Methods:** This was a hospital-based cross-sectional study in which 50 patients with clinically and trichoscopically diagnosed AA cases, and 35 healthy age- and sex-matched controls were studied in summer months. Blood samples were taken from both cases as well as controls and samples were immediately processed by centrifugation (4000 rpm) at room temperature. Plasma 25-hydroxyvitamin D (25(OH)D) was analyzed by chemiluminescence method. A deficiency in Vitamin D was defined as serum 25(OH)D concentrations <30 ng/ml.

**Results:** The mean body mass index in cases was 20.96 ± 1.91, whereas in controls, it was 21.37 ± 1.70 (**P** = 0.31). The mean serum 25(OH)D levels of AA patients was 16.6 ± 5.9 ng/ml, whereas in control group, the mean level was 40.5 ± 5.7, the difference being statistically significant (**P**< 0.001). A significant negative correlation was found between severity of alopecia tool score and Vitamin D level (**P**< 0.001; **r** = −0.730) and also between the number of patches and Vitamin D level (**P**< 0.001, **r** = −0.670).

**Conclusion:** In our study, we found that the levels of 25(OH)D were low in AA patients when compared to healthy controls. Furthermore, there was a significant negative correlation between the levels of serum Vitamin D and severity of AA. Thus, the study suggests the role of Vitamin D in pathogenesis of AA and hence a possible role of Vitamin D supplementation in treatment of same.

**Limitations:** Our study was limited by the lesser number of patients and lack of therapeutic trial of Vitamin D for these patients.

**Key words:** 25-hydroxyvitamin D, alopecia areata, severity of alopecia tool score, Vitamin D receptors

**Introduction**

Alopecia areata (AA) is a common form of nonscarring alopecia characterized by hair loss with no clinical signs of inflammation and can affect scalp and/or any hair-bearing area of the body.[1] It is a common dermatological disorder and accounts for 25% of all alopecia cases.[2] It can occur at any age and there is no sex predilection although some studies show male preponderance.[3] Scalp is the most common site (90%). AA is classified on the basis of extent and pattern of hair loss.[4] It can be patchy AA, alopecia totalis in which entire scalp and body hair such as eyebrows, eyelashes, beard, axillary hair, and pubic hair is affected, and alopecia universalis if the total body hair is involved. Various patterns of AA include reticular, ophiasis, and sisaipho, acute diffuse and total alopecia. The unusual variants include perinaevoid and linear forms.[5]

AA is an autoimmune disease mediated by T-lymphocytes in which autoantigens play an important part in activating T-cells and is associated with other autoimmune...
diseases. Hair follicle is an immune-privileged site where major histocompatibility complex (MHC) Class I and II molecules are not expressed. This immune privilege is collapsed in AA by the presence of increased MHC I and II complexes, decreased immunosuppressive molecules, and higher expression of adhesion molecules (ICAM-2 and ELAM-1) in the perivascular and peribulbar hair follicular epithelium, leading to perifollicular inflammation which causes thin dystrophic hair with miniaturization.

Vitamin D functions such as a hormone and its main sources are through endogenous synthesis in the skin and diet. It has a role in control and regulation of immune mechanisms. It has been demonstrated that Vitamin D receptors (VDRs) are strongly expressed in hair follicles, and lack of VDRs reduces epidermal differentiation and growth of hair follicles.

In view of these points, we designed this study to find the association between Vitamin D level and AA.

Materials and Methods

This was a hospital-based cross-sectional study involving 50 patients of AA, attending our outpatient department (OPD) from May to October 2015. The diagnosis was made on the basis of clinical features and trichoscopy. The control group consisted of 35 age- and sex-matched individuals selected randomly from our OPD with no history of AA.

Exclusion criteria

(1) Patients suffering from any other dermatological condition or any systemic or autoimmune illness.
(2) Other causes of alopecia including scarring alopecia, androgenic alopecia, telogen effluvium, and female pattern hair loss. (3) Patients who had received oral or topical medications during the past 4 weeks (topical, intralesional, or oral steroids, immunosuppressants, Vitamin D supplements, and topical vitamin D analogs).
(4) Pregnant and lactating females. (5) Patients with body mass index (BMI) more than 25 (since there is a relationship between obesity and Vitamin D deficiency) were excluded from the study.

An informed consent was obtained from all patients after explaining to them the nature of the study and the procedures. The study was approved by the ethical committee of the hospital.

History regarding the onset, progression, duration of disease, presence of any other systemic or autoimmune disease, history of any significant drug intake, family history, and approximate number of hours of daily sun exposure was noted.

Demographic, clinical, and laboratory data were recorded in both the groups. Anthropometric measurements including height, weight, and BMI were also recorded. Laboratory tests performed included complete blood count, liver and renal function tests, thyroid function test, and calcium and Vitamin D level.

The trichoscopic findings (DermLite DL3N, California, USA, ×10) for diagnosing AA included black dots, exclamation mark hairs, coulability hairs, and yellow dots. The severity of AA was scored by severity of alopecia tool score (SALT score), and patients were further subgrouped into following SALT subclasses: $S_0$ = no hair loss; $S_1$ = <25; $S_2$ = 26–50; $S_3$ = 51–75; $S_4$ = 76–99; and $S_5$ = 100.

Serum 25-hydroxyvitamin D level analysis

Blood samples were taken from both cases as well as controls after an overnight fast, and samples were immediately processed by centrifugation of 4000 rpm at room temperature. Plasma 25-hydroxyvitamin D (25(OH)D) was analyzed by Chemiluminescence method/kit method (Siemens USA) as per manufacturers protocol, using Siemens ADVIA Centaur Analyzer. The coefficient of variation for interassay analyses was 18.4% at a 25(OH)D level of 16 ng/ml and 11.7% at 48.4 ng/mL. A deficiency in Vitamin D was defined as serum 25(OH)D concentrations <30 ng/ml.

Statistical analysis

Statistical analysis was done using student’s t-test. For comparison of categorical data such as sex and residence, Chi-square test was used. P value < 0.05 was considered statistically significant.

Results

The mean age of cases and controls was 22.4 ± 8.6 and 29.2 ± 7.6, respectively. Among patients, 33 were from urban areas and 17 were from rural areas, whereas in control group, 22 were from urban areas and 13 were from rural areas ($P = 0.765$). The mean duration of the disease was 4.80 ± 4.58 months. The mean BMI in cases was 20.96 ± 1.91, whereas in controls, it was 21.37 ± 1.70 ($P = 0.31$) [Table 1]. Both the cases and controls were normocalcemic, and the difference between the groups was statistically insignificant [Table 1]. Among patients of AA, 29 patients had a single patch and 21 patients had multiple patches. All the lesions were on the scalp. Thirty-eight patients had $S_1$, 12 had $S_2$, whereas there were no patients with $S_3$ to $S_5$ SALT score.

| Table 1: Comparison of Vitamin D level in cases and controls |
|---------------------------------------------------------------|
| **Variable** | **AA patients** | **Healthy controls** | **P** |
| BMI (mg/dL) | 20.96±1.91 | 21.37±1.70 | 0.31 |
| Vitamin D level (ng/ml) | 16.6±5.9 | 25.49±1.02 | <0.001 |
| Residence (U/R) | 33/17 | 22/13 | 0.765 |
| Serum calcium | 8.3±0.3 | 8.5±0.5 | 0.42 |

BMI: Body mass index, U: Urban, R: Rural
The mean serum 25(OH)D concentration of patients with AA was 16.6 ± 5.9 ng/ml, whereas in control group, the mean concentration was 25.49 ± 1.02. The difference in Vitamin D (serum 25(OH)) levels between two groups was statistically significant (P < 0.001).

Among the patients of AA, mean concentration of 25(OH)D in S group was 19.02 ± 4.4 ng/ml whereas the mean concentration in S group was 9.00 ± 2.80 ng/ml (P < 0.001). A significant negative correlation was found between SALT score and Vitamin D level (P < 0.001; r = −0.730) [Table 2]. The patients with single patch alopecia had mean concentration of 25(OH)D of 19.9 ± 4.45 ng/ml whereas those with multiple patches had mean value of 12.0 ± 4.45 ng/ml and the difference was statistically significant (P < 0.001) [Table 3].

A significant negative correlation was found between number of patches and Vitamin D level (P < 0.001, r = −0.670).

Among patients of AA, there was no significant difference in Vitamin D level between men and women (P > 0.05). There was no significant correlation between Vitamin D level and duration of the disease (P > 0.05).

**Discussion**

Our study was designed to determine the relation between Vitamin D level and AA. In our study, we found that the levels of 25(OH)D were low in patients of AA when compared with the healthy controls and the difference was statistically significant (P < 0.001). Furthermore, we found that the levels were much lower in patients with higher SALT score or multiple patches, i.e., a negative correlation was found between the levels of Vitamin D and the severity of AA (determined by SALT score and number of patches).

The mechanism by which Vitamin D deficiency and AA are related is still not clear. Vitamin D has a role in innate and acquired immune systems, and the expression of VDRs and its activating enzyme 1α-hydroxylase on various cells of immune system, for example, macrophages, dendritic cells, and T-cells suggests its role.\[^{14}\] VDR is a member of nuclear hormone receptor family and acts as a ligand-inducible transcription factor that regulates Vitamin D responsive genes.\[^{15}\] Although studies have suggested an important role of VDR in the pathogenesis of AA, we did not evaluate the levels of VDR because of the lack of facility and expertise for the same in our hospital.

Various studies have shown that 1,25(OH)\(_2\)D\(_3\) modulates the activity of dendritic cells.\[^{14}\] It inhibits the differentiation and maturation of dendritic cells; reduces the expression of MHC Class II, CD40, CD80, and CD86; and inhibits the secretion of proinflammatory cytokines such as interleukin (IL)-1, IL-2, IL-6, IL-12, interferon-\(\alpha\), and tumor necrosis factor-\(\alpha\). IL-12 which is an immunostimulatory cytokine involved in shifting the immune system toward the T-helper (Th) 1 phenotype is inhibited by 1,25(OH)\(_2\)D\(_3\), thereby shifting the immune response toward the Th2 phenotype.\[^{16,17}\] The inhibition of the Th1 phenotype and potentiation of the Th2 phenotype has been suggested to be one of the mechanisms, by which 1,25(OH)\(_2\)D\(_3\) suppresses Th1-mediated autoimmune diseases.\[^{14,17}\] In addition, 1,25(OH)\(_2\)D\(_3\) enhances the production and function of T-regulatory cells, which may also help in suppressing autoimmune disorders.

In the present study, the assessment of T-cell function was not feasible because of the nonavailability of the requisite infrastructure.

Our study shows a significant relationship between levels of Vitamin D and AA. Similar results were seen in many other studies as well. In a study by Yilmaz \textit{et al.},\[^{18}\] low serum 25(OH)D and 1,25(OH)\(_2\)D\(_3\) levels were seen in patients with AA compared with healthy controls. Another study by d’Ovidio \textit{et al.}, involving 156 patients with AA and 148 controls, revealed that an insufficiency or deficiency of 25(OH)D was not significantly different between patients with AA and the controls.\[^{19}\] However, a deficiency in 25(OH)D was present in 42.4% of patients, which was significantly higher than the 29.5% observed in the healthy controls. Furthermore, the correlation was not obtained between the levels and the extent of hair loss. Another study by Cerman \textit{et al.} found deficient serum 25(OH)D levels in patients with AA and inverse correlation with disease severity.\[^{20}\] In yet another study by Mahamid \textit{et al.}, a significant correlation was found between Vitamin D deficiency and AA.\[^{21}\]

However, our study has certain limitations. The number of AA cases was less, and our study did not include positive controls as was done in few previous studies. Furthermore, the dietary intake of Vitamin D in patients was not assessed and no therapeutic trial of Vitamin D was given.

**Table 2: Correlation of Vitamin D level with severity of alopecia areata**

| SALT score | Number of patients (%) | Vitamin D level (ng/ml) | P       |
|------------|------------------------|-------------------------|---------|
| S\(_1\)     | 38 (76)                | 19.02 ± 4.4             | <0.001 |
| S\(_2\)     | 12 (24)                | 9.00 ± 2.8              |         |

**Table 3: Correlation of Vitamin D level with number of alopecia patches**

| Number of patches | Number of patients (%) | Vitamin D level (ng/ml) | P       |
|-------------------|------------------------|-------------------------|---------|
| Single patch      | 29 (58)                | 19.9 ± 4.45             | <0.001 |
| Multiple patches  | 21 (42)                | 12 ± 4.45               |         |
Our study was the first of its kind from a temperate area with a high altitude. The levels of exposure to the ultraviolet, seasonal changes and altitude, level of activity, and BMI should be considered to justify the level of Vitamin D in patients in further studies.

Systemic Vitamin D supplementation had been shown to be therapeutically effective in different autoimmune diseases, such as inflammatory bowel disease, Behcet’s disease, rheumatoid arthritis, and systemic lupus erythematosus. Therefore, as we do not know at present whether Vitamin D supplementation would be effective in the therapy of AA or not, further clinical trials are needed to evaluate its efficacy in treating this common dermatosis.

**Conclusion**

In our study, we found that the levels of 25(OH)D were low in patients of AA when compared to healthy controls. Furthermore, there was a negative correlation between the levels and severity of AA assessed by SALT score. Thus, the study suggests the role of Vitamin D in pathogenesis of AA and hence a possible role of Vitamin D supplementation in the treatment of same.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**What is new?**

Vitamin D deficiency may be an important factor in the etiopathogenesis of alopecia areata, and oral Vitamin D supplementation and topical Vitamin D analogs may be used to treat alopecia areata.

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