International Journal of Research in Dermatology
Jain M et al. Int J Res Dermatol. 2021 Nov;7(6):776-781
http://www.ijord.com

DOI: https://dx.doi.org/10.18203/issn.2455-4529.IntJResDermatol20214200

Original Research Article

Evaluation of serum 25-hydroxy vitamin D levels in alopecia areata of scalp: a cross sectional observational study

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Received: 20 September 2021
Accepted: 18 October 2021

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ABSTRACT

Background: Alopecia areata (AA) is a T cell-mediated autoimmune disorder of anagen hair follicle leading to distressing and relapsing non-scarring hair loss. Vitamin D an immunomodulator plays important role in regulating normal hair cycle. Recent evidence suggests inconsistent association between vitamin D deficiency and alopecia areata.
Methods: Hospital-based cross-sectional observational study of forty untreated cases of alopecia areata and forty age and sex-matched healthy controls in 18-45 years of age group recruited from out-patient department. Each patient will undergo a detailed history, clinical examination and SALT (Severity of alopecia tool) scoring. Enhanced chemiluminesence method (Eci) will be used to estimate serum 25-hydroxy vitamin D [25(OH)D].
Results: The mean 25(OH)D level in patients of AA was 12.45±4.80 ng/ml (deficient), while that of controls was 33.73±10.02ng/ml (normal). The difference between the levels of 25(OH)D in patients of AA and controls came out to be statistically significant ($p \leq 0.0001$). A strong negative correlation was seen between SALT score and 25(OH)D level (-0.32), which was found to be statistically significant ($p=0.0462$).
Conclusions: The present study established that vitamin D levels are either insufficient/deficient in alopecia areata and it correlates negatively with severity of SALT (severity of alopecia tool) score.

Keywords: Alopecia areata, 25-hydroxy vitamin D, SALT score

INTRODUCTION

Alopecia areata (AA) is a chronic inflammatory, organ-specific, autoimmune disease characterized by well demarcated, round to oval non-cicatricial hair loss that occurs as a patchy, confluent or diffuse pattern and can attack any hair bearing area of the body.[1] AA can occur in any age group with peak onset at 20-40 years of age group.[2]

The exact cause of AA is still unknown. However, most of the current literature suggests autoimmunity as the major pathogenic process in AA. It is evident by the presence of perilesional or leisonal inflammatory cells, hair follicle-specific autoantibodies in the blood of patients with AA, its association with other autoimmune diseases and the response to treatment with immunosuppressive medications.[1,3]

Vitamin D is a modulator of both innate and adaptive immune system through its varied effects on T and B lymphocytes, dendritic cells and macrophages. All of these expresses Vitamin D receptors (VDRs).[4]

Vitamin D deficiency is suggested to be an environmental trigger for induction of autoimmunity.[5] VDRs are strongly expressed in the key structures of hair follicles i.e. outer root sheath, bulb and dermal papilla and their expression is necessary for the maintenance of normal hair cycle especially for anagen initiation.[4,6] The

deficiency or lack of VDRs reduces epidermal differentiation and hair follicle growth.[7]

There is paucity of literature regarding the role of serum levels 25(OH)D in AA.

The aim of the study was to evaluate serum 25(OH)D levels in patients with AA and correlate with severity.

METHODS

It was a hospital-based cross-sectional observational study involving forty untreated cases of alopecia areata of scalp, between 18-45 years of age, diagnosed clinically by the presence of well defined, round/oval, smooth bald areas of non-scarring hair loss with presence of exclamation mark hair attending our outpatient department of dermatology, venereology and leprology from June 2019 to May 2020.

Forty age and sex matched healthy volunteers who gave their informed bilingual consent for one-time withdrawal of 4 ml of blood sample were included as controls.

Patients with following criterias were excluded- (a) AA of extra-scalp sites only; (b) with other causes of alopecia i.e.; tinea capitis, androgenic alopecia (male or female pattern), scarring alopecia, traction alopecia, and telogen effluvium; (c) on any topical or oral steroid, immunosuppressive drugs, calcium or vitamin D supplementation, or using any photo-protective measures; (d) with history of any autoimmune or systemic diseases; and (e) with Body mass index ≥25.[8]

A well-informed written consent was taken from the patients before inclusion in the study. The study was approved by the ethical committee of hospital. A detailed history and clinical examination of the patients was recorded on specially designed proforma. The severity of AA was graded according to SALT scoring. Various haematological and biochemical investigations were undertaken.

The severity of AA was determined by using Severity of alopecia tool (SALT) devised by Olsen, is determined by visually assessing the amount of terminal hair loss in four areas of the scalp namely, vertex, left temporal, right temporal and occipital area (Figure 1).[9]

Score is determined by visually determining the amount of terminal hair loss in each of the four views of the scalp and adding these together, with a maximum score of100%. Percentage of hair loss in any of these areas is percentage of hair loss multiplied by percent surface area of the scalp in that area. SALT score is the sum of percentage of hair loss in all above mentioned areas.

The final score was calculated as follows.

Final SALT score = area (%) of hair loss in A × 0.18 + area (%) of hair loss in B × 0.18 + area (%) of hair loss in C × 0.40 + area (%) of hair loss in D × 0.24.

Subgrouping of scalp lesion(s) was done as- S1≤25% hair loss; S2=25-50% hair loss; S3=50-75% hair loss; S4=75-99% hair loss; S5= 100% hair loss.

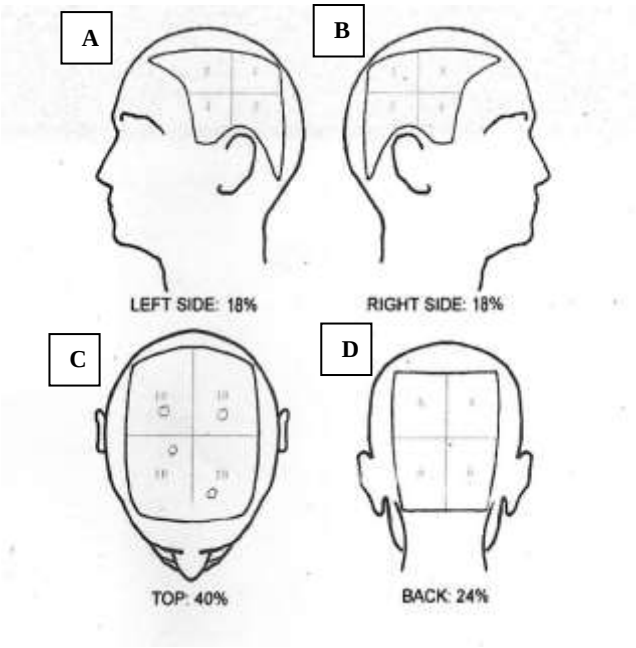

Figure 1 (A-D): Olsen/Canfield tool for the determination of percentage scalp hair loss.

Four ml of venous blood was collected under aseptic conditions, after 12 hours overnight fasting, for estimating 25(OH)D levels. Venous blood was collected in a serum separator tube (BD vaccutainer). The tube was wrapped in aluminium foil and sent to the lab for further processing. Enhanced chemiluminesence method (Eci) was used to estimate serum 25(OH)D. Levels of 25(OH) D was graded as follows.[10]

Table 1: Grading of levels of 25(OH) vitamin D levels.

Level	Range (ng/ml)
Deficient	<10
Insufficient	10-30
Normal	30-60

The data will be analysed by using appropriate statistical methods. Discrete categorical data was represented in the form of either a number and/or a percentage (%).

The normality of quantitative data was checked by measures of Kolmogorov-Smirnov tests of normality. Continuous data was written as either in the form of its mean and standard deviation or in the form of its median, as per the requirement. Quantitative variables were compared using Unpaired t-test/Mann-Whitney test (when the data sets were not normally distributed)

between the two groups. Qualitative variables were correlated using Chi-square test/Fisher's exact test. Spearman correlation coefficients were calculated to see relationship between scores and SALT score. The data was entered in MS excel spreadsheet and analysis was done using Statistical Package for social sciences (SPSS) version 21.0. A p value of <0.05 was considered to indicate statistical significance.

RESULTS

A total of 40 cases and 40 controls satisfying the inclusion and exclusion criteria were included in the study. Baseline clinical characteristics of cases and controls have been illustrated in (Table 2). The majority of cases of AA included in the study (47.5%) were between 21-30 years of age and least number of cases (7.5%) were recorded in age group of >40 years. The mean age noted in the present study was 28.05±7.61 years. The lowest SALT score recorded in our study was 5% while the highest SALT score was seventy-four per cent. In the current study, mean serum 25(OH)D level of patients with AA (12.45±4.80 ng/ml) was significantly lower than that of healthy controls (33.73±10.02 ng/ml) (p<0.0001) (Figure 2).

Mean 25(OH)D levels in cases and controls in various age groups is illustrated in (Table 3). A deficiency was observed in 30% of the patients with AA included in our study (N=12) and none of the healthy controls. Insufficient level of 25(OH)D (10-30 ng/ml) was seen in 70% of the patients with AA included in our study (N=28) and 40.0% of the healthy controls (N=16).

The current study showed significant negative correlation between SALT score and serum 25(OH)D level in the patients with AA i.e.; a gradual decline of 25(OH)D level with increased AA severity (0.04626, -0.32) (Figure 3). The present study showed no significant association between 25(OH)D levels and gender of the patients with AA as well as controls (p=0.49) (Table 4).

Table 2: Baseline clinical and demographic data of cases and controls.

Parameters	Cases (N=40)		Controls (N=40)	
	N	%	N	%
Sex				
Male	21	52.50	22	55.00
Female	19	47.50	18	45.00
Age (in years), mean age	28.05±7.61		27.57±6.24	
Size of AA lesion(s) in cm^2				
1-4	21	52.50	-	-
5-8	15	37.50	-	-
9-12	04	10.00	-	-
Number of alopecia areata lesion(s)				
1-2	10	25	-	-
3-4	17	42.50	-	-
5-6	09	22.50	-	-
7-8	02	5.00	-	-
9-12	02	5.00	-	-
Family history of AA				
Negative	39	97.50	-	-
Positive	01	2.50	-	-
Nail changes in AA				
Negative	39	97.50	-	-
Positive	01	2.50	-	-
SALT score				
S1	28	70	-	-
S2	09	22.50	-	-
S3	03	7.5	-	-
S4	-		-	-
S5	-		-	-
Mean serum vitamin D (in ng/ml)	12.45±4.80		33.73±10.02	
Serum vitamin D (%)				
Normal	-		24	60
Insufficient	28	70	16	40
Deficiency	12	30	-	

Table 3: Mean 25(OH)D levels in cases and controls in various age groups.

Age distribution (years)	25(OH)D (ng/ml)		P value
	Cases	Controls	
≤20	12.13±5.96	28.78±7.11	0.0015
21-30	12.78±4.48	36.06±9.86	<0.0001
31-40	12.03±5.63	27.57±8.40	0.0002
>40	11.33 ± 1.62	38±16.26	0.054

Table 4: Mean 25(OH)D in males and females among cases.

Sex	25(OH)D (ng/ml)	P value
Females	12.21±4.55	0.49
Males	12.66±5.13	

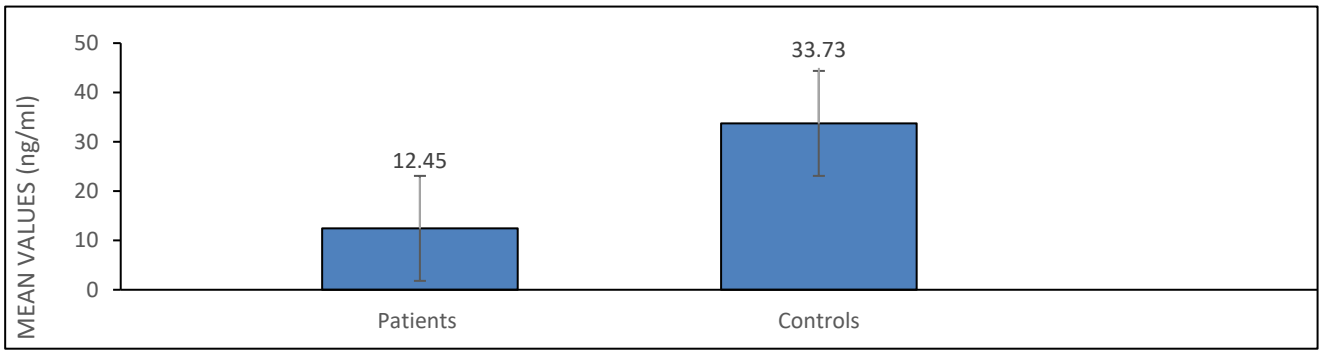

Figure 2: Mean 25(OH)D levels in patients of alopecia areata and control.

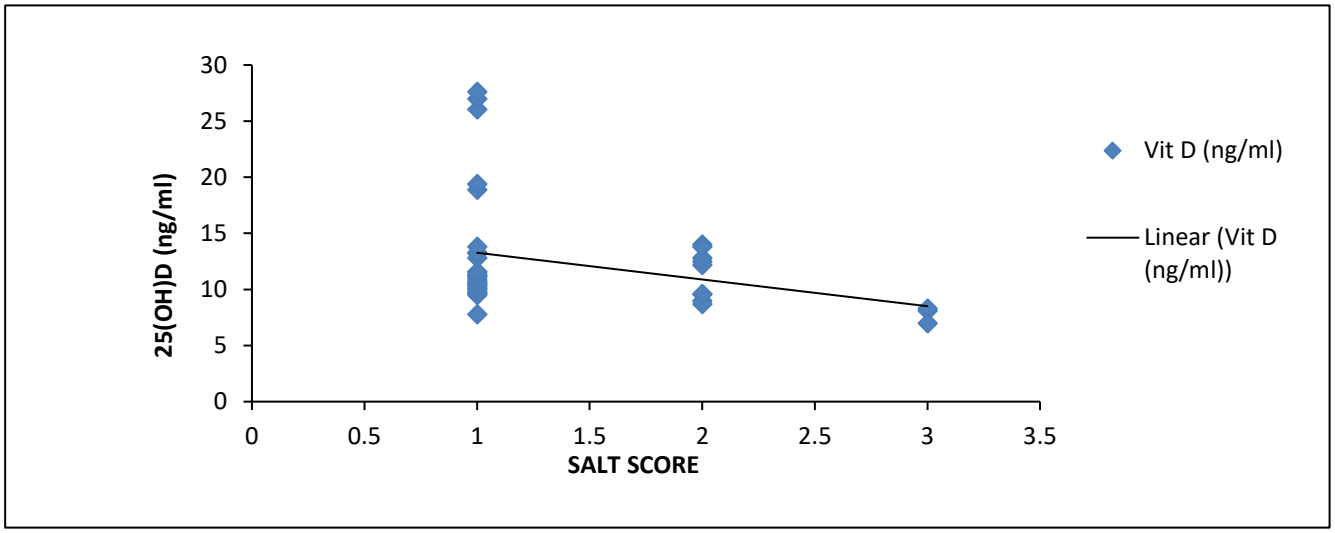

Figure 3: Correlation between SALT score and 25(OH)D (ng/ml).

DISCUSSION

AA is characterised by patchy, confluent or diffuse non-scarring hair loss without any clinical inflammatory signs.[1] Extensive studies have been done regarding the immunopathogenesis of AA, however, recent studies have found an association between low levels of 25(OH)D and AA.[4,7,10-12] Several epidemiological studies have reported associations between vitamin D deficiency and a higher incidence of autoimmune disorders such as Rheumatoid arthritis (RA), Systemic lupus erythematosus (SLE), psoriasis, vitiligo, Multiple sclerosis (MS), Inflammatory bowel disease (IBD), type 1 Diabetes mellitus (DM) and Behcet disease.[13,14]

Arnson et al suggested vitamin D deficiency to be an environmental trigger for induction of autoimmunity, hence vitamin D might have a role in the pathogenesis of AA.[5] Our study sought to investigate the serum levels of 25(OH)D in AA and correlate their levels with the severity of disease as assessed by SALT score. The

majority of cases of AA included in the study (47.5%) were between 21-30 years of age and least number of cases (7.5%) were recorded in age group of >40 years. The mean age noted in the present study was 28.05 years. This is in line with the study done by Mahamid et al on 23 patients of AA where the mean age noted was 24.2 years.[7] In a study by Bakry et al mean age of presentation was 20.70 years among 60 patients of AA included in their study.[15]

In the current study slight male preponderance was seen (M: F=1.10: 1). These findings are consistent with studies by Cerman et al, Mahamid et al and Bakry et al.[4,7,15] The lowest SALT score recorded in our study was 5% while the highest SALT score was seventy-four per cent. Twenty-eight (70.0%) patients were in S1 subgroup while only 3 (7.5%) patients were in S3 subgroup i.e.; they had SALT Score in 51-74% range. Similar findings were observed by Yilmaz et al in their study where maximum number of the patients with AA i.e.; 71.4% were in S1 subgroup.[16] Similarly, Cerman et al in their study on AA used SALT scores and found that 71% of the patients included in their study had SALT score of subgroup S1 while 15% were in subgroup S2 and no patients were in S3 through S5 subgroups.[4] In the current study, mean serum 25(OH)D level of patients with AA (12.45±4.80 ng/ml) was significantly lower than that of healthy controls (33.73±10.02 ng/ml) ($p<0.0001$). In a study, including 86 patients of AA along with 44 patients of vitiligo and 58 healthy persons as controls, Cerman et al reported that serum 25(OH)D levels in patients with AA were significantly lower than those with vitiligo and healthy controls ($p=0.001$ and $p<0.001$, respectively).[4] Similarly, Mahamid et al in a prospective study with 23 consecutive patients diagnosed with AA found a significant correlation between AA and vitamin D deficiency ($p<0.05$) and they were of the opinion that vitamin D deficiency can be a risk factor for AA occurrence.[7]

Bakry et al did a study on serum vitamin D levels in patients with AA (N=60) and documented that serum 25(OH)D levels were significantly lower ($p<0.001$) when compared with healthy controls.[15] Also, in a study involving equal number of patients and controls (N=42), Yilmaz et al reported significantly decreased levels of 25(OH)D and $1,25(OH)_2D_3$ in patients with AA as compared to control group ($p<0.31$).[16]

The current study showed significant negative correlation between SALT score and serum 25(OH)D level in the patients with AA i.e.; a gradual decline of 25(OH)D level with increased AA severity (0.04626, -0.32). This correlation with severity of disease is more meaningful and adds more significance to the proposed association of deficient 25(OH)D levels with AA. This is in accordance with the study by Cerman et al and Bakry et al and these studies also reported an inverse correlation between 25(OH)D level and AA severity.[4,15] The present study showed no significant association between 25(OH)D levels and gender of the patients with AA as well as controls. This is consistent with the findings of Bakry et al who also found no significant association between 25(OH)D levels and gender of patients with AA.[15] Though our study didn't include treatment with vitamin D analogues or oral supplementation but other studies have shown clinical improvement in AA with treatment with topical vitamin D analogues, further supporting the role of vitamin D in AA.

CONCLUSION

On the basis of our study comprising of 40 patients of AA and 40 healthy controls, we may infer that patients with AA have vitamin D insufficiency/deficiency as compared with normal healthy controls, and the same holds an inverse correlation with the severity of AA (SALT) score. Vitamin D levels don't correlate with age, gender, number of lesions, size of lesion and duration of disease. Thus, vitamin D level must be assessed in all cases of alopecia areata and further studies should be contemplated to reiterate the role of vitamin D in AA.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Jain M, Yadav DK, Nyati A, Lal M. Evaluation of serum 25-hydroxy vitamin D levels in alopecia areata of scalp: a cross sectional observational study. Int J Res Dermatol 2021;7:776-81.