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Original Research Article

A Hospital-Based Observational Evaluation of Serum 25-Hydroxy Vitamin D Levels in Alopecia Areata of Scalp

Aswini Kumar Das

Assistant Professor, Department of Skin and VD, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India

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Corresponding author: Dr. Aswini Kumar Das
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Abstract

Aim: 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 conducted in the department of Skin and VD, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India involving fifty untreated cases of alopecia areata of scalp, between 18-50 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 for one year.

Results: A total of 50 cases and 50 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 (45%) were between 21-30 years of age and least number of cases (5%) were recorded in age group of >40 years. The mean age noted in the present study was 25.07±7.40 years.

Conclusion: The trend toward the increased percentage of vitamin D-deficient individuals among AA patients seen in this study may provide insight into the association of vitamin D with AA. The factors that can help determine which AA patients will benefit from vitamin D testing in an AA setting include high SALT scores, younger age, female sex, sun exposure of \30 minutes per day, and lighter skin photo type.

Keywords: alopecia areata; serum 25-hydroxyvitamin D; vitamin D; vitamin D deficiency; vitamin D levels

Introduction

Alopecia areata (AA) is a noncicatricial alopecia that has been postulated to be an autoimmune disease involving one of the few immune-privileged organs, the anagen hair follicle. [1-3] The initial event causing the collapse of the immune-privileged organ in AA patients is still not fully understood. However, it is thought to occur because of reactive oxygen species production, autoantigen production from follicular melanocytes, and T-cell activation. [3,4] Environmental costimulatory factors, such as infection, stress, or trauma, have also been implicated. [5] Recently, vitamin D levels were investigated as one of the factors possibly affecting alopecia areata.

Vitamin D is a fat-soluble hormone that functions mainly in calcium homeostasis via vitamin D receptors, which have been

shown to be widely present in most cells of the body, including the immune system and hair follicles. [6] In the immune system, vitamin D receptors are present in macrophages, T cells, and natural killer cells, key players in maintaining immune privilege. [7,8] In the hair follicle, vitamin D receptors are present in the outer root sheath and mesodermal papilla, where they are thought to initiate anagen. [9] Hence, vitamin D has been hypothesized to play a role in alopecia areata.

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). [10] Vitamin D deficiency is suggested to be an environmental trigger for induction of autoimmunity. [11] 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. [10,12]

The deficiency or lack of VDRs reduces epidermal differentiation and hair follicle growth. [13] There is paucity of literature regarding the role of serum levels 25(OH)D in alopecia areata.

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

Methods

It was a hospital-based cross-sectional observational study conducted in the department of Skin and VD, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India involving fifty untreated cases of alopecia areata of scalp, between 18-50 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 for one year.

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.

Fifty 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 criteria 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. [14]

Methodology

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. [15]

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.

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 chemiluminescence method (ECI) was used to estimate serum 25(OH)D. Levels of 25(OH) D was graded as follows. [16]

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

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

Parameters	Cases (N=50)		Controls (N=50)	
	N	%	N	%
Sex				
Male	32	64	30	60
Female	18	36	20	40
Age (in years), mean age	25.07±7.40		24.48±6.30	
Size of AA lesion(s) in cm2				
1-4	25	50	-	-
5-8	15	30	-	-
9-12	10	20	-	-
Number of alopecia areata lesion(s)				
1-2	10	20	-	-
3-4	20	40	-	-
5-6	10	20	-	-
7-8	5	10	-	-
9-12	5	10	-	-
Family history of AA				
Positive	48	96	-	-

Negative	2	4	-	-
SALT score				
S1	35	70	-	-
S2	10	20	-	-
S3	5	10	-	-
S4	-	-	-	-
S5	-	-	-	-
Mean serum vitamin D (in ng/ml)	12.45±4.80		33.73±10.02	
Serum vitamin D (%)				
Normal	-	-	30	60
Insufficient	35	70	20	40
Deficiency	15	30	-	-

A total of 50 cases and 50 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 (45%) were between 21-30 years of age and least number of cases (5%) were recorded in age group of >40 years. The mean age noted in the present study was 25.07±7.40years. The lowest SALT score recorded in our study was 10% 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).

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	12.18±5.50	25.78±7.13	0.0020
21-30	12.60±4.50	36.14±9.90	<0.0001
31-40	12.06±5.80	27.50±8.40	0.0004
>40	11.44 ± 1.75	36±16.34	0.064

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=15) 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=35) and 40.0% of the healthy controls (N=20).

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

Sex	25(OH)D (ng/ml)	P value
Females	12.48±4.65	0.59
Males	12.56±5.15	

The present study showed no significant association between 25(OH)D levels and gender of the patients with AA as well as controls (p=0.59) (Table 4).

Discussion

Alopecia areata is an autoimmune disease which is characterized by non-scarring hair loss and can affect any hair bearing area of the body. It is caused by CD4+ and CD8+ T cells targeting the hair follicles as an autoimmune mediated skin. [17] Vitamin D is a pro hormone which is synthesized in the skin and regulates the immune response by controlling T and B

lymphocytes. Its deficiency has been established as a risk factor for autoimmune diseases such as systemic lupus erythematosus, psoriasis, vitiligo, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease. [18,19]

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. [20,21]

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.11 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 (45%) were between 21-30 years of age and least number of cases (5%) were recorded in age group of >40 years. The mean age noted in the present study was 25.07 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.13 In a study by Bakry et al mean age of presentation was 20.70 years among 60 patients of AA included in their study. [22]

The lowest SALT score recorded in our study was 10% while the highest SALT score was seventy per cent. Thirty five (70.0%) patients were in S1 subgroup while only 5 (10%) 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. [23] 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. [10]

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. [22] 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)2D3 in patients with AA as compared to control group ($p<0.31$). [23]

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). [24]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. [10,22] The present study showed no significant association between 25(OH)Dlevels 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. [22]

Conclusion

The trend toward the increased percentage of vitamin D-deficient individuals among AA patients seen in this study may provide insight into the association of vitamin D with AA. The factors that can help determine which AA patients will benefit from vitamin D testing in an AA setting include high SALT scores, younger age, female sex, sun exposure of \30 minutes per day, and lighter skin photo type. 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.Future studies can focus on determining vitamin D levels in patients with more severe forms of AA to address this. Further research on vitamin Dsupplementation can also be performed for a subset of AA patients with concomitant vitamin D deficiency.

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