Original Article

A comparative study of serum 25-hydroxy vitamin D level in patients with alopecia areata and healthy control

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Abstract

Background: Alopecia areata (AA) is a type of non-scarring patchy loss of hair in hair-bearing areas and is mostly of autoimmune origin. Vitamin D and its receptor are responsible for a normal hair cycle. Recent studies have shown that vitamin D deficiency is rather frequent among alopecia areata patients.

Objective: To assess serum 25-hydroxy vitamin D level in patients with alopecia areata and to correlate it with the severity of alopecia areata.

Methods: This case-control study included 30 clinically diagnosed cases of alopecia areata and 30 healthy individuals in the department of Dermatology & Venereology, BSMMU, Dhaka. This study was conducted from July 2018 to June 2020. Written informed consent was taken from each patient and interviewed by semi-structured questionnaire. Disease severity was measured by Severity of Alopecia Tool (SALT) score. Five milliliter of venous blood was collected from each patient and control; the sample was preserved and analyzed for 25(OH) D level by automated chemiluminescence immunoassay method in the Department of National Institute of Nuclear Medicine and Allied Sciences, BSMMU. Statistical analysis was carried out by using SPSS software version 23.0. For all statistical tests, a p-value of less than 0.05 was considered significant

Result: The mean serum 25-hydroxy vitamin D levels of cases was 11.30±4 ng/ml, in control group the mean was 19.62±5.09ng/ml, the difference was statistically significant (<0.001). A significant moderate negative correlation was found between SALT score and vitamin D level ($P < 0.024$; $r = -0.411$) but a non-significant weak negative correlation was found between the duration of the disease and vitamin D level ($P < 0.071$; $r = -0.342$)

Conclusion: The serum vitamin D level was low in patients of alopecia areata and normal healthy controls but it is lower in AA patients. There was a significant negative correlation between the levels of serum Vitamin D and the severity of alopecia areata.

Key Words: Alopecia areata, Vitamin D, BSMMU

Introduction

Alopecia areata (AA) is a common form of nonscarring alopecia characterized by hair loss with no clinical signs of inflammation and can affect the scalp and/or any hair-bearing area of the body. [1-2] It can occur at any age. The highest prevalence of alopecia areata is between 30-59 years of age. [3] Family members are affected in 8.7-20% of cases. [4] Scalp is the most common site (90%). AA is classified

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according to the extent and pattern of hair loss. [5] It can be patchy AA, alopecia totalis in which entire scalp and alopecia universalis (AU) where total body hair is involved.[6]

The etiology of AA is not exactly known; however, genetic predisposition, autoimmunity, and environmental factors have been suggested to play a role.[7] Autoimmune etiology has been proposed on the basis of its association with various autoimmune diseases, the presence of autoantibodies, the presence of inflammatory lymphocytes around and within the affected hair follicles and the ability to promote hair regrowth with the use of immunosuppressive agents.[8] The connection between vitamin D deficiency and some autoimmune diseases, has been reported.[9] Therefore, vitamin D deficiency may be a risk factor for the development of alopecia areata.[10]

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. [11-12] IFN-γ, secreted by human CD4+ T cells expressed in alopecia areata lesional skin, is the main element of alopecia areata immunopathogenesis.[13-14] The anagen hair follicle (HF) is normally an immune-privileged (IP) site. IFN-γ contributes to the collapse of the IP site through an increased follicular expression of MHC class I and II molecules.[15] Vitamin D3 significantly inhibits the production of IFN-γ. [16] In inactive SLE, higher levels of IFN-γ was found in lower levels of vitamin D deficient patients and IFN-γ was 150% higher in patients with vitamin D deficiency.[17] So it is justified to assume that vitamin D might play a role in the collapse of IP of the anagen hair bulb.

Cytokines involved in the pathogenesis of AA, most notably interferon (IFN)-γ and interleukin (IL)-15, are dependent on the Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathway.[18] Vitamin D had an inhibitory effect on the JAK/STAT pathway.[19]

Nrf2 is the genes activated by vitamin D.[20] Nrf2 encodes the transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), is defined in the hair follicle and activation of Nrf2 can maintain hair growth during oxidative stress. [21] 1,25(OH)2D3 prevents leptin-induced oxidative stress in human endothelial cells by activating the Nrf2 system. Active vitamin D3 deficiency causes higher oxidative stress through inhibiting transcription of Nrf2 and enhancing DNA damage.[22] Currently, Vitamin D has been shown as an antioxidant.[23]

1,25-dihydroxy vitamin D inhibits the function of Th17 cells, which are potent inducers of autoimmune diseases and it enhances regulatory T cells, which play a key role in suppressing autoimmune responses.[24]

In the light of this information about the role of vitamin D on autoimmunity and hair cycle, it can be hypothesized that vitamin D deficiency may have a role on alopecia areata. The aim of this study was to evaluate the vitamin D status in patients with alopecia areata and investigate its relationship with the severity of alopecia areata.

Methods:

This case-control study was conducted in the Department of Dermatology and Venereology, Bangabandhu Sheikh Mujib Medical University and Department of National Institute of Nuclear Medicine and Allied Sciences, BSMMU, Shahbagh, Dhaka, from July 2018 to June 2020.

A total of 30 patients with alopecia areata were enrolled from the outpatient department (OPD) of dermatology and venereology (case) and the same number of age-sex matched healthy attendants of patients were included (control). Inclusion criteria were i. clinically diagnosed case of alopecia areata, ii. individual of either sex, iii. age >18years, iv. healthy adults who were age and sex matched with the cases and v. participants who were willing to give written consent to undergo the study. Exclusion criteria were: i. pregnancy, ii. lactation, iii. obesity (BMI >25), iv. smoking, v. alcoholism, vi. history of taking medication known to affect serum 25-hydroxy vitamin D level and vii. history of medical conditions known to affect serum 25-hydroxy vitamin D level.

Independent variables were age (year), sex (male/female), occupation, family history, sun exposure/day (minute), total number of alopecia areata patches, size of alopecia areata patches, location of alopecia areata patches, duration of alopecia areata, serum vitamin D level (ng/ml). Dependent variable was SALT score (%).

The study was approved by the institutional review board of BSMMU. All patients and control subjects gave their informed consent to take part in this study. Relevant history was taken regarding present illness, duration of the disease, onset, extension, family history. Every subject was subjected to full

history taking and physical examination for assessment of clinical type and/or pattern of hair loss according to the number of lesions: one patch, two patches, multiple patches, alopecia totalis (AT) or alopecia universalis, and extent/severity of hair loss according to SALT score. The scalp was divided into four areas, namely: (i) vertex: 40% (0.4) of scalp surface area; (ii) right profile of scalp: 18% (0.18) of scalp surface area; (iii) left profile of scalp: 18% (0.18) of scalp surface area; and (iv) posterior aspect of scalp: 24% (0.24) of scalp surface area. The percentage of hair loss in any of these areas was multiplied by the percentage of the surface area of the scalp in that area. SALT score was the sum of the percentage of hair loss in all the above-mentioned areas. Subgrouping of patients into SALT subclasses was done as follows: scalp (S): S0, no hair loss; S1, <25% hair loss; S2, 25–49% hair loss; S3, 50–74% hair loss; S4, 75–99% hair loss; and S5, 100% hair loss. Body (B) hair loss was assessed as: B0, no body hair loss; B1, some body hair loss; and B2, 100% body (Excluding scalp) hair loss.

Five milliliter of venous blood sample was collected from each subject. The serum concentration of 25-hydroxy vitamin D was measured by automated chemiluminescence immunoassay method introduced by siemens and adapted to an ADVIA centaur analyzer in the Department of National Institute of Nuclear Medicine and Allied Sciences, Bangabandhu Sheikh Mujib medical University. Particulars of the patients, SALT score and the serum level of vitamin D were recorded in a preformed data collection sheet.

Statistical analysis was carried out by using the Statistical Package for the Social Sciences (SPSS) software version 23.0 for windows (SPSS Inc, Chicago, Illinois, USA). Quantitative variables were expressed as mean ± standard deviation and categorical variables were expressed as percentages. Independent student t-test was used to compare serum vitamin D level in between patients with alopecia areata and healthy individuals. Chi-Square test was used for comparing other categorical variables. ANOVA test was done to see the difference among the groups of variable. Pearson's correlation coefficient test was used to see the relation between serum vitamin D levels and duration of the diseases in patients. A P-value was considered to be statistically non-significant if >0.05 and statistically significant if ≤ 0.05.

Results:

Table I: Showed that 21(70.0%) patients in the case group and 19(63.3%) in the control group belonged to the age group 21-30 years, second common age group 31-40 years, in cases 20% and 36.7% control. The mean age was found 27.11±5.0 years in the case group and 29.1±4.7 in the control groups. There were no significant differences between the two groups regarding their mean age.

Table-1: Comparison of age between two groups (n=60)

Age (in years)	Group A (Case) (n=30) No. (%)	Group B (Healthy) (n=30) No. (%)	p-value
18-20	3(10.0%)	0(0.0%)	
21-30	21(70.0%)	19(63.3%)	
31-40	6(20.0%)	11(36.7%)	
Total	30(100.0%)	30(100.0%)	
Mean±SD Range (Years)	27.1±5.0 19-40	29.1±4.7 21-38	0.111

Data were expressed as frequency and percentage and mean ± SD

Unpaired student t-test was performed to compare between two groups.

It was observed that 21(70.0%) alopecia areata (AA) patients were male and 9(30.0%) were female in the case group and in the control group 23(76.7%) patients were male and 7(23.3%) were female. Males were predominant in both groups. Statistical difference between the two groups was insignificant.

The majority of patients 19(63.3%) had a duration of disease below 1 year. Mean vitamin D gradually decreased with increased duration of disease, but statistically not significant (p=0.067). Maximum patients 13(43.33%) had multiple alopetic patches followed by one AA patches 8(26.66%), two patches in 6(20.0%) cases, 2(6.6%) alopecia totalis and 1(3.33%) ophiasis cases. Table II shows the number of alopecic patches that were presented in each patient

Table-II Distribution of the study AA patients by number of AA patches (n=30)

Number of AA patches	Frequency	Percentage (%)
One	8	26.66
Two	6	20
Multiple	13	43.33
Ophiasis	1	3.33
Alopecia totalis(AT)	2	6.66
Total	30	100

According to SALT scoring severity of AA, maximum 25 (83.3%) patients had severity S1 (<25%) followed by 6.7% patients had S2 (25-49%), 6.7% had S5 (100.0%) and3.3% had S3 (50-74%) (table III)

Table III: Distribution of the cases according to SALT scoring (n=30)

Disease severity	Frequency	Percentage (%)
S1 (<25%)	25	83.3
S2 (25-49%)	2	6.7
S3 (50-74%)	1	3.3
S4 (75-99%)	0	0.0
S5 (100%)	2	6.7
Total	30	100

Serum mean vitamin D level in S1 (<25%) was 12.3±4.23ng/ml followed by 8.1±0.49 ng/ml in S2(25-49%), 6.20 ng/ml in S3(50-74%) and 4.9±.50 ng/ml in S5 (100%). Mean Vitamin D was significantly different among the type of disease severity and gradually decreased according to disease severity (p=0.043). (table IV).

Table IV: Serum vitamin D level in cases according to SALT scoring (n=30)

Disease severity	Serum vitamin D Mean±SD ng/ml	p-value
S1 (<25%)	12.3±4.23	.43[s]
S2 (25-49%)	8.1±0.49	
S3 (50-74%)	6.20	
S4 (75-99%)	-	
S5 (100%)	4.9±.50	
Total	11.30±4.41	

Data were expressed as mean±SD
p-value measured by ANOVA test, s= significant

Serum vitamin D level was 100% deficient in the case group and in the healthy control group 56.7% patients had vitamin D level deficient and 43.3% insufficient. The difference between the two groups was statistically significant (table V)

Table - V Distribution of the serum Vitamin D status between two groups

Serum Vitamin D (ng/ml)	Group A (Case) (n=30) No. (%)	Group B (Healthy) (n=30) No. (%)	P value
Deficient (<20 ng/ml)	30(100.0%)	17(56.7%)	<0.001
Insufficient (20-30 ng/ml)	0(0.0%)	13(43.3%)	
Total	30(100.0%)	30(100.0%)	

Figures in the parentheses indicate the corresponding percentage;
P value was obtained from Chi-squared Test (x^2)

Pearson's correlation coefficient test was done to see the correlation of serum vitamin D level with the SALT score and duration of AA have shown in the table (VI). There is a significant moderate negative correlation with SALT score (r= -0.411, p=0.024) and an insignificant moderate weak negative correlation with duration of AA (r= -0.342, p=0.071).

Table –VI: Correlation of serum vitamin D (ng/ml) with SALT and duration of AA (n=30)

Serum vitamin D level ng/ml

	r value	p value	
SALT	-0.411	0.024	Significant
Duration of AA	-0.342	0.071	Not significant

Pearson's correlation coefficient test

Discussion

The present study was designed to measure serum

vitamin D levels in patients with alopecia areata and to find out the relationship with severity of the disease. The mean age of the respondents in this study was 27.1±5.0 years. Rehman F et al. reported the mean age of alopecia areata cases was 26 ± 12.89 years in their study.[25] In a study by Siddappa H et al. the mean age was 24.52 ± 10.06 years.[26] Daroach M et al. also found the mean age of alopecia areata patients 28.97±9.96 years.[27] Gade VKV et al. reported the mean age of the cases as 32.73 ±10.43 years.[10] These findings are almost similar to the current study.

In this study male to female ratio was found 2.3:1. Rehman F et al. found the ratio as 2.06:1 in their study.[25] Siddappa H et al. in 2019 reported male to female ratio 2.57:1.[26] Abdel Fatth et al. also found male predominance in their study and the ratio was 3.5:1.[28] Daroach, M. observed male to female ratio as 1.8:1.[27] These findings are almost similar to this current study where male patients were predominant. On the other hand, there are several studies where female alopecia areata cases were predominant. Gade VKV et al. reported the male to female ratio as 1:2.21 in their study.[10] Ghafoor R and Anwar MI also found female dominance and the ratio of male to female was 1:1.5.[29] Globally this ratio is considered as 1:1.[30]

The present study found that maximum patients had multiple patches (43.33%). Ghafoor R and Anwar MI in their study found single patch 6(20%), multiple 15 (50%), ophiasis 3(10%), alopecia totalis (AT) 4(13.33) and alopecia universalis (AU) 2 (6.6%).[29] Erpolat S et al. conducted a case control study with 41 alopecia areata cases where he found 36.6% single patch and 63.4% multiple patches.[31]

This present study reported that the vitamin D level of alopecia areata patients was relatively much lower than the healthy group and was statistically significant (p value<0.001). A similar result was found in other studies. Bhat YJ et al in their study observed a significant lower level of serum vitamin D in comparison to the control (16.6 ± 5.9ng/ml and 25.49 ± 1.02ng/ml; P < 0.001).[32] Siddappa H et al. found the mean serum vitamin D level significantly lower in cases as compared to the controls (18.90 ± 8.32 vs 28.21 ± 18.32 ng/mL; P < 0.001).[26] Mean serum Vit.D level in patients and controls was 7.65 ± 4.50 ng/ml and 15.8 ± 11.47 ng/ml, respectively with a statistically significant difference, P = 0.001 reported by Daroach M.[27] The current study found a high prevalence of vitamin D deficiency in both case and control groups. Daroach M reported 29 (96.7%) patients and 22 (73.3%) controls were found to be Vit. D deficient in their study.[27] Siddappa H et al. found vitamin D deficiency among more cases than controls (64% vs 38%; P < 0.001).[26]

Vitamin D deficiency among the normal population was variably detected in different studies. Mansour et al. demonstrated 90% prevalence of vitamin D deficiency in apparently healthy hospital staff and health care professionals.[33] Zargaret al. found that 82% of healthy subjects had vitamin D deficiency.[34]

The present study measured the severity of alopecia areata of 30 patients by SALT scoring. The mean vitamin D level gradually decreased according to the severity of disease by SALT score and was statistically significant (p value-0.043). The study found a statistically significant negative correlation between serum vitamin D level and SALT scoring (r=-0.411, p= 0.024). Similarly, Siddappa H et al. found a statistically significant inverse correlation between SALT score and serum vitamin D levels (r = -0.298, P < 0.05).[26] A significant negative correlation between the severity of AA assessed by SALT score and Vitamin D levels (P = 0.000; r = −0.474) by Rehman F et al., Cerman AA et al., Attawa EM et al., Bhat YJ et al. and Daroach M et al. found a significant inverse correlation between SALT score and serum vitamin D levels.[25,27,32,35-36]

The duration of disease ranged from 4 months to 10 years with a mean duration of 1.46± 1.81 years. The study observed a gradual declination of mean vitamin D with increasing duration but it was not statistically significant (p value-0.067). In this study, an insignificant negative correlation was found between serum vitamin D level and duration of the disease of alopecia areata patients (P = 0.000; r = −0.474). A similar result was found in the study of Bhat YJ et al. (P = 0.05; r = −0.474).[32] On the other hand a negative significant correlation of serum vitamin D level and duration of the disease of alopecia areata was observed in the study of Daroach M et al. (P =0.03; r = −0.224) and Gade VKV (P = 0.01; r = −0.672).[27,10] So a further large studies incorporating cases of different duration are needed for a definitive conclusion.

Conclusion

This study found that most of the cases of alopecia areata and more than half of the healthy controls were serum vitamin D deficient. The cases of alopecia were relatively more deficient than the healthy controls. There was a negative correlation between the vitamin D levels and the severity of AA.

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Conflict of interest:
No conflicts of interest

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