Determination of serum
25-hydroxyvitamin D levels in patients
with alopecia areata and their
comparison with levels in healthy
controls: A cross-sectional study

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Background: Alopecia areata (AA) has been postulated to be an autoimmune disease affecting the hair
follicles. Because vitamin D receptors are present in the immune system and hair follicles, vitamin D has
been hypothesized to affect the disease.

Objective: The aim of this study was to determine serum 25-hydroxyvitamin D levels and the percentage of
vitamin D deficiency in AA patients and compare them with those in healthy controls in a Philippine tertiary
hospital.

Methods: This cross-sectional study included 29 AA patients and 29 healthy controls. The serum 25-
hydroxyvitamin D levels were determined using the chemiluminescent immunoassay method.

Results: There was no significant difference in the mean vitamin D levels between AA patients
(24.41 ± 6.87 ng/mL) and healthy controls (24.68 ± 6.68 ng/mL) (P = .88). The percentage of patients
with vitamin D deficiency, defined as <20 ng/mL, trended to higher among AA patients (34.4%) than
among healthy controls (17.2%), with an odds ratio of 2.53 (95% CI 0.73-8.65), though this was not
statistically significant.

Limitations: This study involved a limited number of patients in an urbanized area in the Philippines, and
majority of the AA cases seen had mild AA.

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. (JAAD Int
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Key words: alopecia areata; cross-sectional; Philippines; 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

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species production, autoantigen production from follicular melanocytes, and T-cell activation.\textsuperscript{3,4} Environmental costimulatory factors, such as infection, stress, or trauma, have also been implicated.\textsuperscript{5} Recently, vitamin D levels were investigated as one of the factors possibly affecting AA.

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.\textsuperscript{9} In the immune system, vitamin D receptors are present in macrophages, T cells, and natural killer cells, key players in maintaining immune privilege.\textsuperscript{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.\textsuperscript{9} Hence, vitamin D has been hypothesized to play a role in AA.

Currently, there is evidence that the levels of vitamin D are lower in AA patients than in healthy controls.\textsuperscript{10-24} It should also be noted that there are a few studies that did not demonstrate significantly lower levels of vitamin D in AA patients.\textsuperscript{25-28} The increased prevalence of vitamin D deficiency, defined as $<20$ ng/mL or $<30$ ng/mL, depending on the study, was also seen among AA patients.\textsuperscript{12,13,15,19,21-23,25,28} A major limitation of applying these findings is that vitamin D levels are mainly affected by sun exposure combined with other factors, such as age, sex, weight, lifestyle, and diet, all of which vary widely in different regions of the world.\textsuperscript{29-33} Hence, the need for local data on vitamin D levels in AA patients.

The aim of this study was to determine the serum 25-hydroxyvitamin D levels and percentage of vitamin D deficiency in AA patients and compare them with those of healthy controls; the study was conducted at a tertiary hospital in the Philippines. In addition, this study also aimed to determine the correlation between vitamin D levels and various clinical and epidemiologic factors affecting AA.

**METHODS**

A cross-sectional study was conducted at an outpatient clinic at the Department of Dermatology, Philippine General Hospital, University of the Philippines, in Manila, National Capital Region, from December 2017 to July 2018. This was conducted in accordance with the Helsinki declaration and was approved by the University of the Philippines Manila Review Ethics Board. A total of 58 patients were recruited, consisting of 29 patients clinically diagnosed with AA and 29 healthy controls. The sample size was calculated based on the vitamin D levels reported by Mahamid et al,\textsuperscript{12} with an expert value of 0.5 as the correlation coefficient of vitamin D levels and AA characteristics and an α value of 0.05 and power of 80%. Convenience sampling was performed for healthy controls, which were matched based on age, sex, and sun exposure per day ($<30$ minutes or $\geq30$ minutes per day). Holick’s\textsuperscript{34,35} recommendation was used as a guide for determining the cutoff time of sun exposure.\textsuperscript{36} The other parameters noted were body mass index, Fitzpatrick skin phototype, and the presence or absence of photoprotection practices other than sunscreen use (such as umbrella, caps or hats, and long clothing). For AA patients, the additional clinical factors noted were the duration of the disease, clinical subtypes, severity of alopecia tool (SALT) score, and disease activity.\textsuperscript{37,38}

The exclusion criteria, used to account for factors that can affect vitamin D levels, were as follows: (1) a history of treatment within 4 weeks of the following: corticosteroids (systemic, intralesional, and topical), vitamin D and calcium supplements, antiepileptics, antineoplastic drugs, antibiotics ( clotrimazole and rifampicin), antihypertensives, antiretroviral drugs, and endocrine drugs; (2) having the following comorbidities: obesity (body mass index $\geq 30$ kg/m\textsuperscript{2}), dermatologic disorders associated with low vitamin D levels (vitiligo, psoriasis, atopic dermatitis, and chronic urticaria), atopy (bronchial asthma and allergic rhinitis), cancer, diabetes mellitus, thyroid disorders, and other autoimmune diseases (multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and scleroderma or systemic sclerosis); (3) pregnant, nursing women; (4) smokers; and (5) daily sunscreen users.\textsuperscript{35,39,40}

Venous blood samples of the patients were tested for serum 25-hydroxyvitamin D levels using the chemiluminescent immunoassay method. The interpretation of the serum 25-hydroxyvitamin D levels was as follows: vitamin D deficiency at levels of $<20$ ng/mL, vitamin D insufficiency at levels of
21-29 ng/mL, sufficient vitamin D at levels of 30-100 ng/mL.6

The data were analyzed using SAS University Edition. Mean and SD were used to summarize continuous variables, whereas frequency and percentage were used to summarize categorical variables. Baseline characteristics and the primary outcome of serum 25-hydroxyvitamin D level determination between AA patients and healthy controls were analyzed using the 2-sample independent t test and Fisher’s exact test. The association of vitamin D deficiency with AA was analyzed using the Fisher’s exact test and measured using odds ratio calculation. The correlation between vitamin D levels and the various factors was analyzed using the Pearson correlation for continuous variables, Spearman correlation for ordinal variables, and 2-sample independent t test as applicable.

RESULTS

All 58 patients who were deemed eligible underwent vitamin D level determination. There were no dropouts in this study, and all the patients were included in the statistical analysis. The baseline characteristics of the AA patients and healthy controls are summarized in Table I.

There was no statistically significant difference between the vitamin D levels of AA patients and those of healthy controls (Table II). Furthermore, there was no statistically significant association between vitamin D status and AA (Table III). It is of note that the percentage of vitamin D deficiency among AA patients was 34.48% compared with 17.24% among healthy controls, with an odds ratio of 2.53 (95% CI 0.73-8.65) (Table III).

The clinical parameters noted in AA patients and corresponding mean vitamin D levels are summarized in Table IV. Majority of the AA cases seen had patchy AA and a SALT score of S1. Most cases were active and had an acute duration of <3 months. The SALT score had a weakly negative monotonic correlation with the vitamin D levels, whereas the clinical types of AA and disease duration had no correlation with the vitamin D levels (Fig 1). The t test planned for determining the correlation between disease activity and vitamin D levels was not possible because only 1 AA patient had inactive disease.

The epidemiologic parameters and corresponding mean vitamin D levels in AA patients are summarized in Table V and Fig 2. The vitamin D levels were moderately positively correlated with age, lower in females (borderline significant), and very weakly negatively correlated with body mass index (Fig 2, A-C). The vitamin D levels were weakly positively correlated with skin phototype and were lower in patients who had <30 minutes of sun exposure per day (Fig 2, D and E). The vitamin D levels did not differ between those who practiced photoprotection measures apart from sunscreen use and those who did not (Fig 2, F).

DISCUSSION

In this study, there was no difference between the vitamin D levels of AA patients and healthy controls. This may be attributed to the fact that the means of the vitamin D levels in both AA patients and healthy controls were within an insufficient range. In a tropical country like the Philippines, which has abundant sunshine, insufficient vitamin D levels are unexpected in healthy controls. However, vitamin D insufficiency has been shown to be prevalent in the Philippines, with lower levels of vitamin D seen in urbanized areas, younger age groups, and females.31 Additionally, it is of note that in other countries in Southeast Asia, such as Thailand, Malaysia, Singapore, and Indonesia, patients have been shown to have low vitamin D levels in the setting of abundant sunshine.62-67 This observation raises the issue of a possible need for establishing reference ranges for vitamin D levels based on ethnicity and population.

This study also demonstrated that there was a trend of increased odds of vitamin D deficiency in AA patients, though this difference was not statistically significant. This suggests that in populations in which there is high prevalence of low vitamin D levels, analyzing the frequency of vitamin D deficiency can provide data about its association with various diseases. It should also be noted that in this study, AA patients practiced more photoprotection measures than healthy controls, a possible contributing factor to vitamin D deficiency. However, because of the cross-sectional design of this study, the causality of vitamin D deficiency to AA could not be established.

The weak negative correlation of the vitamin D levels with the SALT scores seen in this study should be interpreted with caution because most of the AA cases seen had mild AA. Previous studies have shown lower vitamin D levels with either higher SALT score, severe clinical presentation, or longer disease duration.13,15-18,27 Other studies have not demonstrated the same findings.10,14,15,24,25 The conflicting data can be attributed to the low number

| Abbreviations used: |
|---------------------|
| AA: alopecia areata |
| SALT: severity of alopecia tool |
of patients with severe forms of AA included in the studies. The relatively low prevalence of extensive forms of AA coupled with numerous exclusion criteria might have contributed to this.

In this study, the epidemiologic parameters that demonstrated lower vitamin D levels in AA patients included younger age, female sex, patients who had <30 minutes of sun exposure per day, and lighter skin phototype. Previous studies have also reported lower vitamin D levels in female AA patients.13,14 In contrast, other studies have found no correlation between vitamin D levels and sex, age, or the duration of sun exposure.11,13-15,19,26 Some studies have shown lower vitamin D levels either in males or in older age group.11,14,24 Although these data on the epidemiologic correlation between vitamin D status and AA differ, it should be noted that these studies were performed in various countries, with heterogeneous factors, which ultimately determine the risk of low vitamin D levels. This highlights the importance of conducting local studies. A possible explanation for the trend seen in this study is that younger age groups, comprising the working age group, might be spending more time indoors during work hours. In addition, although most Filipinos have the capacity to tan, a fairer complexion is considered more attractive in the Philippines. This might explain the lower vitamin D levels seen in patients with lighter skin phototypes, which might have been

Table I. Baseline characteristics of alopecia areata patients and healthy controls*

| Epidemiologic factors                        | Alopecia areata patients | Healthy controls | P value |
|----------------------------------------------|--------------------------|------------------|---------|
| Age, mean ± SD (y)                           | 31.48 ± 10.82            | 31.86 ± 10.51    | .89     |
| Age range                                    |                          |                  |         |
| Sex                                          | 19-65                    | 19-64            |         |
| Sex                                          | Males: 10                | Males: 10        | 1       |
|                                              | Females: 19              | Females: 19      |         |
| Average time of sun exposure per wk          | <30 min: 16              | <30 min: 16      | 1       |
|                                              | ≥30 min: 13              | ≥30 min: 13      |         |
| Body mass index (kg/m²)                      | 23.41                    | 24.07            | .30     |
| Skin phototype                               | III: 5                   | III: 3           | .48     |
|                                              | IV: 24                   | IV: 24           |         |
|                                              | V: 2                     |                  |         |
| Photoprotection practices                     | Present: 21              | Present: 13      | .06     |
| Present (umbrella, caps or hats, and long clothing) | Absent: 8               | Absent: 16       |         |

*The patients were matched based on age, sex, and sun exposure per day. The other noted factors included body mass index, skin phototype, and photoprotection practices apart from sunscreen use.

Table II. Serum 25-hydroxyvitamin D levels in alopecia areata patients and healthy controls

| Alopecia areata patients        | Healthy controls        | P value |
|--------------------------------|-------------------------|---------|
| (Mean ± SD, in ng/mL)           | (Mean ± SD, in ng/mL)   |         |
| 24.41 ± 6.87                   | 24.68 ± 6.68            | .88     |

Table III. Percentage of Vitamin D deficiency (<20 ng/mL), insufficiency (21-29 ng/mL), and sufficiency (30-100 ng/mL) in alopecia areata patients and healthy controls

| Vitamin D status     | Alopecia areata patients | Healthy controls | Fisher’s exact test P value |
|----------------------|--------------------------|------------------|-----------------------------|
| Deficiency           | 34.48% (10/29)           | 17.24% (5/29)    | .42                          |
| Insufficiency        | 44.83% (13/29)           | 58.62% (17/29)   |                             |
| Sufficiency          | 20.69% (6/29)            | 24.14% (7/29)    |                             |

Table IV. Characteristics of alopecia areata patients and corresponding mean vitamin D levels

| AA parameter | Number of AA patients | Mean vitamin D level ± SD (ng/mL) |
|--------------|-----------------------|-----------------------------------|
| Clinical type| Patchy                | 25                                |
| Ophiasis     | 2                     | 22.6 ± 6.9                        |
| Totalis      | 1                     | 20.92                             |
| Universalis  | 1                     | 24.68                             |
| SALT score   | S1                    | 20                                |
| S2           | 5                     | 21.02 ± 7.82                      |
| S3           | 2                     | 19.14 ± 5.18                      |
| S4           | 2                     | 22.8 ± 2.66                       |
| Disease activity | Active               | 28                                |
| Inactive     | 1                     | 17.12                             |
| Duration of episode | <3 mo                 | 13                                |
| 3-12 mo      | 11                    | 24.05 ± 6.12                      |
| 12-24 mo     | 3                     | 27.56 ± 10.25                     |
| loss >5 y    | 1                     | 19.52                             |
| scales       |                        | 20.92                             |

SALT, Severity of alopecia tool.
a consequence of sun avoidance. A practical application of these postulations is that simply determining the estimate of sun exposure per day can possibly help predict patients who are at the risk of having lower vitamin D levels.

In 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 phototype. A major limitation of this study is the limited sample size, with most of the AA cases having mild forms of AA. Future studies can focus on determining vitamin D levels in patients with more severe forms of AA to address this. Moreover, this study was conducted at a single tertiary hospital in an urbanized area in the Philippines. Further nationwide studies are needed to determine the true prevalence of vitamin D deficiency among Filipino patients with AA. Further research on vitamin D supplementation can also be performed for a subset of AA patients with concomitant vitamin D deficiency.

**Conflicts of interest**

Dr Gnilo is employed by Abbott as a Program Manager-Clinical Risk Management with salary as compensation. Drs Lizarondo, Gervasio, Chamberlin, and Silva have no conflicts of interest to declare.
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