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

Felix Paolo J. Lizarondo, MD, Mia Katrina R. Gervasio, MD, Charmaine Vanessa S. Chamberlin, MD, Charissa Mia S. Gnilo, MD, and Claudine Y. Silva, MD
Manila, Philippines

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 statsitically 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 2021;5:78-84.)

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

From the Department of Dermatology, Philippine General Hospital, University of the Philippines, Manila.
Funding sources: This study was partially funded by the Philippine Dermatological Society through a research grant given in 2018.
IRB approval status: Approved by the University of the Philippines Manila Review Ethics Board (MED2017-287-01).
Accepted for publication July 28, 2021.
Correspondence to: Felix Paolo J. Lizarondo, MD, Department of Dermatology, Philippine General Hospital, University of the Philippines, Taft Avenue, Manila, Philippines 1000. E-mail: fjlizarondo@up.edu.ph.
2666-3287

https://doi.org/10.1016/j.jdin.2021.07.008

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 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.[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 AA.

CAPSULE SUMMARY

- This study contributes to the developing global picture of the vitamin D status of alopecia areata patients.
- Various factors associated with lower vitamin D levels in alopecia areata patients were investigated in this study, which can help determine patients who will benefit most from vitamin D level determination.

Currently, there is evidence that the levels of vitamin D are lower in AA patients than in healthy controls.[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.[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.[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.[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,[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 ≥30 minutes per day). Holick's[34,35] recommendation was used as a guide for determining the cutoff time of sun exposure.[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.[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 ≥ 30 kg/m^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.[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

Abbreviations used:

AA: alopecia areata
SALT: severity of alopecia tool

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.[41] 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.[42-47] 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

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	19-65	19-64	
Sex	Males: 10 Females: 19	Males: 10 Females: 19	1
Average time of sun exposure per wk	<30 min: 16 ≥30 min: 13	<30 min: 16 ≥30 min: 13	1
Body mass index (kg/m^2)	23.41	24.07	.30
Skin phototype	III: 5 IV: 24	III: 3 IV: 24 V: 2	.48
Photoprotection practices Present (umbrella, caps or hats, and long clothing) Absent	Present: 21 Absent: 8	Present: 13 Absent: 16	.06

*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 (Mean ± SD, in ng/mL)	Healthy controls (Mean ± SD, in ng/mL)	*P* value
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	24.69 ± 7.22
	Ophiasis	2	22.6 ± 6.9
	Totalis	1	20.92
	Universalis	1	24.68
SALT score	S1	20	25.95 ± 6.80
	S2	5	21.02 ± 7.82
	S3	2	19.14 ± 5.18
	S4	2	22.8 ± 2.66
Disease activity	Active	28	24.67 ± 6.85
	Inactive	1	17.12
Duration of current episode of hair loss	<3 mo	13	24.05 ± 6.12
	3-12 mo	11	24.74 ± 7.70
	12-24 mo	3	27.56 ± 10.25
	2-5 y	1	19.52
	>5 y	1	20.92

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

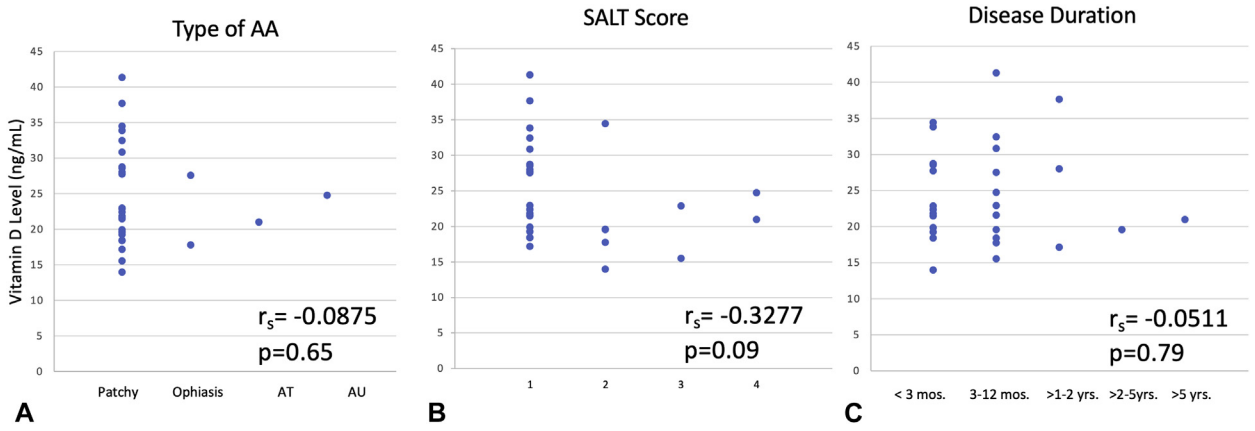

Fig 1. Scatter plots of vitamin D levels seen in various clinical parameters noted in AA patients. **A**, There was no correlation seen between the different clinical types of AA and vitamin D levels, with an r_s of −0.0875 (P = .65). **B**, There was a weakly negative correlation between SALT score and vitamin D levels, with an r_s of −0.3277 (P = .09). **C**, There was no correlation seen between AA duration and vitamin D levels, with an r_s of −0.0511 (P = .79). *AA*, Alopecia areata; r_s, Spearman correlation coefficient; *SALT*, severity of alopecia tool.

Table V. Epidemiologic characteristics of alopecia areata patients and corresponding mean vitamin D levels

Epidemiologic factors		Number of AA patients	Mean vitamin D level ± SD (ng/mL)
Age		29	See note*
Sex	Male	10	27.81 ± 8.35
	Female	19	22.63 ± 5.37
Body mass index		29	See note*
Sun exposure	<30 min per d	16	22.13 ± 5.64
	≥ 30 min per d	13	27.22 ± 7.40
Skin phototype	III	3	23.35 ± 4.10
	IV	24	24.62 ± 7.02
	V	2	31.59 ± 1.15
Photoprotection practices (apart from sunscreen use)	Present (umbrella, caps or hats, and clothing)	22	24.98 ± 7.34
	Absent	7	22.61 ± 5.17

AA, Alopecia areata; *BMI*, body mass index.
*The stratification of age and BMI into groups was not done because both were continuous variables. Hence, the mean vitamin D level is the same as that of AA patient group. The correlation of age and BMI with vitamin D level was analyzed using the Pearson correlation.

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.

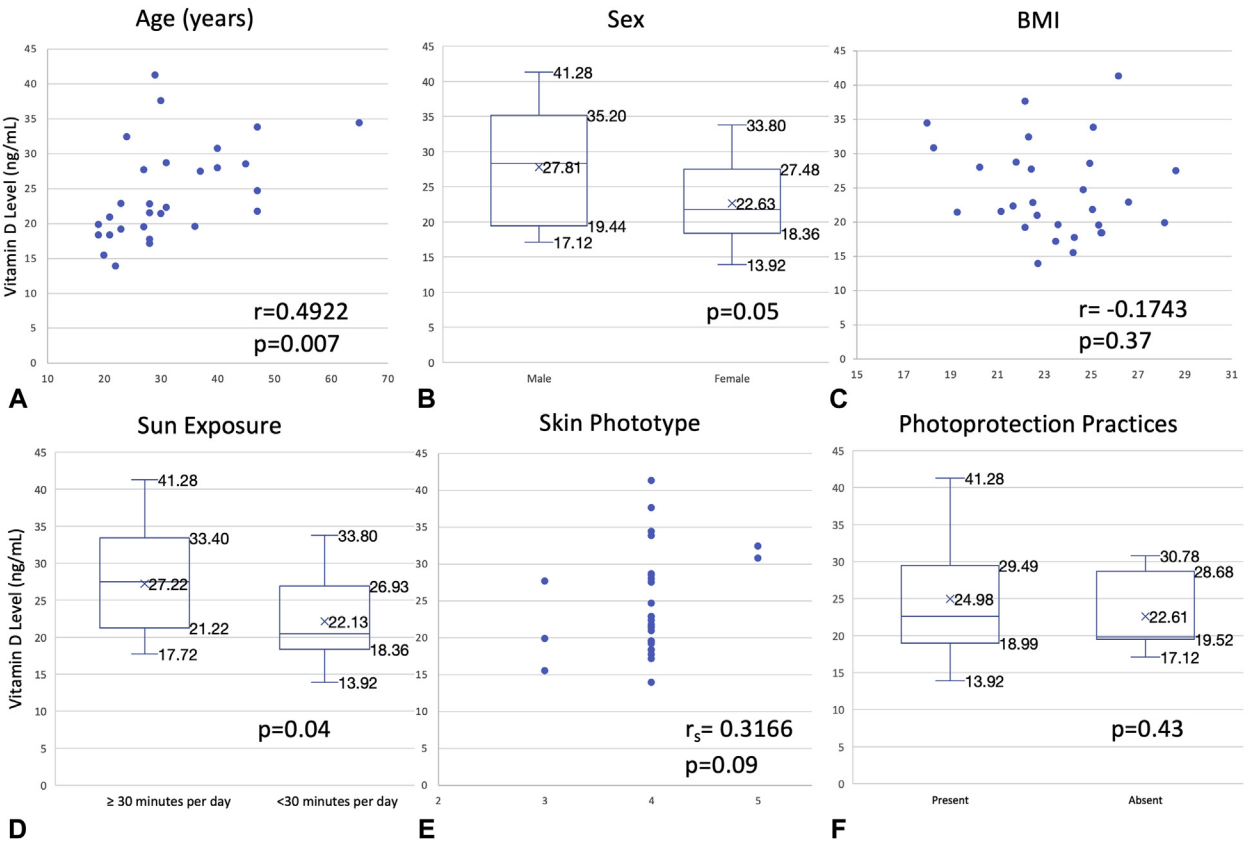

Fig 2. Scatter and box plots of vitamin D levels seen for the various epidemiologic parameters noted in AA patients. **A**, There was a moderately positive correlation between age and vitamin D levels, with an r of 0.4922 (P = .007). **B**, The vitamin D levels in females were borderline significantly lower than those in males (P = .05). **C**, There was a very weak negative correlation between BMI and vitamin D levels, with an r of −0.1743 (P = .37). **D**, The vitamin D levels in AA patients who had <30 minutes of sun exposure per day were significantly lower than those of AA patients who had ≥30 minutes per day (P = .04). **E**, There was a weakly positive correlation between skin phototype and vitamin D levels, with an r_s of 0.3166 (P = .09). **F**, The vitamin D levels of AA patients who had practiced photoprotection measures apart from sunscreen use were not different from those of AA patients who did not practice any form of photoprotection. *AA*, Alopecia areata; *BMI*, body mass index; *r*, Pearson correlation coefficient; r_s, Spearman correlation coefficient.

REFERENCES

1. Sperling L, Sinclair R, Shabrawi-Caelen L. Alopecias. In: Bolognia J, Jorizzo J, Schaffer J, eds. *Dermatology*. 4th ed. Elsevier Saunders; 2018:1162-1174.
2. Ito T. Recent advances in the pathogenesis of autoimmune hair loss disease alopecia areata. *Clin Dev Immunol*. 2013;2013:348546. https://doi.org/10.1155/2013/348546
3. de Berker D, Higgins C, Jahoda C, Christiano A. Biology of hair and nails. In: Bolognia J, Jorizzo J, Schaffer J, eds. *Dermatology*. 3rd ed. Elsevier Saunders; 2012:1075-1092.
4. Rajabi F, Drake LA, Senna MM, Rezaei N. Alopecia areata: a review of disease pathogenesis. *Br J Dermatol*. 2018;179(5):1033-1048. https://doi.org/10.1111/bjd.16808
5. Gilhar A, Schrum AG, Etzioni A, Waldmann H, Paus R. Alopecia areata: animal models illuminate autoimmune pathogenesis and novel immunotherapeutic strategies. *Autoimmun Rev*. 2016;15(7):726-735. https://doi.org/10.1016/j.autrev.2016.03.008
6. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-1930. https://doi.org/10.1210/jc.2011-0385
7. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients*. 2013;5(7):2502-2521. https://doi.org/10.3390/nu5072502
8. Ota K, Dambaeva S, Kim MW, et al. 1,25-Dihydroxy-vitamin D3 regulates NK-cell cytotoxicity, cytokine secretion, and degranulation in women with recurrent pregnancy losses. *Eur J Immunol*. 2015;45(11):3188-3199. https://doi.org/10.1002/eji.201545541
9. Amor KT, Rashid RM, Mirmirani P. Does D matter? The role of vitamin D in hair disorders and hair follicle cycling. *Dermatol Online J*. 2010;16(2):1-3. https://doi.org/10.5070/D38s34p6b7
10. Yilmaz N, Serarslan G, Gokce C. Vitamin D concentrations are decreased in patients with alopecia areata. *Vitam Trace Elem*. 2012;01(04):1-4. https://doi.org/10.4172/2167-0390.1000105
11. El-Mongy NN, El-Nabarawy E, Hassaan SA, Younis ER, Shaker O. Serum 25-hydroxy vitamin D3 level in Egyptian patients with

alopecia areata. *Egypt Women's Dermatol Soc*. 2013;10(1): 37-41. https://doi.org/10.1097/01.EWX.0000419612.74665.2b
12. Mahamid M, Abu-Elhija O, Samamra M, Mahamid A, Nseir W. Association between vitamin D levels and alopecia areata. *Isr Med Assoc J*. 2014;16(6):367-370.
13. Aksu Cerman A, Sarikaya Solak S, Kivanc Altunay I. Vitamin D deficiency in alopecia areata. *Br J Dermatol*. 2014;170(6):1299-1304. https://doi.org/10.1111/bjd.12980
14. Bakry OA, El Farargy SM, El Shafiee MK, Soliman A. Serum vitamin D in patients with alopecia areata. *Indian Dermatol Online J*. 2016;7(5):371-377. https://doi.org/10.4103/2229-5178.190504
15. Attwa E, Kandil AH, Elbalaat W, Samy AM. Assessment of vitamin D level in patients of alopecia areata. *J Clin Investig Dermatol*. 2016;4(2):1-4. https://doi.org/10.13188/2373-1044.1000030
16. Ghafoor R, Anwar MI. Vitamin D deficiency in alopecia areata. *J Coll Physicians Surg Pakistan*. 2017;27(4):200-202.
17. Bhat YJ, Latif I, Malik R, et al. Vitamin D level in alopecia areata. *Indian J Dermatol*. 2017;62(4):407-410. https://doi.org/10.4103/ijd.IJD_677_16
18. Gade VK, Mony A, Munisamy M, Chandrashekar L, Rajappa M. An investigation of vitamin D status in alopecia areata. *Clin Exp Med*. 2018;18(3):577-584. https://doi.org/10.1007/s10238-018-0511-8
19. Siddappa H, Kumar YH, Vivekananda N. Evaluation of association of vitamin D in alopecia areata: a case-control study of 100 patients in a tertiary rural hospital of southern India. *Indian Dermatol Online J*. 2019;10(1):45-49. https://doi.org/10.4103/idoj.IDOJ_84_18
20. Rehman F, Dogra N, Wani MA. Serum vitamin D levels and alopecia areata-a hospital based case-control study from north-India. *Int J Trichology*. 2019;11(2):49-57. https://doi.org/10.4103/ijt.ijt_3_19
21. Tsai T, Huang Y. Vitamin D deficiency in patients with alopecia areata: a systematic review and meta-analysis. *J Am Acad Dermatol*. 2018;78(1):207-209. https://doi.org/10.1016/j.jaad.2017.07.051
22. Lee S, Kim B, Lee C, Lee W. Increased prevalence of vitamin D deficiency in patients with alopecia areata: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol*. 2018;32(7): 1214-1221. https://doi.org/10.1111/jdv.14987
23. Lizarondo FP, Nacianceno PA, Yap-Silva C. Reduced levels of serum 25-hydroxyvitamin D in alopecia areata: a systematic review and meta-analysis. *Acta Med Philipp*. 2019;53(2):152-161.
24. Darwish NM, Marzok HF, Gaballah MA, Abdellatif HE. Serum level of vitamin D in patients with alopecia areata. *Egypt J Basic Appl Sci*. 2017;4(1):9-14. https://doi.org/10.1016/j.ejbas.2016.12.001
25. d'Ovidio R, Vessio M, d'Ovidio FD. Reduced level of 25-hydroxyvitamin D in chronic/relapsing alopecia areata. *Dermatoendocrinology*. 2013;5(2):271-273.
26. Erpolat S, Sarifakioglu E, Ayyildiz A. 25-hydroxyvitamin D status in patients with alopecia areata. *Adv Dermatol Allergol*. 2017;34(3):248-252. https://doi.org/10.5114/ada.2017.67847
27. Unal M, Gonulalan G. Serum vitamin D level is related to disease severity in pediatric alopecia areata. *J Cosmet Dermatol*. 2018;17(1):101-104. https://doi.org/10.1111/jocd.12352
28. Marahatta S, Agrawal S, Khan S. Study on serum vitamin D in alopecia areata patients. *J Nepal Health Res Counc*. 2019;17(1): 21-25. https://doi.org/10.33314/jnhrc.1475
29. Lips P, van Schoor NM, de Jongh RT. Diet, sun, and lifestyle as determinants of vitamin D status. *Ann N Y Acad Sci*. 2014; 1317(1):92-98. https://doi.org/10.1111/nyas.12443
30. Mithal A, Wahl DA, Bonjour JP, et al. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int*. 2009; 20(11):1807-1820. https://doi.org/10.1007/s00198-009-0954-6
31. Lips P. Worldwide status of vitamin D nutrition. *J Steroid Biochem Mol Biol*. 2010;121(1-2):297-300. https://doi.org/10.1016/j.jsbmb.2010.02.021
32. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biol*. 2014; 144(Part A):138-145. https://doi.org/10.1016/j.jsbmb.2013.11.003
33. Edwards MH, Cole ZA, Harvey NC, Cooper C. The global epidemiology of vitamin D status. *J Aging Res Clin Prac*. 2014; 3(3):148-158.
34. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr*. 2004;79(9):362-371. https://doi.org/10.1093/ajcn/79.3.362
35. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-281. https://doi.org/10.1056/NEJMra070553
36. Nimitphong H, Holick MF. Vitamin D status and sun exposure in Southeast Asia. *Dermatoendocrinol*. 2013;5(1):34-37. https://doi.org/10.4161/derm.23873
37. Olsen EA. Investigative guidelines for alopecia areata. *Dermatol Ther*. 2011;24(3):311-319. https://doi.org/10.1111/j.1529-8019.2011.01415.x
38. Mubki T, Rudnicka L, Olszewska M, Shapiro J. Evaluation and diagnosis of the hair loss patient: part II. Trichoscopic and laboratory evaluations. *J Am Acad Dermatol*. 2014;71(3): 431.e1-431.e11. https://doi.org/10.1016/j.jaad.2014.05.008
39. Wadhwa B, Relhan V, Goel K, Kochhar A, Garg V. Vitamin D and skin diseases: a review. *Indian J Dermatol Venereol Leprol*. 2015; 81(4):344-355. https://doi.org/10.4103/0378-6323.159928
40. Agmon-Levin N, Theodor E, Segal RM, Shoenfeld Y. Vitamin D in systemic and organ-specific autoimmune diseases. *Clin Rev Allergy Immunol*. 2013;45(2):256-266. https://doi.org/10.1007/s12016-012-8342-y
41. Angeles-Agdeppa I, Perlas LA, Capanzana MV. Vitamin D status of Filipino adults: evidence from the 8th National Nutrition Survey 2013. *Malays J Nutr*. 2018; 24(3):395-406.
42. Chailurkit L, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. *BMC Public Health*. 2011;11(1):1-7. https://doi.org/10.1186/1471-2458-11-853
43. Rahman SA, Chee WS, Yassin Z, Chan SP. Vitamin D status among postmenopausal Malaysian women. *Asia Pac J Clin Nutr*. 2004;13(3):255-260.
44. Nurbazlin M, Chee WS, Rokiah P, et al. Effects of sun exposure on 25(OH) vitamin D concentration in urban and rural women in Malaysia. *Asia Pac J Clin Nutr*. 2013;22(3): 391-399. https://doi.org/10.6133/apjcn.2013.22.3.15
45. Robien K, Butler LM, Wang R, et al. Genetic and environmental predictors of serum 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore. *Br J Nutr*. 2013;109(3):493-502. https://doi.org/10.1017/S0007114512001675
46. Soesanti F, Pulungan A, Tridjaja B, Batubara JR. Vitamin D profile in healthy children aged 7-12 years old in Indonesia. *Int J Pediatr Endocrinol*. 2013;2013(suppl 1):167. https://doi.org/10.1186/1687-9856-2013-S1-P167
47. Green TJ, Skeaff CM, Rockell JE, et al. Vitamin D status and its association with parathyroid hormone concentrations in women of child-bearing age living in Jakarta and Kuala Lumpur. *Eur J Clin Nutr*. 2008;62(3):373-378. https://doi.org/10.1038/sj.ejcn.1602696