



# Association of Alopecia Areata with Vitamin D and Calcium Levels: A Systematic Review and Meta-analysis

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## ABSTRACT

**Introduction:** To investigate the associations of alopecia areata (AA) with serum vitamin D and calcium levels.

**Methods:** A systematic review of all relevant articles published up to February 2020 in PubMed, Embase, and Cochrane Library databases was conducted. Primary endpoints were serum 25-hydroxyvitamin D [25(OH)D] levels and vitamin D deficiency, and the secondary endpoint was serum calcium level. Odds ratio (OR) and standardized mean difference (SMD) with 95% CI across studies were analyzed.

**Results:** Data on 1585 patients with AA and 1114 controls from 16 case–control studies and three cross-sectional studies were included in

this meta-analysis. A pooled meta-analysis was conducted using the random-effects model because of inter-study heterogeneity (vitamin D level,  $I^2 = 87.90\%$ ; vitamin D deficiency,  $I^2 = 81.10\%$ ; serum calcium level,  $I^2 = 83.80\%$ ). A combined analysis revealed that patients with AA had significantly lower mean serum 25(OH)D level compared with control (WMD  $-9.08$ , 95% CI  $-11.65, -6.50$ ,  $p < 0.001$ ), and were more likely to have vitamin D deficiency (OR 4.14, 95% CI 2.34, 7.35,  $p < 0.001$ ). However, the pooled analysis revealed that patients with AA did not have significantly lower serum calcium levels compared with control (WMD  $-0.17$ , 95% CI  $-0.40, 0.06$ ,  $p = 0.143$ ). Subgroup analysis suggested that matched control, mean age, and country might contribute to the heterogeneity of serum vitamin D level, while study design, matched control, and country might contribute to the heterogeneity of vitamin D deficiency.

**Conclusion:** Deficiency of serum 25(OH)D level, rather than calcium level, was present in patients with AA. Screening for vitamin D deficiency and vitamin D supplementation may be beneficial in the treatment of patients with AA.

**Keywords:** Alopecia areata; Areata; Calcium; Vitamin D; 1,25-Dihydroxyvitamin D; 25-Hydroxyvitamin D

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## Key Summary Points

### Why carry out this study?

Alopecia areata (AA) is a form of non-scarring hair loss characterized by an autoimmune reaction to hair follicles with disordered, shortened hair cycle

Vitamin D deficiency was considered as a risk factor for the development of AA

The relationship between vitamin D deficiency and AA in patients was reported in two previous meta-analyses but not comprehensively evaluated because five subsequent studies were not considered

### What was learned from the study?

Deficiency of serum 25(OH)D level, rather than calcium level, was present in patients with AA. Screening for vitamin D deficiency and vitamin D supplementation may be beneficial in treatment of patients with AA

## INTRODUCTION

Alopecia areata (AA) is a form of non-scarring hair loss characterized by an autoimmune reaction to hair follicles with disordered, shortened hair cycle [1]. The prevalence of AA is 0.1–0.2%, with a calculated lifetime risk of 2% [2]. The age- and sex-adjusted incidence of AA is 20.9 per 100,000 person-years [3]. About 51% of patients with AA are female, and the median age at diagnosis is 33 years, affecting both children and adults, and hair of all colors [3]. Earlier age of onset and male gender appear to have more severe involvement [4]. The hallmark of AA is T cell (CD4<sup>+</sup> and CD8<sup>+</sup>) infiltrates and cytokine production, specifically targeting anaphase hair follicles [5, 6]. Autoantibodies targeting hair follicles, especially against keratin 16 and trichohyalin, are increased in peripheral blood of patients with AA [7].

Although the etiology is different between chemotherapy-induced alopecia (cytotoxic agents killing the rapidly renewing cells in the hair follicles) and AA (autoimmunity), vitamin D deficiency might be considered a risk factor for the development of AA since vitamin D protects hair follicles from chemotherapy-induced alopecia [8, 9]. Keratinocytes possess the enzymatic machinery to synthesize active vitamin D, i.e., 1,25-dihydroxyvitamin D<sub>3</sub> [serum 1,25(OH)D<sub>3</sub>], and express the vitamin D receptor (VDR), which is necessary for the maintenance of the normal hair cycle [10]. Besides, 1,25(OH)D<sub>3</sub> acts as an immunomodulator of innate and adaptive immune functions targeting various immune cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, and dendritic cells [11]. The association between vitamin D and several autoimmune-mediated diseases has been reported, including vitiligo, systemic lupus erythematosus, type I diabetes mellitus, rheumatoid arthritis, psoriasis, multiple sclerosis, and inflammatory bowel disease [12]. Although associations are not causative factors, that might imply that vitamin D deficiency is an environmental trigger for the induction of abnormal autoimmunity [13]. Therefore, 1,25(OH)D<sub>3</sub> modulates both innate and adaptive immune responses by targeting various immune cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, and dendritic cells, among others [11]. In addition, vitamin D synthesis decreases with age [14–16]. Vitamin D deficiency is common in both men and women, and can affect pregnancy outcomes in women and increase the risk of osteoporosis, especially in menopausal women [14–17]. Although the relationship between vitamin D deficiency and AA in patients was reported in two previous meta-analyses [18, 19], this relationship has not been comprehensively evaluated since five subsequent studies were not considered [20–24].

Vitamin D is also a secosteroid hormone, primarily synthesized in epidermal keratinocytes or acquired from the diet, and plays an important role in calcium homeostasis and bone health [25, 26]. Two studies revealed no difference in serum calcium levels between patients with AA and controls despite a statistically significant difference in vitamin D levels [20, 27]. The

presence of 25-hydroxyvitamin D [25(OH)D] deficiency in patients with AA correlated with increased parathyroid hormone (PTH) [24], suggesting a compensatory effect of increased PTH to maintain normal serum calcium in a state of vitamin D deficiency. Darwish et al. showed lower levels of calcium but comparable PTH level [22], while Yilmaz et al. showed comparable levels of both calcium and PTH in patients with AA compared to controls [20].

In this context, the study aimed to evaluate the serum 25(OH)D levels and calcium levels in patients with AA in order to identify their potential roles in the pathogenesis of AA. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

## METHODS

### Search Strategy

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines. An electronic search of the PubMed, Embase, and Cochrane Library databases was performed up to February 18, 2020, without language restrictions. We first screened the abstracts and then chose relevant full-text articles. The reference lists of selected articles were manually searched to identify additional relevant reports.

### Selection Criteria

The inclusion criteria were as follows: (1) diagnoses of AA were based on clinical findings; (2) studies centered on associations of AA with vitamin D and calcium; (3) either serum 25-hydroxyvitamin D levels or vitamin D deficiency or calcium level was available for cases and controls; (4) only the publication with the largest number of participants was included for studies involving overlapping data sets; (5) publication of sufficient information to calculate odds ratios (OR) and weighted mean

difference (WMD); and (6) published in peer-reviewed journals.

The exclusion criteria were as follows: (a) studies without a designated control; (b) the presence of other diseases influencing 25-hydroxyvitamin D and calcium level; (c) summaries, abstracts, case reports, and reviews; (d) studies reported in a language other than English.

### Data Extraction and Assessment of Risk of Bias

Two blinded and independent researchers (Yi Liu and Jing Li) reviewed potentially relevant publications, and any disagreements were resolved by a third senior researcher (Xinfeng Wu). Two investigators independently extracted the following information using a predefined data collection form: author, year of publication, patient and control group characteristics, study design, sample size, gender, country or race, the outcome of interest, etc. Disagreements were resolved by consensus between the two investigators. We attempted to contact the authors for missing primary and secondary outcomes.

The Agency for Healthcare Research and Quality (AHRQ) and Newcastle–Ottawa Scale (NOS) scales were used to independently assess the methodological quality of cross-sectional studies and case–control studies, respectively.

### Outcomes

The primary outcomes were serum 25-hydroxyvitamin D levels and vitamin D deficiency, and the secondary outcome was the serum calcium level. Vitamin D deficiency was defined as serum 25(OH)D level lower than 20 or 30 ng/dL depending on the study.

### Statistical Analysis

The odds ratios (ORs) of having vitamin D deficiency were estimated for each study by comparing patients with AA versus healthy controls and then pooled. The ORs were pooled using the DerSimonian and Laird method if

heterogeneity was present; otherwise, they were pooled using a fixed-effects model. For continuous outcomes, the mean difference of serum 25-hydroxyvitamin D level and calcium level between patients with AA versus healthy controls was estimated for each study and then pooled across studies using weighted mean difference (WMD). Heterogeneity was assessed using  $Q$  statistics, and the degree of heterogeneity was quantified using  $I^2$ . If heterogeneity was detected ( $p < 0.10$  or  $I^2 \geq 50\%$ ), a random-effects model was applied; otherwise, a fixed-effects model was used. In order to check the stability of the result, a sensitivity analysis was performed by sequential deletion of each study. Rosenthal's fail-safe  $N$  was utilized for sensitivity analysis. The risk of publication bias was evaluated via Egger's test, Begg's test, and funnel plots. Subgroup analysis was performed to investigate the potential additional effect of predefined factors. A two-tailed  $p < 0.05$  was considered significant. All statistical analyses in this meta-analysis were performed using the STATA MP 14.0 software (StataCorp, College Station, TX, USA).

## RESULTS

### Characteristics of Included Studies

The flowchart of the study selection process is shown in Fig. 1. There were 19 eligible publications (16 case-control studies and three cross-sectional studies), involving 2699 subjects (1585 AA cases and 1114 controls) [20–24, 27–40]. The sample size of the included studies ranged from 43 to 756, and publication date from 2012 to 2019. The included studies were from eight countries, namely Egypt, India, Israel, Italy, Nepal, Pakistan, Turkey, and the USA. Eleven studies had age- and sex-matched controls. Seventeen, thirteen, and six studies provided data on serum 25(OH)D levels, vitamin D deficiency, and serum calcium level, respectively. Detailed characteristics of the included studies are summarized in Table 1. The integral quality of the included case-control and cross-sectional studies was rated as high (sTables 1 and 2 in the supplementary material).

### Heterogeneity and Publication Bias

During pooled meta-analysis, inter-study heterogeneity ( $I^2 > 50\%$ ) was found in vitamin D level ( $I^2 = 87.90\%$ ,  $p < 0.001$ , Fig. 2), vitamin D deficiency ( $I^2 = 81.10\%$ ,  $p < 0.001$ , Fig. 3), and serum calcium level ( $I^2 = 83.80\%$ ,  $p < 0.001$ , Fig. 4). Therefore, the pooled meta-analysis for these factors was conducted using the random-effects model.

No evidence of publication bias was identified in the meta-analysis of serum vitamin D level (Begg's test,  $p = 0.650$ ; Egger's test,  $p = 0.756$ ) and vitamin D deficiency (Begg's test,  $p = 0.583$ ; Egger's test,  $p = 0.257$ ). Visual inspection of the funnel plots revealed no evidence of publication bias for serum vitamin D levels and vitamin D deficiency (sFigs. 2 and 4 in the supplementary material). Therefore, these data indicated that there was no publication bias in the present meta-analysis, and the results were statistically robust.

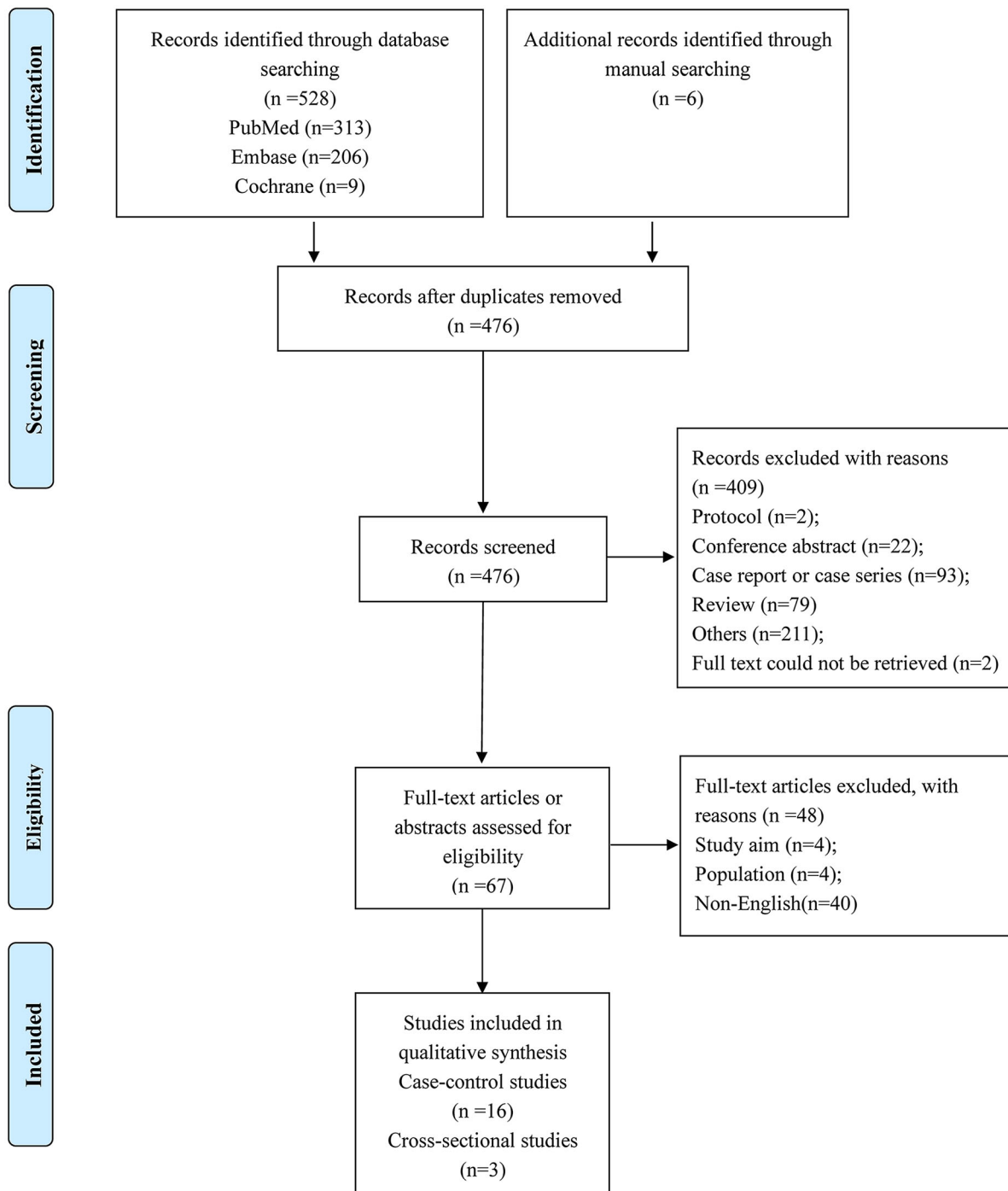
### Meta-analysis Results

According to inter-study heterogeneity by  $Q$  test and  $I^2$  test, the pooled analysis was conducted using the random-effects model for vitamin D level, vitamin D deficiency, and calcium level. Among the 17 studies with serum 25(OH)D level data, the results showed that patients with AA had significantly lower mean serum 25(OH)D level compared with controls (WMD 9.08, 95% CI – 11.66, – 6.50,  $p < 0.001$ , Fig. 2).

Among the 13 studies with vitamin D deficiency data, the meta-analysis suggested that patients with AA were more likely to have vitamin D deficiency with a pooled OR of 4.14 (95% CI 2.34, 7.35,  $p < 0.001$ , Fig. 3). Among the six included studies with serum calcium level data, the findings revealed that patients with AA did not have a statistically lower mean serum calcium level compared with controls (WMD – 0.17, 95% CI – 0.40, 0.06,  $p = 0.143$ , Fig. 4).

### Subgroup Analysis

For serum 25(OH)D levels, similar statistically significant findings were obtained for subgroup



**Fig. 1** Flowchart of literature search and study selection

analyses of study design (WMD of case-control – 9.05, 95% CI – 12.46, – 5.63; WMD of cross-sectional – 9.82, 95% CI – 11.91, – 7.72), sample size (WMD of > 100: – 8.35, 95% CI

– 11.19, – 5.51; WMD of ≤ 100: – 9.59, 95% CI – 13.44, – 5.74), and male ratio (WMD of > 1/2: – 7.79, 95% CI – 11.47, – 4.11; WMD of ≤ 1/2: – 12.03, 95% CI – 16.93, – 7.13)

**Table 1** Characteristics of the 19 studies included in this meta-analysis

Study	Study design	Sample size		Age (year, mean or median)		Male (%)		Country/ Race	Control setting	Outcome of interest	
		Total	AA	Control	AA	Control	AA				
Marahatta S, 2019	Case-control study	60	30	30	28.37±10.070	30.50 ± 9.032	16 (51.6%)	15 (48.4%)	Nepal	Age- and sex-matched healthy controls from hospital staffs	Vitamin D level, Vitamin D deficiency
Rehman F, 2019	Case-control study	270	135	135	26 ± 12.89	26 ± 13.20	91 (67.41%)	91 (67.41%)	India	Age- and sex-matched healthy controls	Vitamin D level, Vitamin D deficiency, serum calcium level
Siddappa H, 2019	Case-control study	200	100	100	24.52 ± 10.06	28.96±11.49	72 (72%)	58 (58%)	India	Age- and sex-matched healthy controls without any history of alopecia areata or other autoimmune/systemic diseases	Vitamin D level, Vitamin D deficiency
Daroach M, 2018	Case-control study	60	30	30	28.9 ± 9.96	31.17 ± 9.43	11 (36.67%)	16 (53.33%)	India	Age- and sex-matched healthy controls	Vitamin D level, Vitamin D deficiency
Gade V.K.V., 2018	Case-control study	90	45	45	32.73 ± 10.43	33.98 ± 8.48	14 (31.1%)	14 (31.1%)	India	Age- and sex-matched healthy volunteers	Vitamin D level, serum calcium level

**Table 1** continued

Study	Study design	Sample size		Age (year, mean or median)		Male (%)		Country/ Race	Control setting	Outcome of interest	
		Total	AA	Control	AA	Control	AA				
Unal M, 2018	Case-control study	54	20	34	12.4 ± 4.2	16.6 ± 0.8	14 (70%)	15 (44.12%)	Turkey	Pediatric healthy controls recruited from individuals who visited to the outpatient clinic	Vitamin D level
Bhat YJ, 2017	Cross-sectional study	85	50	35	22.4 ± 8.6	29.2 ± 7.6	NR	NR	India	Age- and sex-matched individuals selected randomly from outpatient department with no history of AA	Vitamin D level, serum calcium level
Conic R.Z., 2017	Case-control study	756	584	172	35.54 ± 19.28	35.80 ± 15.56	184 (31.50%)	46 (26.75%)	USA	Age-matched patients given a diagnosis of seborrheic dermatitis without hair loss was randomly selected	Vitamin D deficiency
Darwish NMM, 2017	Case-control study	50	30	20	28.67 ± 10	24.8 ± 6	13 (43.3%)	10 (50%)	Egypt	Age- and sex matched healthy controls	Vitamin D level, serum calcium level
Erpolat S, 2017	Case-control study	73	41	32	32.8 ± 7.5	32.7 ± 7.5	26 (63.4%)	18 (56.3%)	Turkey	Healthy control	Vitamin D level, Vitamin D deficiency, serum calcium level

Table 1 continued

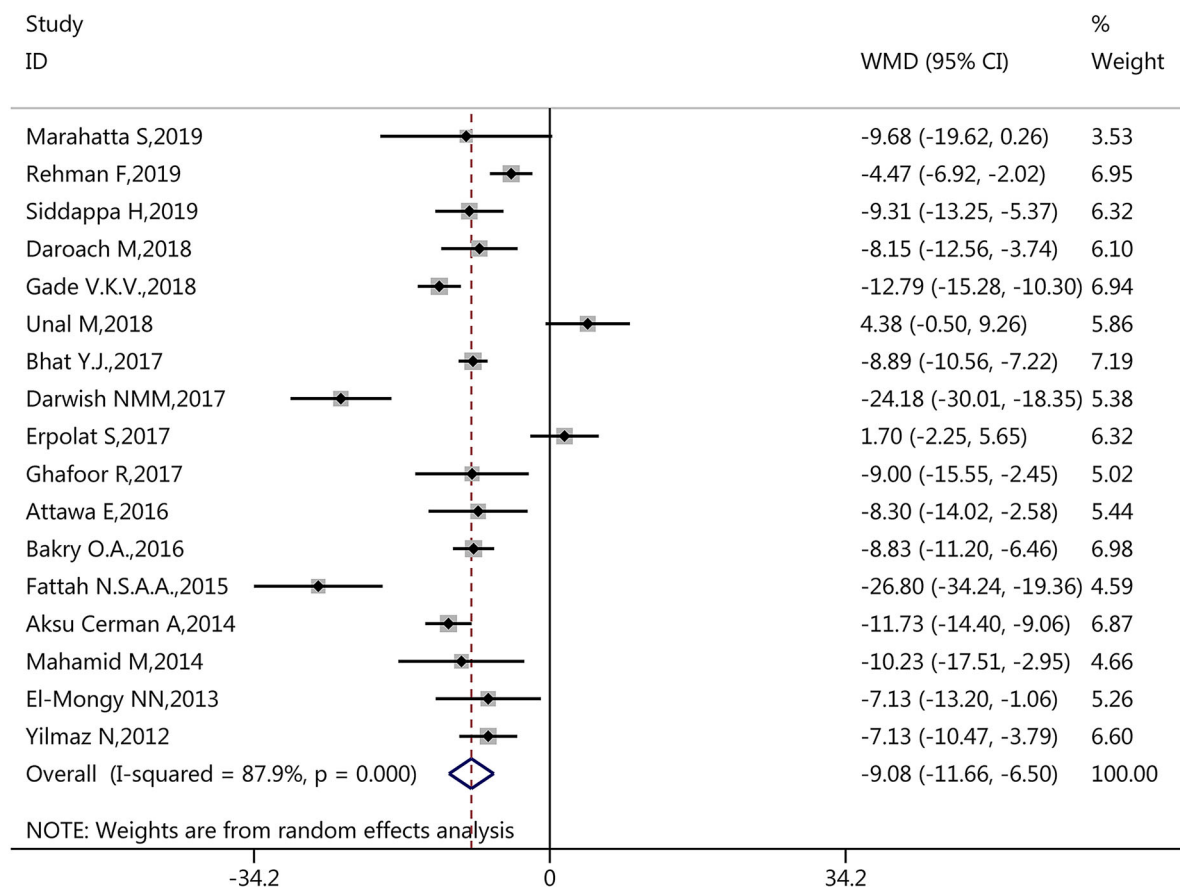
Study	Study design	Sample size		Age (year, mean or median)		Male (%)		Country/ Race	Control setting	Outcome of interest
		Total	AA	Control	AA	Control	AA			
Ghafoor R, 2017	Case-control study	60	30	30	23.77 ± 8.86	24.03 ± 8.62	12 (40%)	12 (40%)	Pakistan Healthy volunteers and patients coming to dermatology department for other disorders like acne, melasma, etc.	Vitamin D level
Artaawa E, 2016	Cross-sectional study	46	23	23	26.44 ± 10.87	29.39 ± 8.10	15 (65.2%)	14 (60.9%)	Egypt Healthy controls	Vitamin D level, Vitamin D deficiency
Bakry O.A., 2016	Case-control study	120	60	60	20.7 ± 10.85	23.71 ± 7.45	36 (60%)	28 (46.7%)	Egypt Age, gender, skin phototype, and body mass index-matched healthy subjects	Vitamin D level, Vitamin D deficiency
Fattah N.S.A.A., 2015	Case-control study	60	30	30	26.8 ± 6.9	25.1 ± 6.9	18 (60%)	18 (60%)	Egypt Healthy volunteers, matched for age, sex, Fitzpatrick skin phototype, the approximate daily amount of vitamin D intake from food source, the occupation (indoor or outdoor), and time of blood sampling	Vitamin D level, Vitamin D deficiency
Aksu Cerman A, 2014	Cross-sectional study	144	86	58	32.21 ± 9.60	32.55 ± 9.78	56 (47.9%)	34 (29.1%)	Turkey Age- and sex-matched volunteers from the hospital staff.	Vitamin D level, Vitamin D deficiency



**Table 1** continued

Study	Study design	Sample size		Age (year, mean or median)		Male (%)		Country/ Race	Control setting	Outcome of interest	
		Total	AA	Control	AA	Control	AA				
Mahamid M, 2014	Case-control study	43	23	20	24.2 ± 12.3	27 ± 11.26	14 (60.87%)	13 (65%)	Israel	Individuals without a history of AA who were enrolled randomly from clinics	Vitamin D level, Vitamin D deficiency
d'Ovidio R, 2013	Case-control study	304	156	148	37.8	34.5	45 (28.85%)	18 (12.16%)	Italy	Healthy individuals	Vitamin D deficiency
El-Mongy NN, 2013	Case-control study	140	70	70	27.79 ± 9.116	30.49 ± 11.059	37 (52.9%)	44 (62.9%)	Egypt	Normal controls	Vitamin D level, Vitamin D deficiency
Yilmaz N, 2012	Case-control study	84	42	42	30.8 ± 8.2	29.3 ± 7.4	14 (33.33%)	13 (30.95)	Turkey	Healthy individuals	Vitamin D level, serum calcium level

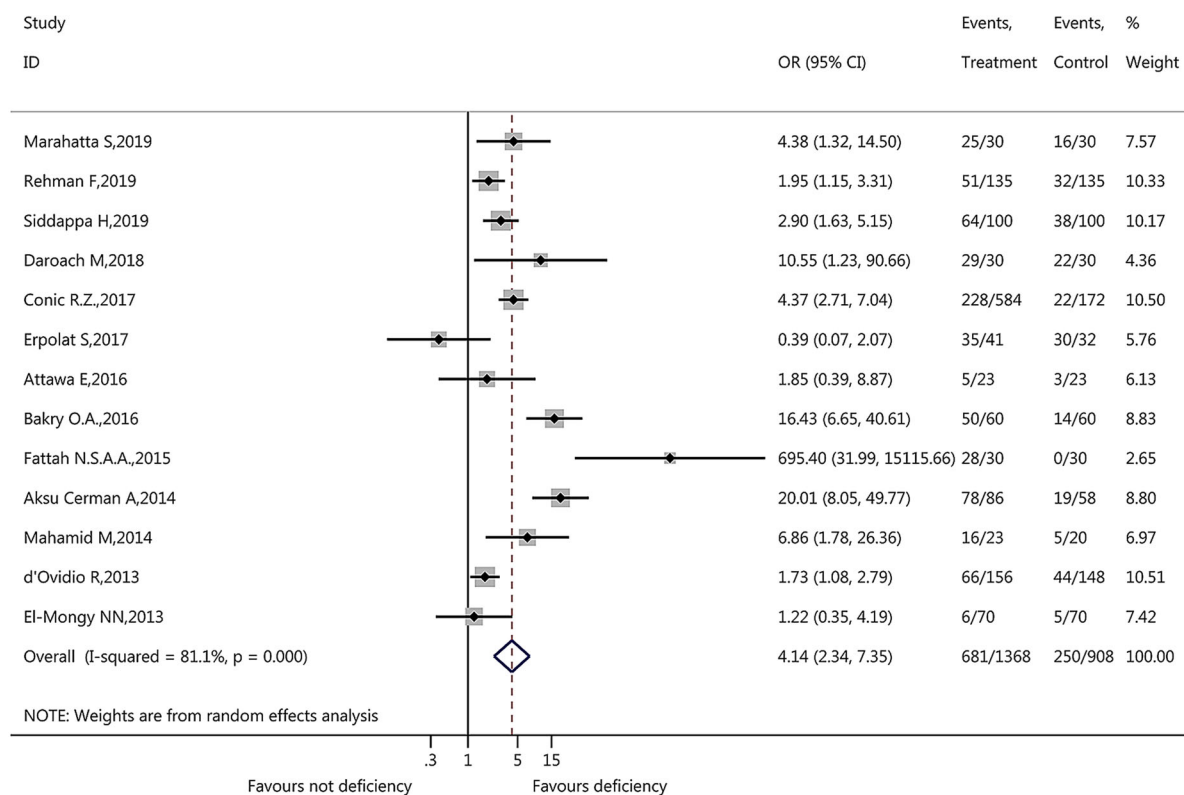
AA Alopecia areata, NR not reported



**Fig. 2** Forest plot of the meta-analysis of serum vitamin D level

(Table 2). However, inconsistent results were found for matched control (WMD of matched control – 11.38, 95% CI – 13.99, – 8.78; WMD of non-matched control – 3.18, 95% CI – 8.35, 1.99), mean age (WMD of > 25 years – 10.06, 95% CI – 12.87, – 7.24; WMD of < 25 years – 3.18, 95% CI – 8.35, 1.99), country (WMD of Nepal – 9.68, 95% CI – 19.62, 0.26, WMD of India – 8.73, 95% CI – 11.59, – 5.87; WMD of Turkey – 3.37, 95% CI – 10.68, 3.94; WMD of Egypt – 14.75, 95% CI – 22.10, – 7.39; WMD of Pakistan – 9.00, 95% CI – 15.55, – 2.45; WMD of Israel – 10.23, 95% CI – 17.51, – 2.95) (Table 2). These findings suggested that matched control, mean age, and country might contribute to a high degree of inter-study heterogeneity in serum vitamin D level.

For vitamin D deficiency, consistent statistically significant findings were identified for subgroup analyses of sample size (OR of > 100: 3.94, 95% CI 2.06, 7.54; OR of ≤ 100: 5.36, 95% CI 1.29, 22.21), male ratio (OR of > 1/2: 4.51, 95% CI 2.05, 9.93; OR of ≤ 1/2: 3.24, 95% CI 1.37, 7.63), and mean age (OR of > 25 years: 3.58, 95% CI 2.04, 6.28; OR of < 25 years: 16.43, 95% CI 6.65, 40.61) (Table 2). However, inconsistent results were found for study design (OR of case–control 3.66, 95% CI 2.06, 6.49; OR of cross-sectional 6.65, 95% CI 0.65, 68.27), matched control (OR of matched control 6.96, 95% CI 3.54, 13.68; OR of non-matched control 1.52, 95% CI 1.00, 2.32), and country (OR of Nepal 4.38, 95% CI 1.32, 14.50, OR of India 2.55, 95% CI 1.54, 4.21; OR of Turkey 2.97, 95%



**Fig. 3** Forest plot of the meta-analysis of vitamin D deficiency

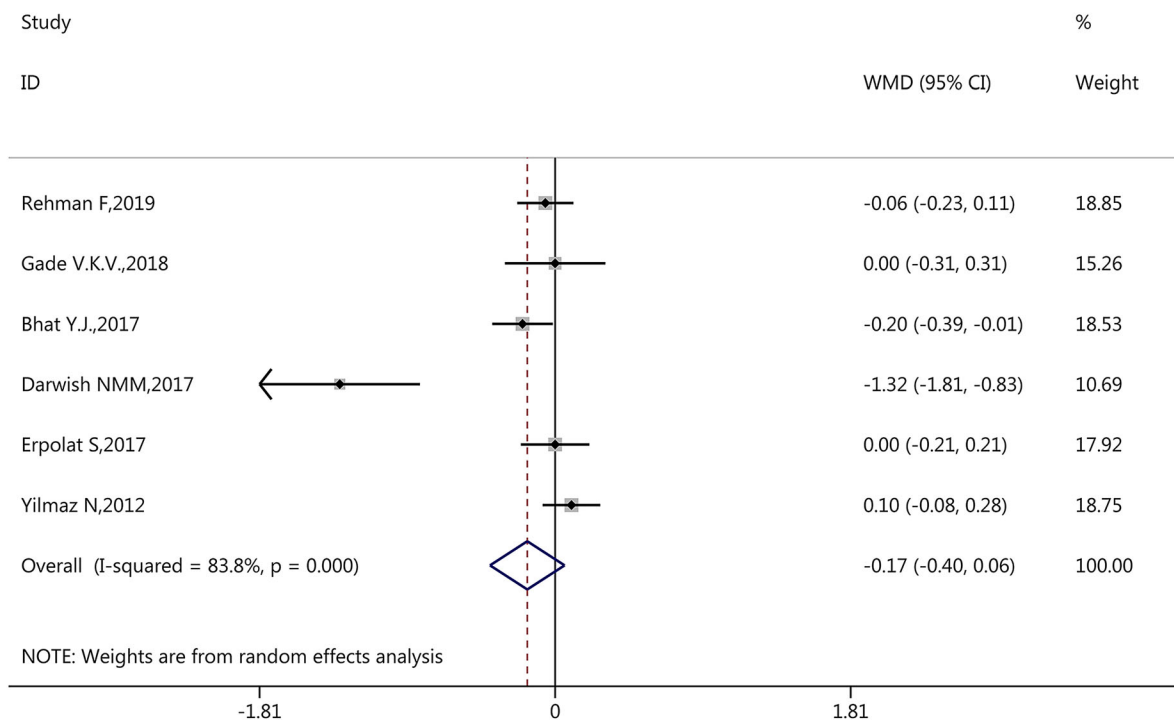
CI 0.06, 147.87; OR of Egypt 8.91, 95% CI 1.20, 66.39; OR of Israel 6.86, 95% CI 1.78, 26.36; OR of Italy 1.73, 95% CI 1.08, 2.79; OR of USA 4.37, 95% CI 2.71, 7.04) (Table 2). These data suggested that study design, matched control, and country might contribute to a high degree of inter-study heterogeneity in vitamin D deficiency.

**Sensitivity Analysis**

A sensitivity analysis was performed to check the robustness of the results. Statistically similar outcomes were obtained for meta-analysis of vitamin D level (sFig. 1 in the supplementary material) and vitamin D deficiency (sFig. 3 in the supplementary material) after sequentially excluding each case–control study, indicating the stability of the present meta-analysis.

**DISCUSSION**

The present systematic review and meta-analysis indicated that patients with AA had lower serum 25(OH)D3 level and higher risk of vitamin D deficiency, but not lower serum calcium level compared with controls. Thus, screening for vitamin D deficiency and supplementation of vitamin D could be beneficial in the treatment of AA. In addition to regulating bone and calcium metabolism, the biologically active metabolite of vitamin D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, can modulate nuclear vitamin D receptor expressed in antigen-presenting cells (APCs) and activated T cells via interference with nuclear transcription factors such as NF-AT and NF-κB or by direct interaction with vitamin D responsive elements in the promoter regions of cytokine genes [41].



**Fig. 4** Forest plot of the meta-analysis of serum calcium level

The beneficial effect of vitamin D<sub>3</sub> is mediated through the prevention of strong Th1 cell responses via the action on APCs, while simultaneously tilting the T cell response towards Th2 dominance [41, 42]. The beneficial effect of vitamin D supplementation was observed in the prevention and treatment of autoimmune-mediated diseases [43–45], but no study was conducted in patients with AA. A 7-year-old patient with AA and reduced vitamin D receptor expression recovered after topical application of calcipotriol, a strong vitamin D analogue [46]. The mechanism of topical application of calcipotriol in the restoration of hair cycle dysfunction is believed to regulate the differentiation of different cells and the expression of Toll-like receptors [44]. Therefore, large prospective studies are required to confirm the role of vitamin D supplementation in the treatment of AA, especially the severe form, which is commonly associated with other autoimmune diseases [47].

This study had two strengths that likely increased the reliability and clinical value of the findings. First, compared with the previous two studies [18, 19], the results of the present study were more reliable as we extensively searched to include all potentially eligible studies. Second, unlike the two previous studies, our pooled analysis of six studies revealed that patients with AA did not have a statistically lower mean serum calcium level compared with controls. A plausible explanation of normal calcium level is the compensatory increase in PTH during vitamin D deficiency. Nevertheless, Darwish et al. revealed lower levels of serum 25(OH)D and calcium but comparable PTH level in patients with AA compared with controls [22]; they also showed a positive linear correlation between serum vitamin D and serum calcium in patients with AA. Yilmaz et al. also found no significant difference in PTH level between patients with AA and controls [20]. Thus, whether there is a compensatory effect of increased PTH and how

**Table 2** Subgroup meta-analysis of serum vitamin D level and vitamin D deficiency

	Vitamin D				Vitamin D deficiency					
	No. of articles	WMD (95%CI)	<i>p</i>	<i>I</i> <sup>2</sup> (%)	<i>p</i> <sub>heterogeneity</sub>	No. of articles	OR (95% CI)	<i>p</i>	<i>I</i> <sup>2</sup> (%)	<i>p</i> <sub>heterogeneity</sub>
Overall	17	- 9.08 (- 11.66, - 6.50)	< 0.001	87.90	< 0.001	13	4.14 (2.34, 7.35)	< 0.001	81.10	< 0.001
Design										
Case-control	14	- 9.05 (- 12.46, - 5.63)	< 0.001	89.70	< 0.001	11	3.66 (2.06, 6.49)	< 0.001	78.80	< 0.001
Cross-sectional	3	- 9.82 (- 11.91, - 7.72)	< 0.001	40.20	0.188	2	6.65 (0.65, 68.27)	0.111	84.90	0.01
Sample size										
≤ 100	12	- 9.59 (- 13.44, - 5.74)	< 0.001	90.50	< 0.001	6	5.36 (1.29, 22.21)	0.021	76.50	0.001
> 100	5	- 8.35 (- 11.19, - 5.51)	< 0.001	75.50	< 0.001	7	3.94 (2.06, 7.54)	< 0.001	85.70	< 0.001
Controls										
Matched	12	- 11.38 (- 13.99, - 8.78)	< 0.001	84.60	< 0.001	9	6.96 (3.54, 13.68)	< 0.001	80.80	< 0.001
Not matched	5	- 3.18 (- 8.35, 1.99)	0.228	84.10	< 0.001	4	1.52 (1.00, 2.32)	0.052	1.10	0.386
Male ratio										
> 1/2	11	- 7.79 (- 11.47, - 4.11)	< 0.001	88.60	< 0.001	10	4.51 (2.05, 9.93)	< 0.001	83.10	< 0.001
≤ 1/2	5	- 12.03 (- 16.93, - 7.13)	< 0.001	86.00	< 0.001	3	3.24 (1.37, 7.63)	0.007	0.3767	0.012
NR	1	- 8.89 (- 10.56, - 7.22)	< 0.001							
Mean age (years)										
> 25	14	- 10.06 (- 12.87, - 7.24)	< 0.001	87.50	< 0.001	12	3.58 (2.04, 6.28)	< 0.001	78.30	< 0.001
≤ 25	3	- 4.49 (- 13.24, 4.25)	0.314	91.40	< 0.001	1	16.43 (6.65, 40.61)	< 0.001		
Country										
Nepal	1	- 9.68 (- 19.62, 0.26)	0.056			1	4.38 (1.32, 14.50)	0.016		
India	5	- 8.73 (- 11.59, - 5.87)	< 0.001	81.80	< 0.001	3	2.55 (1.54, 4.21)	< 0.001	29.10	0.244
Turkey	4	- 3.37 (- 10.68, 3.94)	0.366	94.0	< 0.001	2	2.97 (0.06, 147.87)	0.585	94.1	< 0.001
Egypt	5	- 14.75 (- 22.10, - 7.39)	< 0.001	90.60	< 0.001	4	8.91 (1.20, 66.39)	0.033	86.9	< 0.001

Table 2 continued

	Vitamin D				Vitamin D deficiency					
	No. of articles	WMD (95%CI)	<i>p</i>	<i>I</i> <sup>2</sup> (%)	<i>p</i> <sub>heterogeneity</sub>	No. of articles	OR (95% CI)	<i>p</i>	<i>I</i> <sup>2</sup> (%)	<i>p</i> <sub>heterogeneity</sub>
Pakistan	1	- 9 (- 15.55, - 2.45)	0.007							
Israel	1	- 10.23 (- 17.51, - 2.95)	0.006			1	6.86 (1.78, 26.36)	0.005		
Italy						1	1.73 (1.08, 2.79)	0.023		
USA						1	4.37 (2.71, 7.04)	< 0.001		

WMD weighted mean difference, OR odds ratio, CI confidence interval

it works in patients with AA need further investigation.

This meta-analysis had several potential limitations that must be considered when interpreting the results. First, a meta-analysis is unable to resolve problems with confounding factors that could be inherent in the original studies, although major potential confounders had been adjusted for in most of the included studies. Subgroup analysis suggested that the heterogeneity of serum vitamin D level was partially explained by matched controls, mean age, and country, while heterogeneity of vitamin D deficiency was partly explained by study design, matched controls, and country. Therefore, a random-effects model was used for the synthesis of data in order to mitigate potential variation between patients at an individual patient data level. Second, the results of several subgroups were based on a limited number of studies, and therefore, the possibility of insufficient statistical power cannot be ruled out. For example, the only study on pediatric patients with AA reported no difference in serum 25(OH)D level between pediatric patients with AA and pediatric controls [37]. Hence, caution should be used for generalization of these findings to pediatric patients with AA. Third, serum 25(OH)D concentration might be influenced by numerous factors such as gender, age, ethnicity, comorbidity, diet, sun exposure level, the season of blood sampling, and treatments, such as phototherapy. Therefore, a Mendelian randomization study might be useful to determine the causal association between vitamin D and AA. Fourth, most included studies defined vitamin D deficiency as serum 25(OH)D level below 20 ng/dL, but a few studies defined it as below 30 ng/dL. However, subgroup analysis of different definitions of vitamin D deficiency was consistent with the overall analysis. Finally, only studies published in English were included, and valuable data published in other languages might have been excluded.

## CONCLUSIONS

Deficient serum 25(OH)D level, rather than calcium level, was present in patients with AA.

Screening for vitamin D deficiency and vitamin D supplementation may be beneficial in the treatment of patients with AA. As a result of the limited data, we were unable to comment on the potential additional effect of calcium monitoring.

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