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The role of micronutrients in alopecia areata: A Review

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Abstract

Alopecia areata (AA) is a common, non-scaring form of hair loss caused by immune-mediated attack of the hair follicle. As with other immune-mediated diseases, a complex interplay between environment and genetics is thought to lead to the development of AA. Deficiency of micronutrients such as vitamins and minerals may represent a modifiable risk factor associated with development of AA. Given their role in normal hair follicle development and in immune cell function, a growing number of investigations have sought to determine whether serum levels of these nutrients might differ in AA patients, and whether supplementation of these nutrients might represent a therapeutic option for AA. While current treatment often relies on invasive steroid injections or immunomodulating agents with potentially harmful side-effects, therapy by micronutrient supplementation, whether as a primary modality or as adjunctive treatment, could offer a promising low-risk alternative. However, our review highlights a need for further research in this area, given that the current body of literature largely consists of small case-control studies and case-reports which preclude any definite conclusions for a role of micronutrients in AA. In this comprehensive review of the current literature we found that serum vitamin D, zinc, and folate levels tend to be lower in patients with AA as compared to controls. Evidence is conflicting or insufficient to suggest differences in levels of iron, vitamin B12, copper, magnesium, or selenium. A small number of studies suggest that vitamin A levels may modify the disease. Though understanding of the role for micronutrients in AA is growing, definitive clinical recommendations such as routine serum level testing or therapeutic supplementation, call for additional studies in larger populations and with a prospective design.

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Compliance with ethical standards

Conflicts of interest:

Dr. Qureshi serves as a consultant for Abbvie, Amgen, Centers for Disease Control, Janssen, Merck, Novartis, and Pfizer, and is an investigator for Amgen, Regeneron, and Sanofi. Mr. Thompson, Ms. Mirza, Dr. Park, and Dr. Cho have no conflicts of interest.

1. Introduction

The role of diet and nutrition in dermatologic disease represents an active and growing area of inquiry. Findings in this realm have spurred new evidence-based recommendations for the prevention and treatment of psoriasis, atopic dermatitis, acne, and skin cancer, and have highlighted the need for ongoing investigation [1, 2]. Alopecia areata (AA) is a common immune-mediated condition characterized by non-scarring hair loss. Lifetime incidence of AA ranges from 1.7%–2.1%, with higher prevalence in younger (21–40 years of age) patients but no significant difference in incidence exists between males and females [3]. AA can have profound effects on patients' quality of life, similar to the degree seen in other skin diseases such as psoriasis and atopic dermatitis [4]. Current understanding of AA pathogenesis implicates a collapse of immune-privilege of the hair follicle, with infiltration of CD4+/CD8+ T cells, and an autoimmune mechanism involving melanogensis-associated peptides as autoantigens [5]. Current therapy is therefore targeted to immune-modulation. Options range from relatively benign agents such as topical or injectable steroids to more extensive therapies including oral steroids, phototherapy, methotrexate, and cyclosporine [6].

Micronutrients include vitamins and trace minerals, which though required in only minute amounts, are essential components of our diet. The physiologic roles of these nutrients are highly varied; they function as enzyme cofactors, biologic substrates, and even as hormones [7]. There are multiple reasons to suspect a role for micronutrients in AA. The normal hair follicle cycle depends on micronutrients given their role in cellular turnover, a frequent occurrence in the rapidly dividing hair follicle [8]. Furthermore, some micronutrients reduce oxidative stress, an increasingly suspected contributor to AA pathogenesis [9]. Others, such as vitamin D, might modify the immune response by inhibiting Th1 cell proliferation, the predominant T-helper cell-type in AA [10, 11]. Therefore, a better understanding of the role of these micronutrients could yield breakthroughs in the prevention or treatment of AA. The present body of work has focused primarily on the characterization of serum levels of nutrients and occasionally on the therapeutic uses of such nutrients in the form of supplementation. A review of the current evidence is warranted to gather these findings in hopes of informing patient/physician dietary discussions, to highlight current gaps in knowledge, and to stimulate new hypotheses for the investigation of diet and nutrition and their role in AA.

2 Methods

A search of published literature was conducted and completed in March 2017, to determine which micronutrients have been studied in association with AA. Searches were performed in PubMed and Web of Science with the terms "alopecia AND (areata OR totalis OR universalis)" combined with "vitamin D OR calcipotriol OR calcipotriene", "biotin", "zinc", "iron OR ferritin", "retinoid OR vitamin A OR retinol", "antioxidants OR oxidizing OR oxidative", "vitamin E", "vitamin C", "magnesium", "selenium", "vitamin B12 OR cobalamin", "folate OR folic acid", and "copper". All titles and abstracts were screened to determine whether they addressed the research question: What is the current body of research regarding AA and its association with micronutrients? Articles chosen for inclusion in this review were original articles or case reports providing primary data and involving

human subjects. Selected articles were peer-reviewed and were published in English with the first article dating from 1981.

3 Micronutrients

3.1 Vitamin D

Vitamin D is a fat-soluble vitamin with a primary role in calcium and phosphorus homeostasis and bone health. Serum levels are primarily maintained through the UVB-mediated conversion of 7-dehydrocholesterol in the skin to cholecalciferol, which is hydroxylated in the liver and kidney to the active form of 1,25-dihydroxyvitamin D. Vitamin D can also be obtained from fortified foods or those that are naturally rich (e.g. salmon, sardines, egg yolks) [12, 13]. Once activated, vitamin D functions as a steroid hormone. It first enters the cell via a surface vitamin D receptor (VDR), then complexes with the retinoic acid X receptor and enters the nucleus. This complex binds specific DNA sequences (DNA-responsive elements) attracting transcription factors and thereby enacting expression of vitamin D-responsive genes [13].

Subclinical vitamin D deficiency is common, as reflected by serum measurements in a subset (N = 4495) of respondents to the 2005–2006 National Health and Nutrition Examination Survey; 41.6 percent of US adults had serum 25(OH)D levels below the commonly accepted threshold (20 ng/mL) for deficiency [14, 15]. A growing number of studies have demonstrated associations between 25(OH)D deficiency and extra-skeletal disease [16–20], including autoimmune disease [10]. For example, 25(OH)D levels were lower in patients with a recent diagnosis of systemic lupus as compared to controls, and lower 1,25(OH)D levels have been associated with higher rheumatoid arthritis disease activity [21, 22]. Furthermore, some studies suggest a role for vitamin D supplementation in the prevention and treatment of immune-mediated disease. A prospective study found a 40% reduction in the risk of multiple sclerosis amongst women using supplemental vitamin D [23], and in a recent randomized controlled trial, high-dose vitamin D supplementation was associated with a reduction in thyroid peroxidase antibody levels in patients with autoimmune thyroid disease [24].

Vitamin D has established roles in the normal hair follicle. Murine hair follicle keratinocytes are immunoreactive for the VDR with greatest activity in the anagen stage [25]. In mice homozygous for a VDR knockout mutation, hair loss developed 3 months from birth, with nearly total hair loss at 8 months [26]. Similarly, in mice null for murine-VDR, made to express the human transgene for VDR, there was a prevention of alopecia [27]. Another murine study failed to demonstrate alopecia in wild-type mice nurtured in a UV-free environment with a vitamin D deficient diet. These mice had undetectable levels of circulating 25(OH)D, suggesting a more causative role for the VDR as opposed to its ligand [28]. In humans, the role for vitamin D in the hair follicle is suggested by hair loss in patients with vitamin D-dependent rickets type II. These patients harbor mutations in the VDR gene leading to vitamin D resistance and sparse body hair, often including total scalp and body alopecia [29, 30]. Further, a recent study by Forghani et al. [31] identified novel nonsense mutations in the VDR gene in two such patients resulting in alopecia and type II rickets.

Studies of vitamin D in AA are summarized in (Table 1) [32–44]. Some have specifically examined the role of the VDR. Fawzi et al. recently found lower levels of the VDR in serum and scalp tissue samples in AA patients compared to controls [42]. Polymorphisms of the VDR gene have been shown to increase susceptibility to other autoimmune diseases, including Graves' disease and psoriasis [45, 46]. However, in two studies [36, 37] no such polymorphisms were associated with AA. However, only 25 and 32 patients with AA were studied respectively, and there were no investigations of polymorphisms by racial or ethnic group [36, 37].

A total of five case-control studies [32–35, 43] evaluated serum 25(OH)D levels and AA and found that lower serum vitamin D levels were associated with AA. Most recently, Bakry et al. found lower 25(OH)D levels in 60 AA patients compared to controls in Egypt (deficiency defined as <50 nmol/L). Furthermore, vitamin D levels were inversely associated with increasing severity of AA [43]. A case-control study [32] of 86 AA patients in Turkey similarly identified lower serum 25(OH)D concentrations in AA cases compared to patients with vitiligo and to healthy controls. An inverse association was also shown between 25(OH)D levels and severity of hair loss, using the Severity of Alopecia Tool (SALT) ($P < 0.001$, $r = -0.41$). This study was strengthened by its use of vitiligo as a positive control for autoimmune disease, and was limited by serum measurement only in winter. Another study [33] by Mahamid et al. examined serum vitamin D levels in patients with AA. 25(OH)D levels measured in winter and summer were lower in AA patients compared to controls. Prevalence of vitamin D deficiency (defined as serum levels <20 ng/mL) was 70% in the AA group versus 25% in the control group ($P < 0.05$). Authors also conducted a multivariate analysis demonstrating a positive association (odds ratio (OR) 2.3, 95% confidence interval (CI) 2.2–3.1, $P = 0.02$) between AA and vitamin D insufficiency (defined as serum levels <30 ng/mL). A study [34] of 156 AA patients in Italy included 38 patients with more severe forms of disease including those with full scalp and facial hair loss (alopecia totalis or AT) and those with full body hair loss (alopecia universalis or AU). A higher prevalence of vitamin D deficiency was found in AA patients versus controls. Further, an inverse relationship was observed between serum parathyroid hormone levels and vitamin D levels, suggesting true deficiency of vitamin D. A study in Turkey [35] examined serum vitamin D levels in AA patients, and again found lower serum levels in patients versus controls. These studies did not distinguish vitamin D deficiency as a risk factor versus outcome of AA. In fact, psychosocial stress secondary to AA might lead to sun avoidance, and thereby vitamin D deficiency [32].

Only one recent study [47] has investigated an association between vitamin D and AA in a prospective fashion. Thompson et al. studied survey data regarding lifestyle and medical history from 55,929 women in the Nurses' Health Study. Compiling data on lifestyle factors (e.g. race, body mass index, UV-B flux at residence) known to contribute to vitamin D status, they calculated a vitamin D score as a surrogate of serum vitamin D level for respondents. Authors found no significant difference between the highest versus lowest quartiles of women based on serum vitamin D score and incident AA. Additionally, they found no association between dietary, supplemental, or total vitamin D intake and risk of developing AA.

Calcitriol (synthetic form: calcipotriol) is a vitamin D analog with treatment efficacy in psoriasis, another immune-mediated disease. Its mechanism of action is partially explained by inhibition of T-cell proliferation and a reduction in inflammatory mediator production [48, 49]. Studies (Table 1) [38–41, 44] of topical vitamin D analogs for AA are currently few and results have been inconsistent. Furthermore, interpretation of findings are challenging considering that 34–80% of AA patients will undergo spontaneous recovery without any treatment [50].

The most recent study [44] of topical vitamin D analogs by Narang et al. assessed a twice-daily regimen of topical 0.005% calcipotriol for 22 patients with patchy AA, with no placebo arm. 59.1% of patients had hair re-growth with onset at 4.21 ± 2.13 weeks. Those patients with lowest baseline vitamin D levels experienced the greatest percentage change in SALT scores. The most frequent side-effect reported was skin irritation; others included pruritus, pigmentation, scaling, and folliculitis. A study in Turkey [38] found that after 12 weeks of twice-daily application of 0.005% calcipotriol, SALT scores of 48 AA patients were lower ($P = 0.001$) and hair regrowth of ≥50% was observed in 75% of patients. There was no placebo control in this trial. A study in Korea [39] reported the case of a 7-year-old AA patient who had no improvement with topical 5% minoxidil and 1% hydrocortisone. After application of calcipotriol solution (50 μg/mL daily for 3 months), complete hair regrowth was seen, and there was no relapse of disease in the following 6 months. Furthermore, a punch biopsy of the patch prior to treatment showed a paucity of VDR immunohistochemical staining, but post-treatment, staining was obvious and newly present, suggesting an up-regulation of the VDR. Two other studies were identified as references in these aforementioned studies. However, only abstracts were available for review. In the first, 28 subjects underwent calcipotriol application in conjunction with squaric acid dibutylester sensitization and failed to show potentiation of squaric acid activity in hair regrowth [40]. One other small double-blind, placebo controlled trial of calcipotriol showed no effect. However, only 20 patients were studied, and these were patients with the most severe forms of disease including alopecia totalis or universalis [41].

3.1.1 Conclusions on Vitamin D—Overall, the current literature has consistently demonstrated lower vitamin D levels in patients with AA, the underlying cause of which is not fully understood. The only prospective study to date revealed no association between vitamin D status and risk of developing AA, suggesting that serum deficiency may not modify risk of AA, but instead might arise secondary to AA. Studies of VDR polymorphisms have revealed no suggestive genetic risk factors for AA. Trials of topical vitamin D analogs are promising but inconsistent and have lacked placebo study arms. Finally, no double-blind trials have yet examined oral supplementation as a prevention or treatment strategy.

3.2 Zinc

Zinc is an essential mineral upon which hundreds of enzymes depend for their catalytic activity [51]. For example, alkaline phosphatase is a zinc-dependent enzyme, which has elevated activity in tissues with high proliferative activity, such as the hair follicle [8]. Zinc deficiency can result in extensive hair changes including telogen effluvium (TE) and

induction of thin, brittled hair [52]. Copper/zinc superoxide dismutase is another zinc-dependent enzyme with potent antioxidant effects. Some have speculated that a copper/zinc imbalance might play a role in AA pathogenesis by way of dysregulation of this enzyme and thereby, an imbalance in oxidant/antioxidant activity [53].

Studies of zinc in AA are summarized in (Table 2) [53–56]. Four out of six case-control studies [53–58] to date have identified lower serum zinc levels in patients with AA as compared to controls. Kil et al. [54] included 94 patients with AA, 32 healthy controls, and 208 patients with other common types of hair loss including: male pattern hair loss, female pattern hair loss, and TE. Serum zinc level was lower in all hair loss patients compared to controls without hair loss. Furthermore, only those patients with AA and TE had increased odds of serum zinc levels below 70 micrograms/dL (OR 4.02, 95% CI 1.13–14.31 for AA and OR 4.65, CI 1.12–17.68 for TE). In another study by Abdel Fattah et al. [53], an inverse correlation was found between serum zinc levels and severity of AA, as determined by SALT score. Also, in patients with resistant disease of greater than 6-months duration, there was an inverse correlation between serum zinc levels and duration of AA, suggesting a more prominent role for zinc in difficult-to-treat AA. Two other small case-control studies [55, 56] found lower serum zinc levels in AA patients compared to controls. In contrast to these studies, two [57, 58] case-control studies from Finland (27 AA cases) and Iran (16 AA cases) found no difference in serum zinc levels of AA patients versus controls. The study from Finland revealed minimal differences in serum, red-cell, or 24-hour urine zinc concentrations compared to the general Finish population [58]. The study from Iran [57] also found no differences in serum and hair zinc levels between AA cases and controls.

Studies of oral zinc as treatment for AA have yielded inconsistent results (Table 2) [59–62]. The only double-blind, placebo-controlled trial [59] did not support supplementation for patients with AA. Treatment group took 220 mg of oral zinc sulfate twice daily for 3 months, and though serum and hair concentrations of zinc increased compared to the placebo group, there was no improvement in AA. In contrast, Park et al. [60] showed that in AA patients with serum zinc levels below 70 μg/dL, a regimen of 50 mg zinc gluconate per day led to therapeutic improvement in 9 out of fifteen (60%) patients at 12 weeks. A positive response was more likely in those with mild vs moderate disease and those with fewer patches of hair loss. However, findings in this small study did not reach statistical significance and there was no placebo group. In a study [61] of 18 pediatric AA patients, 9 children were treated on a combination therapy of 100 mg oral zinc aspartate + 0.025% topical clobetasol proprionate + 20 mg biotin per day for 1 year. Nine patients in the control group took 1mg/kg/day of deflazacort for 20 days, tapered to 5 mg per day for one year. Authors noted a trend towards improvement in the treatment group as 3 patients exhibited complete hair regrowth while none exhibited complete hair regrowth in the control group. However, the combination therapy, the use of a different steroid in the control arm, and the lack of a placebo group makes it difficult to definitively proclaim efficacy for the zinc component of therapy. More recently, Lux-Battistelli [62] described a patient with hair loss recurrence after cessation of a 30 mg/day zinc gluconate with psoralen plus ultraviolet A (PUVA) regimen. Upon initiation of a new regimen of zinc gluconate + sulfur amino acids + vitamin D, the patient had complete hair re-growth after 12 months of therapy. A second patient exhibited a similar course with hair loss recurrence after PUVA cessation. The same

combination regimen above, along with re-initiation of PUVA, led to 50% hair regrowth. Though suggestive of an effective therapeutic role for zinc, the combination therapy and complex treatment course makes interpretation of therapeutic effects difficult.

3.2.1 Conclusions on Zinc—To date, most studies of zinc have identified lower serum levels in patients with AA compared to controls. Serum levels also appear to be inversely associated with severity of disease. There is a paucity of evidence surrounding zinc supplementation highlighting the need for additional, double-blinded trials with this mineral as monotherapy. Whether serum zinc levels should be routinely assessed clinically is a question better answered with additional investigation.

3.3 Copper, magnesium, and selenium

Copper, magnesium, and selenium are trace elements which like zinc, exhibit essential physiologic roles which could be implicated in AA. As previously mentioned, copper acts with zinc in the antioxidant enzyme copper/zinc superoxide dismutase [53]. Selenium also contributes to antioxidant defense mechanisms via its interaction with the enzyme glutathione peroxidase [63]. Magnesium acts as a cofactor for over 300 enzyme systems, and plays an important role in nucleotide synthesis, a frequent process in the rapidly dividing hair follicle [64]. A small number of studies have investigated the serum levels of these elements in AA (Table 3) [54–58, 65] and few have identified an association between low levels and AA. In one study [56] of 27 AA patients from Iran, serum and hair copper levels were lower in AA patients compared to controls. However, all other studies [54, 55, 57, 58] of serum copper levels identified no differences between AA patients and controls. Two of these studies [55, 58] also found no differences in magnesium levels. Regarding selenium, two studies have yielded conflicting results. In an Iranian study [65], 29 patients had lower selenium levels compared to controls, while a Finish study found no differences between AA cases and controls [58].

3.3.1 Conclusions on copper, magnesium, and selenium—The functions of these minerals in anti-oxidant defense and nucleotide synthesis suggests they might play a role in the pathophysiology of AA. However, the current paucity of studies of serum levels and supplementation in AA patients precludes any conclusions on their role in the development, progression, and treatment of AA.

3.4 Iron

Iron deficiency remains the most common nutritional deficiency in the world, a sign of which includes chronic diffuse telogen hair loss [66, 67]. Iron serves as a cofactor for ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis [68]. Therefore, as with zinc and magnesium, iron likely exhibits an important role in tissues with high cellular turnover, like the hair follicle matrix. The primary indicator of iron status relied upon in hair loss studies is serum ferritin. Serum levels of this iron-binding protein reflect a patient's total iron storage [69]. In 2005, Trost et al. [67] reviewed studies of iron status and hair loss conditions and described multiple studies with lower ferritin levels in patients with AA, TE, androgenetic alopecia (AGA), and diffuse hair loss. For AA, a total of three studies [68, 70, 71] were reviewed. In contrast to the studies citing low ferritin in hair loss conditions,

authors also found numerous other studies which did not suggest an association between iron deficiency and hair loss. This discrepancy, coupled with the limitation that most studies were conducted only with female participants, led to a conclusion that there was insufficient evidence to recommend screening for iron deficiency in hair loss patients.

In addition to the studies in the Trost review [67], we identified five additional studies [57, 58, 72–74] examining iron status in patients with AA specifically. Only two of the eight total investigations (Table 4) [57, 58, 68, 70–74] supported an association between iron deficiency and AA. In a Scottish cases-only study [71], ferritin levels were low amongst a majority of female but not male patients. More recently in the United States, Kantor et al. [68] examined ferritin levels in women with AA, AT/AU, TE and AGA, compared to controls without hair loss. Mean ferritin levels were lower in patients with AA and AGA, but not in those with TE and AT/AU. A more significant iron deficiency might be expected amongst those with AT/AU. AT/AU could also be a genetically distinct form of AA, the etiology of which is unaffected by iron status. Kantor also suggested that iron deficiency might be an initiating factor, but not a factor in maintaining long-term disease.

While these studies provide compelling evidence for an association between iron deficiency and AA, others have revealed opposite findings. In the largest group (n=52) of AA patients studied to date, an Iranian group [74] found no differences between serum ferritin or serum iron in AA patients vs. controls. A study [72] of 43 AA patients in Turkey yielded similar findings. However, that study population included mostly male subjects (67%), whereas the studies [68, 71] which have supported an association between iron deficiency and AA have been in female subjects. Two case-control studies [57, 58], a case-series [70], and a case report [73] also found no differences in iron status in AA patients compared to controls.

3.4.1 Conclusions on iron—The interaction between a patient's iron status and AA deserves further attention. First, we found no placebo-controlled clinical trials assessing iron supplementation in the treatment of AA. However, it has been hypothesized [68] that correcting serum iron levels would lead to better treatment responses, as shown previously [75] in androgen-dependent alopecia. There is also a need for larger studies with attention to the current discrepancy between findings in females and males. Attention to these research questions will better clarify whether measurement of serum iron status and correction of deficiency should become a mainstay of AA management, a recommendation with as yet insufficient supportive evidence [67].

3.5 B Vitamins

Folate (folic acid or vitamin B_9) as a methyl-group donor, and vitamin B_{12} (cobalamin) as a coenzyme, both contribute to nucleic acid production and thus possess a plausibly important role in the highly-proliferative hair follicle. Folate status can be assessed in multiple ways, with serum folate an indicator of recent dietary intake, and erythrocyte or red blood cell (RBC) folate as an indicator of long-term folate status–analogous to serum iron and ferritin measurements. Vitamin B_{12} can be measured in the serum or plasma, and reflects both intake and stores. When complexed with the protein transcobalamin, it forms

holotranscobalamin, an increasingly recognized marker for early depletion of vitamin B_{12} [76, 77].

At present, there are few studies (Table 5) [72, 73, 78–81] assessing B vitamin status in patients with AA. Yousefi et al. [78] found lower RBC folate levels in 29 Iranian AA patients compared to controls. RBC folate concentrations were also lower in patients with AT/AU versus patchy AA, and SALT score was negatively correlated with RBC folate levels. In a case-control study [79] in Turkey, serum folate, vitamin B_{12}, and holotranscobalamin levels were measured in 75 patients with AA and 54 healthy controls; there were no differences in serum levels of these vitamins. Examining serum folate and vitamin B_{12} levels in 43 Turkish patients with AA, Gonul et al. [72] similarly found no differences compared to controls.

Regarding folate, one explanation for the discrepancy between these null findings and the association identified by Yousefi et al. is that RBC folate is a better indicator of folate stores, while serum levels can fluctuate acutely with dietary intake. Interestingly, in one AA genetic study, Kalkan et al. [80] found that AA patients compared to controls had a higher prevalence of the C677T polymorphism (CT or TT vs. CC genotype) for the enzyme methylenetetrahydrofolate reductase (MTHFR), a key regulator of folate metabolism. The same polymorphism has been associated with other immune-mediated diseases including Graves' disease [82] and multiple sclerosis [83]. Notably, when assessing the serum level of folate and vitamin B_{12} in the same study subjects, authors found no differences between AA patients and controls, despite a relatively large sample size. Regarding vitamin B_{12}, a hypothetical association between AA and B_{12} deficiency is predicated on the autoimmune nature of pernicious anemia (PA), a condition characterized by antibody-induced destruction of gastric parietal cells which produce intrinsic factor, a key protein responsible for downstream intestinal absorption of vitamin B_{12} [84]. AA has been associated with numerous comorbid immune-mediated diseases, and an association with PA would be reason for B_{12} deficiency to be more prevalent in AA patients compared to controls. This principle is evidenced by a case-report [81] of a patient diagnosed with pernicious anemia at age 16 who later developed AA at age 24, and another report [73] of a patient who developed type 1 diabetes at age 18, 9 months later developed AA, and at age 27 was diagnosed with pernicious anemia. However, as mentioned previously, available case-control studies [72, 79, 80] with multiple AA cases did not identify any such differences in B_{12} levels in patients compared to controls.

3.5.1 Conclusions on B vitamins—A few studies suggest associations between AA and low red cell folate levels and MTHFR polymorphisms, and case reports of patients with comorbid AA and pernicious anemia do exist. These investigations suggest that folate or vitamin B_{12} status might modify risk or progression of AA, but multiple contrary studies preclude any clinical recommendations such as serum screening or supplementation of these B vitamins.

3.6 Biotin

Biotin is an important coenzyme for carboxylation reactions and in rare cases of deficiency, patients can develop hair loss [85]. Genetic abnormalities or malabsorption caused by excessive intake of avidin, rich in raw eggs, can result in deficiency of biotin [86]. Supplementation has been successful in the treatment of brittle nails (onychoschisis) [87]. Regarding AA, we identified only one study documenting the use of biotin supplementation for AA. As previously discussed in relation to zinc therapy, Camacho et al. [61] administered a combination of zinc, topical clobetasol, and 20 mg biotin/day, and noticed more complete regrowth in patients in the treatment group (33.3% of patients) as compared to the control group (0%) over a one-year period. However, the combination therapy prohibits any conclusions about the singular efficacy of biotin supplementation. Studies of biotin and AA are few, highlighting a potential area for future research.

3.7 Oxidative stress, antioxidants, and vitamin A

Immune cells are highly sensitive to oxidative damage. For example, they harbor a high proportion of polyunsaturated fatty acids in their plasma membrane, making them susceptible to lipid peroxidation and related damage. They also produce reactive oxygen species (ROS) themselves as part of the immune defense mechanism [88], which can initiate the lipid peroxidation reaction. A growing number of studies have implicated oxidant/antioxidant dysregulation in AA, a disease dependent on immune dysregulation and inflammation. These studies have been recently reviewed [9] with most documenting elevated levels of oxidative stress biomarkers, and reduced levels of protective antioxidant enzymes in patients with AA. Because they act as cofactors for antioxidant enzymes, or as antioxidants themselves, the serum levels of certain micronutrients might represent an important consideration for studying and clinically characterizing AA.

As discussed previously, certain micronutrients play important roles as cofactors for antioxidant enzymes; copper and zinc function with CuZn superoxide dismutase and selenium with glutathione peroxidase [53, 63]. Vitamin E is another nutrient involved in the oxidant/antioxidant pathway, serving an instrumental role in defense against free-radical damage to the plasma membrane [89]. Investigators in Egypt assessed serum and tissue vitamin E levels in 15 patients with AA, finding lower levels in cases compared to healthy controls [90]. In contrast, investigators from Turkey found no differences in vitamin E levels, but did identify lower levels of beta-carotene in AA cases vs. controls [89]. Beta-carotene is a precursor to vitamin A and an antioxidant [63].

Vitamin A describes a family of compounds each with a core backbone and a modified side-chain–for example, a side-chain with a hydroxyl group (retinol) or carboxylic acid group (retinoic acid) [51]. Vitamin A *per se* is not considered an antioxidant, but it has myriad physiological roles including immune modulation [91]. For example, retinoic acid enhances T-cell proliferation and antigen-presenting capacity of dendritic cells, can inhibit B-cell proliferation, and has a role in maintaining gut immune privilege [92, 93]. Duncan et al. [93] recently documented upregulation of genes involved in retinoid metabolism in AA patch biopsies from human subjects and in the AA mouse model C3H/HeJ. Mice fed high levels of vitamin A developed earlier onset of disease, but those which were not fed supplements had

the most severe form of disease. Suo et al. [94] demonstrated similar findings in C3H/HeJ mice, showing a dose-dependent role for vitamin A in the initiation of the anagen hair cycle, which might increase follicle susceptibility to autoimmune destruction. Ultimately, these studies suggest the notion that there exists a certain optimized level of vitamin A, and too little or too much of this compound might favor the development, maintenance, or progression of AA.

3.7.1 Conclusions on oxidative stress, antioxidants, and vitamin A—Oxidative stress and antioxidant dysregulation are increasingly recognized as pathophysiologic players in AA. Micronutrients including zinc, selenium, and copper function as cofactors for antioxidant enzymes, and could presumably play a role in the disease process. In this review, zinc is the only of these nutrients to have sufficient evidence to support that idea. Studies are too few on the role of other antioxidant nutrients including vitamin E, beta-carotene, and vitamin A.

4 Comprehensive conclusions

As with other immune-mediated diseases, AA is thought to arise in those patients with a genetic predisposition, and with contribution from certain environmental risk factors, such as dietary factors. The serum levels of micronutrients can be modifiable through diet or supplementation, thus the motivation for a growing number of micronutrient-focused investigations in patients with AA. Kantor et al. [68] proposed a “threshold hypothesis” for explaining how serum micronutrient levels might contribute to disease. They suggested that in those patients with high heritable risk, patients would develop AA no matter the contribution from serum levels. Further, in those with mild hereditary predisposition, a threshold micronutrient level might exist, under which these sub-optimal micronutrient levels could contribute to development of disease. As we highlighted previously, the actual pathophysiological mechanism by which these sub-threshold levels might contribute to AA include: dysregulation of immune cell function, dysregulation of coenzyme-dependent enzyme function in DNA synthesis, and an imbalance between oxidant and antioxidant activity.

We identified a significant number of studies surrounding these various nutrients and their role in AA. However, numerous limitations highlight the need for additional future studies. First, the majority of aforementioned studies were of a retrospective design, where one cannot rule out the possibility that disease *per se* or lifestyle changes after development of AA might have contributed to the deranged blood levels of micronutrients (reverse causation). For example, some studies identified lower vitamin D levels in patients with AA. In contrast, the only current prospective study [47] found no association between vitamin D and incident AA. This could be explained by environmental factors other than nutrient intake, such as sun avoidance habits secondary to AA-induced psychosocial stress [32]. Prospective studies can avoid this type of concern. Second, almost all reviewed studies were of small size, largely comprising less than 100 AA cases. It is often challenging and costly to obtain blood samples and to assay nutrient levels in large-scale studies. However, dietary intake of these nutrients could be assessed as an alternative and cost-effective way in larger populations to evaluate their roles in AA. Third, certain micronutrients, such as iron, were

investigated primarily only in female subjects, thus, limiting generalizability of findings to males. Fourth, serum levels of these micronutrients may not correctly reflect the bioavailability of corresponding nutrients. For example, while ferritin is the relied-upon marker of iron status, ferritin levels could be affected by infection, inflammation, malignancy or liver damage [67]. Finally, few studies examined the potential role for these compounds as supplements in the treatment of AA. In conclusion, the current body of literature provides a solid foundation from which future studies can address these limitations, in efforts to provide better understanding of the nutritional risk factors and opportunities for treatment in AA.

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Key points

This comprehensive review summarizes what is currently known regarding the role of micronutrients in alopecia areata.

Serum vitamin D, zinc, and folate levels tend to be lower in patients with AA as compared to controls, while evidence is conflicting or insufficient to suggest differences in levels of iron, vitamin B12, copper, magnesium, or selenium. Presently, few studies have investigated micronutrient supplementation as a method of treatment in AA.

To further current knowledge, future studies are needed in larger groups of patients with prospective study design.

Table 1

Studies of vitamin D and alopecia areata [32–44]

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
Vitamin D receptor studies					
Fawzi et al. [42]	2016	Case-control (Hospital- based)	Sex: 12M/8F Age: 26.10 ± 9.431 Country: Egypt	40 (20 AA cases and 20 age- and sex- matched controls from neurosurgery department)	Serum and tissue VDR levels lower in AA cases (serum 9.990 ± 1.6973 ng/mL; tissue 199.710 ± 33.3802 ng/mL) vs controls (serum 13.605 ± 1.6612 ng/mL; tissue 333.910 ± 46.6220) (P = 0.000; P = 0.000)
Serum level studies					
Bakry et al. [43]	2016	Case-control (Not described)	Sex: 36M/24F Age: 20.70 ± 10.85 Country: Egypt	120 (60 AA cases and 60 age-, gender-, and body mass index-matched healthy controls	Serum 25(OH)D lower in AA cases (44.04 ± 15.61 nmol/L) vs controls (66.07 ± 17.40 nmol/L) (P < 0.001) Prevalence of vitamin D deficiency * in AA (83.3%) vs controls (23.3%) (P < 0.001) Inverse association between mean serum vitamin D and disease severity (mild: 58.59 nmol/L; moderate 42.18 nmol/L; severe: 35.39 nmol/L)
Thompson et al. [47]	2016	Prospective cohort	Sex: 55,929F Age: 63.4 ± 6.4 Country: USA	55,929 (133 AA cases)	No difference in hazard ratio (HR) for incident AA between highest vs lowest quartiles of surrogate vitamin D score: multivariate HR 1.08 (95 % CI 0.68–1.73); No difference in HR for AA comparing highest versus lowest quartiles of dietary, supplemental, and total vitamin D intake
Çerman et al. [32]	2014	Case-control (Hospital- based)	Sex: 56M/30F Age: 32.21 ± 9.60 Country: Turkey	188 (86 AA cases, 44 vitiligo cases, and 58 age- and sex-matched controls from volunteer hospital staff)	Serum 25(OH)D lower in AA cases (11.84 ± 6.18 ng/mL) vs vitiligo (16.15 ± 7.93 ng/mL) and vs healthy controls (23.57 ± 9.03 ng/mL) (P = 0.001 and P < 0.001); Prevalence of vitamin D deficiency *; in AA (91%) vs vitiligo (71%) vs controls (33%) (P = 0.003 and P < 0.001); Inverse association between 25(OH)D level and severity of alopecia (P < 0.001, r = −0.409)
Mahamid et al. [33]	2014	Case-control (Hospital- based)	Sex: 14M/9F Age: 24.2 ± 12.3 Country: Israel	43 (23 AA cases and 20 control cases, recruited from clinics and who had no history of AA)	Serum 25(OH)D lower in AA cases vs controls (11.32 ± 10.18 vs 21.55 ± 13.62 ng/mL, P < 0.05); Prevalence of vitamin D deficiency * in AA (69.5%) vs controls (25%) (P < 0.05); Multivariate analysis for vitamin D < 30ng/mL: odds ratio (OR) 2.3 (95% CI, 2.2–3.1, P = 0.02); CRP levels in AA group were elevated (P < 0.05)
d'Ovidio et al. [34]	2013	Case-control (Registry- based)	Sex: 45M/111F Age: 37.8 Country: Italy	304 (156 AA cases enrolled in the National Mediterranean Alopecia Areata Association and 148 controls (no further control detail)	Prevalence of vitamin D deficiency *; in AA (42.4%) vs controls (29.5%) (P < 0.025) Inverse association between Vitamin D and PTH levels (r = −0.24, P < 0.01)
Yilmaz et al. [35]	2012	Case-control (Hospital- based)	Sex: 14M/28F Age: 30.8 ± 8.2	84 (42 AA cases and 42 healthy controls)	Serum 25(OH)D concentration lower in AA cases (33.4 ± 17.7 nmol/L) vs controls (51.2 ± 21.1 nmol/L) (P < 0.001) *

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
			Country: Turkey		
Genetic polymorphism studies					
Akar et al. [37]	2007	Case-control (Not described)	Sex: 30M/2F Age: 24.1 ± 7.5 Country: Turkey	59 (32 AA cases and 27 healthy controls)	Genes studied: *BsmI, ApaI, TaqI* No difference in prevalence of polymorphisms in AA vs. controls
Akar et al. [36]	2004	Case-control (Not described)	Data not available	52 (25 AA cases and 27 healthy controls)	Genes studied: *FokI* No difference in prevalence of polymorphisms in AA vs. controls
Vitamin D treatment studies					
Narang et al. [44]	2017	Clinical trial (no placebo)	Sex : 12M/10F Age : 30.4 ± 10.8 Country: India	22	Regimen: 0.005% calcipotriol lotion applied 2x/day for 12 weeks (or until complete regrowth) Results: 59.1% of patients had hair re-growth with onset at 4.21 ± 2.13 weeks; response stratified by percent change in SALT score: 9 patients with 0% change, 4 patients with <25% change, 3 patients with 26–50% change, 6 patients with >50% change
Çerman et al. [38]	2015	Clinical trial (no placebo)	Sex: 26M/22F Age: 33 ± 11.14 Country: Turkey	48	Regimen: 0.005% calcipotriol cream applied 2x/day for 12 weeks Results: Lower mean SALT score at 12 weeks ($P = 0.001$) Hair regrowth ≥50% in 75% of patients; regrowth ≥75% in 62.5%; complete regrowth in 27.1%
Kim et al. [39]	2012	Case-report	Sex: M Age: 7 Country: Korea	1	Regimen: calcipotriol solution 50 μg/mL applied daily for 3 months Results: Complete hair regrowth at 3 months; no relapse 9 months
Orecchia et al. [40]	2009	Clinical trial	Data not available	28	Results: Failure of calcipotriol to potentiate squaric acid dibutylester effectiveness
Berth-Jones et al. [41]	2009	Clinical trial	Data not available	20	Results: No response to calcipotriol in patients with alopecia totalis and alopecia universalis

AA alopecia areata, *CI* confidence interval, *CRP* c reactive protein, *HR* hazard ratio, *OR* odds ratio, *PTH* parathyroid hormone, *SALT* severity of alopecia tool

*Vitamin D deficiency defined as ≤20 ng/mL or ≤50nmol/L

†BMI and Age given as mean ± standard deviation

Table 2

Studies of zinc and alopecia areata [53–62]

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
Serum level studies					
Abdel Fattah et al. [53]	2016	Case-control (Hospital- based)	Sex: 39M/11F Age: 27 ± 9.53 Country: Egypt	100 (50 AA cases and 50 sex- and age-matched controls)	Serum zinc levels in AA (75.48 ± 11.78 μg/dL) vs. controls (85.7 ± 12.50 μg/dL) (P = 0.001) Inverse correlation between zinc level and 1) severity of AA (P = 0.001, r = −0.573); 2) duration of AA in those with resistant disease * (P = 0.001, r = −0.956)
Dastgheib et al. [57]	2014	Case-control (Not described)	Sex: 16F Age: 26.63 ± 8.53 Country: Iran	43 (16 AA cases and 27 sex- and age-matched healthy controls)	No difference in serum zinc levels or hair zinc levels in AA vs. controls
Kil et al. [54]	2013	Case-control (Hospital- based)	Sex: 44M/50F Age: 37.13 ± 14.86 Country: Korea	126 (94 AA cases and 32 healthy controls); also included 209 patients with MPHL/ FPFL or TE	Serum zinc levels in AA (84.96 ± 24.25 μg/dL), MPHL (87.74 ± 21.20), FPHL 79.61 ± 19.39), TE (84.65 ± 27.23) vs. control (97.94 ± 21.05 μg/dL) (P = 0.01, 0.03, 0.01, 0.03 respectively) Increased odds of serum zinc < 70 ug/dL in AA vs. controls (OR 4.02, 95% CI 1.13–14.31) and TE vs. controls (OR 4.65, CI 1.12–17.68)
Amirnia et al. [56]	2013	Case-control (Hospital- based)	Sex: 3M/24F Age: 66.27 ± 9.90 Country: Iran	54 (27 AA cases and 27 healthy sex- and age-matched controls)	Serum zinc levels in AA (64.25 ± 19.40 μg/dL) vs. control (82.77 ± 5.77 μg/dL) (P < 0.005); Hair zinc levels in AA (98.33 ± 24.25 μg/dL) vs. control (129.51 ± 29.61 μg/dL) (P < 0.005)
Bhat et al. [55]	2009	Case-control (Hospital- based)	Sex: 34M/16F Age: 27.3 Country: India	100 (50 AA cases and 50 healthy sex- and age-matched controls recruited from among hospital employees or individuals accompanying cases, with no skin or systemic disease)	Serum zinc levels in AA (78 ± 7.45 μg/dL) vs. control (88 ± 8.78 μg/dL) (P < 0.05)
Mussalo- Rauhamaa et al. [58]	1986	Case-control (Hospital- based)	Sex: 8M/19F Age: 29 ± 11 Country: Finland	27 AA cases compared to normal Finnish population reference values	No difference in serum zinc levels in AA vs. normal population Compared AA cases to serum zinc values from the normal Finnish population
Zinc as treatment					
Lux-Battistelli C[62]	2015	2 patients	Sex (Age): 1M (16); 1F (31) Country: France	2	Regimen: 30 mg zinc gluconate + sulfur amino acids + vitamin D /day for at least 1 year Results: Progressive hair growth beginning at 3–5 months
Park et al. [60]	2009	Clinical trial (no placebo)	Sex: 10M/5F Age: 29.1 ± 16.2 Country: Korea	15	Regimen: 50 mg /day zinc gluconate for 12 weeks in patients with serum zinc < 70 μg/dL

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
					Results: Marked recovery in 46.7% of patients and partial recovery in 13.3% of patients.
Camacho et al. [61]	1999	Active treatment- controlled	Sex: 10M/8F Age: 8.6 (protocol group); 9.1 (control group) Country: Spain	18 children with no spontaneous remission of their AA (9 in regimen and 9 in control treatment)	Regimen: 100 mg oral zinc aspartate + 0.025% topical clobetasol proprionate + 20 mg biotin /day for 1 year Control treatment: 1mg/kg/day deflazacort for 20 days tapered down to 5mg/day for 1 year Results: Complete regrowth in 33.3% of patients in treatment vs. 0% in controls
Ead RD[59]	1981	Double-blind, placebo- controlled	Data not available	42	Regimen: 220 mg oral zinc sulfate, twice daily for 3 months vs. placebo Results: No improvement in patients on active drug despite increased serum and hair zinc levels

AA alopecia areata, *CI* confidence interval, *OR* odds ratio, *MPHL* male pattern hair loss, *FPHL* female pattern hair loss, *TE* telogen effluvium

† Age given as mean ± standard deviation

* Resistant disease defined as AA lesions with > 6 months duration and in whom three or more therapies were unsuccessful

Table 3

Studies of copper, magnesium, and selenium and alopecia areata [54–58, 65]

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
Copper					
Dastgheib et al.[57]	2014	Case-control (Not described)	Sex: 16F Age: 26.63 ± 8.53 Country: Iran	43 (16 AA female patients and 27 healthy age- matched women)	No difference in serum or hair levels of copper in AA vs. healthy controls
Amirnia et al.[56]	2013	Case-control (Hospital- based)	Sex: 3M/24F Age: 66.27 ± 9.90 Country: Iran	54 (27 AA cases and 27 healthy controls)	Serum copper levels in AA (79.03 ± 28.22 μg/dL) vs. controls (96.77 ± 6.48 μg/dL) (P = 0.002); Hair copper levels in AA (7.91 ± 2.72 μg/dL) vs. controls (10.34 ± 2.3 μg/dL) (P = 0.001) Similar differences seen between androgenic alopecia and healthy controls
Kil et al.[54]	2013	Case-control (Hospital- based)	Sex: 44M/50F Age: 37.13 ± 14.86 Country: Korea	126 (94 AA cases and 32 healthy controls)	No difference in serum levels of copper in AA vs. controls
Bhat et al.[55]	2009	Case-control (Hospital- based)	Sex: 34M/16F Age: 27.3 Country: India	100 (50 AA cases and 50 healthy sex- and age- matched controls recruited from among hospital employees or individuals accompanying patients, with no skin or systemic disease)	No difference in serum levels of copper in AA vs. controls
Mussalo- Rauhamaa et al.[58]	1986	Case-control (Not described)	Sex: 8M/19F Age: 29 ± 11 Country: Finland	27 AA cases compared to normal Finnish population reference values	No difference in serum levels of copper in AA vs. controls; difference in serum copper levels in AA vs. AT and AU (P = 0.02)
Magnesium					
Bhat et al.[55]	2009	Case-control (Hospital- based)	Sex: 34M/16F Age: 27.3 Country: India	100 (50 AA cases and 50 healthy sex- and age- matched controls recruited from among hospital employees or individuals accompanying patients, with no skin or systemic disease)	No difference in serum magnesium levels in AA vs. control
Mussalo- Rauhamaa et al.[58]	1986	Case-control (Not described)	Sex: 8M/19F Age: 29 ± 11 Country: Finland	27 AA cases compared to normal Finnish population reference values	No difference in serum magnesium levels in AA vs. control
Selenium					
Feizy et al.[65]	2008	Case-control (Hospital- based)	Sex: 15M/14F Age: 24.9 ± 10.5 Country: Iran	58 (29 AA cases and 29 sex-, age- and place of residence-matched healthy volunteers)	Serum selenium levels in AA (62.1 ± 13.3 μg/L versus controls (88.3 ± 13.2 μg/L) (P < 0.0005) No association between extent of AA (SALT scores) and serum selenium levels
Mussalo- Rauhamaa et al.[58]	1986	Case-control (Not described)	Sex: 8M/19F Age: 29 ± 11 Country: Finland	27 AA cases compared to normal Finnish population reference values	No difference in serum selenium levels in AA vs. control

AA alopecia areata, *AT* alopecia totalis, *AU* alopecia universalis, *SALT* severity of alopecia tool

† Age given as mean ± standard deviation

Table 4

Studies of iron and alopecia areata [57, 58, 68, 70–74]

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
Dastgheib et al.[57]	2014	Case- control (Not described)	Sex: 16F Age: 26.63 ± 8.53 Country: Iran	43 (16 AA cases and 27 sex- and age- matched healthy controls)	No difference in serum or hair iron levels in AA vs. healthy controls
Gonul et al. [72]	2009	Case- control (Not described)	Sex: 29M/14F Age: 29.1 ± 13.4 Country: Turkey	79 (43 AA cases and 36 healthy sex and age-matched controls)	Low** ferritin levels detected in 4/43 patients; low** iron levels detected in 8/43 patients; No difference between AA and control groups for ferritin and iron levels
Tzellos et al. [73]	2009	Case-report	Sex: 1 M Age: 18 Country: Greece	1 AA	Normal serum ferritin levels
Esfandiarpour et al. [74]	2008	Case- control (Not described)	Sex: 29M/23F Age: 23.52 ± 14.42 Country: Iran	115 (52 AA cases and 63 age-matched healthy controls recruited from blood donors)	No difference in serum ferritin levels or serum iron levels in AA vs. controls
Kantor et al. [68]	2003	Case- control (Hospital- based)	Sex: 24F Age: AA: 34.9 (27.5, 42.3); AT/AU: 53.1 (39.6, 66.6) Country: USA	35 (17 AA, 7 AT/AU cases and 11 controls having neither of the common mutations in the HFE-1 gene for hereditary hemochromatosis)	Serum ferritin levels in AA (24.9 ng/mL [95% CI: 17.2, 32.6]) vs. controls (59.5 ng/mL [40.8, 78.1]) ($P < 0.05$); No difference in serum ferritin in AT/AU vs. controls
White et al. [71]	1994	Case-series	Sex: 9M/21F Age: 31.36 ± 17.34 Country: Scotland	30 AA	14/21 females and 1/9 males were iron deficient*, 20/21 females and 2/9 males had serum ferritin ≤30 μg/L
Boffa et al. [70]	1995	Case-series	Sex: 11M/21F Age: 44.31 ± 14.71 Country: England	32 AA	No evidence of iron deficiency* in male AA patients; prevalence of iron deficiency in AA females comparable to normal population
Mussalo- Rauhamaa et al. [58]	1986	Case- control (Not described)	Sex: 8M/19F Age: 29 ± 11 Country: Finland	27 AA cases compared to normal Finnish population reference values	No difference in serum iron levels in AA vs. normal population

AA alopecia areata, *AT* alopecia totalis, *AU* alopecia universalis

* Iron deficiency defined as serum ferritin ≤ 15 μg/L

** Threshold serum cut-offs not provided

† Age given as mean ± standard deviation or mean (95% CI)

Table 5

Studies of B vitamins (folate and vitamin B_{12}) and alopecia areata [72, 73, 78–81]

Study	Year	Study type (Sample origin)	Demographics (AA cases)†	Sample Size Total (Detail)	Measures and outcomes
Yousefi et al.[78]	2014	Case- control (Hospital- based)	Sex: 12M/17F Age: 24.79 ± 11.93 Country: Iran	61 (29 AA cases and 32 sex- and age-matched healthy controls recruited amongst patients' family members)	RBC folate (median ng/mL/cells (range): AA 167.5 (52.5– 500) vs. controls: 285.5 (97.5–662) ($P < 0.0001$); Negative correlation between SALT score and RBC folate levels (r = −0.41, $P = 0.03$)
Ertugrul et al.[79]	2013	Case- control (Hospital- based)	Sex: 46M/29F Age: 29.2 ± 12.5 Country: Turkey	129 (75 AA cases and 54 controls who had no dermatologic or systemic disease history)	No difference in serum folic acid, vitamin B_{12}, homocysteine, or holotranscobalamin levels in AA vs. controls
Gonul et al.[72]	2009	Case- control (Not described)	Sex: 29M/14F Age: 29.1 ± 13.4 Country: Turkey	79 (43 AA cases and 36 healthy sex and age-matched controls)	No difference in serum folate or vitamin B12 in AA vs. controls
Kalkan et al.[80]	2013	Case- control (Hospital- based)	Sex: 78M/58F Age: 32.27 ± 9.525	266 (136 AA cases and 130 age- and gender-matched controls)	Greater prevalence of MTHFR C677T polymorphisms (CT/TT vs. CC genotype) amongst AA vs. controls ($P < 0.05$) Serum Vitamin B_{12} (pg/mL): CT/TT: 276.28 ± 99.761 vs. CC: 283.94 ± 135.795 ($P = 0.748$) Serum folate (ng/mL): CT/TT: 8.36 ± 2.442 vs. CC: 7.76 ± 2.146 ($P = 0.198$
Zafad et al.[81]	2007	Case-report	Sex: 1M Age: 24 Country: Morocco	1 AA	Patient diagnosed with pernicious anemia associated with hemolytic anemia at age 16 and developed AA at age 24
Tzellos et al. [73]	2009	Case-report	Sex: 1M Age: 18 Country: Greece	1 AA	Low serum vitamin B_{12} (76.8 pg/ml, reference 240–900 pg/mL); normal folate levels

AA alopecia areata, *CI* confidence interval, *OR* odds ratio, *RBC* red blood cell, *SALT* severity of alopecia tool

*Normal serum vitamin B_{12} range 240–900 pg/ml

†Age given as mean ± standard deviation