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RESEARCH ARTICLE

The Effect of Oral Vitamin D on Patients with Alopecia Areata

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Abstract

Background: Patients who had alopecia areata (AA) presented with markedly lower levels of vitamin D3. Adding to that, vitamin D supplementation could have a protective role in human autoimmune illnesses such as AA, so the aim of the study was to identify the role of systemic vitamin D effect on patients with AA in comparison with the other medications, study the effect of combination of vitamin D and other medications on all symptoms of AA and study the C-reactive protein (CRP) and its relationship with AA. Conclusion: The patients with AA who were treated by combination treatment (steroids and vitamin D) had a better response than the patients who were treated by steroids only, and the response of the group of patients with AA who were treated by vitamin D only was not significant. There was non-significant association between CRP and AA.

Keywords: *Vitamin D, Alopecia areata, Vitamin D deficiency.*

Introduction

AA is the most public reason of alopecia prompted by inflammation [1]. It is an autoimmune disorder which has diverse clinical presentations ranging from reversible sparse alopecia to whole scalp hair loss or total body alopecia [2]. Its prevalence is 0.1 to 0.2 %, with a chance of AA occurring during person's life is about 2 %. All ages and entire hair colors can be affected. There is no sex differences [3].Immunological, genetic, Infective and psychological aspects are the most important reasons of AA [4].Basically, AA is diagnosed clinically. The most common presentation of AA is an abrupt loss of hair in limited regions.

The lesion is frequently characterized by a round or ovoid area of hair loss and it can be single or plentiful [5]. Usually any hair containing part of the body may be affected but ninety percent of patches appear on the scalp.

The skin of the area with AA appears normal but sometimes there may be mild redness and atrophy [6]. The regrown hairs usually have absent pigment causing the appearance of fair or white hair [7]. Exclamation mark hairs can be seen frequently along the borders of the alopecia [1]. Nail alterations are found in 10-66% of AA patients [8]. Ocular changes were also found in studies in AA patients [9]. Trichoscopy is an uninvasive way used for the diagnosis and monitoring of the treatment of scalp and hair illnesses [10]. By histological examination of the scalp biopsy specimens of AA, it was concluded that the main finding for confirming the diagnosis is the peribulbar lymphocytic inflammation especially the helper T cells and they are a proof of autoimmunity [11].

A lot of therapeutic choices are used in the treatment of AA [12]. The course of the disease mostly can't be predicted. Regrowth of hair may occur spontaneously. Though, relapse may occur in 85% [7]. Vitamin D is a lipid soluble secosteroid, produced in the epidermis by the keratinocytes [13]. Only the skin that can synthesize, activate vitamin D and expresses its receptor [14].

Usually vitamin D that is gotten from food or synthesized in skin is not active and it must undergo enzymatic activation to produce 1, 25-dihydroxy vitamin D (1, 25(OH) 2D) [15]. Vitamin D receptors (VDRs) were found in keratinocytes in the epidermis and in the

mesenchymal dermal papillary cells [16]. It was reported that vitamin D deficiency could be a triggering factor for the stimulation of autoimmunity resulting in AA [17]. Deficient vitamin D levels had been found to be related to AA in a number of researches; however this had been generally controversial with different results [18].

Subjects and Methods

This study was carried out in Merjan Teaching Hospital in Hilla city from October 2018 to May 2019. Eighty-two patients of AA were included in this study (excluding patients using vitamin D3, calcium supplements, folic acid or iron complements, patients with drug effects on vitamin D3 level such as digoxin and anti-heperlipidemic drugs, pregnancy and lactating women, patients with urinary, hepatic or any gastrointestinal system problems and alcoholic patients), fifteen patients of them didn't return back for follow up while sixty-seven patients continued the follow up.

The patients had different ages and of both sexes. They were diagnosed by specialist dermatologist clinically by loss of hair either in the scalp, eyebrows, eyelashes, beard area, arms or legs. The areas of hair loss were well demarcated and the affected skin appeared normal without scaling or any abnormalities. They were either single or multiple lesions with different sizes and shapes. Oral consent was taken from the patients to participate in this study.

Regarding control group, fifty subjects were included in this study. They were apparently healthy and didn't have any other skin disease or any other disorder. The control group was matched with the patients' groups regarding sex and age.

Full informations regarding history and examination were taken from each patient and a special designed questionnaire sheet was used for this purpose and the following informations were taken: patient's name, age, sex, occupation, address, religion, marital state, mobile number, date of history taking, duration of the disease, progression of the disease whether it is slow or rapid progression, informations regarding the lesion including : (site, size, number and the hair line), nail changes and dystrophy, previous history of AA, medical history which was divided into autoimmune diseases including: (vitiligo, diabetes mellitus, celiac disease, thyroid disorders and systemic lupus erythmatosus), Down syndrome, atopy and others. Other informations included: surgical history, drug history, family history of AA which was divided into previous and recent history and the social history.

The patients were divided into 3 groups (steroids only group: topical +/- intralsional steroids without oral vitamin D3 were used for the treatment of 27 AA patients for 3 months, combination treatment (steroids and vitamin D) group: topical +/- intralsional steroids with oral vitamin D3 were used for the treatment of 27 AA patients for 3 months and vitamin D only group : oral vitamin D3 only were used for the treatment of 13 patients for 3 months).Assessments of the scalp were done by using Severity of Alopecia Tool (SALT) score via visual assessment and further subgrouping of percentage of scalp alopecia, Body alopecia and Nail association.

Procedure for Collecting Blood Samples

Three milliliters of blood were collected from the veins of the antecubital fossa in a gel tube; it was left for 15 minutes for clotting then the serum was separated by centrifugation by 4000 round per minute for 5 minutes. Serum of the controls were collected and put in Appendorf tubes and stored in deep freeze (-80 Centigrade) and analyzed together later. The blood samples were collected at zero time (starting treatment) and at the end of the study (after three months of the treatment).

Measurement of Vitamin D:

Vitamin D was measured by using Vidas apparatus and three kits were used. The kit was consisting of strips and SPR receptacles. 100 microliters of patients' serum were put in the strips then the strips and the SPR were inserted into the apparatus. The apparatus calculated vitamin D level in 40 minutes. Serum of each patient was analyzed on the same day. The following guideline for vitamin D levels had been followed:

- Vitamin D deficiency is less than 20 ng / ml.
- Vitamin D insufficiency is less than 30 ng / ml.
- Normal vitamin D is 30-100 ng / ml.

- Toxic level of vitamin D is more than 100 ng / ml [19].

Vitamin D level was measured at zero time (starting treatment) and at the end of the study (after three months of the treatment).

Measurement of CRP

CRP was measured by using slide test and one kit was used which was consisting of control positive solution, control negative solution, CRP reagent, stirrers and slides. 50 microliters of patients' serum were mixed with 50 microliters of CRP reagent on slides by stirrers and were mixed by circular movements for at least three minutes. Serum of each patient was analyzed on the same day and the result was either positive by the presence of agglutination or negative.

Statistical Analysis

All data were analyzed by Sigma plot version 12 software. Paired T-test was used to compare means between two groups. ONE WAY ANOVA were used to assess significant difference to find the mean differences between three groups or more for collected data. The P value ≤ 0.001 was considered to be statistically significant.

Results

The parameters that were used in our study included serum vitamin D values of the control group and the patients according to the treatment used and serum vitamin D values in relation to the age, sex of the patients and in relation to the severity and duration of the disease as expressed in Table (1) and Table (2) and (3).

Table 1: Serum vitamin D values of the control group and the patients according to treatment groups, age and sex of the patients

Parameters	Pretreatment serum vitamin D level (ng/ml) Mean ± SD	Post treatment serum vitamin D level (ng/ml) Mean ± SD	P value*
Control group	19 ± 7.7		
Steroids only group	14.1 ± 6.2	16.9 ± 5.1	= 0.157
Vitamin D only group	12.3 ± 4.08	48.6 ± 11.1	≤ 0.001
Combination treatment group	12.5 + 4.9	60.4 + 13.8	≤ 0.001
Age ≤ 18 years old	12.3 ± 7.1	53 ± 11.7	› 0.001
Age › 18 years old	12 ± 6.5	37 ± 9.7	
Male sex	12.6 ± 4.9	36.8 ± 5.4	› 0.001
Female sex	20 ± 4.7	30.9 ± 6.2	

*: Significant (P ≤ 0.001)

Table 2: Serum vitamin D values of the patients in relation to the severity of the disease

Salt score	Number of patients	Serum vitamin D levels (ng / ml)
S0	0	0
S1	39	12.8
S2	16	13.1
S3	9	13
S4	3	12.9
S5	0	0

Table 3: Serum vitamin D values of the patients in relation to the duration of the disease

Duration of the disease	Pretreatment serum vitamin D level (ng/ml) Mean ± SD	P value*
‹ 1 month	19.07 ± 5.1	› 0.001
1 month – 6 months	17.97 ± 6.19	
6 months – 1 year	13.65 ± 1.76	
› 1 year	20.1 ± 9.78	

*: Significant (P ≤ 0.001)

Non-significant difference was found in vitamin D levels in relation to the age, sex of the patients, the severity and the duration of the disease

Comparison between Serum Vitamin D Levels in the Pretreatment and Post Treatment States of the Patients and Control:

When we make a comparison between the groups in the pretreatment state, there is a statistically significant difference for control and steroids, vitamin D only and combination treatment groups ($P \leq 0.001$). Also, serum vitamin D levels in the post treatment groups were significantly lower in the steroids only group than in vitamin D only group and the combination treatment group ($P \leq 0.001$) as shown in (Figure 1).

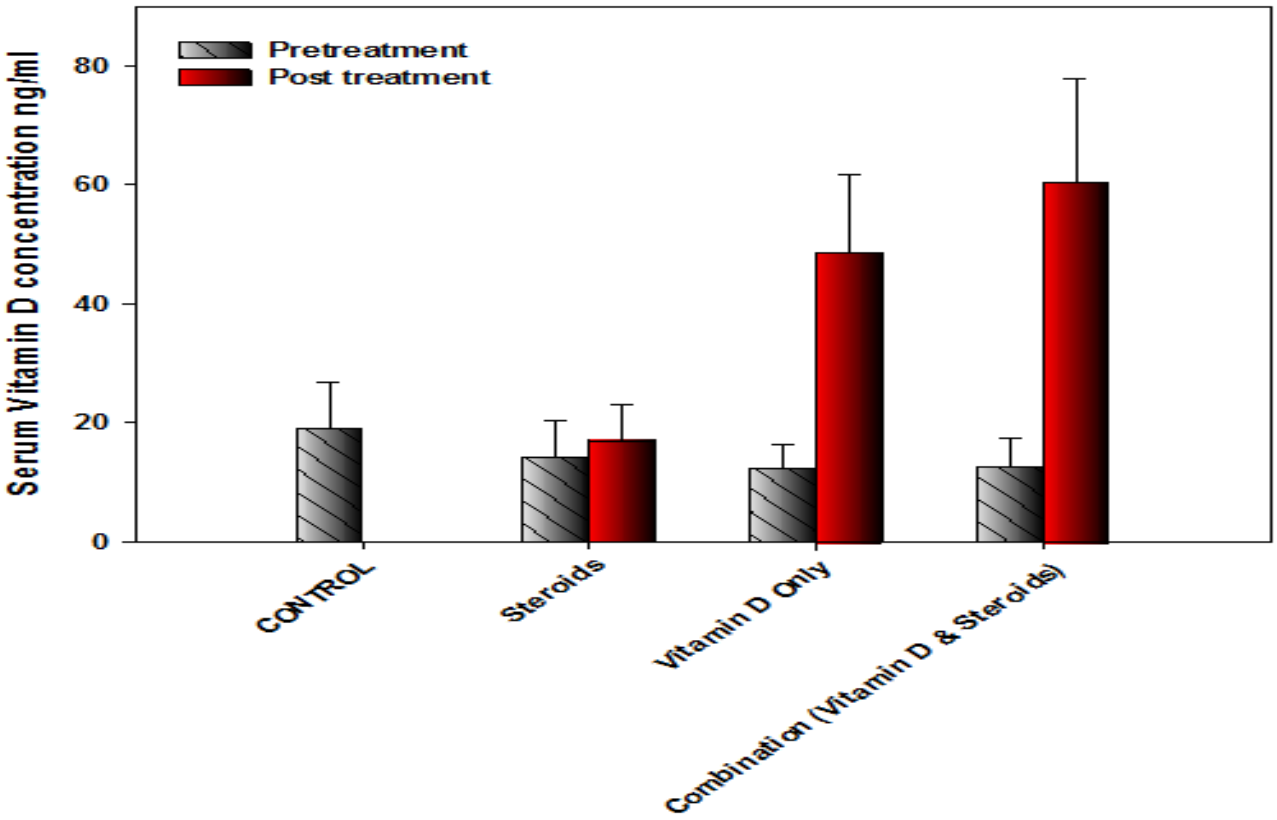

Figure 1: Comparison of vitamin D levels between the control, the steroids only group, vitamin D only group and the combination treatment group in the pretreatment and post treatment states

Clinical Improvement

Regarding clinical response to treatment, according to SALT score, in patients treated by steroids only, 70.37 % of the patients were improved (31.57 % of them had poor improvement, 52.63 % had good improvement and 15.78 % had complete improvement), while 29.62 % of the patients didn't improve. Our results showed that there was no significant clinical response to treatment in patients treated by vitamin D only, 23.07 % of the patients improved, while 76.92 % didn't improve.

There was an obvious clinical response to treatment in patients treated by combination treatment. 88.88 % of the patients were improved (16.66 % of them had poor improvement, 54.16 % had good improvement and 29.16 % had complete improvement) while 11.11 % weren't improved. So, clinically there is a better response in patients treated by combination treatment than the patients treated by steroids only, while there is a non-significant response in patients treated by vitamin D only.

CRP Measurements

Our study found that there were only three patients who had positive CRP measurements (4.47 %) while sixty-four patients had negative CRP measurements (95.52 %). So we suggest that there is a non-significant relationship between CRP and AA.

Discussion and Conclusion

The serum vitamin D measurements of the patients was considerably lesser than the controls and it was statistically significant ($P < 0.001$). This is supported by a study in

India which found a significant difference in vitamin D levels between the cases and the controls [20]. Another study showed that patients with AA showed less serum values of vitamin D by 8.52 ng / dl than the healthy control group, and deficient vitamin D levels were detected in 609 of the 1133 AA patients and in 200 of the 658 persons in the controls [18]. Our study is also parallel to the result of a research in Egypt which revealed a statistically considerable reduction of mean serum vitamin D measurements in patients having AA (14.13 ± 8.72 ng / ml) in comparison to controls (22.43 ± 10.94 ng / ml) with (P value = 0.01) [21].

These results suggest the close relationship between AA and serum vitamin D deficient levels, despite the incompatible results described in some studies. A study in Turkey on children with AA didn't find a significant dissimilarity between vitamin D levels of the patients and control (P > 0.05) [22]. Also other studies in Turkey [23], Iran [24] and Italy [25] had similar findings. This is mostly caused by the little sun exposure due to religious and regional causes. In the present study, the results showed that there is a non-significant difference in vitamin D levels between patients and control with regard to ages of the subjects.

This finding is supported by Karaguzel, *et. al.*, 2018 [22], Darwish, *et. al.,* 2017 [4], Attawa, *et. al.*, 2016 [21], Aksu Cerman, *et. al.*, 2014 [26], Mahamid, *et. al.* 2014 [3], d'Ovidio, *et. al.*, 2013 [25] and Yilmaz, *et. al.*, 2012 [16].We also found that the difference between males and females in vitamin D deficiency was statistically non-significant. This is supported by a research which found that there was a non-significant difference between males and females regarding serum vitamin D levels for both patients (45 males and 111 females) and controls (18 males and 130 females) [25].

An opposite opinion to our study, a study showed that the males patients group had significantly less values of serum vitamin D in comparison to females (p = 0.009) while in the control group, the difference between males and females was non-significant (p = 0.45) [4]. Another study found that the females in both patients (n = 33) and control group (n = 26) had a significant less vitamin D level in females in comparison to males in patients (n = 37) and control group (n = 44) and the P value was (0.005) [27] and similar result was found in other studies including Nassiri, *et. al.*, 2013 [24] and Yilmaz, *et. al.*, 2012 [16].It is thought that these low values of serum vitamin D in females are caused by vitamin D consumption during gestation and breast feeding and the social and cultural issues including the full covering clothes of females in Egypt that prevents the contact with sunlight which is the chief origin of vitamin D production [26].

Our results didn't find an association between deficient levels of vitamin D and the duration of AA. Supporting our results, some studies including Maldonado-Colin, *et. al.*, 2018 [28], Darwish, *et. al.*, 2017 [4], Aksu Cerman, *et. al.*, 2014 [26] and Yilmaz, *et. al.*, 2012 [16] didn't find any significant relationship of vitamin D levels with the duration of AA. While other studies including Unal and Gonulalan, 2018 [23] and Gade, *et. al.*, 2018 [20] revealed a significant opposite correlation between serum values of vitamin D and the duration of the disease.

We revealed that there is no association between the SALT score and serum vitamin D measurements. Darwish, *et. al.*, 2017, also stated that serum vitamin D deficiency wasn't correlated with severity of alopecia [4]. Similarly Maldonado-Colin, *et. al.*, 2018 [28], El- Mongy, *et. al.*, 2013 [27], d'Ovidio, *et. al.*, 2013 [25] and Yilmaz, *et. al.*, 2012 [16] did not find any significant association with severity of AA, probably suggesting vitamin D has a role in triggering the disease but not in its following course.

Inversely, a statistically significant inverse association was found between the SALT score and serum vitamin D levels (P < 0.05) [29]. Also, Unal and Gonulalan, 2018 [23], Bhat, *et. al.*, 2017 [30], Attawa, *et. al.*, 2016 [21] and Aksu Cerman, *et. al.*, 2014 [26] in their studies found a significant opposite relationship between SALT scores and serum vitamin D levels.

Our results showed that patients with AA who were treated by combination treatment (steroids and vitamin D) (60.4 ± 13.8 ng / ml; n = 27) (88.88 % of the patients improved clinically according to SALT score) had a better response than the patients who were treated by steroids only (16.9 ± 5 ng / ml; n = 27) (70.37 % of the patients clinically improved according to SALT score) and this

may be caused by a synergism between vitamin D and steroids. This may show that AA occurs not only due to vitamin D deficiency, but also due to existence of other causative factors, which have not been fully understood. This is supported by a study suggested that vitamin D deficiency is not the only causative agent in AA pathogenesis, but it is associated with other contributory factors, this deficiency can worsen AA severity; therefore vitamin D supplementation can be useful in management of AA in children [23].

Another study detected that the oral vitamin D treatment increases hair regrowth with the higher incidence of total remission in patients having AA with vitamin D deficiency. This conclusion supports the belief that oral vitamin D treatment can be used only to nominate AA patients who also have vitamin D deficiency [22]. Gade, *et. al.* in 2018 suggested that giving vitamin D to AA patients may cause reduction of the disease severity and stimulate remission [20].

We observed that the response of the group of patients with AA who were treated by vitamin D only was not significant (48.6 ± 11.1 ng / ml; n = 13) (23.07 % of the patients improved clinically according to SALT score). This finding is supported by a study which presents a group consisting of ten patients with chronic / relapsing AA with vitamin D deficiency; it stated that the use of vitamin D only in disease management doesn't have a significant hair regrowth. The retrospective nature of the data, small size of the sample and absence of a control group are restrictions [28].The relationship between CRP and AA was investigated in our study and there was no significant association between them. Against our result, a cohort study found that there is an increased CRP level in patients with AA and CRP levels were positively correlated with the duration of the disease but it wasn't related to the severity of hair loss.

This is mostly due to spontaneous regrowth of hair in one site and losing hair in another site, causing a fluctuation in the level of CRP [31]. Another study detected a significant increase in systemic inflammation which is reflected by high CRP levels in patients having AA, in comparison to controls. CRP levels presented a significant positive association with disease severity ($p = 0.001$). CRP also showed a significant positive relationship with duration of the disease ($p = 0.001$) [20]. Multivariate study revealed that CRP values > 1 (odds ratio 3.1, 95% confidence interval 2.6 - 4.2, $P = 0.04$) were related to AA. This finding supports the inflammatory mechanism rather than the immune mechanism in AA [3].

In conclusion, we found that the treatment by combination treatment (steroids and vitamin D) had a better response than the treatment by steroids only, and the treatment by vitamin D only had non-significant response. Also, there was a non-significant relationship between CRP and AA.

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