Serum level of vitamin D3 in vitiligo patients before and after treatment with narrow band UVB. Is there an association with treatment?

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Background: Vitiligo is a pigmentation disorder of the skin characterized by the loss of melanocytes through different mechanisms. Narrow band UVB (NBUVB) is a safe and effective treatment for vitiligo that acts by inducing the synthesis of cytokines involved in melanogenesis. NBUVB appears to be involved in the treatment of vitiligo by increasing the synthesis of vitamin D, which prevents the apoptosis of melanocytes; accordingly, we set out to compare the serum level of vitamin D and its variations following NBUVB treatment according to the degree of response to treatment.

Methods: Thirty-eight patients with vitiligo were subject to phototherapy with NBUVB. Photographs of vitiligo lesions were taken prior to and after completing 60 phototherapy sessions. Further measured were the serum level of 25-hydroxyvitamin D3, VASI score and repigmentation rate before and after the treatment period. Finally, the relationship between the changes in serum vit D3 levels and variations of VASI score was investigated.

Results: Mean serum level of vitamin D3 was 20.78 ng/ml after treatment, which is significantly more compared to the period before the treatment (15.42, P=0.001). Mean VASI score was 5.45 before the treatment, yet was reduced to 2.24 after treatment, which is a significant change (P<0.001); however, the changes in vitamin D3 levels were not significantly correlated with VASI score (P=0.137).

Conclusion: The repigmentation rate in vitiligo lesions are not significantly correlated with serum vitamin D3 levels. Therefore, NBUVB is not likely to improve vitiligo lesions through the increase in serum vitamin D levels.

keywords: vitiligo, vitamin D3, NBUVB

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INTRODUCTION

Vitiligo is a disease characterized by complete and selective loss of interfollicular epidermal melanocytes [1]. Several theories have been proposed regarding the pathogenesis of vitiligo, including autoimmune phenomena, genetic factors, impaired defense against free radicals, accumulation of neurochemicals, psychological factors such as stress and dysfunction of melanocytes [2]. Recently, more and more attention is paid to the role of vitamin D in the pathogenesis of vitiligo and its treatment. Evidence suggests that vitamin D is an immune suppressor agent and its low levels are associated with autoimmune disorders such as vitiligo. However, the reason for the low serum

levels of vitamin D in patients with autoimmune diseases is yet to be known [3]. In the study of Tomita and colleagues, it was shown that vitamin D3 increases the tyrosinase content of cultured human melanocytes [4], suggesting a possible role for vitamin D in the regulation of melanogenesis. UVB has been recognized as an effective and safe treatment for vitiligo. Nevertheless, the mechanism of vitamin D action in the development of pigmentation has not been recognized. UV radiation moderates the abnormal local and systemic immune responses via its immunomodulator effects [5].

NBUVB augments the synthesis of IL-1, TNF-α and LTC-4, which are cytokines capable of inducing mitogenesis, melanogenesis and the migration of melanocytes. In addition, the evidence at hand shows that the UVB part of sunlight (290 to 320 nm) converts 7-dehydrocholesterol to pre-vitamin D 3 in stratum corneum and basal layers, which is a key step in the synthesis of vitamin D3. Vitamin D3 induces melanin production via differentiation and expression of endothelin-B receptor on immature melanocytes in hair follicle bulge; therefore, it seems that at least part of NBUVB-induced repigmentationin vitiligo can be justified through the synthesis of vitamin D3 by NBUVB [6].

The objective of the present study was to evaluate the serum levels of vitamin D in vitiligo patients before and after treatment with NBUVB and its association with repigmentation rates following treatment.

MATERIALS & METHODS

Participants and study design

The study group ggrgrgrda (a total of 38 subjects) was selected by nonrandom and easy sampling according to inclusion and exclusion criteria from vitiligo patients referred to dermatology clinics of Ghaem and Imam Reza hospitals, Mashhad University of Medical Sciences, Mashhad, Iran, from March 2015 to March 2016. They had indication for phototherapy with NBUVB and were enrolled after explaining the objectives and methods. Inclusion criteria were clinical diagnosis of generalized vitiligo (involving more than 10% of body surface area), regular visits and treatment, consultation during autumn and winter, full information about the study and completion of a written consent form. Exclusion criteria were topical treatment with Vitamin D compounds or systemic therapy affecting the level of vitamin D (phototherapy) over the past three months, vitiligo treatment over the past three months, use of vitamin D or calcium supplements, other chronic inflammatory diseases such as multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, diabetes melitus, lupus and lymphoma, photosensitivity, non-melanoma skin cancer, or any history of cancer. The control group was comprised of 38 healthy individuals (without any chronic inflammatory diseases) selected from the companions of other patients that matched the patient's age, daily hours spent in the sun, and body mass index (BMI).

The treatment method, blood sample requirement before and after treatment, and follow-up approach using photography were explained to patients who were enrolled after completing the written consent forms. The level of 25-hydroxy vitamin D3 was measured using Bio Source kit by ELISA. The patients were subject to phototherapy by Waldmann Medical Division Therapy unit NBUVB (1000L,TL01, Germany) for three inconsecutive days based on a typical protocol which commenced with a primary dose of 200mJ/cm^2 (MED for Iranian patients) and increased by 50mJ/cm^2 until the appearance of minimal stable erythema of lesions, which was repeated up to 60 sessions (20 weeks) based on clinical conditions of the patients' lesions. The treated lesions of each patient were comparatively studied, the results of which were recorded in their files; the patients were photographed at the beginning and end of the treatment period. The extent of lesions and the percentage of depigmentation in each of the body parts (head and neck, upper and lower extremities, trunk) were recorded at the beginning and end of the treatment. VASI score was used to assess the rate of response to the treatment (Table 1), and was calculated according to the researchers' and two dermatologists' opinions who were blind to the study.

The level of 25-hydroxy vitamin D was measured before initiating phototherapy treatment and at the end of 60-treatment sessions.

To measure the extent of involvement, the body is divided into four parts: head and neck, upper and lower extremities and trunk (axilla is considered as part of the upper extremities, but groin and

Table 1. Vitiligo Area Severity index

Extent of residual depigmentation	Clinical observation
100	No pigment is present
90	Specks of pigment are present
75	The depigmented area exceeds the pigmented area
50	The depigmented and pigmented areas are equal
25	The pigmented area exceeds the depigmented area
10	Only specks of depigmentation are present
0	No depigmentation

For all body sites: VASI: Σ[hand units]×[residual depigmentation]

buttocks as parts of the lower extremities). Two parameters are checked in each part: the extent of involvement, measured by hand unit (each hand unit is equal to 1%), and the depigmentation rate that is calculated by Table 1. The final score is the product of the extent of involvement of each part by the depigmentation rate. In each patient, VASI score of all body regions was calculated according to the following formula [7]:

$$\sum(Hand\ unit)\times(Residual\ Depigmentation)$$

Finally, the results recorded before and after treatment were compared and analyzed. In addition, before starting phototherapy, serum vitamin D3 levels were compared between patients and normal controls (without vitiligo) matched for age and sex.

Statistical methods

The data were described using descriptive statistics as frequency and mean ± standard deviation in the form of tables and graphs. To compare quantitative variables before and after treatment, the normal distribution of data was primarily checked using Shapiro-Wilk test. In case of normal data distribution, paired samples t-test was used for comparison; nonparametric Wilcoxon test was employed otherwise. To compare the quantitative variables between the two groups, independent samples t-test and an equivalent nonparametric test were used in case of normal and abnormal distribution of data in each group, respectively. To investigate the correlation between variables, Pearson correlation test and Spearman test were utilized for normally and abnormally distributed data, respectively. All the statistical analyses were done using SPSS version 17 and p-value <0.05 was considered as significant. Cohen's kappa coefficient between researchers and experts blind to the study was calculated with a cut off value equal to 0.4.

RESULTS

Table 2 summarizes the demographic data of the 38 patients with vitiligo participating in this study.

Comparison of mean VASI score calculated before and after treatment shows that the overall VASI score was significantly decreased relative to baseline according to the opinion of researchers along with the two dermatologists blind to the study (in both cases P<0.001) (Table 3).

The serum level of vitamin D in patients with vitiligo was 15.4 ± 6.22ng/ml using independent samples t-test, which was significantly lower compared with healthy subjects (21.7 ± 13.39 ng/mL, P=0.012). Comparing the serum levels of vitamin D in patients before and after treatment, it became evident that this variable increased from 15.4 ± 6.22 ng/mL to 20.8 ± 6.03 following treatment, which was significant according to paired samples t-test (P=0.001).

No correlation was found between the changes in serum vitamin D3 levels after treatment with

Table 2. Demographic data of vitiligo patients participating in this study

Demographic information	No.	Percentage
Sex		
Male	9	23.7
Female	29	76.3
Fitzpatrick skin type		
II	2	5.3
III	17	44.7
IV	17	44.7
V	2	5.3
Localization		
Head & neck	19	50
Upper extremity	35	92.1
Lower extremity	36	94.7
Trunk	30	78.9
Age		
28.8±13.39 y (Mean±SD)		
Range (8-53y)		

Table 3. Comparison of mean VASI score calculated prior to and following the treatment according to researchers and two specialists

Calculated criterion	Review period	Number	Mean	Tandard deviation	Statistical comparison*
Total VASI Score (Researchers)	Before treatment	38	5.45	6.782	*P*<0.001
	After treatment	38	2.24	2.681	
Total VASI Score (Dermatologists)	Before treatment	38	5.39	6.891	*P*<0.001
	After treatment	38	2.09	2.710	

*Comparison was done using Wilcoxon test as the basic assumptions of paired t-test were not met

variables of age (p=0.692), gender (p=0.377) and skin type of patients (p=0.831).

Based on the initial levels of vitamin D3, the patients were divided into three groups of sufficient (vitamin D levels 30 ng/mL and higher, 11 patients), insufficient [vitamin D3 level of 15ng/mL (≤30 ng/mL), 19 patients] and deficient (vitamin D3 level of<15ng/mL, 7 patients). The changes the in serum levels of vitamin D3 were higher in patients who had deficiency from the beginning compared with the two other groups. Statistical comparison using one-way ANOVA showed that the difference between the three groups was significant (p=0.001).

To investigate the relationship between the changes in serum vitamin D3 levels before and after treatment with response to NBUVB treatment, the correlation between these two variables and VASI score changes was assessed using Spearman correlation test, (Table 4). As can be seen, there is no correlation between vitamin D3 changes and the variations of the calculated VASI score according to the researchers and dermatologists.

The VASI scores calculated before (correlation coefficient of 0.972 and *P*<0.001) and after (correlation coefficient of 0.976 and *P*<0.001) treatment, and the changes in VASI score during treatment (correlation coefficient of 0.973 and *P*<0.001) were strongly correlated according to the researchers and dermatologists. In addition, patients were divided into five groups in terms of repigmentation rates as follows: 1) poor (0 to 25%), 2) medium (26 to 50%), 3) good (51 to 75%), 4) very good (76 to 90%), 5) excellent (91 to 100%). The results showed that the changes in repigmentation rates had no significant correlation with variations of vitamin D3 levels in any of the groups (*P*=0.86). Neither was there a correlation between vitamin D3 levels before and after treatment with repigmentation (*P*=0.93).

DISCUSSION

Vitiligo is a pigmentation disorder characterized by the loss of melanocytes with a prevalence of 0.5-1% among different races and ethnic groups [8]. Different etiologies, including genetic factors, hyperactive immune system, neurogenic and biochemical damage, autocytotoxicity and adhesion defects of melanocytes have been suggested for vitiligo. Autoimmunity has been considered as a mechanism for vitiligo, such that various systemic autoimmune diseases have been reported in association with vitiligo [9]. Vitamin D deficiency has been proposed as a factor in the pathogenesis of vitiligo and its associated autoimmune disorders. This vitamin is an essential hormone synthesized by the skin involved in skin pigmentation. It increases the synthesis of melanin and melanocyte content of cultured human melanocytes through its anti-apoptotic effect. Moreover, vitamin D reduces the expression of several cytokines that cause vitiligo, and low levels of vitamin D have been observed in vitiligo patients afflicted with other autoimmune diseases. It can therefore be inferred that the use of vitamin D is conducive to inhibiting the destruction of melanocytes and development of vitiligo [10].

Vitamin D analogs have been effective in the treatment of vitiligo in two ways:1) controlling the activation, proliferation and migration of

Table 4. Correlation between vitamin D level changes and variations of VASI score by Spearman Correlation test

Variable	Vitamin D Changes		
	Number	The correlation coefficient (R)	Significance
VASI score changes (Researchers)	38	0.249	0.137
VASI score changes (Dermatologists)	38	0.153	0.367

melanocytes, and augmenting the tyrosinase content of melanocytes, and 2) regulating T-cell activation which reduces the autoimmune damage to melanocytes [11].

UVB acts through the generation of cytokines that stimulate the proliferation of melanocytes, synthesis of melanin and migration of melanocytes. It is involved in the first stage of vitamin D synthesis by increasing the conversion of 7-dehydrocholesterol to pre-vitamin D3 in stratum corneum and basal layer of the skin.

Since vitamin D3 may stimulate the differentiation of immature melanocytes in the bulge of hair follicle and induce melanin production in them, at least part of repigmentation in vitiligo lesions may be justified by vitamin D3 synthesis by UVB [12].

The aim of this study was to show whether or not the serum levels of 25-hydroxy vitamin D3 in vitiligo patients (candidates for phototherapy with NBUVB) would change following consecutive sessions of NBUVB exposure, and if this change is associated with clinical repigmentation rates.

It was observed that the mean serum level of vitamin D3 was 15.4 ± 6.22 ng/ml in patients before treatment with NBUVB, which is significantly lower than the healthy control group with mean vitamin D serum level of 21.7 (P=0.012).

Furthermore, mean serum vitamin D level of patients increased up to 20.8 ± 6.03 ng/ml after treatment, which is significantly higher than its level before treatment (P=0.001), confirming the few studies that have stated that NBUVB augments the serum levels of vitamin D3 [13-16].

The changes in VASI criterion following treatment were significantly reduced relative to before treatment (P<0.001). Furthermore, there was no significant correlation between vitamin D3 levels before treatment with repigmentation rates and after the treatment (P=0.93). There was no correlation between the reduction in VASI criterion and the change in the serum levels of vitamin D3 before and after treatment.

Similarly, the study of Sehrawat revealed no significant relationship between VASI and 25-hydroxy vitamin D before and after the treatment. However, the weak association (six weeks) became stronger with longer-term phototherapy (after 12 weeks) [12]. In addition, the skin type of patients in our study was generally of Fitzpatrick types 3 and 4, while in the foregoing research, it was of 4 and 5 types; moreover, Sehrawat et al. justified their results with the hypothesis that skin color is a major determining factor, preventing the formation of vitamin D3 in the skin. The effect of NBUVB monotherapy was compared with topical Calcipotriol in Akdeniz, Arca and Ada, but the cumulative effect of Calcipotriol was not demonstrated [17-19]. Therefore, UVB appears to be involved in the improvement of vitiligo lesions through a mechanism other than increasing the serum levels of vitamin D.

In this study, we assessed the complications of treatment; including mild and transient burning sensation and itching that were resolved without changing the phototherapy dose. In studies where NBUVB was used in the treatment of vitiligo, mostly short-term similar complications were reported, including erythema (the most common complication), dryness, itching, keratitis and conjunctivitis, none of which led to the discontinuation of the treatment.

It is concluded that NBUVB is not likely to improve vitiligo lesions through elevating the serum vitamin D levels; what is more, the measurement of serum vitamin D levels is not a criterion in the selection of NBUVB as a vitiligo treatment option.

Among the limitations of the current study, mention can be made of the inability to control and monitor the patient's diet, supplements and exposure to sunlight during the daylight.

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