Jundishapur J Nat Pharm Prod. 2018 May; 13(2):e65045. doi: 10.5812/jjnpp.65045.

Published online 2017 December 19. **Research Article**

# Evaluation of Serum Vitamin D Levels in Patients with Vitiligo Undergoing NBUVB (Narrowband UV-B) Therapy

Mohamad Omidian,[1] and Tahereh Asadian[2,*]

[1]Dermatology Department, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Imam Khomeini Hospital, Azadegan Boulevard, Ahvaz, Iran

[*]*Corresponding author*: Tahereh Asadian, Imam Khomeini Hospital, Azadegan Boulevard, Ahvaz, Iran. Tel: +98-6132216504, Fax: +98-6132216504, E-mail: dr.t.asadian@gmail.com

**Received** 2016 May 24; **Revised** 2016 December 03; **Accepted** 2016 December 20.

## Abstract

**Background:** Vitamin D increases the contents of tyrosinase in cultured human melanocytes, which raises its possible role in modulating melanogenesis.
**Objectives:** In this study, the level of 25 (OH) D was measured before and after NBUVB therapy and its relationship with outcome of NBUVB therapy in patients with vitiligo was also assessed.
**Methods:** This study was conducted on 30 patients with generalized vitiligo (less than 30% body surface) and 30 healthy individuals without age and gender limitations. The patients received NBUVB therapy 2 times a week for 15 weeks. We measured the serum vitamin D level and involvement area (VASI score, vitiligo area, and severity index) at baseline and 30 sessions post treatment.
**Results:** The level of vitamin D increased significantly compared to baseline measurements. Vitamin D level changes were not significantly associated with clinical outcome in which VASI score decreased.
**Conclusions:** Vitamin D did not improve clinical outcomes. Thus, we suggest that further studies be conducted to understand the mechanisms of pigmentation due to NBUVB therapy in vitiligo.

*Keywords:* Vitamin D, Vitiligo, Narrow-Band UVB

## 1. Background

Vitiligo is a disorder that leads to the loss of epidermal melanocytes (1). Pigment cell destruction in different individuals can occur by several mechanisms including autoimmune phenomena, genetic disorder, the defect of free radical defense, accumulation of neurochemicals, and chemical substances (4-tertiary butyl phenol), psychological factors, and internal deficiency in melanocytes function. Recently, the role of vitamin D in the pathological mechanism of vitiligo and its probable effect on the treatment of the disease has received more attention (2). Autoimmunity plays an important role in pathogenesis of vitiligo, and it has been found that many autoimmune disorders are associated with low vitamin D levels.

Vitamin D can control the activation, proliferation, and migration of melanocytes. It can also regulate activation of T cells and reduce the damage to autoimmune melanocytes (3). The exact mechanism of NBUVB function in vitiligo is unknown. NBUVB treatment has 2 steps. Firstly, the local and systemic immune systems are balanced against the melanocytes, then, the melanocytes are stimulated to migrate towards epidermis and to make melanin. NBUVB leads to increased synthesis of IL1, TNF$\alpha$, LTC4, and these cytokines cause mitogenesis of the melanocytes, melanogenesis, and migration of the melanocytes (4).

UVB (ultraviolent beam) in the range of 280 to 320 could convert the 7-dehydrocholesterol to vitamin D3. Moreover, several studies have shown that UVB increases the serum vitamin D levels (5). Vitamin D may cause immature melanocytes to produce melanin in the hair follicle bulge by stimulating distinction between them and the effect on the endothelin receptor type B (6). The present study aimed at investigating the relationship between the rate of recovery of patients with their increased vitamin D levels compared to the base value.

## 2. Objectives

Due to the high incidence of vitiligo in our society and its social and economic problems, we decided to conduct this study in the Iranian society to find the relationship between vitamin D levels and vitiligo disease.

## 3. Methods

### 3.1. Study Design and Population

This clinical trial was conducted on 30 patients with generalized vitiligo (less than 30% body surface), with no age and gender limitation, and with equal number of age and gender matched controls, who were referred to Imam Khomeini hospital from April 2015 to March 2016. This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences, and all patients signed an informed consent prior to enrollment.

### 3.2. Inclusion Criteria

Patients with generalized vitiligo (less than 30% body surface), with no age and gender limitation, were included in the study.

### 3.3. Exclusion Criteria

1, Patients treated for vitiligo within 2 recent months; 2, those with concurrent diabetes mellitus; 3, thyroid disorder; 4, skin tumors; 5, other malignancies; 6, photosensitivity, 7, taking drugs of photosensitivity; and 8, accounts of previous intolerance or failure of phototherapy.

### 3.4. Patients

In this study, 35 patients with generalized vitiligo (less than 30% body surface), with no age and gender limitation, were enrolled.

### 3.5. Vitamin D Measurement

Vitamin D level was measured by Electrochemiluminescence (ECL) (7). Of the individuals, 30 were selected as the control group, without restrictions on age and gender, and the level of 25 (OH) D was measured as a basis. We selected the control group from healthy people who referred to the dermatology department of Imam hospital for a check-up.

### 3.6. NBUVB Phototherapy

In vitiligo patients, 25 (OH) D was measured at baseline and after 30 NBUVB therapy sessions. During our study, patients did not receive any vitamin D supplement.

NBUVB phototherapy was given using Waldmann UV 1000 L (TL 01) machine.

### 3.7. Intervention

The intervention group received an initial dose of 0.3 J/cm$^2$, the minimum dose, which makes erythema conditions, and then, it was increased to 20% in each visit until erythematous conditions subside. In the case of symptomatic erythematous skin, such as burning or pain and blister, we discontinued the phototherapy until the symptoms improved. We considered each unit of depigmented hand area as 1%.

The radiation dose for this subgroup was 20% less than the dose of radiation, which may create erythema or blister, and the dose increased by 10% in each session. The NBUVB phototherapy was given twice a week. Genitalia and eyes were covered to be protected against radiation. The VASI (Vitiligo Area and Severity Index) was measured to assess the response of vitiligo to NBUVB therapy at baseline and at the end of the study.

### 3.8. Outcome Measures

Levels of 25 (OH) D and VASI were calculated at 0 (baseline) and after 30 sessions of NBUVB therapy. Baseline [25 (OH) D] levels were measured in controls. The following formula was used to calculate the VASI.

VASI = $\sum$ (hand unit) $\times$ residual depigmentation (2).

The evaluation of depigmentation in a vitiligo patch is presented in Table 1.

### 3.9. Statistical Analysis

Statistical analyses were conducted using SPSS Version 22 (statistical package for the social sciences, version 22, SPSS Inc., Chicago, Illinois, USA). Quantitative variables were summarized with mean $\pm$ SD, and the categorical and nominal variables were presented with frequency (percentage). A P value less than 0.05 was considered statistically significant. Spearman's correlation ($\rho$) and t test were used for statistical analysis and statistical significance was set at P value $\leq$ 0.05.

## 4. Results

In this study, 30 patients with vitiligo and 30 healthy participants were evaluated. A total of 5 patients left the study because of immigration. In the patient group, 12 cases (40%) and in the control group 11 (36.7%) were male (Table 2). The mean age of the patient group was 36.93 $\pm$ 14.5 (range: 7 - 72), and it was 32.03 $\pm$ 15.08 years (range: 13 - 68) in the healthy group (Table 2). We observed that neither the patient group, nor the healthy group had a history of underlying diseases. At baseline, the mean score of vitamin D level in the patient group was 15.30 $\pm$ 14.65 nmol/L,

**Table 1.** Evaluation of Depigmentation in a Vitiligo Patch

| Clinical Evaluation of Pigmentation | Depigmentation's Percentage |
|---|---|
| **No pigment is present.** | 100% |
| **Specks of pigment are present.** | 90% |
| **Depigmented area exceeds the pigmented area.** | 75% |
| **Depigmented and pigmented are equal** | 50% |
| **Pigmented area exceeds the depigmented area.** | 25% |
| **Only specks of depigmentation are present.** | 10% |

and it was 10.71 ± 6.51 nmol/L in the control group, indicating a statistically significant difference (P = 0.01). The end-of-treatment average of vitamin D in the patient group was 25.96 ± 13.03 nmol/L, which was significantly higher than its baseline value (P = 0.03). The Pearson correlation between baseline and end-of-treatment values of vitamin D levels was 0.39.

In the patient group, 14 patients (46.7%) had vitamin D deficiency of (vitamin D level ≤ 10 nmol/L), 13 (43.3 %) inadequate vitamin D (vitamin D level range: 10 - 29 nmol/L), and 3 (10%) sufficient vitamin D (vitamin D level range: 30 - 100 nmol/L), while in the control group, 18 participants (60%) had vitamin D deficiency, 10 (33.3%) insufficient vitamin D, and 2 (6.7%) sufficient level of vitamin D (Table 3).

Table 4 presents the serum vitamin D level at baseline and end-of-treatment and also its correlation with the VASI score. The mean area of the VASI score at baseline (8.61 ± 7.76) was significantly higher than its value at the end-of-treatment (3.81 ± 3.99) (P < 0.0001). Serum vitamin D level had a significant correlation neither at baseline ($\rho$ = -0.3; P value = 0.875), nor at the end-of treatment ($\rho$ = 0.147; P value = 0.439), with the VASI score. The mean score of serum vitamin D level increased significantly at the end-of-treatment (25.96 ± 13.03 nmol/L) compared with baseline (15.30 ± 14.65 nmol/L) (P < 0.0001).

## 5. Discussion

Vitiligo is an acquired common skin disorder that occurs with the loss of skin pigmentation due to a decrease in melanin pigment, which is caused by destruction of melanocytes (8). To date, different theories have been presented about the destruction of melanocyte cells in vitiligo including autoimmune, neurogenic, and metabolic theories (9, 10). Some of the involved factors include imbalances of calcium, Apa1polymorphism of vitamin D receptor, and low levels of serum vitamin D (2). Patients underwent NBUVM therapy 2 times a week for 15 weeks. We measured vitamin D at weeks 0 and 15 (30 sessions) 2 times a week.

Patients undergoing NBUVB therapy showed an increase in serum vitamin D level. However, molecular studies have demonstrated that vitamin D increases the contents of tyrosinase of melanocytes and causes immature melanocytes to produce melanin in the bulge of hair follicle. Therefore, vitamin D modifies melanogenesis in cell surface (2).

Since the circulating levels of 25 (OH) vitamin D change with any changes in the successful therapeutic sessions of NBUVB and because its surface is associated with clinical repigmentation, we became interested in conducting this study.

In our study, the average primary vitamin D in the patient group was 15.30 ± 14.65, and it was 10.71 ± 6.51 in the control group; and the relationship was significant (P value = 0.01). In this regard, the average vitamin D in the patient group was higher than that of the control group, which was inconsistent with the study conducted by Manu S et al. In India, the average vitamin D in the patient group was significantly less than the control group (2). Vitamin D deficiency may be associated with inadequate nutrition and geographic issues; however, the level of 25 (OH) D was not associated with the onset of vitiligo in our society, which is consistent with the study conducted by Xin X in China (11).

We found no correlation between level of serum vitamin D and the VASI score at baseline and at the end-of the treatment. This finding is consistent with a previously published study (11). The present study found that the NBUVB therapy could increase vitamin D level significantly compared with baseline, which confirmed the findings of previous studies (5, 12).

In any case, the role of TNF$\alpha$ and IL1 in melanogenesis is controversial and it has been seen in some studies. Englaro et al. found that TNF$\alpha$ inhibits the incidence and activity of tyrosinase, which is a key enzyme in melanin synthesis. The inhibition of melanogenesis by TNF$\alpha$ is secondary to nuclear factor-KB activation. IL1 causes stimulation of the synthesis of endothelin-1, which plays a role in mitogenesis and melanogenesis. Paradoxically, IL-1$\alpha$ reduces the prolif-

**Table 2.** Demographic Variables of Patient and Control Groups[a]

| Variables | Values | | P Value | |
|---|---|---|---|---|
| | Vitiligo Group | Healthy Group | | |
| **Age, mean ± SD (range), years** | 36.93 ± 14.5 (range: 7 - 72) | 32.03 ± 15.08 years (range: 13 - 68) | | |
| **Gender** | | | P1 | P2 |
| Male | 12 | 11 | | |
| Female | 18 | 19 | | |
| **Mean Vit D level base line** | 15.30 ± 14.65 nmol/L | 10.71 ± 6.51 nmol/L | 0.01 | 0.03 |
| **End of treatment** | 25.96 ± 13.03 nmol/L | | | |

[a] P1, significant level between baselines in vitiligo vs. healthy group; P2, significant level between baselines and end of treatment vitiligo group.

**Table 3.** A Comparison Between Vitamin D Levels in Two Groups of Vitiligo and Healthy Individuals at Baseline Levels of 25 (OH) in the Vitiligo Patients and the Controls[a]

| | Vitiligo Group | | Healthy Group | P Value | |
|---|---|---|---|---|---|
| | | | | P1 | P2 |
| **Vitamin D level** | Baseline | End of treatment | Baseline | 0.579 | 0.03 |
| **Deficient (< 10 nmol/L)** | 14 persons (46.7%) | no person | 18 persons (60%) | | |
| **Insufficient (10 - 29 nmol/L)** | 13 persons (43.3%) | 22 persons (73.3%) | 10 persons (33.3%) | | |
| **Normal (30 - 100 nmol/L)** | 3 persons (10%) | 8 persons (26.7%) | 2 persons (6.7%) | | |

[a] P1, significant level between baselines in vitiligo vs. healthy group; P2, significant level between baselines and end of treatment vitiligo group.

**Table 4.** Serum Vitamin D Level at Baseline and at the End-of-Treatment and Its Correlation with VASI Score in the Patient Group

| | Vitamin D Level | VASI Score | Correlation Coefficient ($\rho$) | P Value |
|---|---|---|---|---|
| **Baseline** | 15.30 ± 14.65 nmol/L | 8.61 ± 7.76 | -0.3 | 0.875 |
| **End-of-treatment** | 25.96 ± 13.03 nmol/L | 3.81 ± 3.99 | 0.147 | 0.439 |

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; $\beta$, beta; ECL, electrochemiluminescence; IL, interleukin; LTC4, leukotriene C4; NBUVB, narrow band ultraviolet bean; PG, prostaglandin; SD, standard deviation; SPSS, statistical package for the social science; TNF$\alpha$, tumor necrosis factor Alfa; UV, ultra violets; VASI score, vitiligo area and severity index.

eration of melanocytes and melanogenesis, while IL-1$\beta$ reduces the tyrosinase activity on melanocytes without any effect on their proliferation. Imokawal et al. revealed that the incidence of endothelin-1, IL1, and tyrosinase in the human keratinocytes increases after UVB therapy in in vitro and in vivo, indicating the role of a possible mechanism in the repigmentation. Another mechanism of phototherapy is the release of PGE2 and pGF2. PGE2 is made in the skin, develops the performance of the melanocytes, regulates the Langerhans cells, and develops mitogenesis of melanocytes (4).

Level of vitamin D decreased after treatment in 3 patients (2 males and 1 female) with the initial normal level of vitamin D, which was at the normal range for male patients, but it was inadequate in the female patient; and this was consistent with previous studies (13, 14).

Such a similar relationship in response to 25 (OH) D to radiation and its relationship with initial level of 25 (OH) D in a study is seen in our sun bed in healthy individuals as well, but it was not associated with body mass index, oral intake of vitamin D, and gender. Furthermore, it was found that when 25 (OH) D level reaches above 100 mol /L, 24-hydroxylase synthesis also increases and 25 (OH) D is inactivated. Only 10% to 15% of the 7-Dehydrocholesterol can be converted into the previtamin D in response to sunlight. Some believe that only 7% of 7-Dehydrocholesterol can be converted into the previtamin D. Thus, it seems that the availability of the substrate may be variable: people with a base level lower than 25 (OH) D, which could also increase in response to UVB, have more availability of substrate compared to those with a higher base level of 25 (OH) (13).

In our study, the average area of secondary involvement (VASI Score) in patients was lower than the initial average area of involvement, indicating the effectiveness of NBUVB therapy in the treatment of vitiligo, which is con-

sistent with previous studies (2, 15).

In accordance with previously published studies, current findings revealed that VASI score at the end-of-treatment decreased significantly compared with baseline. The results indicated that this decline in VASI score was attributed to the effectiveness of NBUVB therapy and increasing the serum vitamin D level, which can be used as adjunct therapy in association with NBUVB therapy.

In conclusion, NBUVB therapy is an effective and safe method for treating vitiligo and can be considered as an appropriate method for targeted patients. We found that vitamin D alone cannot clinically improve vitiligo, and perhaps it can be used as adjuvant therapy to reach the desired results more rapidly. As a final point, to make a better judgment on the role of vitamin D in the treatment of patients with vitiligo, conducting similar studies with larger sample size and long periods are highly recommended.

## Acknowledgments

This study was supported by Ahvaz Jundishapur University of Medical Sciences (grant no: IR.AJUMS.REC.1394.18).

## Footnotes

**Authors' Contribution:** Both authors have equally contributed to the study concept and design, acquisition of data, the analytical methods used, drafting of the manuscript, and critical revision of the manuscript for potent intellectual content.

**Financial Disclosure:** The authors declare that they have no disclosure of financial interests or potential conflicts of interest.

**Funding/Support:** This research was financially supported by the deputy of research and technology, Ahvaz Jundishapur University of Medical Sciences. The authors did not have funding to report.

**Role of the Sponsor:** There was no sponsorship of this study.

## References

1. Boissy R, Nordlund J. Vitiligo, current medical and scientific understanding. *G Ital Dermatol Venereol*. 2011;**146**(1):69–75. [PubMed: 21317859].
2. Sehrawat M, Arora TC, Chauhan A, Kar HK, Poonia A, Jairath V. Correlation of vitamin D levels with pigmentation in vitiligo patients treated with NBUVB therapy. *ISRN Dermatol*. 2014;**2014**:1–6. doi: 10.1155/2014/493213.
3. Li K, Shi Q, Yang L, Li X, Liu L, Wang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. *Br J Dermatol*. 2012;**167**(4):815–21. doi: 10.1111/j.1365-2133.2012.11132.x. [PubMed: 22762534].
4. Parsad D, Bhatnagar A, De D. Narrowband ultraviolet B for the treatment of vitiligo. *Expert Rev Dermatol*. 2014;**5**(4):445–59. doi: 10.1586/edm.10.34.
5. Feldmeyer L, Shojaati G, Spanaus KS, Navarini A, Theler B, Donghi D, et al. Phototherapy with UVB narrowband, UVA/UVBnb, and UVA1 differentially impacts serum 25-hydroxyvitamin-D3. *J Am Acad Dermatol*. 2013;**69**(4):530–6. doi: 10.1016/j.jaad.2013.04.058. [PubMed: 23850091].
6. Watabe H, Soma Y, Kawa Y, Ito M, Ooka S, Ohsumi K, et al. Differentiation of murine melanocyte precursors induced by 1,25-dihydroxyvitamin D3 is associated with the stimulation of endothelin B receptor expression. *J Invest Dermatol*. 2002;**119**(3):583–9. doi: 10.1046/j.1523-1747.2002.00116.x. [PubMed: 12230499].
7. Abdel-Wareth L, Haq A, Turner A, Khan S, Salem A, Mustafa F, et al. Total vitamin D assay comparison of the Roche Diagnostics "Vitamin D total" electrochemiluminescence protein binding assay with the Chromsystems HPLC method in a population with both D2 and D3 forms of vitamin D. *Nutrients*. 2013;**5**(3):971–80. doi: 10.3390/nu5030971. [PubMed: 23525081].
8. Le Poole IC, Das PK, van den Wijngaard RM, Bos JD, Westerhof W. Review of the etiopathomechanism of vitiligo: a convergence theory. *Exp Dermatol*. 1993;**2**(4):145–53. [PubMed: 8162332].
9. Habeef T. *Diagnosis and treatment of skin diseases*. Tehran: Samat Publication; 1998. p. 325–9.
10. Kwinter J, Pelletier J, Khambalia A, Pope E. High-potency steroid use in children with vitiligo: a retrospective study. *J Am Acad Dermatol*. 2007;**56**(2):236–41. doi: 10.1016/j.jaad.2006.08.017. [PubMed: 17224367].
11. Xu X, Fu WW, Wu WY. Serum 25-hydroxyvitamin D deficiency in Chinese patients with vitiligo: a case-control study. *PLoS One*. 2012;**7**(12):52778. doi: 10.1371/journal.pone.0052778. [PubMed: 23300773].
12. Czarnecki D. Narrowband ultraviolet B therapy is an effective means of raising serum vitamin D levels. *Clin Exp Dermatol*. 2008;**33**(2):202. doi: 10.1111/j.1365-2230.2007.02595.x. [PubMed: 18076684].
13. Cicarma E, Mork C, Porojnicu AC, Juzeniene A, Tam TT, Dahlback A, et al. Influence of narrowband UVB phototherapy on vitamin D and folate status. *Exp Dermatol*. 2010;**19**(8):67–72. doi: 10.1111/j.1600-0625.2009.00987.x. [PubMed: 19849714].
14. Bogh MK, Schmedes AV, Philipsen PA, Thieden E, Wulf HC. Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation. *J Invest Dermatol*. 2010;**130**(2):546–53. doi: 10.1038/jid.2009.323. [PubMed: 19812604].
15. Arca E, Tastan HB, Erbil AH, Sezer E, Koc E, Kurumlu Z. Narrow-band ultraviolet B as monotherapy and in combination with topical calcipotriol in the treatment of vitiligo. *J Dermatol*. 2006;**33**(5):338–43. doi: 10.1111/j.1346-8138.2006.00079.x. [PubMed: 16700666].