Effect of narrowband ultraviolet B phototherapy on serum vitamin D levels in patients with vitiligo
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Background
Low vitamin D status has been associated with vitiligo. Narrowband ultraviolet B (NB-UVB) therapy has improved vitamin D balance in psoriasis and atopic dermatitis; however, few data are available on such effect in vitiligo and the relationship of vitamin D levels with disease severity and repigmentation.

Objective
To investigate the influence of NB-UVB phototherapy on vitamin D status in vitiligo patients.

Patients and methods
The serum levels of 25-hydroxyvitamin D were assessed in 28 vitiligo patients before and after exposure to 24 sessions of NB-UVB treatment. Baseline vitamin D levels of patients were compared with those of 20 age and sex-matched healthy participants. Clinical response was evaluated using the vitiligo area scoring index (VASI) scoring system.

Results
Insufficient vitamin D levels (<75 nmol/l) were found in 78.6% of vitiligo patients, compared with 15% of controls. The mean serum vitamin D value was significantly lower than that in controls (P < 0.001). After phototherapy, a significant increase in vitamin D was observed (P < 0.001). The increase in vitamin D was negatively correlated with baseline vitamin D levels. However, there was no significant correlation between vitamin D levels and vitiligo area severity index (VASI) score.

Conclusion
NB-UVB therapy improves low vitamin D levels in vitiligo patients, which may contribute to its therapeutic efficacy.

Keywords:
autoimmune diseases, 25-hydroxyvitamin D, ultraviolet B phototherapy, vitiligo

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Introduction
Vitiligo is a chronic depigmenting disorder characterized by the absence of functional melanocytes in the skin. The etiology and pathogenesis of such disease are still not completely understood [1]. A strong evidence supports an autoimmune cause, together with an underlying genetic predisposition [2]. Vitiligo is frequently associated with systemic autoimmune disorders, including thyroid disease, diabetes, and pernicious anemia [3]. A link has been reported between low vitamin D levels and some autoimmune disorders, such as type I diabetes, rheumatoid arthritis, and multiple sclerosis [4]. Vitiligo could be another example of a relationship between vitamin D deficiency and autoimmunity. Vitiligo has been found to be associated with low levels of 25-hydroxy vitamin D [25(OH) D] (<30 ng/ml). In addition, vitiligo patients who had comorbid autoimmune diseases were found to be more likely to have very low 25 (OH) D levels (<15 ng/ml) [5]. Moreover, some studies have shown differences in vitamin D receptor (VDR) polymorphisms between patients with vitiligo and the general population [6,7].

Besides its well-known effects on bone and calcium homeostasis, vitamin D has been increasingly recognized as a potent regulator of multiple biological functions [8]. More than 90% of vitamin D synthesis is dependent on ultraviolet B (UVB) exposure in the skin (i.e. cutaneous synthesis), and it can also be provided by dietary sources such as fish fat [9]. Vitamin D is considered to be the precursor of a hormone (1,25-dihydroxyvitamin D), which has various biological functions in the skin, such as regulation of cell growth and differentiation and immunomodulation [10]. The role of vitamin D in immune regulation is supported by the expression of VDRs on activated T lymphocytes and the suppressive effect of 1,25-dihydroxyvitamin D in autoimmune conditions [11]. The efficacy of topical treatment with vitamin D analogs has been shown in cutaneous autoimmune diseases, including psoriasis and vitiligo. Vitamin D exerts both stimulatory and protective effects on melanocytes through its action on nuclear VDR on target cells [12]. In addition, vitamin D increases melanogenesis and the tyrosinase content of cultured human melanocytes by its antiapoptotic effect [13], and may act to stimulate the
determination of immature melanocytes in the bulge region of hair follicles to produce melanin [14].

Narrowband ultraviolet B (NB-UVB) phototherapy is a widely used and effective modality in the treatment of vitiligo. The mechanism of action of NB-UVB in patients with vitiligo has not been completely elucidated. Beneficial effects of phototherapy have been demonstrated in a variety of skin disorders. Such therapy results in increased cutaneous vitamin D synthesis, which could be one of its mechanisms of action [15]. Previous studies have shown that full-body NB-UVB is more effective compared with daily oral intake of vitamin D in the treatment of vitamin D deficiency [16]. Moreover, the efficiency of NB-UVB in producing vitamin D has been observed in patients with psoriasis and dermatitis [17].

Given the effectiveness of topical vitamin D compounds on repigmenting vitiligo and the association of low vitamin D levels with autoimmunity and with vitiligo, we sought to determine the influence of NB-UVB phototherapy on serum vitamin D levels in vitiligo patients.

**Patients and methods**

The study was conducted on patients with vitiligo who visited the phototherapy unit of the Department of Dermatology at Assiut University Hospital during the period from December 2012 to April 2013. This period was selected to avoid the possible influence of seasonal variations on vitamin D levels [18]. Pregnant women, patients less than 12 years of age, patients with a history of photosensitivity or photomediated disorders, and patients treated with vitamin D derivatives or receiving phototherapy in the last 3 months were excluded from the study. The study was approved by Assiut Faculty of Medicine Ethical Review Board. Informed consent was obtained from all participants and the study was conducted in compliance with the Declaration of Helsinki Principles.

Forty patients were enrolled in the study. Of these, 12 did not continue treatment. Finally, 28 patients (13 male and 15 female) were available for final assessment. Their ages ranged from 15 to 57 years. Seven patients (25%) were of skin type III, 14 (50%) were of skin type IV, and the remaining seven patients (25%) were of skin type V.

At the first visit, full history taking, general examination, and local examination were carried out for all patients, and disease severity and extent were assessed using the vitiligo area severity index (VASI score). Age, sex, duration of vitiligo, family history of vitiligo, and Fitzpatrick skin phototype were recorded.

All patients were treated with NB-UVB irradiation, which was performed in a Waldmann UV-1001 phototherapy cabin (Waldmann Medizintechnik, Schwenningen, Germany), equipped with nUVB TL-01 tubes (Philips TL-01, Japan; Eindhoven, the Netherlands) with a spectrum from 310 to 315 nm and a maximum wavelength of 311 nm.

Phototherapy was initiated at a dose of 0.2 J/cm² for patients with skin phototypes I or II and at a dose of 0.3 J/cm² for skin phototypes III to V [19]. Subsequently, the dose was increased by 20% per session and then stabilized when minimal erythema developed. Treatment sessions were performed twice weekly on nonconsecutive days with shielding of genitals and eyes, unless they were affected by vitiligo. During treatment, the patients were regularly evaluated for erythema, response to treatment, and any side effects. In case of severe erythema with burning and pain, treatment was stopped until resolution of symptoms and restarted thereafter at the last tolerated dose.

Photographs of the body sites affected by vitiligo were taken for each patient at baseline and after 12 weeks (24 treatments) for evaluation of clinical response using a Sony digital camera (12 megapixels; Minato, Tokyo, Japan). The vitiligo area scoring index (VASI) was used for the assessment of severity and extent of vitiligo and to measure clinical improvement. VASI was calculated at baseline and at the end of 24 NB-UVB sessions. It is based on estimation of the area of vitiligo patches and the extent of repigmentation within these patches over time [20].

The assessment was carried out in five body regions: hands, upper extremities, trunk, lower extremities, and feet. For each region, the VASI was estimated as the product of the area of vitiligo in hand units (~1% of the total body surface area per unit) and the degree of skin depigmentation within each hand unit-measured patch (expressed by percentages of 0, 10, 25, 50, 75, 90, or 100%). The face and neck areas were assessed separately. The total body VASI was then calculated using the following formula:

\[
\text{VASI} = \sum \text{All body sites [hand units]} \times \frac{1}{\text{residual depigmentation}}.
\]

Blood samples were collected before initiation of phototherapy and after exposure to 24 sessions of NB-UVB radiation. Serum 25 (OH) D assay was carried out using a commercial enzyme immunoassay kit (REF. K2110; Immunodagnostik, Benches, Germany) based on competition between 25 (OH) D present in the sample and 25 (OH) D tracer for the binding sites of vitamin D binding protein after extraction of 25 (OH) D from the sample. Serum vitamin D concentration was also assessed for a group of age-matched and sex-matched healthy controls who had no family history of vitiligo, to be compared with baseline levels in vitiligo patients. On the basis estimates of optimal vitamin D level, 75 nmol/l of serum 25 (OH) D is accepted as the lower limit for vitamin D adequacy. Vitamin D insufficiency is considered at levels less than 75 nmol/l (<30 ng/ml) [21].

The percentage of vitamin D change was calculated as the difference in the percentage of change in vitamin D level after phototherapy from the initial baseline level.

**Statistical analysis**

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ± SD or median (range), as...
appropriate, whereas qualitative data were presented as frequency and percentage. For quantitative variables, Student’s t-test analysis was carried out to compare the means of normally distributed data, whereas the Mann–Whitney U-test was used to test the median differences of the data that did not follow normal distribution. Comparison of pretreatment and post-treatment values within group was performed using the Wilcoxon signed-rank test. The χ²-test was used to compare the difference in the distribution of frequencies among different groups. Correlations among variables were studied by means of the Pearson coefficient. A P-value was considered statistically significant when it was less than 0.05.

**Results**

A total of 40 patients with vitiligo were recruited. Among these, 12 did not complete the study because of difficulties in compliance with phototherapy sessions regularly. Twenty-eight patients completed the study and were finally included in the analysis. There were 13 male and 15 female patients, with an age range of 15 to 57 years.

The baseline demographic features and disease characteristics of patients who completed the study are summarized in Table 1.

Baseline serum vitamin D levels of patients were compared with those of 20 healthy age and sex-matched controls. The mean serum 25 (OH) D concentration was significantly lower in patients than in controls (62.93 ± 14.1 vs. 96.01 ± 16.19 nmol/l, respectively; \( P < 0.001 \)). Low vitamin D levels (<75 nmol/l) were observed in 22 (78.6%) patients with vitiligo, compared with three (15%) controls \( (P < 0.001) \).

There were no statistically significant correlations between the baseline vitamin D levels and other variables, including age, skin type, duration, and baseline VASI scores.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>33.21 ± 13.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male/female</td>
</tr>
<tr>
<td>Fitzpatrick skin type [n (%)]</td>
<td>II 7 (25) IV 14 (50) V 7 (25)</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>Median (IQR) (range) 5 (8) (0.3–20)</td>
</tr>
<tr>
<td>Extent of vitiligo (VASI) (%)</td>
<td>Median (IQR) (range) 2.7 (6.23) (0.62–54.2)</td>
</tr>
<tr>
<td>Sites [n (%)]</td>
<td>Hands 21 (75) UL 25 (89.3) Trunk 23 (82.1) LL 25 (89.3) Feet 19 (67.9) Face and neck 10 (35.7)</td>
</tr>
<tr>
<td>Family history [n (%)]</td>
<td>Yes 6 (21.4) No 22 (78.6)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; LL, lower limb; UL, upper limb.

On the basis of initial vitamin D levels, the patients were divided into two groups using a 75 nmol/l cutoff point: group A with low baseline levels of 25 (OH) D (<75 nmol/l, 22 patients) and group B with normal baseline levels of 25 (OH) D (≥75 nmol/l, six patients) [21].

There were statistically nonsignificant differences between the two patient groups as regards age, sex, skin type, disease duration, or baseline VASI score. However, a positive family history of the disease was reported in six out of 22 patients with low baseline level of 25 (OH) D, whereas none of the patients with high baseline level of 25 (OH) D had a family history of vitiligo (Table 2).

As regards clinical response to phototherapy, there was a statistically significant improvement in VASI score from a median of 2.7% (range 0.62–5.425) at baseline to a median of 1.8% (range 0.34–45) after 24 sessions \( (P<0.001) \). The median improvement in the VASI score was 37.5%. The mean cumulative UVB dose after 24 sessions was 31.96 ± 9.92 J/cm².

Compared with baseline, there was a statistically significant increase in the mean serum levels of vitamin D in patients after 24 NB-UVB sessions. Serum 25 (OH) D showed a statistically significant increase \( (P<0.001) \) in group A (with low baseline level), whereas in group B (with high level at baseline) the change in 25 (OH) D was insignificant \( (P = 0.173) \) (Table 3).

Baseline vitamin D level did not show a significant correlation with baseline VASI score. However, a statistically significant negative correlation was observed between the magnitude of vitamin D increase after treatment and the baseline vitamin D level \( (r = -0.626, P<0.001) \) (Fig. 1). In contrast, there was no statistically significant correlation of the improvement in vitamin D with VASI score improvement or total UVB dose (Table 4).

**Discussion**

The present study demonstrated a low vitamin D status in patients with vitiligo and a significant improvement in serum vitamin D levels with NB-UVB phototherapy.

We observed significantly lower levels of vitamin D in vitiligo patients compared with controls. Patients with vitiligo had low levels of 25 (OH) D (<75 nmol/l) more frequently compared with healthy individuals. Similarly, other studies have also reported the association of low vitamin D levels with vitiligo [5,22]. In contrast, a few studies showed no difference in vitamin D levels between vitiligo patients and controls, which was probably due to universal deficiency of vitamin D in their populations [23]. Whether the association of vitiligo with reduced vitamin D levels is because of a role of low vitamin D status in the pathogenesis of the disease or a consequence of the autoimmune disease process needs to be established [5].

In the current study, the baseline vitamin D levels did not show significant correlations with any of age, sex, skin
type, or disease duration. However, interestingly, a positive family history was reported in patients with low vitamin D levels only and not in patients with normal levels. Such finding may point to a relationship between vitamin D deficiency and genetic susceptibility to vitiligo. This is supported by previous studies demonstrating an association of VDR gene polymorphism with 25 (OH) D levels and susceptibility to vitiligo [6,7].

The present study showed no correlation between baseline vitamin D and disease severity based on VASI score assessment. Other studies also showed no relationship between serum 25 (OH) D and body surface area affected in vitiligo [5,22]. This is probably because vitamin D level may be related to disease activity rather than disease severity. Circulating levels of 25 (OH) D were found to be inversely related to the activity of autoimmune diseases [24,25].

NB-UVB therapy is considered the most effective and a safe initial treatment of choice for moderate-to-severe vitiligo. Despite the short period of NB-UVB exposure (24 sessions) in the current study, significant vitiligo repigmentation was observed as shown by significant reduction in VASI scores. Similar findings have been shown by Hamzavi et al. [20], who reported significant clinical improvement to become evident as early as after 2 months of NB-UVB treatment. Moreover, another study reported a reduction in median VASI score by 6.7% after 12 weeks of NB-UVB received three times weekly [26]. It was suggested that earlier responses correlate with higher degrees of repigmentation at the end of treatment [19].

The mechanism of repigmentation induced by NB-UVB in vitiligo is not fully understood. However, the immunomodulatory effect of UVB phototherapy may particularly contribute to its mechanism of action [27]. NB-UVB has been proposed to downregulate the immune attack against melanocytes, as well as stimulate melanocytes to migrate to epidermis and synthesize melanin [28]. It has been suggested that the production of 1, 25 (OH) D occurs in the skin following immunomodulation induced by ultraviolet radiation [29].

In the present study, besides clinical improvement, NB-UVB therapy significantly raised serum vitamin D levels in patients with vitiligo. Vitamin D has proved to have

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Table 2. Demographic and clinical features of patient subgroups categorized according to initial vitamin D status

<table>
<thead>
<tr>
<th></th>
<th>Group A (n=22)</th>
<th>Group B (n=6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.73 ± 12.08</td>
<td>27.67 ± 16.42</td>
<td>0.16*</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>8 (36.4)</td>
<td>5 (83.3)</td>
<td>0.07*</td>
</tr>
<tr>
<td>Fitzpatrick skin type [n (%)]</td>
<td>6 (27.3)</td>
<td>1 (16.7)</td>
<td>0.28*</td>
</tr>
<tr>
<td>III</td>
<td>12 (54.5)</td>
<td>2 (33.3)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>4 (18.2)</td>
<td>3 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>6 (10.8) (0.3–20)</td>
<td>4.5 (3.5) (1–6)</td>
<td>0.23*</td>
</tr>
<tr>
<td>Median (IQR) (range)</td>
<td>2.7 (5.5) (0.62–54.2)</td>
<td>2.8 (19.4) (0.65–25.7)</td>
<td>0.87*</td>
</tr>
<tr>
<td>Extent of vitiligo (VASI) (%)</td>
<td>6 (27.3)</td>
<td>0 (0)</td>
<td>0.37*</td>
</tr>
<tr>
<td>Positive family history [n (%)]</td>
<td>6 (27.3)</td>
<td>0 (0)</td>
<td>0.37*</td>
</tr>
</tbody>
</table>

Group A: low baseline vitamin D levels; group B: normal baseline vitamin D level.
IQR, interquartile range.
*Student’s t-test was used to compare the mean difference between the two groups.
*z²-Analysis was used to compare the difference in proportions.
yThe Mann–Whitney U-test was used to compare the median difference between the two groups.

Table 3. Comparison of serum levels of vitamin D before and after phototherapy

<table>
<thead>
<tr>
<th></th>
<th>Serum vitamin D concentration (nmol/l) (mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Group A</td>
<td>59.4 ± 11.33</td>
<td>76.23 ± 12.61</td>
</tr>
<tr>
<td>Group B</td>
<td>95.53 ± 14.13</td>
<td>94.33 ± 19.31</td>
</tr>
<tr>
<td>Total patients</td>
<td>62.93 ± 14.1</td>
<td>80.11 ± 15.81</td>
</tr>
</tbody>
</table>

Group A: low baseline vitamin D levels; group B: normal baseline vitamin D level.
*P-value < 0.05 is considered statistically significant.

Figure 1.

Correlation between vitamin D level before treatment and percent vitamin D change.
beneficial immunomodulatory effects, and correcting vitamin D deficiency has been proposed to control autoimmunity [30]. Moreover, vitamin D promoted the proliferation and melanin synthesis of cultured human melanocyte [31]. Therefore, correcting vitamin D status in vitiligo patients may at least partially contribute to the therapeutic efficacy of NB-UVB phototherapy in vitiligo.

The efficacy of NB-UVB in correcting vitamin D status has been demonstrated in healthy individuals, as well as in dermatological diseases such as psoriasis and dermatitis. Such beneficial effect on vitamin D was also accompanied by significant improvement in the clinical severity of the skin diseases treated with NB-UVB [15,17,32]. However, the effects of NB-UVB phototherapy on vitamin D levels in patients with vitiligo have not been thoroughly investigated. Recently, a single study [33] has shown that NB-UVB significantly increased circulating levels of 25 (OH) D in vitiligo patients.

Although both clinical improvement and improved vitamin D levels were observed after NB-UVB therapy in the present study, the correlation between clinical improvement and vitamin D response was not statistically significant. A possible explanation is that satisfactory regitmenption with NB-UVB therapy may require up to 1 year, whereas vitamin D correction occurs early in the course of treatment. It was suggested that greater changes in vitamin D serum levels take place after the first 10 exposures [34], which is too short for an adequate clinical response to be achieved. Nevertheless, a larger sample size might help in detecting a statistically significant correlation between repigmentation and vitamin D correction in NB-UVB-treated vitiligo patients.

Interestingly, a significant increase in 25 (OH) D levels together with clinical improvement in vitiligo patients has been demonstrated after 6 months of oral vitamin D treatment [35]. This further supports that vitamin D correction may help reduce disease activity in vitiligo and this can be alternatively achieved with NB-UVB therapy.

In addition, studies have shown that a short course of NB-UVB is more effective in improving vitamin D balance compared with orally given vitamin D in healthy individuals [16,36]. The effect of NB-UVB was still evident 2 months after the treatment course. Similar long-lasting increase in serum vitamin D was also reported after NB-UVB therapy in patients with psoriasis and atopic dermatitis and was equally effective to oral vitamin D [32]. However, there are no published studies comparing the effects of NB-UVB and oral vitamin D substitution on serum 25 (OH) D concentrations in vitiligo patients.

Another important finding in our study is the dependence of the 25 (OH) D increase after NB-UVB on the initial vitamin D status. The increase in vitamin D serum levels was inversely related to the baseline vitamin D levels. Such observation is in accordance with previous studies, which showed similar findings after NB-UVB treatment of psoriasis and atopic dermatitis [15]. This could be explained by the presence of a feedback mechanism that controls vitamin D synthesis. It was claimed that, when 25 (OH) D exceeds 100 nmol/l, the synthesis of 24-hydroxylase also increases, leading to the inactivation of 25 (OH) D [37]. Such effect might be an advantage of NB-UVB over oral vitamin D due to a possible risk of vitamin D overdosing and toxicity that may occur with oral vitamin D [38].

In the present study, vitamin D changes did not correlate with the total UVB dose. Consistent results were reported by other studies [39]. Some studies, in contrast, showed a correlation with UVB dose [15,40]. Such contradiction may be attributed to the pattern of vitamin D change, as it was postulated that vitamin D increase continues to be proportional to UVB dose in the first 3 weeks, and then it reaches a plateau level showing no further increase with additional UVB doses [41].

### Table 4. Correlations between serum levels of vitamin D, VASI scores, and total ultraviolet B doses

<table>
<thead>
<tr>
<th>UVB dose</th>
<th>Baseline VASI score</th>
<th>Baseline vitamin D</th>
<th>Percent VASI improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-value</td>
<td>P-value</td>
<td>r-value</td>
</tr>
<tr>
<td>UVB dose</td>
<td></td>
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</table>

P-value < 0.05 is considered statistically significant.

The present study, as well as the previous studies, supports a role of vitamin D deficiency in the pathogenesis of vitiligo. NB-UVB therapy efficiently corrected the low vitamin D status associated with vitiligo. More studies are required to show how such beneficial effect of NB-UVB can be utilized to maintain vitamin D balance in vitiligo patients. Further studies comparing the effect of NB-UVB phototherapy with that of oral vitamin D intake in vitiligo patients are also warranted.

### Conclusion

The present study, as well as the previous studies, supports a role of vitamin D deficiency in the pathogenesis of vitiligo. NB-UVB therapy efficiently corrected the low vitamin D status associated with vitiligo. More studies are required to show how such beneficial effect of NB-UVB can be utilized to maintain vitamin D balance in vitiligo patients. Further studies comparing the effect of NB-UVB phototherapy with that of oral vitamin D intake in vitiligo patients are also warranted.

### Acknowledgements

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Conflicts of interest
There are no conflicts of interest.

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