

HMB-45 Study Before and After Narrow-Band (311 nm) Ultraviolet B Treatment in Vitiligo

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Background: Vitiligo is an acquired disease in which the loss of functional melanocytes results in depigmented macules and patches. Over the years, wide arrays of markers for melanocytes have been described, including human melanoma black 45 (HMB-45). Narrow-band ultraviolet B (NB-UVB) therapy is one of the therapeutic modalities for vitiligo.

Objectives: We sought to detect HMB-45 staining after 30 sessions of NB-UVB therapy in vitiligo and perivitaliginous skin.

Patients and Methods: All the participants were planned to have 30 sessions of NB-UVB therapy with 724 lamps (FS, 72 T, 12-HO Daavlin MED) at 311 nm wavelengths. The patients underwent skin sampling from lesional and perilesional area before and after 30 sessions of treatment. The skin biopsies were sent to the laboratory for light microscopy and immunohistochemical study. The evaluation of HMB-45 was based on the quantitative method, measuring the number of positive stained cells. Clinical response was defined as repigmentation in three categories: more than 75%; between 40% and 75%; and less than 40%. The data were analyzed using SPSS (version 17).

Results: Twenty-nine patients completed the study. The Wilcoxon test showed a meaningful relation between HMB-45 staining before and after NB-UVB treatment in perilesional skin. We did not find a meaningful relation between HMB-45 staining before and after treatment regarding the mean age, gender, mean duration of disease, and initial lesional area ($P = 0.55$, $P = 0.41$, $P = 0.55$, and $P = 0.87$, respectively). After 30 sessions of NB-UVB therapy, repigmentation was less than 40% in 8 (27.6%), 40 - 75% in 7 (24.1%), and more than 75% in 6 patients.

Conclusions: The HMB-45 stain strength significantly changed after treatment in perilesional skin.

Keywords: HMB45; Vitiligo; NB-UVB; Hypopigmentation; Melanocyte Marker

1. Background

Vitiligo is an acquired disease in which the loss of functional melanocytes results in depigmented macules and patches (1). Several types of vitiligo are defined based on the extension of lesions. The course of disease is unpredictable. There are phases of stabilization in which the process of pigment loss is stopped (2).

The pathogenesis of vitiligo is not well known, but there are different hypotheses, including an inherent defect in the melanocytes, a dysregulation of the immune response, and peripheral nervous system development alteration (3).

Over the years, wide arrays of markers for melanocytes have been described, including human melanoma black 45 (HMB-45). HMB-45 recognizes a 10-kDa antigen localized in premelanosomal vesicles. The antibody was developed from an extract of a lymph-node metastasis of melanoma. This antibody does not stain normal adult melanocytes or intradermal naevi (4). There have been many therapeutic modalities for vitiligo. Medical modalities consist of corticosteroids (oral, topical, and intralesional), oral or topical psoralen plus ultraviolet A, and narrow-band ultraviolet B (NB-UVB) therapy (5).

Westorhof and Nieuwebotova for the first time reported the use of NB-UVB phototherapy for the treatment of vitiligo. The mechanism of the action of NB-UVB in vitiligo is not fully understood. It has been demonstrated that NB-UVB could stimulate the release of basic fibroblast growth factor and endothelin-1 from keratinocytes, both of which induce melanocyte proliferation. Melanocyte migration is also enhanced by NB-UVB via an increased expression of matrix metalloproteinase-2 activity from melanocytes. In addition, NB-UVB can induce immunosuppression and T-lymphocyte apoptosis, both of which can contribute to the repigmentation of vitiliginous lesions (6). Francesco et al. (3) studied the changes in vitiliginous lesions before and after NB-UVB treatment with histochemical methods to identify the possible markers predicting the therapy outcome.

2. Objectives

In light of the Francesco et al. (3) study, we sought to investigate changes in HMB-45 staining before and after NB-UVB treatment in a larger population and different skin types.

3. Patients and Methods

This open study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences and registered (# d/886) in the Vice-Chancellorship of Research Development. All the patients with vitiligo referring to a teaching hospital for treatment were included in the study. Our inclusion criteria were comprised of duration of disease more than one year and lesional area more than 15% of the total body area. The exclusion criteria comprised renal insufficiency; photodermatitis; systemic, autoimmune, or malignant diseases; vitiligo treatment over the preceding 3 months; and current immunosuppressive or immunomodulator medications.

All the participants were planned to have 30 sessions of NB-UVB therapy with 724 lamps (FS, 72 T, 12-HO Daavlin MED) at 311 nm wavelengths. After identifying the minimal erythema dose (MED), the patients received 70% of MED, which was increased by 20% in the next sessions at maximum 500 mJ. If the patients reported erythema, burning, itching, dryness, and scaling, the NB-UVB dose was reduced by 20% or kept constant according to the severity of the symptoms. All the patients underwent skin sampling from lesional and perilesional area before and after 30 sessions of treatment. Skin biopsies were taken with punch biopsy (3 mm) and sutured with nylon thread (#5) and promptly sent to the laboratory for light microscopy and immunohistochemical study. Photography was done at a fixed distance and in light condition for all the patients with a Canon camera (A3300 IS) before phototherapy and then at one, 2, 3 and 4 months after the initiation of treatment.

For immunohistochemical study, the formalin-fixed skin samples after the pretreatment procedure were placed under heat-induced epitope retrieval. The tissue sections were irrigated with DAKO buffer (code K 8010). Then, antibody anti HMB-45 (DAKO-code K 8010/K80 IU) was added and incubated for 40 minutes. The tissue was inserted in high pH solution (code 8010) for 30 minutes, and chromogen was added to it. At the end, slides were prepared by using the counterstaining method. Positive stained cells in 100 cells were counted in 4 high-power fields. Melanocytes were used as our positive and breast tissue as our negative controls. The evaluation of HMB-45 was based on the quantitative method, measuring the number of the positive stained cells. Staining less than 5% was considered negative (-), between 5% and 25% weakly positive (+), 26% and 50% moderately positive (++), and more than 50% strongly positive (+++). Clinical response was defined as repigmentation in three categories: more than 75%; between 40% and 75%; and less than 40%. The data were analyzed using SPSS (version 17) (IL, Chicago).

4. Results

The study was completed by 29 patients, consisting of 11 (37.9%) males and 18 (62.06%) females. The mean age of

the patients was 28.20 ± 12.35 years, and the mean duration of disease was 7.02 ± 6.147 years (6.27 ± 5.53 in the males and 7.77 ± 6.57 in the females). The mean area of vitiligo patches was $33.27 \pm 16.91\%$ of the total body surface according to the rule of nines in skin burn conditions. There was no significant difference between the genders regarding the affected skin area ($P = 0.05$). The HMB-45 staining strength before and after treatment in lesional and perilesional skin is demonstrated in Table 1. The Wilcoxon test showed a meaningful relation between HMB-45 staining before and after NB-UVB treatment in perilesional skin. We did not find a meaningful relation between HMB-45 staining before and after treatment according to the mean age, gender, mean duration of disease, and initial lesional area ($P = 0.55$, $P = 0.41$, $P = 0.55$, and $P = 0.87$, respectively). After 30 sessions of NB-UVB therapy, repigmentation was less than 40% in 8 (27.6%), 40 - 75% in 7 (24.1%), and more than 75% in 6 patients. There were no significant relations between repigmentation and the mean duration of disease and mean age ($P = 0.29$ and $P = 0.52$, respectively). Repigmentation in different skin types is depicted in Table 2. We also looked for the possible existence of inflammatory cells in the lesions after treatment, but no inflammatory cells were reported in H&E stain light microscopy in all the skin samples.

Table 1. HMB-45 before and after NB-UVB Treatment in Lesional and Perilesional Skin ^a

HMB-45 Stain Strength	0	+1	+2	+3
Lesions				
Before treatment	0	0	0	0
After treatment	-	16	13	
Perilesional skin				
Before treatment	-	13	15	1
After treatment		-	15	14

^a Abbreviations: HMB-45, Human melanoma black 45; NB-UVB, Narrow-band ultraviolet B.

Table 2. Repigmentation in Different Skin Types after 30 Sessions of NB-UVB Therapy ^a

Skin Types	Repigmentation			Total
	< 40%	40 - 75%	75%	
I	1	0	0	1
II	3	3	0	6
III	2	2	2	6
IV	2	2	12	16
Total	8	7	14	29

^a Abbreviations: NB-UVB, Narrow-band ultraviolet B.

5. Discussion

Since the first use of NB-UVB phototherapy for the treatment of vitiligo by Westerhof and Nieuweboer-Krobotova, there have been several studies evaluating the effectiveness and mechanism of action of this therapy. Many studies with different methods have shown the effectiveness of NB-UVB therapy for vitiligo (7-12). Several others have investigated the effect of NB-UVB on changes in cytokines and inflammatory factors before and after treatment (2, 11). Moftah et al. (11) studied the peripheral blood in vitiligo patients before and after NB-UVB therapy to assess Foxp3+Tregs and CD4+CD25 and reported that while these cells were higher in the blood of the patients' blood than in that of the healthy control group, they decreased after NB-UVB phototherapy.

De Francesco et al. (3) (2008) studied changes occurring in vitiliginous patches in 14 patients before and after NB-UVB treatment by histochemical and immunohistochemical methods and reported that 42.8% of the subjected obtained 75% repigmentation and that HMB-45 staining following NB-UVB therapy proved moderately positive (++) in 6, weakly positive (+) in 7, and negative (-) in one patient, who presented a poor clinical response. In our study, 75% repigmentation occurred in 27.6% of the subjects: a figure less than that in the De Francesco study, which may be due to the smaller number of treatment sessions. No meaningful relation was found in our study between disease duration, patients' age, and repigmentation rate, indicating that the NB-UVB effect may have no relation with these variables. Our findings showed that the HMB-45 staining rate increased after 30 sessions of phototherapy ($P = 0.0001$). Additionally, there were no statistically meaningful relations between the HMB-45 staining strength and the duration of disease, gender, mean age, and surface area of vitiligo. This may show the independency of the NB-UVB treatment efficacy from background factors such as age and duration of disease. Because of our small study population, we did not find a statistical relationship between the amount of HMB-45 staining and repigmentation rate in lesional and perilesional skin. About 50% of our patients had skin type 4, and repigmentation more than 75% was found in 70% of this group. However, our small sample volume in each skin type group precluded statistical analysis on the repigmentation rate and skin types.

In conclusion, we think that NB-UVB phototherapy is an efficient way to treat vitiligo. It seems that its efficiency is independent of such variables as age, gender, and disease duration. A comprehensive assessment of the role of HMB-45 staining as a predictor marker requires studies with larger populations.

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Authors' Contributions

Dr. Ranjbarry did light microscopy studies, Dr. Karimzadeh carried out the study as his thesis. He assisted in visiting the patients and taking biopsies and gathering data.

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