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Comparison Between (311–312 nm) Narrow Band Ultraviolet-B Phototherapy and (308 nm) Monochromatic Excimer Light Phototherapy in Treatment of Vitiligo: A Histopathological Study

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Abstract

Introduction: Recently, the monochromatic excimer light (MEL) of 308 nm wavelength has shown some advantages in comparison to narrow band ultraviolet B (NB-UVB) for the treatment of vitiligo. To histopathologically compare the early effects of NB-UVB and 308-nm MEL phototherapy on vitiliginous patches using H&E and HMB-45.

Methods: Thirty subjects with non-segmental vitiligo lesions were treated twice a week for 6 weeks with 308-nm MEL, while NB-UVB was used to treat lesions contra laterally. Skin biopsies were taken from lesional areas before and after 6 weeks of treatment by either modality. It was prepared for light microscopy and immunohistochemical study (HMB-45). This study was performed as a clinical trial (Trial registration: http://www.pactr.org; Identifier: PACTR201705002279419)

Results: All lesions before treatment had labeling index (number of pigmented cells/non-pigmented cells) of 0.0 (0%). After treatment the LI for MEL was 4.2 ± 2.6, while for NB-UVB LI it was 0.3 ± 0.7. MEL showed higher statistical significance regarding increase of basal pigmented cells, and significant decrease in vacuolated keratinocytes and basal membrane thickness than NB-UVB.

Conclusion: Although NB-UVB is considered as treatment of choice for vitiligo, MEL is acknowledged as an effective treatment modality for vitiliginous lesions that induces more repigmentation than NB-UVB, and more rapidly, as confirmed by our study.

Keywords: Excimer laser; Haematoxylin & Eosin (H&E); Human melanoma black-45 (HMB -45); Phototherapy; Narrow band ultra-violet B (NB-UVB); Vitiligo.

Introduction

Vitiligo is an idiopathic pigmentary disorder of the skin characterized by sharply demarcated asymptomatic depigmented macules. Its pathogenesis is still unclear. Many mechanisms and theories have been suggested including autoimmunity, auto cytotoxicity, biochemical and neuronal mechanisms.[1]

No universally effective nor curative medical or surgical treatment has been proposed till this day. First line treatments include topical treatments such as corticosteroids and calcineurin inhibitors. While surgical options such as autologous melanocytes transplantation are suggested later on. Phototherapy, including narrow band ultraviolet B (NB-UVB) and monochromatic excimer light (MEL) of wavelengths 311 nm and 308 nm respectively, is considered as a successful method of treatment among those approaches.[2] The cytotoxic T-cells accountable for the destruction of melanocytes and disappearance of melanin are eliminated by phototherapy through apoptosis (diffuse repigmentation) and UVB does stimulate melanocytic proliferation and their migration to the epidermis from nearby follicular units (follicular repigmentation) and perilesional active melanocytes (marginal repigmentation).[3]

NB-UVB indoor cabins have been used to treat vitiligo since the early 1990s, but recently, MEL was used and adapted for the treatment of some dermatological diseases including vitiligo.[4] The 308 nm wavelength delivered by either laser/lamp has shown satisfactory superiority to broad band (BB-UVB) and NB-UVB for clinically treating vitiligo.[5]

Only few researchers studied histopathological changes before and after treatment with NB-UVB.[6] However, to the best of our knowledge, none compared the

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histopathological changes of lesions before and after treatment with MEL and NB-UVB. Therefore, the aim was to study the early effects of both MEL and NB-UVB in vitiliginous lesions, histopathologically, using haematoxylin & eosin (H&E) and immunohistochemically, using human melanoma black-45 (HMB-45) stain.

Methods

All Patients were asked to give full history in regards of medical history, disease duration, as well as family history of vitiligo. Assessment of the skin phototype was done according to Fitzpatrick's classification.

Patients

Our study was conducted on 30 patients with nonsegmental vitiligo with at least 2 symmetrical vitiliginous patches. Their ages ranged from 18–60 years. We excluded patients with lesions located on sun exposed areas (for possible re-pigmentation), past history of dermatological cancer, photosensitivity disorders, immunosuppressive treatment, pregnancy and breastfeeding, phototherapy or topical treatment for the last 6 months prior to the study. Patients were enrolled from the dermatology out-patient clinic of the National Research Centre.

Treatment Plan

Two symmetrical skin lesions were selected from each patient. One was treated with MEL and contra laterally with NB-UVB. During the NB-UVB session, areas already treated by MEL were covered to avoid dual exposure. Treatment sessions were given twice weekly on non-successive days for 6 weeks. For both treatments, the initial dose was almost 70% of the minimal erythematous dose (MED) calculated before treatment. Dose-increases were 40% from treatment numbered 1 to 4, 30% from 4 to 8, and 20% continuously from treatment 8 forwards, until slight erythema was attained. All patients were instructed to avoid any topical treatment.

Phototherapy Sources

NB-UVB was delivered using a Waldmann ramp equipped with 13 Philips®, TL 100W/01 fluorescent tubes (Waldmann Medizintechnik GmbH, Villingen-Schwenningen, Germany) emitting 15 mW/cm^2 at a distance of 20 cm, as measured by a Waldmann -UV Meter -dosimeter, and a TP4 Cosmedico (Technique Médicale, Otterswiller - Saverne, France).

A 308-nm xenon chloride MEL delivery system (Excilite®, Deka Mela, Florence, Italy) was used to irradiate skin with average power density of 50 mW/cm^2 at a tube-to-skin distance of 15 cm and with maximum rectangular irradiating area of 576c m^2 (36 cm × 16 cm).

Biopsy Sampling

Three punch biopsies (5 mm each) were obtained from each patient. One at baseline from vitiliginous skin before starting therapy and one after 6 weeks of therapy from each of the NB-UVB and Excimer light treated areas.

Biopsies were fixated in 10% neutral buffered formalin, routinely processed, embedded in a paraffin block and sectioned by the ordinary microtome into five um thickness cut sections. The resultant sections were mounted on ordinary glass slides and positively charged slides to be subjected to conventional H&E stain and HMB-45 immunohistochemical stain.

Immunohistochemical Staining

Sections on the positively charged slides were prepared for immunohistochemical staining with HMB-45 according to the instructions provided by the manufacturer as follow: monoclonal mouse anti-human HMB-45 protein, which recognized the structural glycoprotein 100 (gp100) associated to immature, premelanosomes-22, 23 (monoclonal antibody, Dako Corp., Carpinteria, CA, USA, in dilution ratio of 1:50, code no: 364S207, USA) to demonstrate HMB-45 expression. Hematoxylin was used as a counter stain.

Histopathological Evaluation

Light microscope with built-on camera (Olympus CX41, Germany) was used to examine and to photograph the H&E stained sections. Both dermal and epidermal layers were examined and reported. Based on to Anbar et al[7] work the following criteria were evaluated: vacuolated degenerated keratinocytes, basal pigmented cells, dermal edema and dermal perivascular inflammatory cells.

Expression of the Marker

HMB-45 normally stains the basal epidermal melanocytes, and the expression is cytoplasmic. The number of positive cells by HMB-45 monoclonal antibody were scored in each section using ×400 magnification in 10 high power fields and the percentage of melanocytes in relation to keratinocytes of the basal cell layer (labelling index) (LI).[8] The mean value of positive cells/HPF was then statistically analyzed in V, NB and MEL areas before and after 6 weeks of therapy. Count of the number of the vacuolated keratinocytes, basal pigmented cells as well as the number of the stained cells were done in 5 random high power fields, counted on the screen using Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Limited, Cambridge, England).

Statistical Analysis

This analysis was conducted by the SPSS program, also known as the statistical program for social sciences for Windows.

For the parametric numeral data mean, standard deviation (±SD) and range were used. As for the non-parametric numerical data, frequency and percentage of non-numerical data: Median and interquartile range (IQR) were applied.

In assessing the statistical significance of the difference of an ordinal variable measured twice for the same study entity, the Wilcoxon signed rank test was applied. McNemar test was used to evaluate the statistical

significance of the difference between a qualitative variable measured for the same study entity. Paired *t* test was performed to consider the statistical significance of the difference between 2 means measured for the same study cluster. *P* value (the level of significance) was considered insignificant where it was higher than 0.05, *P* value of less than 0.05 is significant and *P* value less than 0.01 is highly significant.

Results

Our study included thirty patients with non-segmental vitiligo. Their age was between 18 and 60 years old, with a mean ± SD of 32.5 ± 11.2 years. Three (10%) patients were classified as Fitzpatrick skin type II, 10 (33.3%) were skin type III, 12 (40%) were skin type IV and 5 (16.7%) were skin type V. In this study the disease duration amongst the patients was 6.4 ± 5.4 years with a minimum of 1 year and maximum of 19 years.

Starting dose for MEL treatment was 0.5±0.1 J/cm^2, same as NB-UVB. On the other side the cumulative dose for MEL was 12.7 ± 2.5 J/cm^2, while that for NB-UVB was 15.5 ± 4.2 J/cm^2. Repigmentation showed a superior statistical significance towards MEL in comparison to NB-UVB ($P < 0.001$).

Side effects from both modalities were in the form of erythema, xerosis and blistering. There was no superior significance to any treatment modality in regards to side effects ($P > 0.05$).

Hematoxylin & Eosin Staining Findings

On comparing areas treated with MEL at baseline and after treatment histopathologically with H&E stain, there was a highly significant rise in the number of basal pigmented cells ($P < 0.001$) and a statistically significant shrinkage in basal membrane thickness and number of vacuolated keratinocytes ($P < 0.05$) after treatment. However, no statistically substantial changes were noted in dermal edema and perivascular inflammation. Meanwhile, post treatment with NB-UVB, showed non-significant changes in dermal edema, perivascular inflammation, vacuolated keratinocytes, basal membrane thickness and most importantly basal pigmented cells, which remained the same (Figures 1 and 2).

On comparing both MEL and NB-UVB as regards to the H&E findings, there was superior statistical significance for MEL against NB-UVB concerning basal membrane thickness, vacuolated keratinocytes and basal pigmented cells. There was no superiority to any modality regarding dermal edema and perivascular inflammation.

Immunohistochemistry Findings

All vitiligo lesions before treatment with either NB-UVB or MEL had labelling index (LI) (number of pigmented cells/non pigmented cells) of 0.0 (0%). After treatment (starting eighth session), the labelling index (LI) for MEL was 4.2 ± 2.6 while for NB UVB LI was 0.3 ± 0.7, being more statistically significant for MEL modality ($P < 0.001$; Figure 3).

Discussion

The mechanisms by which UV light improve vitiliginous patches are still being studied, as no single theory has been proved to explain the exact pathogenesis. On the photo biological level, the wavelengths of 311-nm (NB-UVB) and the 308-nm MEL are very similar to each other, and may share their therapeutic effects' theories.[9]

Many studies proved the benefits of 308-nm MEL (laser/lamp) over NB-UVB, regarding the relatively rapid onset of repigmentation, less treatment sessions required for repigmentation and lower cumulative doses.[10]

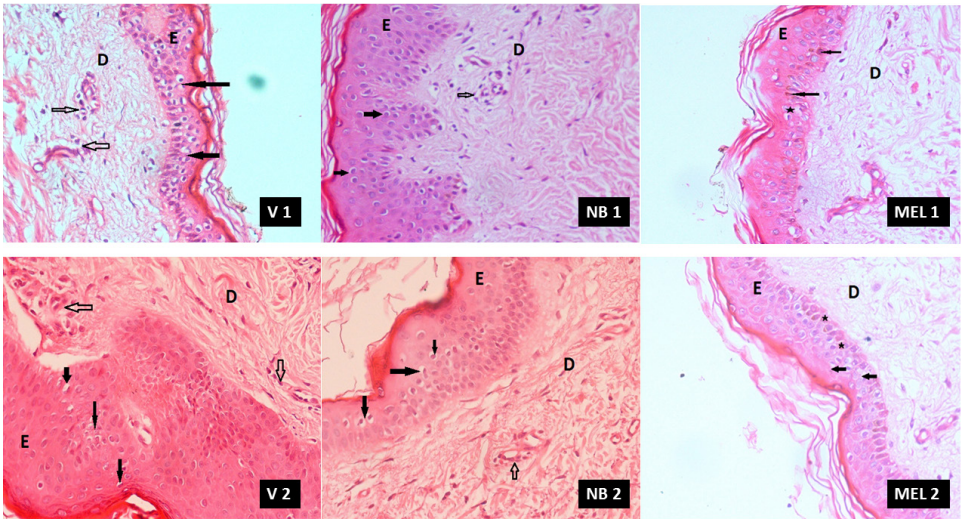

Figure 1. Biopsy Sample Before Treatment Labeled (V1, V2), After Treatment by Narrowband UVB Labelled (NB1, NB2) and After Treatment With Monochromatic Excimer Light (MEL 1, MEL 2). Vitiliginous areas (V1, V2) showed scattered perivascular lymphocytes (hollow arrows), clear basal cells (compact arrows) with moderate dermal edema. After treatment by NB UVB (NB1, NB2), showed similar findings especially the absence of basal melanocytes. While after treatment by MEL (MEL1, MEL2) some improvement was evident by appearance of pigmented basal melanocytes (stars) and less perivascular lymphocytes (hollow arrows) (H&E x 200).

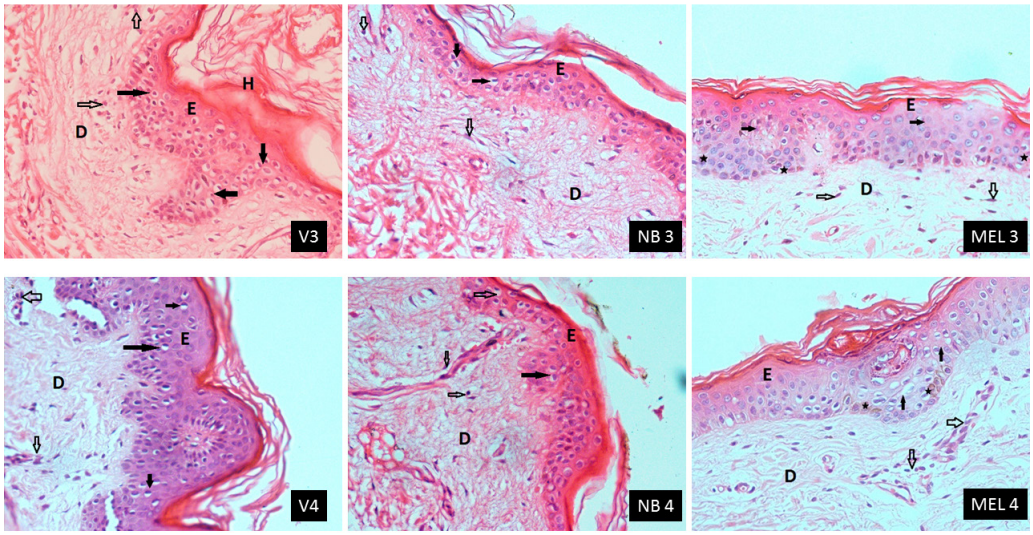

Figure 2. Biopsy Sample Before Treatment Labeled (V3, V4), After Treatment by Narrowband UVB Labelled (NB3, NB4) and After Treatment With Monochromatic Excimer Light (MEL 3, MEL 4). Vitiliginous areas (V3, V4) showed scattered perivascular lymphocytes (hollow arrows), clear basal cells (compact arrows) with moderate dermal edema. After treatment by NB-UVB (NB3, NB4) it showed similar findings, especially absence of basal melanocytes. While after treatment by MEL (MEL3, MEL4) some improvement was evident by appearance of pigmented basal melanocytes (stars) and less perivascular lymphocytes (hollow arrows) (H&E x200).

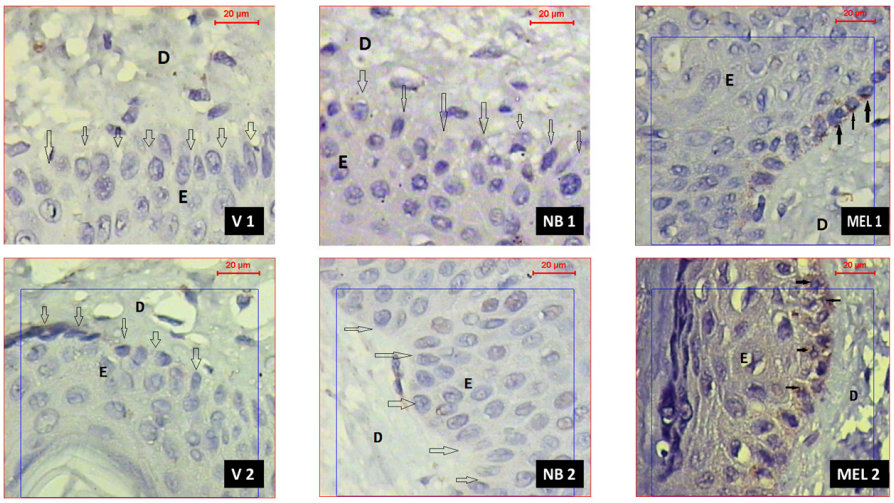

Figure 3. High Power View of the Skin of Before Treatment (V1,V2) Showing Complete Negative Staining of the Basal Cells for HMB45 (the hollow arrows). Labeling Index (LI) = 0%. After treatment by Nb-UVB (NB1,NB2) the biopsy sample did not show any positively stained basal cells for HMB-45 (hollow arrows) LI = 0%. After treatment by MEL (MEL1, MEL2) the slide showed scattered positive basal cells for HMB-45 (compact arrows). MEL 1 LI = 30.8% MEL 2 LI = 50% (HMB-45 x400 [D: dermis, E: epidermis]).

In our study, on the clinical level, repigmentation was detected in the macules treated with 308-nm MEL as early as the eighth session (fourth week) compared to those treated with NB-UVB. There was no superior significance to any treatment modality in regards to side effects, as also confirmed by Linthorst et al.[11]

Our results, however, did not match those of Verhaeghe et al,[12] who led their study on 11 patients only, where 20% achieved repigmentation by NB-UVB after 24 sessions, while none achieved repigmentation by MEL.

To the best of our knowledge and up to date, there are no studies in literature comparing the histopathological correlation between MEL and NB-UVB in the treatment of vitiligo using H&E, and immunohistochemically using HMB-45 stain, as we did. Moosavi et al proposed the possibility of a relationship between the amount of HMB-45 staining and the repigmentation rate in vitiligo patients and could not find such relation.[13]

Regarding H&E staining, there was superior statistical significance between MEL and NB UVB regarding basal membrane thickness, vacuolated keratinocytes, with basal pigmented cells being more evident in MEL treated areas. No superiority in any modality regarding dermal edema and perivascular inflammation, lead to opening

a discussion for the exact mechanism by which phototherapy treats vitiligo. Park et al suggested that the initiation of T-cell apoptosis is higher with (MEL) than with NB-UVB phototherapy.[14]

To conclude, NB-UVB is still the standard phototherapeutic treatment of choice for vitiligo. However, MEL has been recently established as a comparable active treatment option that encourages more repigmentation than NB-UVB in relatively earlier sessions; these findings were confirmed by our histopathological results.

Conflict of Interests

None.

Ethical Considerations

All subjects gave an informed consent to join in this work. The study has been approved by the research ethical committee of the National Research Centre, Giza, Egypt.

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