HISTOPATHOLOGICAL FINDINGS IN LESIONAL AND PERILESIONAL SKIN OF VITILIGO PATIENTS BEFORE AND AFTER NARROW BAND ULTRAVIOLET B PHOTOTHERAPY.

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ABSTRACT

Background: Vitiligo is a skin disease with complex, multifactorial pathogenesis. Abnormalities in surrounding keratinocytes may cause melanocyte death due to deprivation of growth factors. Narrow band ultraviolet B (NB-UVB) is an effective therapeutic option especially in patients with generalized disease.

Objective: The aim of this study was to identify histopathological changes in lesional and perilesional skin of vitiligo patients and the effect of NB-UVB therapy on them.

Methods: Twenty patients were enrolled in this study. They received NB-UVB twice weekly on non-consecutive days for a total of 40 sessions. Skin biopsies from lesional and perilesional skin were obtained from each patient before and after therapy.

Results: After therapy, 10% of patients showed excellent clinical response, 10% showed good response, 40% showed moderate response, 35% showed poor response and 5% showed progressive disease. Before therapy, 50% of patients showed a basal lymphocytic infiltrate with a perivascular lymphocytic infiltrate in both lesional and perilesional skin. 40% of them showed additional hydropic degeneration of lower epidermis with apoptotic keratinocytes in 20% of them. After therapy, these inflammatory changes were significantly reduced (p=0.04).

Conclusion: NB-UVB is an effective method of treatment of vitiligo. This may be due to its immunosuppressive effects. Also, keratinocyte apoptosis may have a role in pathogenesis of vitiligo.

Keywords: Vitiligo; NB-UVB; Keratinocytes; Apoptosis.
Abbreviations: BSA (Body surface area); HCV (Hepatitis C virus); NB-UVB (Narrow band ultraviolet B); VASI (Vitiligo area scoring index); VIDA (Vitiligo disease activity) score.

INTRODUCTION

Vitiligo is an acquired depigmenting skin disease caused by damage of melanocytes. It affects about 0.5 to 2% of general population. Clinically, there are depigmented or hypo-pigmented macules and patches on skin and mucous membranes. The aetiopathogenesis of vitiligo is complex and multifactorial. The disease may result from interplay between several pathogenic factors including autoimmunity, neural dysregulations, and increased oxidative stress in genetically predisposed individuals. There are various therapeutic modalities for vitiligo with varying repigmentation rates. Phototherapy is used as a first-line therapy in patients with extensive disease. NB-UVB is a type of phototherapy which has been found to be as effective as Psoralen-ultraviolet A therapy but with fewer side effects. It can be used in combination with systemic or topical therapies such as corticosteroids, calcineurin inhibitors or calcipotriol. The aim of this study was to evaluate the histopathological changes in lesional and perilesional skin of vitiligo patients before and after NB-UVB phototherapy.

PATIENTS AND METHODS

Twenty patients with generalized non-segmental vitiligo were enrolled in this study.

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They were selected from the outpatient clinic of the Dermatology, Venereology & Andrology Department, Zagazig University Hospitals during the period from December 2015 to December 2017. They were 14 males and 6 females with their ages ranging from 12 to 65 years with a mean of 31.7 ± 18.9 years. The Study was approved by the Institutional Review Board (IRB) at Faculty of Medicine, Zagazig University. Written informed consent was obtained from all participants. The work should be carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

- Inclusion criteria:
  - Generalized non-segmental vitiligo involving ≥ 5% of body surface area (BSA)

- Exclusion criteria:
  1. Patients received topical therapy within the previous month.
  2. Patients received systemic therapy within the previous 3 months.
  3. Patients received phototherapy within the previous 6 months.
  4. Patients with history of skin cancer or Photosensitivity.

Complete history was taken and detailed dermatological examination was done for all patients.

**Assessment of disease activity:**
It was done using the Vitiligo disease activity (VIDA) score, a six-point scale for assessment of disease activity according to patient's own opinion. Grading was as follows: (+4) active disease in last 6 weeks; (+3) active in last 3 months, (+2) active in last 6 months, (+1) active in last 1 year, (0) stable for at least 1 year and (-1) stable for at least 1 year with spontaneous repigmentation.

**Assessment of disease severity:**
It was done using the Vitiligo Area Scoring Index (VASI). First, the body was divided into five regions: hands, upper extremities (including axillae), trunk, feet and lower extremities (including buttocks and inguinal areas). Degree of involvement in each region was determined using the hand unit. One hand unit, including the palm and the palmar surface of all digits, represents approximately 1% of the total BSA. Then, the extent of residual depigmentation was represented by the following percentages: 0, 10%, 25%, 50%, 75%, 90%, or 100%. At 10% depigmentation, only specks of depigmentation were observed; at 25%, the depigmented area was less than the pigmented area; at 50%, the depigmented and pigmented areas were equal; at 75%, the depigmented area was more than the pigmented area; at 90%, specks of pigment were remaining; At 100% depigmentation, no pigment was remaining.

The VASI was derived by multiplying the number of hand units by the percentage of residual depigmentation for each region then summing these values for all body regions as demonstrated in the following formula: 

$$\text{VASI} = \sum [\text{Hand Units}] \times [\text{Residual Depigmentation}]$$

(Possible range; 0–100)

**Narrow-Band UVB Phototherapy:**
All patients received NB-UVB phototherapy twice weekly on non-consecutive days for a total of 40 sessions. This was done using the phototherapy unit GH-8ST (Cosmedico Medizintechnik, Schwenningen, Germany). The device has 8 mercury low pressure lamps (Philips TL 100W/01) with a spectrum of 305-315nm, maximum wavelength of 311nm and irradiance of 6-8 mW/cm². Doses of NB-UVB phototherapy were administered according to ready calibrated tables supplied by the manufacturer.

**Clinical Assessment:**
Patients were monitored before each session and at the end of therapy to determine their clinical response. Photographs of lesions before and after therapy were evaluated by two independent observers. According to percentage of repigmentation, clinical response of patients was graded as follows.

- **Excellent response**: if repigmentation of depigmented lesions was >75% at the end of therapy.
- **Good response**: if repigmentation ranged from 51–75%.
- **Moderate response**: if repigmentation ranged from 26–50%.
- **Poor response**: if repigmentation ranged from 1–25%.
- **Absent response**: if no repigmentation was noted.
Worse response: if progressive depigmentation of lesions was noted. Improvement in VASI score after therapy was graded as follows:

Very much improved: if VASI score decreased by more than 50% at the end of therapy.

Much improved: if VASI score decreased by 25–50%.

Improved: if VASI score decreased by 10–25%.

Minimally improved: if VASI score decreased by less than 10%.

No change: if VASI score decreased by 0%.

Worse: if VASI score increased indicating progression of disease.

Disease activity was determined again at the end of therapy using VIDA score. Any side effects were noted.

Skin biopsy:

Two 5mm punch biopsies were obtained from each vitiligo patient; one from lesional skin and the other from perilesional skin. Two biopsies were obtained from the same site after the end of NB-UVB phototherapy. Site of biopsy was sterilized using alcohol then local anaesthesia (Mepecaine ® 3%) was injected subcutaneously. All specimens were fixed in 10% natural buffered formalin solution. Fixed specimens were then dehydrated in ascending grades of alcohol, cleared in xylol and processed into paraffin blocks. Serial 4 micron thick sections were obtained from each block and stained with Haematoxylin and Eosin (H&E) stain for histological diagnosis.

Statistical Analysis

For data analysis the statistical package SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22 was used. Data was summarized using mean (X), standard deviation (SD), minimum and maximum in quantitative data and using frequency (count; n) and relative frequency (percentage; %) for categorical data. The following tests were used; chi-square test ($\chi^2$), student t-test ($t$), and paired samples t-test. Correlations were done using Spearman correlation coefficient ($r$). In all tests, statistical significance was set at $P \leq 0.05$.

RESULTS

Twenty patients with generalized non-segmental vitiligo were included in this study. They were 14 male patients (70%) and 6 female patients (30%) with their ages ranged from 12 to 65 years with a mean of 31.7±18.9 years.

Four patients (20%) had associated HCV infection and 7 patients (35%) had positive family history of vitiligo.

Triggering factors for development of vitiligo were reported in 10 patients (50%) including; stress in 6 patients (30%), trauma in 3 patients (15%) and burn in 1 patient (5%).

Features of active disease were observed in 11 patients (55%) including; trichrome lesions in 4 patients (20%), confetti-like lesions in 2 patients (10%), positive Koebner's phenomenon in 1 patient (5%) and mixed features in 4 patients (20%).

Clinical response

After completion of 40 sessions of NB-UVB phototherapy, 2 patients (10%) showed excellent clinical response (fig. 1), 2 patients (10%) showed good response, 8 patients (40%) showed moderate response, 7 patients (35%) showed poor response and one patient (5%) showed worse response as the disease was progressive.

There was a statistically significant association between clinical response of patients and VIDA score after therapy ($p=0.008$). Patients with high VIDA score after therapy (indicating highly active disease) showed poor response while those with low VIDA score (indicating less active disease) showed better response (table 1).

After therapy, improvement in VASI score was as follows; 2 patients (10%) showed very much improvement, 4 patients (20%) showed much improvement, 11 patients (55%) showed improvement, 2 patients (10%) showed minimal improvement and one patient (5%) showed worsening of VASI score.

There was a statistically significant difference between VIDA scores before and after therapy ($p=0.02$). Percentage of patients with VIDA score of +4 reduced from 25% before therapy to 10% after therapy. Also, percentage of patients with VIDA score of +2 reduced from 35% before therapy to 20% after therapy. On the other hand, percentage of patients with VIDA score of +1 (indicating
relatively less active disease) increased from 25% before therapy to 55% after therapy. These results indicate significant reduction in disease activity in vitiligo patients after NB-UVB therapy (table 2).

Side effects after therapy had been observed in 6 patients (30%). Two patients (10%) had sunburn-like reaction, one of them showed progressive disease. Two patients (10%) had erythema and 2 patients (10%) had xerosis.

**Histopathological Findings**

Before treatment, histopathological examination of H&E stained sections revealed absent melanin pigment and melanocytes in lesional skin in all 20 patients (100%). After NB-UVB phototherapy, there were variable degrees of partial restoration of melanocytes and melanin pigment in lesional skin in 19 patients (95%) with a single patient showing evident dermal melanophages. Persistent absence of melanin pigment and melanocytes was noted in lesional skin in only one patient (5%) who showed progressive disease.

Before therapy, a basal lymphocytic infiltrate was observed in both lesional and perilesional skin in 10 patients (50%). A perivascular lymphocytic infiltrate was also noted in these patients (figure 2&3). Eight of them (40%) showed additional hydropic degeneration of the lower epidermis and 2 of them (10%) showed apoptotic keratinocytes (figure 4). One patient (5%) showed lymphocytes invading a hair follicle in lesional skin. Following therapy, the inflammatory reaction was persistent in 4 patients (20%). Lymphocytic infiltrate has been significantly reduced after NB-UVB therapy from 50% to 20% in both lesional and perilesional skin ($p=0.04$).
Figure (1): Vitiligo lesions on legs before therapy (A) showing excellent repigmentation response after 40 sessions of NB-UVB phototherapy (B).

Figure (2): Histopathology of lesional skin before therapy showing absent melanocytes and melanin pigment with a basal lymphocytic infiltrate and a perivascular lymphocytic infiltrate (H&E X 400).
Figure (3): **Histopathology of perilesional skin before therapy** showing lymphocytes along the basal layer with a perivascular lymphocytic infiltrate (H&E X 400).

Figure (4): **Histopathology of lesional skin before therapy** showing intracellular hydropic degeneration of lower epidermis (H&E X 400).

**DISCUSSION**

Vitiligo is a skin disease with a great impact on patients' lives. It is characterized by amelanotic macules and patches affecting skin and/or mucosal surfaces. It results from death of melanocytes which may be due to autoimmunity, biochemical disturbances or other abnormalities.

This study included 20 patients with generalized non-segmental vitiligo including 14 male patients (70%) and 6 female patients (30%) with the male to female ratio being 2.33 to 1. This is consistent with previous studies that reported development of vitiligo predominantly in male individuals. In contrast, other studies reported predominant affection of females.

Associated HCV infection was reported in 4 patients (20%). Similarly, HCV has been reported to be significantly more prevalent in vitiligo patients (48.2%) than control (19%). This may indicate a possible causal
relationship between HCV infection and vitiligo.

Seven patients (35%) reported positive family history of vitiligo. This is consistent with a previous study that reported positive family history of vitiligo in 39% of patients. These results may point to the importance of genetic factors in pathogenesis of vitiligo.

Triggering factors were reported in 10 patients (50%). On the contrary, lower incidence of triggering factors (20%) was reported by Lah lou et al. which included psycho-trauma. Similarly, triggering factors were reported in (18%) of 135 patients including trauma (8%), illness (5%), emotional factors and pregnancy (5%) and sever sunburn (2%). This difference may be due to variation in sample size.

Features of active disease were observed in 11 patients (55%) including; trichrome lesions (20%), confetti-like lesions (10%), positive Koebner’s phenomenon (5%) and mixed features (20%). This is consistent with a previous study that reported occurrence of trichrome lesions in (21.27%) of patients. Other studies reported either higher incidence of trichrome lesions (66.3%) or lower incidence (7.8%). These variations may be due to different sample size between these studies.

There are variable therapeutic modalities for vitiligo. NB-UVB has been successfully used in treatment of vitiligo especially patients with generalized disease.

In this study, after receiving 40 sessions of NB-UVB phototherapy, 2 patients (10%) showed excellent clinical response, 2 patients (10%) showed good response, 8 patients (40%) showed moderate response, 7 patients (35%) showed poor response and one patient (5%) showed progressive disease. Similarly, VASI score was very much improved by more than 50% in 2 patients (10%), much improved by 25–50% in 4 patients (20%), improved by 10–25% in 11 patients (55%), minimally improved by less than 10% in 2 patients (10%) and became worse in one patient (5%).

In consistent with these results, Attwa et al. have reported excellent response in 3 patients (15%) after a total of 60 sessions, good response in 5 patients (25%), moderate response in 6 patients (30%) and poor response in 6 patients (30%). Similarly, El-Mofty et al. have reported that nearly 14% of vitiligo patients showed excellent or good response after 50 sessions of NB-UVB therapy and that number increased to nearly 54% after 70 sessions. Slight differences among these studies can be attributed to variable number of sessions.

Side effects of NB-UVB phototherapy were reported in 6 patients (30%) including sunburn-like reaction (10%), erythema (10%) and xerosis (10%). Similarly, Attwa et al. have reported side effects in 5 of their patients (25 %); including mild erythema (15%) and pruritus (10%). These results indicate effectiveness and safety of NB-UVB phototherapy in the treatment of vitiligo.

Melanocytes form a functional unit with neighbouring keratinocytes. While melanocytes deliver melanin to keratinocytes to protect their DNA from UV-induced damage, keratinocytes release several factors that promote survival, proliferation and functions of melanocytes. Moreover, adhesion of melanocytes to adjacent keratinocytes through adhesion molecules, such as E-cadherins, is essential for melanocyte survival. Therefore, abnormalities in keratinocytes may cause loss of melanocytes in vitiligo patients. There is growing evidence that keratinocytes in vitiliginous skin undergo apoptosis that may be due to inflammatory cytokines or oxidative stress.

In this study, histopathological examination of both lesional and perilesional skin revealed lymphocytic infiltrate along the basal layer and in perivascular distribution in (50%) of patients. Hypomelanotic lesions with poorly defined borders (corresponding to active lesions) showed progressive depigmentation in (86%) of patients, epidermal spongiosis with vacuolar degeneration of basal keratinocytes (89%) and clustered CD8+ T cell infiltrate in...
epidermis (89%). On the other hand, the amelanotic lesions with sharply demarcated borders (corresponding to stable lesions) showed progressive depigmentation in only 14%, epidermal spongiosis (14%) and clustered lymphocytic infiltrate in only 5%. In this study, one patient (5%) showed lymphocytes invading a hair follicle in lesional skin. Similarly, focal lichenoid infiltrate surrounding epidermal & follicular melanocytes was detected in evolving vitiliginous lesions. These results indicate affection of keratinocytes in skin of vitiligo patients that may contribute into the pathogenesis of the disease. Moreover, extension of inflammatory changes to apparently normal perilesional skin indicates importance of using therapeutic modalities that have access to pigmented perilesional skin in order to prevent disease progression. NB-UVB phototherapy seems to be a useful modality that may help to achieve this purpose.

In this study, histopathological examination of lesional skin revealed reappearance of melanocytes and melanin pigment in the epidermis with variable degrees in almost all patients (95%) after NB-UVB therapy. Moreover, inflammatory changes significantly subsided in both lesional and perilesional skin in (30%) of patients after therapy. Attwa et al. have also reported similar improvement in histopathological picture of both lesional and perilesional skin after NB-UVB therapy. These results are consistent with other studies that stated that the beneficial effect of NB-UVB in treatment of vitiligo may be attributed to its immunosuppressive properties and its stimulatory effects on melanocyte proliferation and melanogenesis. The immunosuppressive effects of NB-UVB may result from inhibition of pro-inflammatory cytokines and up-regulation of the anti-inflammatory cytokine IL10. Moreover, it causes depletion of Langerhan's cells and apoptosis of T cells through causing DNA damage and stimulation of the pro-apoptotic enzymes, caspases. Stimulationary effects of NB-UVB on melanocyte functions may be due to keratinocyte-derived growth factors such as α-melanocyte stimulating hormone, stem cell factor, endothelins and eicosanoids. Moreover, expression of matrix metalloproteinase-2 is up-regulated leading to activation of melanocyte migration.

LIMITATION
The limitation of this study was that a small number of patients was included. Further large-scaled studies are needed. Other investigatory techniques may be used to evaluate abnormalities of keratinocytes in vitiligo skin.

CONCLUSION
In conclusion, the exact pathogenesis of vitiligo is still not completely understood. Abnormalities of surrounding keratinocytes may contribute to loss of melanocytes in vitiligo patients due to deprivation of keratinocyte-derived growth factors. Further studies are needed to identify these abnormalities and their causes. Extension of pathological changes to apparently normal perilesional skin necessitates use of lines of treatment that reach it. NB-UVB phototherapy is an effective and safe therapeutic modality that can achieve this purpose.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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