# Tacrolimus versus narrowband ultraviolet b in the treatment of vitiligo: a clinical and laboratory evaluation

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**Background**
Etiopathogenesis of vitiligo is unclear. Immunological background has a role. Interleukin 17 (IL-17) may play a role in its pathogenesis. There is no standard treatment regimen for vitiligo; however, calcineurin inhibitors may be an effective treatment option.
**Objective**
The aim was to evaluate tacrolimus versus narrowband ultraviolet B (NB-UVB) in treatment of vitiligo, both clinically and by measurement of IL-17 before and after treatment.
**Patients and methods**
A total of 45 patients with generalized vitiligo were included in the study. Overall, 21 patients were treated with NB-UVB, whereas 24 were treated with tacrolimus for 3 months. Vitiligo area scoring index (VASI) and IL-17 were measured before and after treatment.
**Results**
VASI was significantly improved after both treatments ($P<0.001$ for both groups). IL-17 was significantly decreased after treatment in both study groups ($P<0.001$ for both groups). IL-17 was significantly correlated with VASI.
**Conclusion**
NB-UVB and tacrolimus are both effective in the treatment of vitiligo and in decreasing IL-17. IL-17 may play a role in pathogenesis of vitiligo as its level was decreased significantly after treatment.

**Keywords:**
interleukin 17, narrowband ultraviolet B, tacrolimus, vitiligo

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## Introduction

Vitiligo is a common disorder of pigmentation affecting skin and mucous membrane. Its prevalence is ~1–2% worldwide, with no sex or age predilection [1,2]. The exact etiopathogenesis of vitiligo is not completely understood. Both genetic and acquired factors are supposed to play a role [3]. Theories suggesting destruction of melanocytes such as autoimmune mechanisms and cytotoxic mechanisms are the most acceptable [4].

There are some reports proposing a role of inflammatory cytokines in induction and progression of vitiligo [5,6]. A complex role for a variety of cytokine interactions between keratinocytes, T lymphocytes, and melanocytes was found [7].

The main lymphocytes included in vitiligo pathogenesis are T helper cells (Th), which include four subsets: Th1, Th2, Th17, and regulatory T cells (Tregs). Th1 cells produce interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα). Th2 cells secrete mainly interleukins (IL) such as IL-4, IL-5, and IL-13. Tregs synthesize IL-10 and transforming growth factor-β [8].

The increased frequencies of IFNγ and IL-17/IL-22-producing T cells observed in vitiliginous skin may be owing to production of IL-12 and IL-23 by inflammatory dendritic cells (DCs) [9].

Th17 cells are considered as one of the major pathogenic cell populations underlying the development of many autoimmune diseases, and they are enhanced and stabilized by IL-23 [10]. Thl7 cells produce IL-17, IL-6, IL-21, IL-22, and TNFα and also stimulate keratinocyte release of IL-la, IL-6, and TNFα [11]. Many studies found higher levels of IL-17 in patients with autoimmune and inflammatory diseases such as psoriasis vitiligo than normal participants [12].

Numerous treatment modalities have been used to treat vitiligo, such as corticosteroids, topical immunomodulatory drugs, phototherapy, skin grafts, and camouflage [13]. Narrowband ultraviolet B



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(NB-UVB) phototherapy, targeted ultraviolet B phototherapy, and excimer laser are the most widely used phototherapy for vitiligo nowadays [14]. The efficacy and safety observed with NB-UVB have helped it to replace Psoralen and ultraviolet rays A as the treatment of choice in generalized vitiligo [15].

The exact mechanism of action of NB-UVB in vitiligo is unknown. NB-UVB downregulates the immune attack against the melanocytes, resulting in stimulation of melanocytes to migrate to epidermis and synthesize melanin [16]. It causes a reduction of T cells by inducing apoptosis. NB-UVB impairs in-vitro antigen presentation by both murine DCs and human Langerhans cells [17].

Calcineurin inhibitors, including pimecrolimus and tacrolimus, inhibit the production of T cells and prevent mast cells from releasing proinflammatory mediators [18]. They inhibit calcineurin, resulting in the suppression of proinflammatory cytokine secretion by activated T cells, specifically that of IFNγ, IL-1, IL-2, IL-3, IL-4, IL-5, granulocyte macrophage colony-stimulating factor, and TNFα11,12, which is responsible for the damage of melanocytes, leading to vitiligo [19].

Tacrolimus was found to inhibit expression of IL-17 or TNFα by reducing the proportion of Th17, suggesting the therapeutic effect on Th17-associated diseases such as inflammatory bowel disease, psoriasis, or vitiligo [20].

The aim of this study was to evaluate the efficacy of tacrolimus versus NB-UVB in the treatment of vitiligo both by clinical evaluation and measurement of IL-17.

## Patients and methods

### Patients

This study is a quasiexperimental study (difference in differences – pretreatment and post-treatment comparisons). A total of 50 patients with vitiligo were recruited in this study. They were selected from patients attending the Dermatology Outpatient Clinic, Aswan University, in the period from July 2015 till June 2016. Patients were randomized into two groups, 26 patients who were treated with NB-UVB phototherapy and 24 patients were treated with tacrolimus ointment. Five patients did not complete the study owing to personal reasons, so the final number of patients is 45, with 21 in the first group and 24 in the second group.

Patients who received any topical treatment, systemic treatment, or phototherapy in the previous 6 months; patients with a history of photosensitivity or administration of a drug known frequently to cause photosensitization; patients who showed previous failure owing to intolerance to phototherapy (>100 sessions of phototherapy in the patient's lifetime); patients with epilepsy; pregnant and breast feeding patients; patients with chronic diseases; and patients with autoimmune diseases are excluded from this study.

The study was approved by the Aswan Medical College Institutional Review Board and was carried out according to the principles outlined in the Declaration of Helsinki. All participants signed written informed consent before entering the study. Confidentiality was assured for all participants.

### Methods

Patients included in the study were subjected to complete history taking, complete dermatological examination, and investigation to exclude any other associated immune disease, random blood sugar, complete blood picture analysis, and IL-17 measurement before and after treatment. Vitiligo lesions were examined by an investigator, blinded to the study, before and after treatments. Percentage of affected area was calculated according to vitiligo area scoring index (VASI) [21]. Any adverse effect after treatment was detected.

### Interleukin 17 measurement

IL-17 was measured by enzyme-linked immunosorbent assay technique using Bender MedSystems GmbH (Vienna, Austria). Overall, 5 ml of venous blood was collected in a plain tube from each participant. After clotting, the samples were centrifuged at 3500 rpm for 10 min and then were frozen at −20°C for later use for estimation of IL-17 by enzyme-linked immunosorbent assay technique.

All the microwells were washed at first with washing buffer 400 µl twice before sample addition. Standards were prepared by serial dilution of the vial of standard concentrate in the wells as follows: 100 µl of the sample diluent was added in the first six wells starting from the second well, and then 100 µl of the prepared standard concentrate was placed in the first and second wells.

Repeated mixing of the contents of the second well was done, then 100 µl from the second well was transferred to the third well and mixed well, then

100 µl from the third was aspirated and transferred to fourth well and mixed, and so on till the seventh well, where 100 µl from the seventh well was discarded. That is the standard concentration serial dilution (200, 100, 50, 25, 12.5, 6.25, 3.12 pg/ml). Then 50 µl of the sample diluent was added in the wells assigned for samples. Then 50 µl of the samples was added in sample wells. Then 50 µl of the Biotin conjugate was added into all wells. Then the plate was covered and incubated at room temperature for 2 h. After 2 h, the washing step was done by using the diluted washing reagent (washing concentrate has been included in kit) for five times by repeated aspiration and addition of the washing reagent.

### Treatment

*Narrowband ultraviolet B treatment*

Each patient in the first group received NB-UBV sessions three times weekly on nonconsecutive days for 3 months with eight narrowband UVB lamp (TL01) of Waldmann type F 85/100W-01 (Waldmann, Villingen-Schwenningen, Germany). The initial dose was 0.25 J/cm$^2$. Then the dose was increased at each session by 10–20% with maximum dose of 5 J/cm$^2$. In cases of erythema, pain, or blistering, we temporarily stopped treatment or decreased the dose by 20%.

### Tacrolimus treatment

Each patient in the second group applied topical tacrolimus 0.03% ointment twice daily on the affected area according to the role of fingertip unit for 3 months. A thin layer was applied on the affected area by the patient.

### Statistical analysis

The collected data were verified and coded by the researcher and analyzed by using SPSS/PC, version 21 (IBM Co., Armonk, New York, USA). Descriptive statistics such as mean, SD, frequencies, and percentage were calculated. Test of significances such as $\chi^2$-test was used to compare the difference in distribution of frequencies among different groups. One-way analysis of variance was calculated to test the mean differences in continuous variables between groups. A significant *P* value was considered when it is less than or equal to 0.05.

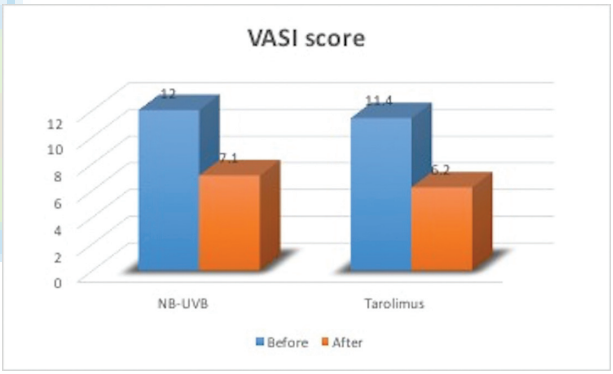
## Results

This study included 45 patients with generalized vitiligo, with average age ranging from 12 to 60 years, and the mean age was 34.9±17.1 years. Overall, 11 (36.7%) of them were males and 19 (63.3%) were females (Table 1). The first group was subjected to NB-UVB phototherapy (46.7%), and the second was treated by tacrolimus (53.3%). There was a nonsignificant difference regarding age and sex distribution between the study groups (*P*=0.365 and 0.317, respectively) (Table 1).

The mean value of VASI at baseline in group I was 12.0±3.2, and was significantly decreased after treatments to reach 7.1±2.9, with *P* value of less than 0.001. The mean value of level of IL-17 at baseline was 33.2±9.5, which significantly decreased after treatment to 28.9±6.9, with *P*=0.019 (Table 1, Figs. 1 and 2).

The VASI in group II was 11.4±3.1 and was significantly decreased to reach 6.2±2.4 after treatment, with *P* value of less than 0.001. IL-17 level was 35.4±12.6 before treatment and significantly decreased to 28.6±5.1 after treatment, with *P* value of less than 0.001 (Table 1, Figs. 1 and 2).

**Figure 1**



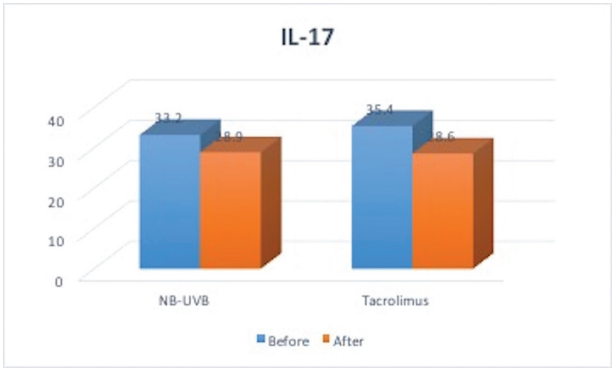

Vitiligo area scoring index score before and after treatment in both study groups.

**Table 1 Comparative analysis of the studied groups (narrowband ultraviolet B vs. tacrolimus)**

| | NB-UVB group (*n*=21) | Tacrolimus group (*n*=24) | *P* value |
|---|---|---|---|
| Age (mean±SD) (years) | 37.9±16.6 | 32.2±17.5 | 0.365* |
| Sex [*n* (%)] | | | |
| Male | 7 (33.3) | 9 (37.5) | 0.317** |
| Female | 14 (66.7) | 15 (62.5) | |
| VASI at baseline | 12.0±3.2 | 11.4±3.1 | 0.682* |
| VASI after treatment | 7.1±2.9 | 6.2±2.4 | 0.281* |
| IL-17 level at Baseline | 33.2±9.5 | 35.4±12.6 | 0.592* |
| IL-17 level after treatment | 28.9±6.9 | 28.6±5.1 | 0.891* |

IL-17, interleukin 17; NB-UVB, narrowband ultraviolet B; VASI, vitiligo area scoring index; *Mann–Whitney *U*-test was used to compare the difference in mean between the two groups; **$\chi^2$-analysis was used to compare the difference in proportions; $P \leq 0.05$, significant.

**Figure 2**




Interleukin 17 level before and after treatment in both study groups.

In comparison between the two study groups, there was a nonsignificant difference in VASI score either before or after treatment, with $P$=0.682 and 0.281, respectively. The level of IL-17 showed a nonsignificant difference between the two study groups either before or after treatments, with $P$=0.508 and 0.804, respectively (Table 2).

**Table 2 Comparison between vitiligo area scoring index and interleukin 17 levels at presentation and after treatment**

| | Baseline | After treatment | *P* value* |
|---|---|---|---|
| NB-UVB group | | | |
| VASI | 12.0±3.2 | 7.1±2.9 | <0.001 |
| IL-17 | 33.2±9.5 | 28.9±6.9 | 0.019 |
| Tacrolimus group | | | |
| VASI | 11.4±3.1 | 6.2±2.4 | <0.001 |
| IL-17 | 35.4±12.7 | 28.6±5.1 | 0.011 |

IL-17, interleukin 17; NB-UVB, narrowband ultraviolet B; VASI, vitiligo area scoring index; *Wilcoxon signed rank test was used to compare the mean difference between the two interval readings; $P$<0.05, significant.

## Discussion

Vitiligo is a multifactorial disorder with a complex pathogenesis based on both genetic and nongenetic factors [1,22]. A significant increase in the expression of variable inflammatory cytokines in patients with vitiligo has been proven. These cytokines have been proposed to play a role in the induction and maintenance of vitiligo [4,5,23]. Thl7 cells produce IL-17, IL-6, IL-21, IL-22, and TNFα and also stimulate keratinocyte release of IL-la, IL-6, and TNFα [5]. IL-la, IL-6, and TNFα inhibit melanocyte proliferation [24]. There is a growing evidence that these cytokines play a role in autoimmune diseases and may have a role in pathogenesis of vitiligo [7,10,11]. IL-17 is produced by Th17 cells. It is a disulfide-linked homodimeric glycoprotein consisting of 155 amino acids with a molecular weight of 35 kDa [24].

In this study, we evaluated the efficacy of tacrolimus versus NB-UVB treatment in vitiligo according to VASI score and serum IL-17 levels.

VSAI has been significantly decreased after treatment with both NB-UVB and tacrolimus. Our results agree with the pervious evidences about the efficacy of NB-UVB in vitiligo [16,17]. In their meta-analysis, Li *et al.* [25] found that adding topical calcineurin inhibitors and vitamin D3 analogs to NB-UVB did not increase treatment outcome except in face and neck lesions.

We found a significant improvement in VASI score after tacrolimus. These findings concede with previous reports about the efficacy of tacrolimus on vitiligo [19]. Pimecrolimus 1% was found to be not effective in the treatment of symmetrical vitiligo [20].

We found high IL-17 levels in patients with vitiligo than normal individuals. This agreed with the previous reports that detected higher levels of IL-17 in patients with vitiligo [26,27]. Serum levels of IL-1, IL-6, and granulocyte macrophage colony-stimulating factor were found to be significantly higher in patients with generalized vitiligo compared with normal controls, suggesting that these cytokines may be involved in the autoimmune mechanism of nonsegmental vitiligo. Moreover, IL-17 itself synergizes with these local inflammatory mediators, which may cause further inhibition of melanocyte proliferation [4,26,27].IL-17 levels were significantly decreased after treatment by either NB-UVB or tacrolimus.

In vitiligo, NB-UVB suppresses IL-17 and IL-22 mRNAs, which strongly correlate with lesion resolution [28]. Moreover, NB-UVB phototherapy lowers peripheral natural killer cell activity, lymphocyte proliferation, and immune regulatory cytokine production by both Th1 (IL-2 and IFNγ) and Th2 (IL-10) T-cell populations [29]. NB-UVB inhibits the local innate inflammatory response to double-stranded RNA (dsRNA) receptors, suggesting a novel mechanism of action of NB-UVB phototherapy in vitiligo [30].

Tacrolimus has been shown to have immuno-modulatory effects by inhibiting activation and maturation of T cells and production of cytokines such as IL-2, IL-4, IL-5, IL-6, TNFα, and IFNγ, so it acts synergistically on IL-23 and so on IL-17 release by Th17 [19].

Many studies have investigated the role of IL-17 in vitiligo pathogenesis. Claire *et al.* [26] postulated that Th17 cells and activated DCs are increased in vitiligo lesions. IL-17, IL-22, and FoxP3 expression have been found to be elevated in tissue and serum of nonsegmental vitiligo [2].

We concluded that both tacrolimus and NB-UVB are effective treatments in patients with vitiligo for the same duration of treatment. VASI score was decreased significantly at the end of both treatments. IL-17 was significantly decreased after treatment by both tacrolimus and NB-UVB. Further studies with a larger sample size and duration of treatment are needed to support our findings.

### Study limitations

The small sample number, lack of control, and short follow-up duration are the main limitations of this work.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1 Ortonne J. Vitiligo and other disorders of hypopigmentation. In: Bolognia J, Jorizzo J, Rapini R, editors. Dermatology. 2nd ed. Spain: Elsevier. 2008. 21:65.

2 Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res 2003; 16:208–214.

3 Zhang XJ, Chen JJ, Liu JB. The genetic concept of vitiligo. J Dermatol Sci 2005; 39:137–146.

4 Le Poole IC, Luiten RM. Autoimmune etiology of generalized vitiligo. Curr Dir Autoimmun 2008; 10:227–243.

5 Basak PY, Adiloglu AK, Ceyhan AM, Tas T, Akkaya VB. The role of helper and regulatory T cells in the pathogenesis of vitiligo. J Am Acad Dermatol 2009; 60:256–260.

6 Rateb AAH, Azzam OA, Rashed LA, El-Guindy NM, Salah El-Din M. The role of nerve growth factor in the pathogenesis of vitiligo. JEWDS 2005; 1:1824.

7 Lambe T, Leung JC, Bouriez-Jones T, Silver K, Makinen K, Crockford TL, *et al.* CD4 T cell-dependent autoimmunity against a melanocyte neo antigen induces spontaneous vitiligo and depends upon Fas-Fas ligand interactions. J Immunol 2006; 177:3055–3062.

8 Levings MK, Bacchetta R, Schulz U, Roncarolo MG. The role of IL-10 and TGF-β in the differentiation and effector function of T regulatory cells. Int Arch Allergy Immunol 2003; 129:263–276.

9 Guttman-Yassky E, Vugmeyster Y, Lowes MA, Chamian F, Kikuchi T, Kagen M, *et al.* Blockade of CD11a by efalizumab in psoriasis patients induces a unique state of T-cell hyporesponsiveness. J Invest Dermatol 2008; 128:1182–1191.

10 Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem 2003; 278:1910–1914.

11 Langrish CL, McKenzie BS, Wilson NJ, de Waal Malefyt R, Kastelein RA, Cua DJ. IL-12 and IL-23: master regulators of innate and adaptive immunity. Immunol Rev 2004; 202:96–105.

12 Bassiouny DA, Shaker O. Role of interleukin-17 in the pathogenesis of vitiligo. Clin Exp Dermatol 2011; 36:292–297.

13 Nordland J, Halder R, Grime S. Management of vitiligo. Dermatol Clin 1993; 11:27–33.

14 Majid I. Vitiligo management: an update. Br J Med Pract 2010; 3:332.

15 Bhatnagar A, Kanwar AJ, Parsad D. Comparison of systemic PUVA and NB-UVB in the treatment of vitiligo: an open prospective study. J Eur Acad Dermatol Venereol 2007; 21:638–642.

16 Parsad D, Bhatnagar A, De Dipankar M. Narrowband ultraviolet B for the treatment of vitiligo. Expert Rev Dermatol 2010; 5:445–459.

17 Carrascosa JM, Tapia G, Bielsa I, Fuente MJ, Ferrandiz C. Effects of narrowband UV-B on pharmacodynamic markers of response to therapy: an immunohistochemical study over sequential samples. J Cutan Pathol 2007; 34:769–776.

18 Seifari H, Farnaghi F, Firooz A, Vasheghani-Farahani A, Alirezaie N-S., Dowlati Y. Pimecrolimus cream in repigmentation of vitiligo. Dermatology 2007; 214:253–259.

19 Dawid M, Veensalu M, Grassberger M, Wolff K. Efficacy and safety of pimecrolimus cream 1% in adult patients with vitiligo: results of randomized, double-blind, vehicle-controlled study. J Dtsch Dermatol Ges 2006; 4: 942–946.

20 Yago T, Nanke Y, Kawamoto M, Yamanaka H, Kotake S. Tacrolimus potently inhibits human osteoclast genesis induced by IL-17 from human monocytes alone and suppresses human Th17 differentiation. Cytokine 2012; 59:252–257.

21 Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: The Vitiligo Area Scoring Index. Arch Dermatol 2004; 140:677–683.

22 Ratsep R, Kingo K, Karelson M, Reimann E, Raud K, Silm H, *et al.* Gene expression study of IL10 family genes in vitiligo skin biopsies, peripheral blood mononuclear cells and sera. Br J Dermatol 2008; 159:1275–1281.

23 Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. J Leukoc Biol 2002; 71:l–8.

24 Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 2008; 8:337–348.

25 Li R, Qiao M, Wang X, Zhao X, Sun Q. Effect of narrow band ultraviolet B phototherapy as monotherapy or combination therapy for vitiligo: a meta-analysis. Photodermatol Photoimmunol Photomed 2017; 33:22–31.

26 Claire QF, Wang CQ, Andres E, Moussai D, Gulati N, Sullivan-Whalen M, *et al.* Th17 cells and activated dendritic cells are increased in vitiligo lesions. PLoS One 2011; 6:e18907.

27 Elela MA, Hegazy RA, Fawzy MM, Rashed LA, Rasheed H. Interleukin 17, interleukin 22 and FoxP3 expression in tissue and serum of non-segmental vitiligo. Eur J Dermatol 2013; 23:350–355.

28 Johnson-Huang LM, Suárez-Fariñas M, Sullivan-Whalen M, Gilleaudeau P, Krueger JG, Lowes MA. Effective narrow-band UVB radiation therapy suppresses the IL-23/IL-17 axis in normalized psoriasis plaques. J Invest Dermatol 2010; 130:2654–2663.

29 Rai R, Srinivas CR. Phototherapy: an Indian perceptive. Indian J Dermatol 2007; 52:169–175.

30 Rácz E, Prens EP, Kant M, Florencia E, Jaspers NGJ, Laman JD. Narrowband ultraviolet B inhibits innate cytosolic double-stranded RNA receptors in psoriatic skin and keratinocytes. Br J Dermatol 2011; 164: 838–847.