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Evaluation Of Serum Il-23 In Active, Stable And Narrow Band Uvb Treated Vitiligo

Omar Al-Mashaykhi[1] , Mohammed Al-Daraji*[2]

1-Dermatology department – Ain Shams University, Cairo, Egypt.

2-Dermatology Unit, Department of Medical Laser Applications, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt,

Abstract

Background: IL-23 is a pro inflammatory cytokine and plays a vital role in many inflammatory diseases and cancers, biologics targeting IL-23 have been tried in many diseases.

Objectives: the aim of this study was to evaluate serum levels of IL-23 in vitiligo patients also to determine its possible relation to disease activity, body surface area and duration.

Subjects and methods: this study was a case control study which was carried out on 40 patients diagnosed as non-segmental vitiligo and 30 age and sex matched healthy controls, all patients were subjected to 40 NB-UVB sessions (3 sessions /week) and the serum level of IL-23 was measured by ELISA before and after treatment.

Results: serum IL-23 before and after treatment by NB-UVB among active vitiligo patients showed a high significant reduction ($p<0.01$), the same results was present comparing active vitiligo cases with the controls, a significant correlation was found between serum IL-23 in all cases before NB-UVB treatment and body surface area ($p<0.05$). **Conclusions:** alteration of serum IL-23 may play an important role in the immune-pathogenesis of non-segmental vitiligo, serum IL-23 may be useful as a marker of activity in non-segmental vitiligo as well as therapeutic response marker.

Keywords: Interleukin-23, Vitiligo, VETI score, NB-UVB.

Introduction:

Vitiligo is a common, specific, often heritable, acquired disorder characterized by well circumscribed milky white cutaneous macules devoid of identifiable functional melanocytes in the epidermis due to multifactorial and overlapping pathogenic mechanisms[1]

The pathogenesis of vitiligo is complex, the exact pathogenesis is not well known. It is a multifactorial disease involving the interplay of several factors **[2]**. Prevalent hypotheses include the autoimmune, genetic, neural, self-destruction, growth factor deficiency, viral, and convergence theories, which have served as the basis for treatment formulations **[3].**

The main clinical feature of the disease is the presence of white macules of different shapes and sizes which are symmetric or asymmetric over the body **[2].**

The involvement of Th-17 cells in the pathogeneses of vitiligo has been suggested by many authors, who found increased serum levels of the cytokines produced by Th-17 cells. **[4]**IL-17 and IL-22 seem significantly associated with vitiligo **[5].** High levels of IL-17 have been observed in lesional skin and serum of patients affected by the disease **[6].** IL-17 imbalance suggests its involvement in the pathogenesis of vitiligo and supports the hypothesis that skewing of the immune system toward Th-1 or Th-17 confirm the autoimmune nature of vitiligo **[7].**

IL-23 induces the differentiation of Th-17 cells in a pro inflammatory context, especially in the presence of transforming growth factor (TGF)-b and interleukin-6 (IL-6) **[8]**. IL-23 receptors are expressed by inflammatory macrophages, which are activated by an unknown mechanism to produce IL-1, TNF-a, and IL-23. IL-23 Seems to have a central role in autoimmunity **[9].**

Narrow-bandultra violet B radiation (NB-UVB) therapy offers one of the most effective treatment modalities for vitiligo but the mechanism of action of NB-UVBisnot well understoodfollicle [10]. The NB-UVB therapy induces perifollicula rpigmentation suggesting that it influence melanocyte reserve in theouter root sheath of hair follicle [10]. However, a two step effectof NB-UVB has been suggested but may also occur simultaneously [11].

Clinical trials for anti Il-23 and IL-17 have been tried in many diseases with different levels of success rate example: Psoriasis, Psoriatic arthritis, Crohn's disease, Ankylosing spondylitis, Rheumatoid arthritis, Multiple sclerosis, Graft-versus-host disease, Atopic dermatitis, Hidradenitis suppurativa, Primary biliary cirrhosis, Sarcoidosis, Systemic lupus erythematosus, Behçet's disease, Asthma and Type 1 diabetes **[12].**However, to our knowledge no study involved anti IL-23 in vitiligo till now.

The effect of NB-UVB on T helper and Treg cytokines in active vitiligo has not been studied widely and the purpose of this study was to evaluate serum IL-23 levels in active, stable and NB-UVB treated vitiligo patients

Materials and methods:

This case control study included 40 patients presented with vitiligo (active-stable), diagnosed on the basis of typical clinical features, were selected as patient group. Thirty age and sex matched apparently healthy individuals were also included representing the control group. All patients were selected from the dermatology outpatient clinic of vitiligo, Ain Shams University Hospital in the period from May 2019 till June 2020. Informed verbal consents were taken before inclusion of the patients into the study according to Ethical Committee of Scientific Research.

Patients with clinical diagnosis of non-segmental vitiligo according to clinical picture and wood's light examination with age range was 12-65 years and both sexes were included.

Patients with autoimmune diseases e.g. hypo and hyperthyroidism, autoimmune thyroiditis, type 1 diabetes mellitus, pernicious anemia, rheumatoid arthritis and systemic lupus erythromatosus. or any other dermatological disease that cause abnormal pigmentation. Pregnancy or lactation. Patients with phototoxic reactions related to phototherapy or photosensitivity or photomediated disorders as albinism, or dermatomyositis**,** also patients

with previous skin cancer or pre malignant skin lesions or taking immunosuppressive drugs such as methotrexate. Age below 12 years or above 65 years were excluded.
Healthy individuals with no symptoms or signs of vitiligo and were selected from the working staff of Ain Shams University hospitalas a control group.
Patients were divided into two groups A and B (20 patients each) as follows:
Group A: active N.S. vitiligo.
Group B: stable N.S. vitiligo**.**
Group C: (control) 30 healthy age and sex matched subjects.
All patients were subjected to 40 NB-UVB sessions (3 sessions /week) and the serum level of IL-23 was measured by ELISA before and after treatment.

Phototherapy:
The machine used was UV-100L Waldman (Germany) lighting and was equipped with UVB lamps (TL01 lamp) which have physical irradiance values of 7-10 mW / cm^2 and biological effective (erythematous) irradiance 0.4-0.6 mW / cm^2. The initial dose was 0.25 J/cm^2 as vitiliginous skin is similar to type 1 skin, it is natural to believe that the dosimetry should have been uniform irrespective of the skin phototype of the patient**.** The dose was increased at each session by 10%-20% with maximum dose of 5 J/cm^2. When symptomatic erythema, burning pain or blistering developed, the irradiation dose was decreased by 20% or the session was skipped **[13].**

Specimen collection and sampling:
The detection method was based on enzyme-linked immunosorbent assay (ELISA) (Human IL-23 ELISA Ready-SET-Go!) Catalog Number: 88-7237.Sensitivity: 10pg/mL. Standard Curve Range: 10pg/ml. As 20 blood samples from the active vitiligo group before NB- UVB treatment, 20 blood samples from the stable vitiligo cases, And another 20 blood samples from the active group (group A) after NB UVB treatment by 40 NB-UVB sessions 3times/week. With 30 blood samples from matched healthy controls.
Five ml of whole blood was collected in ethylene diamine tetra-acetic acid (EDTA) containing tubes from every participant in all groups. The sample was allowed for clotting then centrifuged at 3000 rpm and the plasma separated within <1 h from sample collection and then stored at –20°C until ELISA investigations. The 90 stored plasma samples were allowed to reach room temperature (20–25°C) once before analysis, to avoid repeated melting/freeze cycles.

Clinical assessment of Vitiligo:
Patients with vitiligo were assessed according to vitiligo extension tensity index **(VETI)** scoring system **[14].**

Statistical Analysis:
The collected data was revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science **(SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001).** Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Results:

Comparison between levels of s.IL-23 before and after NB-UVB treatment in all patients.
Before NB-UVB treatment the level of serum IL-23 in all patients ranged from 10 – 190 pg/ml (mean 57.47 ± 36.84) pg/ml while the level after NB-UVB treatment ranged from 20-80 pg/ml (mean of 53.65 ±18.48) pg/ml.
Statistical comparison revealed that the difference was not significant (P=0.388) **(Table 1).**

Table (1): Comparison between levels of S.IL-23 before and after NB-UVB treatment in all patients (active +stable).

Patients	S. IL.23 (PG/mL)		Independent t-test	
	Mean ± SD	Range	t-test	P-value
Before	57.47 ± 36.84	10 – 190	0.863	0.388 *
After	53.65 ±18.48	20 – 80		

Independent t-test *:non significant

Comparison between the patient group (active + stable) and control group before NB-UVB treatment as regards S. IL-23 level
The level of serum IL-23 in all patients (active + stable) vitiligo before NB-UVB treatment ranged from 10 – 190 pg/ml (mean 57.47 ± 36.84) pg/ml while in healthy controls ranged from 10-88 pg/ml (mean 35.50 ± 20.21) pg/ml.

Comparison revealed that the difference was significant (P=0.005) **(Table 2).**

Table (2): Comparison between the patient group (active + stable) and control group before NB-UVB treatment as regards S. IL-23 level.

Groups	S. IL.23 (PG/mL)		Independent t-test	
	Mean ± SD	Range	T	P-value
Active + Stable	57.47 ± 36.84	10 – 190	2.934	0.005 *
Control	35.50 ± 20.21	0 – 88		

Independent t-test *highly significant

Comparison between patients with active and stable vitiligo as regards serum IL-23 level before NB:UVB

The level of serum IL-23 in patients with active vitiligo before NB-UVB treatment ranged from 35 – 190 pg/ml (mean76.39 ± 37.55) pg/ml while in stable vitiligo ranged from 10- 112 pg/ml (mean of 40.45 ±27.14) pg/ml.

Comparison revealed that the difference was significant (P=0.002) **(Table 3)**

Table (3): Comparison between patients with active and stable vitiligo as regards serum IL-23 level before NB:UVB.

Activity	S. IL.23 (PG/mL)		Independent t-test	
	Mean ±SD	Range	t-test	P-value
Active	76.39 ± 37.55	35 –190	3.406	0.002 *
Stable	40.45 ±27.14	10 –112		

Independent t-test * significant

Comparison between levels of S. IL-23 before and after NB-UVB treatment in active vitiligo group

The level of serum IL-23 in patients with active vitiligo before NB-UVB treatment ranged from **35 – 190** pg/ml (mean**76.39 ± 37.55**)pg/ml while after treatment ranged from 20-80 pg/ml (mean **53.65 ±18.48**) pg/ml.

Comparison revealed that the difference was a significant (P=0.021) **(Table 4).**

Table(4): Comparison between levels of S. IL-23 before and after NB-UVB treatment inactive vitiligo group.

Active group	S. IL.23 (PG/mL)		Independent t-test	
	Mean ±SD	Range	t-test	P-value
Before	76.39 ± 37.55	35 –190	2.406	0.021*
After	53.65 ±18.48	20 –80		

Independent t-test * significant

Comparison between the levels of s.IL-23 in stable and NB-UVB treated vitiligo

Before NB-UVB treatment the level of serum IL-23 in patients with stable vitiligo ranged

from10 –112 pg/ml (mean40.45 ±27.14) pg/ml while after NB-UVB treatment ranged from 20-80 pg/ml (mean 53.65 ±18.48) pg/ml.

Comparison revealed that the difference was not significant (P=0.080) **(Table5).**

Table(5): Comparison between the levels of S. IL-23 in stable and NB-UVB treated vitiligo.

Groups	S. IL.23 PG/mL		Independent t-test	
	Mean ±SD	Range	t-test	P-value
Stable	40.45 ±27.14	10 –112	1.798	0.080*
Treated	53.65 ±18.48	20 –80		

Independent t-test *non significant.

Comparison between patients with active vitiligo and controls as regards serum IL-23 level before NB-UVB treatment

The level of serum IL-23 in patients with active vitiligo before NB-UVB treatment ranged from 35 –190 pg/ml (mean76.39 ± 37.55) pg/ml while in controls ranged from 10-88 pg/ml (mean 35.50 ± 20.21)pg/ml .Comparison revealed that the difference was highly significant (P=0.00) **(Table 6)**.

Table (6): Comparison between patients with active vitiligo and controls as regards serum IL-23 level before NB-UVB treatment.

Groups	S. IL.23PG/mL		Independent t-test	
	Mean ± SD	Range	t-test	P-value
Control	35.50 ± 20.21	10 – 88	4.915	0.000*
Active	76.39 ± 37.55	35 – 190		

Independent t-test *highly significant.

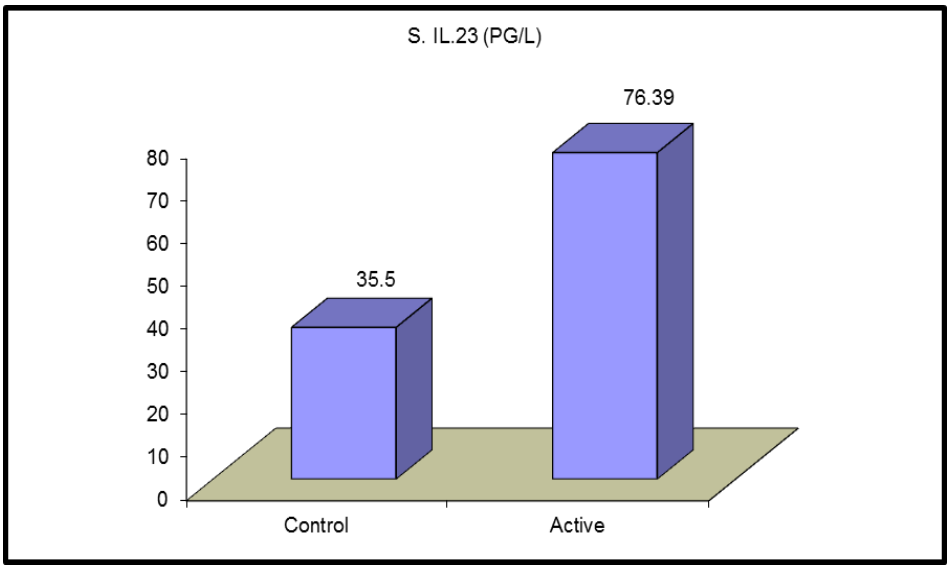

Figure (1): Comparison between patients with active vitiligo and controls as regards serum IL-23 level before NB:UVB treatment.

Comparison between patients with stable vitiligo and controls as regards serum IL-23 level

The level of serum IL-23 in patients with stable vitiligo ranged from 10 – 112 pg/ml (mean 40.45 ± 27.14) pg/ml while in controls ranged from 10-88 pg/ml (mean 35.50±20.21) pg/ml.

Comparison revealed that the difference was not significant (P=0.463) **(Table 7).**

Table (7): Comparison between patients with stable vitiligo and controls as regards serum IL-23level.

Groups	S. IL.23PG/mL		Independent t-test	
	Mean ± SD	Range	t-test	P-value
Control	35.50 ± 20.21	10 – 88	0.739	0.463*
Stable	40.45 ± 27.14	10 – 112		

Independent t-test *non significant.

Comparison between the levels of s.IL-23 in active vitiligo after NB-UVB treatment and controls

The level of serum IL-23 in patients with active vitiligo afterNB-UVBtreatmentrangedfrom20–80pg/ml(mean53.65 ± 18.48) pg/ml while in controls ranged from 10-88 pg/ml (mean 35.50 ± 20.21)pg/ml.

Comparison revealed that the difference was significant (P=0.002) **(Table 8**

Table (8): Comparison between patients with active vitiligo and controls as regards serum IL-23 level after NB-UVB treatment.

Groups	S. IL.23PG/mL		Independent t-test	
	Mean ±SD	Range	t-test	P-value
Control	35.50 ± 20.21	10 – 88	3.217	0.002*
Treated (active)	53.65 ±18.48	20 –80		

Independent t-test * significant

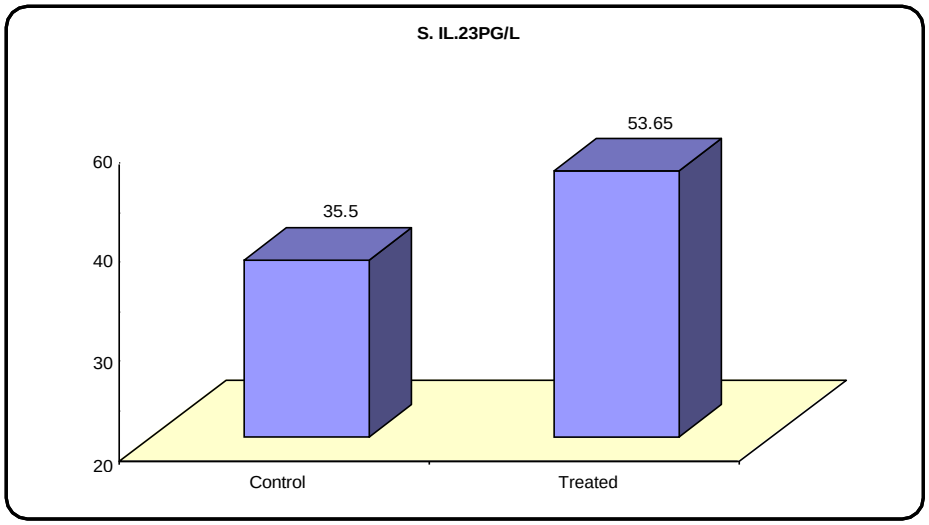

Figure (2): Comparison between patients with active vitiligo and controls as regards serum IL-23 level after NB-UVB treatment.

Discussion:

Vitiligo is an acquired skin disorder caused by the disappearance of pigment cells from the epidermis that gives rise to well defined white patches which are often symmetrically distributed. It occurs worldwide in about 0.1-2% of different population and it occurs as frequently in males as it does in females. The exact cause is unknown, but might involve genetic factors, autoimmunity, neurologic factors, toxic metabolites, and lack of melanocyte growth factors [15].

There are limited studies related to the role of IL-23 in the pathogenesis of vitiligo that were performed on few numbers of vitiligo patients which lead to contradictory results [12]. In our study we tried to evaluate the role of IL-23 in active, stable and NB-UVB treated vitiligo in comparison with healthy controls to determine the potential participation of IL-23 in the pathogenesis of vitiligo.

The present work represented a case control study which was carried out on 40 subjects; twenty patients with active vitiligo, twenty with stable vitiligo and thirty age and sex

matched healthy controls. All patients were subjected to 40 NB-UVB sessions, 3 sessions/week (13-week) and serum IL-23 levels were measured by ELISA technique before and after treatment.

In the current study, before treatment all vitiligo patients had a significant higher serum IL-23 levels compared to healthy controls. Serum IL-23 levels in active vitiligo patients was significantly higher than that of healthy subjects, however stable vitiligo patients had a non significant difference. The fore mentioned results denoted the potencial participation of altered IL-23 levels in the pathogenesis of vitiligo which might be through inciation and/or mantainance of inflammation that influences the disease activity.

Our findings were consistent with **[16]**who reported an increased s.IL-23 levels in vitiligo patients compared to controls and a positive correlation between s.IL-23 levels with disease activity and body surface area.

We agreed with **[17]**who found significant high levels of serum IL-23 levels in vitiligo patients as compared to controls.

In contrast, **Cengiz et al. [18]**and **Osman et al. [19]**reported non significant difference of the levels of serum IL-23 between patients and controls. These contradictory results can be explained by the fact that both studies included both segmental and non segmental vitiligo in their work and segmental vitiligo might have a different aetio pathological scenario **[20]**which might affect interpretation of results. **Osman et al. [19]**haven't excluded patients that were on topical or systemic treatment and they didn’t classify patients according to disease activity (active or stable), so they might accidently include more stable patients in their patients samples, all of which might have significantly modified serum levels of IL-23.

A highly significant reduction in the s.IL-23 levels in active vitiligo patients before versus after NB-UVB treatment was detected which might demonstrate the role of NB-UVB as an immunomodulatory agent. **Tembhre et al.** reported similar effect of NB-UVB but on IL-17 levels, interestingly that significant difference was lost when we added stable vitiligo patients to the active group and compared them with the NB-UVB treated group which might further confirm the potential role of IL-23 in the disease activity[21].

It is worth mentioning that in NB-UVB treated vitiligo patients the serum.IL-23 levels were still significantly higher than those of the controls. On the other hand, there was non-significant higher s.IL-23 levels in NB-UVB treated vitiligo patients than stable vitiligo patients. That might be explained by the short treatment duration, or the need of combination therapy to attain full control on cytokine milieu in vitiligo.

Regarding **VETI** score we agreed with other studies **[16,17,22]**that reported the efficacy of NB-UVB treatment and its influence on the disease extent and/or severity. All patients were improved after NB-UVB sessions with a significant reduction in **VETI** score in both active vitiligo group and stable vitiligo group. These results might strengthen the value of NB-UVB treatment as an effective therapy in non-segmental vitiligo.

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