



Expression of E-cadherin in Vitiligo Patients before and after Narrow Band UVB

Abd ELaziz Abdelsalam EL Refaie^a, Hager Ezzat Gaber^a and Mona ELSayed Ahmed Abdellatif^a

^a Dermatology and Andrology department, Faculty of Medicine, Beni-Suef University, Egypt

Abstract:

Background: Vitiligo is the most common chronic acquired depigmenting disorder, resulting from the loss of melanocytes from the basal epidermal layer. Ecad is the major adhesion molecule mediating melanocyte–keratinocyte interactions and is essential for both the establishment of epithelial structures during development and numerous dynamic processes such as differentiation, polarity, proliferation, and migration during morphogenesis and homeostasis. **The aim of this work** : is to estimate the level of E- cadherin in serum of vitiligo patients before and after NB-UVB therapy as compared to normal control persons, to evaluate the role of E-cadherin in the pathogenesis of this disease . **Patients and methods:** The level of E-cadherin was detected in 30 venous blood samples of vitiligo patients before (group I) and after treatment with NB-UVB (group II) and 30 healthy controls using ELISA. Informed consent was obtained from the participants in this study after ethical committee approval from Dermatology Department of Medicine, Beni Suef University . **Results:** Serum level of E- cadherin was significantly higher in vitiligo patients before exposure to narrow band UVB compared to healthy controls and became lower in vitiligo patients after exposure to narrow band UVB but still higher than control. No detected relation was found between serum level of E- cadherin and patients' age, skin type, symmetry ,vitiligo type ,last time of new lesions and VIDA (p-value >0.05). In addition, Serum Level of E-cadherin was significantly higher in Vitiligo patients with negative as compared with positive family history; with a statistically significant (p-value= 0.045),and slight negative significant linear correlation between serum level of E-cadherin and percentage of vitiligo in studied Vitiligo patients; (p=0.045). **Conclusion** :E- cadherin may play a role in the pathogenesis of vitiligo. **Recommendations:** On the basis of our findings in this study and in conjunction with that from previous studies, we suggest that: 1) Additional studies on large number of cases in association with assessment of E-cadherin to evaluate its exact role in vitiligo pathogenesis. 2) Study gene polymorphism of E-cadherin gene and association with vitiligo. 3) Performing experimental study to evaluate the effect of E- cadherin up-regulation on vitiligo patients' response to NB-UVB and other modalities of therapy.

Keywords: Vitiligo; E cadherin; Narrow band UVB ; ELISA.

1. Introduction:

Vitiligo is the most common acquired type of leukoderma, causing significant social and psychological difficulties in all patients, the hallmark of the disease is white patches of the skin [1]. The major classifications of vitiligo are non-segmental or generalized vitiligo, segmental vitiligo, and mixed vitiligo according to international consensus [2].

The etiology of vitiligo remains poorly understood, but progressive disease is clearly characterized by inflammatory infiltrates at the margin of lesions, suggesting an immune-mediated acceleration phase common to all forms of vitiligo [3].

Several studies have detected serum antibodies directed melanocyte antigens in vitiligo patients, and a correlation has been found between such antibodies and disease activity [4].

Nonetheless, other theories have been suggested regarding causes for depigmentation, including deficient adhesion proteins, increased presence of oxidative stress, and neurogenic factors [5].

The molecular basis is unknown but may involve trauma induced inflammation and cell adhesion defects [6]. In keratinocytes in depigmented vitiligo lesions, the expression of two molecules implicated in cell – cell adhesion. Discoidin domain receptor tyrosine kinase 1 (DDR1), also known as CD167a, and E-cadherin (Ecad), appeared to be weaker than normal [7].

Cadherins are a family of Ca - dependent transmembrane proteins mediating specific hemophilic cell – cell adhesion. (E cad) is the major adhesion molecule mediating melanocyte–keratinocyte interactions [8].

E-Cadherin dependent cell–cell adhesion is sensitive to environmental redox status and is highly dependent on Ca²⁺ level [9].

Both redox status and Ca²⁺ level are dysregulated in the epidermis of vitiligo patients, eventually implicating role of E-Cad in pathogenesis of vitiligo [10]. Ecad is essential for both the establishment of epithelial structures during development and numerous dynamic processes such as differentiation, polarity, proliferation, and migration during morphogenesis and homeostasis [8].

Current treatment modalities are directed towards stopping progression of the disease and achieving repigmentation. Therapies include topical or systemic corticosteroids, topical immunomodulators, photo (chemo) therapy, surgery, and depigmentation of normally pigmented skin. In photo (chemo) therapy, narrowband ultraviolet-B therapy (NB-UVB) seems to be superior to psoralen ultraviolet-A therapy (PUVA) and broadband UVB. The exact mechanism of Narrow Band UVB (NB – UVB) induced pigmentation still remains unknown [11]. [12], first provided encouraging results in favor of NB –UVB in vitiligo in which 63% of their patients achieved 75% or greater repigmentation after 12 months of twice-weekly therapy when

compared with 46% of patients achieving similar degree of repigmentation with topical PUVA [12].

A recent retrospective study that assessed treatment outcome and persistence of repigmentation from twice-weekly NB-UVB concluded that persistence of repigmentation was seen in 80% of patients even after a year of stopping therapy [13].

2. Patients and Methods:

This was a cohort study performed in dermatology outpatient at Beni-Suef University hospital within six months from 11/2019 -4/2020.

The present study included 30 Egyptian vitiligo patients from both sexes. They were recruited from dermatology outpatient clinic at Beni-Suef University hospital. Patients were diagnosed by detailed history, clinical examination and confirmed by skin biopsies. Thirty healthy controls were chosen randomly from relatives of patients and others who came for cosmetic procedures .

Informed consent was obtained from the participants in this study after ethical committee approval from Dermatology Department, Faculty of Medicine, Beni-Suef University .

2.1 Inclusion criteria:

1. Patients with vitiligo 20-50 years before and after exposure to narrow band UVB.
2. Both males and females will be included.
3. Age and sex matched controls.

Exclusion criteria:

1. Pregnant females and children.
2. Patients receiving any systemic treatment or topical immunomodulatory for vitiligo within the past one month.
3. Patients with associated systemic or dermatological diseases.

2.2 The studied subjects were divided into three groups as follows:

1. Group I: (n = 30) vitiligo patients who didn't receive any treatment for at least one month before the start of study.
2. Group II: (n = 30) vitiligo patients after the end of narrow band ultraviolet B course (3sessions/ week for 6months or when repigmentation occurs).
- 3) Group III: (n = 30) healthy individuals as a control group.

Patients were assessed clinically to determine vitiligo type, skin type, extent and sites of vitiligo. The vitiligo disease activity score (VIDA) was utilized to evaluate the disease activity .

Estimation of the serum level of E-cadherin using ELISA:

- A. Venous blood samples were taken from vitiligo patients & control
- B. Narrow band ultraviolet B therapy was administered as three sessions / week for six months or when repigmentation occurs (if earlier).

C. Note: the dose of NB-UVB was 0.5 joule in the first three sessions and then increased by 0.1 joule every three sessions. The dose of narrow band was adjusted by minimal erythema dose. Second venous blood sample was taken from patients after the end of narrow band ultraviolet B course.

The VIDA is a scoring system based on the observer's subjective impression of the patient's present disease activity within the indicated time periods, as follows ;

- Active in the past 6 weeks (score +4)
- Active in the past 3 months (score +3)
- Active in the past 6 months (score +2)
- Active in the past year (score +1)
- Stable for at least 1 year (score 0)
- Stable for at least 1 year with spontaneous repigmentation (score -1)

"Active" is defined as the expansion of existing lesions or the appearance of new lesions. "Stable" refers to having no new lesions and no progression of existing lesions for at least 1 year.

Statistical methodology:

The collected data was tabulated, coded and then statistically analyzed using statistical package for social sciences (SPSS) computer software (version 25), IBM software, USA, for Windows.

Continuous variables were presented as mean values \pm standard deviation (SD), and categorical variables were presented as percentages.

Comparisons among qualitative data were done using chi-squared test and Fisher test. For quantitative data; independent sample t-test was used to elucidate significance among the cases and control group means, paired sample-t test was used to examine the difference in mean pre-and post-narrow band UVB among studied cases. Differences were considered significant at $P \leq 0.05$.

Pearson's correlation analysis was done to evaluate linear relationship between serum level of E-cadherin and other parameters. . Correlation graphs were drawn only for significant correlation which is considered significant at $P \leq 0.05$.

Correlation is considered positive (direct correlation) when r (correlation coefficient) had a + signal and negative (inverse correlation) in case of - signal and it is considered:

- Weak when $r = 0 - 0.35$,
- Moderate when $r = >0.35 - 0.65$; and
- Strong when $r = > 0.65$.

3. Results:

The current study included 30 Vitiligo patients from both sexes. They all presented to dermatology department at Beni-Suef University hospital. The Vitiligo patients were 11 males and 19 females patients, their age ranged from 20 to 50 years, the average age was; 32.10 ± 11.4 . And 30 healthy controls were taken, they were age and sex matched to the Vitiligo cases.

Table (1): Relation between Serum Level of E-cadherin and patients gender in studied Vitiligo patients ; (N= 30):

E-cadherin	Gender	N	Mean	SD	Minimum	Maximum	p-value
	Male	11	447.25	132.80	236.20	647.60	
	Female	19	481.88	110.65	249.30	748.70	

**p-value >0.05 is considered non-significant by independent sample t-test*

As demonstrated in table (15); Serum Level of E-cadherin was slightly higher in female Vitiligo patients as compared with males; however no statistically significant difference was detected in relation between gender and Serum Level of E-cadherin (p-value= 0.449).

Table (2): Relation between Serum Level of E-cadherin and vitiligo type in studied Vitiligo patients; (N= 30):

E-cadherin	Type of vitiligo	N	Mean	SD	Minimum	Maximum	p-value
	Generalized	25	466.01	119.18	236.20	748.70	
	Localized	5	485.04	125.22	318.20	647.60	

**p-value >0.05 is considered non-significant by independent sample t-test.*

Table (2) demonstrates no detected relation between Serum Level of E-cadherin and locality of lesion in Vitiligo patients; p-value >0.05

Table (3): Relation between Serum Level of E-cadherin and Symmetry of Lesions in studied Vitiligo patients; (N= 30):

E-cadherin	Symmetry	N	Mean	SD	Minimum	Maximum	p-value
	Asymmetrical	12	494.27	80.58	339.30	645.30	
	Symmetrical	18	452.46	137.43	236.20	748.70	

**p-value >0.05 is considered non-significant by independent sample t-test.*

Table (3) demonstrates no detected relation between Serum Level of E-cadherin and symmetry of lesion in Vitiligo patients; p-value >0.05

Table (4): Relation between Serum Level of E-cadherin and Type of Skin in studied Vitiligo patients ; (N= 30):

E-cadherin	Type of Skin	N	Mean	SD	Minimum	Maximum	p-value
	III	6	471.18	134.536	249.30	645.30	0.993
	IV	23	468.12	119.548	236.20	748.70	
	V	1	481.50	.	481.50	481.50	

*p-value >0.05 is considered non-significant by One Way ANOVA-test.

Table (4) demonstrates no detected relation between Serum Level of E-cadherin and skin type in Vitiligo patients; p-value >0.05

Table (5): Relation between Serum Level of E-cadherin and Vitiligo Type in studied Vitiligo patients; (N= 30):

E-cadherin	Vitiligo Type	N	Mean	SD	Minimum	Maximum	p-value
	Vulgaris	15	446.95	144.61	236.20	748.70	0.416
	ACRAL	2	539.60	8.62	533.50	545.70	
	Mixed	8	521.12	63.79	439.20	645.30	
	Focal	1	561.20		561.20	561.20	
	Segmental	2	386.65	96.80	318.20	455.10	
	Universalis	2	394.25	46.45	361.40	427.10	

*p-value >0.05 is considered non-significant by One Way ANOVA-test.

Table (5) demonstrates no detected relation between Serum Level of E-cadherin and Vitiligo type in Vitiligo patients; p-value >0.05.

Table (6): Correlation between Serum Level of E-cadherin and Percentage of Vitiligo in studied Vitiligo patients; (N= 30):

Serum Level of E-cadherin	Percentage of Vitiligo	
	r = -0.368	p-value = 0.045*

r Pearson correlation coefficient analysis

Table (6) demonstrates slight negative significant linear correlation between Serum Level of E-cadherin and Percentage of Vitiligo in studied Vitiligo patients; (r= -0.368, p=0.045)

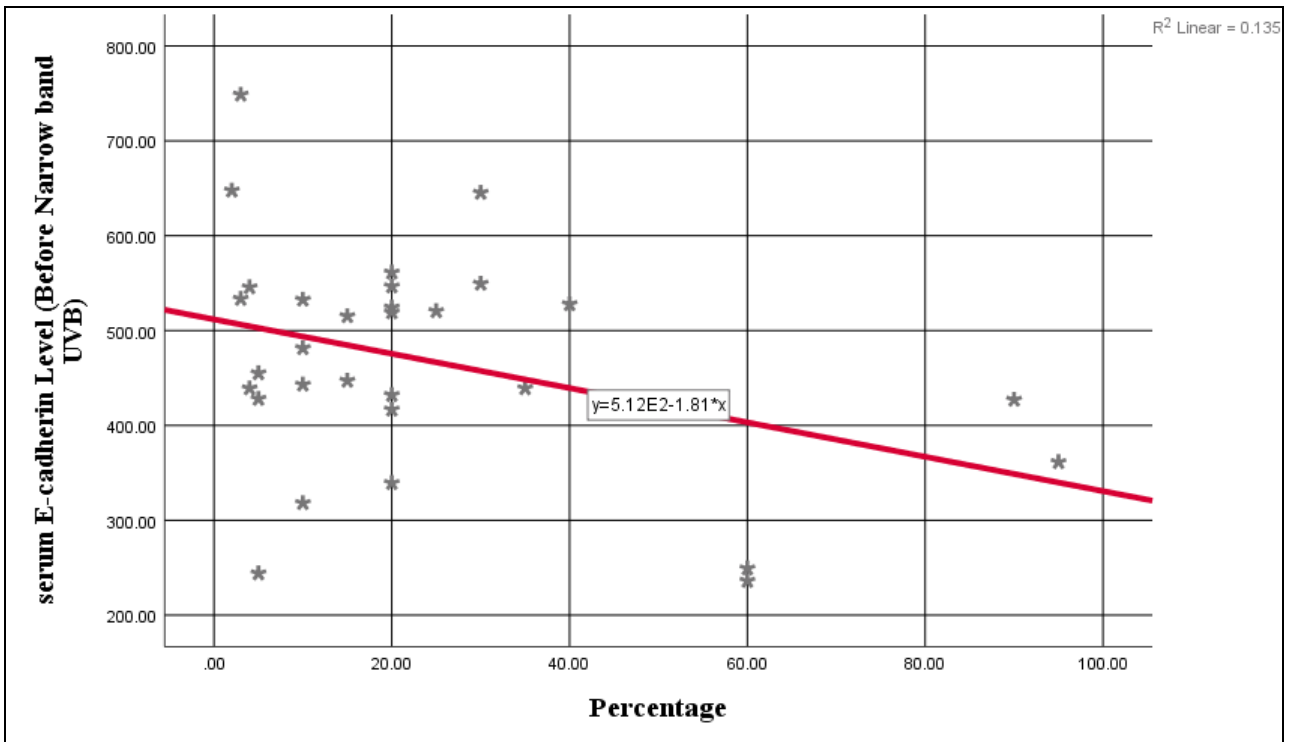


Fig (1): Correlation between Serum Level of E-cadherin and Percentage of Vitiligo in studied Vitiligo patients

Table (7): Correlation between Serum Level of E-cadherin and Vitiligo disease activity (VIDA) in studied Vitiligo patients; (N= 30):

	Vitiligo disease activity (VIDA)	
Serum Level of E-cadherin	$r = 0.154$	$p\text{-value} = 0.416$

r Pearson correlation coefficient analysis

Table (7) demonstrates no detected significant linear correlation between Serum Level of E-cadherin and Vitiligo disease activity (VIDA) in studied Vitiligo patients; ($p\text{-value} > 0$).

Table (8): Serum Level of E-cadherin before and after exposure to narrow band UVB in Vitiligo patients:

E-cadherin		Mean	SD	Minimum	Maximum	p-value
	Before UVB	469.18	118.2	236.70	748.70	
After UVB	147.99	38.36	108.70	235.20		

* $p\text{-value} > 0.05$ is considered non-significant by paired sample t-test.

As demonstrated in table (8); Serum Level of E-cadherin decreased significantly after exposure to narrow band UVB in Vitiligo patients; the mean Serum Level of E-cadherin values (469.18 vs. 147.99) before and after exposure to narrow band UVB in Vitiligo patients respectively with a statistically significant p-value

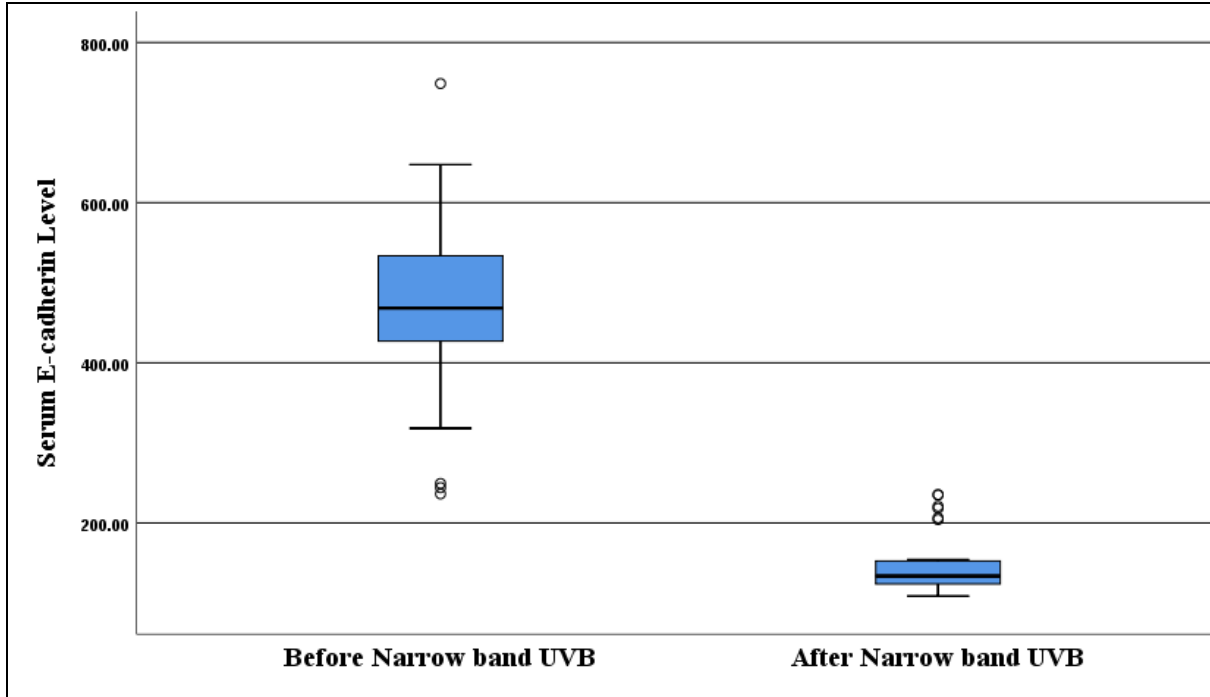


Fig (2): Serum Level of E-cadherin before and after exposure to narrow band UVB in Vitiligo patients

4. Discussion:

Vitiligo is the most common acquired type of leukoderma, causing significant social and psychological difficulties in all patients. The hallmark of the disease is white patches of the skin [1].

The characteristic lesion is a totally amelanotic, non scaly chalky-white macule with distinct margins [14]. Vitiligo should not be dismissed as a cosmetic or insignificant disease, as its effects can be psychologically

devastating, often with a considerable burden on daily life [15].

It is a multifactorial disorder characterized by the Loss of functional melanocytes [16]. Multiple mechanisms Have been proposed for melanocyte destruction in vitiligo. These include genetic, autoimmune responses, oxidative stress, generation of inflammatory mediators and melanocyte detachment mechanisms [15].

Cadherins are a family Ca^{2+} of dependent transmembrane proteins mediating specific homophilic cell–cell adhesion. Ecad is the major adhesion molecule mediating melanocyte–keratinocyte interactions. Ecad is essential for both the establishment of epithelial structures during development and numerous dynamic processes such as differentiation, polarity, proliferation, and migration during morphogenesis and homeostasis [8].

Homophilic Ecad cell–cell adhesion is sensitive to environmental redox status and is highly dependent on Ca^{2+} levels [17]. Both redox status and Ca^{2+} level are deregulated in the epidermis of vitiligo patients [10], implicating eventually Ecad in vitiligo pathogenesis. The stabilization of melanocytes in the basal layer of the epidermis is dependent on the adhesion protein E- cadherin [18].

Impaired cell-surface E-cadherin expression was recently shown in the non lesional skin of patients with vitiligo[5]. Several mechanisms could be involved in such a disruption, such as inhibition of its expression, internalization in the endosomal structure, or cell surface cleavage into a soluble form (soluble E-cadherin). E-cadherin cleavage may be induced by several proteases, including MMPs such as MMP-3, MMP-7, and MMP-9, A disintegrin, and MMP domain–containing protein10 (ADAM10), which are all known to be involved in extracellular matrix

remodeling and cell migration in various physiologic and pathologic processes [19].

It has also been suggested that a mechanical detachment of melanocytes followed by transepidermal elimination is the probable explanation for the mechanism of the depigmentation observed during the mechanical friction [20].

As regards clinical data, the present study results showed that the average age of vitiligo patients was 32.10 years consistent with a study of [21] where the average age was 25.9 years old.

We found that the time at which the last new lesion appeared has a mean period of 5.08 months. In addition, the percentage of the affected area of vitiligo skin patients has a mean value of 23.53% which was in accordance to [22] , who reported that the extent of disease had a mean value of 22%.

The female to male ratio in this study was 2:1. Most of the other reports shown that males and females were affected with almost in the same percentages, for instance, [23] showed that among 150 recruited vitiligo patients, 103 (68.7%) were females and 47 (31.3%) were males. The number of female vitiligo patients were found to be higher than male because women notice the change in appearance and approach the doctors sooner than men and of the social stigma in the community, young females tend to report earlier due to matrimonial anxiety [24].

Nearly two-thirds of the studied patients were not-working (66.7%) while (33.3%) were working. The majority of the studied Vitiligo cases had no family history of the disease (26 cases (86.7%)); while only 4 cases (13.3%) had a positive family history of Vitiligo. Percentages were reported in a previous study of [25] were 67.5 % had no family history of the disease (27) cases while 13 cases (32.5%) had a positive family history of Vitiligo. In our study vitiligo vulgaris (50.0%) was the most common type which was similar to another study of [21], while different from another study of [25] in which the focal type as the most common. In addition, the most encountered skin type in our study was type 4, and this was in consistent with [23], but in contrast to who [26] reported type 3 as the commonest one in vitiligo.

All the studied Vitiligo cases in the present study had bilateral lesions. This agreed with [24] who found that majority of patients had bilateral distribution. Moreover, the results of the current study showed that 56.7% patients had lesions involving face, and 63.3% showed hands and feet were affected. This was in accordance to [27] who documented 41.1% of cases with hands or feet involvement, and 55.1% with facial involvement. In our study, serum Level of E-cadherin was significantly higher in vitiligo patients as compared with healthy controls before exposure to narrow band (group 1), it's level decreased significantly after exposure to narrow band UVB in (group

2). Serum Level of E-cadherin was still significantly higher in vitiligo patients as compared with healthy controls even after exposure to narrow band UVB.

This was consistent with [28] who reported that the CDH1 CC genotype was found to be significantly associated with the risk of developing vitiligo using real-time polymerase chain reaction (RT-PCR) ($p=0.005$). In addition, This was in accordance to [5] who reported that there was altered E-cadherin expression in nonlesional skin biopsy distant from the depigmentation macules, mainly the buttock area, in 29 vitiligo patients. Previous studies demonstrated that Ecad is required for melanocyte resistance to mechanical and oxidative stress, establishing a link between silent, cell-autonomous defects in vitiligo melanocytes and known environmental stressors accelerating disease expression as in the study of [5].

For subgroup analysis, according to the sex; there was non-statistically significant difference in e cadherin level between the cases and the control groups regarding sex (p -value= 0.449) The result of [28] supported this current study. using blood samples from 152 patients and 152 controls showed that there was non-statistically significant difference in e cadherin level between the cases and the control groups regarding sex (p -value> 0.05).

On studying the correlation between serum Level of E-cadherin and other clinical data (locality, symmetry, Type of Skin, patients'

age, history of last new lesion and vitiligo disease activity (VIDA) demonstrates no detected significant linear correlation. The study demonstrates slight negative significant linear correlation between Serum Level of E-cadherin and Percentage of Vitiligo in studied Vitiligo patients; ($r = -0.368$, $p = 0.045$) while different from another study of [5] and [28] that there was no significant correlation.

In a previous study, showed that the type 1 cytokines IFN- γ and TNF- α induce melanocyte detachment in both in vitro and in vivo models, and this effect is dependent on the inhibition of E-cadherin gene expression, internalization of E-cadherin, and its cleavage through the release of MMP-9 by keratinocytes. Strikingly, MMP-9 inhibitors and JAK inhibitors prevented the release of soluble E-cadherin, leading to the stabilization of melanocytes in the basal layer of the epidermis. (Boukhedouni., et al 2020).

The restoration of normal expression of sE cadherin by NB-UVB could be partially responsible for improvement of vitiligo, translated in the current study by the significant decrease of its serum level after the NB-UVB sessions.

5. Conclusion and Recommendations:

E-cadherin may play a role in the pathogenesis of vitiligo and NB-UVB may have a role in regulation of E-cadherin expression.

II- Recommendations

On the basis of our findings in this study and in conjunction with that from previous studies, we suggest that:

- 1) Additional studies on large number of cases in association with assessment of E-cadherin to evaluate its exact role in vitiligo pathogenesis.
- 2) Study gene polymorphism of E-cadherin gene and association with vitiligo.
- 3) Performing experimental study to evaluate the effect of E-cadherin up-regulation on vitiligo patients' response to NB-UVB and other modalities of therapy.

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