

## E-Cadherin and Hydrogen Peroxide in Vitiliginous Skin

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### ABSTRACT

**Background:** Vitiligo is a chronic acquired skin disorder that causes loss of its colour. The definite cause is unknown but may be due to genetic factors, immune system changes or stress exposure. Oxidative stress may cause melanocytic destruction through its damaging effects on the cell components.

**Objective:** Measurement of E-cadherin and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) level in vitiligo patients versus controls.

**Patients and Methods:** This case-control study involved 20 sex and age matched participants, 10 active non segmental vitiligo (NSV) patients, and 10 non vitiliginous controls, all recruited from the Dermatology Department, Ain Shams University and New Cairo Police Academy Hospital from March 2018 till March 2021. Skin biopsies were taken from four different anatomical sites in cases and controls for immune-histochemical evaluation of E-cadherin and biochemical measurement of H<sub>2</sub>O<sub>2</sub>.

**Results:** H<sub>2</sub>O<sub>2</sub> showed statistically significant higher values, while E-cadherin had significantly lower staining in patients than controls.

**Conclusion:** Higher H<sub>2</sub>O<sub>2</sub> level, in vitiligo patients, is suggestive of oxidative stress. Current study may indicate that vitiligo is not just a disease of melanocytes, but keratinocytes may also play a role, as well. Keratinocytes in depigmented epidermis may have a changed microenvironment, as evidenced by a lack of cell-to-cell adhesion between keratinocytes and melanocytes, as well as between keratinocytes, disrupting the skin pigmentary system.

**Keywords:** E-Cadherin, Hydrogen Peroxide, Vitiligo and Oxidative Stress.

### INTRODUCTION

Vitiligo is an acquired skin depigmentation affecting about 1% of people,<sup>[1,2]</sup> due to destruction of melanocytes<sup>[3]</sup>. It may be segmental, non-segmental or mixed<sup>[4]</sup>. Pathogenesis hypotheses of vitiligo include: autoimmune<sup>[5]</sup>, neural<sup>[6]</sup>, and defective free radical defense interfering with melanogenesis<sup>[7,8]</sup>. The main epidermal faulty cellular adhesion function may be involved, according to the melanocytotoxicity idea<sup>[9,10]</sup>. Catenins and cadherins mediate interactions between keratinocytes and melanocytes<sup>[11]</sup>. Environmental redox state affects cell-cell adhesion that is dependent on E-cadherin<sup>[12,13]</sup>.

Auto-cytotoxic hypothesis assumes oxidative stress, as initiative to melanocyte degeneration<sup>[14]</sup>, having hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) buildup in the patients' epidermis who are actively experiencing vitiligo<sup>[15,16]</sup>, which is more observed in perilesional skin, suggesting its role in initiating depigmentation process<sup>[17]</sup>. Reactive oxygen species (ROS) cause lipid peroxidation, deoxyribonucleic acid modification, and inflammatory cytokines secretion<sup>[18]</sup>.

We aimed at evaluating the immunohistochemical staining of tissue E-cadherin in skin samples from active non segmental vitiligo (NSV) patients, in different anatomical areas, in comparison to controls, and measuring H<sub>2</sub>O<sub>2</sub> levels in the same samples in an attempt to find a possible causal relationship between E-cadherin and H<sub>2</sub>O<sub>2</sub> in the pathogenesis of vitiligo.

### PATIENTS AND METHODS

This case control study was issued in the Department of Dermatology, Venereology and Andrology, Ain Shams

University Hospitals and New Cairo Police Academy Hospital from March 2018 till March 2021.

This study included twenty subjects who were divided into two groups:

**Group I:** Ten active NSV patients recruited from the outpatient clinic and **Group II:** Ten non vitiliginous controls (attended the dermatology clinic for cosmetic issues).

We included patients with active NSV whose vitiligo disease activity (VIDA) score was 3 and 4<sup>[19]</sup>, Fitzpatrick skin types II, III and IV, who did not receive any systemic treatment or phototherapy for vitiligo at least for 3 months or topical treatment for 2 months before the current study.

We excluded; segmental and stable vitiligo patients i.e., there has been no advancement of current disease or the emergence of new lesions in the previous six months, those on systemic or topical vitiligo treatment, patients suffering any concomitant dermatological diseases or receiving antioxidants.

Complete dermatological examination was done to exclude other diseases and VIDA score assessment based on a six-point scale according to **Njoo et al.**<sup>[19]</sup>, was done for evaluating vitiligo activity; [+4: activity of 6 weeks or less period, +3: activity of 6 weeks to 3 months, +2: activity of 3 to 6 months, +1: activity of 6 to 12 months, 0: stable at least for 1 year and -1: stable at least for 1 year with spontaneous repigmentation].

Four punch biopsies (5 mm) were taken from each patient and control: non-lesional skin without any sign of inflammation, [face (post auricular), distal acral sites, trunk and lower limb (around the knee)].

Two extra punch biopsies (3 mm) were taken only from five patients from peri-lesional skin of face and trunk for immune-histochemical comparison with control skin and non-lesional skin of cases as regards E-cadherin level.

Local anesthetic (1-2% lignocaine) was infiltrated around the skin to be biopsied. Each biopsy was cut into two halves. Formaldehyde 10% was used to fix half of the specimens, routinely processed and embedded in paraffin. From the paraffin blocks, serial 5-micron thick slices were cut, stained with hematoxylin and eosin for routine histological analysis, and then immunostaining, the other half was fixed in phosphate buffer saline (PBS) and had been frozen at -16C° for biochemical evaluation of H<sub>2</sub>O<sub>2</sub>.

The slices were placed on positively charged slides and stained with an E-cadherin monoclonal antibody kit for immunohistochemical analysis (clone 36B5 mouse (Monoclonal Antibody), Specific to E-cadherin, from Thermo Fisher scientific, UK.

The image analyzer Leica Q win V.3 programme was installed on a computer at the Histology Department of Ain Shams University's Faculty of Medicine. A Leica DM2500 microscope (Wetzlar, Germany) was linked to the computer. To quantify stain intensity, four distinct captured non-overlapping high power fields (x20) were obtained for each specimen; [minimal (+), moderate (++) or strong (+++)] according to **Bahnassy et al.** [20].

Biochemical estimation of H<sub>2</sub>O<sub>2</sub> using OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit (Colorimetric), from Cell BioLabs, Inc., San Diego, USA. The OxiSelect™ Hydrogen Peroxide/Peroxidase test Kit is a colorimetric quantitative test for hydrogen peroxide or peroxidase.

Approximately, 0.2 mg of tissue was dissected to remove any fatty tissue, then tissue examined for the level of H<sub>2</sub>O<sub>2</sub> was homogenized in 0.5 ml of PBS, pH 7.4 using tissue Lyser (Qiagen; Hilden, Germany); the homogenate was centrifuged at 10000xg (x gravity) for 20 minutes, then the supernatant was used for measurement of H<sub>2</sub>O<sub>2</sub> using colorimetric assay.

#### **Ethical approval:**

**The Ethics Committee of the Ain Shams University's Faculty of Medicine granted the study approval. All participants signed an informing consent after a thorough explanation of the goals of the study and submitted to detailed personal and past history questions including previous**

**medications and periods of activity. The Helsinki Declaration was followed throughout the study's conduct.**

#### **Statistical Analysis**

After gathering, editing, coding, and entering the data, SPSS version 23 was used. The quantitative data were shown as median with inter-quartile range (IQR) if non-parametric, and mean±standard deviation, and ranges when parametric. The independent t-test was used to compare quantitative data between two independent groups with a parametric distribution, while the Mann-Whitney test was used to compare data with a non-parametric distribution. One Way ANOVA was used, followed by Post-Hoc analysis, to compare quantitative data with parametric distribution between more than two independent groups. Gender was presented as percentages and numbers and was compared across the groups by Fisher exact test. To determine the correlation between two quantitative factors within the same group, Spearman correlation coefficients were employed. A P value of < 0.05 was deemed significant.

#### **RESULTS**

A total of 10 cases with active NSV and 10 controls were included in the current case control study; both were matched for age and sex. Patients' ages ranged between (11 - 48) years with mean and SD; (26.50 ± 13.31) years, while for controls, it ranged between (15 - 52) years with mean and SD; (28.00 ± 11.40) years. Among case group there were 4 males (40%) and 6 females (60%), while in control group there were 7 males (70%) and 3 females (30%). Patients' duration of the disease ranged between (5-30) years with a mean and SD; (13.60 ± 8.63) years. Patients' VIDA score ranged between (3 and 4) with mean and SD; (3.60 ± 0.52).

The current study revealed a non-significant statistical difference in E-cadherin densities among different anatomical areas [non-lesional; face, distal acral sites, trunk and lower limb skin and peri-lesional (post auricular and trunk skin)] in cases' group. In controls, there was also the same, non-significant statistical difference between E-cadherin densities in different anatomical sites.

**Table 1** shows a highly statistically significant decrease in E-cadherin density in cases compared to controls in different anatomical sites.

**Table (1):** Comparison between control and cases as regards E-cadherin density in different anatomical sites

E-cadherin %		Control group	Cases group	P-value
		No. = 10	No. = 10	
Non-lesional Face (postauricular)	Mean ± SD	79.78 ± 3.63	71.40 ± 4.24	<0.001**
	Range	71.24 – 84.39	64.7 – 78.96	
Non-lesional Distal Acral sites	Mean ± SD	78.74 ± 4.41	72.24 ± 4.62	0.005**
	Range	70.58 – 84.46	65.96 – 80.01	
Non-lesional Trunk	Mean ± SD	79.51 ± 5.40	72.12 ± 5.42	0.007**
	Range	70.45 – 86.33	64.7 – 83.32	
Non-lesional Lower limb (knees)	Mean ± SD	80.80 ± 4.42	72.13 ± 5.01	<0.001**
	Range	73.57 – 87.21	65.96 – 79.59	
Peri-lesional Face (postauricular)	Mean ± SD	79.71 ± 3.63	72.40 ± 5.52	0.004**
	Range	71.24 – 84.39	65.69 – 79.55	
Peri-lesional Trunk	Mean ± SD	79.69 ± 5.40	70.06 ± 6.58	0.002**
	Range	70.45 – 86.33	65.32 – 81.32	
Average E-cadherin	Mean ± SD	79.70 ± 2.86	71.70 ± 4.25	<0.001**
	Range	75.62 – 85.46	66.45 – 80.46	

HS: Highly significant

There was highly statistically significant decrease in E-cadherin density in non-lesional and peri-lesional skin of the vitiligo patients compared to healthy controls, however, a non-statistically significant difference was found between non-lesional and peri-lesional skin of vitiligo patients (**Table 2**).

**Table (2):** Comparison between E-cadherin density in controls' skin versus non-lesional and peri-lesional skin of cases

E-cadherin %	Control group	Cases group (non-lesional)	Cases group (peri-lesional)	P-value
Mean ± SD	79.70 ± 2.86	71.97 ± 4.38	71.23 ± 5.80	<0.001**
Range	75.62 – 85.46	66.59 – 80.47	66.16 – 80.44	
<b>Post hoc analysis</b>				
<b>Control vs non-lesional group</b>		<b>Control vs peri-lesional group</b>		<b>Non-lesional vs peri-lesional</b>
0.0001**		0.002**		0.785

\*\* : Highly significant

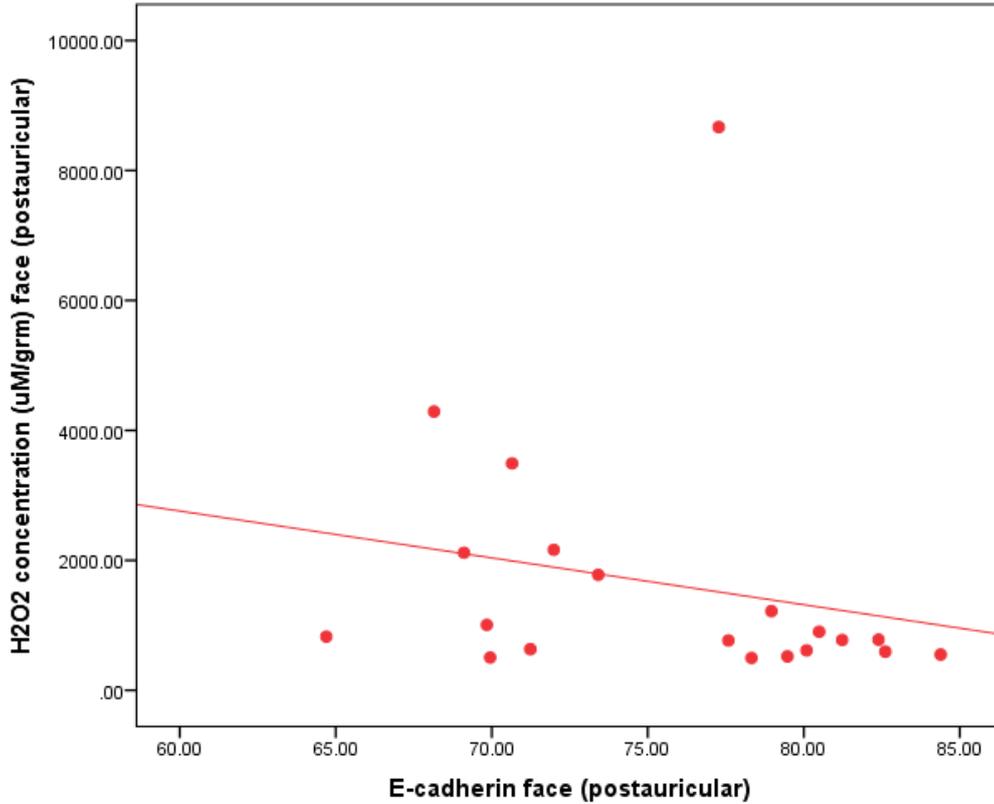
There was a highly statistically significant increase in average H<sub>2</sub>O<sub>2</sub> concentrations in non-lesional skin of vitiligo patients in comparison to healthy controls, in the same anatomical sites. There was also, a highly statistically significant increase in H<sub>2</sub>O<sub>2</sub> concentrations in non-lesional skin of vitiligo patients, from face and distal acral sites (sun and trauma exposed areas) compared to healthy controls, as well as, a statistically significant increase in non-lesional skin from trunk and lower limb (sun and trauma less exposed areas) compared to healthy controls, (**Table 3**).

**Table (3):** Comparison between control and cases as regards H<sub>2</sub>O<sub>2</sub> concentration in different anatomical sites

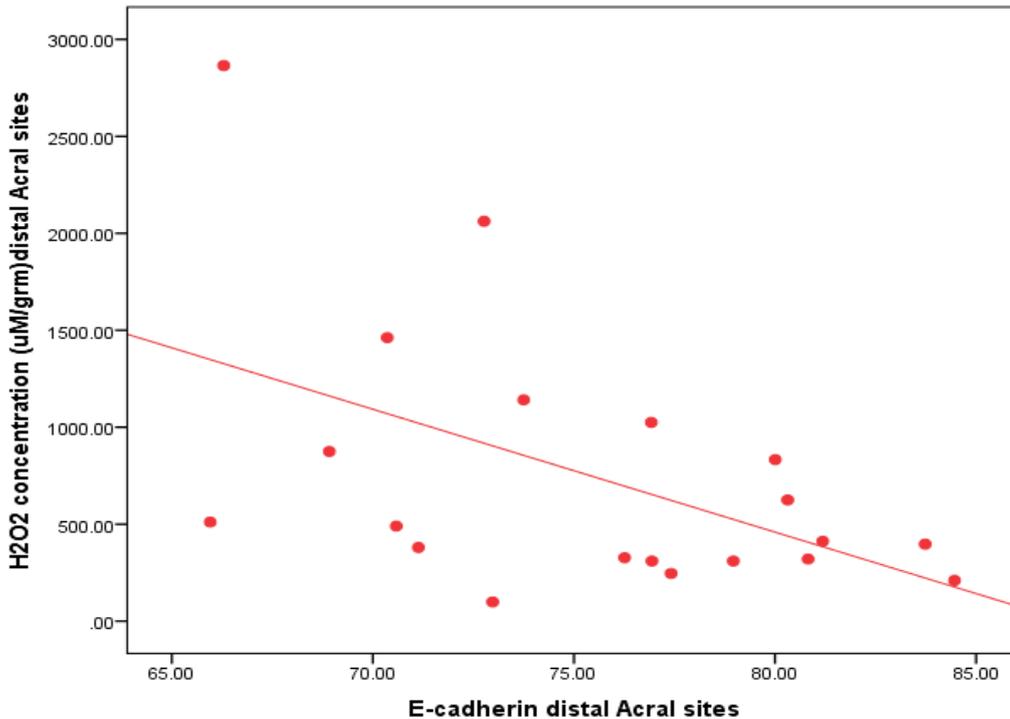
H <sub>2</sub> O <sub>2</sub> concentration (uM/grm)		Control group	Cases group	P-value
		No. = 10	No. = 10	
Non-lesional Face (post-auricular)	Median (IQR)	623.5 (550 – 774)	1947.5 (1007 – 3492)	0.002**
	Range	497 – 901	505 – 8668	
Non-lesional Distal Acral sites	Median (IQR)	315 (246 – 412)	950 (511 – 1462)	0.002**
	Range	99 – 625	327 – 2865	
Non-lesional Trunk	Median (IQR)	267 (193 – 377)	470 (399 – 849)	0.010*
	Range	97 – 809	156 – 1251	
Non-lesional Lower limb (knees)	Median (IQR)	161.5 (101 – 204)	300 (181 – 592)	0.019*
	Range	73 – 320	139 – 707	
Average H <sub>2</sub> O <sub>2</sub>	Median (IQR)	339.5 (322.5 – 450.75)	821 (588.75 – 1540.75)	0.001**
	Range	203.75 – 563	393.75 – 2907.25	

Median, IQR and range: non-parametric test. \*: Significant, \*\*: Highly significant

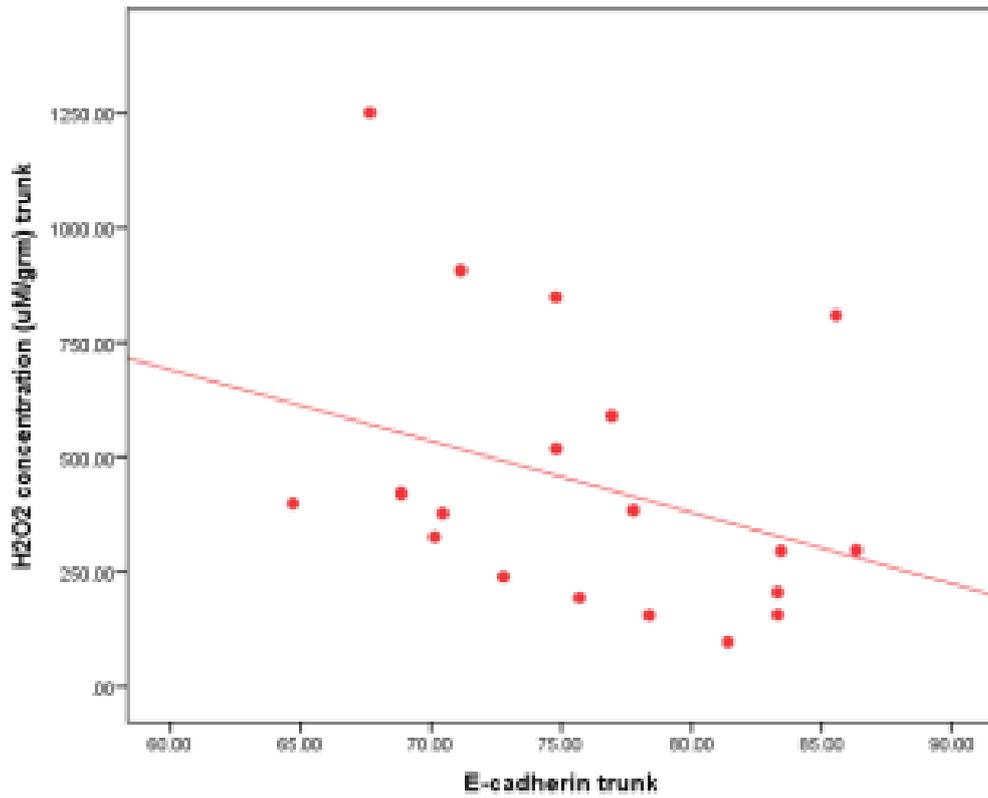
Controls showed statistically non-significant correlation between average  $H_2O_2$  concentrations and E-cadherin density in different anatomical sites, (P: 0.987 and r: 0.006), using Spearman correlation coefficient. On the other hand, cases showed statistically significant negative correlation between  $H_2O_2$  concentrations and E-cadherin density in different anatomical sites (**Figures 1-5**).



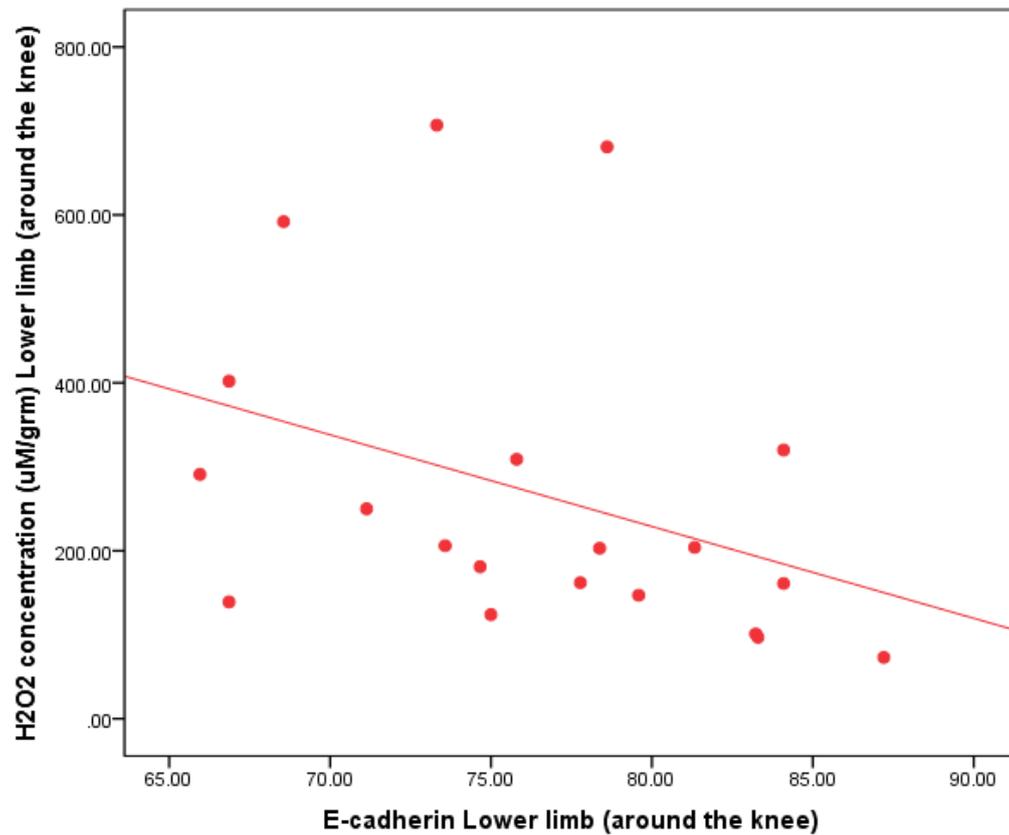
**Figure (1):** Scatter plots for negative correlation between  $H_2O_2$  concentration and E-cadherin density in face (post-auricular skin), (P: 0.043 and r: -0.457)



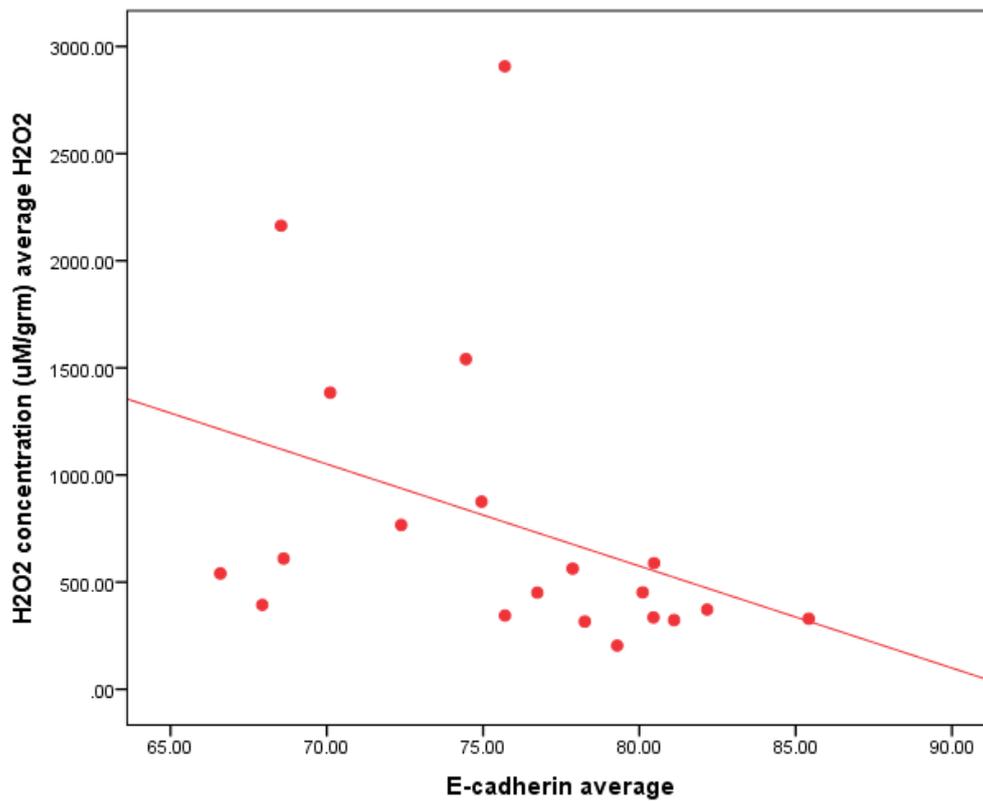
**Figure (2):** Scatter plots for negative correlation between  $H_2O_2$  concentration and E-cadherin density in skin of distal acral sites, (P: 0.028 and r: -0.490)



**Figure (3):** Scatter plots for negative correlation between H<sub>2</sub>O<sub>2</sub> concentration and E-cadherin density in skin of trunk, (P: 0.047 and r: -0.449)

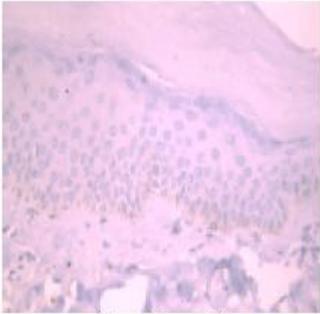
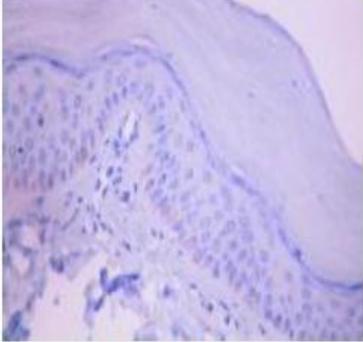
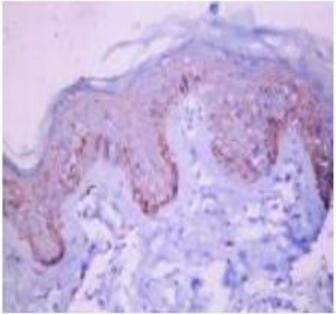
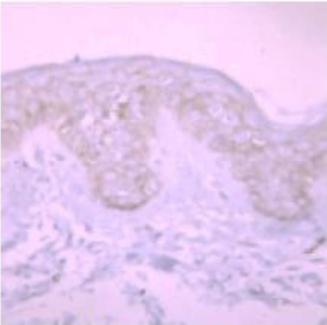
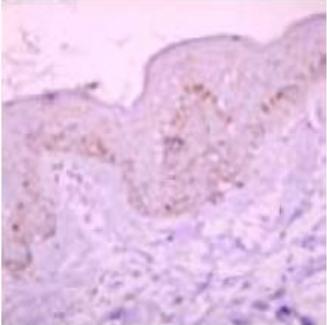
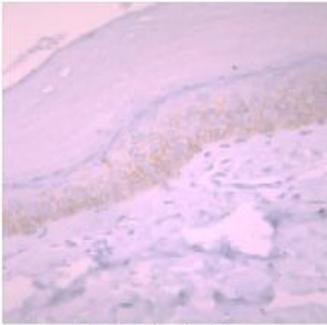
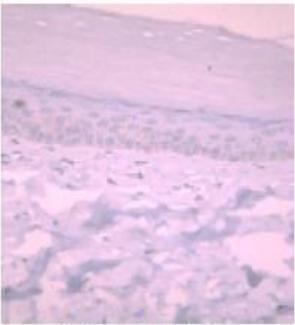
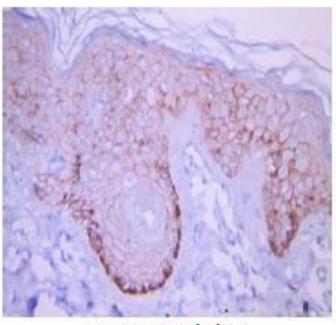
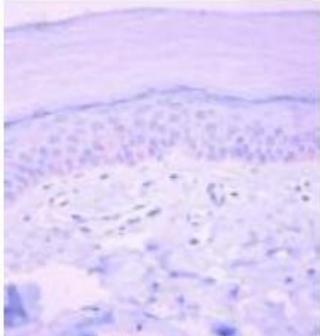
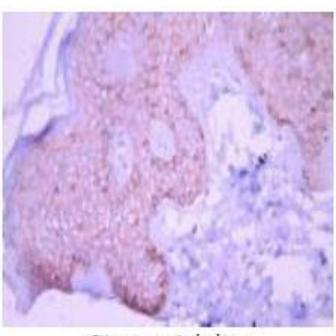


**Figure (4):** Scatter plots for negative correlation between H<sub>2</sub>O<sub>2</sub> concentration and E-cadherin density in skin of lower limb (P: 0.041 and r: -0.460)



**Figure (5):** Scatter plots for negative correlation between average H<sub>2</sub>O<sub>2</sub> concentration and E-cadherin density, (P: 0.006 and r: -0.594)

**Figure (6)** shows strong epidermal staining of E-cadherin, in different anatomical sites in controls, while vitiligo cases showed minimal to moderate staining of E-cadherin in non-lesional sites and minimal staining in peri-lesional sites.

	<b>Non-lesional</b>	<b>Peri-Lesional</b>	<b>Control</b>
<b>Face</b>	 <p>Minimal staining</p>	 <p>Minimal staining</p>	 <p>Strong staining</p>
<b>Distal acral sites</b>	 <p>Moderate staining</p>		 <p>Strong staining</p>
<b>Trunk</b>	 <p>Moderate staining</p>	 <p>Minimal staining</p>	 <p>Strong staining</p>
<b>Lower limb</b>	 <p>Minimal staining</p>		 <p>Strong staining</p>

**Figure (6):** samples from skin, immunohistochemically stained for E-cadherin in cases (non-lesional and peri-lesional) versus control in different anatomical sites (high power x20).

## DISCUSSION

It has been proposed that the first pathogenic event in melanocyte degeneration is a defective free radical equilibrium that leads to increased oxidative stress [8,14]. The redox state of the environment affects cell-cell adhesion that is reliant on E-cadherin [16]. According to the melano-cytology theory, vitiligo is caused by a primary epidermal faulty cellular adhesion that results in the loss of melanocytes [21].

**Wagner et al.** [16] detailed examination of vitiligo patients' clinically normal pigmented skin revealed aberrant distribution of E-cadherin in the membrane. The basal layer of the epidermis's reduced melanocyte to keratinocyte ratio and supra-basal position were linked to low E-cadherin levels.

**Faria et al.** [22], suggested that a reduction in laminin and beta-1 integrin was the cause of the defect in cellular adhesion in lesional vitiligo skin compared to non-lesional skin, but not E-cadherin. While, **Grill et al.** [23] took biopsies from normal appearing skin of 10 SV and 10 NSV patients as well as 10 healthy controls they found that, in comparison to controls with normal pigmented skin, both NSV and SV had the similar immune-histochemical decrease in membrane E-cadherin expression in melanocytes.

The current study did not show any anatomical variations in E-cadherin expression both in vitiligo as well as in healthy control skin. This suggests that anatomical variations do not play a role in vitiligo pathogenesis. To the best of our knowledge, no studies were conducted comparing adhesion molecules expression in different anatomical sites in controls and vitiligo cases. On the other hand, E-cadherin showed statistically significant lower density in non-lesional and peri-lesional skin of active vitiligo patients compared to the same anatomical areas in controls, which means that the decrease in E-cadherin is present in active cases even away from the lesions.

We also aimed to measure tissue H<sub>2</sub>O<sub>2</sub> levels in the same anatomical areas. H<sub>2</sub>O<sub>2</sub> levels showed statistically significant higher values in non-lesional skin specimens in active vitiligo patients compared to controls. This indicates that increased oxidative stress is generalized in vitiligo and not confined to lesional and perilesional skin.

Interestingly, H<sub>2</sub>O<sub>2</sub> levels showed statistically highly significant increase in the post-auricular facial skin, followed by the acral skin in patients versus controls (P: 0.002). Post-auricular skin is not heavily sun-exposed, but may also, be trauma liable area like the acral skin of the hands. While in hidden and protected areas; trunk and lower limbs, H<sub>2</sub>O<sub>2</sub> showed statistically significant higher values in cases versus controls (P: 0.010 and 0.019 respectively), suggesting that sun and mechanical trauma in exposed parts may represent stimulants for oxidative stress [15,24].

The majority of research in vitiligo patients reported a decrease in overall anti-oxidative capacity, although the oxidative stress index was greater,

indicating a dysregulated pro-oxidant/anti-oxidant balance. There is conflicting information about H<sub>2</sub>O<sub>2</sub> levels in vitiligo sufferers, with the majority pointing to higher levels [25].

In an attempt to find a possible causal relationship between H<sub>2</sub>O<sub>2</sub> levels and E-cadherin density in the pathogenesis of vitiligo, we correlated their levels in the study samples. No statistically significant correlations were found between their values in healthy controls; however, a significant statistically negative correlation between H<sub>2</sub>O<sub>2</sub> levels and E-cadherin density was detected in non-lesional vitiligo skin samples, adding more evidence to the previous findings.

**Wagner et al.** [16] reported that there is growing proof linking oxidative stress to the etiology of vitiligo. Compared to keratinocytes, which have more antioxidant content, melanocytes are far more vulnerable to oxidative stress. Cell-cell adhesion is disrupted by high H<sub>2</sub>O<sub>2</sub> concentration because it destabilises E-cadherin at the membrane.

**Ardigo et al.** [26] stated that depigmentation only happens in mice with E-cadherin-deficient melanocytes when mechanical stress is applied, such as when the tail skin of the animals is repeatedly brushed, and in a model of rebuilt epidermis with normal melanocytes when H<sub>2</sub>O<sub>2</sub> exposure causes E-cadherin destabilisation. Thus, providing an explanation for both acral involvement and the Koebner phenomenon in vitiligo, in which trauma results in melanocytes lacking in E-cadherin.

## CONCLUSION

Therefore, our study proposes that defective melanocytes-keratinocyte adhesion resulting from deficient E-cadherin could be enhanced by altered oxidative state evidenced by increased H<sub>2</sub>O<sub>2</sub> level in non-lesional skin of patients with vitiligo, which may lead to detachment of epidermal melanocytes. There were no anatomical variations in normal and vitiligo-prone skin that could explain the reasons for the known patterns of non-segmental vitiligo.

## RECOMMENDATIONS

We recommend further studies to assess the role of anti-oxidants on E-cadherin expression in vitiligo and the anatomical distribution of other adhesion molecules that bind melanocytes to the basement membrane, as well as to keratinocytes, to verify their possible role in vitiligo.

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