The Egyptian Journal of Hospital Medicine (October 2017) Vol.69 (3), Page 2159-2164

Oxidative Stress in Segmental Versus Non Segmental Vitiligo

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ABSTRACT

Background: vitiligo is a coetaneous autoimmune disease, cosmetically non-acceptable to patients with higher Fitzpatrick skin type because of the contrast between lesional and non-lesional skin**.** Oxidative stress through H_2O_2 that is converted by superoxide dismutase (SOD) into H_2O and super oxide anion (O^-) which affects directly on preopiomelanocortin (POMC) system in the epidermis of vitiligo patients which plays the key role in melanogenesis**.**

Aim of the work: this study aimed to measure H_2O_2 in segmental vs. non-segmental vitiligo. This was done through colorimetric evaluation of H_2O_2 in the epidermis of patients with vitiligo and it was focused on the role of the oxidative stress in the pathogenesis of vitiligo.

Patients and methods: this pilot study was included 30 patients recruited from the outpatient clinic of Dermatology and Venereology at Ain Shams University Hospital and outpatient clinic of Al Haud Al Marsoud Hospital. They were all suffered from vitiligo. Fifteen of them had non-segmental and the other 15 had segmental vitiligo. Each group was subdivided into active and stationary. The active disease was defined as appearance of a new lesion and/or extension of preexisting lesions in less than one year**.**

Results: there was a significant increase of H_2O_2 level in the lesional vs. non-lesional skin in all number of patients, NSV patients, NSs patients, NSa, and SVs, but not in SVa patients. The H_2O_2 raised in NSV patients when compared to SV patients. There was a non-significant rise of H_2O_2, also in patients with SVa. There was a significant increase in the epidermal H_2O_2 level in the NSV patients above that of SV patients.

Conclusion: this study suggested that oxidative stress was present in both SV and NSV. This might open a window for several studies concerning the patients of SV and whether the oxidative stress is primary or secondary. Also whether these patients might benefit from antioxidant medications or not.

Keywords: oxidative stress, segmental versus, non segmental vitiligo, H_2O_2.

INTRODUCTION

Vitiligo is a cutaneous autoimmune disease, cosmetically non-acceptable to patients with higher Fitzpatrick skin type because of the contrast between the lesional and non-lesional skin **(1).** The major theories discussing the pathogenesis of vitiligo are :1- the biochemical theory**,** 2- the neural theory, 3- the autoimmune theory **(2)** and 4- the oxidative stress theory which implies that there is defect in free radical defense, interfering with the synthesis of melanin **(2,3).**

The pathogenesis is complicated and involves multiple factors; however, the exact pathogenesis is not known. **Lerner *et al.*** firstly supposed the neural theory and then theory involved reactive oxygen species (ROS), the autoimmune theory and the melanocytorrhagy theory have been proposed **(4).** Patients with vitiligo were found to have marked elevation of GTP-cyclohydrolase activity, that leads to excessive production of 6-tetrahydrobiopterin ($6BH_4$) which results in increased epidermal catecholamine synthesis **(5).** Catecholamines compete with tyrosine on the enzyme tyrosinase, then become hydrolyzed during this process liberation of H_2O_2 occurs **(6).** The high epidermal catecholamines especially norepinephrine causes vasoconstriction of the vitiliginous skin resulting into hypoxia which predisposes to more oxidative stress **(7).** The increased internal and external phenol/catechol concentration competes with tyrosine on tyrosinase. This results into liberation of reactive quinones. These reactive quinones bind to tyrosinase giving rise to a new antigen that is carried by Langerhans cells to the regional lymph nodes and stimulates the proliferation of cytotoxic T cells. These cytotoxic T cells are the first step in melanocytes killing **(8).**

Oxidative stress through H_2O_2 that is converted by superoxide dismutase (SOD) into H_2O and super oxide anion (O^-) affects directly on preopiomelanocortin (POMC) system in the epidermis of vitiligo patients which plays the key role in melanogenesis **(9).**

Dammak *et al.*(10) reported higher epidermal levels for markers of oxidative stress in patients with active course of non-segmental vitiligo (NSV) than in those with stationary course. The former study assessed catalase (CAT), SOD, and glutathione peroxidase (GPx).

Received:14 / 8 /2017 DOI : 10.12816/0041075

Accepted: 23 / 8 /2017

This study aimed to measure H_2O_2 in segmental vs. non-segmental vitiligo. This was done through colorimetric evaluation of H_2O_2 in the epidermis of patients with vitiligo and this was focused on the role of the oxidative stress in the pathogenesis of vitiligo.

PATIENTS and METHODS

PATIENTS

This pilot study included 30 patients recruited from the outpatient clinic of Dermatology and Venereology at Ain Shams University Hospital and outpatient clinic of Al Haud Al Marsoud Hospital. They were all suffering from vitiligo. Fifteen of them had non-segmental and the other 15 had segmental vitiligo. Each group was subdivided into active and stationary. The active disease was defined as appearance of a new lesion and/or extension of preexisting lesions in less than one year**.** All patients were diagnosed by clinical examination and examination by Wood's light.

Inclusion criteria

1. Patients with segmental vitiligo.
2. Patients with non-segmental vitiligo.

Exclusion criteria

1. Patients on systemic antioxidants
2. Patients receiving phototherapy or photochemotherapy.

METHODS

Each patient was subjected to complete history taking, clinical and dermatological examination.

History

- Personal history: age, name, sex, special habits, residence and marital status.
- Present history: onset, course, duration, history of previous treatment of the disease.
- History of activity of vitiligo: appearance of new lesions or\ and widening of preexisting one and duration of activity.
- History of any systemic disease.
- Family history

Examination

- General examination for signs of any systemic diseases.
- Local examination of the lesion regarding site, size, distribution, and signs of response to treatment within the lesion.
- Examination of the edge of the lesion for pigmentation and\or inflammation.
- Wood's light examination.

Biopsy

Two biopsies were taken from each patient. One from the normal skin and the other was from the margin of the lesion. The non-lesional biopsy was not less than 15 cm away a lesion. Local anesthetic (1-2% lignocaine) was infiltrated around the skin to be biopsied.

A three mm punch biopsies were taken one from the margin of lesional skin and the others from non-lesional skin at least 15cm away from the lesion.

The specimen was preserved in -60c refrigerator then the H_2O_2 was measured by Cell Biolabs' OxiSelect™ Hydrogen Peroxide Assay Kit Cell Biolabs Inc. USA which is a quantitative assay for measuring hydrogen peroxides from both aqueous and lipid solutions. The kit employs a simple HTS-compatible assay for measuring hydrogen peroxide concentrations in the biological samples without any need for pretreatment. It can measure aqueous hydrogen peroxides and lipid hydrogen peroxides and no extraction step was needed for lipid assay format. Absorbance values are proportional to the hydrogen peroxide levels within the samples. The kit has a detection sensitivity limit of 1 μM. Each kit provided sufficient reagents to perform up to 500 assays.

The study was approved by the Ethics Board of Ain Shams University.

Analytical statistics

1. Student T Test was used to assess the statistical significance of the difference between two studied group means.
2. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between the two studied groups.
2. Paired t-test was used to assess the statistical significance of the difference between two means measured twice for the same study group
3. Wilcoxon signed rank test was used assess the statistical significance of the difference of an ordinal variable (score) measured twice for the same study group.

Interquartile range

It is the difference between the upper and lower quartile values in each result.

RESULTS

Comparison of H_2O_2 level between lesional and non-lesional skin of all patients

Using student T test the H_2O_2 level in the lesional skin it was 41.3+/-53.42 ($p< 0.01$) (highly significant) while in the non-lesional skin was 27.44+/-5.2 with +/-SD 53.42 in the lesional skin and 5.20 in the non-lesional skin **(Table 1).**

Table 1: comparison of H_2O_2 level among all cases

	Mean	±SD	Minimum	Maximum	Median	IQR*	
Lesional skin	41.30	53.42	24.12	320.00	28.90	26.70	35.31
Non-lesional skin	27.44	5.20	23.09	47.94	26.51	24.42	27.59

*Interquartile range.

Table 2: H_2O_2 level in lesional vs. non-lesional skin of all patients.

	Mean	±SD	Median	P	Sig
lesional	41.30	53.42	28.90	0.001	**HS**
Non-lesional skin	27.44	5.20	26.51		

Comparison between H_2O_2 level in lesional skin vs. non-lesional skin in the segmental vitiligo patients group

We assayed H_2O_2 level in lesional skin in segmental vitiligo (SV) patients and it was 28.66+/-3.99, while in non-lesional skin it was 26.38+/-2.55, (p=0.01) showing highly significant increase of H_2O_2 in the lesional skin above non-lesional skin of the SV patients with a SD +/- 2.55 in non-lesional skin and 3.99 in lesional skin **(Table 3).**

Table 3: comparison between H_2O_2 level in lesional vs. non-lesional skin in SV patients.

	Mean	±SD	Median	P	Sig
Lesional skin	28.66	3.99	27.83	0.01	**HS**
Non-lesional skin	26.38	2.55	26.38		

Student T test was used as an analytical method to assess the significance.

Comparison in H_2O_2 level between lesional vs. non-lesional skin in all non-segmental vitiligo (NSV) patients group

Using Wilcoxon analytical test, there was a highly significant difference in H_2O_2 level with the lesional skin having higher H_2O_2 level than the non-lesional skin (H_2O_2 level lesional skin was 53.93+/-74.52 while in non-lesional skin it was 28.49+/-6.86, (p=0.001) **(Table 4).**

Table 4: comparison between H_2O_2 level in the lesional vs. non-lesional skin in non-segmental vitiligo patients

	Mean	±SD	Median	P	Sig
lesional skin	53.49	74.52	31.64	0.001	**HS**
Non-lesional skin	28.49	6.86	26.77		

Comparison between H_2O_2 level in lesional and non-lesional skin in active segmental vitiligo (SVa) patients

There was no significant difference regarding H_2O_2 level in the lesional skin in comparison with the non-lesional skin in SVa patients (p= 0.057) using student T test as an analytical statistics. **(Table 5).**

Table 5: comparison in H_2O_2 level between the lesional and non-lesional skin in active segmental vitiligo (SVa) patients

	Mean	±SD	Median	P	Sig
Lesional skin	27.05	3.71	25.53	.057	NS
Non-lesional skin	25.04	1.52	24.48		

Comparison between lesional and non-lesional skin in active non-segmental (NSa) vitiligo patients as regards H_2O_2 level.

Comparing H_2O_2 level in the lesional skin of patients with active non-segmental vitiligo (NSa) with the non-lesional skin of patients of the same disease group showed that there was a significant difference in the H_2O_2 level (p=0.018) with a significant increase in the lesional skin (**Table 6).**

Table 6: Comparison of H_2O_2 level in the lesional vs. non-lesional skin in NSa.

	Mean	±SD	Median	P	Sig
Lesional skin	80.67	106.51	33.67	.018	**S**
Non-lesional skin	29.37	8.34	27.19		

Comparison between H_2O_2 level in lesional vs. non-lesional skin in stationary segmental vitiligo (SVs) patients

Using student T test there was significant difference in H_2O_2 level with the lesional skin 30.50 while the non-lesional skin was 27.90 **(Table 7).**

Table 7: comparison between lesional and non-lesional skin in SVs.

	Mean	±SD	Median	P	Sig
lesional skin	30.50	3.70	29.86	.018	S
Non-Lesion skin	27.90	2.73	27.31		

Comparison between H_2O_2 level in lesional vs. non-lesional skin in stationary non-segmental vitiligo (NSs) patients:

There was a highly significant difference in the H_2O_2 level in lesional skin compared to the non-lesional skin of the NSs patients (p=0.007) using student T test **(Table 8).**

Table 8: comparison between H_2O_2 level in lesional and non-lesional skin in NSs patients.

	Mean	±SD	Median	P	Sig
lesional skin	30.53	6.56	28.04	.007	**HS**
Non-Lesional skin	27.73	5.74	24.82		

Student T test was used in the assessment of the significance of this group.

Comparison between H_2O_2 level between active and stationary segmental vitiligo (SV) cases regarding lesional and non-lesional skin

While, in case of H_2O_2 level between lesional skin in SVs vs. SVa there was a non-significant statistical difference with the p value was 0.095.

There was a significant increase in the H_2O_2 level in the non-lesional skin of patients with SVs when compared to the H_2O_2 level of non-lesional skin in patients with SVa (p= 0.024). **(Table 9).**

The lesional skin of SVs patients has non-significant increase in the H_2O_2 level above the lesional skin of SVa patient group; while regarding H_2O_2 level in the non-lesional skin, the non-lesional skin of SVs patients have higher H_2O_2 level than that of SVa.

Table 9: comparison between H_2O_2 level between SVa and SVs patients regarding lesional and non-lesional skin

	Activity						P*	Sig
	Stationary			Active				
	Mean	±SD	Median	Mean	±SD	Median		
lesional skin	30.50	3.70	29.86	27.05	3.71	25.53	.095	**S**
Non-Lesional skin	27.90	2.73	27.31	25.04	1.52	24.48	.024	NS

Comparison between H_2O_2 level in NSa vs. stationary NSs each lesional and non-lesional skin

Using paired T test and Wilcoxon signed rank test showed that the statistical significance between the non-lesional skin in patients with NSa and non-lesional skin. In patients with NSs was found to be non-significant with the p value 0.662; while the H_2O_2 level in the lesional skin of patients with NSa when compared to that of patients with NSs was found to be significantly increased in those with NSa with the p value was 0.021 and the SD was+/- 106.51. **(Table 10).**

Table 10: comparison between H_2O_2 level between NSs and NSa patients both lesional and non-lesional skin

	Activity						P	Sig
	Stationary			Active				
	Mean	±SD	Median	Mean	±SD	Median		
Lesional skin	30.53	6.56	28.04	80.67	106.51	33.67	0.021	S
Non-Lesional skin	27.73	5.74	24.82	29.87	80.34	27.19	0.662	**NS**

DISCUSSION

Vitiligo is known as a disorder in pigmentation due to the anatomical disappearance of epidermal melanocytes by histopathological examination leading to the disappearance of the skin color and the development of the depigmented patches characteristic for the vitiligo[(8)].Two subtypes of vitiligo are known, the non- segmental and the segmental vitiligo. The non-segmental subtype is more or less bilateral and develops in adulthood with a progressive course while the segmental type is unilateral in distribution with early onset during childhood [(11)]. Several theories for the pathogenesis of SV have been described, which are the neural theory, somatic mosaicism and the microvascular skin homing of immune cells, whether leading to an autoimmune destruction of melanocytes or not [(12)].

The neural theory was the main accepted one for the pathogenesis of SV for years. The theory suggested local neuronal abnormalities (e.g. subacute encephalitis, spinal cord lesions). It is mainly based on the old concept that SV lesions follows a dermatomal distribution [(13)].

The theory of microvascular skin homing suggested that the midline delineation in the unilateral lesions of SV represents the cytotoxic T cells patterns of migration from a specific lymph nodes along the microvascular system [(15)]. Our study showed high H_2O_2 level in the lesional skin of SV patients, this indicates that oxidative stress might play an important role in the initiation of SV. As these lesions were found to be not followed the dermatomal lines strictly [(13)], so SV lesions might represent a different population of the melanocytes which represent different metabolism and antigenicity. This opinion is supported by the Somatic Mosaicism Theory [(14)].

Schallreuter *et al.*[(16)] demonstrated high epidermal H_2O_2 and also $ONOO^-$ in strictly SV (SSV) patients as well as in the segmental part of mixed vitiligo without including healthy controls nor fixation of the skin type. There was no differentiation between active and stationary lesions of the SV in the forementioned study.This implies the importance of the disturbed redox balance in the initiation and progression of vitiligo either non-segmental or segmental [(15)]. Surprisingly, there was a non-significant difference in H_2O_2 level in patients with SVa between lesional and non-lesional skin, however, the accurate differentiation between active and stationary SV is not always clear. There was a significant difference between the lesional skin of NSV and that of SV regarding H_2O_2 level being higher in the lesional skin of NSV and this refers to that the disturbed redox balance plays an important role in the NSV than its role in SV that results from different population of melanocytes during embryogenesis. **Schallreuter *et al.*** [(16)] induced repigmentation in patients with NSV by the Dead Sea climatotherapy and topical application of pseudocatalase (PC-KUS) to remove oxidative stress and this was the results of our study. There was a shooting level of H_2O_2 in patient no.21 who had NSa with duration of vitiligo 5 years, duration of activity 5 months and history of smoking from 17 years. This patient also had high antithyroid antibody titres and thus he had autoimmune state raising the H_2O_2 titre [(17)]. Another option for the measurement of H_2O_2 could be oxidative stress measurement device, an optical analyzer which has a light source and a light detector which is used for the measurement of an optical property of a special

medium to generate optical measurement data. The device has a processor, which analyzes the optical measurement data and can generate a value for one or more oxidative stress component. The data for the presence of an oxidative stress dependent disease can be measured and calculated **(15).**

CONCLUSION

In conclusion, this study suggested that oxidative stress was present in both SV and NSV. This might open a window for several studies concerning the patients of SV and whether the oxidative stress is primary or secondary. Also whether these patients might benefit from antioxidant medications or not.

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