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Evaluation of Serum Level of Glutathione Peroxidase Activity in Vitiligo Patients

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Abstract

Background: Vitiligo is an acquired disorder with loss of epidermal melanocytes. Oxidative stress is thought to play a significant role in the pathogenesis of vitiligo. Glutathione Peroxidase (GPx) is one of the most important antioxidant enzymes.

Aim of Study: To estimate serum levels of Glutathione Peroxidase activity in patients with vitiligo to assess its role in the disease activity.

Patients and Methods: This study included (60) patients with vitiligo and (20) healthy individuals served as a control group. They were collected from the Outpatient Clinic of Dermatology and Venereology Department Tanta University Hospital. Serum levels of glutathione peroxidase activity were estimated in the patients and control group.

Results: Serum GPx activity level showed a statistically significant increase in vitiligo patients compared to control group.

Conclusion: Oxidative stress is thought to play a significant role in the pathogenesis of vitiligo represented by significantly increased GPx activity level in vitiligo patients.

Key Words: *Vitiligo – Glutathione peroxidase.*

Introduction

VITILIGO is an acquired disease with circumscribed depigmented macules and patches due to loss of functioning melanocytes. Vitiligo may develop anywhere on the body [1]. Sites that are normally relatively hyperpigmented such as the face, dorsal aspect of the hands, axillae, umbilicus, nipples, sacral, inguinal and anogenital regions are the common regions. The incidence is 0.1% to 2.0% worldwide [2].

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There are two types of vitiligo: The first is Segmental Vitiligo (SV) and the second is Non-Segmental vitiligo (NSV). Non-segmental vitiligo is classified into (generalized, universal, focal, acrofacial, and mucosal vitiligo). Both sexes may be affected. In vitiligo, there is absence of melanocytes in the skin lesion due to its destruction [3].

Vitiligo is a complex phenomenon in which genetic and non-genetic factors are encountered. In vitiligenous skin, there is no functional melanocytes.Destruction of melanocytes leads to loss of histochemically recognizable melanocytes [1].

Oxidative stress has an important factor in the pathogenesis of vitiligo, according to the self-destructive theory of melanocytes in the pathogenesis of vitiligo [4]. During several physiological and pathological processes, there is production of several free radicals like Reactive Oxygen Species (ROS), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and nitric oxide [5]. These free radicals are scavenged by antioxidant enzymes and non-enzymatic antioxidants constantly. Antioxidant enzymes such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione reductase, and Catalase (CAT), and non-enzymatic antioxidant as Beta-carotene, Vitamin C, and Vitamin E. In oxidative stress, antioxidant activity is decreased leading to increase in free radicals, which destroy cellular compounds like protein, carbohydrate, DNA, and lipids [6].

Glutathione Peroxidase (GPx) is a family of enzymes which has peroxidase activity. Its main action is to defend cells from oxidative injury by reducing free H_2O_2 to H_2O and reducing lipid hydroperoxides to their corresponding alcohols [7].

In humans, there are 8 identified isoforms of glutathione peroxidase (GPx1-8). GPx1, GPx3, and GPx4 are the most well described [8].

GPx-1 is an antioxidant enzyme which is the most plentiful in human tissues as it is found in the cytosol of most cells including RBCs. GPx1 is counteracting oxidative stress enzyme as it is the principal enzyme that decreases H_2O_2 and other hydroperoxides in cytosol and mitochondria [9].

GPx-3, known as plasma glutathione peroxidase (GPx-P) or extracellular glutathione, occurs in the plasma as a glycoprotein and is considered the most useful extracellular antioxidant enzyme [9].

Membrane-bound glutathione peroxidase GPx-4 (phospholipids hydroperoxide GPx) decreases esterified lipids as it has the ability to decrease lipid-hydroperoxides inside biological membranes [9].

Patients and Methods

Patients:

This study included (60) patients with Vitiligo collected from the Outpatient Clinic of Dermatology and Venereology Department Tanta University Hospital (from February 2017 to February 2018). They were assessed according to VASI and VIDA scores. In addition to, (20) healthy individuals with matched age and sex served as a control group.

The study was approved by Research Ethics Committee at Faculty of Medicine, Tanta University, approval code (31373/02/17).

Inclusion criteria:

1- Newly diagnosed cases with Vitiligo.

2- Cases previously diagnosed and treated but stopped treatment for 3 months before enrollment in this study.

Exclusion criteria:

1- Patients under treatment (systemic, topical or phototherapy).

2- Patients who have systemic diseases (DM, thyroid disease, autoimmune disorders).

3- Patients who have a concomitant dermatological disease.

4- Pregnancy and lactation.

5- Obesity and BMI more than thirty.

6- Patients receiving drugs affecting lipid values.

Method:

All studied individuals were subjected to:

1- Complete history taking.

2- General and dermatological examination to exclude any systemic or dermatological diseases.

3- Ocular and audiological examination to exclude any associations with vitiligo.

4- Wood's lamp examination.

5- VASI and VIDA scores were assessed.

6- Photographs to the affected sites.

7- Written informed consent was taken before starting.

Sampling:

Seven ml of venous blood was withdrawn from each subject after fasting 10-12 hours. Two ml was delivered to EDTA tube for complete blood picture. The remaining blood was delivered to a plain tube and centrifuged. The serum was separated and divided into two aliquots. One aliquot used to determine liver, renal functions, glucose level and lipid profile at once. The remaining aliquot was stored at 20ºC until assay of glutathione peroxidase activity level.

Serum level of Glutathione Peroxidase activity level (GPx) by Enzyme-Linked Immunosorbent Assay method.

Results

This study included 60 patients with vitiligo and 20 healthy persons with matched age and sex served as control group. Patients' demographic data, vitiligo type, duration of the disease, family history, activity, Vitiligo Area Severity Index (VASI) and Vitiligo Disease Activity Score (VIDA) discussed in (Tables 1,2).

Serum GPx activity level showed a statistically significant increase in vitiligo patients compared to control group ($p<0.001$) group (Table 3) and Graph (1). While the difference between segmental and nonsegmental groups was statistically non-significant.

In both segmental and non-segmental groups, the difference between serum activity level of GPx in active and stable cases was statistically non-significant group (Table 5) and Graph (2).

The correlations between serum activity level of GPx and clinical data including (activity, age, sex, duration, VASI and VIDA) in each group were statistically non-significant (Table 4).

Table (1): Patients' demographic data.

	Cases (n=60)		Cases (n=20)		Test of sig.	*p*
	No.	%	No.	%		
Sex:						
Male	18	30.0	8	40.0	χ^2=	0.408
Female	42	70.0	12	60.0	0.684	
Age (years):						
Min.-max.	7.0-60.0		10.0-60.0		U=	0.718
Mean ± SD	29.95±14.45		29.35±16.94		567.50	
Median	27.50		26.0			
BMI (kg/m^2):						
Min.-max.	19.20-29.10		19.80-29.10		*t*=	0.560
Mean ± SD	24.60±2.79		24.17±2.87		0.586	
Median	25.40		24.95			

p : χ^2 and *p*-values for Chi square test for comparing between the two groups.
U, *p* : U and *p*-values for Mann Whitney test for comparing between the two groups.
t, *p* : *t* and *p*-values for student *t*-test for comparing between the two groups.

Table (2): Patients' data.

Vitiligo type	No.	%
Segmental	15	25.0
Non-segmental	45	75.0
• Generalized	20	33.3
• Localized	10	16.7
• Acrofacial	9	15.0
• Acral	6	10.0

	Segmental (n=15)		Non-segmental (n=45)	
	No.	%	No.	%
Duration (years):			31	68.9
<5	10	66.7	14	31.1
≥5	5	33.3	0.08-32.0	
Min.-max.	0.13-22.0		4.04±6.07	
Mean ± SD.	5.67±8.67		1.50	
Median	1.0			
Family history:				
Negative	13	86.7	29	64.4
Positive	2	13.3	16	35.6
Activity:				
Active	11	73.3	32	71.1
Stable	4	26.7	13	28.9
VA SI (%):				
Min.-max.	10.0-30.0		10.0-90.0	
Mean ± SD.	20.67±6.23		32.22±22.55	
Median	20.0		20.0	
VIDA:				
Min.-max.	0.0-2.0		0.0-4.0	
Mean ± SD.	1.20±0.86		1.56±1.32	
Median	1.0		1.0	

Table (3): Comparison between the different studied groups according to GPx.

	Segmental (n=15)	Non-segmental (n=45)	Control (n=20)	H	*p*
GPx (mU/ml):					
• Min.-max.	6.0-124.0	6.0-74.0	3.0-50.0	25.464*	<0.001*
• Mean ± SD	39.87±42.69	18.03±12.40	9.45±10.0		
• Median	19.0	15.0	8.0		
• Sig. bet. Grps	*p*1=0.199, *p*2<0.001*, *p*3<0.001*				

F, *p* : F and *p*-values for ANOVA test, Sig. bet. grps was done using Post Hoc Test (LSD).
H, *p* : H and *p*-values for Kruskal Wallis test, Sig. bet. grps was done using Post Hoc Test (Dunn's multiple comparisons test).
p_1 : *p*-value for comparing between segmental and non-segmental.
p_2 : *p*-value for comparing between segmental and control.
p_3 : *p*-value for comparing between non segmental and control.
* : Statistically significant at $p \leq 0.05$.

Table (4): Correlation between GPx and clinical data in each group.

	GPx (mU/ml)							
	Total cases (n=60)		Segmental (n=15)		Non-segmental (n=45)		Control (n=20)	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
• Age (years)	0.058	0.660	0.238	0.393	0.051	0.741	–0.217	0.357
• Sex (female)	–0.102	0.438	–0.312	0.257	–0.002	0.990	–0.089	0.708
• BMI (kg/m^2)	0.024	0.853	0.324	0.239	–0.007	0.966	0.089	0.708
• Duration (years)	0.037	0.779	–0.229	0.411	0.059	0.700		
• Stability	0.175	0.180	0.282	0.309	0.108	0.481		
• Family history	–0.243	0.061	–0.229	0.412	–0.235	0.121		–
• VASI (%)	–0.174	0.182	–0.384	0.157	–0.106	0.487		–
• VIDA	0.077	0.558	0.174	0.534	0.115	0.452		–

r_s : Spearman coefficient.
* : Statistically significant at $p \leq 0.05$.

Table (5): Relation between activity and GPx in each group.

	Activity			
	Segmental (n=15)		Non-segmental (n=45)	
	Active (n=11)	Stable (n=4)	Active (n=32)	Stable (n=13)
GPx (m U/m l):				
Min.-max.	6.0-88.0	17.0-124.0	6.0-40.0	10.0-74.0
Mean ± SD.	28.73±29.91	70.50±61.78	15.73±7.08	23.68±19.63
Median	19.02	70.50	15.0	15.0
U (*p*)	14.00 (0.292)		179.50 (0.474)	

t, *p* : *t* and *p*-values for Student *t*-test.
U, *p* : U and *p*-values for Mann Whitney test.
* : Statistically significant at $p \leq 0.05$.

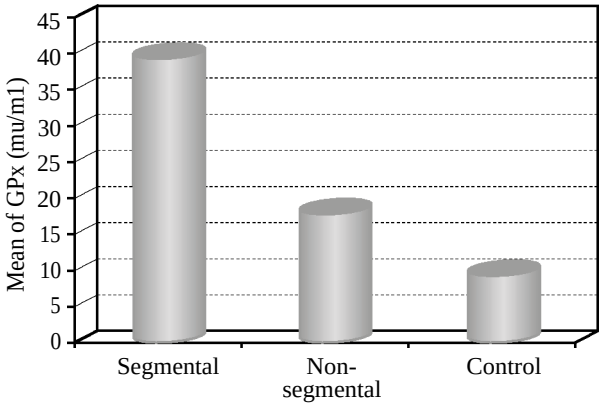

Fig. (1): Comparison between the different studied groups according to GPx.

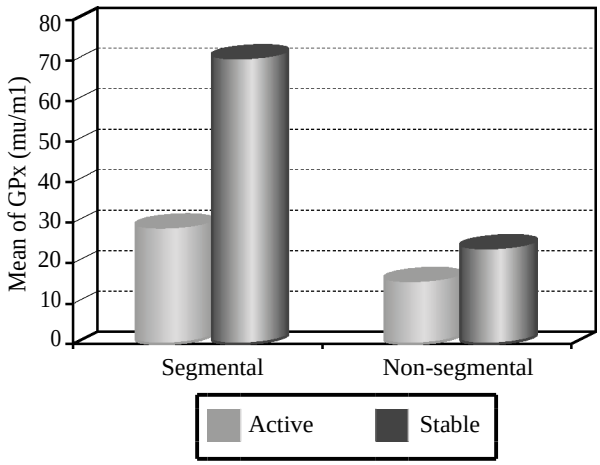

Fig. (2): Relation between activity and GPx (mU/ml) in each group.

Discussion

Vitiligo is an acquired disease of pigmentation in which epidermal melanocytes are absent. It involves mostly the skin and occasionally the mucosa and hair. Oxidative stress has a big role in the pathogenesis of vitiligo. Glutathione Peroxidases (GPx) are important antioxidant enzymes that defend against oxidative stress [10].

As regards serum activity level of Glutathione peroxidase, our study showed a statistically significant increase in serum activity level of Glutathione peroxidase in vitiligo patients both segmental and non-segmental groups than the control group.

By reviewing literature,we have found wide controversial variations in the studies which measured GPx activity.

In agreement with our study, Kamel et al., [11] and Ozturk et al., [12] revealed, an increase in the level of GPx in plasma of vitiligo patients compared to control group. Kamel et al., explained this increase in order to face the increased level of ROS (hydrogen peroxide and lipid peroxide) that has been produced in vitiligo. Ozturk et al., explained their results by lysis of erythrocytes due to increased ROS level in erythrocytes releasing its GPx in plasma increasing plasma GPx level.

Contrary to our results, Zedan et al., [13], Jalel and Hamdaoui [14] and Khan et al., [15] showed a statistically significant decrease in serum GPx activity level in the patients with vitiligo compared to the healthy controls. They explained their results that the low GPx levels in patients with vitiligo may be due to its consumption in neutralizing the increasing levels of the free radicals as GPx not only degrades hydrogen peroxide but also has the ability to neutralize lipid hydroperoxides [16].

Other studies showed that there were no statistically significant differences between serum levels of GPx activity in vitiligo and controls as in the studies conducted by, Barikbin et al., [17] and Batçiog˘lu et al., [18]. They explained their results that melanocyte damage in vitiligo is probably linked to mechanisms other than a disturbance in GPx activity.

Measurement of GPx activity level has shown wide controversies and big contrast. This contrast may be due to different methodologies used for analysis of GPx activity. The complex interaction of biochemical, environmental, and immunologic events, may account for these contrasting results [13].

Some studies examined its plasma level, other studies examined GPx activity in erythrocytes. Several studies showed decreased its activity in erythrocytes like those conducted by (Metta et al., [19]; Ines et al., [4]; Karsli et al., [20] and Dammak et al., [21]. However, another study conducted by Hazneciet et al., [7] didn't find any difference between the erythrocyte GPx levels among the vitiligo patients and healthy controls.

Moreover, epidermal level of Gpx was measured by Kamel et al., [11] and Yildirim et al., [5] revealed an increase in level of GPx in skin of vitiligo patients than the control group and this was in contrast to Passi et al., [22] in which their study on active vitiligo patients revealed decreased epidermal activity of GPx compared to control group.

In our study, the correlations between serum activity level of GPx and clinical data including (activity, age, sex, duration, VASI, VIDA) and lipid profile in each group were statistically non-significant similar to Zedan et al., [13] who found that the correlations between GPx activity level

and the clinical features were statistically non-significant although their results showed statistically significant decrease in GPx activity level.

Conclusion:

Oxidative stress is thought to play a significant role in the pathogenesis of vitiligo through imbalance of oxidant-antioxidant systems including significantly increased GPx activity level in vitiligo patients compared to control group in the present study. So antioxidants may have a role in the treatment of vitiligo.

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تقييم مستوى الجلوتاثيون بيروكسيديز فى مصل مرضى البهاق

يعد مرض البهاق أضطراب جلدى مكتسب يتميز بوجود بقع محددة ناقصة الصبغة تصيب الجلد والشعر والأغشية المخاطية. بالرغم من كثرة الأبحاث يظل سبب حدوث البهاق غير معروف.

الهدف من الدراسة هو قياس مستوى نشاط الجلوتاثيون بيروكسيديز فى مصل مرضى البهاق لتقييم دوره فى المرضى وعلاقته بتطور المرض ونشاطه.

تم أختيار ستين مريضاً ممن يعانون من البهاق وعشرين من الأفراد الأصحاء المتوافقين فى العمر والجنس كمجموعة ضابطة وتم تجميعهم عشوائياً من المترددين على العيادة الخارجية للأمراض الجلدية والتناسلية بمستشفيات جامعة طنطا. لقد خضع كل الأشخاص المشاركين بهذا البحث إلى أخذ التاريخ المرضى كاملاً. فحص إكلينيكى وجلدى شامل. تم قياس مستوى نشاط الجلوتاثيون بيروكسيديز بعد أخذ موافقة كتابية من المشاركين.

نتائج البحث: وجدت زيادة ذات دلالة إحصائية فى مستوى نشاط أنزيم الجلوتاثيون بيروكسيديز فى مصل مرضى البهاق القطعى وغير القطعى عنه فى المجموعة الضابطة. ولكن لم تلاحظ علاقة ذات دلالة إحصائية بينه وبين (مستوى دهون، البيانات الإكلينيكية).

الأستنتاج من هذه الدراسة أنه من المحتمل حدوث مرض البهاق بواسطة عملية الإجهاد التأكسدى ممثلاً هذا بزيادة نشاط أنزيم الجلوتاثيون بيروكسيديز فى الدراسة الحالية.