Evaluation of Oxidative Stress in Patients with Vitiligo in Najaf/ Iraq

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Abstract

Background: It has been suggested that, in vitiligo there was a systemic imbalance of both enzymatic and non-enzymatic antioxidants which could be the source of damage of the melanocytes. Generation of reactive oxygen species and lipid peroxides is associated with this imbalance which could be the possible pathogenic factors in this hypopigmentary disorder.

Objective: The aim of the present study was to determine the levels of antioxidant parameters; glutathione peroxidase (GSH-Px), glutathione (GSH), catalase (CAT) and the level of lipid peroxidation marker Malandialdehyde (MDA) in patients with vitiligo in comparison with healthy controls matched for age and sex and to correlate these levels with the activity , extent of the disease and with age and sex of the patients, aiming to clarify the role of systemic oxidative stress in pathogenesis of vitiligo.

الخلاصة

يعتبر افتراض وجود خلل في النظام العام للمواد المضادة للاكسدة الانزيمية واللاانزيمية في مرض البهاق وهو مصدر تدمير للخلايا الصبغية ويعتبر تولد الاوكسجين الحر ونواتج اكسدة الدهون المصاحبة لهذا الخلل هما العاملان المرضيان الممكنان في هذا المرض .

الهدف من الدراسة

كان الهدف من هذه الدراسة هو تقييم مستويات مضادات الاكسدة مثل انزيم كلوتاثايون بيروكسيديز ومستوى الكلوتاثايون والكتاليز ونسبة ثنائي الدهايد المالون وهو علامة على تاكسد دهون الخلايا في الدم لمرض البهاق ومقارنة ذلك بمجموعة ضابطة من الاصحاء متماثلة في السن والجنس وكذلك دراسة مدى ارتباط هذه النتائج بمدى انتشار المرض وتوضيح دور الاجهاد التاكسدي لكيفية حدوث المرض .

عدد المرضى والمجموعة الضابطة

اجريت هذه الدراسة على (50) مريضا يعانون من مرض البهاق (29 انثى , 21 ذكر) تتراوح اعمارهم (10-55) سنة وثلاثون (30) شخصا اصحاء كمجموعة ضابطة . وثم تقييم مستويات مضادات الاكسدة (كلوتاثايون بيروكسيديز , كلوتاثايون والكتاليز) وكذلك ثنائي الدهايد المالون في الدم للمرضى والمجموعة الضابطة وتم تقسيم المرض الى مجموعة تعاني من البهاق واسع الانتشار (30 مريضا) ومجموعة تعاني من البهاق المحدود (20 مريضا) وتبعا للنشاط المرضي الى مجموعة بها مرض نشط (32 شخصا) ومجموعة مرض ثابت (18 شخصا). وقد اظهرت هذه الدراسة وجود انخفاض ملحوظ ذو دلالة احصائية في مستويات كلوتاثايون بيروكسيديز والكلوتاثايون والكتاليز في مرض البهاق من المجموعة الضابطة وكذلك وجود زيادة معنوية ذات دلالة احصائية في مستوى ثنائي الدهايد المالون في مصل مرض البهاق عن المجموعة الضابطة . وبالنسبة لنشاط وانتشار المرض لا يوجد فرق ذو دلالة احصائية معنوية في مستوى انزيم الكلوتاثايون بيروكسيديز والكلوتاثايون والكتاليز وثنائي الدهايد المالون سواء بين المرض المنتشر والمحدود او النشط والثابت وكذلك بالنسبة للعمر والجنس في مجموعة الدراسة من المرضى .

النتائج

نستنتج من هذه الدراسة انخفاض مستوى مضادات الاكسدة وزيادة مستوى ثنائي الدهايد المالون كمؤشر لاكسدة الدهون يشير الى وجود اجهاد تاكسدي عام يؤدي الى تحلل الخلايا الصبغية وهذا يلقي الضوء على اهمية الاجهاد التاكسدي العام في حدوث مرض البهاق .

التوصيات

اصبح من الضروري اجراء دراسات اخرى لتحديد كيفية حدوث الاجهاد التاكسدي المسؤول عن هذا الخلل واستخدام مضادات الاكسدة كعلاج لمرض البهاق .

Introduction

Vitiligo is an idiopathic, acquired depigmenting skin disorder characterized by milky white patches of different sizes and shapes. It affects 1-2% of the world population [1,2]. Besides the most popular autoimmune theory, several studies have shown the involvement of oxidative stress in the pathogenesis of this disease[3]. Furthermore, histological examination of involved and uninvolved skin of patients with vitiligo shows vacillation to variable degrees in the total epidermis, which could be indicative of lipid peroxidation[4,5] .

Antioxidant is a substance that when present at low concentrations compared to that of an oxidizable substance, it can act by scavenging biologically important reactive oxygen species (O_2, H_2O_2, OH, peroxyl) by preventing their formation or by repairing the damage that they do . Fig (1) [6] The antioxidants can be divided into either endogenous antioxidants present normally in biological system or exogenous antioxidants which can be administrated exogenously [7]. The endogenous antioxidant substances include enzymatic such as super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and non- enzymatic such as vitamin E (α - tochopheral), and reduced glutathione (GSH) [8] .

Oxidative stress may be induced by increasing the generation of reactive oxygen species (ROS) and other free radicals [6]. The generation of ROS is known to be associated with a decrease in antioxidants levels[9]. Many studies revealed blood changes of oxidants-antioxidants in vitiligo patients [2,10,11]. Therefore, this study was conducted to evaluate the changes in serum levels of some oxidants (Malondialdehyde) (MDA) and antioxidants (glutathione, catalase activity and glutathione peroxidase enzyme activity) in some subgroups of vitiligo patients and make a comparison between them.

Patients and control subjects

Sera of fifty patients with vitiligo include 23 males and 27 females were obtained from dermatology department in Al - Sader Medical Teaching City / Najaf/ Iraq with age ranged between (10 – 55) years and the results were compared with another 30 healthy individuals with same age and range. Patients suffered from other disease interferes with data excluded in the current study. The study was carried out at the Department of Biochemistry. College of Medicine, University of Kufa.

Material and Method

Chemical and Apparatus

All laboratory chemical and reagents were of analar grade . Trichleroacetic acid ,was obtained from Hopking Williams, Thiobarbituric acid from Merek Germany Co. ltd, reduced glutathione from Biochemical's Co. Ltd, Hydrogen peroxide from Merek CO. Ltd, (di-potassium hydrogen phosphate, potassium dihydrogen phosphate and di-sodium hydrogen phosphate from Merek Germany Co.ltd) were used during our study.

Blood specimens

Disposable syringes and needles were used for blood collection . Blood samples were obtained from patients and control group by vein puncture. Sample were allowed to clot at 37 ^{0}C then centrifuged at 3000 Xg for 10 minutes. Sera were removed and stored at -20 ^{0}C until analysis time. The patients were divided according to extent of the disease into two groups, group with localized vitiligo (21-p) patients and generalized (29 - p). Groups are subdivided into active disease (32-p) patients was defined on basis of progression or appearance of new lesions with in the last three months , while stable phase (18-p) patients was characterized on the basis of lack of progression or appearance of new lesions in the last six months [12] and according to the duration

of disease into 3 groups , group 1(less than 1 year , group 2 (1-10 years) and group 3 (more than 10 years).

Methods

GSH-Px was assayed according to the procedure Rotruck etal with some modification[13], Glutathione was assayed as described by Burits etal [14] (by spectrophtometric assay based on 5,5-dithiobis - nitrobenzoic acid). Catalase CAT activity assayed as described by Aebi H[15]. Malondialdehyde was assayed using thiobarbituric reactive substances method described by Guidet B and Shah [16].

Biostatical analysis

The results were expressed as mean ± SD. Students t-test was used for comparison of results of patients and the control group. Significant variation was considered when p value was less than < 0.05. The correlate between the values of oxidative stress parameters and various factor (different groups, age, duration) were performed by the liner regression analysis (Table 3).

Results

The levels of GSH-Px, GSH, CAT were significantly lower in vitiligo patients compared to controls (Table 1). Furthermore, serum MDA was significantly higher in vitiligo patients compared to controls (Table 1). There was a highly significant inverse correlation between plasma levels of CAT, GSH and serum levels of MDA (Table 3). Regarding the activity and extent of disease (Table 5,6), there was insignificant difference in serum levels of GSH-Px, GSH , CAT& MDA either in generalized or localized vitiligo whether it was in active or stable course. There was insignificant difference in the serum levels of MDA, GSH-PX, GSH and CAT regarding the age, sex and duration of the disease in the studied patients (Table 4,2).

Discussion

Vitiligo is an idiopathic acquired circumscribed hypomelanotic skin disorder resulting from loss of pigment forming melanocytes[17]. There are three hypothesis to explain vitiligo, the immune hypothesis , the neural hypothesis, and the self destructive hypothesis[18,19]. Oxidative stress acts as the triggering event in melanocyte degeneration in vitiligo [20,21]. Maresca et al[22] had reported an imbalance of both enzymatic and non enzymatic antioxidants and suggested that this imbalance could be either the source or consequence of peroxidative damage of melanocytes. Glutathione peroxidase (GSH-Px) is an antioxidant enzyme that protects the membranes and essential proteins from potential damaging effect of reactive oxygen and lipid peroxide. It converts H_2O_2 and other peroxides into water [23,6] .

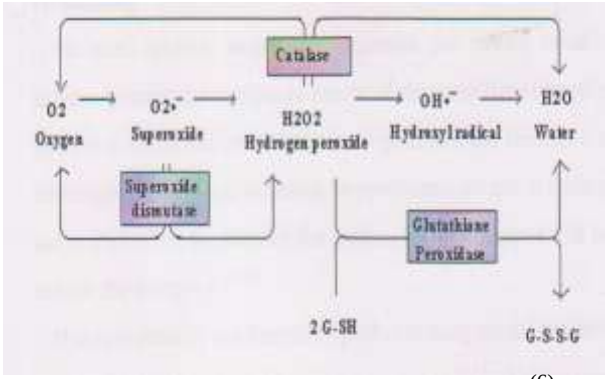

Fig 1. Action of antioxidant enzymes [6].

Table 1. Serum glutathione peroxides (GPx), reduce glutathione (GSH) , catalase (CAT) , malondieldehyde (MDA) and control group

parameters	groups	mean	± SD	Range	P
GPx	C	12.78	4.06	7.74 – 16.88	
(U /ml)	P	6235	1.309	1.01 – 7.7	< 0.001
GSH	C	283	45	141 – 566	
(mmol/L)	P	157	30	79 – 314	< 0.001
CAT	C	55	2.85	51 – 60	
(k / ml)	P	20.6	5.65	10.1 – 28.7	< 0.001
MDA	C	1.07	0.29	0.5 – 1.46	
(µM)	P	3.43	1.21	1.65 – 7.4	< 0.001

C = control = 30 healthy

P = patients = 50 vitiligo

Based on the results obtained in this study, the serum glutathione peroxidase activity was significantly lower in vitiligo patients in comparison to controls (P value = 0.001). This finding is consistent with the results that obtained previously [2,3,9,11,24]. GSH-Px has been observed to have a higher affinity for H_2O_2 than catalase [21]. For this reason, GSH-Px activities may be diminished in our vitiligo patients to compensate the increase in free radicals and H_2O_2. Furthermore, Hasse et al.[25] reported that the accumulation of millimolar concentration of H_2O_2 leads to influence of antioxidant enzymes.

In contrary to the curent results, Picardo et al.[20] and Passi et al.[21] had found that there was a non significant changes of GSH-Px activity in blood and skin of vitiligo patients respectively. Moreover, Yildrim et al.[26] had found a significant high level of GSH-Px activity in vitiliginous skin.

Regarding patients' age, the serum GSH-Px activity had been decreased in all age groups of vitiligo patients with no significant difference among different age groups (Table 2). These results was in accordance with the results obtained by Agrawal et al.[2] and Beazley et al.[3] who found that erythrocyte and serum GSH-Px activity were low in vitiligo patients without significant difference among different age groups (Table 2). glutathione (GSH) plays an important role in the prevention of radical mediated injury to the body. During oxidative stress, GSH levels decline and oxidized glutathione increases[27] (Fig 1).

In the present study plasma GSH levels were significantly lower in vitiligo patients compared to controls (P value = 0.001) (Table 1). This finding was consistent with the results obtained by other investigators [2,9,21,28], who had found a significant lower serum and tissue GSH levels in vitiligo patients in comparison to controls (Table 1). The decreased level of GSH in vitiligo may be explained by more production of free radicals which leads to consumption of antioxidant defense system including GSH. On the other hand, our result disagree with the results obtained by Picardo et al.[20] who had found no significant difference in GSH levels in vitiligo patients and controls. Human cells posses an efficient antioxidant system for protection against and control of the toxic effects of the free radicals. Assessment of serum antioxidants may give an idea about the oxidative status of the body[29].

In the present study, there was a highly significant decrease in the plasma levels of catalase (CAT) in vitiligo patients in comparison to controls (P value= 0.001) (Table 1). This result was in agreement with the previous studies[2,3,25,28,30] that indicated that there were a significant decrease in both enzymatic antioxidant such as glutathione peroxidase, catalase, glucose-6- phosphate dehydrogenase and non enzymatic antioxidants as glutathione, in vitiligo patients. The significant lower level of catalase may be attributed to the

decrease in both enzymatic and non enzymatic antioxidants in the form of decreased levels of glutathione peroxidase enzyme activity and levels of glutathione. Furthermore, a non significant changes in serum antioxidant in vitiligo patients was also reported [20,22,31,32]. Regarding patients' age and sex, it has been evident from our study that the plasma levels of catalase had non significant difference between different age and sex of studied groups (Table 2).

Lipid peroxidation involves the membrane associated poly-unsaturated fatty acids of phospholipids as a major manifestation of oxidative stress[20]. Malondialdehyde (MDA) is an end product of lipid peroxidation and an indicator of oxidative stress[23,6].

Table 2. Serum glutathione peroxides (GPx), reduce glutathione (GSH) , catalase (CAT) , malondieldehyde (MDA) and control group according to different age

Parameter	Groups	Mean	± SD	range	P
GPx (U / ml)	P1(10 – 25)	3.46	0.62	0.4 – 3.2	NS
	C1(10 – 25)	4.2	1.25		
	P2(26 - 40)	2.118	0.655	0.5 – 3.8	< 0.001
	C2(26 – 40)	4.39	1.6		
	P3(41 – 55)	1.87	0.42	0.3 – 2.4	<0.005
	C3(41 – 55)	3.19	1.24		
GSH (µmol/L)	P1(10 – 25)	157	30	79 – 314	< 0.001
	C1(10 – 25)	283	45	141 – 566	
	P2(26 - 40)	135	31	77 – 270	< 0.001
	C2(26 – 40)	272	54	136 – 544	
	P3(41 – 55)	125	41	65 – 250	<0.005
	C3(41 – 55)	296	74	148 – 592	
CAT (k/ml)	P1(10 – 25)	23.45	5.85	10.45 – 31.35	< 0.001
	C1(10 – 25)	53.59	1.95	50.5 – 57	
	P2(26 - 40)	19.99	5.45	10.1 – 28.7	<0.005
	C2(26 – 40)	55.45	3.1	51.2 – 60.3	
	P3(41 – 55)	17.47	5.4	10.35 – 28	< 0.001
	C3(41 – 55)	55.25	1.75	51.4 – 60	
MDA (µM)	P1(10 – 25)	2.93	1.27	0.48 – 4.9	< 0.001
	C1(10 – 25)	0.9	0.22	0.59 – 1.31	
	P2(26 - 40)	3.45	1.07	1.6 – 6.06	< 0.001
	C2(26 – 40)	1.025	0.33	0.51 – 1.44	
	P3(41 – 55)	4.07	1.08	1.85 – 6.15	< 0.001
	C3(41 – 55)	1.15	0.275	0.675 – 1.5	

P = number of patients p1 = 25 p2 = 15 p3 = 10

C = number of controls c 1 = 10 c2 = 10 c3 = 10

NS = non significance

SD = standard diviation

Table(3): Correlation of Glutathione peroxidase (GSH-Px),Reduced glutathione(GSH), Catalase (CAT) activities and Malondialdehyde in age related groups of vitiligo patient and the control group.

Parameter		r	P
GPx (U / ml)	Patient	- 0.46	< 0.005
	Control	0.22	NS
GSH (µmol / L)	Patient	- 0.39	< 0.005
	Control	0.20	NS
CAT (k / ml)	Patient	- 0.33	< 0.005
	Control	0.16	NS
MDA (µM)	Patient	0.41	< 0.005
	Control	014	NS

NS = nonsignificance

Table 4 : Serum antioxidant & serum MDA in vitiligo patients regarding the duration

Parameters		< 1 year N = 25	1 – 10 year N = 15	> 10 years N = 10	P
GPx (U / ml)	Mean ± SD	2.57 ± 0.84	3.8 ± 0.95	2.82 ± 0.7	0.09 NS
GSH (µmol / L)	Mean ± SD	122 ± 24.4	118 ± 16.86	110.6 ± 22.12	0.110 NS
CAT (k / ml)	Mean ± SD	22.4 ± 3.7	18.6 ± 3.1	17.6 ± 2.9	0.056 NS
MDA (µM)	Mean ± SD	2.57 ± 1.29	3.15 ± 0.8	3.8 ± 0.95	0.422 NS

NS = nonsignificance
N = number of patient

Table 5 : Variation in the level serum of antioxidants and serum MDA according to extent of vitiligo patients

Parameters		Localized vitiligo N = 21	Generalized vitiligo N = 29	P
GPx (U / ml)	Mean ± SD	2.88 ± 0.82	3.38 ± 1.1	0.708 NS
GSH (µmol / L)	Mean ± SD	160.8 ± 26	158 ± 17.8	0.59 NS
CAT (k / ml)	Mean ± SD	18.6 ± 4.7	20.5 ± 5.13	0.59 NS
MDA (µM)	Mean ± SD	3.3 ± 1.1	3.9 ± 1.3	0.708 NS

NS = nonsignificance

Table 6 : Variation in the level serum of antioxidants and serum MDA in vitiligo patients according to activity of the disease

Parameters		active vitiligo N = 32	stable vitiligo N = 18	P
GPx (U / ml)	Mean ± SD	2.73 ± 0.68	3.2 ± 0.64	0.77 NS
GSH (µmol / L)	Mean ± SD	155 ± 25.8	145 ± 26	0.22 NS
CAT (k / ml)	Mean ± SD	20.8 ± 4.16	18.8 ± 3.56	0.7 NS
MDA (µM)	Mean ± SD	3.33 ± 1.12	3.6 ± 0.9	0.613 NS

NS = nonsignificance

In our study, we have found a highly significant increase in the serum levels of MDA in vitiligo patients in comparison to control (p value = 0.001) (Table 1). Similar result was also reported by many investigators [2,9,11,26,33]. However, Picardo et al.[20] reported a non-significant changes in serum (MDA) of vitiligo patients. The high lipid peroxide levels in vitiligo patients could be explained by the fact that GSH-PX neutralizes lipid hydroperoxides. So low levels of GSH-PX in vitiligo patients could lead to oxidative stress, which is evident in high lipid peroxide (MDA levels) in these patients [2].

In this study, there was inverse significant correlation between serum MDA and plasma glutathione, which is evident in high lipid peroxide (MDA levels) glutathione levels and CAT. These data could further prove that low glutathione level and low CAT are associated with increased lipid peroxidation which may cause melanocyte destruction. These results were in accordance with many other studies [2,9].

Regarding the clinical extent and activity of vitiligo, it had been evident from our study that serum antioxidant parameters; GSH-Px enzyme activity, GSH levels and CAT showed no significant difference between generalized and localized vitiligo whether it was in active or stable course. This finding supports the concept of the important role of systemic imbalance in serum oxidants antioxidants (oxidative stress), in the pathogenesis of vitiligo regardless the extent and activity of the disease. Furthermore, lipid peroxidation marker, serum MDA showed also no significant difference between generalized and localized vitiligo whether it was active or stable course. These finding were consistent with the result of Agrawal et al.[2]. Moreover, Yildirim et al.[9] and Yildrim et al.[26] also reported that there was a role of oxidative stress in pathogenesis of vitiligo, among studied patients of generalized stable viitiligo .

From the results it is concluded that the low level of serum antioxidant parameters (GSH-Px, GSH and CAT) and the high serum level of Lipid peroxide marker (MDA) indicated that there was systemic oxidative stress which is probably the possible cause in melanocyte degeneration. This highlights the important role of systemic oxidative stress in the pathogenesis of both active and stable vitiligo. Further studies will be necessary to determine the pathogenesis of oxidative stress responsible for this oxidative imbalance and the use of antioxidant supplementations as a treatment in vitiligo.

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