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Study on the Oxidant and Antioxidant Status in Vitiligo Patients

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Abstract

Backgrounds: The aetiology of vitiligo is still unknown. Several hypotheses have been proposed to explain vitiligo: genetic neural, immunological, self destructive, convergence hypothesis and oxidative stress hypothesis

The current study is concerned with the oxidative stress hypothesis and how oxidants and antioxidants affect the pathogenesis of vitiligo. So, our aim is to determine the role of malondialdehyde and glutathione in the pathogenesis of vitiligo. The amount of malondialdehyde (oxidant) and glutathione (antioxidant) were measured in serum and in skin tissue in 30 vitiligo cases and 20 healthy controls

Results: The study showed significant changes between patients and controls in glutathione level in blood and tissue samples. Also there were significant changes between patients and controls in malondialdehyde in blood and in tissue samples favoring that glutathione and malondialdehyde play a role in the pathogenesis of vitiligo.

Introduction

Vitiligo is a relatively common, acquired pigmentary disorder characterized by areas of depigmented skin resulting from loss of epidermal melanocytes (Odom *et al.,* 2000)

It affects between 1 % and 4 % of the general population, males and females are equally affected and it can develop at any age, but in approximately half of all vitiligo cases onset is before age of 20 years (Koca *et al*., 2004).

The aetiology of vitiligo is still unknown. Several hypotheses have been proposed to explain vitiligo: genetic, neural, immunological, self destructive, convergence hypothesis and oxidative stress hypothesis (Odom *et al*., 2000).

Oxidative stress could act as the initial triggering event in melanocytes degeneration. Free radicals (FRS) are atoms or molecules [e.g. superoxide, hydrogen peroxide & nitricoxide] that occur during several physiological and pathological processes (Weller, 1999).

Free radicals can damage cell compounds such as protein, carbohydrate, DNA and particularly lipid (Knight, 1995).

Insufficient antioxidant protection or excess production of reactive oxygen species (ROS) causes oxidative damage. The balance between oxidative damage and antioxidant enzyme systems appears to determine the physiological and pathological effects of ROS (Passi *et al.*,1998).

The aim of this work is to determine the role of oxidant *(malondialdehyde)* and antioxidant *(glutathione)* In the pathogenesis of vitiligo. This will be done through estimating their levels in blood and tissue of patients with vitiligo.

Patients and Methods

The present study was carried out at out-patient clinic of dermatology department of El Zahraa Hospital of Al- Azhar University. It includes two groups.

Group I: thirty patients suffering from vitiligo, 20 females and ten males with mean age of 32.4 ±14.6 years. They classified further into two subgroups: subgroup A: included 18 patients with generalized vitiligo, 14 females and 4 males with mean age of 30.4 ± 13.5 years and

with skin type II or IV. Two of them were smokers, 16 with history of psychic trauma and 8 with depression.

Subgroup B: included 12 patients with acrofacial vitiligo, 6 females and 6 males with mean age of 30 ± 12.3 years and skin phototype III or IV. Four were smokers and 8 with history of psychic trauma.

Group ll: included twenty healthy individuals as control, 12 males and 8 females with mean age 34.1 ±13.2 years, and skin phototype III or IV.

All patients and controls has no history of previous treatment of vitiligo for at least 8 weeks prior to inclusion in the study, no liver or heart disease, no recent or previous chemotherapy, no long term drug use for any chronic disease.

For all of them we had measured levels of glutathione and malondialdehyde in serum and skin tissue.

Estimation of GSH in blood was carried out according to the method of Toth *et a1*.(1986) which is based on the reduction cleav-age of 5- 5' dithiobis (2-nitrobenzoic acid).

Steps

1. Two ml: H2S04 (0.08) were added to 0.25 ml fresh blood, mixed well by vortexing and left for ten minutes.
2. 0.25 ml sodium tungestate was added and mixed well and left over night at 2- 8°c.
3. Mixture was centrifuged for 10 minutes at 5000rpm.
4. 0.3 ml of supernatant was added to 1 ml Tris buffer and 100ml DTN13 and mixed well.
5. A glutathione standard curve was constructed and absorbance was deter-mined spectrophotometrically at 14 nm against a blank.

Estimation of serum level of MDA as a marker of lipid peroxidation: this was done according to the methods of Sat oh (1978).

Steps:

1. 2.5 ml of 20 mg/ dl TCA were added to 0.5ml serum and the tube was left to stand for 10 minutes at room tempe-rature then centrifuged and participate was washed once with 0.05 sulfuric acid.
2. 2.5 ml of 0.05 M sulfuric acid and 3 ml of TBA were added to this precipitate.
3. Heating in boiling water for 30 minu-tes, and then cooling. The resulting chromogen was extracted with 4.0 ml of n-butyl alcohol by shaking. The absorbance was determined at wavele-ngth of 530nm and concentrations of MDA were obtained from a standard curve.

- **Determination of tissue levels of glutathione and MDA:**

- Tissue processing:

Tissues were minced and homog-enized in a lysis buffer containing Tris- Hcl [10 mM PH 7.5], NaCI (150 mM) triton x-100 (1% v/v), and PMSF (I OmM). The homogenized tissues were centrifuged for 45 min. at 4 °c and I5.000g supernatants were used for biochemical studies (Osmak *et aI*., 1997).

Determination of MDA in supernatants: was done according to the method of Oh Kawa *et al*., (1979). Which is based on spectroph-otometric measurement of the color that occurred during the reaction of thio barbituric acid with MDA.

-Determination of intracellular glutathione in the supernatant:

This was measured by the modified Teitz method (1969). The GSH content was measured by its reaction with DTNB. The yellow color developed was monitored spectrophotometrically at 412 nm valves were normalized according to the total protein assessed according to Lowry (1969).

Results

The present study included 30 patients with vitiligo and 20 healthy volunteers. Eighteen had generalized vitiligo and 12 had acrofacial vitiligo. Their ages ranged between 9- 60 years with mean of 32.4± 14.6 years. They were 10 males and 20 females.

Control group included 12 males and 8 females with mean age of 34.1 ± 13.2 years.

The important points of the medical history of our patients are epresented in table (1), and the clinical criteria of lesions are illustrated in table (2).

There was no significant statistical difference in levels of glutathione in blood [table (3)] or in MDA in blood [table (4)] between smokers and non smokers, presence or absence of family history of vitiligo, presence or absence of isomorphic phenomenon, or skin phototype III or IV.

But there was significant lower level of glutathione in blood in vitiligo patient compared to the control group (p value< 0.001) (table 5).

There was significantly higher level in MDA in blood in vitiligo patients compared to the control group (Table 5).

Tissue level of GSH in patients group was significantly lower than that in control group, and also the mean of MDA tissue level in patient group was higher than that in control group. We estimate GSH and MDA tissue levels according to total body protein (table 6).

There was significantly lower level of GSH in lesional samples compared to perilesional samples, while the mean of MDA levels of Lesional samples was significantly higher in lesional samples compared to perilesional ones (table 7).

We found that there is a good correlation between blood level of both GSH and MDA and lesional tissue samp;les, and between blood level of both GSH and MDA and perilesional tissue samples (table 8).

Table (1): Medical history of patients

Variable		number	percent
I. Occupation	Barker	1	3.3
	Dentist	1	3.3
	Farmer	2	6.7
	House wife	15	50.0
	Manual worker	7	23.1
	Sales man	4	13.3
II. Special habits	a) Smoking	6	20.0
	B) diet with antioxidant	15	50.0
Ill. Emotional upset		16	53.3
IV. Depression		8	26.7
V.History Course	Stable	6	20.0
	Progressive	24	80.0
Relation to season	+ve	11	36.7
	-ve	19	63.3
Hi story of vitiligo treatment	+ve	13	43.3
VI. Systems review	- Ocular manifestation	5	16.7
	- Diabetes mellitus	4	13.3
	- Alopecia areata	5	16.7
	- Premature graying of hair	1	3.3
VII. Family history	positive	12	40.6

Table (2): Local dermatological examination

Variable			number	percent
Clinical aspect	Distribution	Acroficial	12	40.0
		Generalized	18	60.0
	Borders	Hypopigmented	27	90.0
		Hyperpigmented	3	10.0
	Isomorphic phenomenon		11	36.7
Skin phototype		III	18	60.0
		IV	12	40.0

Table (3): Level of glutathione in blood between vitiligo patients and control [GSH umol/ gHb]

Variable		Number of cases	Mean	SD	p-value
Smoking	Yes	6	3.66	0.36	0.204
	No	24	3.23	0.71	
Diet containing antioxidant	Yes	15	3.11	0.72	0.045
	No	15	3.53	0.56	
Ocular manrestation	Yes	5	2.78	0.64	0.111
	No	25	3.42	0.63	
Family history of vitiligo	Yes	12	3.18	0.74	0.464
	No	18	3.41	0.62	
Distribution	Acrofacial	12	3.55	0.48	0.172
	generalized	18	3.16	0.74	
Isomorphic phenomenon	Yes	11	3.51	0.50	0.237
	No	19	3.21	0.74	
Skin type	III	21	3.25	0.77	0.788
	IV	9	3.47	0.32	

P- Value $\leq$ 0.05 is considered significant

Table (4): Blood level of MDA in vitiligo patients (MDA/umol/gHb).

Variable		Number of cases	Mean	SD	p-value
Smoking	Yes	6	6.37	0.90	0.344
	No	24	6.39	0.99	
Diet containing antioxidant	Yes	15	6.87	1.17	0.984
	No	15	6.77	0.80	
Ocular manifestation	Yes	5	7.48	0.77	0.210
	No	25	6.69	0.98	
Family history of vitiligo	Yes	12	6.93	1.13	0.646
	No	18	6.42	0.91	
Distribution	Acrofacial	12	7.08	1.04	0.075
	generalized	18	6.84	0.88	
Isomorphic phenomenon	Yes	11	6.84	0.96	0.798
	No	19	6.81	1.03	
Skin type	III	21	6.92	1.02	0.541
	IV	9	6.58	0.9 2	

p- Value $\leq$ 0.05 is considered significant

Table (5): Blood level of GSH and MDA in patients and control group .

Variable	Blood level of GSH in patients		Control		p- value
	Mean	*SD*	*Mean*	*SD*	
GSH (umol/ ghb)	3.32	0.67	4.74	0.62	< 0.001
Hb (K/ml)	12.01	6.74	11.90	0.97	0.850
MDA (nmol/ml)	6.82	0.99	5.01	0.73	< 0.001

p- Value $\leq$ 0.05 is considered significant

Table (6): Level of Tissue GSH and MDA in patients and control

Variable	Level of Tissue GSH & MDA in patients		Control		p- value
	Mean	*SD*	*Mean*	*SD*	
GSH (nmol/ mgb)	8.98	1.21	13.04	1.32	0.002
MDA(nmol/ mgb)	4.46	0.91	1.93	0.40	0.002
Total protein (mf(/ml)	6.35	1.00	6.75	0.69	0.961

p- Value $\leq$ 0.05 is considered significant

Table (7): Lesional and perilsional levels of GSH and MDA

Variable	Lesion		Perilesion		Difference		p- value
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
GSH (nmol/ mgb)	8.98	1.21	10.61	0.82	-1.63	0.62	0.007
MDA(nmol/ mgb)	4.46	0.91	3.23	0.46	1.24	0.79	0.006
Total protein (mJf/ml)	6.35	1.00	6.30	0.35	0.05	0.86	0.280

Table (8): Comparison between level of MDA and GSH levels in blood and tissues.

Variable	GSH (nmol/ mg)		MDA (nmol/ mg)		Total J!rotein
	Lesion	*Perilesion*	*Lesion*	*Perilesion*	*Lesion*
Perilesion	0.884	-	0.507	-	0.540
Lesion	0.133	0.338	0.704	0.246	

Discussion

Vitiligo is an acquired skin disorder characterized by sharply demarcated depigmented lesions of variable size and shape, which have the tendency to increase in size during the patient's life time. Histology shows a complete absence of melanocytes in the lesions *(Njoo and Westerhof, 2001)*.

The aetiology of vitiligo is still unknown and there are many theories to explain it *(Koca et al., 2004)*. Studying the oxidative stress theory, we concentrate on free radicals (FRs) which are atoms or molecules that occur during several physiological and pathology process. Free radicals can damage cell compounds such as protein, carbohydrate, DNA and particularly lipid *(Yildirim et al., 2004)*.

Recent studies have shown that FRs were increased and the antioxidant systems were insufficient in vitiligo *(Beazley et al.,1999)*.

Glutathione is an antioxidant agent and malondialdehyde is an end- product of lipid peroxidation induced by reactive oxygen species *(Latha et aI., 2001)*.

In this study, we found that levels of blood GSH of patients with vitiligo were significantly lower than those of controls. This finding agree with Deep Ali and his co- workers 2004. Who found high statistically significant differences between the two groups.

It is explained on the basis that glutathione act as antioxidant that scavenges free radicals and other reactive species *(Wu et al., 2004)*.

There was no statistically significant differences between smokers and non-smokers patients as regard levels of glutathione. But glutathione levels in patients having adequate antioxidant diet is more than that of patients having inadequate one, this finding suggests the role of antioxidant in the treatment of vitiligo. Dietary antioxidant supplementation especially in old age can increase cell mediated immunity, which are already decreased by oxidative stress with age *(Devasagayam et al.,2004)*.

In the present study the level of glutathione in tissue of patients and control were measured and it was found that glutathione level is lower in patient's tissue than that of controls. The difference was statistically significant. This agrees with Passi and his co- workers (1998) who found high statistically significant differences between the two groups.

These results support the role of glutathione as antioxidant. Also we found that the blood and tissue levels of GSH in patients with generalized vitiligo were significantly lower than those of healthy matched persons.

Also, we obtained a higher levels of MDA in sera of vitiligo patients than in control group. The difference was statistically significant. This agrees with Koca and his coworkers (2004) who found high statistically significant differences between the two groups.

We found that levels of MDA is higher in tissue of patients than I control persons. The difference was statistically significant. This finding agree with Yildirim and his co- workers (2003) who found high statistically significant differences between the two groups. Also, agree with Koca and his coworkers (2004) who found high statistically significant differences between the two groups.

High level of MDA in vitiligo patient's skin lesion can be explained on the basis that keratinocytes appear to be a source of reactive oxygen species that may affect neighboring skin cells and melanocytes. As a result it may influence the process of melanogenesis and contribute to the progression of vitiliginous lesions *(lvaniva et al., 2005)*.

These results support the role of MDA in lipid peroxidation in both, melanocytes and keratinocytes that could be inactive of peroxidative damage in vitiligo patients (Koca *et a1*., 2004).

Tissue levels of MDA in patients with generalized vitiligo were significantly higher than those of healthy matched controls.

The lowered levels of GSH in blood of vitiligo patients and in tissue samples and elevated levels of MDA in serum of vitiligo patients and in tissue samples reflect oxidative stress states that can lead to cell injury and death.

So our results appear to support the oxidative stress hypothesis of the disease, which provide as explanation for generalized vitiligo, where the toxic free radical accumulate and damage melanocytes.

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دراسة فى المؤكسدات ومضادات الأكسدة فى مرضى البهاق

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البهاق مرض مكتسب يسبب تشوهات جلدية ذات رقعات نتيجة لغياب مكسبات اللون نظريات الإصابة بمرض البهاق غير مفهومة بالكامل نظرية ضغط المؤكسدات تفترض تواجد الشوارد الحرة نتيجة عدم التوازن بين المؤكسدات ومضادات الأكسدة تتسبب فى تدمير الخلايا.

ينتج التأكسد شوارد حرة سامة وهو ينسا أما عن نقص مضادات الأكسدة أو زيادة تصنيع مخلفات أوكسجين حرة.

مضادات المؤكسدات أما داخلية كتنفس هوائي طبيعي او خارجية مثل أوكسيد النيتروجين . وينتج الشوارد الحرة أما عن طريق تفاعلات التأكسد الذاتي او عن طريق نظام الأنزيمات مولدات الأكسيجين فوق الأحادي او كمخلفات حرة عن الخلايا الأكولة او كجزء من التمثل الغذائي الطبيعي أو كرد فعل لاشعة ذرية .

وقد هدفنا فى هذا العمل الى استبيان ما اذا كان زيادة معدل إنتاج هذه المخالفات ونقص معدل مضادات التأكسد له علاقة بمرض البهاق ام لا.

اجرى هذا البحث بالعيادة الخارجية لقسم الأمراض الجلدية والتناسلية بمستشفى الزهراء الجامعي على 30 حالة يعانون من مرض البهاق الكلى منهم 18 يعانون من بهاق كلى و12 يعانون من بهاق طرفى كلى و 20 شخص أصحاء يكونون المجموعة الضابطة وقد خضع جميع المشتركين فى البحث الى معرفة التاريخ المرضى مع عمل فحص اكلينيكى شامل مع التركيز على نقاط هامة منها : التعرض لعوامل الأكسدة كالتدخين والأمراض المزمنة والتعرض لأزمات نفسية حادة او حوادث او إصابات مشابهة فى العائلة ، وقد أجرى لهم جميعا فحص معملى لقياس نسبة المالون دهى الدهيت والجلوتاثايون فى الدم و أنسجة الجلد وأوضحت الدراسة ان مستويات هذه المواد لم تتأثر بالتدخين او النظام الغذائي او التعرض لزمات النفسية او وجود تاريخ مرضى فى العائلة لمرض البهاق .

وأوضحت الدراسة انخفاض مستوى الجلوتاثايون فى الدم وأنسجة الجلد بالمقرنة بالعينات الضابطة وكلك هنالك فرق واضح بين مرضى البهاق مع من يعادلهم فى العينة الضابطة فى مستوى مادة المالون دهى الدهيت فى الدم وأنسجة الجلد وهذه النتائج تعزز نظرية ضغط المؤكسدات كعامل مثبت لمرض البهاق .