Assessment of the Serum Paraoxonase1 Level and its Relation to Total Oxidant Status and Disease Activity in Vitiligo Patients

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ABSTRACT

Background: Vitiligo is an acquired pigmentary disorder of unknown etiology, affecting approximately 1 % of the world population, without predilection for race or sex. It is characterized by white macules and patches, whose size increases over time, due to the loss of melanocytes. Vitiligo can appear at any time, and it significantly impairs the patients' quality-of-life.

Aim of the Work: The aim of the work was to to evaluate the serum level of paraoxonase1 in patients with vitiligo and its relation to total oxidant status and disease activity in vitiligous patients.

Patients and Methods: This is a case control study which included 48 vitiligo patients and 48 age- and sex-matched controls. The patients recruited from the Outpatient Dermatology Clinic at Ain Shams University Hospitals during the period from August 2020 till March 2021.

Results: The observation that oxidative stress is linked to the disease activity was also manifested in this work as we found a statistically positive correlation between the serum TOS levels and VIDA score which reflected the positive correlation between oxidative stress and vitiligo activity. Our study found that there was a statistically significant higher oxidative stress reflected by the high serum TOS levels among the vitiligo compared to controls. This finding supports the aforementioned hypothesis and it also came in accordance with the observations of high oxidative stress state in vitiligo patients which was repeatedly reported in many studies. Confirming the low levels of PON1 among vitiligo patients. Furthermore, we also reported the significant inverse relation between PON1 levels and the oxidative stress reflected by the serum TOS levels in vitiligo patients. This reflected the reduced protective antioxidant mechanisms in vitiligo patient making them more vulnerable to oxidative stress

Conclusion: Vitiligo is probably mediated through a process of oxidative stress and imbalance of oxidant–antioxidant system. Oxidative stress present in vitiligo disease can be determined by measurement of serum PON1 in these patients. Oxidative stress may play an important role in the pathogenesis of vitiligo. Melanocyte damage in vitiligo might be linked to oxidative stress. The finding of a PON1 decrease in vitiligo patients emphasises the underlying hypothesis in the progression of the disease, and it can highlight the effect of free radicals and leading oxidative damage in vitiligo disease. However, further, larger studies are necessary to confirm our results.

Keywords: Serum Paraoxonase1 Level; Total Oxidant Status; Vitiligo

INTRODUCTION

Vitiligo is an acquired pigmentary disorder of unknown etiology, affecting approximately 1 % of the world population, without predilection for race or sex. It is characterized by white macules and patches, whose size increases over time, due to the loss of melanocytes. Vitiligo can appear at any time, and it significantly impairs the patients' quality-of-life ***(Alikhan et al., 2011; Taieb et al., 2013; Lotti et al., 2014).***

Multiple pathogenetic factors have been proposed to clarify the etiology of vitiligo, including the neural theory, genetic predisposition, impaired anti-oxidative defense and the autoimmune theory ***(Alikhan et al., 2011).***

One of the important hypotheses in the pathogenesis of vitiligo is the oxidative stress hypothesis, which is based on the reality of the formation of some toxic metabolites throughout pigment biosynthesis ***(Yesilova et al., 2012).***

Oxidative stress may play an essential role in activating subsequent autoimmune responses related to vitiligo ***(Xie et al., 2016).*** Reactive oxygen species (ROS) are induced by multi-factors and as impaired antioxidant defenses, show the loss of melanocyte redox homeostasis, and therefore, the stressed melanocytes generate damage-associated molecular patterns (DAMPs) or autoantigens that then initiate innate immunity and adaptive immunity, leading to the dysfunction and death of melanocytes via an inflammatory cascade ***(Richmond et al., 2013).***

ROS can damage key lipid, protein, and enzyme systems involved in melanogenesis, and they also impair protein-repair mechanisms ***(Hasse et al., 2004; Glassman, 2011).*** Apart from direct or indirect evidence of elevated ROS in vitiligo patients, there is also evidence of deficient antioxidants ***(Beazley et al., 1999; Schallreuter et al., 1999; Hasse et al., 2004; Sravani et al., 2009).***

Measurement of total oxidant status (TOS), using a recently established method, better reflects the global effects of various oxidants in an organism ***(Erel, 2005; Esen et al., 2012).***

Paraoxonase (PON)-1 is an antioxidant enzyme and a member of the PON enzyme family, comprising PON-1, PON-2, and PON-3 that degrade bioactive oxidized lipids and are thus antiatherogenic***(Marsillach et al., 2011).*** PON-1, an esterase carried by high-density lipoprotein, is known to exert a protective effect against oxidative damage of cells and lipoproteins, playing an anti-inflammatory and antiatherogenic role ***(Ferretti et al., 2005).*** PON-1 mRNA is restricted to adult kidney, liver, and colon and fetal liver, whereas PON-2 mRNA is more widely distributed in adult human brain, heart, kidney, spleen, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, salivary, thyroid and adrenal glands, pancreas, skin, and bone marrow, and fetal brain and liver ***(Mackness et al., 2010).***

PON1 has two main roles: detoxifying organophosphate compounds, such as paraoxon, and protecting low-density lipoprotein by hydrolysis of lipid peroxides ***(Atasoy H et al., 2015).*** Reduced serum PON1 activity has been reported to be associated with some diseases under oxidative stress and inflammation conditions ***(Esen et al., 2015).*** Antioxidants have a protective role in the development of some autoimmune diseases like psoriasis, vitiligo, and alopecia aerate ***(Ramadan et al., 2013).***

Paraoxonase 1 can be used as an indicator in determining oxidative stress existent in the pathogenesis of vitiligo diseases ***(Yesilova et al., 2012).***

Imbalance of oxidant/antioxidant associated with hydrogen peroxide accumulation and low CAT levels in the blood and epidermis of patients with vitiligo was reported ***(Beazley et al., 1999; Schallreuter et al., 1999; Arican et al., 2008; Sravani et al., 2009; Glassman 2011).***

AIM OF THE WORK

The aim of the work was to to evaluate the serum level of paraoxonase1 in patients with vitiligo and its relation to total oxidant status and disease activity in vitiligous patients.

PATIENTS AND METHODS

This is a case control study which included 48 vitiligo patients and 48 age-and sex-matched controls. The patients recruited from the Outpatient Dermatology Clinic at Ain Shams University Hospitals during the period from August 2020 till March 2021.

The study protocol: After the approval of Ethics Committee of Faculty of Medicine, Ain Shams University (Approval number: FMASU M S 364 / 2020), all participants signed an informed consent after explaining for them the objective of the study, benefits and risks.

Patients: Fourty-eight patients with vitiligo were included in this study. Twenty-four were considered active cases while the other 24 were non active vitiligo patients. The study also included 48 age-and sex-matched healthy adults as a control group. The vitiligo activity and tensity were assessed by VIDA and VETI scores respectively.

Sample Size: Using pass2 program for sample size calculation and according to previous literature the expected mean level of 108 Paraoxonase1 in cases=108.70+36.19 and in controls=151.26+40.35(UIL) sample size of 48cases and 48 controls achieves>99% power to detect difference between 2 groups setting***(Yesilova et al., 2012)***.

Inclusion criteria: Patients with non-segmental vitiligo, age of patients between 18 and 60 years old, 24 active vitiligo patients with activity within the last 6 months (VIDA score ≥ 2) and 24 patients with non-active vitiligo within the last 6 months (VIDA score <2), both sexes were included and patients were either newly diagnosed or off treatment.

Exclusion criteria: Pregnant, lactating females and children<18 years old, use of topical therapy for vitiligo one month prior to enrolment, use of systemic treatment or phototherapy 3 month prior to enrolment and patients in which the serum paraoxonase1 or total oxidant status level might be disturbed like;Patients with metabolic syndrome ***(Usta et al., 2011; Senti et al., 2003).*** Neoplastic diseases ***(Reuter et al., 2010).*** Kidney diseases ***(Small et al., 2012).*** Medications e.g Vitamins supplements ***(Khassaf et al., 2003);*** calcium, systemic steroid, anticonvulsants, antiretroviral therapy ***(Akay et al., 2003).***

All patients were subjected to the following:

Full medical history taking with special emphysis on: Personal history: including name, age, sex and special habits of medical importance, drug history, past history of heavy smoking, diabetes, hypothyroidism, autoimmune diseases, cardiovascular disease, pulmonary disease, acute inflammation and cancer to detect diseases of the exclusion criteria, family history regarding similar condition in the first degree relatives and analysis of the complaint of patients and history of the present illness with special emphasis on onset, course and duration of the disease (with special attention to activity and distribution of the lesions).

Clinical examination: General examination: The patient skin phototype, weight, and height were recorded and body mass index (BMI) was calculated according to the equation (weight (kg) ÷ height^2 (m^2)).

Dermatological examination: All patients were examined by Wood's lamp for detection of early lesions. Skin, scalp and mucus membranes were examined carefully for depigmented patches and white hair.

Vitiligo type where determined according to: Distribution: segmental and non-segmental vitiligo, site: generalized, combined, acrofacial and genital.

Vitiligo severity and tensity were assessed by VIDA and VETI scores respectively. Collecting serum samples to detect levels of PON1 and TOS. All data were filed and documented.

The vitiligo scores used in the study:

Disease Activity (VIDA) score: VIDA score is a six-point scale for evaluating vitiligo activity. Individuals, own opinion is the base in VIDA score. Lower VIDA scores indicate less activity. VIDA score based on patients' opinion divided in 6 stages: Grading is as follows: +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. -1: stable at least for 1 year with spontaneous repigmentation ***(Njoo et al., 1999).***

We considered cases with a recent activity within the last 6 months (VIDA score ≥+2) were active cases, while patients with lower VIDA score (VIDA <+2) were considered as non-active cases.

Vitiligo extent tensity index (VETI) Score: The VETI score is a new system that proposes to measure the extent of vitiligo by a numerical score and combines analysis of extensity and severity of vitiligo and produce a constant and reproducible number like PASI. The percentage of extension involvement (p) is evaluated using the rule of nines **(figure 1)** ***(Feily, 2014).***

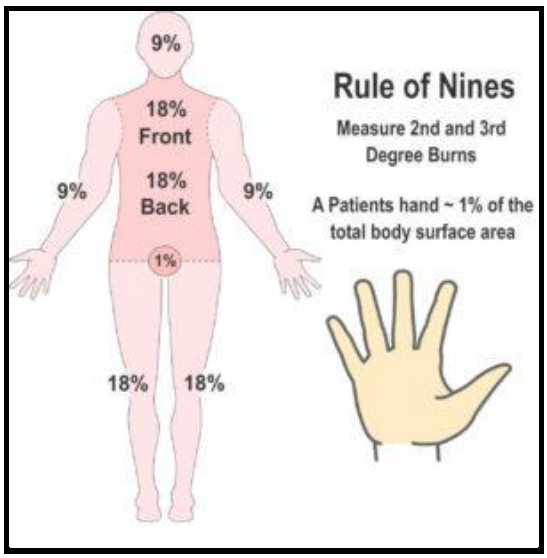

Figure (1): Rule of nines in burn assessment
(Damin Rispoli, 2010)

Already used in burn assessment, five sites affected, head (h), upper limbs (u), trunk (t) and lower limbs (l) and genitalia (g) are separately scored by using five stages of disease tensity (T): Stage 0: Normal skin, stage 1: Hypopigmentation (including trichorme and homogeneous lighter pigmentation), stage 2: Complete depigmentation with black hair and with perifollicular pigmentation, stage 3: Complete depigmentation with black hair and without perifollicular pigmentation, stage 4: Complete depigmentation with compound of white and black hair with/without perifollicular pigmentation and stage 5: Complete depigmentation plus significant hair whitening ***(Feily, 2014).***

VETI score: (Percentage of head involvement × grade of tensity) + 4(Percentage of trunk involvement × grade of tensity) + 2(Percentage of upper limbs involvement × grade of tensity) + 4(Percentage of lower limbs involvement × grade of tensity) + 0.1(Percentage of genitalia involvement × grade of tensity)

VETI= (Ph x Th) + (Pt x Tt)4 + (Pu x Tu)2 + (Pl xTl)4 + (Pg x Tg)0.1

P: percentage of involvement **T:** tensity

(Feily, 2014)

The coefficients reported in this formula are based on percent of skin surface by the rule of nines. Accordingly, the coefficient of head is 1 (9:9=1), trunk and lower limb is 4 (36:9=4), upper limb is 2 (18:9=2) and genitalia is almost 0.1(1:9= 0.1). The maximum score is 55.5 ***(Feily, 2014).***

Methods:

Sample collection, handling and storage: Five ml of venous blood were withdrawn from each patient and control subjects. The blood samples were collected using plain tubes (for TOS and PON1). After collection, blood was allowed to clot for 10-20 minutes at room temperature before centrifugation for 20 min at 2000-3000 RPM, and then the serum was separated by a pipette and

kept in one Eppendorf tube labeled with the number of the subject. Specimens were kept at -20°C to preserve until analysis, repeated freeze-thaw cycles were avoided.

Assessment of serum PON1 and TOS levels: Serum level of PON1 and TOS where assessed by ELISA technique. Human Paraoxonase1 ELISA (Enzyme-Linked Immunosorbent Assay) kit (Cat.No E1172Hu) and Human Total Oxidant Status ELISA kit (Cat.No E1599Hu) are sandwich kits for the accurate quantitative detection of human paraoxonase1 (PON1) and Total Oxidant Status (TOS) in serum respectively.

Assay principle: The assay depends on Enzyme-linked Immunosorbent Assay (ELISA). The plates had been pre-coated with human PON1 and TOS antibodies in separate plates for each marker. PON1 and TOS present in the sample were added and binds to antibodies coated on the well, and then biotinylated humen PON1 and TOS antibodies were added and bind to PON1 and TOS, respectively in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated PON1 and TOS antibodies. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was added and color developed in proportion to the amount of humen PON1 and TOS. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Assay Procedure: All samples and standards were prepared as instructed and all reagents were brought to room temperature before use, the number of stripes determined by that of samples to be tested added by that of standards, 50µl of standard to standard well and 40µl of sample to sample well were added. Then 10 µl of anti-PON1 antibody/anti-TOS antibody was added separately to different sample well, then 50µl streptavidin-HRP was added to both sample and standard wells, all wells were covered then shaked gently to mix them up and incubated at 37℃ for 60 minutes, each plate was washed five times with 0.35 µl wash buffer for 30 seconds to 1 minute for each wash, 50µl substrate A solution and 50µl substrate B solution were added to each well and shaked gently to mix them up then incubated for 10 minutes at 37℃ for 60 minutes away from light for color development, 50µl stop solution was added to each well to stop the reaction (the blue color changed into yellow immediately at that moment) and optical indensity (O.D) value was determined at 450 nm using a microplate reader 10 minutes later.

Statistical analysis: Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23.

Descriptive statistics: The quantitative data were presented as mean, standard deviations and ranges when parametric while median with inter-quartile range (IQR) when non parametric. Also qualitative variables were presented as number and percentage.

Analytical statistics: The comparison between groups regarding qualitative data was done by using **Chi-square test** and/or **Fisher exact test** only when the expected count in any cell found less than 5.

The comparison between two independent groups regarding quantitative data with parametric distribution was done by using **Independent t-test.**

The comparison between two independent groups regarding quantitative data with non-parametric distribution was done by using **Mann-Whitney test** while the comparison between more than two independent groups regarding quantitative data with non-parametric distribution was done by using **Kruskall-Wallis test.**

Spearman correlation coefficient was used to assess the correlation between two quantitative parameters in the same group.

Receiver operating characteristic curve (ROC) was used to assess the best cut off points for PON1 and TOS with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC).

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P > 0.05: Non significant (NS), P < 0.05: Significant (S) and P < 0.01: Highly significant (HS).

RESULTS

Table (1): Socio-demographic characters of the study participants

Variable		Vitiligo Patients (N=48)		Controls (N=48)		Test value	P-value	Sig.
		No.	%	No.	%			
Age (years)	Mean± SD	34.0± 13.13		34.81± 11.90		^{Z}MWU= 0.510	0.610	NS
	Median	32.50		33.0				
	Range	18.0- 60.0		18.0- 60.0				
Sex	Male	28	58.3%	24	50.0%	X^2= 0.671	0.413	NS
	Female	20	41.7%	24	50.0%			
BMI (Kg/m^2)	Mean± SD	24.91± 13.41		24.94± 11.90		^{Z}MWU= 0.132	0.895	NS
	Median	24.70		25.20				
	Range	17.50- 31.60		17.50- 31.66				
Skin phenotypes	Type I	0	0.0%	0	0.0%	X^2= 1.326	0.515	NS
	Type II	0	0.0%	0	0.0%			
	Type III	16	33.3%	13	27.1%			
	Type IV	31	64.6%	32	66.7%			
	Type V	0	0.0%	0	0.0%			
	Type VI	1	2.1%	3	6.3%			

P value< 0.05 is significant, SD: Standard deviation, IQR: Interquartile range, ^{Z}MWU *= Mann- Whitney U test,* X^2= Chi- Square test.

Table (2): Clinical characteristics of vitiligo disease among the included patients

Variables		Vitiligo patients (No=48)
Vitiligo types (No., %)	Segmental	0 (0.0 %)
	Non-segmental	48 (100 %)
Family history (No., %)	Yes	9 (18.8%)
	No	39 (81.3%)
Duration of vitiligo (years)	Mean± SD	4.73± 6.15
	Median	3.0
	Range	1– 40
Affected site (No., %)	Acrofacial	10 (20.8%)
	Genital	2 (4.2%)
	Combined (≥2- 5 sites)	27 (56.3%)
	Generalized (≥6 sites)	9 (18.8%)
Activity (No., %)	Non- active (VIDA <+2)	24 (50.0%)
	Active (VIDA ≥+2)	24 (50.0%)
VETI score	Mean± SD	3.82± 2.90
	Range	0.20– 11.10

Table (3): Comparison between vitiligo patients and healthy controls regarding PON1 serum level and TOSL serum level

Variable		Vitiligo Patients (N=48)		Controls (N=48)		Test value	P-value	Sig.
		No.	%	No.	%			
PON1 Level (U/L)	Mean± SD	6.49± 3.70		29.62± 12.74		^{Z}MWU= 8.398	**<0.001**	HS
	Range	2.0- 14.0		12.0- 60.0				
TOS level (µmol/L)	Mean± SD	9.77± 5.80		1.26± 0.32		^{Z}MWU= 8.028	**<0.001**	HS
	Range	1.20- 24.0		0.50- 2.0				

P value< 0.05 is significant, SD: Standard deviation, IQR: Interquartile range, U= Mann- Whitney U test, X^2= Chi-Square test

Table (4): ROC curve for PON and TOS levels in detection of vitiligo disease

parameters	Cutoff value	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
PON1 Level (U/L)	≤ 13.0	0.997	97.92%	95.83%	95.92%	97.88%	99.70%	<0.001
TOS level (µmol/L)	>2.0	0.988	97.92%	100.00%	100.00%	97.96%	99.00%	<0.001

PPV= Positive Predictive Value, NPV= Negative Predictive Value, AUC= Area Under Curve

Table (5): Comparison between active and non-active vitiligo as regard TOS level and PON1 level

Variable	Active vitiligo (N=24)		Non active vitiligo (N=24)		Test value	P-value	Sig.
	Mean	± SD	Mean	± SD			
TOS level (µmol/L)	13.77	5.43	5.78	2.44	0.495	**<0.001**	**HS**
PON1 Level (U/L)	3.33	.86	9.65	2.54	0.592	**<0.001**	**HS**

*P value< 0.05 is significant, SD: Standard deviation, * Mann- Whitney U test,*

Table (6): Validity of PON1 Level and TOS level in detection of vitiligo disease

parameters	Cutoff value	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
PON1 Level (U/L)	≤ 13.0	0.997	97.92%	95.83%	95.92%	97.88%	99.70%	<0.001
TOS level (µmol/L)	>2.0	0.988	97.92%	100.00%	100.00%	97.96%	99.00%	<0.001

PPV= Positive Predictive Value, NPV= Negative Predictive Value, AUC= Area Under Curve

Table (7): Correlation between PON1 level and TOS level, VIDA and VETI score in patients group

	PON1 level		TOS level	
	r	p- value	r	p- value
TOS level	-0.793	**<0.001 (HS)**	NA	**NA**
VIDA score	-0.819**	**<0.001(HS)**	0.742**	**<0.001 (HS)**
VETI score	-0.276	0.058 (NS)	0.321	**0.022 (S)**

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS),
r: spearman correlation coefficient
IMA: ischemia-modified albumin, **TOS:** total oxidant status, **VIDA score:** vitiligo disease activity score, **VETI score:** vitiligo extent tensity index score.

DISCUSSION

Vitiligo is an idiopathic, acquired, circumscribed, hypomelanotic skin disorder, characterized by milky white patches of different sizes and shapes. It is due to the destruction of melanocytes resulting in the absence of pigment production of the skin and mucosal surfaces. The worldwide incidence of vitiligo is reported to be 0.1–2% ***(Jain et al., 2011).***

Vitiligo is a multifactorial disorder due to genetic and environmental factors ***(Hamzavi at al., 2015).*** Different theories have been proposed for the pathogenesis of vitiligo in which autoimmunity in genetically susceptible individuals is considered to play an important role. The autoimmune mediated destruction of melanocytes is a well-accepted theory and currently is the leading hypothesis in vitiligo pathogenesis. This immune reaction is mediated by cellular and humoral immunity and cytokines ***(Handjani et al., 2017).***

One of the important hypotheses in the pathogenesis of vitiligo is the oxidative stress hypothesis, which is based on the reality of the formation of some toxic metabolites throughout pigment biosynthesis ***(Arora & Kumaran, 2017).*** Oxidative stress results from the overproduction of pro‑oxidant species in cells and/or reduction of cellular antioxidant capacity. It can damage nucleic acids, lipids, and proteins, leading to mutagenesis or cell death ***(Denat et al., 2014).***

According to autocytotoxic hypothesis, oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration. Oxidative stress is defined as a disruption of the delicate balance between the formation of reactive oxygen species and the antioxidant defense system ***(Bakry et al., 2020).***

Our study found that there was a statistically significant higher oxidative stress reflected by the high serum TOS levels among the vitiligo compared to controls. This finding supports the aforementioned hypothesis and it also came in accordance with the observations of high oxidative stress state in vitiligo patients which was repeatedly reported in many studies such as; ***El-Farargy et al. (2021)*** who investigated serum PON1 as an evidence of oxidative stress between 20 patients with active vitiligo and 20 healthy control.

Yesilova et al. (2012) who investigated serum PON1, Arylestrase, catalase, ceruloplasmine, Total oxidant capacity (TOC), Total antioxidant capacity (TAC), Oxidative stress index (OSI) as an evidence of oxidative stress between vitiligo patient and healthy control group**.**

Seçkin et al. (2015) who investigated the (PON1) gen polymorphism among vitiligo patients and control group.

Hussein et al. (2009) who investigated the levels of vitamin B12, folic acid, oxidative stress (lipid peroxidation;MDA), total antioxidant capacity (TAC), Paraoxanase (PON1)in serum of patients with vitiligo compared to matches control group

The oxidative stress has been linked to the vitiligo activity by ***Jain et al. (2011)*** who reported that SOD was significantly increased in the active stage of vitiligo.

The level of MDA, which is a marker of oxidative stress and a bioproduct of lipid peroxidation, was higher in vitiligo patients than in the controls. Active and generalized disease can increase the level of MDA, which is correlated with increased oxidative stress of tissue ***(Yildirim et al., 2004; Ines et al., 2006; Dammak et al., 2009)***.

The observation that oxidative stress is linked to the disease activity was also manifested in this work as we found a statistically positive correlation between the serum TOS levels and VIDA score which reflected the positive correlation between oxidative stress and vitiligo activity.

Oxidative stress was first noted in vitiligo from the presence of high levels of H_2O_2 in affected skin, and disturbed reactive oxygen species (ROS) homeostasis was demonstrated in tissue and blood of patients with vitiligo, especially when the disease was active ***(Arican, Kurutas 2008; Glassman, 2011***).

(ROS) can damage key lipid, protein, and enzyme systems involved in melanogenesis, and they also impair protein-repair mechanisms***(Hasse et al., 2004; Glassman, 2011***).

Epidermal ROS sources may be endogenous, such as radicals formed due to activated neutrophils or enzyme activities, such as NADPH oxidase, xsantin oxidase, lipooxigenase, or nitric oxide synthases; it also may be exogenous, such as ultraviolet rays, which are prooxidative stimulants, atmospheric gases, microorganisms, pollution, and xsenobiotics ***(Glassman, 2011; Jain et al., 2011).***

The formation of oxidative stress was observed during the melanin biosynthesis of intermediator products that have direct toxic effects on melanocytes, such as 3, 4-dihydroxyphenylalanine, dopachrome, and 5, 6-dihydroxyindole.[31] It is considered that melanocyte destruction occurs in patients with vitiligo due to the accumulation of toxic compounds (H_2O_2) and inhibition of detoxification mechanisms (inhibits CAT activity), leading to oxidative stress ***(Yildirim, 2004; Khan et al., 2009; Jalel, Hamdaoui, 2009; Glassman, 2011).***

Human paraoxonase‑1 (PON1) is a Ca^{2+}-dependent esterase synthesized in the liver. PON1 is related to high‑density lipoprotein. PON1 has two main roles: detoxifying organophosphate compounds, such as paraoxon, and protecting low‑density lipoprotein by hydrolysis of lipid peroxides ***(Atasoy et al., 2015).***

Reduced serum PON1 activity has been reported to be associated with some diseases under oxidative stress and inflammation conditions (***Esen et al., 2015)***. Antioxidants have a protective role in the development of some autoimmune diseases like psoriasis, vitiligo, and alopecia aerate ***(Ramadan et al., 2013).*** PON1 is an antioxidant enzyme and a member of the PON enzyme family, comprising PON1, PON2, and PON3 that degrade bioactive oxidized lipids and are thus antiatherogenic ***(Marsillach et al., 2011).***

Based on the PON1 role as a protective of oxidative stress ***(Tsuzura et al. 2004; Costa et al. 2005; Gur et al. 2006; Vasdev et al. 2006)*** and the established role of oxidative stress in vitiligo patients ***(Karaca et al., 2009; Yesilova et al 2012; Karsli et al., 2014; El-Farargy et al., 2021)*** we studied the serum PON1 in vitiligo patients and its possible relation to the disease activity and the oxidative stress state in vitiligo patients. We found that the serum PON1 levels were highly statistically significantly reduced in vitiligo patients compared to the controls. Our findings were in agreement with the reported observations of ***Hussein et al. (2009), Yesilova et al. (2012), Ramadan et al. (2013), and El-Farargy et al. (2021).***

Hussein and his colleagues examined the role and serum levels of vitamin B12, folic acid, PON1, oxidative stress; MDA and TAC in patients with active generalized vitiligo, in addition to controls and found significant increase in oxidative stress biomarkers; MDA, while marked reduction in TAC and PON1 levels in vitiligo patients compared to matches control and so Disturbance in oxidant/ antioxidant balance**.**

Yesilova et al. in 2012 supported these findings through investigating serum levels of paraoxonase 1, arylesterase, catalase, ceruloplasmin, total antioxidant capacity, and oxidative stress index in patients with active lesions of generalized vitiligo, as well as in matched, healthy controls and reporting lower levels of PON1 and ARE, ceruloplasmin antioxidant enzymes, TAC and increased serum TOC levels and OSI in generalized vitiligo patients compared with the control group**.**

Also**, *Ramadan et al. in 2013*** reported that All patients with psoriasis, alopecia areata and vitiligo showed significantly lower levels of both PON1 and vitamin E in tissue and serum than the controls which denotes the role of oxidative stress in pathogenesis of these autoimmune diseases.

This finding was also detected by ***El-Farargy et al. (2021)*** who estimated the serum paraoxonase1(PON1) level among 20 patients with generalized active vitiligo and 20 healthy controle group and found decreased level of serum (PON1) in patients than in control group**.**

Our study joined these studies in confirming the low levels of PON1 among vitiligo patients. Furthermore, we also reported the significant inverse relation between PON1 levels and the oxidative stress reflected by the serum TOS levels in vitiligo patients. This reflected the reduced protective antioxidant mechanisms in vitiligo patient making them more vulnerable to oxidative stress compared to controls with the agreement with our result was the study of ***Yesilova and his colleage at 2012*** who compared the relation between serum (PON1)level and (TOS)level in active vitiligo patients compared to healthy control group which showed decreased level of (PON1) and increase (TOS) in vitiligo patients than that of control group.

The paraoxonase (PON) family of antioxidant enzymes degrade oxidized phospholipids, and have been shown to be deficient in many disease states ***(Costa et al. 2005)*** and it has aprotective role aginst oxidative stress ***(Tsuzura et al. 2004; Costa et al. 2005; Gur et al. 2006; Vasdev et al. 2006)***.Oxidative stress has a role in the pathogenesis of vitiligo ***(****Karaca* ***et al., 2009; Yesilova et al 2012; Karsli et al., 2014; El-Farargy et al., 2021)*** so paraoxonase enzyme protect aginst vitiligo disease ***(Yesilova et al 2012; El-Farargy et al., 2021).*** Its deficiency leads to more H2O2 accumulation and so destruction of melanocytes ***(Yesilova et al 2012).***

We also compared the serum PON1 levels between active and non-active vitiligo cases and we found that active vitiligo cases had significantly lower PON1 levels compared to non-active cases. This was confirmed by the negative significant correlation between the serum PON1 levels and VIDA score To our knowledge no previous studies reported the values of PON1serum level to compare between active and nonactive vitiligo but the study by ***Yesilova et al. (2012); Ramadan et al. (2013)***; ***El-Farargy et al. (2021)*** compared between active vitiligo and healthy control group.

CONCLUSION

Vitiligo is probably mediated through a process of oxidative stress and imbalance of oxidant–antioxidant system. Oxidative stress present in vitiligo disease can be determined by measurement of serum PON1 in these patients. Oxidative stress may play an important role in the pathogenesis of vitiligo. Melanocyte damage in vitiligo might be linked to oxidative stress. The finding of a PON1 decrease in vitiligo patients emphasises the underlying hypothesis in the progression of the disease, and it can highlight the effect of free radicals and leading oxidative damage in vitiligo disease. However, further, larger studies are necessary to confirm our results.

Moreover, we believe that larger sample size with various populations would give more accurate results with the relationships between early diagnoses of the disease, the variation among populations with vitiligo gene polymorphism, and development of vitiligo treatment

REFERENCES

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