A Comparative Study of Oxidant- Antioxidant Status in Blood and Tissue in Vitiligo Patients

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Abstract:

Background: The etiology of vitiligo is still unknown. So there are many hypotheses to explain its pathogenesis like genetic, autoimmune, neural, biochemical, auto-cytotoxic and oxidative stress theories. Oxidative stress is a disorder in pro and anti-oxidants balance in which pro-oxidants predominate; also it is a factor that initiates pathogenesis of melanocytes’ degeneration.

Objective: The aim of the present work was to study the impact of some oxidative stress markers in the pathogenesis of vitiligo.

Patients: The study was carried out on 20 vitiligo patients and 10 controls with matched age and sex.

Methods: Estimation of some markers of oxidative stress as nitric oxide (NO), Malondialdehyde (MDA), superoxide dismutase (SOD) and of glutathione peroxidase activity (GPx) in the blood and tissue of vitiliginous patients compared to controls.

Results: the level of oxidative stress was more obvious in the vitiliginous lesion than that of the systemic circulation.

Conclusions: From the results of this study, we can depend on measurement of oxidative stress in the affected skin rather than the plasma.
Introduction:

Vitiligo is an acquired cutaneous disease characterized by pearly white patches of variable shapes and sizes that have a tendency to increase in size centrifugally [1]. It usually begins in childhood or early adulthood but in most of the cases it occurs before 20 years of age and the incidence decreases with increasing age [1,2]. It affects 0.5 to 2% of the world's population [1,3] with no sex predilection but usually women are more affected than men because of their greater medical attention for cosmetic problems [4]. The clinical picture consists of one or more well demarcated white macules or patches progressing in size and number and they are generally asymptomatic [2,5].

The diagnosis of vitiligo is mainly clinical by the presence of achromatic macules and normal skin coexisting in the same individual [1,2]. Wood’s light; which is a lamp of 35 nm causes a bluish white fluorescence in the affected skin [5].

The etiology is still unknown so there are several hypotheses concerning the pathogenesis of vitiligo: genetic, autoimmune, neural, auto-cytotoxic, biochemical and oxidative stress theories [1,2,3,4,5].

In general oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage [6,7].

Reactive oxygen species (ROS) are produced as byproducts of melanogenesis in melanocytes, and controlled in the epidermis by several antioxidant enzymes such as catalase and glutathione peroxidase [6,8,9]. Oxidative stress is thought to be the initial pathogenic event in melanocyte destruction [10]. In oxidative stress, there is insufficient antioxidant activity leading to excessive accumulation of free radicals, which damage cellular compounds such as proteins, carbohydrates, DNA and lipids. Vitiligo is accompanied by oxidative stress where there is overproduction of \( \text{H}_2\text{O}_2 \) that is cytotoxic to melanocytes by several mechanisms [11,12,13].

Aim of the work:

The aim of the present work was to study the impact of some oxidative stress markers in the pathogenesis of vitiligo.

Patients:

The study was carried out on 20 patients with vitiligo and 10 controls with matched age and sex. The twenty patients (Group I) with vitiligo were attending the dermatology outpatient clinic of the main Alexandria University Hospital and the ten control subjects (Group II) were chosen without any clinical evidence or family history of vitiligo or history of any autoimmune disease (attending the out patient clinic of surgical department of the main Alexandria University Hospital to do minor surgical procedures).

Patients with the following criteria were excluded from the study: patients with any concomitant dermatological diseases, history of the use of any topical or systemic treatment for vitiligo at least for a previous month, active and passive smokers, history of intake of vitamins and anti-inflammatory drugs at least for a previous month, history of chronic liver, kidney or
cardiovascular disease, history of excessive exercise apart from daily life activities and pregnancy.

**Methods:**

After obtaining consent from the participants, Blood samples as well as skin specimens were taken from patients and control groups and it was immediately carried in an ice box to the Department of Biochemistry, and were subjected to the following: estimation of nitric oxide (NO) (one measure for cellular oxidants) by colorimetric determination of nitrite \cite{13,14}, estimation of malondialdehyde (MDA) (a measure for lipid peroxidation) by colorimetric method \cite{13,15}, estimation of superoxide dismutase (SOD) (antioxidant enzyme) activity by colorimetric method \cite{13,16} and estimation of glutathione peroxidase activity (GPx) (antioxidant enzyme) by spectro-photometric method \cite{13,17}.

**Statistical Analysis:**

The statistical analysis was performed by using Student t test. The differences were considered significant when \( P < 0.05 \).

**Results:**

The study sample constituted from 20 vitiligo patients attending the dermatology outpatient clinic of Alexandria University Hospital and 10 control subjects with no history of vitiligo or any other autoimmune disease.

Mean studied age of vitiligo patients was 44.10 ± 13.42 (range 23-67 years) and that of controls was 41.50 ± 10.56 (range 23-56 years) and by using t-test there was no significant difference (t=0.534, p=0.598). Nearly half of the sample were females (11 cases, 55%) and 9 were males (45%) and also the control group consisted of 6 females (60%) and four males (40%); so the two groups were age and sex matched.

When vitiligo patients were classified according to Fitzpatrik classification of skin types, 7 cases (35%) were skin type III and 13 cases (65%) were skin type IV. All cases showed an insidious onset. The duration of the disease ranged from four months to 50 years with a mean and SD of 12.80 ± 15.56.

Comparison of vitiligo patients according to the duration of lesion showed that five patients (25%) had recent lesions ranging from one month to one year, five patients (25%) had old lesions ranging from one year to three years and 10 patients (25%) had older lesions for more than three years.

Comparison of vitiligo patients according to stability of the disease showed that 16 cases were progressive (70%) and four cases were stable (30%).

As regards precipitating factors, 18 cases (90%) gave history of stress in the form of life stress as loss of money, job and death of a close relative.

Five cases (25%) complained that exposure to the sun worsens and inflames their disease. Nineteen cases (95%) were generalized and only one case (5%) was localized.
The majority of cases (13 cases, 65%) showed a negative family history of vitiligo and the remaining 7 cases (35%) showed a positive family history.

**Nitric Oxide (NO): one measure for cellular oxidants**

Nitric oxide was measured as its metabolite nitrite. Plasma concentration level in vitiligo patients (Group I) ranged from 1.02- 9.43 µmol/L with a mean value of 3.82 ± 2.75 µmol/L, while plasma concentration level in control group (Group II) ranged from 1.43- 13.52 µmol/L with a mean value of 7.10 ± 3.67 µmol/L.

Concentration of nitrite in skin specimen of vitiligo patients (Group I) ranged from 1.08-7.29 µmol/mg protein with a mean value of 3.65 ± 2.13 µmol/mg protein while its concentration in skin specimen of control group (Group II) ranged from 0.02- 5.35 µmol/mg protein with a mean value of 2.79 ± 1.78 µmol/mg protein. Comparison between the mean values of nitrite in skin specimens of the two studied groups showed an increased value in vitiligo patients compared to control but statistical comparison using t-test \([t=1.094, P=0.283]\) showed no significant difference.

**Malondialdehyde (MDA): a measure for lipid peroxidation**

Plasma concentration level in vitiligo patients (Group I) ranged from 1.62- 8.04 nmol/ml with a mean value of 4.66 ± 1.82 nmol/ml, while plasma concentration level in group II ranged from 3.30- 7.84 with a mean value of 5.45 ± 1.30 nmol/ml. Statistical comparison between the mean values of plasma in the 2 studied groups using t- test \([t=1.213, P=0.235]\) showed no significant difference.

Concentration level of MDA in skin specimen of vitiligo patients (Group I) ranged from 2.03-11.14 nmol/mg protein with a mean value of 5.10 ± 2.28 nmol/mg protein, while concentration level in skin specimen of control group (Group II) ranged from 1.34- 5.50 nmol/mg protein with a mean value of 3.31 ± 1.22 nmol/mg protein. Statistical comparison between the mean values of concentration in skin specimens of the two studied groups using t-test \([t=2.319, P=0.028]\) showed a significant increase in vitiligo patients compared to the control group.

**Superoxide dismutase (SOD): antioxidant enzyme**

Plasma concentration level in vitiligo patients (Group I) ranged from 10.14-96.28 with a mean value of 43.16 ± 28.48 U/ml, while plasma concentration level in control (Group II) ranged from 20.27- 81.08 U/ml with a mean value of 54.15 ± 21.72 U/ml. Statistical comparison between the mean values of plasma of the two studied groups using t- test \([t=1.072, P=0.293]\) showed no significant difference.

Concentration level of SOD in skin specimen of vitiligo patients (Group I) ranged from 27.64 - 155.93 U/mg protein with a mean value of 83.98 ± 43.99 U/mg protein, while concentration level of SOD in skin specimen of control group (Group II) ranged from 41.93-176.08 U/mg protein and on statistical comparison between the mean values of SOD of the two studied groups using t-test \([t=1.799, P=0.083]\) showed no significant difference.

**Glutathione peroxidase (GPx): antioxidant enzyme**

Plasma concentration level of vitiligo patients (Group I) ranged from 19.45-97.27 mU/ml with a mean value of 50.44 ± 27.14 mU/ml, while plasma concentration level in control (Group II)
ranged from 19.45 – 58.36 mU/ml with a mean value of 38.42 ± 14.22 mU/ml. Comparison between the mean values of plasma GPx of the two studied groups showed an increase in patients compared to control group but statistical comparison using t-test [t=1.592,P=0.123] showed no significant difference.

Concentration of GPx in skin specimen of vitiligo patients (Group I) ranged from 15.82 - 97.27 mU/mg with a mean value of 46.33 ± 23.07 mU/mg protein, while its concentration in skin specimen of control group (Group II) ranged from 15.82-51.65 mU/mg protein with a mean value of 31.54 ± 14.79 m/mg protein. Comparison between the concentration levels of GPx in skin specimens of the two studied groups showed an increase in patients compared to control group but statistical comparison using t-test [t=1.838,P=0.077] showed no significant difference.

**Discussion:**

The etiology of vitiligo is still unknown. So there are many hypotheses to explain its pathogenesis. One of the hypotheses to explain vitiligo is the self-destructive theory of melanocytes, which suggests a role for oxidative stress [6,7,8,9,10]. Oxidative stress is thought to be the initial pathogenic event in melanocyte destruction [7,18]. This is why in this study it was decided to measure the levels of two antioxidants (SOD, GPx), an oxidant (NO), and a product of lipid peroxidation (MDA) both in plasma and in skin specimens of vitiligo patients to evaluate the role of oxidative stress in the pathogenesis of vitiligo.

**Nitric oxide (NO)**

Nitric oxide (NO) is a free radical that is generated in cells from L-arginine by nitric oxide synthetase (NOS) enzymes [19]. It was recorded that there is an increase in NOS in inflammatory conditions and this contributes to the cytotoxic action of inflammatory cellular infiltrates around normal human melanocytes [20,21].

The present study revealed an increase in the level of Nitrite in patients than control when measured in skin specimen while the plasma level is more in control than in patients.

Other studies showed higher levels of NO in patients compared to control group as Koca and Armatcu [22], Yildirim et al [23] and Hazneci and karabalut [24] who found higher serum NO levels in generalized vitiligo and this was explained by the fact that NO could lead to auto-destruction of normal human melanocytes [25] and also due to the fact that the production of NO species reduced the attachment of melanocytes to the extracellular matrix leading to depigmentation [20]. Moreover, NOS requires tetrahydrobiopterin (BH4) as a co-factor in the synthesis of NO and as in vitiligo, there is accumulation of 6BH4 [26]. It might be responsible for the high NOS activity and therefore high levels of NO. Also, NO is a free radical and inherently reactive and mediate cellular toxicity by damaging critical metabolic enzymes and by reacting with O2- to form an even more potent oxidant, peroxynitrite (ONOO-). So, NO plays a major role as a triggering agent in the patho-physiology of vitiligo especially when elevated in the skin rather than in the plasma.

**Malondialdehyde (MDA)**

MDA is an end-product of lipid peroxidation induced by reactive oxygen species (ROS). It is
well correlated with the degree of lipid peroxidation and is an indicator of oxidative stress [13].

The present study revealed no significant difference in the level of MDA when measured in plasma between vitiligo patients and control group. However in skin specimen, there was a significant increase in vitiligo patients compared to control group. This increase indicates high level of lipid peroxidation due to exposure of ROS in vitiliginous lesion than that in the systemic circulation.

In agreement with this result, Picardo et al [27] found normal serum MDA level in combined types of vitiligo. However other studies showed higher serum MDA in patients compared to control as Yousry et al [28] and Yildirim et al [23].

Yildirim et al [13] and Dammak et al [29] found statistically significant high levels of MDA in tissues of vitiligo patients compared to control group and they explained this that it is a condition of oxidative stress. Moreover, Dammak et al added that lipid peroxidation in the cellular membrane of melanocytes may play an important role in the rate of depigmentation observed in the skin of patients with active vitiligo.

**Superoxide dismutase (SOD)**

Superoxide dismutase (SOD) is a group of metallo-enzymes that scavenges superoxide radicals and reduces their toxicity. It is an antioxidant that dismutates the O$_2^-$ anion to form O$_2$ and H$_2$O$_2$ [30].

The present study revealed a decrease in the level of SOD in vitiligo patients than control group both in plasma and skin specimens but this decrease was statistically insignificant. Such decrease may be due to its utilization in detoxication of released ROS (superoxide anion).

In agreement with the present study, Koca et al [30], found significantly lower levels of serum SOD activity in vitiligo patients compared to healthy control. They explained this by the fact that superoxide and hydroxyl radicals are the most important radicals in peroxidation and decreased SOD activity could be responsible for the increase of superoxide radicals which may explain the increased level of MDA [30]. However, Picardo et al [27] and Passi et al [31] reported that there were no differences in the levels of erythrocyte SOD activity in patient with active vitiligo versus control. On the other hand, many studies showed an increase in SOD in erythrocytes [25], in peripheral mononuclear cells [32], in the serum [23] and in whole blood [33] in vitiligo patients. They suggested that the increased SOD activity in vitiligo patients might be an adaptation to the increased oxidative stress evident in these individuals and their result suggested that prominent oxidative stress, particularly in the presence of high levels of O$_2^-$ levels leads to high levels of SOD followed by high amounts of H$_2$O$_2$. High levels of H$_2$O$_2$ and O$_2^-$ might result in destruction of defective melanocytes in vitiligo patients. Moreover, another study was done to compare segmental and non segmental vitiligo, they found significantly higher erythrocyte SOD activity in both types of vitiligo patients compared with control with no significant change in segmental compared with non-segmental vitiligo. [13,29,34,35,36]

In addition, other study showed high levels of SOD in vitiliginous and non vitiliginous skin of vitiligo patients than the levels of normal skin of control. They stated that these results supported the role of oxidative stress in the pathogenesis of vitiligo and indicated that oxidative stress is not a localized phenomenon but a more generalized process. This may be one of the explanations for
developing newer lesions in vitiligo patients in the course of the disease [37].

**Glutathione peroxidase (GPx)**

Glutathione peroxidase (GPx) is another antioxidant that converts H₂O₂ and other peroxides into H₂O. Also it catalyzes the reduction of hydroperoxides in the presence of glutathione [38]. It protects the membranes and essential proteins from potential damaging effect of reactive oxygen and lipid peroxide [39].

The present study revealed an increase in the level of GPx in plasma and skin specimens of vitiligo patients compared to control group. This increase is in order to face the increased level of ROS (hydrogen peroxide and lipid peroxide) that has been produced in vitiligo.

In agreement with this study, another study showed statistically significant increase in the level of plasma GPx in vitiligo patients compared to control group [40].

However some studies showed no significant difference between vitiligo patients and control in plasma level of GPx [24,30,31,33] and other studies showed a significant decrease in the level of plasma GPx in vitiligo patients compared to controls [36,40,41]. Agrawal et al [42] explained that low levels of GPx in vitiliginous patients neutralizes lipid hydroperoxides which could lead to oxidative stress. Also, Yousry et al [28] added that GPx has a higher affinity for H₂O₂. So GPx activities may be decreased in vitiligo patients to compensate the increase in free radicals and H₂O₂. Furthermore Hasse et al [43] reported that the accumulation of millimolar concentrations of H₂O₂ leads to affection of antioxidant enzymes as proved by low blood GPx activity of vitiligo patients regardless their age group. Also Agrawal et al [29] and Beazley et al [44] found low erythrocytes and serum GPx activity in vitiligo patients without significant difference among different age groups.

In skin specimens, the study revealed an increase in level of GPx in vitiligo patients compared to control group.

In agreement with results obtained in this study, Yildirim et al [13] who found significantly higher levels of GPx in the tissues of patients with generalized vitiligo compared to control group. However, another study showed lower levels of GPx in the epidermis of both lesional and non-lesional skin of vitiligo patients [24] and during active phases of vitiligo indicating an imbalance of antioxidants in the epidermis of vitiligo patients [45].

In this study, it was found that there was no relation between oxidant/antioxidant parameters and skin types, stability or duration of the disease, family history or effect of stress and sun exposure.

**Conclusions:**

- Oxidative stress has a role in the pathogenesis of vitiligo

- From the results of this study, the level of oxidative stress was more obvious in the vitiliginous lesion than that of the systemic circulation so we can depend on measurement of oxidative stress in the affected skin rather than the plasma.
• An important question may be raised, such imbalance between oxidants and antioxidants mechanisms, is it a primary etiological factor or secondary to other possible etiological factors for development of vitiligo? This may need further investigations.

References


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