



The relationship between liver enzymes, antioxidants, and ABO blood types in Vitiligo patients

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Abstract

Vitiligo is a skin disorder resulting from the destruction of melanocytes, leading to their cessation of melanin production, the pigment responsible for skin color, and its transformation into white patches on the skin. It is considered an autoimmune condition, resulting from an immune disorder, genetic predisposition, psychological stress, or environmental factors such as sunburn. Vitiligo affects males and females of all ages and is not limited to a specific body weight. However, individuals with vitiligo exhibit differences in liver enzymes and antioxidant levels. This study investigated the relationship between antioxidants, liver enzymes, and blood types. Forty serum samples were collected (20 from healthy individuals and 20 from individuals with vitiligo). Demographic and epidemiological characteristics, including age, sex, and weight, were measured, and no significant differences were observed between the two groups. The enzymes (AST, ALT, ALP, GGT) were studied, and a significant increase in all liver enzymes was observed in the patient group compared to the control group, according to the p-value. Our study recorded imbalances in antioxidants (SOD, CAT, MDA, GSH, TAC), which were statistically significant. SOD, CAT, GSH, and TAC all showed a significant decrease compared to the control group, while the antioxidant MDA in the patients showed a significant decrease compared to the control. The reason for the decrease in antioxidant levels is attributed to oxidative stress (resulting from free radicals ROS, which depletes antioxidants) and a deficiency in nutrients such as (vitamin C and E, in addition to a deficiency in selenium, zinc, and alpha-lipoic acid), which are essential elements for the function of antioxidants. The current study of blood types did not observe any significant differences in predisposition to vitiligo. Similarly, we did not find any correlation between the four blood types and liver enzymes in patients, nor were there any differences between the four blood types and antioxidant levels. This suggests that the disease is not related to blood type but is purely an autoimmune disease. We studied Pearson's correlation coefficient between liver enzymes and antioxidants, and the enzymes showed some

correlations with antioxidants, which play a role in the dysfunction of melanocytes, the occurrence of oxidative stress, and the impact on the liver and bile ducts.

Keyword: Liver Enzymes, Antioxidants, ABO Blood Groups, Vitiligo Patients.

Introduction

Vitiligo is a chronic skin disorder resulting from the destruction of melanocytes (the cells that produce skin pigment), leading to a cessation of melanin production, the pigment responsible for skin color, this results in the appearance of painless, milky-white patches on the skin, it is considered an autoimmune condition and is not contagious. Genetics, psychological stress, or environmental factors such as sunburn may play a role in triggering the onset of vitiligo (**Touni et al., 2023**).

Vitiligo is characterized by loss of skin pigmentation in the form of patches that can appear on any part of the body. It affects approximately 1% of the world's population, with little difference in prevalence based on gender, race, or geographic region, as in ancient times, vitiligo negatively impacts patients' quality of life by reducing their self-confidence and causing significant psychological distress, this decline in quality of life is comparable to other debilitating skin conditions such as psoriasis and eczema. Vitiligo skin lesions are visible signs of the disease, causing shame, anxiety, and depression, visible areas such as the hands and face are usually affected, and patients often fear the spread and worsening of the disease in these areas (**Frisoli et al., 2020**).

Vitiligo is classified into two main types: First, non-segmental vitiligo, which includes Vitiligo Areata (where the number of visible patches on the body ranges from 1 to 2, and is further divided into (localized, partial, and focal), as well as Acrofacial Vitiligo (where patches are present on the face, lips, hands, and feet), generalized vitiligo (which is divided into generalized and universal and is present throughout the body), and mucosal vitiligo (which is specific to the mucous membranes). Second, segmental vitiligo, where patches are distributed linearly along the body (**Mohta, 2026**). Morphologic features of vitiligo include Trichrome Vitiligo, which is identified by the presence of a narrow or wide median between the vitiligo patches and the surrounding normal skin. Vitiligo is a disease, heterogeneous and unstable, and Quadric-chrom vitiligo, in which the vitiligo patches appear quadric in color and can be clearly seen in dark skin, as hyperpigmentation is observed at the edges of the vitiligo patches, and pentachrome vitiligo, in which this type is rare, the patient appears sequential in white, brown, and blue-gray in the area of hyperpigmentation and normal skin, and the black race are the individuals most predisposed to this type of disorder, and Blue vitiligo, in which blue vitiligo patches usually appear in the area resulting from the inflammation specific to hyperpigmentation, and these patches develop extensively as a result of the progression of the disease after hyperpigmentation resulting from acquired immunodeficiency diseases, and inflammatory vitiligo, in which the occurrence of these changes may reveal the occurrence of inflammation in the vitiligo patches with frequent itching that may cause some treatments to be followed (**Joge et al., 2022; Müge Acar., 2023**).

Vitiligo is a multifactorial disease resulting from complex interactions between genetic and environmental factors. Data point to the pivotal role of immune cells and their mediators in the immunopathological mechanism of vitiligo. Key immune cells include type I helper T cells, cytotoxic T cells, regulatory T cells, resident memory cells, dendritic cells, natural killer cells, and ILC-1 cells. Furthermore, key mediators such as interferon-gamma (IFN- γ), chemokines CXCL9 (CXCL9) and

CXCL10 (CXCL10), and interleukin-15 (IL-15) are involved in the etiology of vitiligo. These mediators attack CD8⁺ T cells and NK cells, which then directly target and destroy melanocytes in healthy epidermis. These melanocytes are responsible for melanin production. After the destruction of melanocytes, the area remains depigmented, resulting in a white patch (vitiligo patch). The cell is damaged and empty because the immune system mistakenly identifies the pigment cells as foreign and attacks and destroys them, leading to loss of skin color in those areas. In addition, non-immune cells, such as keratinocytes, fibroblasts, and stem cells, contribute to the development of vitiligo. However, the precise events leading to the development of vitiligo are still not fully understood. Oxidative stress is currently considered the initial event that activates innate immunity in the early stages of vitiligo (**Liu *et al.*, 2024**). Genetic factors, in conjunction with environmental factors, induce stress in melanocytes and keratinocytes, which manifests as endoplasmic reticulum stress and the release of reactive oxygen species by melanocytes that have the ability to alter cellular DNA, proteins, and lipids (**Speeckaert *et al.*, 2023**). This leads to the production of many damage-associated molecular patterns, such as melanocyte-specific antigens, miRNAs, and heat shock proteins. Inducible heat shock protein 70 (iHSP70) is of great importance in linking innate and acquired immunity, as it stimulates the transformation of dendritic cells into active antigen-presenting cells. Dendritic cells (DCs) present pigment cell-derived peptides to T cells, leading to their differentiation and production of interferon-gamma (IFN- γ) (**Hlača *et al.*, 2022**).

Oxidative stress plays a role in the skin acting as a barrier organ that provides protection against external stressors, ultraviolet radiation, microorganisms, and oxidizing chemicals are examples of external stimuli that target pigment cells and cause the production of reactive oxygen species, therefore, pigment cells use multiple mechanisms to maintain oxidative-reductive balance, antioxidants, including superoxide dismutase, catalase, glutathione, vitamin C, and vitamin E, may counteract the effects of reactive oxygen species (**Xuan *et al.*, 2022**). However, recent studies have shown an imbalance in antioxidants, with elevated levels of superoxide dismutase, decreased levels of catalase, and increased lipid peroxidation in vitiligo patients, in addition, elevated levels of reactive oxygen species have been found in both affected and unaffected skin in vitiligo patients. Melanocytes in vitiligo exhibit several structural defects that impair their ability to resist oxidative stress, including endoplasmic reticulum dilation, mitochondrial dysfunction, and abnormal melanosome structure, endoplasmic reticulum dilation occurs as a result of the accumulation of misfolded proteins, which perpetuates endoplasmic reticulum stress and oxidative stress (**Bialczyk *et al.*, 2023**).

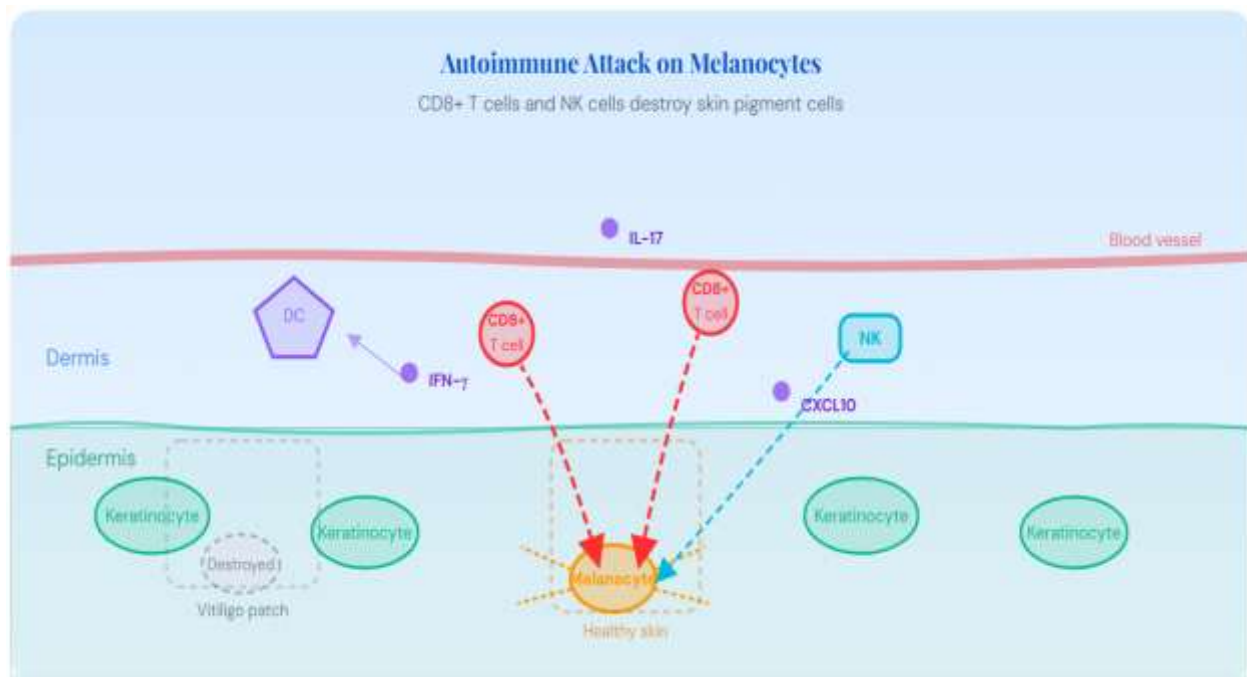


Fig (1-1) illustrates the autoimmune attack on pigment cells in vitiligo patients. The image shows cytotoxic CD8+ T cells directly attacking pigment cells (red), while pigment cells are attacked under the direction of CXCL10 by natural killer (NK) cells (green), and the immune response is coordinated and the remaining cells are activated by dendritic cells (DCs) (purple) The melanocyte, which is responsible for producing skin pigment (melanin), is being destroyed (yellow).

Materials and Material

2-1: Sample Collection

5 ml of peripheral venous blood was drawn from 40 patients participating in the study (20 from healthy individuals and 20 from individuals with vitiligo) who were attending dermatology clinics at Kirkuk General Hospital and private clinics. Collection was performed using sterile venous blood collection instruments and single-use plastic syringes. Each sample was divided into two tubes, containing 4 ml of blood without anticoagulant, and centrifuged for 15 minutes at 5000 rpm to obtain serum for antioxidant and liver enzyme evaluation. One ml was used for blood typing in the affected individuals. Patients with other conditions (hypertension, diabetes, and other autoimmune diseases) were excluded.

2-2: Liver Enzyme Levels (ALT, AST, ALP, GGT) Estimation

Liver Enzyme Levels were determined using an ELISA test kit according to the instructions of the Chinese manufacturer (**Elabscience**).

2-3: Antioxidant Levels (SOD, CAT, MDA, GSH, TAC) Estimation

Serum antioxidant levels were measured using the (**Elabscience**) colorimetric kit, according to the manufacturer's instructions.

2-4: ABO Blood Type Testing

The ABO blood types of participating patients were determined according to the kit's manufacturer, ATLAS Jordan.

2-5: Statistical Analysis

SPSS version 25 was used, along with independent t-tests, to compare continuous variables between the two groups (vitiligo patients and control group): age, body mass index, liver enzymes, and antioxidant parameters. The chi-square test (χ^2) was used to determine the distribution of sex (male/female) and ABO blood types between the two groups. One-way ANOVA was used to compare liver enzymes and antioxidant parameters among the four blood groups of patients, and Pearson Correlation was used to study the relationship between liver enzymes and antioxidant parameters according to the significance level ($P < 0.05$) and ($P < 0.01$).

Results

1- Epidemiological and demographic characteristics

The results of the demographic and epidemiological study, as shown in Table (1), indicate that age, gender, and weight did not show any significant differences between the group of patients and those infected, according to the p-value.

Table (1) shows some epidemiological and demographic characteristics

Characteristic	Vitiligo patients (n=20)	Control group (n=20)	Chi-square t / χ^2	p-value
Age (years)	32.4 ± 10.2	31.8 ± 9.7	0.21	0.834
Males, n(%)	9 (45%)	10 (50%)	0.10	0.752
Females, n(%)	11 (55%)	10 (50%)	0.10	0.752
BMI (kg/m ²)	25.1 ± 3.8	24.7 ± 3.5	0.38	0.706
Disease duration (yrs)	5.3 ± 3.1	—	—	—

Values represent the mean ± standard deviation (Mean ± SD) or the number and percentage. The independent t-test was used for continuous variables, and the chi-square test for categorical variables.

2 -Liver enzymes and their relationship to vitiligo

The results of the study, as shown in Table (2), revealed a statistically significant difference between the values of liver enzymes in patients with vitiligo compared to the control group. The ALT enzyme showed a significant increase in patients (38.6 ± 9.2 U/L) compared to healthy individuals (22.4 ± 6.1 U/L). AST levels in patients showed a clear significant increase (41.3 ± 10.5 U/L) compared to the control group (24.1 ± 7.3 U/L). ALP levels showed a significant increase (112.4 ± 22.8 U/L) compared to the control group (88.6 ± 18.3 U/L). GGT enzyme showed a significant increase in patients (34.7 ± 8.4 U/L) compared to healthy individuals (21.3 ± 6.2 U/L).

Table 2(Comparison of liver enzymes between the two groups of patients and healthy

Liver Enzyme	Vitiligo patients (Mean ± SD)	Contrail group (Mean ± SD)	t-value	P-value
ALT (U/L)	38.6 ± 9.2	22.4 ± 6.1	7.18	< 0.001 **
AST (U/L)	41.3 ± 10.5	24.1 ± 7.3	6.52	< 0.001 **

ALP (U/L)	112.4 ± 22.8	88.6 ± 18.3	3.96	< 0.001 **
GGT (U/L)	34.7 ± 8.4	21.3 ± 6.2	6.13	< 0.001 **

**Highly statistically significant (P < 0.001). Independent t-test. Normal range: ALT: 7–56 U/L, AST: 10–40 U/L, ALP: 44–147 U/L, GGT: 9–48 U/L.

3-Antioxidants and their relationship to vitiligo

The results of our study, as shown in Table (3), revealed the differences in antioxidant parameters among those with vitiligo. Superoxide dismutase (SOD) showed a significant decrease in those with vitiligo, 48.2 ± 11.4 U/mL, compared to the control group, 72.6 ± 13.8 U/mL. Catalase (CAT) also showed a significant decrease in the patient group, 31.5 ± 8.7 U/mL, compared to the control group, 54.3 ± 11.2 U/mL. Malondialdehyde (MDA) levels showed a significant increase in patients, 5.84 ± 1.32 nmol/mL, compared to the control group, 2.41 ± 0.78 nmol/mL. Glutathione (GSH) levels showed a clear significant decrease in patients, 412.6 ± 88.5 µmol/L, compared to the control group, 618.4 ± 102.3 µmol/L. Finally, the total antioxidant capacity (TAC) in patients showed a significant decrease of 0.82 ± 0.21 µmol/L compared to the control group of 1.54 ± 0.32 µmol/L.

Table (3): Comparison of antioxidant parameters between the two groups of patients and healthy

Antioxidant Parameter	Vitiligo patients (Mean ± SD)	Contrail group (Mean ± SD)	t-value	P-value
SOD (U/mL)	48.2 ± 11.4	72.6 ± 13.8	6.67	< 0.001 **
CAT (U/mL)	31.5 ± 8.7	54.3 ± 11.2	7.85	< 0.001 **
MDA (nmol/mL)	5.84 ± 1.32	2.41 ± 0.78	11.09	< 0.001 **
GSH (µmol/L)	412.6 ± 88.5	618.4 ± 102.3	7.42	< 0.001 **
TAC (mmol/L)	0.82 ± 0.21	1.54 ± 0.32	9.16	< 0.001 **

** Highly statistically significant (P < 0.001). SOD, CAT, GSH and TAC are lower in patients (indicating oxidative stress), while MDA is higher (considered an indicator of oxidative damage).

4- ABO blood group and its relationship to vitiligo

Our study indicated, according to Table (4) which shows the distribution of blood types among patients with vitiligo compared to the control group, that the blood types of the patients were recorded as type A 7 (35%), followed by type B and O, then AB 5 (25%), 5 (25%), and then 3 (15%) respectively. No significant differences were recorded between the blood types and the control group according to the P-value.

Table (4): ABO Blood group distribution between the two groups of patients and healthy

Blood Group	Vitiligo patients n (%)	Contrail group n (%)	Total	Chi square χ^2	P-value
A	7 (35%)	6 (30%)	13 (32.5%)	0.13	0.719
B	5 (25%)	4 (20%)	9 (22.5%)	0.15	0.698
AB	3 (15%)	4 (20%)	7 (17.5%)	0.18	0.673
O	5 (25%)	6 (30%)	11 (27.5%)	0.13	0.719
Total	20 (100%)	20 (100%)	40 (100%)	0.39*	0.821*

* Chi-square test value for all blood types compared between the two groups. There were no statistically significant differences in the distribution of blood types ($P > 0.05$).

5- Liver Enzymes in Vitiligo Patients by ABO Blood Group

The results of our study, according to Table (5), indicate the distribution of liver enzymes according to the ABO blood group patterns, where no significant differences were recorded in liver enzymes between the four blood groups A, B, AB, O according to the P-value.

Table (5): Liver Enzymes in Vitiligo Patients by ABO Blood Group

Blood Group	ALT (U/L) Mean $\pm$ SD	AST (U/L) Mean $\pm$ SD	ALP (U/L) Mean $\pm$ SD	GGT (U/L) Mean $\pm$ SD
A (n=7)	39.2 $\pm$ 8.4	42.1 $\pm$ 9.8	114.2 $\pm$ 21.6	35.8 $\pm$ 7.9
B (n=5)	37.8 $\pm$ 10.1	40.5 $\pm$ 11.2	110.8 $\pm$ 24.3	33.9 $\pm$ 9.1
AB (n=3)	40.1 $\pm$ 9.6	43.8 $\pm$ 10.4	116.7 $\pm$ 19.8	36.4 $\pm$ 8.6
O (n=5)	37.1 $\pm$ 9.8	39.8 $\pm$ 10.7	109.4 $\pm$ 23.1	33.2 $\pm$ 8.8
ANOVA (F)	0.42	0.38	0.31	0.29
P-value	0.741	0.768	0.817	0.833

One-way ANOVA. There were no statistically significant differences in liver enzymes between different blood types ($P > 0.05$).

6- Antioxidant Parameters in Vitiligo Patients by ABO Blood Group

The results of the study, according to Table (5), indicate the distribution of liver enzymes according to the ABO blood group patterns among those with vitiligo. The liver enzymes did not record any significant differences between the antioxidants SOD, CAT, MDA, GSH, and TAC according to the P-value.

Table 6: Antioxidant Parameters in Vitiligo Patients by ABO Blood Group

Blood Group	SOD (U/mL)	CAT (U/mL)	MDA (nmol/mL)	GSH (μmol/L)	TAC (mmol/L)
A (n=7)	47.1 $\pm$ 10.8	30.8 $\pm$ 8.2	5.92 $\pm$ 1.28	408.3 $\pm$ 91.2	0.80 $\pm$ 0.19
B (n=5)	49.3 $\pm$ 12.1	32.1 $\pm$ 9.4	5.78 $\pm$ 1.41	418.7 $\pm$ 86.4	0.84 $\pm$ 0.22
AB (n=3)	46.8 $\pm$ 11.6	30.2 $\pm$ 8.9	5.96 $\pm$ 1.38	405.1 $\pm$ 93.8	0.81 $\pm$ 0.20
O (n=5)	49.8 $\pm$ 11.9	32.8 $\pm$ 8.6	5.71 $\pm$ 1.29	421.4 $\pm$ 88.1	0.85 $\pm$ 0.23
ANOVA (F)	0.27	0.31	0.22	0.18	0.24
P-value	0.847	0.817	0.881	0.911	0.868

One-way ANOVA. There were no statistically significant differences in antioxidants between blood types ($P > 0.05$).

7- The relationship between liver enzymes and antioxidants in vitiligo patients

Table (5) shows the relationship between liver enzymes AST, ALT, ALP and the antioxidants SOD, CAT, MDA, GSH, and TAC according to the P-value. The results showed that the relationship of the liver enzyme ALT with SOD was -0.612, meaning that as the antioxidant ALT increases and the level of SOD decreases, the body's oxidative defense weakens. The relationship of the enzyme with CAT was recorded at -0.588, indicating a strong inverse relationship that suggests catalase enzyme breakdown. The

relationship of the enzyme with MDA was recorded at +0.724, which is the strongest correlation in the table, indicating oxidative stress. The relationship of the liver enzyme with GSH was recorded at -0.541, and the decrease in glutathione indicated antioxidant depletion. The relationship of the enzyme with the antioxidant TAC was recorded at -0.663, indicating a decrease in the total energy of antioxidants. The liver enzyme AST showed the same correlations as above, indicating liver cell damage. Its correlation with MDA was -0.598, indicating high oxidative stress. The enzyme ALP showed moderate correlations with TAC and MDA, indicating the bile ducts are affected by oxidative stress. The enzyme GGT showed correlations with TAC and MDA, indicating that it is less affected by oxidative stress.

Table (7): Pearson correlation between liver enzymes and antioxidants

Liver enzyme	Antioxidant	correlation coefficient (r)	p-value	Significance
ALT	SOD	-0.612	< 0.001	**
ALT	CAT	-0.588	0.006	**
ALT	MDA	+0.724	< 0.001	**
ALT	GSH	-0.541	0.014	*
ALT	TAC	-0.663	< 0.001	**
AST	SOD	-0.598	0.005	**
AST	CAT	-0.572	0.008	**
AST	MDA	+0.701	< 0.001	**
AST	GSH	-0.527	0.017	*
AST	TAC	-0.648	0.002	**
ALP	MDA	+0.534	0.015	*
ALP	TAC	-0.481	0.032	*
GGT	MDA	+0.512	0.021	*
GGT	TAC	-0.468	0.037	*

**P < 0.01 is highly significant, *P < 0.05 is moderately significant. A negative correlation (-) indicates decreased antioxidant activity with increased enzyme activity. A positive correlation (+) with MDA indicates increased oxidative stress.

Discussion

Our study revealed no statistically significant differences between certain demographic characteristics (age, gender, body weight) and vitiligo, indicating that vitiligo can appear at any age. Although the difference between males and females was not statistically significant, with females reporting slightly higher rates than males, this is attributed to hormonal changes and higher rates of autoimmune diseases in women. Our study agrees with **Haulrig et al. (2024)**, who observed no differences in vitiligo incidence between males and females, noting an equal distribution of the condition in both groups. If the disease is hereditary, vitiligo does not depend on genes linked to the X or Y chromosome; it is an autosomal, not sex-linked, gene (meaning both males and females are predisposed to the disease). Mutations occur in genes such as NLRP1, FOXP3, and PTPN22, affecting both sexes. Furthermore, the CD8 T-cell immune response targets specific pigment cells rather than a systemic system, thus minimizing its impact on hormones. Nationality. A study by **Mahajan et al., (2019)** indicated that vitiligo affects both sexes and

all age groups, regardless of geographical, environmental, living conditions, lifestyles, or ethnicity. It usually begins in early adulthood and progresses slowly in most cases. Physical trauma is the main triggering factor. Patients with a first-degree relative with vitiligo may have a slightly higher chance of developing it at a younger age compared to others, but the difference is not substantial.

Here, our study agrees with **O'Haga *et al.*, (2023)**, who explained that there does not appear to be a relationship between the appearance of vitiligo and body mass index (BMI) classification in the areas of the eyelids, lips, mouth, back, hips, genitals, legs, and knees. The study results indicated a significant increase in the liver enzymes ALT, AST, ALP, and GGT in individuals with vitiligo. Elevated ALT is a sensitive indicator of liver cell damage and oxidative stress, which impairs liver function in patients. Elevated AST levels demonstrate liver dysfunction, not merely coincidental, as they are associated with hydrogen peroxide accumulation resulting from CAT enzyme deficiency in these patients. Elevated ALP levels, associated with bile ducts and cell membranes, indicate inflammation of the bile ducts and cell membranes due to oxidation. GGT enzyme abnormalities point to oxidative stress (as an immune mechanism) and a deficiency of the essential antioxidant GSH. This suggests that vitiligo results from oxidative stress and liver dysfunction (**van Beek *et al.*, 2013**). Our study also revealed decreased levels of the antioxidants SOD, CAT, GSH, and TAC, indicating high oxidative stress and its impact on liver enzymes, leading to their depletion. The study agreed with (**Bakry *et al.*, 2020**) that SOD is the first line of defense for antioxidants against free radicals, and that its decrease in the infected group indicates that the melanocytes have lost their protection from the toxic superoxide anion, thus leading to their destruction and causing oxidative stress. A decrease in CAT levels, as indicated by the study (**Nandi *et al.*, 2019**), shows that CAT is responsible for breaking down H₂O₂ in the skin into water and oxygen, and that its decrease means the accumulation of H₂O₂ in the skin, which leads to melanocyte toxicity. A decrease in GSH levels, as explained by **Handy & Loscalzo, (2022)**, shows that GSH is an antioxidant that works within the cell, and its decrease indicates its consumption when it confronts oxidative stress, with the cells being unable to self-regenerate and a decline in the glutathione peroxidase enzyme that is associated with it. An increase in TAC levels, as indicated by the study (**Siddiqua, 2018**), shows that TAC reveals the body's defensive ability towards oxidation, and its decrease means exhaustion of the oxidative defense and that it is systemically burdened and exhausted, which proves that vitiligo is not a skin disease.

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