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Serum Heme Oxygenase-1 and Antioxidant Enzyme Levels in Vitiligo: Relationship with Disease Activity and Response to Narrowband UVB Phototherapy. A Prospective Study

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Abstract

Oxidative stress contributes to melanocyte loss in vitiligo. Heme oxygenase-1 (HO-1), a major Nrf2-regulated cytoprotective enzyme, protects melanocytes against oxidative injury, but data on circulating HO-1 in relation to clinical features and phototherapy remain limited. We compared serum HO-1, catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA) and nitric oxide (NO) between patients with vitiligo and healthy controls, and evaluated their clinical associations and changes after narrowband ultraviolet B (NB-UVB) phototherapy. This prospective study enrolled 45 adults with non-segmental vitiligo and 35 age- and sex-matched healthy controls. Disease activity and extent were assessed with the VIDA and VASI scores. HO-1 was measured by ELISA and the other markers spectrophotometrically. Eight patients had paired measurements after three months of NB-UVB. Compared with controls, patients had higher CAT ($p = 0.021$) and lower MDA ($p = 0.004$) and NO ($p = 0.015$) levels; GPx differed significantly ($p = 0.012$), whereas serum HO-1 did not ($p = 0.309$). No marker differed between active ($n = 31$) and stable ($n = 14$) disease. MDA correlated positively with VASI ($r = 0.337$), whereas CAT and GPx were not correlated with MDA or NO. After NB-UVB, serum HO-1 increased significantly (median 0.49 to 0.80 ng/mL; $p = 0.036$) and VIDA decreased ($p = 0.014$); changes in the other markers were not significant. Higher CAT with lower MDA and NO levels may reflect an adaptive systemic antioxidant response, while the association of MDA with disease extent indicates ongoing lipid peroxidation. The rise in serum HO-1 after NB-UVB suggests that HO-1 induction may contribute to the effect of phototherapy and warrants confirmation in larger studies.

INTRODUCTION

Vitiligo is an acquired, chronic pigmentary disorder characterised by the selective loss of functional melanocytes from the epidermis and, occasionally, the hair follicles, resulting in sharply demarcated, chalk-white depigmented macules and patches involving the skin, hair and mucous membranes [2, 9]. The disease is often progressive and imposes a considerable psychosocial burden [9].

The pathogenesis of vitiligo is multifactorial and involves genetic susceptibility, environmental triggers and autoimmunity. Oxidative stress is considered a key early event that damages melanocytes and initiates the autoimmune response [2, 7]. Reactive oxygen species (ROS) are generated through both endogenous and exogenous pathways; besides mitochondrial metabolism, melanogenesis itself is an important endogenous source of ROS, which renders melanocytes particularly vulnerable [7]. Vitiligo melanocytes show increased sensitivity to peroxidative agents, and mitochondrial impairment has been described in peripheral blood mononuclear cells during the active phase of the disease, indicating that the redox imbalance is not restricted to the skin [6, 21].

Under physiological conditions, ROS are neutralised by enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), and by non-enzymatic scavengers. When ROS production exceeds this capacity, lipid peroxidation, reflected by malondialdehyde (MDA), and oxidative damage to proteins and DNA ensue, ultimately leading to melanocyte dysfunction and

apoptosis [7]. Nitric oxide (NO), a reactive nitrogen species, may also contribute to melanocyte injury. However, studies of circulating oxidant and antioxidant markers in vitiligo have yielded heterogeneous results [29].

The nuclear factor erythroid 2–related factor 2 (Nrf2) pathway is one of the principal defence mechanisms activated under oxidative stress. Upon activation, Nrf2 dissociates from its repressor Kelch-like ECH-associated protein 1 (Keap1), translocates to the nucleus and binds to antioxidant response elements (ARE), inducing the transcription of cytoprotective and detoxifying enzymes [13]. Heme oxygenase-1 (HO-1), a key inducible target of this pathway, degrades heme into ferrous iron, carbon monoxide and biliverdin, which is subsequently converted into bilirubin; together, these products exert antioxidant, anti-inflammatory and anti-apoptotic effects [26]. HO-1 protects human melanocytes against hydrogen peroxide (H_2O_2)-induced oxidative damage via the Nrf2–ARE pathway [14], and impaired Nrf2 activation with reduced HO-1 expression has been demonstrated in vitiligo melanocytes, together with decreased serum HO-1 levels in patients [15]. Moreover, H_2O_2 accumulates in the epidermis of patients with vitiligo [27], polymorphisms in the Nrf2 promoter have been associated with disease susceptibility and serum HO-1 activity [28], and HO-1 has been implicated in the impaired function of regulatory T cells in vitiligo [33].

The main goals of vitiligo treatment are to halt disease progression and to achieve repigmentation. Current options include topical and systemic immunosuppressive agents, phototherapy and, for stable refractory disease, surgical approaches [4]. Narrowband ultraviolet B (NB-UVB) phototherapy is a first-line treatment for generalised vitiligo owing to its efficacy and favourable safety profile in both children and adults [4]. Beyond its immunomodulatory effects, ultraviolet radiation is a potent inducer of HO-1 [5, 18], and HO-1 expression increases in the skin of patients who respond to psoralen plus ultraviolet A (PUVA) therapy [8]. Nevertheless, whether NB-UVB modifies circulating oxidative stress markers and HO-1 levels in patients with vitiligo remains insufficiently clarified [16].

In this study, we aimed to (i) compare serum HO-1, CAT, GPx, MDA and NO levels between patients with vitiligo and healthy controls; (ii) investigate their relationship with demographic and clinical characteristics, including disease activity and extent; and (iii) evaluate their changes after NB-UVB phototherapy. To our knowledge, changes in serum HO-1 after NB-UVB in vitiligo have not been reported previously.

MATERIALS AND METHODS

Study design and ethics

This prospective case–control study, including a longitudinal phototherapy subgroup, was conducted at the Department of Dermatology and Venereology, Ankara University Faculty of Medicine. Participants were enrolled between June 2024 and June 2025, and follow-up of the phototherapy subgroup was completed in August 2025. The study protocol was approved by the Ankara University Faculty of

Medicine Human Research Ethics Committee (approval no. İ04-265-23), and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Participants

Consecutive adult patients with non-segmental vitiligo presenting to our outpatient clinic were evaluated for eligibility. The diagnosis was based on clinical examination and Wood's lamp findings and classified according to international consensus [10]. Patients were excluded if they were younger than 18 years; had received systemic corticosteroids within the previous 6 months or topical corticosteroids within the previous 3 months; had received any phototherapy previously; were taking antioxidant supplements; or had contraindications to phototherapy. The control group consisted of age- and sex-matched healthy volunteers with no known systemic or dermatological disease, who did not smoke or consume alcohol and were not taking any medication. The sample size was calculated a priori with G*Power 3.1.9 on the basis of the primary hypothesis, the difference in serum HO-1 levels between patients and controls. With an effect size of d = 0.685, a two-sided α of 0.05 and a power of 80%, a minimum of 35 participants per group was required.

Clinical assessment

Age, sex, body mass index (BMI), age at disease onset, disease duration, smoking and alcohol use, family history of vitiligo and concomitant autoimmune diseases were recorded on a standardised form. Involvement of the face, trunk, acral areas, genital region and hair was documented. Disease extent was assessed with the Vitiligo Area Scoring Index (VASI; range 0–100) [11], and disease activity with the Vitiligo Disease Activity score (VIDA; range − 1 to + 4) [23]. Patients with a VIDA score ≥ + 1 (disease activity within the preceding year) were classified as having active vitiligo, and those with a VIDA score ≤ 0 as having stable vitiligo.

Phototherapy

Phototherapy was offered to eligible patients and administered at the phototherapy unit of the Department of Dermatology and Venereology, Ankara University Faculty of Medicine, using the unit's NB-UVB devices. NB-UVB (311 nm) was given three times weekly for three months, for a total of 36 sessions. Eight patients completed 36 sessions of NB-UVB; the remaining 37 patients declined phototherapy, were not considered suitable, or did not complete the planned course. VASI and VIDA scores were reassessed at the end of treatment.

Sample collection

Venous blood samples were collected into plain (red-top) serum tubes from all participants at the initial evaluation and, in the phototherapy subgroup, again at the end of the third month of treatment, after an overnight fast, and were processed at the biochemistry laboratory of our hospital. Serum was separated and stored at − 80°C until analysis, and all samples were analysed together in a single batch at the end of the study.

Biochemical analyses

Serum NO was determined indirectly: nitrate was reduced to nitrite using metallic cadmium, followed by formation of a coloured azo compound via the Griess reaction. The absorbance of the resulting compound was measured spectrophotometrically at 540 nm, and results were expressed as μmol/L.

MDA levels were measured using the thiobarbituric acid (TBA) reaction, in which MDA reacts with TBA to form a coloured complex with maximum absorbance at 532 nm. Results were expressed as nmol/mL.

GPx activity was determined by monitoring the oxidation of NADPH to $NADP^+$ during the reduction of oxidised glutathione catalysed by the enzyme; the decrease in NADPH absorbance was measured spectrophotometrically at 340 nm. CAT activity was assessed on the basis of enzymatic decomposition of H_2O_2, monitored as the decrease in absorbance at 240 nm. Enzyme activities were expressed as IU/mL.

Serum HO-1 was measured with a commercial sandwich ELISA (Human Heme Oxygenase 1 ELISA Kit, E0932Hu, 96T; BT LAB, Shanghai, China; detection range 0.1–9 ng/mL, sensitivity 0.05 ng/mL; intra-assay CV 3.0–5.8%, inter-assay CV < 10%) according to the manufacturer's instructions and expressed as ng/mL.

Statistical analysis

Statistical analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as numbers and percentages, and continuous variables as mean ± standard deviation (SD) and median (minimum–maximum). The normality of distributions was assessed with the Shapiro–Wilk test. Age, which was normally distributed, was compared between patients and controls using the independent-samples t test. As most other variables were not normally distributed, two independent groups were compared using the Mann–Whitney U test, and three groups (active vitiligo, stable vitiligo and controls) using the Kruskal–Wallis test followed by pairwise Mann–Whitney U tests. Pre- and post-treatment values were compared using the Wilcoxon signed-rank test. Categorical variables were compared using the chi-square or Fisher's exact test, as appropriate. Correlations between continuous variables were assessed using Spearman's rank correlation coefficient. A two-sided p value < 0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics

A total of 45 patients with vitiligo and 35 healthy controls were included. The groups were comparable in terms of age (45.91 ± 16.69 vs. 45.09 ± 16.49 years; p = 0.826) and sex distribution (female, 57.8% vs. 51.4%; p = 0.571) (Table 1).

Table 1
Demographic and clinical characteristics of patients with vitiligo and healthy controls

Characteristic	Vitiligo (n = 45)	Control (n = 35)	p
Sex, n (%)			0.571[a]
Female	26 (57.8)	18 (51.4)	
Male	19 (42.2)	17 (48.6)	
Age, years, mean ± SD (min–max)	45.91 ± 16.69 (18–85)	45.09 ± 16.49 (18–78)	0.826[b]
BMI, kg/m², mean ± SD (min–max)	27.36 ± 5.04 (18.9–37.8)	–	
Smoking, n (%)	12 (26.7)	0	
Alcohol use, n (%)	2 (4.4)	0	
Family history of vitiligo, n (%)	17 (37.8)	–	
Concomitant autoimmune disease, n (%)	15 (33.3)	–	
Age at onset, years, mean ± SD	26.67 ± 12.74	–	
median (min–max)	26 (4–57)		
Disease duration, years, mean ± SD	19.24 ± 15.53	–	
median (min–max)	17 (0–59)		
Disease duration group, n (%)			
≤3 years	11 (24.4)	–	
3–10 years	5 (11.1)	–	
≥10 years	29 (64.4)	–	
VIDA, mean ± SD	2.38 ± 1.74	–	
median (min–max)	3 (0–4)		
VIDA score, n (%)			
+4	18 (40.0)	–	
+3	10 (22.2)	–	

BMI, body mass index; SD, standard deviation; VASI, Vitiligo Area Scoring Index; VIDA, Vitiligo Disease Activity score. [a] Chi-square test. [b] Independent-samples t test. Controls were non-smokers and did not consume alcohol by definition. Active disease was defined as VIDA ≥ + 1 (n = 31, 68.9%) and stable disease as VIDA ≤ 0 (n = 14, 31.1%).

Characteristic	Vitiligo (n = 45)	Control (n = 35)	p
+2	2 (4.4)	–	
+1	1 (2.2)	–	
0	14 (31.1)	–	
VASI, mean ± SD	21.16 ± 30.32	–	
median (min–max)	8.5 (0.3–100)		

BMI, body mass index; SD, standard deviation; VASI, Vitiligo Area Scoring Index; VIDA, Vitiligo Disease Activity score. [a] Chi-square test. [b] Independent-samples t test. Controls were non-smokers and did not consume alcohol by definition. Active disease was defined as VIDA ≥ + 1 (n = 31, 68.9%) and stable disease as VIDA ≤ 0 (n = 14, 31.1%).

In the vitiligo group, the mean BMI was 27.36 ± 5.04 kg/m². Twelve patients (26.7%) were smokers and two (4.4%) consumed alcohol. A family history of vitiligo was present in 17 patients (37.8%), and 15 (33.3%) had at least one concomitant autoimmune disease, including alopecia areata, Hashimoto's thyroiditis, diabetes mellitus, inflammatory bowel disease and psoriasis. The mean age at onset was 26.67 ± 12.74 years, and the median disease duration was 17 years (range 0–59). Disease duration was ≤ 3 years in 11 patients (24.4%), 3–10 years in 5 (11.1%) and ≥ 10 years in 29 (64.4%).

The mean VIDA score was 2.38 ± 1.74 (median 3; range 0–4): 18 patients (40.0%) had a score of + 4, 10 (22.2%) + 3, 2 (4.4%) + 2, 1 (2.2%) + 1 and 14 (31.1%) 0. Accordingly, 31 patients (68.9%) had active and 14 (31.1%) had stable disease. The mean VASI score was 21.16 ± 30.32 (median 8.5; range 0.3–100). Active and stable patients did not differ in terms of sex, age, BMI, smoking, family history, concomitant autoimmune disease, age at onset or disease duration (all $p > 0.05$).

Associations with clinical characteristics

VASI scores were significantly higher in patients with a concomitant autoimmune disease than in those without (median 14.5 vs. 2.75; $p = 0.042$) and differed according to disease duration ($p = 0.030$), being highest in patients with a duration of ≥ 10 years (median 11.59 vs. 1.89 for ≤ 3 years and 1.95 for 3–10 years). VIDA scores also differed across duration groups ($p = 0.047$). VASI was not significantly correlated with age, BMI, age at onset or disease duration as a continuous variable ($r = 0.252$; $p = 0.095$), and VIDA was not associated with any demographic variable (all $p > 0.05$).

Among serum parameters, NO levels were higher in men than in women (median 30.5 vs. 25.4 µmol/L; $p = 0.033$), and HO-1 levels differed across disease duration groups ($p = 0.047$), with the lowest values in patients with a duration of 3–10 years (median 0.28 ng/mL, n = 5) compared with ≤ 3 years (1.13 ng/mL) and ≥ 10 years (0.93 ng/mL). No other associations were found between serum parameters and sex, age, BMI, smoking, family history, autoimmune disease, age at onset or disease duration (all $p > 0.05$).

Comparison between patients with vitiligo and controls

Serum HO-1 levels did not differ significantly between patients and controls (median 0.93 vs. 0.48 ng/mL; p = 0.309). CAT activity was significantly higher in patients (median 7.20 vs. 4.32 IU/mL; p = 0.021), whereas MDA (median 2.10 vs. 2.82 nmol/mL; p = 0.004) and NO (median 27.50 vs. 30.25 µmol/L; p = 0.015) levels were significantly lower than in controls. GPx activity also differed significantly (p = 0.012); although the mean was higher in patients owing to a small number of markedly elevated values, the median was lower (87.84 vs. 113.76 IU/mL) (Table 2).

Table 2
Serum oxidant and antioxidant parameters in patients with vitiligo and healthy controls

Parameter	**Vitiligo (n = 45)** **mean ± SD** **median (min–max)**	**Control (n = 35)** **mean ± SD** **median (min–max)**	**p**
HO-1 (ng/mL)	1.54 ± 2.04 0.93 (0.05–8.92)	2.14 ± 3.16 0.48 (0.01–9.60)	0.309
CAT (IU/mL)	8.67 ± 7.79 7.20 (0–36.00)	5.68 ± 6.48 4.32 (0–36.00)	**0.021**
GPx (IU/mL)	124.77 ± 189.51 87.84 (61.92–1353.60)	111.91 ± 26.86 113.76 (69.12–164.16)	**0.012**
MDA (nmol/mL)	2.26 ± 0.73 2.10 (1.37–4.52)	2.84 ± 0.91 2.82 (1.45–4.84)	**0.004**
NO (µmol/L)	28.38 ± 6.89 27.50 (16.25–52.00)	32.86 ± 9.50 30.25 (19.50–67.50)	**0.015**

CAT, catalase; GPx, glutathione peroxidase; HO-1, heme oxygenase-1; MDA, malondialdehyde; NO, nitric oxide; SD, standard deviation. p values were obtained using the Mann–Whitney U test; bold values indicate statistical significance.

Relationship with disease activity

The comparison of VASI scores and serum parameters among active vitiligo, stable vitiligo and control groups is presented in Table 3. The VASI score was significantly higher in stable than in active vitiligo (40.08 ± 43.10 vs. 12.61 ± 17.30; p = 0.004). None of the serum parameters differed significantly between active and stable disease (all p > 0.05).

Table 3
VASI scores and serum parameters in active vitiligo, stable vitiligo and control groups

Parameter	Active vitiligo (n = 31) mean ± SD median (min–max)	Stable vitiligo (n = 14) mean ± SD median (min–max)	Control (n = 35) mean ± SD median (min–max)	p1	p2	p3
VASI	12.61 ± 17.30 3.57 (0–73)	40.08 ± 43.10 14.25 (1–100)	–	**0.004**	–	–
HO-1 (ng/mL)	1.35 ± 1.87 0.77 (0.05–6.78)	1.95 ± 2.40 0.98 (0.43–8.92)	2.14 ± 3.16 0.48 (0.01–9.60)	0.125	0.224	0.117
CAT (IU/mL)	8.36 ± 7.42 7.20 (0–34.56)	9.36 ± 8.80 7.20 (1.44–36.00)	5.68 ± 6.48 4.32 (0–36.00)	0.730	0.065	0.059
GPx (IU/mL)	136.99 ± 227.83 87.84 (61.92–1353.60)	97.71 ± 25.02 92.88 (67.68–146.88)	111.91 ± 26.86 113.76 (69.12–164.16)	0.912	**0.044**	0.090
MDA (nmol/mL)	2.31 ± 0.76 2.10 (1.37–4.52)	2.14 ± 0.67 1.94 (1.53–4.11)	2.84 ± 0.91 2.82 (1.45–4.84)	0.514	**0.012**	**0.011**
NO (µmol/L)	28.09 ± 7.02 27.50 (16.25–52.00)	29.02 ± 6.82 27.75 (21.75–44.50)	32.86 ± 9.50 30.25 (19.50–67.50)	0.704	**0.047**	0.160

CAT, catalase; GPx, glutathione peroxidase; HO-1, heme oxygenase-1; MDA, malondialdehyde; NO, nitric oxide; SD, standard deviation; VASI, Vitiligo Area Scoring Index. p values were obtained using the Mann–Whitney U test; bold values indicate statistical significance. p1, active vs. stable vitiligo; p2, active vitiligo vs. control; p3, stable vitiligo vs. control.

Compared with controls, patients with active vitiligo had significantly lower MDA (p = 0.012) and NO (p = 0.047) levels and a significantly different GPx distribution (p = 0.044), with a lower median value (87.84 vs. 113.76 IU/mL). HO-1 and CAT levels did not differ between active vitiligo and controls (p = 0.224 and p = 0.065, respectively). Patients with stable vitiligo had significantly lower MDA levels than controls (p = 0.011), whereas HO-1, CAT, GPx and NO levels did not differ significantly (all p > 0.05).

Correlation analyses

In the vitiligo group, MDA levels were positively correlated with VASI scores ($r = 0.337$; $p < 0.05$), and CAT activity was positively correlated with GPx activity ($r = 0.364$; $p < 0.05$) (Table 4). CAT and GPx activities were not correlated with MDA ($r = 0.024$ and $r = 0.050$) or NO ($r = 0.077$ and $r = 0.001$) levels, and HO-1 was not correlated with any other parameter. No significant correlations were found between VIDA scores and serum parameters, including NO ($r = -0.047$).

Table 4
Correlations between clinical scores and serum parameters in patients with vitiligo (n = 45)

VIDA	VASI	VIDA	HO-1	CAT	GPx	MDA
	−0.200					
HO-1	−0.033	−0.174				
CAT	0.039	−0.290	0.130			
GPx	−0.099	−0.067	0.138	**0.364***		
MDA	**0.337***	0.122	0.052	0.024	0.050	
NO	−0.094	−0.047	−0.075	0.077	0.001	0.098

Values are Spearman's rank correlation coefficients (r). *p < 0.05. CAT, catalase; GPx, glutathione peroxidase; HO-1, heme oxygenase-1; MDA, malondialdehyde; NO, nitric oxide; VASI, Vitiligo Area Scoring Index; VIDA, Vitiligo Disease Activity score.

Changes after NB-UVB phototherapy

The phototherapy subgroup comprised five women and three men, with a mean age of 44.67 ± 11.52 years and a median disease duration of 9 years (range 0–43); one patient smoked and two had a concomitant autoimmune disease. Paired results are shown in Table 5. Serum HO-1 levels increased significantly after treatment (median 0.49 vs. 0.80 ng/mL; mean 0.64 ± 0.72 vs. 1.18 ± 1.40 ng/mL; $p = 0.036$). MDA levels decreased (median 2.14 vs. 1.94 nmol/mL; $p = 0.204$), and NO levels remained unchanged ($p = 0.889$). Changes in CAT and GPx were not significant ($p = 0.182$ and $p = 0.398$), and their mean values were dominated by single extreme measurements: the mean CAT increase reflected one post-treatment value of 100.8 IU/mL, whereas the median decreased slightly (5.76 vs. 5.04 IU/mL), and the mean GPx decrease reflected one baseline value of 1353.6 IU/mL, whereas the median increased (84.96 vs. 94.32 IU/mL). Clinically, the median VIDA score decreased from 4 to 3 ($p = 0.014$), and the VASI score decreased from a median of 13.05 to 11.94, a change of borderline significance ($p = 0.050$).

Table 5
Serum parameters and clinical scores before and after NB-UVB phototherapy (n = 8)

Parameter	Before treatment mean ± SD median (min–max)	After treatment mean ± SD median (min–max)	p
HO-1 (ng/mL)	0.64 ± 0.72 0.49 (0.05–2.29)	1.18 ± 1.40 0.80 (0.14–4.52)	**0.036**
CAT (IU/mL)	5.58 ± 3.21 5.76 (1.44–11.52)	21.06 ± 33.61 5.04 (1.44–100.80)	0.182
GPx (IU/mL)	239.64 ± 450.04 84.96 (67.70–1353.60)	95.58 ± 24.18 94.32 (63.36–132.48)	0.398
MDA (nmol/mL)	2.31 ± 0.77 2.14 (1.45–3.79)	1.92 ± 0.57 1.94 (0.81–2.66)	0.204
NO (µmol/L)	29.00 ± 5.75 27.63 (22.75–38.50)	28.38 ± 4.63 28.25 (22.75–35.75)	0.889
VASI	18.11 ± 18.10 13.05 (1.20–50.00)	14.20 ± 14.38 11.94 (1.00–40.00)	0.050
VIDA, median	4	3	**0.014**

CAT, catalase; GPx, glutathione peroxidase; HO-1, heme oxygenase-1; MDA, malondialdehyde; NB-UVB, narrowband ultraviolet B; NO, nitric oxide; SD, standard deviation; VASI, Vitiligo Area Scoring Index; VIDA, Vitiligo Disease Activity score. p values were obtained using the Wilcoxon signed-rank test; bold values indicate statistical significance.

Within this subgroup, baseline MDA levels were strongly correlated with VASI ($r = 0.833$) and NO ($r = 0.738$), and post-treatment HO-1 levels were inversely correlated with GPx ($r = -0.857$) (all $p < 0.05$); given the very small sample, these correlations should be interpreted with caution.

DISCUSSION

In this prospective study, patients with vitiligo had higher serum CAT activity and lower MDA and NO levels than healthy controls, whereas serum HO-1 levels did not differ significantly. None of the oxidant or antioxidant markers distinguished active from stable disease, but MDA levels increased with disease extent. Notably, serum HO-1 increased significantly after three months of NB-UVB phototherapy, in parallel with a significant reduction in disease activity.

Most previous studies have reported reduced or unchanged antioxidant enzyme activity in vitiligo. A systematic review and meta-analysis found decreased CAT, together with elevated SOD and increased lipid peroxidation [29]; serum CAT and GPx activities were lower in patients with active and stable vitiligo in the studies of Kassab et al. and Mulayim et al. [17, 22], and impaired blood CAT activity has recently been reported [25], whereas erythrocyte CAT activity was unchanged in other cohorts [1, 12]. GPx findings are particularly heterogeneous: plasma GPx activity was increased in one study [24], erythrocyte GPx activity was decreased in others [1, 12, 32], and a meta-analysis of 23 studies found no overall difference between patients and controls, although lower serum and plasma levels emerged in subgroup analyses [31]. The higher CAT activity observed in our cohort, together with the positive correlation between CAT and GPx, suggests a coordinated upregulation of antioxidant enzymes, possibly as an adaptive response to chronic oxidative load. Most of our patients had long-standing disease (64.4% for ≥ 10 years), and adaptive induction of antioxidant enzymes over time may partly explain the discrepancy with studies including patients with shorter disease duration. Differences in sample type (serum, plasma, erythrocytes or tissue), analytical methods, ethnicity and disease stage further hamper comparisons between studies [29]. With regard to GPx, the mean value in our patients was influenced by a few very high values, whereas the median was lower than in controls; the direction of the GPx difference should therefore be interpreted with caution.

In contrast to many studies reporting increased MDA in serum, erythrocytes or tissue of patients with vitiligo [1, 12, 17, 19, 20, 22, 32], MDA levels were lower in our patients, both in active and in stable disease, than in controls. Findings on circulating NO are similarly inconsistent: serum NO was increased in some studies [22, 32] and unchanged in others [19]. At the group level, lower MDA and NO levels together with higher CAT activity could be consistent with an upregulated antioxidant system limiting lipid peroxidation and redox-sensitive NO production. However, CAT and GPx activities were not correlated with MDA or NO at the individual level; therefore, a direct causal relationship between the enhanced antioxidant response and lower oxidant levels cannot be inferred. Moreover, MDA was positively correlated with VASI, in line with the findings of Li et al. [20], indicating that lipid peroxidation still increases with disease extent. Lower NO levels might also reflect consumption of NO through its reaction with superoxide to form peroxynitrite, or differences in dietary nitrate intake, sun exposure or pre-analytical conditions, which were not controlled in this study. It should also be noted that serum NO concentrations and tissue expression of NO synthase isoforms reflect different biological compartments and are not directly comparable.

None of the markers differed between active and stable vitiligo. Similarly, Kassab et al. found no difference in serum MDA between active and stable disease, although antioxidant enzyme activities were lower in active disease [17], whereas Ines et al. reported a more pronounced oxidant–antioxidant imbalance in active vitiligo [12]. The relatively small stable group (n = 14) and the reliance of VIDA on patient-reported history may have limited our ability to detect differences. Interestingly, VASI scores were higher in stable than in active disease, underlining that disease extent and disease activity represent distinct clinical dimensions. VASI was also higher in patients with longer disease duration and in those

with concomitant autoimmune disease, consistent with previous observations linking disease duration with comorbidity and disease extent [30].

Jian et al. reported significantly lower serum HO-1 levels in 113 patients with vitiligo than in controls [15], and Song et al. found lower serum HO-1 activity in patients [28]; decreased serum levels of HO-1 metabolites, including bilirubin, carboxyhaemoglobin and iron, have also been described [33]. In our study, serum HO-1 did not differ significantly between patients and controls or between active and stable disease. Wide inter-individual variability, the smaller sample size, ethnic differences and the measurement of HO-1 protein concentration rather than enzymatic activity may account for this discrepancy. Furthermore, circulating HO-1 levels may not mirror HO-1 expression in lesional melanocytes and keratinocytes. HO-1 levels differed across disease duration groups, with the lowest values in patients with a duration of 3–10 years; however, this group included only five patients, and the finding should be regarded as exploratory.

Ultraviolet radiation is a well-established inducer of HO-1 [5, 18]. Elassiuty et al. demonstrated upregulation of HO-1 in the skin of patients successfully treated with PUVA and showed that HO-1 overexpression protects melanocytes from stress-induced cell death [8]. In a mouse model of vitiligo, the repigmenting effect of combined NB-UVB and adipose-derived stem cell therapy was abolished by Nrf2 inhibition [3], and Karsli et al. reported decreased erythrocyte MDA and increased GPx activity after NB-UVB in patients with vitiligo [16]. Our findings extend these observations to the systemic level: serum HO-1 increased significantly after NB-UVB, accompanied by a significant decrease in VIDA. MDA levels decreased after treatment, in line with previous reports [16], although not significantly, whereas the apparent changes in mean CAT and GPx were driven by single extreme values and were not reflected in the medians. Increased HO-1 might contribute to the therapeutic effect of phototherapy through its antioxidant and anti-apoptotic actions and through immunomodulatory effects, including the restoration of regulatory T-cell function [26, 33]. Whether serum HO-1 could serve as a biomarker of response to phototherapy remains to be determined in larger studies correlating its changes with objective repigmentation.

This study has several limitations. First, the phototherapy subgroup was small (n = 8), was not randomly assigned and lacked an untreated comparison group with repeated sampling: most remaining patients declined phototherapy, were not considered suitable, or did not complete the planned course. Therefore, the observed changes cannot be attributed with certainty to NB-UVB rather than to time, seasonal variation or selection. Second, this was a single-centre study with a relatively small stable vitiligo group, and no correction was applied for multiple comparisons, which increases the risk of type I error. Third, biomarkers were measured only in serum, without parallel tissue analysis, and HO-1 was measured as protein concentration rather than enzymatic activity; Nrf2 activation was not assessed. Fourth, potential confounders such as diet, sun exposure, season and smoking were not controlled; in particular, controls were non-smokers, whereas 26.7% of patients smoked. Finally, VIDA is based on patient recall, and the three-month follow-up may have been too short to capture the full extent of repigmentation.

CONCLUSION

Patients with vitiligo showed higher serum CAT activity and lower MDA and NO levels than healthy controls, which may reflect an adaptive systemic antioxidant response, although enzyme activities were not correlated with oxidant levels at the individual level. Serum HO-1 levels did not differ significantly from those of controls, and oxidant and antioxidant markers did not distinguish active from stable disease, whereas MDA increased with disease extent. Serum HO-1 increased significantly after NB-UVB phototherapy, in parallel with reduced disease activity, suggesting that HO-1 induction may contribute to the therapeutic effect of phototherapy. Larger, multicentre prospective studies combining serum and tissue measurements are needed to clarify the role of HO-1 and to determine its potential as a biomarker of treatment response.

DECLARATIONS

Competing interests:

The authors declare that they have no conflict of interest.

Ethics approval:

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ankara University Faculty of Medicine Human Research Ethics Committee (approval no. İ04-265-23).

Informed consent:

Written informed consent was obtained from all participants.

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Author Contribution

G.N.Ö.D. conceptualised the study, recruited patients, collected clinical data, performed the formal analysis and wrote the original draft. G.F.T. and S.E.Ü. performed the biochemical analyses and reviewed the manuscript. N.K. conceptualised the study, supervised the work and reviewed the manuscript. All authors approved the final version.

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Presentation:

Part of this study will be presented as an oral presentation at the 34th National Dermatology Congress, Antalya, Türkiye, 21–25 October 2026.

Data availability:

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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