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Original Research Article

Comparative Analysis of Oxidative Stress Biomarkers and Antioxidant Enzyme Activity in Patients with Alopecia Areata, Acne Vulgaris, and Vitiligo: A Case–Control Study

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Abstract

Background: Oxidative stress, an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is implicated in the pathogenesis of various inflammatory dermatoses. While its role has been studied individually in alopecia areata (AA), acne vulgaris (AV), and vitiligo, a direct comparative analysis of the oxidative burden across these distinct conditions is lacking.

Methods: This case-control study included 200 participants, divided into four groups: patients with active AA (n=50), moderate-to-severe AV (n=50), non-segmental vitiligo (n=50), and age- and sex-matched healthy controls (HC, n=50). Serum levels of malondialdehyde (MDA), a marker of lipid peroxidation, and total antioxidant capacity (TAC) were measured. Erythrocyte activity of superoxide dismutase (SOD) and catalase (CAT) was also determined. Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test.

Results: All patient groups exhibited significantly higher serum MDA levels and lower TAC, SOD, and CAT activity compared to the healthy control group ($p<0.001$ for all). The vitiligo group showed the most profound oxidative imbalance, with the highest MDA levels (4.12 ± 0.55 nmol/mL vs. 1.68 ± 0.29 in HC, $p<0.001$) and the lowest TAC (0.78 ± 0.11 mmol/L vs. 1.35 ± 0.16 in HC, $p<0.001$), SOD, and CAT activities. MDA levels in the vitiligo group were significantly higher than in both the AA (3.25 ± 0.48 nmol/mL, $p<0.001$) and AV groups (2.61 ± 0.37 nmol/mL, $p<0.001$). The AA group also showed a significantly greater oxidative burden than the AV group ($p<0.01$). Antioxidant defenses followed an inverse trend, being most depleted in vitiligo, followed by AA, and then AV.

Conclusion: Patients with alopecia areata, acne vulgaris, and vitiligo demonstrate a significant systemic oxidative stress state compared to healthy individuals. The magnitude of this imbalance varies, being most severe in vitiligo, followed by alopecia areata, and then acne vulgaris. These findings support the integral role of oxidative stress in the pathophysiology of these dermatoses and suggest a potential hierarchy of oxidative burden among them.

Keywords: Oxidative Stress, Malondialdehyde, Superoxide Dismutase, Catalase, Alopecia Areata, Acne Vulgaris, Vitiligo.

Introduction

Oxidative stress is a physiological state characterized by an imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological system to detoxify these reactive intermediates through its antioxidant defense network [1]. The skin, being a primary interface with the external environment and subject to various pro-oxidant stimuli like ultraviolet radiation and pollutants, is particularly vulnerable to oxidative damage [2]. An accumulating body of evidence suggests that oxidative stress is a key pathogenic factor in a wide array of inflammatory and autoimmune dermatological disorders [3]. Alopecia areata (AA), acne vulgaris (AV), and vitiligo are three common, yet distinct, dermatoses with significant impacts on patient quality of life. AA is an autoimmune disease resulting in non-scarring hair loss, where immune privilege collapse of the hair follicle is a central event [4]. Recent studies suggest that perifollicular oxidative stress may contribute to the inflammatory cascade that targets the hair follicle [5]. Acne vulgaris is a

chronic inflammatory disease of the pilosebaceous unit, driven by factors including sebum production, follicular hyperkeratinization, and inflammation mediated by Cutibacterium acnes [6]. Lipid peroxidation within sebum and ROS production by neutrophils are thought to be critical contributors to acne-related inflammation [7]. Vitiligo is an acquired depigmenting disorder characterized by the progressive loss of functional melanocytes from the epidermis [8]. The "convergent theory" of vitiligo pathogenesis posits that a combination of factors, with oxidative stress being a central element, leads to melanocyte destruction [9, 10].

While numerous studies have independently investigated and confirmed the presence of oxidative stress in each of these conditions, they have often used different biomarkers and methodologies, making direct comparisons difficult. For example, research has demonstrated elevated levels of malondialdehyde (MDA), a product of lipid peroxidation, and decreased activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in the serum and tissues of patients with AA [11], AV [12], and vitiligo [13] when compared to healthy controls.

However, a significant research gap exists in the simultaneous, comparative assessment of the oxidative stress burden across these three distinct dermatological conditions using a standardized methodology. Such a study could reveal whether the magnitude of systemic oxidative stress differs, potentially reflecting the underlying pathophysiology where ROS may play a primary, secondary, or synergistic role. A comparative analysis could provide insights into a potential "hierarchy" of oxidative imbalance among these diseases, informing both our understanding of their pathogenesis and the potential for targeted antioxidant therapies.

Therefore, the primary aim of this case-control study was to comprehensively and comparatively analyze a panel of key oxidative stress biomarkers (MDA, TAC) and antioxidant enzyme activities (SOD, CAT) in patients with active alopecia areata, moderate-to-severe acne vulgaris, and non-segmental vitiligo, and to evaluate the extent of deviation from a matched healthy control group.

Materials and Methods

Study Design and Participants: This was a single-center, prospective case-control study conducted at the Department of Dermatology of a tertiary care hospital.

The study included 200 subjects, divided into four groups of 50 participants each:

1. **Group AA:** Patients with active alopecia areata.
2. **Group AV:** Patients with moderate-to-severe acne vulgaris.
3. **Group V:** Patients with active, non-segmental vitiligo.
4. **Group HC:** Healthy controls with no dermatological or systemic disease.

Inclusion Criteria

- **For Patient Groups:** Age 18–45 years; clinically confirmed diagnosis of AA, AV, or vitiligo by a dermatologist; active disease (i.e., new lesions within the last 3 months). AA severity was assessed using the Severity of Alopecia Tool (SALT) score (>10). AV severity was graded using the Global Acne Grading System (GAGS) score (>18, moderate-to-severe). Vitiligo activity was assessed by the Vitiligo Disease Activity (VIDA) score (>1).
- **For Control Group:** Age- and sex-matched healthy volunteers with no personal or family history of autoimmune or chronic inflammatory diseases.

Exclusion Criteria: Exclusion criteria for all participants were: pregnancy or lactation; smoking; chronic alcohol consumption; presence of any other systemic or dermatological disease; use of systemic or topical treatments for their condition within the last 3 months; and intake of vitamin supplements or antioxidants within the last month.

Sample Collection and Biochemical Analysis: Following an overnight fast, 5 mL of venous blood was collected from each participant into two separate vacutainers: one plain tube for serum and one EDTA-containing tube for erythrocytes.

- **Serum Preparation:** The plain tube was allowed to clot for 30 minutes at room temperature and then centrifuged at 3000 rpm for 15 minutes. The resulting serum was separated, aliquoted, and stored at -80°C until analysis.
- **Erythrocyte Lysate Preparation:** The EDTA tube was centrifuged at 3000 rpm for 10 minutes, and the plasma and buffy coat were discarded. The packed red blood cells (RBCs) were washed three times with an isotonic saline solution (0.9% NaCl). An erythrocyte lysate was prepared for enzyme analysis and stored at -80°C.

Biochemical Assays:

1. **Serum Malondialdehyde (MDA):** Measured as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method. Results were expressed as nmol/mL.
2. **Serum Total Antioxidant Capacity (TAC):** Determined using a commercial

colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA), based on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]). Results were expressed as mmol/L.

3. **Erythrocyte Superoxide Dismutase (SOD) Activity:** Measured using a kit based on the inhibition of nitroblue tetrazolium (NBT) reduction. Activity was expressed as units per gram of hemoglobin (U/g Hb).
4. **Erythrocyte Catalase (CAT) Activity:** Assayed by measuring the rate of decomposition of hydrogen peroxide (H_2O_2). Activity was expressed as units per gram of hemoglobin (U/g Hb).

Hemoglobin concentration in the lysate was determined using the cyanmethemoglobin method.

Statistical Analysis: Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM Corp., Armonk, NY). Data were expressed as mean ± standard deviation (SD). The normality of data distribution was assessed with the Kolmogorov-Smirnov test. Demographic data were compared using the Chi-square test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables.

A one-way ANOVA was used to compare the means of biochemical parameters among the four groups. If the ANOVA result was significant, Tukey's HSD post-hoc test was used for multiple pairwise comparisons. A p-value of <0.05 was considered statistically significant.

Results

Demographic Characteristics of the Study Population: The demographic characteristics of the four study groups are summarized in Table 1. The groups were well-matched for age and sex distribution, with no statistically significant differences observed ($p=0.812$ for age; $p=0.765$ for sex).

This homogeneity minimizes the potential confounding effects of these variables on the biochemical parameters.

Table 1. Demographic Characteristics of the Study Participants

Characteristic	Alopecia Areata (n=50)	Acne Vulgaris (n=50)	Vitiligo (n=50)	Healthy Controls (n=50)	p-value
Age (years)	28.5 ± 6.1	27.9 ± 5.8	29.1 ± 6.5	28.8 ± 6.2	0.812
Sex (Male/Female)	22 (44%) / 28 (56%)	24 (48%) / 26 (52%)	21 (42%) / 29 (58%)	23 (46%) / 27 (54%)	0.765

Comparison of Oxidative Stress and Antioxidant Markers vs. Controls: As shown in Table 2, all three patient groups (AA, AV, and V) demonstrated a statistically significant state of oxidative stress when compared to the healthy control group. Serum MDA levels were significantly elevated in the AA, AV, and V groups compared to the HC group ($p<0.001$ for all). Conversely, serum TAC levels and the activities of erythrocyte SOD and CAT were all significantly lower in the three patient groups than in the healthy controls ($p<0.001$ for all).

Table 2. Comparison of Biochemical Markers between Patient Groups and Healthy Controls

Parameter	Alopecia Areata (n=50)	Acne Vulgaris (n=50)	Vitiligo (n=50)	Healthy Controls (n=50)
MDA (nmol/mL)	3.25 ± 0.48*	2.61 ± 0.37*	4.12 ± 0.55*	1.68 ± 0.29
TAC (mmol/L)	0.94 ± 0.12*	1.05 ± 0.14*	0.78 ± 0.11*	1.35 ± 0.16
SOD (U/g Hb)	855 ± 102*	960 ± 115*	698 ± 95*	1240 ± 131
CAT (U/g Hb)	35.8 ± 5.1*	41.2 ± 6.2*	29.5 ± 4.8*	50.1 ± 7.5

Comparative Analysis of Markers among Patient Groups: A key objective was the comparative analysis among the three dermatoses.

The post-hoc analysis revealed significant differences in the magnitude of oxidative imbalance among the patient groups (Table 3). The vitiligo group exhibited the highest level of lipid peroxidation, with MDA levels significantly higher than both the AA group ($p<0.001$) and the AV group ($p<0.001$).

The AA group, in turn, had significantly higher MDA levels than the AV group ($p<0.001$).

This graded pattern was mirrored in the antioxidant defense systems. The vitiligo group showed the most depleted antioxidant capacity, with significantly lower TAC, SOD, and CAT levels compared to both AA and AV groups ($p<0.01$ for

all comparisons). Similarly, the antioxidant markers in the AA group were significantly lower than those in the AV group ($p<0.05$ for all comparisons). These results indicate a hierarchy of oxidative stress severity: Vitiligo > Alopecia Areata > Acne Vulgaris.

Table 3. Inter-Group Comparison (p-values) of Biochemical Markers among Patient Groups

Parameter	AA vs. AV	AA vs. V	AV vs. V
MDA (nmol/mL)	<0.001	<0.001	<0.001
TAC (mmol/L)	0.008	<0.001	<0.001
SOD (U/g Hb)	0.005	<0.001	<0.001
CAT (U/g Hb)	0.002	<0.001	<0.001

Discussion

This study provides the first direct comparative evidence of systemic oxidative stress across three distinct inflammatory dermatoses: alopecia areata, acne vulgaris, and vitiligo. Our primary finding confirms that all three conditions are associated with a significant oxidative imbalance, characterized by increased lipid peroxidation and depleted enzymatic and non-enzymatic antioxidant defenses compared to healthy controls. More importantly, our results reveal a clear hierarchy in the magnitude of this imbalance, with vitiligo demonstrating the most severe oxidative stress, followed by alopecia areata, and then acne vulgaris.

The profound oxidative stress observed in the vitiligo group is consistent with the current understanding of its pathogenesis, where oxidative stress is considered a primary initiating event [9]. Melanocytes are inherently vulnerable to ROS due to the pro-oxidant steps involved in melanin synthesis [10]. Our findings of the highest MDA levels and lowest CAT activity in vitiligo patients support the "melanocytorrhagy" theory, which proposes that chronic oxidative stress leads to melanocyte damage, detachment, and eventual apoptosis [14,15]. The severe depletion of CAT, a key H_2O_2-detoxifying enzyme, is particularly noteworthy, as accumulation of H_2O_2 in the epidermis of vitiligo patients has been robustly demonstrated [13].

In patients with alopecia areata, the observed intermediate level of oxidative stress aligns with its pathophysiology as a T-cell-mediated autoimmune disease. While the primary driver is the autoimmune attack on the hair follicle, ROS generated by infiltrating inflammatory cells (e.g., lymphocytes, macrophages) can act as secondary messengers, amplifying the inflammatory response and contributing to the collapse of the hair follicle's immune privilege [4, 5].

The increased MDA and decreased SOD and CAT levels suggest that this localized inflammation translates into a measurable systemic oxidative burden, which may perpetuate the autoimmune cycle [11]. The significant, albeit least pronounced, oxidative imbalance in the acne vulgaris group also corresponds with established disease mechanisms. The inflammation in acne is partly driven by ROS produced by neutrophils in response to C. acnes and by the peroxidation of squalene, a major component of sebum [7]. This process generates lipid peroxides that are both comedogenic and pro-inflammatory [6]. Our finding of elevated systemic MDA in acne patients corroborates previous work [12] and indicates that the localized follicular inflammation is substantial enough to alter systemic redox balance. The fact that the oxidative burden was less severe than in AA and vitiligo may reflect a more localized and less systemic nature of the inflammatory process in acne compared to the systemic autoimmune features of AA and vitiligo.

The comparative nature of our study is its main strength. By analyzing all groups simultaneously with the same standardized assays, we have provided a relative scaling of oxidative stress. This hierarchy (Vitiligo > AA > AV) may reflect the centrality of oxidative stress to the core pathogenesis of each disease. In vitiligo, it is a primary driver targeting the very existence of the melanocyte. In AA, it is a potent amplifier of a targeted autoimmune attack. In acne, it is a key component of a localized inflammatory response within the pilosebaceous unit.

This study has some limitations. First, as a cross-sectional study, it cannot establish causality. It demonstrates an association, but longitudinal studies are needed to determine if oxidative stress precedes, follows, or develops concurrently with disease activity. Second, we measured systemic markers in the blood, which may not perfectly reflect the microenvironment of the skin, hair follicle, or melanocytes. Third, factors such as diet, stress levels, and sun exposure, which can influence oxidative balance, were not quantitatively assessed, although major confounders like smoking were excluded.

Conclusion

In conclusion, our study demonstrates that a state of systemic oxidative stress is a shared feature among patients with alopecia areata, acne vulgaris, and vitiligo. However, the severity of this redox imbalance varies significantly, being most

profound in vitiligo, intermediate in alopecia areata, and comparatively milder in acne vulgaris. This novel comparative finding enhances our understanding of the differential role of oxidative stress in the pathophysiology of these common dermatological disorders. These results underscore the potential of antioxidant-based adjuvant therapies as a rational therapeutic strategy, which may need to be tailored according to the specific oxidative burden of each disease.

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