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Comparative Study of Oxidative Stress Markers, Clinical Chemistry Indicators, and Neurological Biomarkers Implicated in Disease Progression in Progressive and Stable Vitiligo

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Abstract

Vitiligo progression is thought to reflect a convergence of redox imbalance, metabolic susceptibility, and neuro-immune dysregulation. This prospective comparative study enrolled 180 adults with nonsegmental vitiligo, stratified at baseline into progressive (new or enlarging lesions within 6 months; n=90) and stable disease (no change for ≥12 months; n=90). The objective was to quantify between-group differences in oxidative stress markers (malondialdehyde, total antioxidant capacity, superoxide dismutase, catalase, glutathione peroxidase, and 8-hydroxy-2′-deoxyguanosine), clinical chemistry indicators linked to comorbidity and autoimmunity (TSH, anti-TPO, 25-hydroxyvitamin D, fasting glucose, lipid profile, high-sensitivity C-reactive protein), and neurological biomarkers implicated in cutaneous neuro-immune signaling (S100B, substance P, neuropeptide Y, nerve growth factor). Compared with stable vitiligo, progressive disease demonstrated higher malondialdehyde (3.72±0.81 vs 2.91±0.69 µmol/L, $p<0.001$) and 8-OHdG (12.8±3.7 vs 9.9±3.1 ng/mL, $p<0.001$), with lower total antioxidant capacity (1.08±0.23 vs 1.29±0.25 mmol Trolox eq/L, $p<0.001$) and reduced enzymatic antioxidants (SOD, catalase, glutathione peroxidase; all $p\leq0.002$). Progressive disease showed higher S100B (98.4±33.1 vs 74.6±28.7 pg/mL, $p<0.001$), substance P (172±54 vs 139±49 pg/mL, $p<0.001$), and neuropeptide Y (86.5±21.3 vs 76.4±19.8 pg/mL, $p=0.001$). Independent predictors of progression were malondialdehyde, total antioxidant capacity, S100B, and anti-TPO positivity (AUC 0.86; optimal

cut-off combining malondialdehyde >3.3 μmol/L and S100B >85 pg/mL yielded sensitivity 78% and specificity 80%). Novel emphasis is provided on a combined redox–neurocutaneous signature that discriminates progressive from stable disease beyond clinical indices, suggesting biomarker-anchored risk stratification may refine surveillance and therapeutic timing. Keywords: vitiligo, oxidative stress, S100B

Introduction

Vitiligo is a common depigmenting disorder with a dynamic clinical course that alternates between quiescence and bursts of expansion. Although clinical scoring systems capture extent and recent activity, they incompletely map the molecular currents that presage progression. A compelling body of work implicates oxidative stress as an upstream catalyst for melanocyte vulnerability, with accumulating reactive oxygen species promoting lipid peroxidation, mitochondrial strain, and DNA injury. These redox disturbances in epidermal and perifollicular niches coincide with antigen exposure and danger signaling that can precipitate adaptive immunity. Yet, the tempo of disease in an individual patient likely depends on more than redox pressure alone; systemic metabolic status and neuro-immune crosstalk within skin further contour the threshold for lesion expansion. Parsing this tripartite axis—oxidative, metabolic-autoimmune, and neurocutaneous—offers a path to a biologically grounded definition of activity.1-6

Redox imbalance is detectable in circulation and skin. Canonical readouts include malondialdehyde as a terminal product of lipid peroxidation; 8-hydroxy-2′-deoxyguanosine as a fingerprint of oxidative DNA damage; and total antioxidant capacity that reflects the net ability of plasma constituents to neutralize reactive species. Enzymatic defenses—superoxide dismutase, catalase, and glutathione peroxidase—operate in tandem to detoxify superoxide and hydrogen peroxide. In vitiligo, several studies have described diminished enzyme activities and reduced antioxidant pools, accompanied by increased oxidative adducts. This pattern is biologically plausible in an epithelium in which melanogenesis itself imposes oxidative load. Whether the magnitude of redox derangement stratifies risk of near-term spread is a clinically consequential question with immediate implications for counseling and follow-up cadence.7-8

The metabolic and autoimmune milieu also modulates vulnerability. Autoimmune thyroid disease often clusters with vitiligo, and thyroid autoantibodies may flag heightened autoimmune tone. Abnormalities in vitamin D, dyslipidemia, and low-grade inflammation can condition innate and adaptive responses and shape keratinocyte-melanocyte dialog. Vitamin D insufficiency is variably associated with vitiligo; its immunomodulatory role, effects on melanogenesis, and influence on keratinocyte differentiation could theoretically dampen resilience against oxidative cues. In parallel, high-sensitivity C-reactive protein and atherogenic lipid fractions may index systemic inflammatory drive, which can spill into cutaneous immune circuits. The contribution of fasting glucose and insulin resistance remains debated but integrates with oxidative stress biology through mitochondrial reactive oxygen species and NADPH oxidase activity.9-10

A third pillar of activity in vitiligo is neurocutaneous communication. Peripheral sensory and autonomic fibers release neuropeptides that influence vasomotor tone, immune cell trafficking, and melanocyte behavior. Substance P, a prototypic neuropeptide, can amplify local inflammation and alter melanocyte dendricity. Neuropeptide Y interfaces with stress pathways and immune cells; its accumulation in lesional skin has been described alongside keratinocyte release. S100B, a calcium-binding alarmin expressed by melanocytes and glial cells, is released upon cellular stress and injury; circulating levels may reflect melanocyte distress and danger signaling to antigen-presenting cells. Nerve growth factor, while trophic, also signals stress responses in keratinocytes and may reshape neuro-immune balance. A hypothesis emerges that progressive vitiligo is accompanied by a neuropeptide-rich microenvironment that cooperates with oxidative stress to sustain inflammatory recruitment and antigen presentation.

Contemporary translational studies argue for multimodal biomarker panels to move beyond single-analyte narratives. An integrative signature that spans redox, metabolic-autoimmune, and neurocutaneous axes could improve classification of active disease and predict near-term progression. Such a signature would be clinically valuable if it demonstrated reproducible differences between progressive and stable vitiligo, added discrimination beyond routine clinical indices, and yielded pragmatic thresholds to inform monitoring. The present study set out to assemble such a panel and to evaluate its performance head-to-head in progressive versus stable disease using standardized assays and prespecified analytic criteria.

Finally, any biomarker proposition must be tempered by feasibility and equity. Assays for malondialdehyde, total antioxidant capacity, and enzymatic antioxidants are widely available and affordable; ELISAs for S100B, substance P, neuropeptide Y, and nerve growth factor are technically straightforward in most diagnostic laboratories. Thyroid function and autoantibodies are routine. A panel constructed from these components could be incorporated into dermatology workflows with modest cost and rapid turnaround, enabling risk tracking during periods of watchful waiting or phototherapy. The aim here was not to supplant clinical acumen but to supply quantifiable context that sharpens the recognition of covert activity and informs timing of escalations, including systemic immunomodulators or targeted antioxidant strategies.

Methodology

This was a prospective, observational comparative study conducted at a Department of Neurology, Sialkot Medical College tertiary dermatology center over 14 months. Adults aged 18–60 years with nonsegmental vitiligo were consecutively screened. Inclusion required Fitzpatrick phototypes II–V, absence of systemic immunosuppressants or phototherapy in the prior 8 weeks, and ability to complete baseline and 12-week evaluations. Progressive disease was defined as appearance of new lesions or ≥10% expansion of any index lesion within 6 months, confirmed by standardized photographs; stable disease required no change for ≥12 months by patient report and clinical comparison. Exclusion criteria comprised segmental vitiligo, pregnancy or lactation, active infection, chronic inflammatory dermatoses, hepatic or renal failure, uncontrolled diabetes (HbA1c ≥8.5%), known neurologic disorders, or current antioxidant supplementation exceeding daily recommended allowances. After eligibility confirmation, verbal informed consent was obtained and documented per institutional policy; confidentiality was preserved using coded identifiers, and the protocol received ethics approval. Sample size was computed in Epi Info (version 7) for a two-sided t test to detect a mean difference in malondialdehyde of 0.4 μmol/L between groups (assumed SD 0.8), α=0.05, power=0.80, yielding 82 per arm; anticipating 10% attrition, a target of 90 per arm (total 180) was set. Fasting morning blood was collected for malondialdehyde (thiobarbituric acid reactive substances), total antioxidant capacity (Trolox-equivalent assay), enzymatic antioxidants (erythrocyte SOD, catalase, glutathione peroxidase), 8-hydroxy-2′-deoxyguanosine (ELISA), TSH, anti-TPO, 25-hydroxyvitamin D, fasting glucose, lipid profile,

and high-sensitivity C-reactive protein. Neurological biomarkers included S100B, substance P, neuropeptide Y, and nerve growth factor quantified by validated ELISAs with duplicate measures. Disease extent and activity were scored using VASI and VIDA. Preanalytical variables were standardized: fasting ≥8 hours, sampling between 8–10 a.m., immediate plasma separation, and storage at −80°C. Statistical analysis used Shapiro–Wilk tests, independent-samples t tests or Mann–Whitney U as appropriate, and Benjamini–Hochberg correction for multiple comparisons within each biomarker domain. Pearson or Spearman correlations related biomarkers to VASI and VIDA. A multivariable logistic regression modeled progression status with covariates chosen a priori (age, sex, BMI, phototype, smoking), and stepwise addition of biomarker blocks; variance inflation was checked to avoid collinearity. Discrimination was quantified by area under the ROC curve with bootstrapped 95% CIs, and optimal cut-offs were derived by Youden index. Two-tailed $p<0.05$ was considered significant.

Results

Table 1. Baseline demographic and clinical characteristics

Variable	Progressive (n=90)	Stable (n=90)	p value
Age, years, mean±SD	34.6±9.8	35.2±10.1	0.69
Female, n (%)	48 (53.3)	46 (51.1)	0.77
BMI, kg/m², mean±SD	24.9±3.6	24.6±3.8	0.60
Phototype III–IV, n (%)	62 (68.9)	60 (66.7)	0.76
Current smoker, n (%)	14 (15.6)	12 (13.3)	0.66
VASI, mean±SD	6.7±3.4	6.2±3.1	0.28
VIDA, median (IQR)	+3 (2–4)	−1 (−1 to 0)	<0.001

Groups were well balanced on demographics and extent; activity indices confirmed the intended stratification.

Table 2. Oxidative stress and clinical chemistry indicators

Analyte	**Progressive mean±SD**	**Stable mean±SD**	**p value**
Malondialdehyde, µmol/L	3.72±0.81	2.91±0.69	<0.001
8-OHdG, ng/mL	12.8±3.7	9.9±3.1	<0.001
Total antioxidant capacity, mmol Trolox eq/L	1.08±0.23	1.29±0.25	<0.001
Erythrocyte SOD, U/mg Hb	911±182	1028±201	0.002
Catalase, kU/g Hb	42.1±9.7	47.6±10.2	0.001
Glutathione peroxidase, U/g Hb	38.5±8.3	43.2±9.1	0.001
hs-CRP, mg/L	2.7±1.6	2.0±1.3	0.004
TSH, mIU/L	2.38±1.12	2.11±0.98	0.15
Anti-TPO positive, n (%)	22 (24.4)	11 (12.2)	0.04
25-OH vitamin D, ng/mL	21.3±7.8	24.1±8.1	0.01
Fasting glucose, mg/dL	96.8±12.1	93.4±10.7	0.03
LDL-C, mg/dL	121±31	112±28	0.02

Progressive vitiligo displayed a high-oxidative, low-antioxidant profile with modestly greater systemic inflammation and metabolic susceptibility; anti-TPO positivity was enriched.

Table 3. Neurological biomarkers and multivariable model for progression

Variable	**Progressive mean±SD or n (%)**	**Stable mean±SD or n (%)**	**p value**
S100B, pg/mL	98.4±33.1	74.6±28.7	<0.001
Substance P, pg/mL	172±54	139±49	<0.001
Neuropeptide Y, pg/mL	86.5±21.3	76.4±19.8	0.001

Variable	**Progressive mean±SD or n (%)**	**Stable mean±SD or n (%)**	**p value**
Nerve growth factor, pg/mL	44.2±15.6	39.1±14.3	0.01
Logistic model AUC (95% CI)	0.86 (0.81–0.91)	—	—
Independent predictors (OR, 95% CI, p)	Malondialdehyde (1.92 per 0.5 µmol/L, 1.40–2.63, <0.001); Total antioxidant capacity (0.58 per 0.1 mmol/L, 0.44–0.77, <0.001); S100B (1.36 per 20 pg/mL, 1.12–1.67, 0.002); Anti-TPO positive (2.11, 1.01–4.41, 0.047)	—	—

Neurocutaneous biomarkers were significantly higher in progressive disease. A combined model integrating oxidative and neurological markers plus anti-TPO discriminated progression with strong accuracy.

Discussion

This comparative analysis demonstrates that progressive vitiligo is characterized by a coherent biochemical signature that integrates heightened lipid peroxidation, amplified DNA oxidative injury, depleted antioxidant reserves, and enrichment of neurocutaneous danger signals. The breadth and magnitude of between-group differences, accompanied by robust p values after multiple-comparison control, affirm biologic rather than stochastic separation. The data advance a pragmatic construct in which redox load and neuropeptide-alarmin activity cooperate to maintain an inflamed microenvironment conducive to lesion expansion.11-13

The oxidative profile in progressive disease—higher malondialdehyde and 8-OHdG accompanied by lower total antioxidant capacity and reduced SOD, catalase, and glutathione peroxidase—aligns with the concept that melanocytes at the advancing edge encounter sustained reactive oxygen species pressure. Lipid peroxidation products are immunogenic and can prime dendritic cells,

while DNA oxidation feeds into innate sensing pathways. The finding that antioxidant enzymes are systematically depressed suggests either consumption, transcriptional repression, or post-translational inactivation in the face of chronic oxidative cues. These results support a role for antioxidant augmentation as an adjunct in active disease, not as monotherapy but as a means to lower the threshold for immune quiescence.14-16

Convergence of autoimmune and metabolic indicators with the redox signature adds clinical texture. The over-representation of anti-TPO positivity in progressive disease indicates an immune tone that may be permissive of cutaneous autoimmunity. Low-grade systemic inflammation (hs-CRP) and modestly adverse lipids and fasting glucose underscore a systemic milieu that can perpetuate reactive oxygen species generation and vascular-immune crosstalk. The absence of a significant TSH difference highlights that antibody positivity, rather than overt thyroid dysfunction, may be the more relevant signal of risk. Vitamin D insufficiency, while not uniformly causal, plausibly diminishes epithelial resilience and melanocyte support.17-19

Neurocutaneous biomarkers distinguished progressive disease with clarity. Elevated S100B, substance P, neuropeptide Y, and nerve growth factor in progressive cases indicate active neuro-immune signaling in the skin. S100B serves as a damage-associated molecular pattern; its elevation coheres with melanocyte stress at disease margins. Substance P and neuropeptide Y, released from sensory and sympathetic fibers, can recruit and activate immune cells, alter keratinocyte cytokine profiles, and modulate melanocyte dendricity and survival. The net effect is a feed-forward loop in which stress, neuropeptides, and immunity reinforce oxidation and antigen exposure. The independent predictive value of S100B in multivariable modeling suggests that it functions as an integrative reporter of melanocyte distress beyond generic inflammation.20

The multivariable framework demonstrated strong discrimination for progression, with an area under the curve of 0.86 using a compact set—malondialdehyde, total antioxidant capacity, S100B, and anti-TPO. This parsimony is clinically advantageous. Thresholds derived from the Youden index yield interpretable cut-offs that can be operationalized in clinics to inform visit frequency, imaging, or early consideration of therapies aimed at tamping down activity. Importantly, these

biomarkers do not replace clinical examination or patient-reported activity but rather scaffold decision-making when physical changes are subtle.

A pathophysiological schema emerges in which redox pressure primes melanocytes and keratinocytes to release alarmins, while neuropeptide flux enhances immune recruitment and epithelial stress responses. Systemic metabolic and autoimmune factors then tune the set point for activation. Such a schema rationalizes combined therapeutic strategies: phototherapy or immunomodulation to interrupt adaptive immunity, together with targeted antioxidant support and stress-axis modulation to quiet upstream triggers. The present results justify trials that allocate interventions according to biomarker-defined risk, testing whether lowering malondialdehyde or S100B trajectories translates to durable clinical stability.

This work has practical implications for trial design. Enrollment based on clinical activity alone risks heterogeneity; anchoring eligibility to biochemical activity may enrich for progressors and increase power to detect treatment effects. Biomarkers also provide quantitative secondary endpoints that are temporally sensitive and may change earlier than lesion size, enabling adaptive designs. Assay feasibility and reproducibility in routine laboratories support external validity.

Limitations include the single-center setting, modest follow-up horizon, and absence of lesional skin assays to localize biomarker sources. Although preanalytical variables were standardized, diet, stress, and subclinical infections could influence measured levels. Phototype distribution was balanced but not exhaustive of global diversity. Finally, causality cannot be inferred; the observed signature accompanies progression but requires interventional studies to confirm mechanistic roles.

Conclusion

A composite redox–neurocutaneous–autoimmune signature robustly distinguishes progressive from stable vitiligo and yields clinically usable thresholds for risk stratification. Integration of malondialdehyde, total antioxidant capacity, S100B, and anti-TPO meaningfully improves discrimination beyond clinical indices. Biomarker-informed monitoring and intervention sequencing merit prospective validation to curb lesion expansion and consolidate stability.

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