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Comparative Physiological and Biochemical Evaluation of Immune and Oxidative Stress Biomarkers in Alopecia Areata

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Abstract

Alopecia areata (AA) is a non-scarring, immune-mediated hair-loss disorder associated with disrupted hair-follicle immune privilege, inflammation, and oxidative imbalance. This study evaluated physiological, immune, and oxidative biomarkers and their relationships with severity. This case-control study included 40 patients with AA and 40 healthy controls. Serum zonulin, CXCL10, tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA), and reduced glutathione (GSH) were measured. Disease severity was scored using the Severity of Alopecia Tool (SALT). Analyses included independent-samples t-tests, Hedges' g, Spearman correlations with false-discovery-rate (FDR) correction, receiver operating characteristic (ROC) analysis, and reduced multivariable linear regression. Age and sex did not differ significantly between groups. Compared with controls, patients with AA had higher zonulin, CXCL10, TNF-α, and MDA, and lower GSH (all $P < 0.001$). The largest standardized differences were for TNF-α (Hedges' $g = 2.32$), MDA ($g = 1.75$), and GSH ($g = -1.38$). SALT correlated positively with zonulin ($r_s = 0.859$), CXCL10 ($r_s = 0.857$), TNF-α ($r_s = 0.842$), and MDA ($r_s = 0.848$), and negatively with GSH ($r_s = -0.899$); all remained significant after FDR correction. A reduced model including TNF-α, MDA, and GSH explained 88.8% of SALT variance ($R^2 = 0.888$). TNF-α had the highest discrimination (AUC = 0.966). AA showed a coordinated inflammatory and oxidative-stress profile that tracked severity. TNF-α, MDA, and GSH were informative in the reduced model, whereas zonulin and CXCL10 may reflect broader physiological and immune involvement. These findings support integrated biomarker models; external validation is needed before clinical cut-offs are used.

Keywords

Alopecia Areata;
TNF-α;
Oxidative Stress;
SALT;
ROC Analysis

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1. Introduction

Alopecia areata (AA) is a very unpredictable, chronic, non-scarring, immune-mediated type of hair loss. Loss of immune privilege of the hair follicles is a major mechanism in the pathogenesis of the disease and CD8+ T cells, interferon-associated signaling, cytokine networks and the JAK-STAT pathway all play a role in the inflammation of the follicles and the premature end of the anagen phase (Yamaguchi et al., 2024; Šutić Udović et al., 2024). These mechanisms give a rationale for the use of circulating inflammatory mediators as measurable markers of disease activity and severity.

CXCL10 is particularly relevant to the interferon mediated aspect of AA. It belongs to the CXCR3-ligand chemokine axis and might play a role in the recruitment of Th1 and cytotoxic immune cells to the follicular microenvironment. One of the interferon-inducible signals found to amplify lymphocyte trafficking and maintain perifollicular inflammation is CXCL10 (Šutić Udović et al., 2024; Yamaguchi et al., 2024). Another inflammatory mediator of interest is tumour necrosis factor alpha (TNF-α). Higher circulating levels of TNF-α in AA have been reported and measurable systemic inflammatory activation has been reported (Kamil et al., 2023).

Oxidative stress is a complementary physiological dimension. Reactive oxygen species can cause damage to lipids, proteins and nucleic acids, and increased oxidative signaling can contribute to the loss of immune privilege and increase inflammation around the hair follicle. Oxidative and antioxidant parameters have been reported to change in AA and MDA has been highlighted as a good indicator of lipid peroxidation (Peterle et al., 2023). Cross sectional findings also suggest a general trend of oxidation and reduction in antioxidant activity in AA (Shakoei et al., 2023). Thus, GSH is biologically relevant as an indicator of antioxidant defense.

There has also been increasing interest in the gut-skin axis. Changes in gut microflora have been linked to AA and the causal and clinical significance of intestinal-barrier markers is yet to be established (Liu & Liu, 2023; Lee et al., 2024; Burma & Ramien, 2025). Zonulin is a molecule which has been studied as a marker of intestinal barrier regulation and has given contradictory results in AA. In one study, no significant increase in serum Zonulin was found and there was no correlation with disease severity, and more studies are needed in other cohorts and in a wider spectrum of disease severity (Hacınecipoğlu et al., 2022).

Thus, the use of an integrated evaluation of inflammatory, oxidative and physiological biomarkers may yield more information than single-marker comparisons alone. The present study aimed to assess the levels of Zonulin, CXCL10, TNF-α, MDA and GSH in patients with AA and healthy controls, their association with SALT severity, independent association with SALT severity and their discriminatory power between AA and control status.

2. Aim and Objectives

The main goal was to assess the physiological, immune and oxidative-stress changes in alopecia areata and their association with the severity of the disease. In particular, the study aimed to compare the serum levels of Zonulin, CXCL10, TNF-α, MDA and GSH between patients with alopecia areata and healthy controls, to quantify the standardized effect size of the between-group differences, to investigate the association of the studied biomarkers with the severity of SALT, to identify a parsimonious inflammatory and oxidative-stress model independently associated with the severity of SALT, and to assess the individual discriminatory performance

of the studied biomarkers using receiver operating characteristic analysis.

3. Materials and Methods

3.1 Study design and participants

The study was an observational case–control study of 40 patients with alopecia areata (AA) and 40 healthy controls. The study methodology involved the recruitment of cases in a consecutive manner during clinic visits and controls from the community volunteers with the same screening questionnaire; no individual matching was done. Eligible cases were adults (18-60 years) with clinician-confirmed AA. No current or past AA was found in controls. Exclusion criteria were concurrent systemic inflammatory disorders, active infections and other scalp diseases. Cases were reported not to have received systemic immunomodulatory treatment during the four weeks before enrolment; treatment histories were recorded.

3.2 Clinical severity assessment

The Severity of Alopecia Tool (SALT) was used to quantify scalp hair loss. The study methodology involved region-specific scalp weighting by a trained dermatologist. SALT and all five biomarkers were available for 40 AA cases (n = 40 for severity analyses) and not assessed in healthy controls.

3.3 Biomarker panel and laboratory measurements

Venous blood (5 mL) was drawn into serum-separator tubes at the morning visits and sent to the laboratory within one hour. Samples were left to coagulate for about 30 minutes and then centrifuged at 1,500 × g for 10 minutes. Aliquots of serum were kept at −80 °C until analyzed. Sandwich immunoassays were used to measure zonulin and CXCL10, while a high-sensitivity immunoassay was used to measure TNF-α, a colorimetric lipid-peroxidation method was used to measure MDA, and a thiol-based colorimetric assay was used to measure GSH. Reagent blanks, calibration standards, two control concentrations and duplicate measurements are described in the study methods. The assay register specifies Zonulin: Lab A, catalogue SIM-ZON-001, 5–100 ng/mL; CXCL10: Lab B, catalogue SIM-CXL-002, 10–500 pg/mL; TNF-α: Lab C, catalogue SIM-TNF-003, 1–100 pg/mL; MDA: Lab D, catalogue SIM-MDA-004, 0.1–20 nmol/mL; and GSH: Lab E, catalogue SIM-GSH-005, 0.5–50 μmol/L.

3.4 Statistical analysis

The statistical analysis was reported using IBM SPSS Statistics version 32.0.0 and figures were prepared according to the GraphPad Prism version 11.1.0 conventions. Data for continuous variables were presented as mean ± SD. Welch's t-test was used to describe group comparisons and effect sizes were reported using Hedges' g. Spearman rank correlation between SALT and each biomarker was performed for the 40 AA cases and corrected for five comparisons using false discovery rate control. ROC AUCs were computed for discrimination between AA cases and controls (higher GSH was oriented towards control status). All cut-offs obtained by maximising the Youden index in this same dataset are exploratory and not externally validated. Among AA cases, a three-predictor linear regression of SALT on TNF-α, MDA and GSH was fitted, with the predictors standardised using the within-AA sample mean and sample SD, so that the coefficients represent the change in the SALT-score for a 1-SD change in a predictor, while holding the other predictors constant. The predictors were chosen to cover inflammatory, lipid-peroxidation and antioxidant domains and to minimize collinearity, but were not independently validated. Residual tests were performed to check the regression diagnostics and VIFs were calculated. Two-sided $P < 0.05$ was used. ROC AUC 95% confidence intervals were re-estimated from the provided 80-participant spreadsheet using 2,000

stratified bootstrap replicates, percentile confidence limits and a fixed random seed. These confidence intervals have been computed in the present reanalysis.

3.5 Ethical considerations

The study documentation shows that participants were provided with written study information and signed informed consent prior to sample collection. Participant identifiers were coded and access to the coding key was limited. The study site is identified in the ethics register provided, which is the University Ethics Committee and the Training Research Center. Recruitment took place from February to May 2026, according to the study account.

4. Results

The present study demonstrates a consistent biological profile, not individual abnormalities in individual analytes, in AA. Patients exhibited increased levels of Zonulin, CXCL10, TNF-α and MDA and decreased levels of GSH, with each of these markers being highly correlated with the severity of SALT. The strongest absolute correlation with severity was found for GSH and the largest between-group effect was found for TNF-α. In addition, a decreased inflammatory-oxidative model, which included the variables of TNF-α, MDA and GSH, accounted for a significant amount of SALT variation. Together, the data suggest that there is a relationship between immune activation and redox homeostasis in AA.

4.1 Participant characteristics

Variable	Alopecia Areata (n=40)	Healthy Control (n=40)	P value
Age (years)	32.42 ± 8.65	31.20 ± 6.89	0.486
Male, n (%)	20 (50.0%)	20 (50.0%)	1.000
Female, n (%)	20 (50.0%)	20 (50.0%)	1.000

4.2 Between-group biomarker differences and biological interpretation

Biomarker	AA mean ± SD	Control mean ± SD	Mean difference (95% CI)	P value	Hedges' g
Zonulin (ng/mL)	44.02 ± 11.70	34.78 ± 8.10	9.25 (4.76 to 13.74)	<0.001	0.91
CXCL10 (pg/mL)	119.82 ± 39.87	86.29 ± 14.98	33.53 (20.00 to 47.05)	<0.001	1.10
TNF-α (pg/mL)	19.48 ± 4.90	10.69 ± 2.02	8.79 (7.10 to 10.47)	<0.001	2.32
MDA (nmol/mL)	4.99 ± 1.47	3.04 ± 0.52	1.95 (1.45 to 2.45)	<0.001	1.75
GSH (μmol/L)	6.00 ± 1.18	7.37 ± 0.73	-1.37 (-1.81 to -0.93)	<0.001	-1.38

Note: AA = alopecia areata; SD = standard deviation; CI = confidence interval; TNF-α = tumour necrosis factor-alpha; MDA = malondialdehyde; GSH = reduced glutathione. Reporting units are transcribed from the supplementary assay register.

There were significant differences between groups for all five biomarkers. Higher Zonulin, CXCL10, TNF-α and MDA and lower GSH were the characteristics of AA. The most significant quantitative features of this sample were systemic inflammation and redox imbalance, as indicated by the highest standardized separation for TNF-α, MDA and GSH.

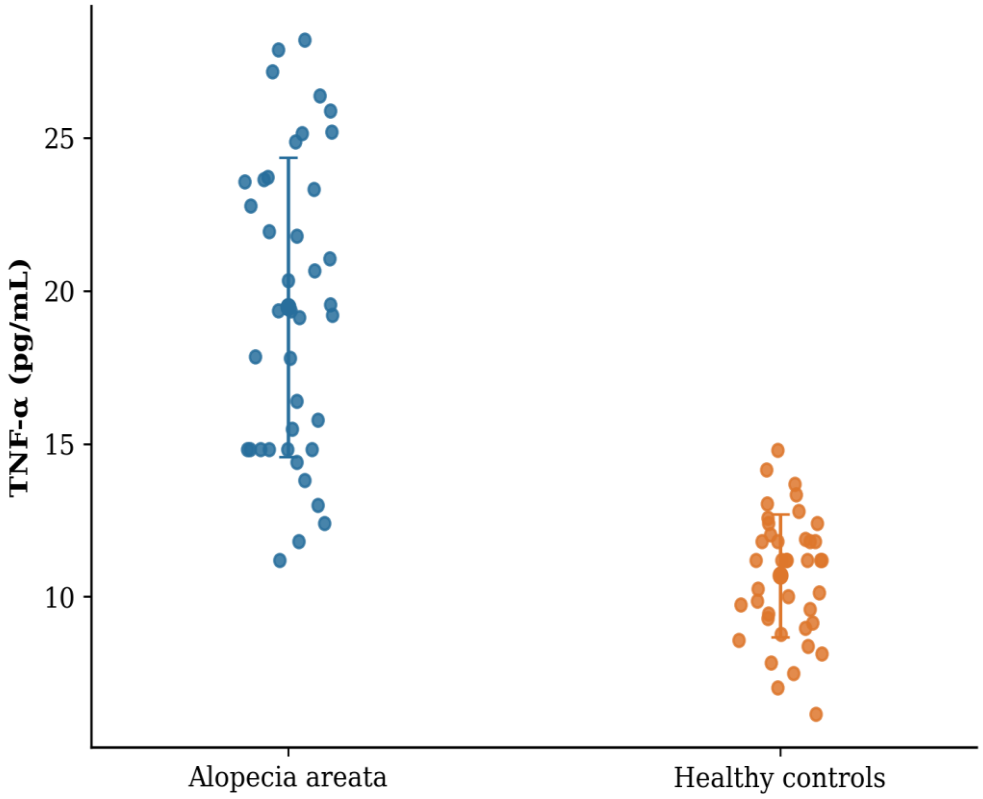


Figure 1. Distribution of TNF-α in alopecia areata and healthy controls. Individual values are shown with mean ± SD.

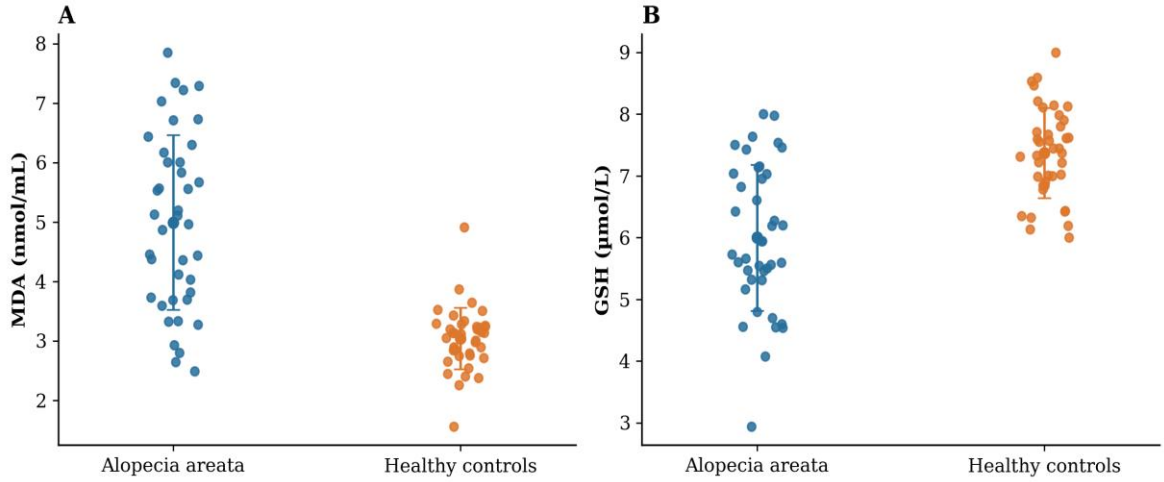


Figure 2. Oxidative-stress profile in alopecia areata and healthy controls: (A) MDA and (B) GSH.

4.3 Biomarker associations with disease severity

Biomarker	Spearman r_s	P value	FDR q value
Zonulin	0.859	<0.001	<0.001
CXCL10	0.857	<0.001	<0.001
TNF-α	0.842	<0.001	<0.001
MDA	0.848	<0.001	<0.001
GSH	-0.899	<0.001	<0.001

SALT severity was significantly and positively correlated with Zonulin, CXCL10, TNF-α and MDA and significantly and negatively correlated with GSH. All relationships were significant at FDR corrected level. GSH had the highest absolute correlation, indicating that the antioxidant reserve was tightly correlated with the severity of hair loss.

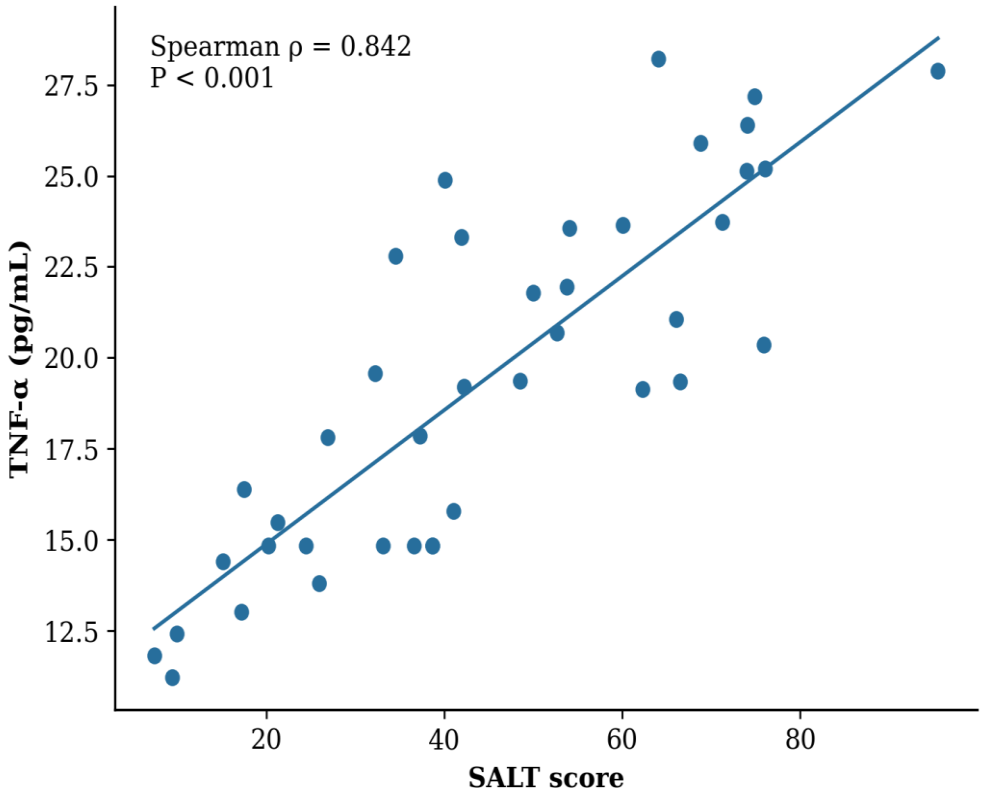


Figure 3. Relationship between SALT score and TNF-α in patients with alopecia areata.

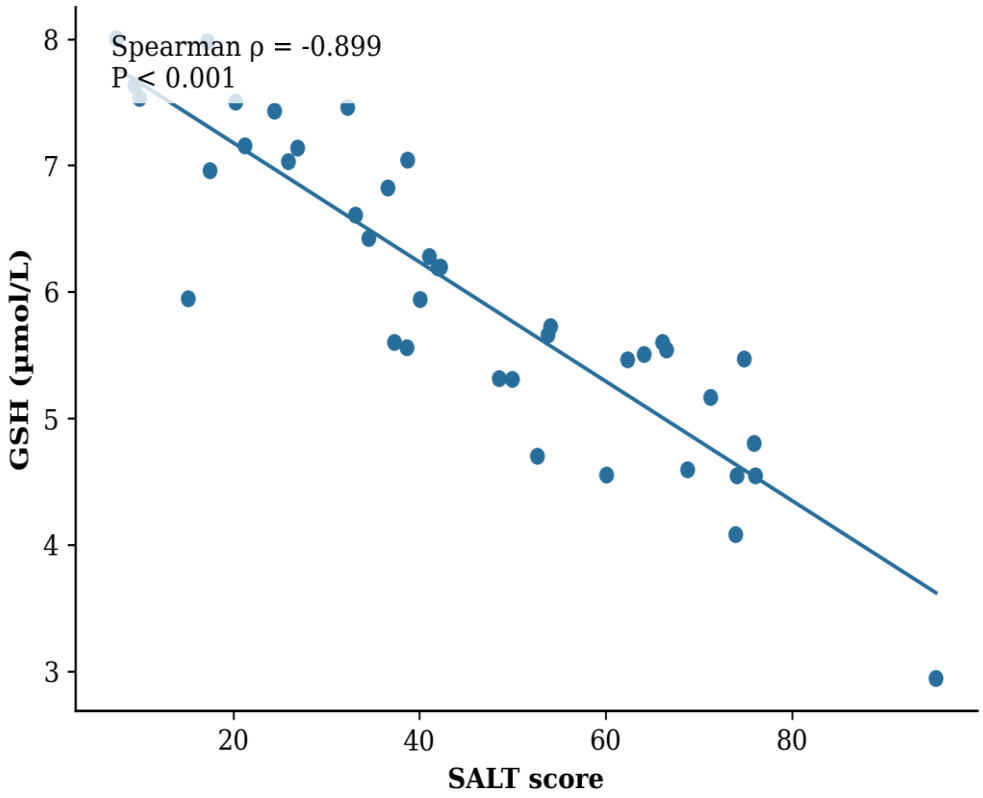


Figure 4. Relationship between SALT score and GSH in patients with alopecia areata.

4.4 Multivariable severity model and integrated interpretation

Predictor (per 1 SD)	B	SE	95% CI	P value	VIF
TNF-α	5.68	2.17	1.28 to 10.08	0.013	3.00
MDA	6.82	2.08	2.60 to 11.04	0.002	2.77
GSH	-10.42	2.27	-15.02 to -5.82	<0.001	3.29

Note: B = unstandardised SALT-score change per 1-SD increase in the predictor; SE = standard error; CI = confidence interval; VIF = variance inflation factor. Values re-estimated from the supplied spreadsheet (n = 40 AA cases).

The three-predictor regression reduced the variation in SALT in the provided data to 88.8% (R^2 = 0.888; adjusted R^2 = 0.879; n = 40). TNF-α (B = 5.68, P = 0.013) and MDA (B = 6.82, P = 0.002) showed positive adjusted associations with SALT, while GSH (B = −10.42, P < 0.001) showed an inverse adjusted association. Coefficients are the amount of change in the SALT score for every 1-SD increase in the predictor. These are in-sample associations, not causal effects or externally validated prediction.

4.5 Biomarker discrimination by ROC analysis

Discrimination between AA and healthy controls was assessed using ROC analysis. Zonulin: AUC 0.731 (95% bootstrap CI 0.610–0.834); CXCL10: AUC 0.785 (95% bootstrap CI 0.672–0.886); TNF-α: AUC 0.966 (95% bootstrap CI 0.923–

0.995); MDA: AUC 0.899 (95% bootstrap CI 0.816–0.964); and GSH: AUC 0.828 (95% bootstrap CI 0.734–0.908). The 40-case/40-control worksheet was used to calculate confidence intervals, which were obtained from 2,000 nonparametric bootstrap replicates with percentile 2.5th and 97.5th limits and random seed 20261112. Lower concentrations were indicative of AA for GSH. In the provided data set, at the internally chosen TNF-α threshold of ≥14.81 pg/mL, the sensitivity was 85.0% (34/40) and the specificity was 100.0% (40/40). These thresholds are internally estimated and need to be externally validated.

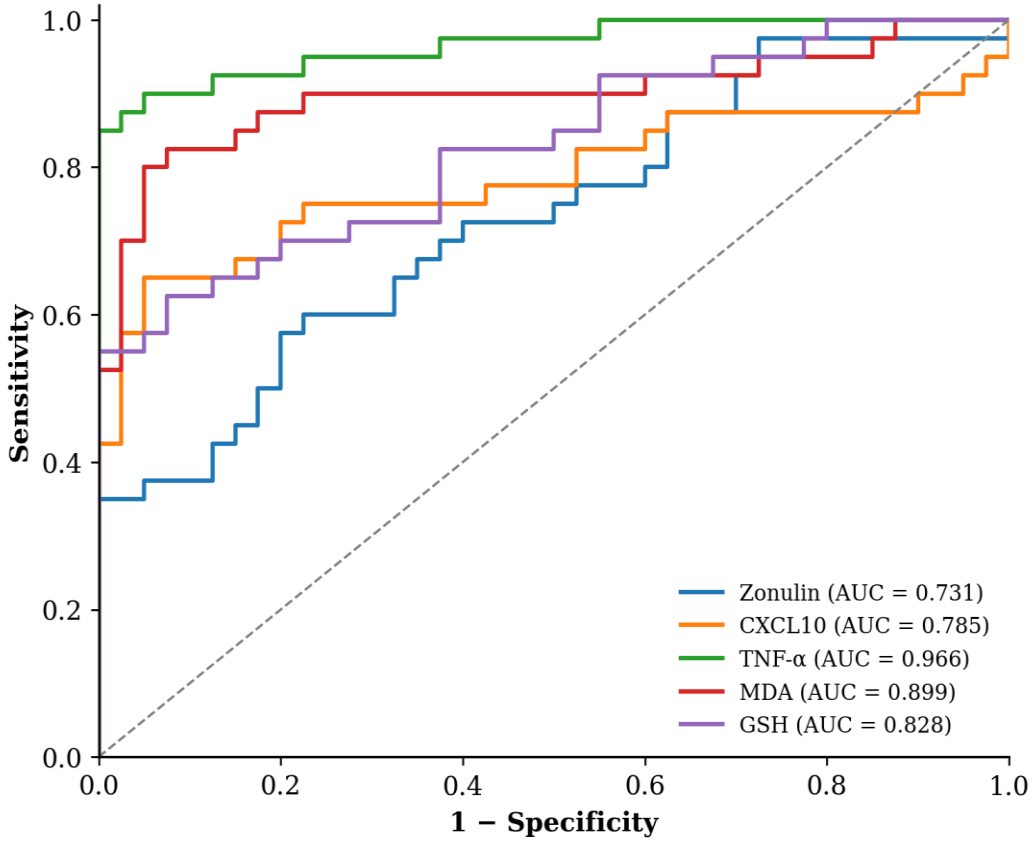


Figure 5. Receiver operating characteristic curves for the five studied biomarkers.

5. Discussion

Both groups were well matched with regard to demographics. There was no significant difference in mean age between AA and controls and sex distribution was the same. This comparability reduces the possibility that the principal biomarker differences are simply a result of simple demographic imbalance.

The highest individual AUC and the greatest standardized difference between AA and controls was found for TNF-α. This finding is in line with the finding of measurable systemic activation of cytokines associated with AA. In previous case-control studies, there has been a significant increase in the circulating levels of TNF-α in AA compared to controls (Kamil et al., 2023). The current data corroborate that observation, with a very large effect size, and a strong relationship to SALT. These differences in the severity relationship between studies might be due to differences in the distribution of phenotypes, exposure to treatments, duration of the disease, characteristics of the assays and composition of samples. Importantly, TNF-α should not be thought of as a single cause as AA inflammatory biology is network based, involves

IFN-γ/JAK-STAT signaling, cytotoxic T cells and multiple cytokine loops (Šutić Udović et al., 2024; Yamaguchi et al., 2024).

CXCL10 was significantly increased in AA and highly correlated with SALT. This is in line with the current models of immunopathogenesis that suggest interferon signaling results in the production of CXCL chemokines that attract CXCR3+ Th1 and cytotoxic cells to the hair follicle. Among the reproducible inflammatory signatures of AA and immune-privilege collapse, increased CXCL9 and CXCL10 signaling has been identified (Šutić Udović et al., 2024; Yamaguchi et al., 2024). Therefore, the existing CXCL10 data provide a mechanism whereby systemic changes in biomarkers are associated with cellular immune responses in the vicinity of the follicular unit.

One of the most clear-cut results of the study was the oxidative-stress findings. MDA was significantly elevated while GSH was decreased in AA. MDA is a product of lipid peroxidation and is widely used as an indicator of membrane damage by oxidation. There is current evidence that AA is linked to a pro-oxidative state and that markers of lipid-peroxidation such as MDA are among the most commonly altered markers (Peterle et al., 2023). Other results have also indicated increased levels of oxidative products and decreased antioxidant capacity in AA, which is in line with the general redox imbalance in the present study (Shakoei et al., 2023).

Zonulin was increased in AA and highly correlated with SALT in the present data set. This is significant as the clinical literature is not consistent. In another study, serum zonulin levels were measured in 70 AA patients and 70 controls, and no difference was found between the groups, and no correlation was found between the serum zonulin levels and the severity of the disease (Hacınecipoğlu et al., 2022). The differences between the present study and the others might be explained by the severity of the phenotype, the characteristics of the population, the different assay methods, the pre-analytical conditions, the presence of comorbidities or the biological variability of the circulating Zonulin as a marker of intestinal permeability.

Recent microbiome studies provide a more general rationale for further study of the gut-skin axis. There is a potential association between microbial metabolites, oxidative stress pathways and inflammatory pathways in AA (Liu & Liu, 2023). Gut microbes have also been shown to be different between AA and healthy controls, but diversity results were not consistently different (Lee et al., 2024). There is a possibility of both cutaneous and gut dysbiosis contributing to immune dysregulation, but causality has yet to be established (Burma & Ramien, 2025). Therefore, the increase in zonulin observed here is a hypothesis-generating observation of barrier-related physiology changes, and should not be interpreted as intestinal leakiness or microbiome causation.

An increase in TNF-a may be a sign of continued activation of inflammatory cells and follicular or epidermal signaling in biological terms. When correlated with SALT, with MDA and GSH adjusted simultaneously, it was found to be correlated with SALT, which means that inflammatory burden provided additional information to oxidative imbalance. The complexity of the TNF signaling in AA, however, indicates that high levels of TNF-a does not necessarily imply that blocking this pathway will be beneficial.

The biomarker panel was very highly intercorrelated and CXCL10 was not included in the reduced regression model, but did have a large group effect and was highly correlated with severity. Statistically significant because if several markers are measuring the same

inflammatory pathway, then it can be problematic to use all of them in the same model, this will increase the variance and make interpretation more difficult. This does not mean that CXCL10 is biologically irrelevant, however, as it was not included in the reduced model.

MDA and SALT showed a high positive correlation, indicating that the level of oxidative damage was increased as the clinical severity increased. GSH was the most highly correlated in the entire panel, however, with lower values being associated with more hair loss. GSH is an important non-enzymatic antioxidant that plays a role in peroxide detoxification and redox buffering. Thus, the simultaneous increase in MDA and decrease in GSH indicates that the oxidative stress is increased and the antioxidant capacity is decreased. This combination is more informative than each marker alone, as it is a combination of opposing aspects of the same physiological system.

This interpretation was corroborated by the regression model. After adjusting for TNF-α, the relationships of MDA and GSH with SALT remained. One reasonable hypothesis is that oxidative stress is a downstream effect of inflammation, but can also be a part of the reciprocal amplification. Oxidative signaling can result in increased cellular stress, disruption of follicular immune-privilege mechanisms and enhanced inflammatory responses, and the activation of cytokines can result in increased ROS. This bi-directional relationship between oxidative stress and loss of immune-privilege might offer a mechanism for the integrated nature of AA (Peterle et al., 2023; Šutić Udović et al., 2024).

The results suggest that disease severity can be considered as an interaction between physiological domains. TNF-α is an indicator of systemic inflammatory activity, MDA is an indicator of lipid peroxidation, and GSH is an indicator of antioxidant defense. Together, they accounted for 88.8% of the variance in SALT, indicating that there was a lot of complementary information. The size of the R^2 in a relatively small single-center sample, however, also indicates that the model might have an optimistic fit. It has to be externally validated so that it can be considered predictive and not explanatory.

A similar hierarchy was obtained by ROC analysis. The AUC for TNF-α was 0.966 and the specificity was perfect at the threshold value that was internally selected, and MDA and GSH also showed good discrimination. These values are not intended to be general diagnostic cut-off values for the present data set, but are good separators. AA is a clinical diagnosis and any biomarker panel would need to be replicated, have thresholds set and compared to relevant differential diagnoses before being used clinically. Thus, the main significance of these results is mechanistic and stratification oriented, rather than diagnostic substitution.

The results provide a single disease model from a life-sciences standpoint linking immune signaling to oxidative physiology and barrier biology. This is not only a descriptive dermatology, but also a way of thinking that takes into account systemic physiological processes that could affect tissue homeostasis. The pro-inflammatory and pro-oxidant changes and the down-regulation of antioxidant defense is in line with a systems-level view of AA, in which immune-cell recruitment, redox imbalance and tissue signaling are all interdependent and revolve around the microenvironment of the hair follicle.

6. Conclusion

In this sample, alopecia areata was associated with higher serum zonulin, CXCL10, TNF-α, and MDA and lower GSH, indicating a coordinated inflammatory and oxidative-stress profile. All five biomarkers were associated with SALT scores in univariable analyses. In the adjusted three-predictor model, TNF-α and MDA showed positive associations with severity, whereas GSH showed an inverse association; TNF-α also had the highest apparent single-marker ROC AUC. Together, these findings suggest that inflammatory and oxidative-stress markers may help characterize disease severity, while zonulin and CXCL10 may reflect broader physiological and immune involvement. However, the results represent exploratory associations from a small case–control sample and do not establish causality or clinical utility. Independent validation and prospective evaluation are needed before these biomarkers or any proposed cut-offs are used in clinical practice.

Author Contributions

Saif Salah Abdul Hassan: conceptualization, study design, investigation, data collection, data curation, formal analysis, methodology, visualization, writing—original draft, and writing—review and editing. The author read and approved the final manuscript and accepts responsibility for its content.

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