Medical Science Journal for Advance Research

Vol. 7, No. 2, June 2026
e-ISSN: 2774-4892

Serum S100B and CTLA-4 as Diagnostic and Immunoregulatory Biomarkers in Vitiligo Patients

Haider Chyad Lafta AL-Janahi*[1], and Israa Abdulwahid Dheeb[2]

[1,2]*Department of Medical Microbiology, College of Medicine, University of Al-Qadisiyah, Iraq.*

***Corresponding Author Email:** hhader785@gmail.com

ABSTRACT

Background: *Vitiligo is an acquired autoimmune depigmenting disease in which melanocyte damage, inflammatory amplification and defective immune tolerance interact.* ***The Aim:*** *This study evaluated serum S100B and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) as diagnostic and immunoregulatory biomarkers in vitiligo.* ***Methods:*** *A case-control study included 60 patients with vitiligo and 60 apparently healthy controls. Serum S100B, CTLA-4, IL-10, IL-17 and TGF-beta were measured by ELISA, while CD4, CD8, CD25, CD27 and FoxP3 were assessed by flow cytometry. Group comparisons, receiver operating characteristic (ROC) analysis and correlation testing were performed.* ***Results:*** *Serum S100B was significantly higher in patients than controls (0.960 +/- 0.06 vs. 0.508 +/- 0.11, P<0.001), whereas CTLA-4 was significantly lower (0.145 +/- 0.02 vs. 0.242 +/- 0.04, P<0.001). S100B showed good diagnostic performance (AUC=0.905, sensitivity=90.0%, specificity=91.7% at >0.89), while CTLA-4 showed excellent performance (AUC=0.989, sensitivity=96.7%, specificity=95.0% at <0.171). In patients, S100B correlated positively with IL-17 and CD27 and negatively with TGF-beta, IL-10 and FoxP3. CTLA-4 correlated positively with TGF-beta and FoxP3 and negatively with IL-17 and CD27.* ***Conclusion:*** *Vitiligo was characterized by an increased S100B signal and reduced CTLA-4, supporting a pattern of alarmin-driven inflammation with impaired regulatory control. Combined evaluation may improve immunological stratification, but external validation is required.*

Keywords: Vitiligo; S100B; CTLA-4; alarmins; Regulatory T cells; ELISA.

Article Information

Received: April 28, 2026; Revised: May 29, 2026; Online: June 2026y

INTRODUCTION

Vitiligo is a chronic acquired depigmenting disorder caused by selective loss or functional exhaustion of epidermal melanocytes. Although its clinical presentation is cutaneous, its biology is systemic and immune-mediated. Current models describe vitiligo as the product of genetic susceptibility, oxidative stress, innate immune activation and adaptive cytotoxic responses directed against melanocyte antigens [1-3]. The disease is therefore best interpreted as an immune-inflammatory disorder rather than a purely cosmetic condition.

Cytotoxic T-cell activity is central to melanocyte destruction. Lesional and circulating CD8+ T cells produce effector molecules and cytokines that favor melanocyte apoptosis, while regulatory T cells (Tregs) normally restrain autoreactive responses and maintain peripheral tolerance [4,5]. Disturbance of this balance is reflected by increased inflammatory cytokines, particularly Th17-related mediators, and reduced immunosuppressive signals such as IL-10, TGF-beta and FoxP3-associated Treg activity [5,6].

https://doi.org/10.46966/msjar.v7i2.413

S100B is a calcium-binding protein that may behave as an alarmin when released during cellular stress or tissue injury. Alarmins can activate innate and adaptive immunity and amplify autoimmune inflammation [7,8]. In vitiligo, S100B has been proposed as a marker of disease activity, melanocyte injury and Langerhans-cell-related inflammatory remodeling [9-12]. In contrast, CTLA-4 is an inhibitory immune checkpoint expressed mainly by T cells and Tregs. By competing with CD28 for CD80/CD86 binding, CTLA-4 limits excessive T-cell activation and contributes to self-tolerance [13,14]. Lower CTLA-4 activity or unfavorable CTLA-4 genetic variation has been associated with autoimmune susceptibility, including vitiligo [15].

Despite growing interest in these molecules, the combined interpretation of S100B as an alarmin-associated marker and CTLA-4 as a regulatory checkpoint marker remains insufficiently defined in vitiligo. The present study aimed to evaluate serum S100B and CTLA-4 levels in vitiligo patients compared with healthy controls, determine their diagnostic accuracy, and analyze their relationships with inflammatory cytokines and cellular immune markers.

METHODS

Study design and participants. This case-control study was conducted from November 2024 to January 2025 in Al-Najaf, Iraq. The final analyzed sample included 60 clinically diagnosed vitiligo patients and 60 apparently healthy controls. Diagnosis was made clinically by dermatology specialists. Controls had no history of systemic disease and were clinically considered healthy. All participants provided informed consent, and the study procedures were conducted according to the ethical approval procedures of the participating hospital setting.

Eligibility criteria. Vitiligo patients were eligible when they had not received treatment during the three months preceding blood analysis. Patients receiving treatment, patients with diabetes mellitus, other autoimmune diseases, atopic dermatitis, inflammatory skin diseases, or pregnancy were excluded. Healthy controls with chronic disease or refusal to participate were not included.

Clinical assessment and sample collection. Demographic and clinical information included age, sex, family history, exposure to stress, disease duration and distribution of involved body sites. Five milliliters of venous blood were collected. Serum was separated after clotting and centrifugation and stored at -20°C until ELISA testing. EDTA-anticoagulated blood was used for flow-cytometric evaluation of cellular immune markers.

Laboratory assays. Serum IL-10, IL-17, TGF-beta, CTLA-4 and S100B were measured by sandwich ELISA using commercially available BT Lab kits (China), according to the manufacturer-based protocol. Optical density was read at 450 nm and concentrations were calculated from standard curves. Flow cytometry was performed using a BriCyte E6 instrument to estimate CD4, CD8, CD25, CD27 and FoxP3-positive cell markers. Approximately 10,000 cells were analyzed for each sample after antibody staining and erythrocyte lysis.

Statistical analysis. Data were analyzed using SPSS version 26 and Microsoft Excel. Distribution was evaluated by Kolmogorov-Smirnov testing. Quantitative variables were expressed as mean +/- standard deviation. Independent-samples t-test or non-parametric alternatives were used according to distribution, while chi-square testing was used for categorical variables. ROC analysis estimated diagnostic performance, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC). Correlations were evaluated using appropriate correlation

coefficients. P values <0.05 were considered statistically significant.

RESULTS

The study included equal numbers of vitiligo patients and controls (60 per group). The groups were comparable in age and sex distribution, reducing demographic imbalance as a source of bias. Mean age was 29.80 +/- 9.71 years in patients and 28.83 +/- 9.82 years in controls (P=0.536). Females represented 55.0% of the patient group and 60.0% of controls (P=0.580). Among patients, 18.3% had positive family history, 61.7% had disease duration of at least five years and 51.6% were classified as severe cases.

Table 1. Demographic and clinical profile of the study population.

Variable	Vitiligo patients (n=60)	Healthy controls (n=60)	P value
Age, years (mean +/- SD)	29.80 +/- 9.71	28.83 +/- 9.82	0.536
Male sex, n (%)	27 (45.0%)	24 (40.0%)	0.580
Female sex, n (%)	33 (55.0%)	36 (60.0%)	0.580
Positive family history, n (%)	11 (18.3%)	-	-
Disease duration >=5 years, n (%)	37 (61.7%)	-	-
Stress reported, n (%)	19 (31.7%)	-	-
Severe vitiligo, n (%)	31 (51.6%)	-	0.001
All body parts involved, n (%)	32 (53.3%)	-	-

SD: standard deviation. P values compare patients and controls where applicable; Severity distribution P value refers to patient-group severity categories.

Serum CTLA-4 was markedly lower in vitiligo patients than controls, while serum S100B was markedly higher. Both differences were statistically significant at P<0.001. ROC analysis showed that CTLA-4 had excellent diagnostic discrimination, with an AUC of 0.989. Because CTLA-4 was reduced in patients, a disease-positive threshold below 0.171 gave 96.7% sensitivity and 95.0% specificity. S100B also demonstrated strong diagnostic performance, with an AUC of 0.905; a disease-positive threshold above 0.89 gave 90.0% sensitivity and 91.7% specificity.

Table 2. Serum S100B and CTLA-4 levels and diagnostic accuracy.

Markers	Patients mean +/- SD (range)	Controls mean +/- SD (range)	P value	Disease-positive cutoff	AUC (95% CI)	Sensitivity	Specificity
CTLA-4	0.145 +/- 0.02 (0.11-0.18)	0.242 +/- 0.04 (0.16-0.33)	<0.001	<0.171	0.989 (0.969-1.000)	96.7%	95.0%
S100B	0.960 +/- 0.06 (0.83-1.21)	0.508 +/- 0.11 (0.40-0.99)	<0.001	>0.89	0.905 (0.828-0.982)	90.0%	91.7%

AUC: area under the ROC curve; CI: confidence interval. Cutoff direction is presented according to the observed disease-associated direction of change.

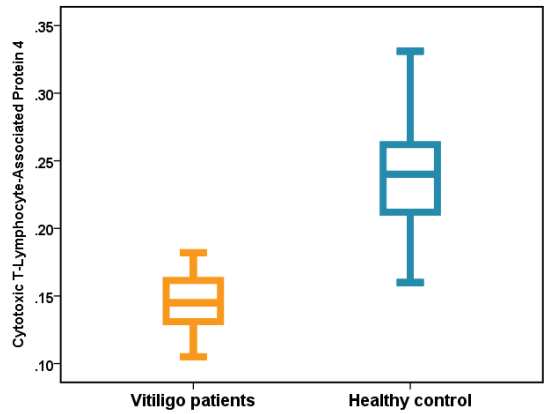

Figure 1. Mean CTLA-4 level in vitiligo patients and healthy controls.

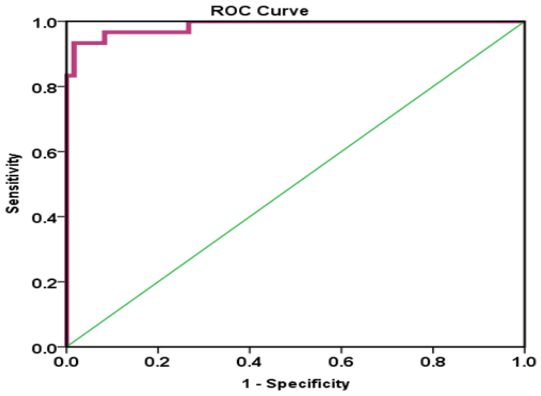

Figure 2. ROC curve for CTLA-4 in distinguishing vitiligo patients from healthy controls.

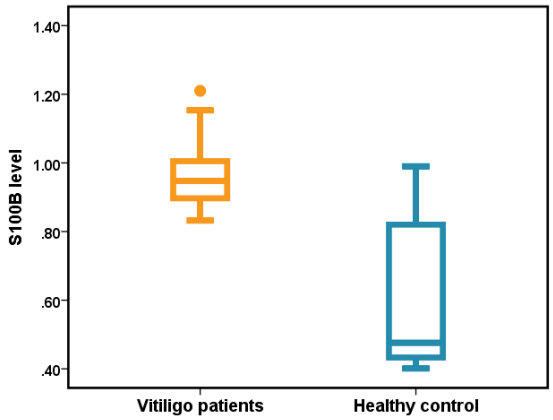

Figure 3. Mean S100B level in vitiligo patients and healthy controls.

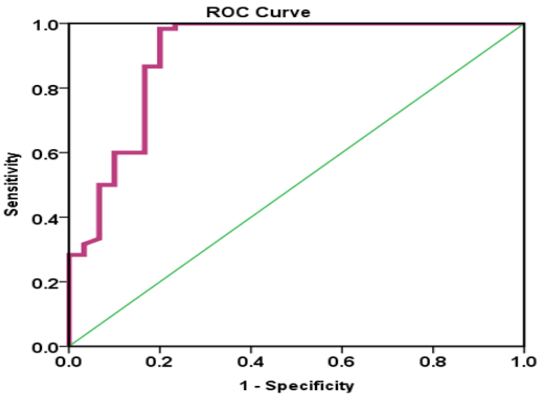

Figure 4. ROC curve for S100B in distinguishing vitiligo patients from healthy controls.

Figures were selected because they directly document the between-group differences and ROC behavior of the two article markers, CTLA-4 and S100B.

Subgroup analyses showed no significant differences in S100B or CTLA-4 levels according to family history, disease duration or severity category. CTLA-4 levels were 0.136 +/- 0.01 in patients with positive family history and 0.147 +/- 0.02 in those with negative family history (P=0.157); they were 0.142 +/- 0.02 in disease duration <5 years and 0.147 +/- 0.02 in duration >=5 years (P=0.392). S100B levels were also comparable across family history (P=0.896), duration (P=0.474) and severity (P=0.736).

Table 3. Subgroup distribution of CTLA-4 and S100B according to family history, disease duration and severity in vitiligo patients.

Subgroup variable	Category	CTLA-4 level	S100B level
Family history	Positive	0.136 ± 0.01	0.95 ± 0.04
Family history	Negative	0.147 ± 0.02; P=0.157	0.96 ± 0.06; P=0.896
Disease duration	<5 years	0.142 ± 0.02	0.97 ± 0.07
Disease duration	≥5 years	0.147 ± 0.02; P=0.392	0.96 ± 0.06; P=0.474
Disease severity	Mild	0.142 ± 0.02	0.98 ± 0.07
Disease severity	Moderate	0.159 ± 0.03	0.98 ± 0.08
Disease severity	Severe	0.145 ± 0.02; P=0.082	0.94 ± 0.06; P=0.736

Values are presented as mean ± SD. P values refer to comparisons within each subgroup variable. These data were condensed from Chapter Three tables 3-40 to 3-42 and 3-46 to 3-48.

Correlation analysis revealed two opposing immunological patterns. CTLA-4 correlated positively with regulatory mediators, including TGF-beta and FoxP3, but negatively with IL-17 and CD27. S100B showed the inverse inflammatory pattern, correlating positively with IL-17 and CD27 and negatively with TGF-beta, IL-10 and FoxP3.

Table 4. Significant correlations of CTLA-4 and S100B with immune markers in vitiligo patients.

Index markers	Correlated parameter	r	P value	Interpretation
CTLA-4	TGF-beta	0.466	0.010	Positive regulatory association
CTLA-4	FoxP3	0.477	0.008	Positive Treg-related association
CTLA-4	IL-17	-0.550	0.001	Inverse inflammatory association
CTLA-4	CD27	-0.463	0.011	Inverse T-cell activation association
S100B	IL-17	0.531	0.001	Positive Th17-related association
S100B	CD27	0.529	0.001	Positive T-cell activation association
S100B	TGF-beta	-0.522	0.001	Inverse regulatory association
S100B	IL-10	-0.604	0.001	Inverse anti-inflammatory association
S100B	FoxP3	-0.562	0.001	Inverse Treg-related association

DISCUSSION

This study identifies a clear bidirectional biomarker pattern in vitiligo: increased S100B and decreased CTLA-4. The magnitude and direction of these changes are biologically coherent. S100B elevation supports the concept that melanocyte stress and tissue injury release danger-associated signals capable of amplifying cutaneous and systemic inflammation. In parallel, reduced CTLA-4 suggests weakening of a major inhibitory checkpoint required to limit autoreactive T-cell activation.

The S100B finding is consistent with the alarmin model of vitiligo. Alarmins are released during cellular injury and can bridge innate and adaptive immune activation [7,8]. Previous reports have proposed S100B as a disease-activity marker in non-segmental vitiligo and showed increased circulating alarmins in active disease [9-11]. The present result extends this concept by demonstrating that S100B was not merely higher in patients, but also positively linked to IL-17 and CD27. IL-17 is strongly implicated in inflammatory and autoimmune skin disease and has been repeatedly associated with vitiligo activity [16,17]. CD27 reflects T-cell activation and survival signaling, and its positive relationship with S100B supports an active immune-amplification state.

The inverse correlations of S100B with TGF-beta, IL-10 and FoxP3 strengthen the interpretation that high S100B is coupled to impaired regulatory restraint. IL-10 and TGF-beta are key anti-inflammatory mediators, and FoxP3 is central to Treg identity and suppressive function. Therefore, increased

S100B in the present patients may represent more than a passive damage marker; it may indicate a microenvironment in which danger signaling, Th17 polarization and reduced tolerance occur together.

CTLA-4 showed the opposite pattern. Its lower serum level in patients and its strong diagnostic AUC suggest that the CTLA-4 axis is substantially altered in this vitiligo cohort. Mechanistically, CTLA-4 down-modulates T-cell activation by interfering with CD28-mediated co-stimulation and by supporting Treg-mediated suppression [13,14]. The positive correlations of CTLA-4 with TGF-beta and FoxP3 observed here fit this biology, while the negative correlations with IL-17 and CD27 indicate that reduced checkpoint activity accompanies stronger inflammatory and T-cell activation signals. These findings align with evidence linking CTLA-4 variants or expression changes to autoimmune disorders and vitiligo susceptibility [15].

The diagnostic results are striking, particularly for CTLA-4. An AUC of 0.989 indicates excellent discrimination between cases and controls, and S100B also showed good discrimination. However, diagnostic accuracy from a single-center case-control design should not be overinterpreted. The separation between patients and healthy controls may be wider than that encountered in routine clinical settings, where inflammatory dermatoses, autoimmune comorbidities and treatment status can affect biomarker levels. Thus, these markers are best considered candidate adjunctive biomarkers rather than stand-alone diagnostic tests.

The lack of significant variation in S100B and CTLA-4 across severity, duration and family-history subgroups requires cautious interpretation. It may mean that these markers reflect the presence of the immunological disease state more than clinical extent at a single time point. Alternatively, subgroup sample sizes, especially the moderate-severity and positive-family-history groups, may have limited power to detect differences. Longitudinal follow-up would be stronger than cross-sectional subgrouping for testing whether S100B rises with activity or whether CTLA-4 recovery parallels disease stabilization.

The main strengths of this study are the balanced patient-control design, simultaneous measurement of soluble biomarkers and cellular immune markers, and integration of ROC and correlation analyses. The main limitations are the single-center design, modest sample size, absence of longitudinal follow-up, lack of post-treatment assessment and absence of external validation. Future studies should test combined S100B/CTLA-4 panels with IL-17, TGF-beta and Treg markers in larger cohorts and should evaluate whether these markers predict progression or response to therapy.

CONCLUSION

Vitiligo patients in this case-control study showed significantly increased serum S100B and significantly decreased CTLA-4 compared with healthy controls. S100B was linked to IL-17/CD27-driven inflammatory activation and inversely related to regulatory mediators, whereas CTLA-4 was positively associated with TGF-beta and FoxP3 and negatively associated with inflammatory activation. These results support a model in which vitiligo involves simultaneous alarmin-driven immune amplification and defective checkpoint-mediated tolerance. S100B and CTLA-4 may serve as useful adjunct biomarkers for immunological stratification of vitiligo, but their clinical use requires independent validation in larger and longitudinal cohorts.

REFERENCES

1. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. Lancet. 2015;386:74-84.

2. Hlaca N, Zagar T, Kastelan M, Brajac I, Prpic-Massari L. Current concepts of vitiligo immunopathogenesis. Biomedicines. 2022;10(7):1639.

3. Marchioro HZ, Silva de Castro CC, Fava VM, Sakiyama PH, et al. Update on the pathogenesis of vitiligo. An Bras Dermatol. 2022;97(4):478-490.

4. Riding RL, Harris JE. The role of memory CD8+ T cells in vitiligo. J Immunol. 2019;203(1):11-19.

5. Giri PS, Dwivedi M, Laddha NC, Begum R, et al. Altered expression of nuclear factor of activated T cells, forkhead box P3, and immune-suppressive genes in regulatory T cells of generalized vitiligo patients. Pigment Cell Melanoma Res. 2020;33(4):566-578.

6. Custurone P, Di Bartolomeo L, Irrera N, Borgia F, et al. Role of cytokines in vitiligo: pathogenesis and possible targets for old and new treatments. Int J Mol Sci. 2021;22(21):11429.

7. Danieli MG, Antonelli E, Piga MA, Claudi I, et al. Alarmins in autoimmune diseases. Autoimmun Rev. 2022;21:103142.

8. Murao A, Aziz M, Wang H, Brenner M, Wang P. Release mechanisms of major DAMPs. Apoptosis. 2021;26:152-162.

9. Speeckaert R, Voet S, Hoste E, van Geel N. S100B is a potential disease activity marker in nonsegmental vitiligo. J Invest Dermatol. 2017;137:1445-1453.

10. Abd El-Halim R, Abo Khodier H, Fouda IF, Abd El-Samee HS. Evaluation of S100B serum level as a biomarker of disease activity in vitiligo patients. IJMA. 2019;1(1):37-41.

11. He K, Wu W, Wang X, Dai W, Wang S, Li C, Li S. Circulatory levels of alarmins in patients with non-segmental vitiligo: potential biomarkers for disease diagnosis and activity/severity assessment. Front Immunol. 2022;13:1069196.

12. Yang F, Yang L, Teng L, Zhang H, Katayama I. Morphological alterations and increased S100B expression in epidermal Langerhans cells detected in skin from patients with progressive vitiligo. Life. 2021;11:579.

13. Verma N, Burns SO, Walker LSK, Sansom DM. Immune deficiency and autoimmunity in patients with CTLA-4 (CD152) mutations. Clin Exp Immunol. 2017;190(1):1-7.

14. Kim GR, Choi JM. Current understanding of cytotoxic T lymphocyte antigen-4 (CTLA-4) signaling in T-cell biology and disease therapy. Mol Cells. 2022;45(8):513-521.

15. Gouda NS, Fawzy MS, Toraih EA. Impact of cytotoxic T-lymphocyte-associated protein 4 codon 17 variant and expression on vitiligo risk. J Clin Lab Anal. 2021;35(6):e23777.

16. Acharya P, Mathur M. Interleukin-17 level in patients with vitiligo: a systematic review and meta-analysis. Australas J Dermatol. 2020;61:e208-e212.

17. Bhardwaj S, Rani S, Srivastava N, Kumar R, Parsad D. Increased systemic and epidermal levels of IL-17A and IL-1beta promotes progression of non-segmental vitiligo. Cytokine. 2017;91:153-161.