

Clinical significance of circulating regulatory T Cells, soluble CD25, and CXCL9 in assessing disease activity of vitiligo

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Abstract

Vitiligo is an autoimmune skin disorder characterized by T-cell-mediated melanocyte destruction and loss of immune tolerance. Dysregulation of regulatory T cells and altered chemokine signaling have been implicated; however, their relationship with disease activity remains unclear. Therefore, this study aimed to evaluate peripheral immune cell subsets, particularly Tregs, sCD25, and CXCL9 biomarkers in patients with vitiligo and their relationship with disease activity. This study included 44 patients with non-segmental vitiligo and 44 matched healthy controls. Peripheral blood lymphocyte subsets and regulatory T cells were analyzed by flow cytometry. Serum CXCL9 and sCD25 were measured by ELISA. Patients were diagnosed with non-segmental vitiligo based on clinical evaluation. Disease severity and activity were assessed using the VASI and VIDA scores. Peripheral lymphocyte subsets were generally preserved; however, CD4⁺ T cells were reduced in patients compared with healthy controls, with a lower CD4/CD8 ratio. Regulatory T cells (CD4⁺CD25⁺FOXP3⁺) were significantly decreased in percentage of patients compared with healthy controls. Treg frequency correlated positively with the VIDA score. Serum CXCL9 and soluble CD25 showed no significant differences between study groups; however, they both correlated inversely with disease activity. Neither marker correlated with the VASI score. Vitiligo is associated with selective immune dysregulation characterized by reduced CD4⁺ T-cell levels and a decreased Treg proportion. Although CXCL9 and sCD25 do not distinguish vitiligo patients from healthy controls, their correlation with disease activity suggests potential utility as dynamic biomarkers.

Keywords: Vitiligo, Regulatory T cells, CD4⁺CD25⁺FOXP3⁺, soluble CD25, CXCL9, VIDA, VASI.

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Introduction

Vitiligo is an acquired, polygenic depigmenting condition characterized by milky white skin

patches¹ and is categorized into two forms, segmental (SV) and non-segmental (NSV),² with NSV being more common and potentially

associated with autoimmune diseases such as Hashimoto's thyroiditis.³ The pathophysiology of vitiligo is broad and complex, although the particular pathways connecting genetic predisposition, melanocyte destruction, and lack of immunological tolerance remain poorly understood.⁴ The autoimmune destruction of melanocytes in the skin of patients with vitiligo, induced by melanocyte-specific CD8⁺ T cells, illustrates the role of cell-mediated immunity.⁵ The Fas–Fas ligand (Fas–FasL)-dependent death of melanocytes by autoreactive CD4⁺ T lymphocytes highlights their crucial involvement in the pathogenesis of vitiligo.⁶ Thus, the presence of these autoreactive CD8⁺ and CD4⁺ T cells in vitiligo patients' skin and blood samples indicates a dysregulation of the regulatory T cells (Tregs) system, which can inhibit these cells.⁷

Self-reactive CD8⁺ and CD4⁺ T cells rely heavily on Treg cells to maintain immunological tolerance.⁸ A previous study has reported that patients with vitiligo had altered Treg cell number and function.⁹ Adoptive transfer of Treg cells, which prevents the progression of vitiligo, has previously demonstrated the role of Tregs in the disease, while Tregs from patients with vitiligo fail to suppress the proliferation and activation of cytotoxic T lymphocytes.¹⁰

Forkhead box Protein 3 (FOXP3) is a key transcriptional regulator of Tregs that facilitates Treg cell function by inhibiting the release of the cytokines interleukin-2 (IL2) and interleukin-4 (IL4) and upregulating the Treg cell markers cytotoxic T-lymphocyte antigen-4 (CTLA-4) and CD25.¹¹

CD4 T cells co-expressing the IL-2 receptor α -chain (CD4 CD25) appear to play a critical role in maintaining T cell homeostasis, limiting the proliferation of effector T cells and thereby preserving self-tolerance.¹² CD4⁺ CD25⁺ FOXP3⁺ Tregs play a crucial role in self-tolerance and immune response regulation under both normal and pathological settings.¹³ Several studies have reported conflicting results regarding the levels and /or function of Tregs in patients with vitiligo.^{14,15,16} Soluble CD25 (sCD25), a circulating IL-2 receptor α -chain, is a component of the high-affinity IL-2 receptor complex and modulates immunological responses dependent

on IL-2.¹⁷ sCD25, derived mostly from activated T cells and Tregs, is detectable in serum, especially in inflammatory and autoimmune conditions, confirming its significance as a biomarker of immunological activation.¹⁸ In vitiligo, increased sCD25 indicates immunological activation within a complex, cytokine-driven pathophysiology involving both innate and adaptive immunity.¹⁹

Peripheral blood from patients with vitiligo contains a higher number of autoreactive T cells. Generally, cytotoxic T-lymphocytes (CTLs) move into the skin in response to chemokine signals. Chemokines are key inflammatory mediators involved in many autoimmune reactions.²⁰ C-X-C motif chemokine ligand 9 (CXCL9) is linked to the T-helper 1 (Th1) pattern and is identified as one of the most critical chemokine axes that drives T-cell migration in many autoimmune and inflammatory disorders. In vitiligo, CXCL9 has been found to induce melanocyte-specific CTLs to invade the basal layer of the epidermis and attack melanocytes, resulting in melanin deficit.²¹ Although previous studies have investigated the roles of Tregs, sCD25, and CXCL9 in vitiligo, their clinical value as biomarkers of disease activity remains uncertain. Moreover, few studies have evaluated these biomarkers simultaneously in patients with non-segmental vitiligo. Therefore, further investigation is warranted to clarify their potential role in disease assessment and monitoring. The present study aimed to evaluate the immunological mechanisms underlying vitiligo by assessing peripheral CD4⁺CD25⁺FOXP3⁺ Treg cells, serum soluble CD25, and CXCL9 levels, and to investigate their association with disease activity as potential biomarkers of immune dysregulation and disease progression.

Subjects and Methods

This study was conducted at the Department of Clinical Pathology, Faculty of Medicine, Assiut University, Egypt. Patients were consecutively recruited from the Dermatology Outpatient Clinic, Assiut University Hospitals, a tertiary referral center serving Upper Egypt. Clinical evaluation was performed in the Dermatology Department, while laboratory investigations and

immunological analyses were conducted at the Department of Clinical Pathology. Participant confidentiality was maintained throughout the study. All data were anonymized before analysis by assigning unique study identification codes. Only the research team had access to the coded data, which was securely stored. All findings were reported in aggregate form to protect participant privacy.

This case-control study included 44 patients with vitiligo and 44 age- and sex-matched healthy controls. The sample size was calculated using G*power version 3.1.9.2.¹¹ Patients with clinically diagnosed NSV were enrolled, while those receiving recent systemic therapy within the previous 3 months, or with systemic autoimmune diseases or type 1 diabetes mellitus, were excluded. All participants underwent detailed history taking and clinical examination. Diagnosis of non-segmental vitiligo was based on clinical evaluation and confirmed by Wood's lamp examination, with assessment of lesion type, distribution, and extent. The extent of vitiligo was assessed using the Vitiligo Area Scoring Index (VASI) score,²² while disease activity was evaluated using the Vitiligo Disease Activity (VIDA) score.²³

Blood sampling and measurement

A total of 10 mL of venous blood was collected from each participant under aseptic conditions. Blood samples were collected into EDTA tubes for complete blood count (CBC) and flow cytometric analysis, and into plain tubes for serum separation. After centrifugation, the serum was separated and divided into three aliquots. The first aliquot was used for routine laboratory investigations, including thyroid-stimulating hormone (TSH), C-reactive protein (CRP), and rheumatoid factor (RF). TSH was measured using the ADVIA Centaur XPT Immunoassay System (Siemens Healthineers, Germany), while CRP and RF were measured using the ADVIA 1800 Chemistry System (Siemens Healthineers, Germany). The second aliquot was used to detect antinuclear antibodies (ANA) using the AESKU HEp-2 Diagnostics indirect immunofluorescence assay for the qualitative determination of IgG antibodies against HEp-2 cells (REF S51.100,

AESKU.DIAGNOSTICS, Germany). The third aliquot was stored at -80°C until the analysis of sCD25 and CXCL9 using an enzyme-linked immunosorbent assay (ELISA), according to the manufacturers' instructions. A complete blood count (CBC) was performed using the ADVIA 2120i Hematology System (Siemens Healthineers, Germany). Flow cytometric analysis was performed using the CytoFLEX Flow Cytometer (Beckman Coulter, USA) to assess lymphocyte subsets, including CD4⁺CD25⁺FOXP3⁺ Treg cells and B lymphocytes.

Flow Cytometric Analysis of T Lymphocyte Subsets and Treg Cells

Peripheral blood was collected in EDTA tubes and processed within 24 hours at room temperature ($20\text{--}25^{\circ}\text{C}$). Cells were stained with fluorochrome-conjugated monoclonal antibodies targeting CD3 (Phycoerythrin-Cyanin 5.5, PC5.5; Ref: B49203; Beckman Coulter, France), CD4 (Fluorescein Isothiocyanate, FITC; Ref: IM0448U; Beckman Coulter, France), CD8 (Phycoerythrin, PE; Ref: IM0452U; Beckman Coulter, France), CD19 (Phycoerythrin-Cyanin, PC; Ref: IM2643U; Beckman Coulter, France), CD25 (Allophycocyanin, APC; Ref: B09684; Beckman Coulter, France), and FOXP3 (Phycoerythrin, PE; Ref: B46031; Beckman Coulter, France). PBS 1X (Cat. No. PK768; ABC-SCIENTIFIC CO, China) was used as a leukocyte stabilizing wash buffer (pH 7.4). Red blood cell lysing solution was prepared using 8.2 gm of ammonium chloride (Cat. No:10977, Chem-Lab, Egypt), 0.84 g of sodium bicarbonate (Ref: E003608, El Nasr Chemicals Co., ADWIC, Egypt), and 0.029 g of EDTA (Ref: E002811, El Nasr Chemicals Co., ADWIC, Egypt). The former components were dissolved in distilled water, the volume was made up to 1 liter, and the pH was adjusted to 7.2 ± 0.2 . A fresh 1X working solution was prepared daily. Intracellular FOXP3 staining was performed using the Intra Prep Permeabilization Kit (Ref. No. IM2389; IMMUNOTECH SAS, France). Multicolor flow cytometry was performed using the Beckman Coulter Cyto-FLEX, acquiring 20,000–30,000 events per sample at 400–500 cells/sec. Lymphocytes were gated based on forward and side scatter (FSC/SSC) to exclude debris and

dead cells. T cells were identified as CD3⁺ and subdivided into CD4⁺ and CD8⁺ subsets, and the CD4/CD8 ratio was calculated. B cells were identified as CD19⁺ within the lymphocyte gate. CD4⁺ T cells were gated from the lymphocyte

population; the population of T regs was obtained by calculating the percentage of CD25⁺FOXP3⁺ double-positive cells within the CD4⁺ gate from peripheral blood of patients with vitiligo and healthy controls, as shown in Fig. 1.

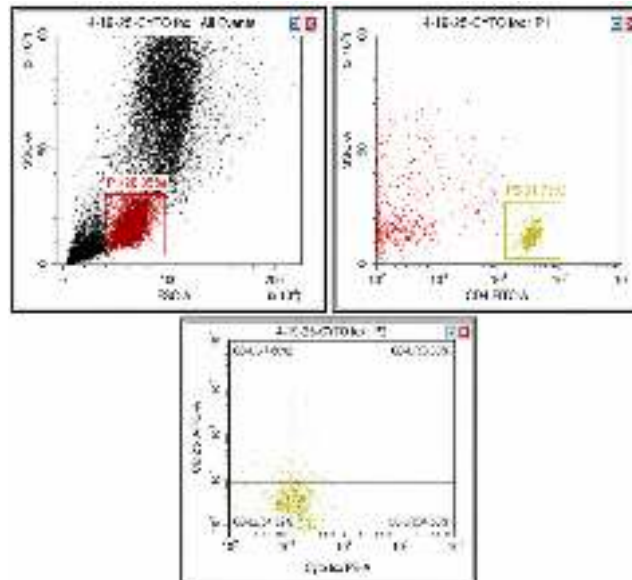


Figure 1. Example of flow cytometric analysis of Treg cells in peripheral blood in the patient of vitiligo. Lymphocytes were first gated based on FSC/SSC (P1, 26.05%). With this gate, CD4⁺ T cells were identified (P2, 31.73%). Treg cells were defined as CD4⁺CD25⁺FOXP3⁺ cells, representing 3.30% of CD4⁺ lymphocytes.

Quantitative Measurement of Serum CXCL9 and CD25 by ELISA

The concentrations of human CXCL9 and CD25 in serum samples were determined using commercial ELISA kits (CXCL9: Catalog No. SG-15706; CD25: Catalog No. SG-10728; Sino-Gene-Clon Biotech Co., Ltd., China) following the manufacturer's protocols. The assay measurement ranges were 0–1350 pg./mL for CXCL9 and 0–9 µg/L for CD25. Serum samples were assayed at a five-fold dilution, and the obtained concentrations were corrected by multiplying the measured values by a factor of 5. The plates were read at an absorbance of 450 nm using a Stat Fax 200 microplate reader (Awareness Technology, Inc., USA).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26. Data were expressed as means $\pm$ SD, medians (ranges), or frequencies (%), as appropriate. Quantitative variables were tested for normality using the Shapiro-Wilk test.

Comparisons were performed using the independent t-test, Mann–Whitney U test, and Chi-square test, while correlations were assessed using Spearman's correlation coefficient. Comparisons among the three study groups (stable vitiligo, active vitiligo, and healthy controls) were performed using the Kruskal–Wallis test. ROC curve analysis was used to evaluate the diagnostic performance of CD4⁺CD25⁺FOXP3⁺ Treg cells, soluble CD25, and CXCL9 in relation to disease activity. A *p*-value < 0.05 was considered statistically significant. To identify independent predictors of the study outcome, relevant multivariable binary logistic regression analysis was performed. Variables considered clinically and/or statistically significant in the univariate analysis were entered into the regression model. The results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A two-tailed *p*-value < 0.05 was considered statistically significant.

Results

The study included 44 patients and 44 matched healthy controls. There were no significant

differences between the groups regarding gender or age distribution, as shown in Table 1. Clinical Characteristics of the Patient Group, as shown in Table (2).

Table 1. Demographic data of the groups studied.

	Patients (n= 44)		Controls (n= 44)		<i>p</i> -value
	No.	%	No.	%	
Gender: †					
Male	18	40.9%	18	40.9%	NS
Female	26	59.1%	26	59.1%	
Age: (years) ‡					
Mean ± SD	33.73 ± 11.41		36.18 ± 9.90		NS
Median (Range)	34.0 (18.0-55.0)		34.5 (18.0-55.0)		

SD, standard deviation. † Chi-square test. ‡ Independent samples t-test. $p > 0.05$ is not significant (NS).

Table 2. Clinical characteristics of the vitiligo patients.

Clinical data	No. (n= 44)	%
Age at onset (years)		
Mean ± SD	25.11 ± 13.59	
Median (Range)	24.0 (4.0-52.0)	
Duration of disease (years)		
Mean ± SD	8.68 ± 9.60	
Median (Range)	6.5 (0.08-51.0)	
Duration of last activity (months)		
Mean ± SD	4.95 ± 3.58	
Median (Range)	4.0 (1.0-12.0)	
New lesions	29	65.9%
Expansion of old lesion	15	34.1%
Koebner	7	15.9%
Depigmentation of repigmented lesion	10	22.7%
VIDA score:		
Mean ± SD	2.23 ± 1.22	
Median (Range)	2.0 (0.0-4.0)	
VASI score:		
Mean ± SD	4.95 ± 6.50	
Median (Range)	2.63 (0.01-33.50)	
Family history:		
Positive	13	29.5%
Negative	31	70.5%

SD, standard deviation; VIDA, Vitiligo Disease Activity score; VASI, Vitiligo Area Scoring Index.

Most patients had active disease (72.7%). VIDA +2 was the most frequent score (31.8%), indicating disease activity within the previous 3–

6 months, while stable disease (no new lesions for at least 1 year) was observed in 9.1% of patients, as shown in Table 3.

Table 3. Distribution of VIDA Scores among the Studied Patients.

VIDA score	No. (44)	%
0: Stable for at least 1 year	4	9.1%
+1: Activity of 6 to 12 months	8	18.2%
+2: Activity of 3 to 6 months	14	31.8%
+3: Activity of 6 weeks to 3 months	10	22.7%
+4: Activity of 6 weeks or less	8	18.2%

VIDA, Vitiligo Disease Activity score.

CBC parameters showed no significant differences between patients and healthy controls in total leukocyte count, hemoglobin

level, or platelet count. Similarly, the lymphocyte percentage was comparable between the two groups, as shown in Table 4.

Table 4. Complete blood picture results in vitiligo patients and healthy controls.

	Patients (n= 44)	Controls (n= 44)	p-value ‡
WBC 10 ³ /μl			
Mean ± SD	6.51 ± 1.97	6.43 ± 2.55	NS
Median (Range)	6.6 (1.1-10.8)	5.8 (3.5-13.8)	
HB g/dl			
Mean ± SD	13.29 ± 1.38	13.38 ± 1.50	NS
Median (Range)	13.5 (8.7-15.8)	13.7 (10.4-17.8)	
PLTs 10 ³ / μl			
Mean ± SD	284.25 ± 72.99	266.32 ± 74.55	NS
Median (Range)	275.5 (121.0-450.0)	260.5 (131.0-464.0)	
Lymphocyte %			
Mean ± SD	35.25 ± 8.17	36.94 ± 9.96	NS
Median (Range)	34.2 (19.4-52.8)	37.6 (18.2-54.8)	

SD, standard deviation; WBC, white blood cell count; Hb, hemoglobin; PLTs, platelets. ‡ Independent samples t-test.

p > 0.05 is not significant (NS).

Markers of systemic inflammation and autoimmunity were also evaluated. RF and CRP levels were significantly higher in patients with vitiligo than in healthy controls. In contrast, no significant difference was observed in serum

TSH levels between the two groups. ANA results were negative in all participants, with no ANA-positive cases detected in either group, as shown in Table 5.

Table 5. Comparison of autoimmune and inflammatory markers between patients and healthy controls.

	Patients (n= 44)	Controls (n= 44)	p-value ‡
Rheumatoid Factor (up to 14 IU/ml)			
Mean ± SD	6.55 ± 9.21	3.53 ± 2.83	0.018
Median (Range)	4.7 (0.1-52.5)	2.9 (0.1-10.7)	
C-Reactive protein (up to 6 mg/L)			
Mean ± SD	3.11 ± 3.52	1.67 ± 1.89	0.018
Median (Range)	2.1 (0.1-18.3)	1.1 (0.0-7.6)	

Table 5. Continued.

	Patients (n= 44)	Controls (n= 44)	p-value ‡
TSH (0.55-4.8 mIU/ml)			
Mean ± SD	2.18 ± 1.27	1.90 ± 0.95	NS
Median (Range)	2.0 (0.4-6.3)	1.8 (0.5-4.7)	
Anti-Nuclear Abs: No. (%)			
Negative	44 (100.0%)	44 (100.0%)	--

SD, standard deviation; TSH, thyroid-stimulating hormone; ANA, antinuclear antibodies

‡ Mann–Whitney *U* test. *p* > 0.05 is not significant (NS).

Patients showed a significant reduction in CD4⁺ T-cells compared with healthy controls, with a corresponding decrease in the CD4/CD8 ratio. No significant differences were observed in CD3⁺

or CD8⁺ T-cell percentages. Similarly, the percentage of CD19⁺ B-cells was comparable between the two groups, as shown in Table 6.

Table 6. Immunophenotypic analysis of peripheral blood T- and B-lymphocyte subsets in vitiligo patients compared with healthy controls.

	Patients (n= 44)	Controls (n= 44)	p-value
CD3 (%) †			
Mean ± SD	62.87 ± 9.75	66.55 ± 9.90	NS
Median (Range)	63.4 (36.0-81.9)	66.9 (44.0-82.0)	
CD4 (%) †			
Mean ± SD	31.97 ± 8.15	36.16 ± 8.03	0.017
Median (Range)	31.9 (16.0-57.8)	35.0 (18.0-57.0)	
CD8 (%) †			
Mean ± SD	30.70 ± 6.09	28.36 ± 6.82	NS
Median (Range)	30.9 (18.0-47.2)	28.0 (15.0-48.0)	
CD4/CD8 Ratio ‡			
Mean ± SD	1.04 ± 0.35	1.32 ± 0.51	0.014
Median (Range)	1.0 (0.5-2.3)	1.1 (0.6-2.7)	
CD19 (%) ‡			
Mean ± SD	13.19 ± 5.49	12.68 ± 3.79	NS
Median (Range)	12.9 (5.2-26.0)	12.1 (7.0-23.5)	

SD, standard deviation, † Independent samples t-test., ‡ Mann–Whitney *U* test. *p* > 0.05 is not significant (NS).

Patients showed a significant reduction in Treg cells, with lower CD4⁺CD25⁺FOXP3⁺ percentages compared with healthy controls. No significant

differences were observed in serum soluble CD25 levels or CXCL9 concentrations between the two groups, as shown in Table 7.

Table 7. Comparison of Treg Cells (CD4⁺CD25⁺FOXP3⁺) and serum biomarkers (sCD25 and CXCL9) between vitiligo patients and healthy controls.

	Patients (n= 44)	Controls (n= 44)	p-value ‡
CD4+CD25+FOXP3+%:			
Mean ± SD	4.79 ± 1.40	9.12 ± 1.66	0.000
Median (Range)	4.5 (2.7-7.8)	9.0 (6.1-13.2)	
Soluble CD25 µg/L:			
Mean ± SD	1.95 ± 1.85	2.00 ± 1.96	NS
Median (Range)	1.5 (0.0-7.0)	1.5 (0.5-8.0)	

Table 7. Continued..

	Patients (n= 44)	Controls (n= 44)	<i>p</i> -value ‡
CXCL9 pg/ml:			
Mean ± SD	739.76 ± 397.06	610.38 ± 412.22	NS
Median (Range)	807.5 (222-1816)	617.5 (40-1280)	

SD, standard deviation. ‡Mann–Whitney *U* test. *p* > 0.05 is not significant (NS).

Patients with stable and active vitiligo showed significantly lower CD4⁺CD25⁺FOXP3⁺ Treg cell percentages than healthy controls. Serum CXCL9 levels were significantly higher in patients with

stable vitiligo than in both active vitiligo and healthy controls, whereas soluble CD25 levels did not differ significantly among the three groups, as shown in Table 8.

Table 8. Comparison of CD4⁺CD25⁺FOXP3⁺ Treg cells, soluble CD25, and CXCL9 levels among patients with stable vitiligo, active vitiligo, and healthy controls.

	Stable vitiligo	Active vitiligo	Controls	<i>p</i> -value ¹	<i>p</i> -value ²	<i>p</i> -value ³	<i>p</i> -value ⁴
	Median (Range)	Median (Range)	Median (Range)				
CD4 ⁺ CD25 ⁺ FOXP3 ⁺ %	3.9 (2.8-6.1)	4.7 (2.7-7.8)	9.0 (6.1-13.2)	0.000	NS	0.000	0.000
Soluble CD25 µg/L	1.75 (0.5-7.0)	1.5 (0.0-7.0)	1.5 (0.5-8.0)	NS	NS	NS	NS
CXCL9 pg./ml	908.25 (298.5-1816.0)	600.5 (222.0-1394.0)	617.5 (40.0-1280.0)	NS	0.041	0.026	NS

p 1: Comparison among all groups (Kruskal–Walli's test).

p 2: Comparison between Stable vitiligo and Active vitiligo Mann–Whitney test.

p 3: Comparison between Stable vitiligo and Control Mann–Whitney test.

p 4: Comparison between Active vitiligo and Control Mann–Whitney test. *p* > 0.05 is not significant (NS).

A significant positive correlation was observed between CD4⁺CD25⁺FOXP3⁺ Treg percentage and the VIDA score, whereas no correlation was found with the VASI score. Serum soluble CD25 showed a significant negative correlation with

the VIDA score, and CXCL9 levels were also inversely correlated with the VIDA score. No significant correlations were detected between either marker and the VASI score, as shown in Table 9.

Table 9. Correlation of CD4⁺CD25⁺FOXP3⁺%, Soluble CD25 and CXCL9 with VIDA and VASI Scores in Vitiligo Patients.

	VIDA score		VASI score	
	r- value	<i>p</i> -value	r- value	<i>p</i> -value
CD4 ⁺ CD25 ⁺ FOXP3 ⁺ %	0.731	0.013	-0.203	NS
Soluble CD25 µg/L	-0.401	0.007	0.006	NS
CXCL9 pg./ml	-0.308	0.042	0.167	NS

r, correlation coefficient; VIDA, Vitiligo Disease Activity score; VASI, Vitiligo Area Scoring Index.

Spearman's rank correlation coefficient. *p* > 0.05 is not significant (NS).

ROC curve analysis was performed to evaluate the ability of CD4⁺CD25⁺FOXP3⁺%, soluble CD25, and CXCL9 to discriminate disease activity status. CXCL9 showed the best detection of

disease activity (AUC = 0.702), followed by CD4⁺CD25⁺FOXP3⁺% and soluble CD25, although none of the markers reached statistical significance, as shown in Table 10.

Table 10. ROC curve analysis of CD4⁺CD25⁺FOXP3⁺%, sCD25 and CXCL9 in relation to disease activity.

Variable	Cut-off	Sensitivity	Specificity	+PV	-PV	AUC	p-value
CD4 ⁺ CD25 ⁺ FOXP3 ⁺ %	> 4.4	62.50	75.00	87.0	42.9	0.671	NS
sCD25 µg/L	≤ 1	46.88	83.33	88.2	37.0	0.663	NS
CXCL9 pg/ml	≤ 889	68.75	66.67	84.6	44.4	0.702	

ROC, receiver operating characteristic; AUC, area under the curve; +PV, positive predictive value; -PV, negative predictive value. $p > 0.05$ is not significant (NS).

Among the variables included in the study, only TSH and the percentage of CD4⁺CD25⁺FOXP3⁺ Tregs remained significantly associated with the study outcome. Higher TSH levels were independently associated with increased odds of the outcome (OR = 3.671, 95% CI: 1.052–12.811, $p = 0.041$), whereas higher percentages

of CD4⁺CD25⁺FOXP3⁺ Treg cells were independently associated with decreased odds of the outcome (OR = 0.090, 95% CI: 0.022–0.362, $p = 0.001$). No significant independent associations were observed for age, rheumatoid factors, C-reactive protein, CXCL9, or soluble CD25 ($p > 0.05$), as shown in Table 11.

Table 11. Multivariable binary logistic regression analysis of independent predictors of the study outcome.

Variables	p-value	OR	95% C.I.	
			Lower	Upper
Age (years)	NS	1.031	0.932	1.140
Rheumatoid Factor U/ml	NS	0.986	0.619	1.572
C-Reactive protein mg/L	NS	1.568	0.863	2.850
TSH mIU/ml	0.041	3.671	1.052	12.811
CD4 ⁺ CD25 ⁺ FOXP3 ⁺ %	0.001	0.090	0.022	0.362
CXCL9 pg/ml	NS	1.000	0.997	1.004
Soluble CD25 µg/L	NS	1.050	0.507	2.176

OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; RF, rheumatoid factor; CRP, C-reactive protein. $p > 0.05$ is not significant (NS).

Discussion

To make appropriate therapeutic decisions in vitiligo, it is essential to distinguish stable from active disease, particularly when considering surgical therapy or aiming for early control of progressive disease. Vitiligo Disease Activity score is a validated score designed to evaluate disease stability/activity,²⁴ however, the outcome may be inaccurate as this subjective score depends on patients' own reports, opinions, and memory. Regarding clinical examination, some signs can be highly suggestive of vitiligo activity/stability; however, there is a lack of a reliable scoring system to confirm activity/stability clinically.²⁵ In the

present study, the mean age of patients with vitiligo was 33.73 ± 11.41 years, which is in line with the mean ages reported by Nejad et al., 2013²⁶ and Amer et al., 2019,²⁷ who reported mean ages of 30.8 and 28.11 years, respectively. However, in a study by Saudi et al., 2021,²⁸ the mean age of patients was 43.53 ± 14.35 years. These data reinforce that vitiligo can occur at any age.

In this study, the mean age at disease onset was 25.11 ± 13.59 years, indicating considerable inter-individual variability in disease onset. This is in accordance with the finding of Bhardwaj et al., 2020,²⁹ who reported that the mean age at onset of vitiligo was 20.8 ± 10.0 years. On the contrary, Martins et al., 2020³⁰ have found that

the average age of onset of nonsegmental vitiligo was 6.1 ± 3.1 years.

In the present study, we observed that females (59.1%) were more frequently affected than males (40.9%). This is in accordance with the findings of Martins et al., 2020³⁰ and Saudi et al., 2021,²⁸ who reported that the incidence of vitiligo was higher among females. This may be attributed to females being more concerned about the disease and its distressing cosmetic impact than males, leading to earlier and more frequent presentations of females to dermatology clinics. In addition, autoimmune diseases are more common among females.³¹

In the present study, nearly one-third of patients (29.5%) reported a positive family history, exceeding the rates reported by Gharib et al., 2021²¹, who documented a family history in 20% of patients, and Giri et al., 2020,¹¹ who reported that 15% of patients had a positive family history. This variation may be attributed to differences in genetic background and ethnic composition among populations. The higher familial aggregation observed in this study supports the role of vitiligo-associated susceptibility genes, including HLA, NLRP1, PTPN22, and CTLA4, which are implicated in immune regulation and melanocyte autoimmunity.³²

Markers related to systemic inflammation and autoimmunity were further analyzed. In the present study, CRP levels were significantly elevated in the patient group compared with age-matched and sex-matched healthy controls. Similarly, Aryanian et al., 2022,³³ investigated the role of CRP in vitiligo and reported significantly higher CRP levels in patients compared with healthy controls. Conversely, Namazi et al., 2018,³⁴ reported no statistically significant difference between the two groups, and found that the local inflammation caused by vitiligo had no impact on serum CRP levels. It is important to note that CRP levels can be affected by various factors such as smoking, infections, inflammation, and cancer.³⁵

RF has been investigated in vitiligo as part of the broader concept of autoimmune disease clustering. In this study, rheumatoid factor concentrations were significantly higher in patients with vitiligo than in healthy controls.

This finding is in agreement with Gonggryp and Kalla, 1992,³⁶ who reported coexistence between vitiligo and rheumatoid arthritis. However, although vitiligo is frequently associated with other autoimmune diseases, including rheumatoid arthritis, most studies have reported normal RF levels in patients with vitiligo without clinical arthritis.³⁷ This suggests that RF positivity is not a characteristic feature of vitiligo itself but may reflect coexisting autoimmune conditions or an increased risk of future autoimmune diseases rather than the cutaneous disease alone.

Numerous researchers have studied the association between thyroid disorders and vitiligo. In the present study, no statistically significant difference was observed in serum TSH levels between patients with vitiligo and healthy controls. This finding is consistent with Elhwari et al., 2024,³⁸ who found no significant differences in TSH levels between the vitiligo patients and the healthy control group. On the other hand, Daneshpazhooh et al., 2006,³⁹ reported that blood TSH levels were significantly higher in patients with vitiligo. Therefore, based on the findings of this study, patients with vitiligo are not at an increased risk of thyroid disorders (functional or autoimmune) compared with healthy individuals.

In the present study, the percentage of CD19⁺ B-cells did not differ significantly between patients with vitiligo and healthy controls. This is consistent with Willemsen et al., 2022,⁴⁰ who reported comparable proportions of total B-cells in patients with vitiligo and healthy controls. However, Raam et al. 2018,⁴¹ demonstrated reduced naïve B-cell subsets.

No statistically significant differences were detected between patients with vitiligo and healthy controls in the percentage of CD3⁺ T-cells. This result is consistent with the observations of Sherif & Enas, 2021.⁴² These observations support the concept that systemic T-cell homeostasis remains intact in vitiligo, despite ongoing cutaneous immune activity. Conversely, Mohsen et al., 2025⁴³, reported decreased total T lymphocyte counts in patients with vitiligo.

Notably, in our study, we observed a significant reduction in the proportion of CD4⁺ T lymphocytes in patients with vitiligo compared with healthy controls, suggesting an imbalance in T-cell subsets. This observation is consistent with reports by Yamauchi et al., 2002⁴⁴ and Nigam et al., 2011⁴⁵, who similarly documented decreased CD4⁺ T-cell percentages in patients with vitiligo.

In parallel, we detected a non-statistically significant increase in CD8⁺ T lymphocytes in patients with vitiligo compared with healthy controls. This observation aligns with the results of Nigam et al., 2011⁴⁵ and Dwivedi et al., 2013,¹⁴ thereby supporting the cytotoxic T-cell-mediated pathogenesis of melanocyte destruction. In our study, the CD4/CD8 ratio was significantly lower in patients with vitiligo than in the control group. This finding is in agreement with studies by Gunduz et al., 2004⁴⁶ and Dwivedi et al., 2013.¹⁴ Nevertheless, Pichler et al., 2009⁴⁷ did not observe a reduction in the percentage of CD4⁺ T cells or in the CD4⁺/CD8⁺ ratio.

The present study demonstrated a significant reduction in the percentage of CD4⁺CD25⁺FOXP3⁺ Treg cells in patients with vitiligo. This finding is consistent with Dwivedi et al., 2013¹⁴ and Ben Ahmed et al., 2012.¹⁵ Nevertheless, Moftah et al., 2014⁴⁸ and Tembhre et al., 2013,⁴⁹ reported variable Treg levels, suggesting heterogeneity related to disease activity, population differences, and study design.

Regarding disease activity, the present study demonstrated a positive correlation between circulating CD4⁺CD25⁺FOXP3⁺ Treg cells and the VIDA score. However, Moftah et al., 2014,⁴⁸ found no clear association between Treg levels and disease activity. On the other hand, previous work by Dwivedi et al., 2013,¹⁴ showed a negative correlation between Treg cells and the VIDA score. Importantly, Treg cells in active vitiligo may be functionally impaired despite numerical variation, indicating that increased frequency does not necessarily reflect effective immune regulation.⁵⁰

This discrepancy reflects the distinction between quantitative and functional Treg alterations. In active autoimmune inflammation,

compensatory expansion of circulating Treg cells may occur; however, these cells are often functionally deficient and unable to adequately suppress autoreactive immune responses. Overall, these findings suggest that vitiligo is characterized by both quantitative and qualitative defects in Treg cell function.⁵¹

Soluble CD25 (sCD25) reflects T-cell activation status in peripheral blood.⁵² However, its relationship with vitiligo activity remains controversial.⁵⁰ In the present study, serum sCD25 levels did not differ significantly between patients with vitiligo and healthy controls. The lack of difference between groups suggests that sCD25 is not a diagnostic biomarker for vitiligo. Interestingly, sCD25 showed a significant negative correlation with the VIDA score. These findings differ from those of Speeckaert et al., 2016,⁵³ who found an association between elevated sCD25 levels and disease activity.

ROC analysis in patients with vitiligo showed that sCD25 had fair discriminatory power for disease activity; however, this association was not statistically significant. However, its inverse correlation with the VIDA score may reflect compensatory immune regulation during active disease rather than a purely pro-inflammatory state. Overall, these findings support the role of sCD25 as a marker of immune regulatory dynamics rather than a direct indicator of vitiligo activity.

In the present study, serum CXCL9 levels were higher in patients with vitiligo than healthy controls, the difference was not statistically significant. This agrees with Lin et al., 2021⁵⁴ and Rashighi et al., 2014,⁵⁵ who also reported no significant intergroup differences. While Yang et al., 2018²⁰ reported that serum CXCL9 levels were lower in patients with vitiligo than in healthy controls, the difference was not statistically significant. Nevertheless, Zhang et al., 2019⁵⁶ and El-Domyati et al., 2022⁵⁷ reported significantly elevated CXCL9 levels in patients with vitiligo. These inconsistencies may be explained by differences in study designs, sample sizes, disease activity, treatment status, and methodological or population-related factors.

In the present study, serum CXCL9 concentrations showed a significant inverse

correlation with the VIDA score. However, Abdallah et al., 2024⁵⁸ and Mahgoub et al., 2025,⁵⁹ found no statistically significant correlation between serum CXCL9 levels and the VIDA score in patients with Vitiligo. In contrast, Aulakh et al., 2025,⁶⁰ reported that CXCL9 showed highly significant positive correlations with the VIDA scores. The inverse relationship between CXCL9 and disease activity may reflect chemokine sequestration and increased tissue migration during active disease, leading to reduced circulating levels. This finding is supported by Lin et al., 2021,⁵⁴ who reported higher CXCL9 expression in lesional skin compared with serum, suggesting a significant role in local recruitment of autoreactive T cells during active vitiligo.

ROC curve analysis demonstrated that serum CXCL9 had the strongest association with disease activity in patients with vitiligo; however, this association did not reach statistical significance, indicating moderate discrimination accuracy.

This study was limited by its small sample size and single-center design, which may affect the generalizability of the findings. In addition, the evaluation of peripheral blood markers may not fully reflect local immune changes in vitiligo lesions. Furthermore, larger multicenter studies with longitudinal follow-up are needed to validate these findings. Also, potential confounding factors, particularly smoking status and autoimmune thyroid antibodies, were not evaluated. Future studies should account for these variables to better clarify their potential influence on immune biomarker levels.

In conclusion: Our findings suggest that vitiligo is associated with selective immune dysregulation rather than global immune deficiency. Peripheral lymphocyte populations were preserved; however, patients with vitiligo showed a significant reduction in CD4⁺ T-cell proportions and a decreased CD4⁺/CD8⁺ ratio, indicating cytotoxic immune dominance. Treg cells were significantly reduced, confirming impaired immune tolerance, while their positive correlation with disease activity suggests a compensatory but functionally defective regulatory response. Although serum CXCL9 and soluble CD25 levels in patients with vitiligo were

not significantly different from healthy controls, both showed inverse correlations with disease activity, reflecting altered chemokine trafficking and immune regulatory dynamics. Collectively, these findings highlight immune imbalance as a central mechanism in vitiligo pathogenesis and suggest the rationale for targeted immunomodulatory therapies.

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Author Contributions

WMT performed the study administration and contributed to the conception and design of the study. ERM conducted the clinical evaluation of patients and collected the clinical data. TTH developed the methodology, interpreted the data, and prepared the final manuscript. DTK performed the data analysis and interpretation. JHM conducted the practical work and wrote the original draft of the manuscript. MAF collected the clinical data.

Declaration of Conflicting Interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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Ethical approval

The study was approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University, IRB (17200787), on 14 November 2022.

Informed consent

Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and the study was registered at ClinicalTrials.gov (NCT05569096).

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