Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology,
E-ISSN (2090-2506)
Volume 33 (2), April, 2026
Pages: 33–42.
www.Ejimmunology.org
https://doi.org/10.55133/eji.330204

Evaluation of CD19+ CD24+CD27 B cell and serum level of interleukin 10 and interleukin 35 in patients with systemic lupus erythematosus

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Abstract

Regulatory B cells (Bregs) maintain immune homeostasis through anti-inflammatory cytokine production, yet their role in systemic lupus erythematosus (SLE) remains poorly understood. This study evaluated concentrations of CD19+CD24 high CD27+ regulatory B cell frequencies and serum interleukin-10 (IL-10) and interleukin-35 (IL-35) in 40 SLE patients compared to 25 normal controls. Patients in this cross-sectional observational study were stratified by SLE Disease Activity Index (SLEDAI) scores into Group I: SLE patients with active disease (≥5) and Group II: SLE patients with inactive disease (≤4) disease groups. CD19+CD24highCD27+ B cells were quantified by flow cytometry, while serum IL-10 and IL-35 levels by enzyme-linked immunosorbent assays. Active SLE patients demonstrated significantly reduced CD19+CD24 highCD27+ B cells (23.20×10^6) compared to controls (56.40×10^6, p<0.001). IL-35 levels were markedly decreased in active disease patients (54.04±8.10 pg/ml) versus patients with inactive disease (83.38±20.05 pg/ml) and controls (89.04±10.86 pg/ml, p<0.001). Conversely, IL-10 concentrations were elevated in SLE active patients (4.44±1.27 pg/ml) compared to inactive patients and controls (1.95±0.56 and 2.03±0.61 pg/ml, respectively, p<0.001). Strong correlations were observed between regulatory B cell counts and SLEDAI scores (r=-0.547, p<0.001). In conclusion, active SLE is characterized by impaired regulatory B cell populations and dysregulated cytokine profiles, with reduced IL-35 and elevated IL-10 levels, correlated with disease activity.

Keywords: SLE, Regulatory B cells, IL-10, IL-35, Autoimmunity, Immune Regulation

Date received: 02 December 2025; **accepted:** 02 March 2026

Introduction

Systemic lupus erythematosus (SLE) represents a complex chronic autoimmune disorder characterized by dysregulated autoantibody production and multisystem organ involvement affecting the skin, joints, cardiovascular system, kidneys, and central nervous system.[1] The pathogenesis of SLE involves aberrant B lymphocyte activation and function, which plays a fundamental role in disease initiation and progression.[2] However, emerging evidence suggests that specific B cell subsets possess regulatory properties that may counterbalance the inflammatory processes characteristic of autoimmune diseases.[3]

Regulatory B cells (Bregs) constitute a distinct immunosuppressive population responsible for maintaining immune homeostasis and tolerance.[4] These cells demonstrate therapeutic potential in various autoimmune conditions, including type 1 diabetes mellitus and collagen-induced arthritis, through their capacity to attenuate inflammatory responses.[5, 6]

The immunoregulatory function of Bregs is primarily mediated through the production of anti-inflammatory cytokines, particularly transforming growth factor-beta (TGF-β), interleukin-10 (IL-10), and interleukin-35 (IL-35), which collectively suppress tissue inflammation and promote immune tolerance.[7, 8]

IL-10 functions as a critical immunomodulatory cytokine that directly inhibits T cell activation while suppressing pro-inflammatory cytokine expression in effector T cells, thereby establishing regulatory control within the immune system.[9, 10] Similarly, IL-35 contributes significantly to Breg-mediated immune regulation and inflammation resolution.[11] The functional relationship between IL-10 and IL-35 in Breg biology is particularly noteworthy, as IL-35 appears essential for optimal IL-10+ Breg regulatory activity.[12] Furthermore, IL-35 demonstrates the capacity to induce regulatory phenotypes in both T and B cell populations, highlighting its pivotal role in Breg-mediated immunity.

In human peripheral blood, CD19+IL-10+ Bregs can be phenotypically characterized as CD19+CD24highCD27+ B cells.[13] This surface marker combination has gained widespread acceptance as a reliable identifier for human Bregs in clinical research.[14] Recent investigations demonstrated that reduced frequencies of CD19+CD24highCD27+ B cells correlate with increased susceptibility to autoimmune diseases, including Graves' disease, Crohn's disease, and rheumatoid arthritis.[15-17] Additionally, these cells serve as valuable biomarkers for predicting therapeutic responses to biologic treatments in rheumatoid arthritis patients.[18]

Despite these advances, the relationship between CD19+CD24highCD27+ B cells and associated cytokines in refractory SLE patients remains inadequately characterized.[19] This study aimed to evaluate the frequency of CD19+CD24highCD27+ Bregs and serum concentrations of IL-10 and IL-35 in SLE patients compared to normal controls, while examining correlations between these parameters and clinical manifestations of SLE.

Patients and Methods

This observational analytical cross-sectional study included 40 patients diagnosed with SLE based on SLE International Collaborating Clinics (SLICC) Criteria for SLE 2012,[20] who were recruited from the outpatient clinic, Tanta University Hospitals, Egypt during the period from July 2024 to December 2024. Additionally, 25 normal control subjects, matched for age, sex, and educational level, with no evidence of autoimmune diseases or active infections, were enrolled.

Exclusion criteria included malignancies, immunodeficiency states, cardiac diseases, pregnancy or lactation, use of antihistamines, seropositivity for human immunodeficiency virus or hepatitis C virus, history of hypertension, diabetes mellitus, cardiovascular diseases, impaired hepatic or renal function, and concurrent inflammatory conditions.

Disease activity was assessed using the SLE Disease Activity Index (SLEDAI).[21] Patients were stratified into two groups based on their SLEDAI scores: Group I: included those with scores ≥5, classified as having active disease, while Group II: patients with scores ≤4, categorized as having inactive disease.[22]

All participants underwent comprehensive clinical evaluation including detailed history taking with documentation of age at onset and disease duration, followed by complete physical examination. Laboratory investigations were performed to assess disease parameters, including complete blood count, urinalysis, C-reactive protein, erythrocyte sedimentation rate, liver function tests, serum creatinine, blood urea nitrogen, serum complement levels (C3 and C4), immunoglobulin concentrations (IgG, IgM, and IgA). Data for detection of anti-dsDNA antibodies, and 24-hour urinary protein quantification were recorded from patients' hospital records.

Specific Immunological Analyses

Flow cytometric analysis of peripheral blood samples was performed to determine the proportion of CD19+ CD24high CD27+ Bregs on BD FACSCanto™ II (Flow cytometry machine). The monoclonal immunofluoescent antibodies were used for B-cell phenotyping as follows: CD19-PC7 (PE Cyanine7), CD24-PE (phyco-ertyhrin) and CD27 -FITC (fluorescein isothiocyanate)

Serum concentration of IL-10 and IL-35 were measured using an enzyme-linked immunosorbent assay (ELISA) technique (Abcam SimpleStep ELISA). The assay typically employs a sandwich ELISA method, where a capture antibody is pre-coated on a 96-well plate. Samples were added, followed by a biotin-conjugated detection antibody and horseradish peroxidase-conjugated avidin (HRP-avidin), producing a color change proportional to the IL-10 and IL-35 concentrations. The optical density (O.D.) was measured at 450 nm using a microplate reader.

Statistical Analysis

Data were analyzed using a statistical package for the social sciences (SPSS, version 20, IBM Corp, Armonk, NY, 2011). Categorical variables are presented as numbers and percentages. Chi-square or Fisher's Exact test (if >20% of cells had expected count <5) were used to assess group differences. Monte Carlo correction was also applied in such cases. Continuous data were tested for normality using the Shapiro-Wilk test and expressed as range, mean, standard deviation, median, and interquartile range. Analysis of variance (ANOVA) with Tukey's Post Hoc test was used to compare four groups. Student's t-test or Mann-Whitney test compared to two groups. Kruskal-Wallis with Dunn's test was used to compare multiple non-normally distributed groups. Pearson's coefficient was used to assess correlations. Significance was considered at $p \leq 0.05$.

Results

Active SLE patients (Group I) demonstrated significantly reduced CD24hiCD27+/CD19+ B cells (23.20×10^6) compared to controls (56.40×10^6, $p<0.001$). IL-35 levels were markedly decreased in patients with active disease (54.04±8.10 pg/ml) versus inactive disease patients (83.38±20.05 pg/ml) and controls (89.04±10.86 pg/ml, $p<0.001$). Conversely, IL-10 concentrations were elevated in active SLE patients (4.44±1.27 pg/ml) compared to both inactive patients and controls (1.95±0.56 and 2.03±0.61 pg/ml, respectively, $p<0.001$). Table 1

Table 1. Demographic data and biomarker analysis of the systemic lupus erythematosus (SLE) patients and controls.

		Group I (n = 20)	Group II (n = 20)	Group III (n=25)	*p* value
Sex	Male	3 (15%)	0 (0%)	2 (8%)	NS
	Female	17 (85%)	20 (100%)	23 (92%)	
Age (years)		30.95 ± 9.69	44.75 ± 12.43	36.12 ± 8.87	<0.001
CD24hiCD27+/CD19+ B cells ($\times 10^6$)		23.20 (15.45 – 28.06)	25.37 (15.48 – 35.53)	56.40 (44.92 – 69.45)	<0.001
		*p*1=NS, *p*2>0.001, *p*3>0.001			
Interleukin (IL)-35 (pg/ml)		54.04 ± 8.10	83.38 ± 20.05	89.04 ± 10.86	<0.001
		*p*1>0.001, *p*2>0.001, *p*3=0.579			
IL-10 (pg/ml)		4.44 ± 1.27	1.95 ± 0.56	2.03 ± 0.61	<0.001
		*p*1>0.001, *p*2>0.001, *p*3=NS			

Data are presented as mean ± SD, median (IQR), or frequency (%). *p*1: between I, II, *p*2: between II, III, and *p*3: between I, III. $p > 0.05$ is not significant (NS).

Active SLE patients exhibited extensive organ involvement with increased anti- dsDNA antibodies (90%) and low complement (70%) being predominant laboratory findings. Oral ulcers occurred in 70% of active cases versus 10% in inactive disease. Pulse prednisolone therapy was exclusively administered to active patients (60%), while oral prednisolone predominated in inactive cases (70%). Cyclophosphamide treatment was restricted to active disease (50%), whereas azathioprine was more frequently prescribed for inactive patients (85% versus 35% in active disease, $p<0.001$), Table 2.

Table 2. Clinical manifestations and treatment patterns of the 40 systemic lupus erythematosus (SLE) patients.

		Group I (n = 20)	Group II (n = 20)	*p* value
Disease duration (years)		3 (2 – 5)	4 (2 –7.5)	NS
Glucocorticoids	No	0 (0%)	6 (30%)	<0.001
	Oral Prednisolone	8 (40%)	14 (70%)	
	Pulse Prednisolone	12 (60%)	0 (0%)	
Immunosuppressants	Azathioprine	7 (35%)	17 (85%)	0.001
	Cyclophosphamide	10 (50%)	0 (0%)	
	MMF	3 (15%)	3 (15%)	
Seizers		2 (10%)	0 (0%)	-
Psychosis		1 (5%)	0 (0%)	-
Organic brain syndrome		3 (15%)	0 (0%)	-
Visual disturbance		2 (10%)	0 (0%)	-
Cranial nerve disorders		2 (10%)	0 (0%)	-
Lupus headache		1 (5%)	0 (0%)	-
Cerebrovascular accidents		0 (0%)	0 (0%)	-
Vasculitis		4 (20%)	0 (0%)	-

Table 2. Continued.

	Group I (n = 20)	Group II (n = 20)	*p* value
Arthritis	5 (25%)	0 (0%)	-
Myositis	2 (10%)	0 (0%)	-
Urinary casts	6 (30%)	0 (0%)	-
Hematuria	1 (5%)	0 (0%)	-
Proteinuria	7 (35%)	0 (0%)	-
Pyuria	4 (20%)	0 (0%)	-
Rash	8 (40%)	3 (15%)	-
Alopecia	8 (40%)	1 (5%)	-
Oral ulcers	14 (70%)	2 (10%)	-
Pleurisy	5 (25%)	2 (10%)	-
Pericarditis	4 (20%)	2 (10%)	-
Low complement	14 (70%)	2 (10%)	-
Increased DNA	18 (90%)	2 (10%)	-
Fever	6 (30%)	4 (20%)	-
Thrombocytopenia	2 (10%)	6 (30%)	-
Leukopenia	4 (20%)	4 (20%)	-

MMF: Mycophenolate mofetil, Data are presented as median (IQR), or frequency (%). $p > 0.05$ is not significant (NS).

CD24hiCD27+/CD19+ B cell counts demonstrated strong inverse correlation with SLEDAI scores (r=-0.547, $p<0.001$) and IL-10 levels (r=-0.551, $p<0.001$), indicating reduced regulatory B cell populations in severe SLE disease. Positive correlation was observed with IL-35 concentrations (r=0.456, $p=0.003$), suggesting coordinated anti-inflammatory responses. Age and disease duration showed no significant associations with regulatory B cell numbers, indicating that disease activity rather than chronicity influences these immunoregulatory populations, Table 3.

Table 3. Correlational analysis of regulatory B cells with different study parameters.

SLE patients (n = 40)	CD24hiCD27+/CD19+ B cells (×10^6)	
	Correlation	*p* value
Age (years)	-0.156	NS
Disease duration (years)	-0.101	NS
SLEDAI score	-0.547	<0.001
Interleukin (IL)-35 (pg/ml)	0.456	0.003
IL-10 (pg/ml)	-0.551	<0.001

SLE: Systemic lupus erythematosus; SLEDAI: SLE Disease Activity Index. $p > 0.05$ is not significant (NS).

Immunosuppressive therapy significantly influenced regulatory B cell populations (p=0.011). Patients receiving cyclophosphamide demonstrated the lowest CD24hiCD27+/CD19+ B cell counts (15.45×10^6) followed by Mycophenolate mofetil (MMF) recipients (17.64×10^6). Azathioprine-treated patients maintained higher levels (25.85×10^6). Glucocorticoid administration showed marginal effects, with untreated patients exhibiting the highest regulatory B cell counts (42.89×10^6). Sex differences were not statistically significant, indicating that treatment effects supersede demographic influences, Table 4.

Table 4. Impact of treatment on regulatory B cells in Systemic lupus erythematosus (SLE) patients.

SLE patients (n = 40)		N	CD24hiCD27+/CD19+ B cells ($\times10^6$)	*p* value
Sex	Male	3	21 (2.5 – 30.22)	NS
	Female	37	25.4 (5.1 – 51.7)	
Glucocorticoids	No	6	42.89 (5.1 – 51.7)	NS
	Oral Prednisolone	22	25.42 (5.32 – 40.22)	
	Pulse Prednisolone	12	17.31 (2.5 – 45.96)	
Immunosuppressants	Azathioprine	24	25.85 (5.1 – 51.7)	0.011
	Cyclophosphamide	10	15.45 (2.5 – 25.76)	
	MMF	6	17.64 (5.7 – 45.96)	

MMF: Mycophenolate mofetil; Data are presented as median (IQR). $p > 0.05$ is not significant (NS).

IL-35 and IL-10 exhibited opposing relationships with disease activity. IL-35 demonstrated strong negative correlation with SLEDAI scores (r=-0.876, p<0.001), while IL-10 showed positive correlation (r=0.961, p<0.001). Age was negatively correlated with IL-10 levels (r=-0.355, p=0.025), suggesting age-related modulation of inflammatory responses. Disease duration showed no significant correlations with either cytokine, emphasizing that current disease activity rather than chronicity determines cytokine profiles in SLE patients, Table 5.

Table 5. Correlations of cytokines with parameters of patients with systemic lupus erythematosus (SLE) disease.

SLE patients (n = 40)	Interleukin (IL)-35 (pg/ml)		IL-10 (pg/ml)	
	Correlation	*p* value	Correlation	*p* value
Age (years)	0.279	NS	-0.355	0.025
Disease duration (years)	0.139	NS	-0.147	NS
SLEDAI score	-0.876	<0.001	0.961	<0.001

SLEDAI: SLE Disease Activity Index. $p > 0.05$ is not significant (NS).

Glucocorticoid therapy significantly influenced both cytokines (p<0.001). Patients without glucocorticoids maintained highest IL-35 levels (94.30±13.64 pg/ml) and lowest IL-10 concentrations (1.57±0.61 pg/ml). Pulse prednisolone therapy resulted in lowest IL-35 (51.88±8.60 pg/ml) and highest IL-10 levels (4.96±1.39 pg/ml). Immunosuppressive agents showed similar patterns, with cyclophosphamide producing lowest IL-35 (49.07±3.90 pg/ml) and highest IL-10 concentrations (5.31±1.23 pg/ml), indicating treatment-induced cytokine profile modifications reflecting disease activity modulation, Table 6.

Table 6. Effects of treatment on cytokine expression in the systemic lupus erythematosus disease (SLE) patients.

SLE patients (n = 40)		N	IL-35 (pg/ml)	IL-10 (pg/ml)
Sex	Male	3	51.59 ± 6.94	4.88 ± 1.94
	Female	37	70.10 ± 21.37	3.06 ± 1.51
p value			NS	NS
Glucocorticoids	No	6	94.30 ± 13.64	1.57 ± 0.61
	Oral Prednisolone	22	70.91 ± 19.89	2.68 ± 0.89
	Pulse Prednisolone	12	51.88 ± 8.60	4.96 ± 1.39

IL: Interleukin, Data are presented as mean ± SD. $p > 0.05$ is not significant (NS).

Discussion

SLE represents a complex autoimmune disorder characterized by dysregulated immune responses, where the balance between pro-inflammatory and regulatory mechanisms becomes fundamentally disrupted, leading to persistent inflammation and multi-organ involvement that defines the clinical heterogeneity of this condition.[23]

The observed reduction in $CD19^+CD24^{hi}CD27^+$ Bregs in active SLE patients represents a critical immunoregulatory defect that aligns with established pathophysiological mechanisms in lupus. Our findings demonstrated a significant decrease in these regulatory populations in active disease (23.20×10^6) compared to normal controls (56.40×10^6), corroborating previous investigations by Jin et al., 2013,[19] who reported comparable reductions in Chinese patients with new-onset SLE disease (8.22 ± 3.48% vs. 31.67 ± 5.53% in controls). Similarly, Xiong et al., 2022[24] documented decreased absolute counts of $CD19^+CD24^{hi}CD27^+$ B cells in both active and inactive SLE patients, reinforcing the concept that regulatory B cell depletion constitutes a fundamental immunological aberration in lupus pathogenesis. This regulatory B cell subset, identified by Iwata et al. 2011[13] as IL-10-competent B10 cells representing approximately 5% of blood B cells in healthy individuals, appears to be preferentially affected in autoimmune conditions, with their depletion potentially contributing to the loss of peripheral tolerance characteristic of SLE disease.

The predominance of severe clinical manifestations in active SLE patients, particularly the high frequency of anti-double stranded DNA (dsDNA) antibodies (90%) and hypocomplementemia (70%), reflects the underlying immunological dysregulation associated with regulatory B cell depletion. These clinical patterns align with the inverse correlation between regulatory B cell counts and disease activity scores observed in our cohort, supporting previous findings by Wang et al. 2014[25] who demonstrated negative correlations between $IL\text{-}10^+$ B cells and SLEDAI scores. The differential treatment patterns observed, with cyclophosphamide restriction to active disease and azathioprine predominance in inactive cases, reflect current therapeutic approaches targeting the inflammatory cascade. The study by Nunez et al. 2023[26] demonstrated that B cell depletion therapy resulted in significant reductions in IL-10 levels three months post-infusion, potentially reflecting the elimination of regulatory $CD19^+CD24^{hi}CD27^+$ B cells, which are critical IL-10 producers.

The strong inverse correlation between $CD19^+CD24^{hi}CD27^+$ B cell counts and SLEDAI scores (r=-0.547) demonstrates the direct relationship between regulatory B cell depletion and disease severity, supporting their potential utility as biomarkers for SLE disease monitoring. This relationship corroborates findings by Jin et al. 2013[19] who reported that primary $CD24^{hi}CD27^+CD19^+$ B cells inversely correlated with SLEDAI scores and that patients with arthritis and hematologic disorders exhibited lower regulatory B cell frequencies. The absence of significant correlations with age and disease duration suggests that current immunological status, rather than chronological factors, determines regulatory B cell populations. The study by Salomon et al. 2017[18] demonstrated that higher baseline levels of $CD24^{hi}CD27^{+}$ Bregs were associated with Disease Activity Score 28 remission in rheumatoid arthritis patients, indicating that preserved regulatory B cell populations may predict better therapeutic responses across autoimmune conditions. The positive correlation with IL-35 concentrations (r=0.456) observed in our study suggests coordinated anti-inflammatory mechanisms, as both Bregs and IL-35 contribute to immune suppression through overlapping pathways involving regulatory T cell induction and inflammatory cytokine inhibition.

The differential impact of immunosuppressive therapies on regulatory B cell populations reveals important mechanistic insights into treatment effects beyond simple disease activity suppression. Cyclophosphamide treatment resulted in the lowest $CD19^{+}CD24^{hi}CD27^{+}$ B cell counts (15.45×10^6), followed by mycophenolate mofetil (17.64×10^6), while azathioprine-treated patients maintained relatively higher levels (25.85×10^6). These findings suggested that certain immunosuppressive agents may preferentially affect regulatory B cell subsets, potentially explaining differential clinical responses observed in practice. The study by Blair et al. 2010[27] demonstrated that SLE-derived $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs exhibit impaired regulatory function with reduced IL-10 production, suggesting that quantitative depletion compounds qualitative functional defects in lupus patients.

The marked reduction in IL-35 levels in active SLE patients (54.04±8.10 pg/ml) compared to inactive patients and controls represented a significant anti-inflammatory defect that correlates with disease severity and regulatory B cell depletion. This cytokine deficiency aligns with findings by Xiong et al. 2022[24] who reported decreased IL-35 concentrations in active SLE (60.91 ± 15.81 pg/ml) compared to inactive disease and controls, with particularly low levels in anti-dsDNA positive and nephritis subgroups. The strong negative correlation with SLEDAI scores (r=-0.876) demonstrated that IL-35 is a potential biomarker for SLE disease activity and therapeutic monitoring. The study by Collison et al. 2007[28] identified IL-35 as a novel inhibitory cytokine specifically produced by regulatory T cells and required for maximal suppressive activity, while Dambuza et al. 2017[29] demonstrated that IL-35 converts resting B and T cells into IL-10-producing regulatory populations.

The elevated IL-10 concentrations in active SLE patients (4.44±1.27 pg/ml) compared to inactive patients and controls, despite regulatory B cell depletion, represents a paradoxical finding that reflects functional rather than quantitative cytokine abnormalities in SLE. This elevation corroborates multiple previous studies, including Wang et al. 2017[30] who reported significantly higher serum IL-10 levels in SLE patients with positive correlations to inflammatory markers, and Xiong et al. 2022[24] who demonstrated elevated IL-10 in active disease, particularly in anti-dsDNA positive and nephritis subgroups. The strong positive correlation with SLEDAI scores (r=0.961) suggests that IL-10 elevation may reflect inflammatory compensation rather than effective immunoregulation. The study by Yuan et al. 2011[31] demonstrated that the anti-inflammatory function of IL-10 is impaired in SLE monocytes with long-term immune complex exposure, showing reduced suppressive effects on tumor necrosis factor-alpha (TNF-α) and IL-6 production despite normal IL-10 receptor expression. The study by Cui et al. 2011[32] further supported this concept by demonstrating downregulated IL-10 receptor expression on $CD4^{+}$ T cells from lupus nephritis patients with delayed and reduced STAT-3 phosphorylation, indicating compromised IL-10 signaling pathways.

The differential effects of therapeutic interventions on IL-35 and IL-10 levels demonstrated treatment-specific mechanisms beyond general immunosuppression. Pulse prednisolone therapy resulted in the lowest IL-35 levels and highest IL-10 concentrations, while untreated patients maintained the most favorable cytokine profile with highest IL-35 and lowest IL-10 levels. Cyclophosphamide treatment produced the most pronounced cytokine dysregulation with lowest IL-35 and highest IL-10 concentrations, suggesting that intensive immunosuppression may paradoxically worsen certain immunoregulatory pathways. These findings align with the regulatory B cell depletion patterns observed with different treatments, supporting the concept that therapeutic agents differentially affect various components of the immunoregulatory network. The study by Nie et al. 2021[33] demonstrated that IL-35 treatment suppressed type 2 inflammation-inducing cytokines in allergic rhinitis models, suggesting that IL-35 supplementation might represent a therapeutic approach for restoring anti-inflammatory balance in autoimmune conditions. The age-related negative correlation with IL-10 levels (r=-0.355) suggests that inflammatory responses may be modulated by demographic factors, potentially influencing treatment responses in different patient populations.

The limitation of the current study included the small sample size from a single center, may limit generalizability of the study findings. The cross-sectional design prevents establishing causal relationships between biomarkers and SLE disease activity.

In conclusion, active SLE patients exhibited significantly reduced CD19+CD24hiCD27+ Breg cells and IL-35 levels but elevated IL-10, correlating strongly with SLEDAI scores. Breg frequency inversely links with IL-10 and disease severity, while positively associating with IL-35. Immunosuppressants—notably cyclophosphamide—further deplete Bregs and dysregulate cytokines. These findings underscore Breg/IL-35 deficiency and inconsistent IL-10 elevation as hallmarks of SLE activity, highlighting their biomarker potential for monitoring disease progression and treatment impact.

Author Contributions

All authors contributed to the study conception and design. NSMA, RMAA and HMAA performed material preparation, data collection and analysis. MSE, SAT, EE, EME, BSK, RMA, RSA wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Ethical Committee of Tanta University, Tanta, Egypt.

Informed consent

A written informed consent was obtained from each study participant.

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