Interleukin biomarkers as predictive tools for lupus nephritis grade and disease activity in systemic lupus erythematosus

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Abstract

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple organs, particularly the kidneys. Interleukin (IL) biomarkers including IL-10 and IL17/23 axis play an important role in SLE pathogenesis.

Objectives: To investigate the predictive value of IL-17, IL-23, and IL-10 biomarkers in detecting lupus nephritis (LN) class in SLE cases.

Methods: This is a case-control study involving 160 individuals: 100 patients with SLE (80 LN patients who had a recent report of kidney biopsy in the two months prior to the study +20 non renal SLE patients), and 60 age- and sex-matched healthy volunteers. All participants were subjected to clinical and laboratory studies, as well as the evaluation of their IL-17, IL-23, and IL-10 biomarkers.

Results: IL-17, IL-23, and IL-10 were significantly elevated in SLE patients (p-value < 0.001), especially in cases with high disease activity (p-value < 0.001). Moreover, these biomarkers were considerably higher in LN patients (p-value < 0.001), particularly among class III and IV LN (p-value < 0.001) and in cases with high nephritis activity index (p-value < 0.001). ROC curve analysis revealed precise cutoff points of IL-17, IL-23, and IL-10 levels in each renal histopathological class with high sensitivity and specificity.

Conclusion: IL-17, IL-23, and IL-10 biomarkers are higher in SLE patients and are correlated with SLE Disease Activity Index (SLEDAI). They are more prevalent in individuals with LN, particularly in cases with high activity index and with more aggressive classes (in renal classes III and IV). These biomarkers might function as indicators for detecting LN activity and as predictors of LN class.

Keywords: Systemic Lupus erythematosus; Lupus Nephritis; Interluekin-17, 23, and 10; Disease Activity Index-lupus nephritis histopathological class.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease manifested by a wide range of clinical signs and symptoms[1]. One common and severe symptom of SLE is kidney involvement, notably lupus nephritis (LN). The estimated prevalence of LN is between 35% and 60%, and its clinical manifestation ranges from mild condition to rapidly progressing nephropathy[2,3,4,5]. In spite the advancement in our understanding of LN pathogenesis and the improvement of effective treating plans, only 50%–70% of patients experience remission and LN remains a leading cause of death and disability in SLE cases[6].

The most reliable method for assessing LN, confirming the diagnosis, identifying the kind and extent of kidney tissue damage, as well as the glomerular activity and chronicity indices, is still a kidney biopsy[7,8]. However, this approach is invasive, and it is not practical to perform recurrent biopsies for LN assessing. Therefore, there is an obvious need for new biomarkers that can distinguish between different classes of LN, LN activity grades, and severity indices, predict renal flares, and assess the responses to treatment and the progression of the disease. Certain biomarkers serve as viable alternative, or complementary less invasive methods for managing LN. These biomarkers could be indicators of pathological processes and response to treatment [9,10].

Conventional laboratory biomarkers encompass immunological serology tests such as anti-double-stranded DNA (Anti-dsDNA) and levels of complement, along with renal disease-related parameters regarding proteinuria assessed via 24-hour urine protein excretion or the urine protein/creatinine ratio (uPCR), urinary sediment, and glomerular filtration rate (GFR)[10]. These are the tried-and-true measures for evaluating LN clinically. However, they could not identify the onset of LN flares and its progression, along with restricted sensitivity and specificity to distinguish active disease from chronic organ damage, so, development of management strategies is essential[11, 12].

Th17 cells are a subset of T-cells that developed from CD4+ T-cells under the influence of IL-6, IL-21, and IL-1. Their primary cytokine is IL-17. Other cells, including T-cell receptor- (TCR-) γδ and TCR-αβ double negative (DN) T-cells (CD3 + CD4 + CD8)[13], also generate IL-17. In addition to inducing other cytokines, IL-17 has potent proinflammatory effects[12], encourages the invasion of inflammatory cells, and increases the infiltration T-lymphocytes[14]. It has been shown that IL-17 and B-cell activating factor (BAFF) collaborate to enhance the proliferation B-cell lymphocytes and the production of antibodies[15]. Patients with SLE have higher serum levels of IL-17 along

with increased Th17 cells[16,17]. Furthermore, IL-17 has been found in interstitial infiltrating T-lymphocytes and renal glomeruli[18,19].

The IL-23/IL-17 axis is established when antigen-presenting cells produce IL-23, which stimulates the growth of Th17 cells and is essential for their maintenance[20]. Previous research on LN patients revealed high levels of IL-23 in their blood, and lupus-prone animals showed an increase in T-cells expressing high amounts of both IL-17 and IL-23 receptors[19]. Nephritis in mice has been demonstrated to be induced by IL-23-treated lymphocytes; however, in mouse lupus models, nephritis development is prevented by reducing IL-23-receptor[21].

Monocytes and, lymphocytes produce the cytokine IL-10, which has pleiotropic effects on inflammation and immunological modulation. It improves B-cell development, survival, proliferation, and generation of antibodies; these actions contribute to autoimmune disorders. Although IL-10 is anti-inflammatory cytokine, little is known about its effects in SLE patients[22]. A deeper understanding of cytokines could provide a new therapeutic approaches and the discovery of new biomarkers of disease activity, as well as a greater awareness of LN pathogenesis.

MCP-1, TWEAK, NGAL, and uric acid are among the diagnostic biomarkers that showed a strong ability to differentiate between patients with LN from those without. Furthermore, several cytokines and chemokines, such as cell adhesion molecules (CAMs), IL-10, IL-17, MCP-1, and IP-10, are valuable in monitoring the activity of LN. Patients with active LN have been shown to have elevated MCP-1 urine levels (uMCP-1) that is a chemotactic factor that encourages the migration of leukocyte to the kidney (23,24).

Therefore, we aimed to assess the usefulness of IL-17, IL-23, and IL-10 as predictive tools for detecting LN class, as well as, the association of each cytokine with SLE and LN disease activity.

Materials and Methods

Study design and participants

A case-control study was conducted on 100 SLE patients and 60 age- and sex-matched healthy volunteers. The study was conducted at the outpatient clinics of rheumatology, rehabilitation, and internal medicine, as well as the inpatient rheumatology unit, at Menoufia University Hospital from October 2021 to October 2023. Data was collected simultaneously with patient recruitment. Patients were selected based on consecutive enrollment during their clinic visits. Inclusion criteria were as follows: patients diagnosed with SLE and meeting the Systemic Lupus International Collaborating Clinics (SLICC) criteria for classifying SLE[25] and patients with LN who

had a recent report of kidney biopsy (within two months before the study) (Figure 1). The reporting of this study conforms to STROBE guidelines (26). In contrast, the exclusion criteria were as follows: history of smoking, alcohol drinking, pregnancy, cancer, diabetes, hypertension, endocrine disorders, metabolic syndrome, or cardiovascular disease.

All subjects provide written informed consent, and the study was authorized by the Menoufia University Faculty of Medicine ethical committees under IRB number 42024INTM14.

Clinical and Laboratory Assessment

In this study, the following laboratory investigations were performed on the cases: complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), kidney function tests (serum urea and creatinine), albumin, anti-nuclear antibody (ANA), anti-dsDNA, and protein creatinine ratio.

Moreover, the patients underwent a full clinical examination and history taking. Disease activity was monitored using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2000)[27]. Kidney biopsy reports for LN patients included their histopathology class, the activity and chronicity indices (done by an independent pathologist based on the International Society of Nephrology/Renal Pathology Society classification system)[28,29].

Samples collection and measurements

In total, 7 mL of venous blood was withdrawn from the cubital vein of each participant and then distributed as follows: 1 mL of blood was placed in a citrate tube for the measurement of ESR, and 2 mL of blood was placed in an EDTA tube to determine the CBC using the Sysmex XT-1800i automated hematology analyzer (Sysmex, Japan).

Centrifugation was used to separate the serum from the remaining 4 mL to perform further biochemical examination. The Cobas c501 Auto analyzer (Roche, Germany) was used to assess liver and kidney functions, while the Cobas e601 Auto analyzer (Roche, Germany) was employed to measure CRP. To assess anti-dsDNA antibodies, we utilized the Algeria Automated Analyzer and an ELISA kit (Catalog #MBS269122). The threshold for positive anti-dsDNA antibody results was established at 30 IU/mL. ANA testing was performed using indirect immunofluorescence (IIF), with a cutoff value of a titer of 1:160 or greater.

Additionally, we employed a BioTekELx800 UV-Vis microplate reader (Biotek Instruments, USA) following the manufacturer's guidelines to analyze cytokines IL-17A, IL-23, and IL-10 using ELISA kits from the International Trade Company, Shanghai, China. The catalog numbers for the specific cytokines were Catalog#201-12-0048, Catalog# SEA384Hu, and Catalog# RD 194572200 R. Each result was expressed as pg/mL ± standard deviation (SD).

Urine samples were collected in sterile containers and analyzed using a Beckman Instruments Synchron CX 9 ALX analyzer (Fullerton, CA, USA) to determine the albumin-to-creatinine ratio (ACR) along with urine creatinine and albumin levels[30].

Sample size

A cluster sample included all patients meeting inclusion criteria, collected from outpatient clinics of rheumatology, rehabilitation, and internal medicine departments, as well as the inpatient rheumatology unit at the Faculty of Medicine, Menoufia University, during the period from October 2021 to October 2023; additionally, almost half of the patients' number was added as a control group.

Statistical analysis

SPSS (Statistical Package for Social Sciences), version 20, was used to tabulate and evaluate the data that had been reported (SPSS Inc., Chicago, IL, USA). Mean ± standard deviation (SD) and range were used to characterize quantitative data, while percentages and numbers were employed to represent categorical data. Two sets of quantitative data that were normally distributed were compared using the Student *t*-test, while the Mann–Whitney *U* test was for non-normally distributed data. Moreover, Kruskal–Wallis test was utilized to compare quantitative data in more than two subgroups of non-normally distributed data. The receiver operating characteristics (ROC) curve was utilized to evaluate the sensitivity and specificity of the investigated markers for predicting a given event. A value of ≤0.05 was deemed significant for the *P* value[31].

Results

The present study revealed no statistically significant differences in age or sex between the SLE patients and controls. Concurrently, 70% of SLE patients were females. The study participants' mean age was 36.6 ± 7.25 years for cases and 34.73 ± 6.47 years for controls. Patients with SLE had average disease duration of 8.84 ± 3.75 years. Compared to controls, SLE patients had significantly higher blood levels of IL-17, IL-23, and IL-10 (Table I).

In total, 85% of SLE cases were receiving steroids and 40% were on hydroxychloroquine. On the other hand, 30% were on mycophenolate mofetil, 46% on azathioprine, and 17% on cyclophosphamide.

The descriptive statistics of the laboratory data related to ESR, CRP, CBC (HB, WBCs, and platelets) among the SLE cases under study are displayed in Table II. Mean serum levels of creatinine urea, and albumin were 0.96 ± 0.30, 39.60 ± 13.98, and 33.82 ± 6.95, respectively.

The mean anti-dsDNA was 221.85 ± 171.10, and 96% of cases showed ANA positivity. In terms of SLEDAI, about 16% of the cases had mild disease activity, 47% had moderate disease activity, 28% had severe disease activity, and 9% had very severe disease activity.

In this study, 80% of the SLE cases had LN. Based on the renal histopathological classes found in the renal biopsy results, the patients were categorized as follows: 10% of the cases had class I nephropathy, 31.2% had class II, 47.5% had class III, and 11.2% had class IV. Additionally, approximately 51.2% of patients with LN had a high activity index (12-24), whereas 48.8% had a low (0-5)/moderate (6-11) activity index (Table II). Compared to SLE patients with moderate disease activity, individuals with high disease activity had significantly higher serum levels of IL-17, IL-23, and IL-10 biomarkers (Table III).

The current study's results showed that blood levels of the biomarkers IL-17, IL-23, and IL-10 increased significantly at more aggressive classes of LN. Class IV demonstrated the highest values, whereas class I showed the lowest (Table III).

According to the current study, serum urea, creatinine, albumin, ANA, anti-dsDNA titer, and the biomarkers IL-17, IL-23, and IL-10 were significantly elevated in cases of LN compared to non-LN cases. Additionally, renal patients exhibiting a high renal disease activity index displayed higher levels of these markers compared to those with moderate-to-low renal activity index (Tables IV and V).

Figure 2 (A, B, C, and D) illustrates the effectiveness of IL-17, IL-23, and IL-10 at varying levels in the prediction of LN histopathology classes. Specifically, at a cutoff point of 57.5, IL-17 serum level was found to be able to distinguish between cases of LN (class I) and non-nephritis cases with a sensitivity of 88.8% and a specificity of 65%. In contrast, the cutoff points for class II, III, and IV nephritis were 60.5, 85.5, and 98.5, exhibiting sensitivity and specificity of 88% and 75, 89.5% and 76%, and 77.8% and 84.2%, respectively.

The blood level of IL-23 demonstrated that, at a cutoff point of 142, it could differentiate between cases of LN (class I) and non- nephritis cases with a sensitivity of 85% and a specificity of 100%. For class II, the required value must exceed 146.5, indicating a sensitivity of 80% and a specificity of 75%. Class III was anticipated at a cutoff value of 205, with a sensitivity of 76.3% and a specificity of 92%. At the threshold above 267.5, class IV was predicted with a sensitivity of 77.8% and a specificity of 97.4% (Table VI).

Finally, in cases of LN, the serum concentration of IL-10 may signify various types of renal impairment: A value ranging from 18.5% to 20.5% may indicate class I, with 86.3.8% sensitivity and 75% specificity. Values ranging from 20.5 to 26.5 exhibit a sensitivity of 68% and a specificity of 75% for class II prediction. The ≥26.5–<33.5 values provide a sensitivity of 81.6% and a

specificity of 68% for predicting class III, whereas ≥33.5values demonstrate 100% sensitivity and 92.1% specificity for identifying class IV **(Table VI).**

Discussion

Numerous interleukins have a role in the pathophysiology of LN, and may serve as therapeutic targets for renal involvement in lupus patients[1-3]. In light of renal histopathology being the gold standard for determining the classes of LN, our goal was to investigate the usefulness of IL-17, IL-23, and IL-10 biomarkers as predictors for detecting LN class and their relationship with SLE and LN disease activity.

Prior studies documented elevated IL-17, IL-23, and IL-10 levels in SLE patients and their relationship with disease activity, renal involvement, LN activity, and renal histopathology[32-41]. However, none of these studies identified the exact cutoff points that can predict LN class. This study is the first to report precise cutoff points for IL-10, IL-17, and IL-23 at which we could predict LN class, so we suggest using them as a noninvasive substitute for kidney biopsy, particularly for individuals unable to undergo the procedure.

The current investigation found a statistically significant difference in the IL-17, IL-23, and IL-10 levels between the cases and controls; cases with high versus low disease activity regarding SLEDAI; cases with LN versus those without LN; and patients with nephritis who had a high renal activity index versus those who had a moderate-to-low renal activity index.

According other researches, SLE patients had higher levels of IL-17, IL-23, and IL-10 biomarkers than controls, and this is consistent with our findings. Additionally, previous studies revealed that Th17 cells and IL-17 biomarkers are implicated in several SLE pathological pathways, including autoantibodies synthesis, B-cells activation, the induction of vascular inflammation, and leukocytes recruiting, all of which contribute to the inflammation persistence and glomerular damage[32,33,36-38,40,41].

Other studies suggested that the autoantibodies synthesis by activated B-cells triggers the secretion of IL-23 by dendritic cells, inducing the formation of more IL-17. Moreover, IL-10 has been observed to inhibit the activation of T-cells and macrophages and the production of proinflammatory cytokines on immune cells in vitro. Furthermore, IL-10 promotes B-cell survival, proliferation, differentiation, and antibody production[10] and reduces autoreactive B-cell apoptosis by up regulating Bcl-2 expression, leading to enhanced production of autoantibodies in SLE[35,42-44].

Other results that corroborated our findings demonstrated a significant positive relationship between IL-17 levels and SLEDAI scores. They also, demonstrated that the elevated serum IL-10 levels were found to be considerably associated with the high SLEDAI scores[41, 45].

Consistent with our findings, additional investigations revealed that peripheral blood mononuclear cells have been shown to upregulate IL-23, leukocytes co-stimulated with IL-23 in patients with LN produce IL-17A, and IL-17 is included in the onset and progressing of kidney disease. Furthermore, IL-17, IL-23, and IL-10 levels were elevated in patients with high LN activity, suggesting that these markers may be useful biomarkers in detecting LN activity[44, 46-48]. Moreover, Dedong *et al.*[48] indicated that IL-17 and IL-23 may serve as alternative biomarkers for the diagnosis of LN, evaluating its activity, and assessing its response to treatment.

The current study demonstrated a significant statistical difference between blood levels of IL-17, IL-23, and IL-10 in different renal histopathological classes at distinct cutoff values for each class of LN (from class I to class IV, as there were no cases of class V nephritis). According to Chen *et al.*[45] and Santa Cruz *et al.*[49], who support our findings, patients with more progressive class of LN (class IV and V LN) reported a higher percentage of Th17 cells in the circulation. Moreover, there is strong evidence supporting the role of the IL-17/23 axis in the pathophysiology of LN, with both cytokines serving as predicting biomarkers of LN disease activity and progression. In contrast, Zeid *et al.*[22] discovered insignificant differences in the blood level of IL10 between the various clinical groups.

Zickert and colleagues[50] reported that patients with chronic active nephritis (classes III, IV, V) had greater levels of IL-17. While, those who did not see improvement had higher IL-23 levels. They concluded that individuals with LN who exhibited no improvement in BILAG (The British Isles Lupus Assessment Group) had greater IL-23 levels, indicating that a subset of these patients possessed a Th17 phenotype, which may influence their treatment response and serve as a biomarker for inadequate therapeutic efficacy[50]. They also concluded that a high baseline IL-17 predicted an unfavorable histopathological response.

The Van Vollenhoven trial, which evaluated the effectiveness of ustekinumab (Interleukin 17 inhibitor) in patients with SLE, demonstrated significant clinical improvements for patients treated with ustekinumab in the first two phases of the trial compared with the placebo group. While a phase III study did not report enough evidence to support the continued use of ustekinumab as a treatment for lupus[51].

While, Cesaroni *et al.,* study suggested that the effectiveness of ustekinumab in treating SLE patients depends on the blockage of IL-12 that mainly affects the mechanism of action of ustekinumab[52].

On another hand in a case report described by Dai et al that studied the effect of secukinumab (IL-17A inhibitor) in a patient diagnosed with psoriasis and systemic lupus erythematous, significant improvement in all clinical symptoms related to joint pain, stiffness, dorsal erythema, skin manifestations and laboratory measurements of SLE, and psoriasis was reported after treatment with secukinumab[53].

Other studies have documented that IL-23 blockers are a significant therapeutic target for managing SLE patients, as IL-23 is responsible for the basic features of lupus, involving the growth of dendritic T cells, decreased IL-2 levels, and enhanced IL-17 formation[54,46].

Based on our findings, we recommend further clinical studies to consider the IL-17/23 axis and IL-10 blockers as a treatment option for SLE and LN. Furthermore, it is important to study the protective effects of IL-17/23 inhibitors in preventing the onset of LN in SLE patients and in halting the progression of early-stage LN.

Limitations

This article lacks an evaluation of the effect of medications on outcomes, which is a limitation of this study.

Conclusion

IL-17, IL-23, and IL-10 biomarkers are higher in SLE patients and are correlated with SLE disease activity (SLEDAI). They are more prevalent in individuals with LN, particularly in cases with high activity index and with more aggressive classes of LN (in renal classes III and IV). They also might function as biomarkers for detecting LN activity and as predictors of LN class. These biomarkers may serve as a sensitive and accurate alternative to renal biopsy, especially in cases where this invasive procedure is impractical.

Tables and Figures

Table I – Demographic data, IL-17, IL-23, and IL-10 levels among participants of the study groups.

	Cases **N = 100**	**Control** **N = 60**	Test	P value
Age (years) Mean ± SD Range	 36.6 ± 7.25 25–54	 34.73 ± 6.47 22–47	*t*-test 1.69	 0.09
Gender Male Female	 30 (30.0) 70 (70.0)	 14 (23.3) 36 (76.7)	X^2 0.84	 0.36
Disease duration (years)	8.84 ± 3.75 2–18			
IL 17 (pg/mL) Mean ± SD Range	 75.16 ± 22.76 15–105	 24.27 ± 8.72 12–45	U 9.75	 <0.001
IL-23 (pg/mL) Mean ± SD Range	 187.26 ± 56.47 88–290	 26.87 ± 8.20 2–39	*t*-test 27.92	 <0.001
IL-10 (pg/mL) Mean ± SD Range	 25.13 ± 9.52 4–45	 3.96 ± 1.11 2–6	U 10.39	 <0.001

X^2 = chi-square; U = Mann–Whitney *U* test; IL = interleukin; SD= standard deviation; pg/mL = picogram/milliliter.

Table II - Descriptive statistics of the laboratory data among the studied cases.

	The studied systemic lupus cases **N = 100**
ESR (mm/hr)	56.66±22.06 20 – 98
CRP (mg/dL)	27.53±16.12 4 – 70
Hb (mg/dL)	11.20±1.27 7.5 – 13
WBCs (cells/mL)	5246.0±1817.0 2000 – 9200
Platelets (cells/mL)	279.69±68.99 109 – 410
Urinary protein/creatinine ratio (uPCR) g/mg	1.20±0.82 0.1 – 3.3
Serum creatinine (mg/dL)	0.96±0.30 0.5 – 1.5
Serum urea (mg/dL)	39.60±13.98 20 – 69
Anti-dsDNA (U/mL)	221.85±171.10 15 – 540
ANA **+ ve** **- ve**	 96 (96%) 4 (4%)
Serum albumin (g/l)	33.82±6.95 20 – 54
SLEDAI	

Mild (1–5)	16 (16.0)
Moderate (6–10)	47 (47.0)
Severe (11–19)	28 (28.0)
Very severe (≥20)	9 (9.0)
Renal histopathology	N = 80
Class I	8 (10.0%)
Class II	25 (31.2%)
Class III	38 (47.5%)
Class IV	9 (11.2%)
Renal tissue activity index	N = 80
High activity (12-24)	41 (51.2)
Low(0-5)/moderate activity (6-11)	39 (48.8)

ESR = Erythrocyte sedimentation rate; CRP = C-reactive protein; HB = hemoglobin, WBCS = white blood cells; ANA = anti-nuclear antibody, Anti-dsDNA = anti-double-stranded deoxyribonucleic acid; SLEDAI = SLE Disease Activity Index; uPCR = urinary protein/creatinine ratio; U/mL = unit/milliliter; g/l= gram/liter.

Table III - Serum levels of IL-17, IL-23, and IL-10 in relation to disease activity (SLEDAI) and among different renal histopathology classes.

	SLEDAI				K test	P value
	Mild (1–5) N = 16	**Moderate (6–10) N = 47**	**Severe (11–19) N = 28**	**Very severe (≥20) N = 9**		
IL-17 (pg/mL)						
Mean ± SD	42.88 ± 22.02	73.53 ± 16.12	88.86 ± 13.52	98.44 ± 5.05	53.24	<0.001
Range	15–80	34–97	37–102	90–105		
IL-23 (pg/mL)						
Mean ± SD	138.75 ± 37.72	167.55 ± 47.99	223.0 ± 36.42	265.22 ± 27.75	44.01	<0.001
Range	88–210	88–265	110–279	210–290		
IL-10 (pg/mL)						

Mean ± SD Range	17.44 ± 8.37 4–29	23.02 ± 7.31 9–35	28.39 ± 7.87 9–44	39.67± 7.43 27–45	29.18	<0.001
	Renal histopathology (biopsy)				K test	P value
	Class I **N = 8**	**Class II** **N = 25**	**Class III** **N = 38**	**Class IV** **N = 9**		
IL-17 (pg/mL) Mean ± SD Range	49.5 ± 21.57 19–89	73.88 ± 13.12 37–90	90.74 ± 6.96 69–100	98.5 ± 5.15 90–105	46.42	<0.001
IL-23 (pg/mL) Mean ± SD Range	137.13 ± 16.90 110–170	178.32 ± 27.29 127–210	223.18 ± 37.58 140–269	264.0 ± 34.14 200–290	43.68	<0.001
IL-10 (pg/mL) Mean ± SD Range	17.50 ± 4.81 7–22	23.24 ± 5.96 12–33	29.31 ± 5.0 16 - 43	42.33 ± 3.67 34–45	43.4	<0.001

K test = Kruskal–Wallis test; SLEDAI = SLE Disease Activity Index; IL = interleukin; SD = standard deviation; pg/mL = picogram/milliliter

Table IV - Comparison between SLE cases with and without lupus nephritis regarding laboratory-measured parameters.

	Systemic lupus nephritis among cases			
	Present **N = 80**	**Absent** **N = 20**	Test	P value
Urinary protein/creatinine ratio (uPCR) g/mg Mean ± SD Range	1.46 ± 0.73 0.2–3.3	0.20 ± 0.10 0.1–0.6	U 6.81	<0.001
Serum creatinine (**mg/dL**)			*t*-test	

Mean ± SD Range	1.03 ± 0.28 0.5–1.5	0.68 ± 0.15 0.5–1	5.57	<0.001
Serum urea (**mg/dL**) Mean ± SD Range	 42.28 ± 13.99 20–69	 28.88 ± 7.27 20–48	t-test 5.94	 <0.001
Albumin g/l Mean ± SD Range	 33.13 ± 6.58 20–45	 36.60 ± 7.86 22–54	t-test 2.03	 0.045
Anti-dsDNA (**U/ml**) Mean ± SD Range	 266.06 ± 161.91 19–540	 45.0 ± 78.89 15–170	U 5.52	 <0.001
ANA +ve **-ve**	 80(100%) 0(0%)	 16(80%) 4(20%)	X^2 16.67	 <0.001
IL 17 (pg/mL**)** Mean ± SD Range	 82.22 ± 17.74 19–105	 46.90 ± 18.46 15–78	U 5.83	 <0.001
IL 23 (pg/mL**)** Mean ± SD Range	 205.15 ± 47.73 110–290	 115.70 ± 20.06 88–140	*t*-test 12.83	 <0.001
IL 10 (pg/mL**)** Mean ± SD Range	 27.70 ± 8.27 7–45	 14.85 ± 7.0 4–29	U 5.25	 <0.001

ANA = anti-nuclear antibody; Anti-dsDNA = anti-double-stranded deoxyribonucleic acid; IL = interleukin; uPCR = urinary protein/creatinine ratio; SD = standard deviation; pg/mL **=** picogram/milliliter**;** U/mL = unit/milliliter.

Table 5 - Comparison between cases with high renal disease activity and cases with low/moderate renal disease activity regarding the measured parameters.

	Disease activity among cases		Test	P value
	High activity (12-24) **N = 41**	**Low (0-5)/moderate activity(6-11)** **N = 39**		
Urinary Protein/creatin ratio (uPCR) g/mg Mean ± SD Range	1.94 ± 0.65 0.2 – 3.3	0.94 ± 0.35 0.4–2.1	U 6.39	<0.001*
Serum creatinine (**mg/dL**) Mean ± SD Range	1.07 ± 0.29 0.5–1.5	0.98 ± 0.26 0.5–1.5	*t*-test 1.52	0.13
Serum urea (**mg/dL**) Mean ± SD Range	46.86 ± 14.79 20–69	37.46 ± 11.41 20–66	*t*-test 3.17	0.002*
Albumin Mean ± SD Range	32.94 ± 6.84 20–44	33.33 ± 6.38 20–45	0.27	0.79
Anti-dsDNA Mean ± SD Range	324.58 ± 150.48 22–540	204.54 ± 151.93 19–540	U 3.31	0.001*
IL-17 (pg/mL) Mean ± SD Range	93.68 ± 5.95 77–105	70.18 ± 18.06 19–90	*t*-test 7.73	<0.001*
IL-23 (pg/mL) Mean ± SD	234.51 ± 40.01	174.28 ± 33.82	*t*-test 7.25	<0.001*

Range	115–290	110–240		
IL-10 (pg/mL) Mean ± SD Range	 31.15 ± 7.43 16 - 45	 24.08 ± 7.61 7–44	*t*-test 4.21	 <0.001*
Renal histopathology Class I Class II Class III Class IV	 0 (0.0) 3 (7.3) 30 (73.2) 8 (19.5)	 8 (20.5) 22 (56.4) 8 (20.5) 1 (2.6)	X^2 40.6	 <0.001*

The quantitative data was described as mean ± SD and range. ANA = anti-nuclear antibody; Anti-dsDNA= anti-double-stranded deoxyribonucleic acid; pg/mL = picogram/milliliter.

Table VI - The validity of IL-17, IL-23, and IL-10 for prediction of classes of renal histopathology.

	AUC	P-value	Cut-off point	Sensitivity	Specificity
Differentiate between patients with nephritis and those without					
IL-17 (pg/mL)	0.92	<0.00	57.5	88.8%	65%
IL-23 (pg/mL)	0.94	<0.001	142	85.0%	100%
IL-10 (pg/mL)	0.88	<0.001	18.5	86.3%	75%
Prediction of class II					
IL-17 (pg/mL)	0.83	0.006	60.5	88%	75.0%
IL-23 (pg/mL)	0.87	0.002	146.5	80%	75%
IL-10 (pg/mL)	0.77	0.02	20.5	68%	75%
Prediction of class III					
IL-17 (pg/mL)	0.86	<0.001	85.5	89.5%	76%
IL-23 (pg/mL)	0.85	<0.001	205	76.3%	92%

IL-10 (pg/mL)	0.78	<0.001	26.5	81.6%	68.0
Prediction of class IV					
IL-17 (pg/mL)	0.86	0.001	98.5	77.8%	84.2%
IL-23 (pg/mL)	0.83	0.002	267.5	77.8%	97.4%
IL-10 (pg/mL)	0.98	<0.001	33.5	100%	92.1%

IL = interleukin; AUC = area under the curve; pg/mL = picogram/milliliter.

Figure 1 – Flow diagram of study participants throughout the study.

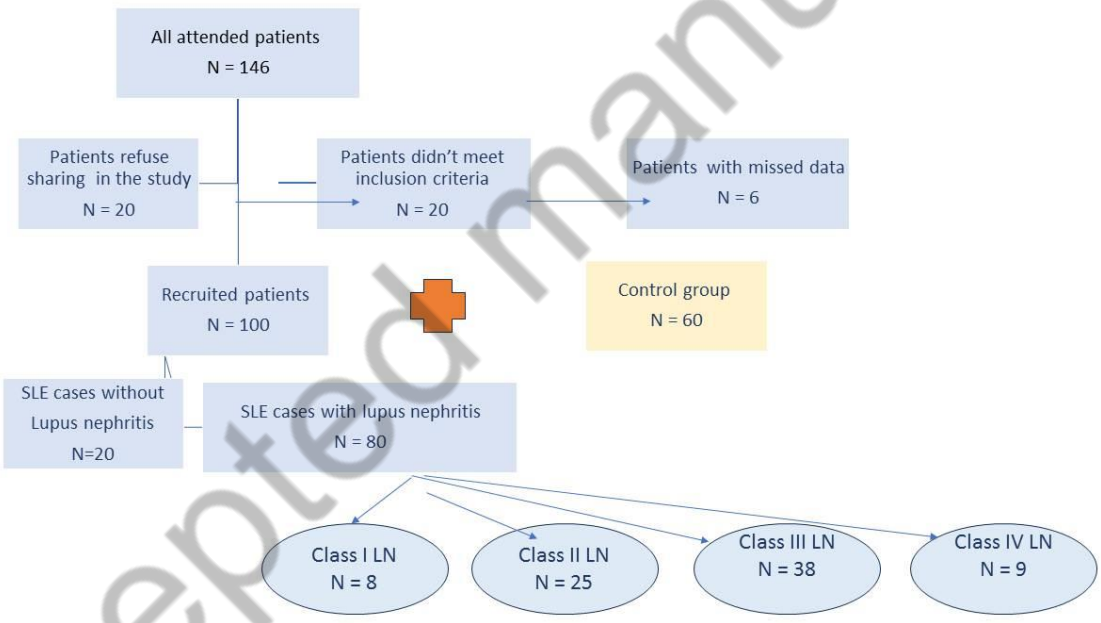
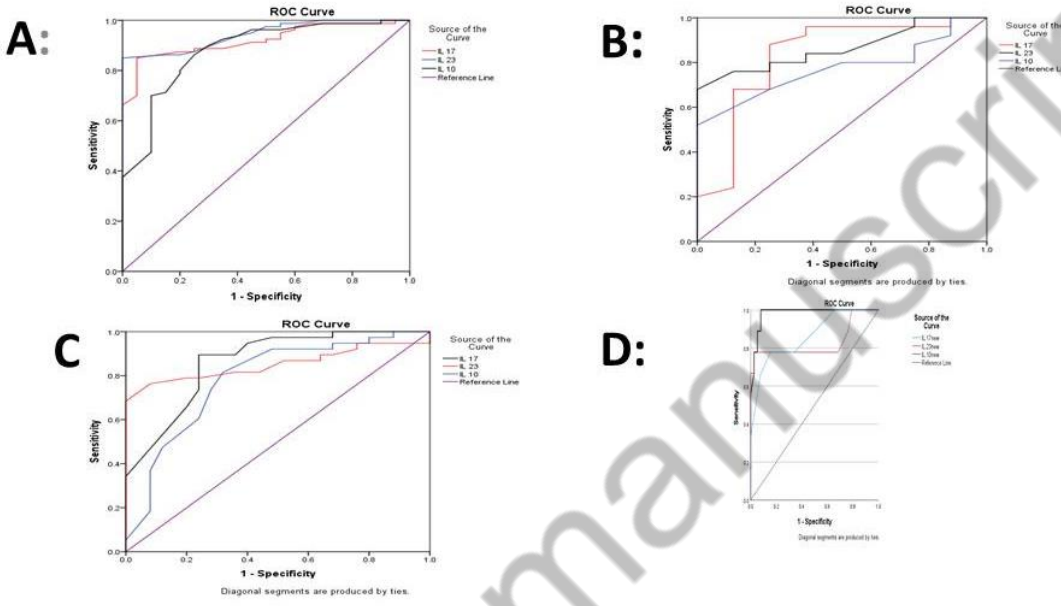


Figure 2 - A. ROC curve analysis of interleukins (IL-17, IL-23, IL-10) for prediction of systemic lupus nephritis from systemic lupus without nephritis. **B.** ROC curve analysis of interleukin (IL-17, IL-23, IL-10) for prediction of class II renal affection. **C.** ROC curve analysis of interleukin (IL-17, IL-23, IL-10) for prediction of class III renal affection. **D.** ROC curve analysis of interleukin (IL-17, IL-23, IL-10) for prediction of class IV renal affection.



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