Al-Azhar International Medical Journal

Volume 5 | Issue 6 Article 54

7-1-2024
Section: Internal Medicine

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How to Cite This Article

Farahat, Mohamed Nabil Raafat; Hassan, Mohamed Hassan Attia; Assem, Ahmed Ali; and El Nasherty, Moataz Mohamed Ahmed (2024) "Serum Interleukin-35 as a Non-Invasive Biomarker in Lupus Nephritis Patients," *Al-Azhar International Medical Journal*: Vol. 5: Iss. 6, Article 54.
DOI: https://doi.org/10.58675/2682-339X.2514

ORIGINAL ARTICLE

Serum Interleukin-35 as a Non-Invasive Biomarker in Lupus Nephritis Patients

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Abstract

Background: ***As one of the most significant organ manifestations of systemic lupus erythematosus (SLE), lupus nephritis (LN) is classified as a subtype of glomerulonephritis.***

Aim and objectives: ***To assess serum IL-35 levels as a non-invasive marker in SLE cases without nephritis.***

Patients and methods: ***Using a random systematic method, this cross-sectional research was done on 80 cases selected from attendees of internal medicine clinics at Al Azhar University Hospitals. Cases were separated into three groups: Group (A): [Active group] 30 cases with current onset active LN, Group (B): [Inactive group] 30 SLE cases without renal disease and Group (C): [Control group] 20 patients of healthy matched age & sex as the control group.***

Results: ***There was statistically significant variance amongst (Group A and group C) and among (Group B and group C) concerning serum IL-35. There was a statistically significant difference between the active LN group and SLE patients without renal disease group regarding all renal biomarkers.***

Conclusion: ***IL-35 can be a biomarker for renal involvement in patients with LN. IL-35 expression was inhibited either directly or indirectly by a mechanism at some point throughout the progression of LN. Therefore, IL-35 exerts a beneficial influence on LN.***

Keywords: Serum Interleukin-35; Lupus Nephritis; SLE

1. Introduction

Systemic lupus erythematosus (SLE) is a classic autoimmune disorder where the immune system produces antibodies against the cell nucleus, leading to a wide range of clinical symptoms. The main pathology observations in individuals with SLE consist of inflammation, vasculitis, deposition of immunological complexes, and vasculopathy. The precise cause of SLE is not yet understood.[1]

As a form of glomerulonephritis, lupus nephritis (LN) is among SLE's most serious organ manifestations. The histological classification of LN into six distinct categories reflects the range of signs and degrees of renal involvement in SLE. The majority of people diagnosed with SLE who acquire LN typically do so over five years. Nevertheless, it is common for LN to manifest at later stages. Often, LN serves as the initial indication that leads to the diagnosis of SLE.[2]

LN is commonly managed by immunosuppressive medications involving glucocorticoids, mycophenolate mofetil (MMF), or cyclophosphamide.[3]

Nevertheless, traditional immunosuppressive therapies may not consistently provide positive outcomes, and among cases who do react, 35% may experience a recurrence. Additionally, 5–20 per cent of individuals with LN develop ESKD within ten years after the first occurrence, and drug-induced toxicity remains a worry. Undoubtedly, the timely and precise identification of LN and immediate commencement of treatment are crucial to avert the advancement of the illness.[4,5]

Interleukin-35 (IL-35) is a newly revealed member of the IL-12 family and is believed to significantly impact the control of the immunological response and the development of autoimmune diseases.[6]

Accepted 21 June 2024.
Available online 31 June 2024

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https://doi.org/10.58675/2682-339X.2514

IL-35 comprises IL-12A (p35) and Epstein-Barr virus induced 3 (EBI3). It has two known biological functions: inhibiting the proliferation of T cells and transforming naive T cells into a population of induced Treg cells that produce IL-35, known as iTr35.[7,8]

This work aimed to measure serum IL-35 levels as a noninvasive marker in patients with and without nephritis who have SLE.

2. Patients and methods

This cross-sectional research was done on 80 cases selected from attendees of internal medicine clinics at Al Azhar University Hospitals using a random systematic method. Cases were separated into three groups: Group (A): [Active group] 30 cases with current onset active LN, Group (B): [Inactive group] 30 SLE patients without renal disease and Group (C): [Control group] 20 patients of healthy matched Age & gender as control group. In addition to obtaining written informed permission, the protocol for the research was approved by the local ethics commission.

Sample Size: This study draws upon research done by He et al.,[9]. The sample size was calculated utilizing Epi Info STATCALC, taking into account the following assumptions: - A confidence level of 95% is utilized, with a power of 80% for both sides. The estimated odds ratio has an error margin of 5%, resulting in a value of 1.115. The ultimate maximum sample size extracted from the Epi-Info output was 49. Consequently, the sample size was augmented to 80 cases to account for potential dropouts throughout the follow-up period.

Inclusion criteria: The enrolled patients must be at least 18 years old and have autoantibody-positive SLE, as shown by antinuclear antibody titers of at least 1:80, the presence of anti-double-stranded DNA antibodies, or both. Additionally, they needed to meet the categorization criteria for SLE set by the American College of Rheumatology. LN cases were characterized by chronic proteinuria (≥ 0.5 g/24 h), cellular casts or persistent hematuria, or by biopsy.

Exclusion criteria: People with certain autoimmune or rheumatic disorders, kidney illnesses other than LN, infections, or any other co-morbidities of malignant tumours.

Method:

All patients underwent Complete history taking, physical examinations, and investigational studies.

The glomerular filtration rate was calculated utilizing the Cockcroft-Gault equations (eGFR) based on serum creatinine levels. The Cockcroft-Gault equation measured the glomerular filtration rate (eGFR) concerning serum creatinine levels. The equation is named after the researchers who developed it: Cockcroft and Gault. The formula is as follows:

Ccr=72×Scr(mg/100mL)/ (140-age) (wtkg)

eGFR (mL/min) = (140 - age) x (weight in kg) x (0.85 if female) / (72 x serum creatinine in mg/dL)

In this equation, Age refers to the patient's Age in years, and Weight is the Weight of the patient in kilograms. If the patient is female, you multiply by 0.85 to account for differences in muscle mass between males and females. Serum creatinine is the level of creatinine in the blood, measured in milligrams per deciliter (mg/dL).

Assessment of lupus nephritis:

LN was assessed by proteinuria (≥ 0.5 g/24 h) or hematuria in the presence of cellular cast or by biopsy.

Disease activity assessment: The measurement of SLE disease activity was done by the systemic lupus erythematosus (SLEDAI-2k) Gladman et al.[10] were obtained throughout diagnosis visits or hospital records. When the SLEDAI-2k score is more than 4, it is considered active SLE. It concerns the renal affection components documented in the SLEDAI-2k index and the ACR renal criteria.

Serum IL-35 estimation by ELISA technique:

Assay type: Sandwich-ELISA

Devise: chem well Elisa fully automation

Blood samples: Each patient had 10 millilitres of peripheral venous blood drawn into plain tubes between 8 and 9 in the morning following a 12-hour fast. Then, the samples were centrifuged, and the serum was separated and stored at -20 °C until tested. We prepared Standards and Samples by creating a known concentration stock solution of IL-35 standard, making a series of dilutions for the standard curve, and diluting serum samples accordingly. Then, we added IL-35 standards and serum samples to a pre-coated microliter plate. Samples were incubated to a specific temperature to allow IL-35 binding "Incubation" [1]. Then, we removed the unbound material and washed the plate to remove non-specifically bound substances "Washing" [2]. Then, an enzyme-conjugated IL-35 detection antibody was added to the plate and incubated to allow binding "Detection" [3]. Then, the plate was washed again to remove the unbounded detection antibody. Then, we added a substrate solution for a colourimetric reaction and incubated it in the dark "Substrate Reaction" [4]. Then, we used a microplate reader to measure the absorbance at an appropriate wavelength (450 ±10nm) and to create a standard curve based on IL-35 standard absorbance" Measurement" [5]. Finally, IL-35 concentrations in serum samples were interpolated using the standard curve, and the final concentrations were calculated based on dilution factors. "Data Analysis" [6].

Ethical Consideration

There was strict confidentiality about the information that was gathered from the

participants. Any report or publication that pertains to this study did not include any identifying information on the individuals who participated in the research. The participants in this research were explained the risk-benefit analysis, besides the goal and definition of the research, before they were allowed to participate. We were successful in obtaining an informed consent.

3. Results

There was no significant variance in age and sex amongst the Active LN group & SLE cases without renal disease

Table 1. Comparison between Active lupus group and SLE patients without renal disease regarding Demographic Data and patient's evaluations

	ACTIVE LUPUS NEPHRITIS (N = 30)	SLE PATIENTS WITHOUT RENAL DISEASE (N = 30)	P. VALUE
AGE (YEARS)	34.8 ± 5.97	34.03 ± 5.82	0.62237
MEDIAN (RANGE)	32 (28-45)	32.5 (27-45)	
SEX			
• FEMALE	27 (90%)	26 (86.67%)	0.69361
• MALE	3 (10%)	4 (13.33%)	

There was statistically significant variance amongst active LN & controls as regard CysC, eGFR and serum IL-35 and no statistically significant difference as regard RBP.

Table 2. Comparison between Active LN group and controls regarding Renal Biomarkers

	ACTIVE LUPUS NEPHRITIS (N = 30)	CONTROL (N = 20)	P. VALUE
RBP (MG/L)	42.53 ± 7.56	44.8 ± 3.08	0.21839
MEDIAN (RANGE)	41.5 (30-58)	45 (40-50)	
CYSC (MG/L)	1.47 ± 0.25	4.36 ± 2.56	<0.0001*
MEDIAN (RANGE)	1.45 (1-1.87)	4.5 (0.9-9)	
EGFR (ML/MIN/1.73 M^2)	75.07 ± 5.1	90.35 ± 8.5	<0.0001*
MEDIAN (RANGE)	76 (68-87)	92 (74-99)	
SERUM IL-35 (PG/ML)	772.43 ± 109.2	256.9 ± 55.87	<0.0001*
MEDIAN (RANGE)	764 (567-934)	234 (187-398)	

RBP (Retinol-Binding Protein), CysC (Cystatin C), eGFR (Estimated Glomerular Filtration Rate),

There was statistically significant distinction among SLE cases without renal disease & controls as regard RBP, CysC, eGFR and serum IL-35

Table 3. Comparison between SLE patients without renal disease and controls regarding Renal Biomarkers

	SLE PATIENTS WITHOUT RENAL DISEASE (N = 30)	CONTROL (N = 20)	P. VALUE
RBP (MG/L	32.33 ± 3.93	44.8 ± 3.08	<0.0001*
MEDIAN (RANGE)	32 (26-42)	45 (40-50)	
CYSC (MG/L)	1.2 ± 0.08	4.36 ± 2.56	<0.0001*
MEDIAN (RANGE)	1.2 (1-1.3)	4.5 (0.9-9)	
EGFR (ML/MIN/1.73 M^2)	100.9 ± 11.1	90.35 ± 8.5	0.00092*
MEDIAN (RANGE)	100 (76-119)	92 (74-99)	
SERUM IL-35 (PG/ML)	345.57 ± 62.33	256.9 ± 55.87	0.00001*
MEDIAN (RANGE)	343 (239-439)	234 (187-398)	

According to disease characteristics, our findings reported that there was no statistically significant variance amongst the active LN group and SLE cases without renal disease concerning disease duration, SLEDAI-2k (SLEDAI 2000) scores, the presence, and levels of anti-dsDNA antibodies.

Table 4. Comparison between Active lupus group and SLE patients without renal disease regarding Disease Characteristics

	ACTIVE LUPUS NEPHRITIS (N = 30)	SLE PATIENTS WITHOUT RENAL DISEASE (N = 30)	P. VALUE
DISEASE DURATION \MN	30.3 ± 11.23	28.13 ± 10.49	0.45074
MEDIAN (RANGE)	27 (18-55)	26.5 (16-60)	
SLEDAI-2 K	9.3 ± 1.07	9.23 ± 1.26	0.82851
MEDIAN (RANGE)	9 (8-12)	9 (7-12)	
ANTI-DSDNA (+/– POSITIVE IF MORE THAN 75 UNITE)	24 (80%)	21 (70%)	0.37964
ANTI-DSDNA (IU/ML)	463.92 ± 122.08	423.71 ± 132.9	0.3071
MEDIAN (RANGE)	417.5 (298-643)	420 (199-654)	

SLEDAI-2 k: Systemic Lupus Erythematosus Disease Activity Index-2k, anti-dsDNA: Anti-double-stranded DNA antibodies

There was highly statistically significant variance amongst Active LN & SLE cases without renal diseases as regard IgG and IgM and statistically significant difference as regard IgA.

Table 5. Comparison between Active lupus group and SLE patients without renal disease regarding Immunoglobulins

	ACTIVE LUPUS NEPHRITIS (N = 30)	SLE PATIENTS WITHOUT RENAL DISEASE (N = 30)	P. VALUE
IGG (G/L)	13.01 ± 3.47	18.46 ± 1.48	<0.0001*
MEDIAN (RANGE)	12.7 (8.7-18.5)	18.7 (15.7-20.9)	
IGA (G/L)	2.29 ± 0.51	2.78 ± 0.67	0.0028*
MEDIAN (RANGE)	2.4 (1.5-3.2)	2.7 (1.9-4)	
IGM (G/L)	0.87 ± 0.14	1.2 ± 0.18	<0.0001*
MEDIAN (RANGE)	0.87 (0.56-1.1)	1.18 (0.9-1.87)	

IgG (Immunoglobulin G), IgA (Immunoglobulin A), IgM (Immunoglobulin M)

There was highly statistically significant variance amongst active LN group & SLE cases without renal disease as regard ALB, BUN and BUA and statistically significant difference as regard Scr.

Table 6. Comparison between Active lupus group and SLE patients without renal disease regarding Renal Function Tests

	ACTIVE LUPUS NEPHRITIS (N = 30)	SLE PATIENTS WITHOUT RENAL DISEASE (N = 30)	P. VALUE
ALB (G/DL)	2.72 ± 0.15	3.66 ± 0.19	<0.0001*
MEDIAN (RANGE)	2.7 (2.5-3)	3.7 (3.3-4)	
BUN (MG/DL)	17.13 ± 2.65	11.92 ± 2.28	<0.0001*
MEDIAN (RANGE)	17.5 (12-22)	11.25 (8.9-17.6)	
SCR (MG/DL)	0.9 ± 0.15	0.78 ± 0.09	0.0004*
MEDIAN (RANGE)	0.93 (0.6-1.1)	0.78 (0.6-0.98)	
BUA (/MG/DL)	3.4 ± 0.52	2.56 ± 0.29	<0.0001*
MEDIAN (RANGE)	3.26 (2.69-4.65)	2.55 (2.1-3.0)	

ALB (Albumin), BUN (Blood Urea Nitrogen), Scr (Serum Creatinine), BUA (Blood Uric Acid)

There was highly statistically significant variance among active LN group & SLE cases without renal disease group as regard all renal biomarkers

Table 7. Comparison between Active lupus group and SLE cases without renal disease concerning Renal Biomarkers

	ACTIVE LUPUS NEPHRITIS (N = 30)	SLE PATIENTS WITHOUT RENAL DISEASE (N = 30)	P. VALUE
RBP (MG/L)	42.53 ± 7.56	32.33 ± 3.93	<0.0001*
MEDIAN (RANGE)	41.5 (30-58)	32 (26-42)	
CYSC (MG/L)	1.47 ± 0.25	1.2 ± 0.08	<0.0001*
MEDIAN (RANGE)	1.45 (1-1.87)	1.2 (1-1.3)	
EGFR (ML/MIN/1.73 M^2)	75.07 ± 5.1	100.9 ± 11.1	<0.0001*
MEDIAN (RANGE)	76 (68-87)	100 (76-119)	
SERUM IL-35 (PG/ML)	772.43 ± 109.2	345.57 ± 62.33	<0.0001*
MEDIAN (RANGE)	764 (567-934)	343 (239-439)	

Disease duration was significantly associated ESR. SLEDAI-2 k showed significant negative correlation with Scr.

Table 8. Correlation between Disease duration and SLEDAI-2 k with different parameters.

	DISEASE DURATION		SLEDAI-2 K	
	r	P. Value	r	P. Value
DISEASE DURATION			0.192935	0.13969
SLEDAI-2 K	0.192935	0.13969		
AGE	.533**	0.00001	0.189321	0.1474
SEX				
FEMALE	-.416**	0.00095	-0.05041	0.70207
MALE	.416**	0.00095	0.050413	0.70207
ANTI-DSDNA	0.198274	0.12885	-0.19787	0.12965
ANTI-DSDNA	0.097529	0.5239	-0.13521	0.37587
C3	-0.04113	0.75502	-0.14561	0.26696
C4	0.117189	0.37254	0.029138	0.82509
ESR	.686**	<0.0001	-0.11175	0.39528
CRP	0.1444	0.271	-0.0759	0.56433
IGG	0.175354	0.18021	0.232194	0.07423
IGA	0.070107	0.59453	-0.2122	0.10358
IGM	-0.06524	0.62042	0.228381	0.07924
ALB	-0.02069	0.87532	0.065785	0.6175
BUN	0.066143	0.61559	0.095421	0.46831
SCR	0.144755	0.26981	-.308*	0.0167
BUA	0.242937	0.06144	0.050167	0.70346
RBP	0.100256	0.44596	0.079738	0.54477
CYSC	0.062062	0.63759	0.217117	0.09564
EGFR	-0.03986	0.76237	0.02322	0.86022
SERUM IL-35	0.105332	0.42315	-0.00148	0.99103

r: Pearson Correlation, C3 (Complement C3), C4 (Complement C4), ESR (Erythrocyte Sedimentation Rate), CRP (C-Reactive Protein)

4. Discussion

There was no significant variance in age and sex amongst the Active LN group and SLE cases without renal disease.

The present study agreed with He et al.,[9] who aimed to assess the clinical significance of serum IL-35 levels in individuals with SLE with and without nephritis. Their research was done on 120 individuals with SLE, of which 80 had LN, and 40 had SLE without nephritis. They disclosed that in terms of gender and age, there was not a statistically significant distinction among the two groups under research.

According to disease characteristics, our findings reported no statistically significant variance amongst the active LN group and SLE cases without renal disease concerning disease duration, SLEDAI-2k (SLEDAI 2000) scores, and the presence and levels of anti-dsDNA antibodies.

Similarly, our results were consistent with those of He et al.,[9] who revealed no statistically significant variance among the two studied groups concerning the duration of disease, SLEDAI-2 k scores, and anti-dsDNA.

The present study reported that the active LN group displayed significantly lower levels of IgG, IgA, and IgM than SLE patients without renal disease, suggesting possible immunological alterations.

Similarly, the current study is consistent with He et al.,[9] who discovered that the LN group had markedly lesser serum concentrations of IgG (P = 0.001), IgA (P = 0.009), IgM (P = 0.007) contrasted with SLE without nephritis group.

Regarding renal markers, our results demonstrated that the active LN group showed significantly lesser levels of ALB and Scr but significantly greater levels of BUN and BUA compared to SLE patients without renal disease, possibly indicating impaired kidney function.

Similarly, our findings were consistent with Abdel-Rehim et al.,[11] who revealed that lupus cases with established renal disease (group II) had significantly greater levels of BUN and Creatinine when contrasted with SLE cases with silent LN (group I).[4]

There was a statistically significant distinction regarding all renal biomarkers between the active LN group and the SLE patients without renal disease group.

The current study is consistent with Nassif et

al.,[12] who demonstrated that LN patients had significantly higher levels of serum IL-35 ($p < 0.001$) than those without LN and controls.

The current research revealed that disease duration was significantly correlated with ESR, and SLEDAI-2 k displayed a significant negative relationship with Scr.

The current study agreed with Li et al.[13] who showed that The levels of IL-35 were markedly elevated in SLE cases with an SLEDAI score of ≥ 8 contrasted with individuals with an SLEDAI score of < 8 (85.29 vs. 54.57, Z = −4.314, P = 0.000). The levels of serum IL-35 were associated with SLEDAI scores (r = 0.326, P = 0.000) & anti-double-stranded DNA antibodies (r = 0.214, P = 0.010). The blood levels of IL-35 exhibited a substantial drop in a group of 19 individuals with SLE following therapy, which was in line with the level of disease activity.

4. Conclusion

The ActiveLN group exhibited significantly greater RBP, CysC, and Serum IL-35 levels than SLE cases without renal disease, which may indicate reduced kidney and inflammatory activity in the former group. We also reported that the disease period significantly correlated with ESR & SLEDAI-2 k, which showed a significant negative correlation with Scr. We concluded that IL-35 is a promising biomarker for detecting renal involvement in individuals with LN. IL-35 expression was inhibited, either directly or indirectly, by several mechanisms throughout the progression of LN. Therefore, it is important to acknowledge that IL-35 has an advantageous impact on LN. Additional research is essential to clarify the precise impact and signalling mechanism of IL-35 in LN, which might potentially lead to novel treatment interventions for LN.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article

Funding

No Funds : Yes

Conflicts of interest

There are no conflicts of interest.

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