Serum Soluble Interleukin-2 Receptor Alpha in Systemic Lupus Erythematosus

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Original Paper

Introduction. This study aimed at determination of circulating soluble interleukin-2 receptor (IL-2 R) alpha in the sera of patients with systemic lupus erythematosus (SLE) and correlating the level of expression of these receptors with the SLE disease activity.
Materials and Methods. The study included 55 patients with SLE and 20 healthy volunteers as controls. The following investigations were done: serum complement component 3, complement 4, erythrocyte sedimentation rate, complete blood count, serum creatinine, creatinine clearance, 24-hour urinary protein, urinalysis, and serum soluble IL-2R alpha level. Kidney biopsy was performed and examined with light microscopy for patients with lupus nephritis by a single pathologist blinded to the clinical activity of the disease. The results were analysed in relation to the clinical activity index of systemic lupus activity measure (SLAM).
Results. The study showed that levels of soluble IL-2R alpha were significantly higher in the total group of patients with SLE compared to the controls ($P < .001$). Furthermore, serum IL-2R alpha levels were significantly higher in patients with lupus nephritis than those without nephritis. There were strong positive correlations between IL-2R alpha levels and the SLAM score, histological activity index, erythrocyte sedimentation rate, and 24-hour urinary protein excretion. Also, significant inverse correlations with complement 3 and packed cell volume was observed (r = 0.738; r = 0.669; r = 0.328; r = 0.705; r = -0.444; r = -0.420, respectively).
Conclusions. Serum soluble IL-2R alpha is a reliable marker of disease activity in patients with SLE and could be used as an indicator of early renal involvement with the possibility of using it for follow-up.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a classic autoimmune disorder characterized by involvement of multiple organs and presence of multiple B-lymphocyte and T-lymphocyte abnormalities. Immune complex deposition and subsequent activation of the complement system are involved in the pathogenesis of the disease. There is a broad spectrum of renal involvement which is present in about 75% of the patients with SLE.[1] Routine serological tests used to monitor patients with SLE (ie, serum anti-double-stranded DNA antibody levels, immune complexes, and complement components) have suboptimal correlations with the clinical status.[2] It has been found that activated T cells and B cells release both interleukin-2 and a

soluble form of interleukin-2 receptor (sIL-2R). The serum IL-2R level thus has been used as a marker for disease activity in a number of conditions associated with T-cell and B-cell activation, including collagen vascular diseases, infections, organ transplantation, and neoplastic diseases.[3-5] Marked elevation of sIL-2R has been reported in patients with variable hematologic malignancies, such as adult T-cell leukemia, hairy cell leukemia, and lymphocytic leukemia.[5] The aim of this study was to evaluate the usefulness of measuring the levels of soluble IL-2R alpha subunit in the sera of patients with SLE, to correlate its level with SLE disease activity, and to assess its value as an early indicator of renal involvement.

MATERIALS AND METHODS

Fifty-five patients with SLE admitted to the departments of internal medicine and dermatology were enrolled in this prospective study. They fulfilled 4 or more of the revised *American Collage of Rheumatology* criteria[6] for diagnosis of SLE. The exclusion criteria were hematological malignancies, viral infections, pulmonary disorders, psychiatric diseases, documented sepsis, and autoimmune disorders other than SLE. In addition, 20 healthy volunteers (employees and blood donors at Tanta University hospitals) were included. Informed written consent was obtained from all the patients and control participants.

The patients and controls were divided into 3 groups: group 1 comprised 20 healthy volunteers, matched for age and sex with the patients groups. They were 19 women (95%) and 1 man (5%), with their ages ranged from 15 to 52 years (mean age, 24.2 ± 8.9 years). Group 2 consisted of 20 patients with SLE but without lupus nephritis. They had serum creatinine levels less than 1.2 mg/dL. They were 19 women (95%) and 1 man (5%). Their mean age was 27.8 ± 10.6 years (range, 15 to 52 years). Group 3 comprised 35 patients with SLE and lupus nephritis according to revised *American Collage of Rheumatology* criteria.[6] Their serum creatinine levels were at least 0.4 mg/dL above the reference level without any other specific cause. They were 33 women (94.3%) and 2 men (5.7%). Their mean age was 28.1 ± 7.3 years (range, 15 to 37 years).

All of the patients were assessed using the SLE activity measure (SLAM).[7] Laboratory investigations were carried out in all of the participants in the three groups, including complete blood count, serum creatinine, erythrocyte sedimentation rate (ESR),[8] serum antinuclear antibody by indirect immunofluorescence using Hep-2 cells (Sanofi Diagnostics Pasteur Inc, Minnesota, USA),[9] anti-double-stranded DNA by indirect immunofluorescence on *Crithidia luciliae* (Sanofi Diagnostics Pasteur Inc, Minnesota, USA),[9] serum complement 3 (C3) and complement 4 (C4) by nephelometry (Behring GmbH, Marburg, Germany),[10] complete urine analysis, 24-hour urinary protein excretion (UPE),[11] and creatinine clearance.[12] In addition, soluble IL-2R alpha subunit concentrations in serum were determined (Quantikine, R&D System Inc, Minneapolis, USA).

Abdominal ultrasonography was done for all of the patients, and percutaneous kidney biopsy was done for all of the patients in group 3. The world Health Organization (WHO) classification system was used for staging,[13] and activity and chronicity indexes were determined whenever feasible.[14]

The procedures were done in accordance with the ethical standards of Tanta University Hospitals (Tanta, Egypt) on human experimentation.

Statistical Analyses

Statistical analyses were done by the SPSS software (Statistical Package for the Social Sciences, version 9.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were expressed as mean ± standard deviation. The *t* test, analysis of variance, and the chi-square test were used where applicable. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for IL-2R alpha as a predictor of renal involvement. The SLE disease activity determined with SLAM score was used as the gold standard. Simple linear regression analysis was used to assess the correlations. *P* values less than .05 were considered of significance.

RESULTS

Biochemical Parameters

Antinuclear antibody was positive in all of the studied patients. Meanwhile, anti-double-stranded DNA was positive in 46 of 55 patients (83.6%). Erythrocyte sedimentation rate was significantly higher in the patients of groups 2 and 3 compared to the controls in group 1 ($P < .001$). Packed cell

volume (PCV) and lymphocytic count were significantly lower in groups 2 and 3 compared to controls ($P < .001$, $P = .04$, and $P = .002$, respectively). There were significant differences between the patients in group 3 and the other two groups concerning depressed C3, creatinine clearance, serum creatinine, 24-hour UPE, and urinary erythrocyte and leukocyte counts, while no significant difference was observed between group 1 and group 2. Serum soluble IL-2R alpha levels were significantly higher in the total group of patients with SLE than the controls. Soluble IL-2R alpha levels were significantly higher in patients with nephritis than those without nephritis ($P < .001$). Table 1 depicts the above parameters in the three groups. There were no significant differences in soluble IL-2R alpha levels between the controls and patients with SLE but not nephritis ($P = .84$; Figure 1).

The SLE disease activity determined with the SLAM score was used as the gold standard, and the serum soluble IL-2R alpha showed a sensitivity of 93.0%, a specificity of 77.7%, a positive predictive value of 91.1%, and a negative predictive value of 82.4%. There were no statistical significant difference among the studied groups regarding C4, platelet count, and leukocyte count.

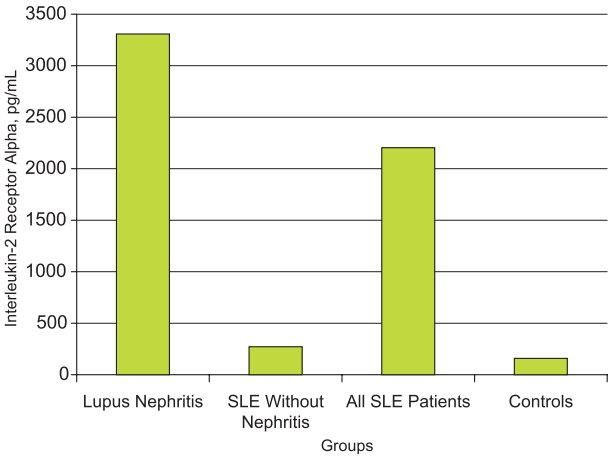
Kidney Biopsy in Patients With Lupus Nephritis

Nonproliferative glomerulonephritis (WHO class II) was present in 6 out of 35 patients with lupus nephritis (17.1%); meanwhile, WHO class III (focal proliferative glomerulonephritis)

Table 1. Studied Parameters in Healthy Individuals (Group 1) and Patients With and Without Nephritis (Groups 2 and 3, Respectively)*

Parameter	Group 1	Group 2	Group 3	*P*†	LSD‡
sIL-2R alpha, pg/mL	162.0 ± 49.7 (80 to 252)	271.4 ± 203.4 (80 to 1000)	3279.4 ± 2426.2 (140 to 6900)	< .001	G1 versus G3 G2 versus G3
C3, mg/dL	118.3 ± 35.0 (92 to 190)	97.7 ± 37.1 (33 to 190)	59.5 ± 43.9 (19.1 to 189)	< .001	G1 versus G3 G2 versus G3
C4, mg/dL	17.7 ± 7.2 (10 to 40)	15.7 ± 6.1 (6 to 30)	13.6 ± 13.8 (4 to 50)	.58	…
UPE, g/24 h	0.078 ± 0.032 (0.01 to 0.15)	0.120 ± 0.074 (0.06 to 0.30)	1.710 ± 1.050 (0.60 to 3.70)	< .001	G1 versus G3 G2 versus G3
Creatinine clearance, mL/min	122.30 ± 20.34 (82.0 to 156.0)	105.05 ± 27.41 (82.0 to 132.0)	70.36 ± 40.81 (21.3 to 159.0)	< .001	G1 versus G3 G2 versus G3
Serum creatinine, mg/dL	0.79 ± 0.22 (0.4 to 1.1)	0.90 ± 0.14 (0.7 to 1.2)	1.74 ± 0.80 (0.8 to 4.0)	< .001	G1 versus G3 G2 versus G3
Lymphocyte count, × 10^9/L	1.92 ± 0.47 (1.5 to 3.1)	1.52 ± 0.56 (0.6 to 2.7)	1.39 ± 0.68 (0.4 to 3.1)	.009	G1 versus G2 G1 versus G3
Leukocyte count, × 10^9/L	6.50 ± 2.80 (4.0 to 11.0)	6.35 ± 2.95 (3.4 to 12.0)	5.22 ± 1.94 (2.7 to 11.2)	.08	…
Platelet count, × 10^9/L	266.3 ± 41.5 (197 to 352)	222.0 ± 112.8 (70 to 399)	233.8 ± 101.5 (110 to 400)	.29	…
PCV, %	40.56 ± 3.75 (32.6 to 45.8)	33.09 ± 8.49 (21.5 to 51)	27.74 ± 4.52 (20.0 to 38.0)	< .001	All Groups
ESR, mm/h	7.8 ± 3.9 (3 to 15)	68.7 ± 42.9 (20 to 130)	83.9 ± 27.5 (36 to 150)	< .001	G1 versus G2 G1 versus G3

*sIL-2R indicates soluble interleukin-2; LSD, least significant difference; C3, complement 3; C4, complement 4; UPE, urinary protein excretion; PCV, packed cell volume; ESR, erythrocyte sedimentation rate; and G, group.
†Analysis of variance.
‡Significant differences between the groups. Ellipses indicate no significant difference was shown by LSD test.



Soluble interleukin-2 receptor alpha levels in the controls, total patients with systemic lupus erythematosus (SLE), patients with SLE and without nephritis, and patients with lupus nephritis.

was diagnosed in 9 out of 35 kidney biopsies from these patients (25.7%), and 20 out of 35 patients showed WHO class IV (diffuse proliferative glomerulonephritis) in their kidney biopsies (57.1%).

Interleukin-2 Receptor Alpha and Systemic Lupus Erythematosus Activity

Strong positive correlations between soluble IL-2R alpha levels and the SLAM score, histological activity index, ESR, and 24-hour UPE were seen. On the other hand, strong inverse correlations were observed between soluble IL-2R alpha levels and C3 and PCV. On the contrary, no significant correlations were found between IL-2R alpha levels and C4, platelet count, leukocyte count, lymphocytes, serum creatinine, and histological chronicity index (Table 2).

Table 2. Correlations Between Soluble Interleukin-2 Receptor Alpha and Different Parameters Studied in Patients With Systemic Lupus Erythematosus*

Parameter	r	*P*
C3	-0.444	.001
C4	-0.250	.07
ESR	0.328	.01
PCV	-0.420	.001
Platelet count	0.056	.69
Leukocyte count	-0.243	.07
Lymphocyte count	-0.082	.55
Serum creatinine	0.230	.09
24-hour UPE	0.705	< .001
SLAM score	0.738	< .001
Activity index (n=29)	0.669	< .001
Chronicity index (n=29)	-0.046	.81

*C3 indicates complement 3; C4, complement 4; ESR, erythrocyte sedimentation rate; PCV, packed cell volume; UPE, urinary protein excretion; and SLAM, systemic lupus activity measure.

DISCUSSION

The heterogenecity of clinical manifestations and the fluctuating course of the SLE disease produce difficulty in assessing the need for and the response to treatment. Central to this problem is the measurement of disease activity, and in particular, the differentiation between reversible activity and irreversible organ damage. There are various approaches to the measurement of disease activity in SLE. These include monitoring of certain laboratory tests, assessment of clinical features, or various combinations of these two.[15] In this study, the serum level of sIL-2R was found to be significantly higher in the total group of patients with SLE than in the controls. Most of this elevation could be attributed to the patients with nephritis, whose sIL-2R levels were significantly higher than patients without nephritis; on the other hand, no significant difference was observed in the soluble IL-2R levels between the controls and SLE patients without nephritis. This agrees with Laut and colleagues and Campen and colleagues who have reported markedly elevated IL-2R levels in patients with very active disease, moderate elevation in those with mildly active disease, and normal levels in patients with inactive disease.[16,17] Other previous studies have reported that IL-2R levels were higher in patients with SLE than that in controls.[18-20] The concentration of soluble IL-2R in active SLE was higher than that in inactive SLE.

Several published studies have evaluated IL-2R levels in relation to other serologic tests in SLE. Decreased levels of C3 and C4,[16,17,20,21] elevated global disease activity,[20,22] elevated ESR,[23] and increased proteinuria[16] correlated with elevated sIL-2R levels. The present study confirmed these findings, in which elevated soluble IL-2R alpha levels were found to strongly correlate with decreased levels of C3 and PCV, elevated SLAM score, proteinuria, and higher ESRs. While these studies suggest a positive correlation between IL-2R levels and the global disease activity, other investigators found no significant correlation between IL-2R and lupus activity index at the time of disease exacerbation with patients studied prospectively.[24] In addition, no correlation was noted between IL-2R levels and

any specific organ system manifestation at the time of maximal disease activity.[24] The reasons for the conflicting results of these studies are not clear, but it might be the different patient selection and disease activity criteria. The present study demonstrated that soluble IL-2R levels in patients with SLE and proliferative glomerulonephritis were significantly higher than that in those with nonproliferative glomerulonephritis. This is in agreement with the study by Laut and coworkers who stated that the mean soluble IL-2R levels were significantly higher in the group of patients with diffuse or focal proliferative glomerulonephritis than in the group of patients with membranous nephropathy or mesangial changes.[16]

We also correlated soluble IL-2R levels with the histological activity index. This correlation was statistically significant. However, there was no significant correlation between the histological chronicity index and IL-2R level. This is again in agreement with the results Laut and coworkers reported[16]; they found significant positive correlations between IL-2R levels and the activity index. Meanwhile, they found a significant correlation between IL-2R levels and the histological chronicity index. This strong correlation may reflect the long-term chronic inflammatory state seen in these patients.

CONCLUSIONS

Based on the previous results, comparative studies, and correlations, we demonstrated that serum soluble IL-2R alpha is a reliable marker of disease activity in patients with SLE and could be used as an indicator of renal involvement with the possibility of using it for follow-up and monitoring patients with lupus nephritis during the treatment course.

CONFLICT OF INTEREST

None declared.

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