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Original Article

Relationship between serum soluble interleukin 7 receptor and disease activity in patients with lupus nephritis

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Abstract: The aim of this study was to determine serum levels of soluble IL-7R (sIL-7R) in systemic lupus erythematosus (SLE) patients with lupus nephritis and its correlation with disease activity. For this cross-sectional study, 66 patients (20 males and 46 females) with lupus nephritis and 56 age-matched healthy controls (18 males and 38 females) were recruited. Serum sIL-7R levels (ng/mL) were determined by a sandwich ELISA kit. Disease activity was measured by SLE disease activity index (SLEDAI) score, 24 h proteinuria, serum creatinine levels, glomerular filtration rate (GFR), and levels of complement C3 (C3), C4, and C1q. We found that serum sIL-7R level was 67.7% significantly higher in patients with lupus nephritis (1693.6 ± 812.4 vs. 2785.5 ± 1173.5 ng/mL, P<0.0005). Patients with active nephritis (n=32) had significantly higher serum sIL-7R than those in disease remission (n=34) (3255.4 ± 1583.5 vs. 2186.5 ± 1062.8 ng/mL, P=0.002). Serum sIL-7R levels correlated significantly with disease activity markers with $|r|$ ranging from 0.330 to 0.445 (all P<0.05). Higher serum sIL-7R levels were associated with lower C3, C4, C1q, and GFR and with higher 24 h proteinuria, serum creatinine levels, and SLEDAI scores. In conclusion, our results indicate that sIL-7R could be a valuable marker of disease activity in lupus nephritis.

Keywords: Lupus nephritis, serum soluble interleukin 7 receptor, disease activity

Introduction

Lupus nephritis is a common symptom in systemic lupus erythematosus (SLE) that affects 30-60% of adult and up to 70% of children with SLE [1]. It is characterized by an inflammatory response caused by the glomerular deposition of immune complex. Treatment for lupus nephritis is usually based on histological subtypes and requires immunosuppressants to preserve renal function. Despite advances in treatment regimen, rate of complete remission in proliferative disease remains <50%, and a sizable number of patients with stage III-V lupus nephritis will still exhibit progressive inflammation and some degree of renal damage [2]. Even in patients who achieve complete or partial remission, flare of nephritis can still occur and its occurrence does not always depend on renal markers of disease activity. This reflects an incomplete understanding of the disease pathogenesis and a need for novel markers for monitoring lupus nephritis.

Interleukin-7 (IL-7) belongs to the IL-2 family and is mainly produced by stromal cells, fibroblastic reticular cells, and keratinocytes [3, 4]. Its major role involves T cell survival and function [5]. Studies have shown that polymorphisms in IL-7 receptor α (IL-7Rα) are associated with increased risk in multiple autoimmune conditions and blocking IL-7 in mouse models of multiple sclerosis [6, 7], type 1 diabetes [8, 9], and rheumatoid arthritis (RA) [10-12] has therapeutic benefit. IL-7 pathway has been implicated in the pathogenesis of SLE [13, 14]. IL-7 can induce IL-7Rα$^+$ T cells to secret Th1 and Th17-associated cytokines, such as interferon γ and IL-17, which are involved in the pathogenesis of SLE [15, 16].

In this case-control study, we aimed to determine serum levels of soluble IL-7R (sIL-7R) in

Table 1. Results of 24 h proteinuria, serum creatinine, GFR, and serum sIL-7R in the study cohort

Variables	Patients with lupus nephritis (n=66)	Healthy controls (n=56)	*p*-value	Active lupus nephritis (n=32)	Lupus nephritis in remission (n=34)	*p*-value
24 h proteinuria, g/24 h	2.13 ± 0.51	0.08 ± 0.01	**<0.0005**	2.42 ± 0.43	2.11 ± 0.40	**0.004**
Serum creatinine, μmol/L	239.5 ± 128.6	62.5 ± 12.9	**<0.0005**	262.4 ± 68.6	208.34 ± 57.2	**0.001**
glomerular filtration rate, ml/min	35.6 ± 9.6	91.2 ± 13.5	**<0.0005**	31.3 ± 12.5	43.9 ± 10.2	**<0.0005**
Serum sIL-7R, ng/mL	2785.5 ± 1173.5	1693.6 ± 812.4	**<0.0005**	3255.4 ± 1583.5	2186.5 ± 1062.8	**0.002**

Results are mean ± SD. Boldface indicate P<0.05. sIL-7R: soluble Interleukin 7 receptor.

patients with lupus nephritis and its correlation with disease activity. Results may provide evidence for sIL-7R as a marker for local inflammation in lupus nephritis.

Patients and methods

Patients and controls

For this cross-sectional study, 66 patients (20 males and 46 females) with lupus nephritis were recruited at the nephrology outpatient clinic at the First People's Hospital of Jining, China, between October 2012 and May 2015. All patients fulfilled the 1997 revised criteria for the classification of SLE [17] and had a confirmed diagnosis of nephritis by kidney biopsy [2]. Exclusion criteria were: 1) severe comorbidities including other autoimmune diseases such as dermatomyositis, Sjögren's syndrome, mixed connective tissue disease, and RA, severe infection, and malignancies; 2) unconscious or mental disorders which precluded an informed consent; 3) women who were pregnant or breastfeeding; and 4) age <18 years or ≥80 years old. Mean (SD) age of the patients was 37.6 (4.1) years old (range: 22-75 years) and mean (SD) disease duration was 4.3 (2.1) years (range: 0.5-12 years). Thirty-two patients were classified as with active disease and 34 patients were in remission. A group of 56 apparently healthy subjects (18 males and 38 females) were recruited as controls. Mean (SD) age of the controls was 37.4 (4.2) years (range: 20-72 years). There was no significant difference in age and gender between the two groups (both P>0.05). The study was approved by Research Ethics Committee of the hospital and all participants provided written informed consent. All study procedures were conducted according to the World Medical Association Declaration of Helsinki.

Disease characteristics and sIL-7R

Disease activity was measured by SLE Disease Activity Index (SLEDAI) with higher score indicating higher disease activity [18]. Other measures of disease activity included serum complement component 3 (C3), C4, C1q, 24 hours proteinuria (g/24 h), serum creatinine (μmol/L), and estimate GFR (ml/min). Assays for C3, C4, and C1q were purchased from Eykits (Shanghai, China) and measured by iChem-340 (Icubio, Shenzhen, China). Twenty-four-hour Proteinuria was measured by IMMAGE 800 (Beckman Coulter, Brea, CA, US). Kits for serum creatinine levels were purchased from Biomars (Beijing, China). The patients were intravenously bolus injected with 185 MBq/1 mL of (99) Tc (m)-DTPA 20 min after drinking 300 mL of water. Renal dynamic imaging was performed and the image was processed according to standard procedure (Gates' method) to obtain Glomerular Filtration Rate (GFR).

Fasting blood sample were collected for sera. Serum sIL-7R levels (ng/mL) were determined by a sandwich ELISA kit (Yinggong Inc., Shanghai, China). In this kit, the first anti-IL-7R antibody served as the capture antibody; the sIL-7R was detected with a biotinylated anti-IL-7R antibody generated in species other than that for producing the IL-7R capture antibody. Streptavidin-HRP was applied to determine the abundance of antigen-antibody binding as previously reported [19].

Statistical analyses

Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS, version 19.0, IBM Corp., Armonk, NY, US). Results were presented as mean ± SD. Comparisons in serum sIL-7R, C3, C4, C1q, serum creatinine, and GFR levels between patients and controls and between patient with active disease and those in remission were performed using two-sample *t*-test. Pearson or Spearson correlation analyses were performed to determine the correlation between serum sIL-7R levels and levels of C3, C4, C1q, serum

Table 2. Results of complements levels and SLEDAI scores in patients with active lupus nephritis and patient with disease in remission

Variables	Active lupus nephritis (n=32)	Lupus nephritis in remission (n=34)	*p*-value
Complement 3, g/L	0.33 ± 0.12	0.68 ± 0.20	**<0.0005**
Complement 4, g/L	0.10 ± 0.03	0.17 ± 0.03	**<0.0005**
Complement 1q, g/L	0.10 ± 0.01	0.13 ± 0.02	**<0.0005**
SLEDAI score	12.5 ± 3.5	2.3 ± 1.0	**<0.0005**

Results are mean ± SD. Boldface indicate P<0.05. SLEDAI: systemic lupus erythematosus disease activity index.

Table 3. Correlation coefficients between serum sIL-7R and markers of disease activity

Variables	*r*	*p*-value
SLEDAI score	0.445	**<0.05**
Complement 3	-0.33	**<0.05**
Complement 4	-0.421	**<0.05**
Complement 1q	-0.368	**<0.05**
24 h proteinuria	0.359	**<0.05**
Serum creatinine	0.371	**<0.05**
Glomerular filtration rate	-0.335	**<0.05**

Boldface indicate P<0.05. SLEDAI: systemic lupus erythematosus disease activity index.

creatinine, and GFR. All analyses were two-tailed and a *p*-value <0.05 was considered statistically significant.

Results

Disease characteristics

Compared with healthy controls, patients with lupus nephritis had significantly impaired renal function, presented as significantly higher 24 h proteinuria, higher serum creatinine levels, and lower GFR (**Table 1**). Patients with active nephritis had poorer renal function than those in disease remission. C3, C4, C1q, and GFR were significantly lower and 24 h proteinuria and serum creatinine levels significantly higher in patients with active lupus nephritis (**Tables 1** and **2**). Total SLEDAI scores were also significantly higher in patients with active lupus nephritis (12.5 ± 3.5 vs. 2.3 ± 1.0, P<0.0005).

Serum sIL-7R levels

Compared with healthy controls, serum sIL-7R level was 67.7% significantly higher in patients with lupus nephritis (1693.6 ± 812.4 vs. 2785.5 ± 1173.5 ng/mL, P< 0.0005) (**Table 1**). Patients with active nephritis had significantly higher serum sIL-7R than those in disease remission (3255.4 ± 1583.5 vs. 2186.5 ± 1062.8 ng/mL, P=0.002).

Correlations between serum sIL-1R levels and disease activity

Table 3 shows the correlation coefficient between serum sIL-7R levels and markers of disease activity. Serum sIL-7R levels correlated significantly with disease activity markers with |*r*| ranging from 0.330 to 0.445 (all P<0.05). Higher serum sIL-7R levels were associated with lower C3, C4, C1q, and GFR and associated with higher 24 h proteinuria, serum creatinine levels, and SLEDAI scores.

Discussion

In this study, we investigated serum levels of sIL-7R in patients with lupus nephritis and its correlation with disease activity. We found that patients with lupus nephritis had significantly higher serum levels of sIL-7R and levels were significantly higher in those with active disease. Higher serum levels of sIL-7R correlated significantly with higher disease activity and poorer renal function. Our results indicate that sIL-7R has the potential as a novel biomarker for disease activity in lupus nephritis.

Lupus nephritis is initiated in most cases by the glomerular deposition of immune complexes, such as circulating anti-nuclear, anti-C1q, and cross reactive anti-glomerular autoantibodies [1]. Consequences of deposition of immune complexes are production of a large variety of inflammatory mediators in the nephritic kidney and a subsequent infiltration of inflammatory cells through glomerular or interstitial blood vessels [20]. Damage of renal tissues accumulates as inflammation progresses. Initiation and monitoring of treatment in lupus nephritis reply on histologic subtyping and disease activity markers. However, the commonly used disease activity markers for SLE, such as anti-ds DNA, C3, and C4 may not sufficiently reflect flare of nephritis in SLE [21] and kidney-specific measurements such as quantification of proteinuria or GRF could be biased in long-stand-

ing diseases as they could reflect glomerular damage rather than active inflammation.

In this study, we investigated the potential of using sIL-7R as a marker for disease activity in lupus nephritis. sIL-7R is produced by alternatively splicing the full-length IL-7R mRNA [5]. Recent studies have demonstrated an important role of IL-7 pathway in autoimmune diseases and its potential as a therapeutic target. In human, polymorphisms in IL-7Rα confers enhanced susceptibility to multiple sclerosis [6, 7, 22, 23] and type 1 diabetes [8, 9]. Elevated serum sIL-7R levels have been reported in patients with RA [10, 12]. Studies have also shown that IL-7 pathway could be involved in the pathogenesis of SLE. In MRL-Fas^{lpr} lupus-predisposed mice, proliferation of T cells was associated with increased production of IL-7 due to expansion of fibroblastic reticular cells [14]. Blockade of IL-7R reduced T cell activation and autoimmune manifestations.

Our results add to the evidence that IL-7 pathway may be involved in the pathogenesis of lupus nephritis. Significantly higher levels of sIL-7R were found in patients with lupus nephritis when compared with healthy controls and the levels were higher in patients with active disease. The levels of sIL-7R reflected a more active disease with higher levels associated with various disease activity markers, including levels of complements (C3, C4, and C1q), as well as kidney-specific markers. Our results are in line with those reported in two previous studies [24, 25]. A study by Badot et al found that serum sIL-7R levels were strongly raised in patients with lupus nephritis and the levels decreased upon successful treatment [24]. Immunohistochemistry on kidney biopsy samples showed abundant perivascular IL-7R expression, accompanied by expression of TNFα in the surrounding tissue. A recent study by Chi et al also reported that serum levels of sIL-7R were significantly higher in SLE patients with nephritis in comparison with healthy controls or SLE patients without nephritis [25]. The levels of serum sIL-7R correlated significantly with SLEDAI scores and levels of anti-C1q antibodies. Collectively, these findings support the involvement of IL-7 pathway in the initiation and progression of lupus nephritis and that sIL-7R might be valuable marker of disease activity in lupus nephritis.

Our study has several limitations. First, as a cross-sectional study, causal relationship between sIL-7R could not be readily established. Longitudinal studies with larger sample size, particularly studies examining changes of sIL-7R levels after treatment will be required to confirm our findings. Second, expression of IL-7R mRNA in peripheral blood mononuclear cells (PBMCs) was not assessed in our study. However, previous studies have shown that there was no significant change in IL-7R mRNA in PBMCs in patients with lupus nephritis compared with healthy controls [24, 25]. On the other hand, IL-7R was found to be abundantly expressed in kidney perivascular cells, indicating an activation of renal cells [24].

In conclusion, patients with lupus nephritis had significantly higher serum levels of sIL-7R and levels were significantly higher in those with active disease. Higher serum levels of sIL-7R correlated significantly with higher disease activity and poorer renal function. Our results indicate that sIL-7R could be a valuable marker of disease activity in lupus nephritis.

Disclosure of conflict of interest

None.

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