

Urinary CXCL10: a marker of nephritis in lupus patients

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SUMMARY

Systemic lupus erythematosus (SLE) is a connective tissue disease characterized by the formation of autoantibodies and immune complexes. Lupus nephritis is one of the hallmark features of SLE. CXCL10 is a chemokine secreted by IFN γ -stimulated endothelial cells and has been shown to be involved in the pathological processes of autoimmune diseases. The objective was to measure urinary CXCL10 in SLE patients, to compare levels between nephritis and non-nephritis groups and to study its correlation with other variables.

Sixty lupus patients were enrolled in our trial. Thirty patients had lupus nephritis and the other 30 were without evidence of lupus nephritis. Thirty healthy subjects were willing to participate as a healthy control group. Renal biopsy was performed for lupus nephritis group. Urinary CXCL10 was measured using the ELISA technique. Serum creatinine, C3, C4 and 24 h urinary proteins were measured. Lupus activity was assessed using systemic lupus erythematosus disease activity index (SLEDAI) scoring system. Renal activity was measured using renal activity scoring system. CXCL10 was significantly higher in lupus nephritis patients than in lupus patients without nephritis. CXCL10 was significantly correlated with renal activity score, 24 hours urinary proteins and the SLEDAI score. It is highly valid predictor of SLE nephritis with high sensitivity and specificity. CXCL 10 a highly sensitive and specific non-invasive diagnostic tool for lupus nephritis patients.

Key words: CXCL10; lupus nephritis.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a connective tissue disease characterized by the formation of autoantibodies and immune complexes (1). Lupus nephritis (LN) is one of the hallmark features of SLE, seen in 40-60% of patients (2). It is an important cause of morbidity and mortality. One approach to improve outcome is to diagnose patients early. Late diagnosis of LN correlates with a higher frequency of renal insufficiency and end stage renal disease, underlining the importance of early diagnosis. While kidney biopsy is a valuable tool, it is an invasive procedure that is not always feasible and cannot be performed repeatedly. Moreover, it may not be representative, as only a limited number of glomeruli are sampled (3).

CXCL10 is a chemokine also known as IFN γ -inducible protein 10 (IP-10); it is secreted by IFN γ -stimulated endothelial cells, fibro-

blasts and monocytes. CXCL10 promotes migration of T cells to sites of inflammation and is also known to play a role in the down-regulation of angiogenesis, together with its receptor, CXCR3 (CXCR3) (4). CXCL10 is highly expressed in a wide range of human diseases. It has been shown to be involved in the pathological processes of three main human disorders namely infectious, inflammatory and autoimmune diseases (5). In SLE patients, CXCL10 levels in serum are highly elevated and correlate with levels of disease activity (6). Interestingly, urinary expression of messenger RNA (mRNA) for both CXCR3 and CXCL10 has been shown to correlate with nephritis activity, being up-regulated in patients with active lupus nephritis and not detectable in healthy control subjects (7).

Objectives

The aim of the current study was to measure urinary CXCL10 in a cohort of SLE

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patients, to compare its level between nephritis and non-nephritis groups and to study its correlation with other variables including 24 h urinary proteins, SLEDAI score, C3, C4, serum creatinine, renal activity score and renal biopsy grading.

■ MATERIALS AND METHODS

In our case control study, we recruited 60 patients diagnosed as having systemic lupus erythematosus who fulfilled the American College of Rheumatology (ACR) classification criteria for SLE (8). Patients were divided into two groups. Group one: involved 30 patients with evidence of lupus nephritis as having 24 h urinary protein >0.5 gm or active urinary sediment in the form of either microscopic or macroscopic hematuria or white cell cast with or without elevated serum creatinine. Group two: involved 30 SLE patients without any evidence of lupus nephritis.

These two groups were compared with 30 healthy age- and sex-matched control subjects. All patients were subjected to careful history taking, full clinical examination and routine laboratory investigations. SLE disease activity was assessed by the systemic lupus erythematosus disease activity index (SLEDAI) (9). A complete hemogram, chemistry, urinalysis, 24 h urinary proteins and serological tests (C3 and C4) were performed all patients. Anti-dsDNA and anti C1q was measured for all patients. Renal biopsy was performed only for SLE patients with clinical evidence of nephritis. Histological grading was done according to ISN-RPS criteria by 2003 (10).

The study was approved from hospital ethical committee and a written informed consent was taken from all individuals who agreed to contribute in the study after full information about the study and the related procedures.

Urinary CXCL10 determination

CXCL10 levels in urine were determined by sandwich enzyme-linked immunosorbent assay (ELISA) (Opt EIA Systems, BD Biosciences, San Diego, CA, USA) ac-

ording to the manufacturer's instructions. Levels were measured within 5 days of performing kidney biopsy.

Renal activity score

Renal activity score was measured using the following criteria: proteinuria 0.5-1 gm/day =3 points, proteinuria >1-3 gm/day =5 points, proteinuria >3 gm/day =11 points, urine red blood cell count ≥ 5 /hpf =3 points, urine white blood cell count ≥ 5 /hpf =1 point (11).

Statistical analysis

For statistical analysis, statistical software SPSS 10.0 (SPSS, Chicago, IL, USA) was employed. Quantitative data were expressed as mean \pm SD. For comparison between patients and controls, and comparison of clinical features and pathological data of patients, the one way ANOVA analysis of variance and Chi-square test were used. For correlation between CXCL10 *versus* other variables, correlation coefficient test was used. Statistical significance was considered as $p < 0.05$.

■ RESULTS

Study population

Demographic, clinical, and some relevant laboratory characteristics of the patients are summarized in Table I. In general, the study group comprised 60 SLE patients (55 females, 5 males) with a mean age of 23 ± 5.5 years. There was no statistically significant difference in age, gender and disease duration between groups. SLEDAI score was calculated including the renal activity score in both SLE groups. There was significantly higher SLEDAI score in lupus nephritis group *versus* non-nephritis lupus group. There was significantly higher proteinuria and serum creatinine level in lupus nephritis group.

Urinary CXCL10

A statistically highly significant elevation in the level of urinary CXCL10 in lupus nephritis group *versus* non-nephritis lupus group was observed (Table I).

Table I - Demographic, laboratory and activity score data in nephritis versus non-nephritis lupus patients.

Variables	Lupus nephritis N=30	Non-nephritis lupus N=30	Controls N=30	P
Age	22.8±3	23.7±4	24.4±1.6	>0.05
Gender	3 (10%)	2 (6.7%)	2 (6.7%)	>0.05
Male	27 (90%)	28 (92.3%)	28 (93.3%)	
Female				
Disease duration (years)	6.8±3.3	7.4±4	-	>0.05
SLEDAI score	12.8±2.5	6.8±1.1	-	<0.001
Renal activity score	7.8±2.9	-	-	-
24 urinary protein (g)	2.1±1	0.24±0.08	0.11±0.03	<0.001
C3 (mg %)	35.6±11	119±10.8	109±11	<0.001
C4 (mg %)	5.2±1.4	22.9±3	21.2±2.4	<0.001
Creatinine (mg %)	1.3±0.3	0.82±0.2	0.79±0.3	<0.001
CXCL10 (Pg/dL)	270.5±62	70.6±12	30.5±16	<0.001

SLEDAI score, systemic lupus erythematosus disease activity index; C3, complement 3; C4, complement 4.

Renal biopsy

Tables II and III summarize the renal biopsy results in studied lupus nephritis patients.

Correlation between CXCL10 versus other variables among lupus nephritis group and non-nephritis lupus group

Tables IV and V demonstrate correlation between CXCL10 versus and other variables among lupus nephritis group and non-nephritis lupus group, respectively.

In lupus nephritis group, there was a statistically significant positive correlation between CXCL10 and urinary protein level,

Table II - Renal biopsy histopathology in lupus nephritis patients.

Renal biopsy grade	No	%
Diffuse proliferative	16	53.3%
Focal proliferative	6	20%
Mesangial proliferative	8	26.7%

Table III - Distribution of renal biopsy grading in lupus nephritis patients.

Renal biopsy grade	No	%
I	0	0
II	8	26.7%
III	6	20%
IV	16	53.3%
G	11	68.8%
S	5	31.2%

G, global; S segmental.

Table IV - Correlation between CXCL10 versus other variables among nephritis group.

Variables	CXCL10	
	r	P
24 urinary protein	0.72	<0.001
SLEDAI	0.45	<0.001
C3	-0.42	<0.001
C4	-0.14	>0.05
C1q	-0.03	>0.05
Cr	0.09	>0.05
Renal activity score	0.68	<0.001
Biopsy grades	0.84	<0.001

SLEDAI score, systemic lupus erythematosus disease activity index; C3, complement 3; C4, complement 4; Cr, creatinine.

SLEDAI, renal activity score and grade of renal biopsy, with an inverse correlation with C3.

In non-nephritis lupus group, statistically significant inverse correlation between CXCL10 with C4, without any significant correlation with other variables.

Validity of CXCL10 in prediction of nephritis

Using lupus nephritis (present or absent) and CXCL10 (positive or negative using best cut off of 93) as dichotomous variables, Table VI shows that CXCL10 was found to be a highly valid predictor of SLE nephritis with high sensitivity (100%) and specificity (98%).

Table V - Correlation between CXCL10 versus other variables among non nephritis group.

Variables	CXCL10	
	r	P
24 urinary protein	0.12	>0.05
SLEDAI	0.10	>0.05
C3	-0.15	>0.05
C4	-0.43	<0.001
C1q	-0.15	>0.05
Cr	0.16	>0.05
Renal activity score	0.22	>0.05

SLEDAI score, systemic lupus erythematosus disease activity index; C3, complement 3; C4, complement 4; Cr, creatinine.

Table VI - Validity of CXCL10 in prediction of nephritis.

Variables	CXCL10 Pg/dL
Best cut off	93
AUC	0.100
Sensitivity	100%
Specificity	98%
PPV	100%
NPV	96%

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

■ DISCUSSION

The present study addresses the urinary level of CXCL10 in a cohort of lupus nephritis *versus* non lupus nephritis patients and discloses the correlations with other variables including 24 h urinary proteins, SLEDAI score, C3, C4, serum creatinine, renal activity score and renal biopsy grading.

We found a significant higher urinary level of CXCL10 in lupus nephritis group compared to non-nephritis group. Furthermore, we found a significant in our trial to correlation between urinary level of CXCL10 and patients' variables.

In the nephritis group, there was statistically significant positive correlation between CXCL10 and 24 h urinary protein, SLEDAI, renal activity score, and grade of renal biopsy. However, an inverse correlation was detected with C3. Furthermore, we

could not reveal any correlation with either C4 or serum creatinine. On the other hand, in non-nephritis lupus patients, the only correlation was an inverse correlation was detected with C4. Apart from the correlation detected with C4, these correlations appeared to be logic in respect of linking CXCL10 with disease activity in general and renal involvement in particular. With the limitation that our study is a cross-sectional rather than a longitudinal one and it has a limited number of patients, CXCL10 may be suggested as a valid marker in prediction of nephritis in lupus patients, with a sensitivity of 100% and a specificity of 98%. Further longitudinal studies are required to verify this suggestion.

It postulated that, clinically evident renal disease occurs in approximately half of the patients with SLE (12). Pathogenesis of lupus nephritis is multifactorial, most importantly immune complex deposition (13). In the normal kidney, production of inflammatory chemokines is low, but is significantly increased under pathophysiological circumstances such as ischemia, toxin exposure, or acute inflammation (14). CXCL10, one of the first chemokines identified, directs the trafficking of activated effector CD4⁺ and CD8⁺ T lymphocytes and other effector lymphocytes. CXCR3 is a chemokine receptor that is preferentially expressed on the surface of Th1 T cells. The receptor is activated by three related chemokines: IP-10/CXCL10, Mig/CXCL9, and I-TAC/CXCL11. CXCR3 has been localized to infiltrating effector T cells of the Th1 type in a wide variety of human inflammatory diseases, including renal transplant rejection, glomerulonephritis, rheumatoid arthritis, and multiple sclerosis (15). Interestingly, IP-10 gene expression appeared to have consistent changes in relation to the histological class of lupus nephritis and correlated with the histological activity index. These findings suggest a specific role of these genes in the pathogenesis of lupus nephritis (16).

In renal allograft recipients it was found that for clinical and subclinical pathologies, urinary CXCL10 correlated well with the extent of tubulo-interstitial inflamma-

tion (17). Ho et al. (18) performed a study which validated urinary CXCL10 as a non-invasive, sensitive, and specific marker for tubulitis in an independent cohort. It was also found to distinguish borderline, sub-clinical and clinical tubulitis from normal histology, and interstitial fibrosis and tubular atrophy. Further, peripheral blood mononuclear cells from SLE patients were shown to produce higher amounts of IP-10 in patients with active lupus nephritis as compared to healthy controls (19). Thus, in patients with active lupus, there seems to be increased production of the CXCL10 chemokine at the disease site, which may contribute to disease pathogenesis and increased urinary level.

Several previous studies tried to investigate levels of CXCL10 in SLE patients. Our study results are found to be consistent with work done by Avihingsanon et al. (7). In that study, pre-biopsy urine samples were collected from 26 LN patients over a period of 6 months. It showed that the level of CXCL10 mRNAs in urine could distinguish class IV LN from others and that it was reduced in response to treatment. Also, Abujam et al. (20) showed increased urinary level of CXCL10 in active *versus* non active lupus nephritis. It comprised 136 patients with SLE including 78 active (46 active renal and 32 active non-renal). A previous study found active SLE patients to have increased levels of serum CXCL10 compared to non-active SLE patients, rheumatoid arthritis patients and healthy controls (21). Another study in which 40 SLE patients with renal disease, and 40 patients without renal disease were recruited, showed that plasma concentrations of CXCL10 were higher in SLE patients than in healthy individuals (22). Thomas et al reported that urinary IP-10 mRNA is a significantly better test for class IV lupus nephritis. Furthermore, patients who responded to therapy had significantly lower levels of IP-10, suggesting that IP-10 can be used as a barometer for treatment efficacy (23). To our knowledge, our study is amongst the earliest to address the correlation between CXCL10 and each of 24 hour urinary proteins, renal

activity score, and SLEDAI score. Bauer *et al.* concluded that monitoring serum chemokine levels - including CXCL10- in SLE may improve assessment of current disease activity, the prediction of future flare, and overall clinical decision-making (24). A further longitudinal study may be required to validate its role as a biomarker for prediction and following up patients with lupus nephritis.

Finally, we can conclude that, urinary CXCL10 level is elevated in lupus nephritis patients, and was found to be linked with SLE disease activity in general and renal involvement in particular. It is a sensitive and specific marker for diagnosis of lupus nephritis, with a sensitivity of 100% and specificity of 98%.

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