

Original article

Assessment of lupus nephritis by measuring urinary retinol binding protein.

Background: Renal disease is a common manifestation of systemic lupus erythematosus (SLE). Both glomerular and tubular functions could be affected. The tubular function can be measured by different methods including urinary retinol binding protein (RBP).

Objective: The study was aimed to assess the urinary RBP level in SLE patients with and without evidence of renal disease and to determine whether its measurement would be of value in early diagnosis and subsequent monitoring of renal disease in SLE.

Methods: We studied 22 female patients with SLE aged between 6 and 18 years (mean \pm SD: 14.4 \pm 3.6 years) in comparison to 18 healthy age and sex matched subjects. Patients were categorized into two groups: Group I (non-renal SLE patients) which included 7 patients, who had no clinical or laboratory evidence of renal disease; Group II (renal SLE patients) which included 15 patients with lupus nephritis. They were subjected to determination of disease activity using the SLE Disease Activity Index (SLEDAI) and laboratory investigations including complete urine analysis, ESR, serum ANA, anti-DNA and C3 in SLE patients, and corrected creatinine clearance, urinary total protein, urinary microalbumin, and urinary RBP by ELISA.

Results: The urinary RBP (mg/g Cr) was significantly higher in SLE patients as a whole than controls. It was higher in renal patients than both non-renal patients and controls (1.1 \pm 0.32, 0.75 \pm 0.15, 0.5 \pm 0.08 respectively, $t = 3.6$, $p < 0.001$; $t = 7.11$, $p < 0.001$ respectively). Also, it was higher in non-renal patients than controls ($t = 4.1$, $p < 0.001$). Urinary RBP was inversely correlated to corrected creatinine clearance ($r = -0.55$, $p < 0.05$) and positively correlated to SLEDAI score, ESR, total protein and albumin in urine ($r = 0.38$, $p < 0.05$; $r = 0.41$, $p < 0.05$; $r = 0.64$, $p < 0.05$; $r = 0.58$, $p < 0.05$ respectively). From the 7 non-renal SLE patients who had urinary total protein < 0.2 gm/24 hrs and no increase of albumin in urine, there were 5 patients (71.4%) with increased urinary RBP. The diagnostic sensitivity of urinary RBP, total protein and albumin in urine were 82%, 59% and 77% respectively. So, RBP held the best predictive value among other parameters in this study. From a prognostic point of view, Z score analysis of studied parameters revealed the importance of RBP in the follow up of non-renal SLE patients.

Conclusion: Urinary RBP is increased in SLE patients whether demonstrating evidence of renal disease or not. The increased urinary RBP in a large proportion of patients who had no other evidence of renal involvement could reflect early subclinical nephropathy. In renal SLE patients, RBP correlated positively to other parameters of disease activity and severity such as SLEDAI score, urinary total protein and albumin, and correlated negatively to corrected creatinine clearance. So its measurement seems to be useful in early diagnosis and subsequent monitoring of renal disease activity in SLE.

Key words: retinol binding protein, SLE, nephritis, urine, children.

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INTRODUCTION

Morphological renal changes are present in almost all patients with systemic lupus erythematosus (SLE). Yet, only 50-70% develop clinical renal disease¹. Renal biopsy may also show histological

finding of early nephropathy even in absence of any clinical findings². A proportion of these patients with clinically silent nephropathy will later go to develop overt renal disease.

Urine protein measurement has been used to screen for the presence of renal disease either by quantitative measurement of total protein or dipstick analysis. Now it is possible to measure specific proteins as markers of glomerular and tubular disease to facilitate the earlier detection of renal disease^{3,4}. The few reports describing the use of specific urine protein measurement in SLE are conflicting. Parving et al.⁵ did not find any significant difference in the urinary excretion of either albumin or beta₂-microglobulin when compared with the control. Conversely, Terai et al.⁶ found that urinary albumin concentration was significantly higher in SLE patients with normal renal function than in controls.

Retinol binding protein (RBP) is one of the low molecular weight proteins. RBP is freely filtered by the glomerulus and is then almost completely absorbed and catabolized by proximal tubular cells. Any increase in urine excretion of RBP is highly specific of renal tubular disease⁷.

The aim of this study was to assess urinary RBP in SLE patients with and without evidence of renal disease. Also, we sought to anticipate the value of urinary RBP in detection of subclinical nephropathy and if its measurement would have a role in the early diagnosis and subsequent monitoring of renal disease activity in SLE.

METHODS

This study was conducted on 22 patients with SLE who were followed up at the Pediatric Allergy and Immunology Unit, Children's Hospital, Ain Shams University. All of them were fulfilling the 1982 American College of Rheumatology Revised Criteria for the diagnosis of SLE⁸. They were all females, their ages ranged between 6 and 18 years (mean: 14.4±3.6 years). The mean duration of illness was 4.4±2.8 years (range 0.4-11 years).

Patients were categorized into two groups:

Group I (non-renal SLE patients): It included seven patients who had no clinical symptoms of renal disease. All had negative protein dipstick tests, protein in urine < 0.2 gm/24 hrs, and no evidence of microscopic or macroscopic hematuria, pyuria, or urinary casts, and normal serum creatinine concentrations.

Group II (renal SLE patients): It included fifteen patients with lupus nephritis. Renal disease was diagnosed by the presence of one or more of the following: protein in urine ≥ 0.2 gm/24 hrs, hematuria, pyuria, urinary casts (red cell, hemoglobin, granular, tubular or mixed casts) and/or abnormal serum creatinine concentrations.

Eight patients were diagnosed as lupus nephrotic syndrome with proteinuria > 2 gm/24 hrs. Patients were excluded from the study if their serum creatinine was > 3 mg/dL or if they had urinary tract infection. Twenty-one patients were receiving prednisone (1-2 mg/Kg/day) either alone (n= 13) or in combination with other immunosuppressives, predominantly intravenous cyclophosphamide (n= 5) or azathioprine (n = 4).

Control group: Results of the previous two groups were compared with those of 18 age and sex matched clinically healthy subjects as the control group. Their ages ranged between 10 and 18 years with a mean age of 14.4±2.5 years.

Methods

Subjects in the study underwent the following:

- 1- **History taking:** laying stress on age, duration of the disease, urinary symptoms, SLE manifestations (e.g. joint pains, rash, cutaneous photosensitivity, Raynaud's phenomenon, CNS symptoms including seizures), symptoms of hypertension as vomiting, headache, blurred vision, urine volume per 24 hrs and the type of therapy received by the patients.
- 2- **Clinical examination** including anthropometric measures for the weight, height and surface area by using nomogram for the latter⁹, blood pressure measurement, skin rash distribution, joint affection, chest and heart examination, abdominal examination for hepatosplenomegaly and CNS examination especially for the level of consciousness, motor and sensory systems.
- 3- **Assessment of SLE activity** was done by using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)¹⁰.
- 4- **Collection of samples:**
 - a- Urine samples: twenty-four hours urine sample was taken for estimation of urinary creatinine, total protein, microalbumin and RBP. The urine sample was collected in sterile containers with no preservatives. It was stored at - 20°C through the time of collection. On the other hand, complete urine analysis was done on a freshly collected sample.
 - b- Blood samples: six ml of venous blood were collected. Four ml were transferred into a clean dry tube and left to clot. Prompt separation of serum was carried out by centrifugation at 1500 rpm and used for creatinine assay, serum ANA, anti- DNA and C3 assay. Another two milliliters were collected on Na citrate for ESR.

5- *Laboratory investigations: including*

- a- Complete microscopic urine analysis for WBCs, RBCs and casts.
- b- Erythrocyte sedimentation rate (Westergren method)
- c- Assay of serum and urinary creatinine were carried out on Synchron CX7 autoanalyzer (Beckman Instruments, Brea, California, USA) by modified rate Jaffe method¹¹. The corrected creatinine clearance was then calculated.
- d- Assay of serum ANA, anti- DNA for SLE patients. They were detected by indirect immunofluorescence supplied by IMMCO Diagnostics (USA)¹². Also estimation of serum C3 was done by quantitative determination using the turbidimetry (Behringwerke Diagnostics, Marburg, Germany)¹³.
- e- Determination of urinary total protein (TP) by the turbidimetric method using TP kit supplied by Stanbio (Stanbio Laboratory Inc., San Antonio, TX, USA).
- f- Determination of urinary microalbumin by the single radial immunodiffusion method, using VLC-partigen kit supplied by Behring (Behring Werk, Ag-Hamburg: Institutio Behring, S.P.A. 67019 Scopito)¹⁵.
- g- Determination of RBP: This was measured by the enzyme-linked immunosorbent assay (ELISA) kit for RBP supplied by United Biotec Inc., 10010 Pianeer Way C, Mountain Diew CA, USA. The principle of this assay is sandwich enzyme technique, where the constituted standard or diluted sample was added to microtitre plate wells precoated with antibody specific for human RBP. After this incubation step, the plate is washed and an antibody conjugated to horse-radish peroxidase is added for a further incubation. Following a final wash step, substrate solution is incubated in the wells resulting in a colored product which can be measured at 450 nm after quenching with acid. The color intensity is proportional to the amount of RBP present.

Urine total protein, albumin in urine and urinary RBP concentrations were expressed as a ratio to creatinine to correct for variations in urine flow rate.

6- *Statistical methods:*

The results were analyzed by commercially available computers software package (StatView, Abacus Concepts, Inc, Berkley, CA, USA). Data are given as mean and standard deviation (SD). As most of data were skewedly distributed, logistic t test was used. The degree of association between the various variables was evaluated using

Spearman's rank correlation coefficient (r). The diagnostic reliability testing was done through calculation of diagnostic sensitivity and specificity to elucidate the best cut off value for each parameter. Z score analysis was done to examine the prognostic utilities of the studied parameters and to find which parameter can be used as a prognostic marker.

RESULTS

Comparison between SLE patients and controls

The results of this study showed that urinary RBP, total protein and albumin were significantly higher in SLE patients compiled as one group than controls. Non-renal SLE patients and controls were quite comparable in terms of corrected creatinine clearance, total protein and albumin in urine. However, urinary RBP was significantly higher in non-renal SLE patients than controls. The corrected creatinine clearance, urinary total protein, albumin and RBP were significantly higher in renal SLE patients than controls (table 1).

Comparison between non-renal and renal SLE patients

There was no significant difference between non-renal and renal SLE patients as regards age and disease duration. However, SLEDAI score, urinary total protein, albumin and RBP were significantly higher in renal than non-renal patients. While the corrected creatinine clearance was significantly lower in renal than non-renal SLE patients. (table 2).

Urinary RBP was significantly higher in renal SLE patients than both non-renal SLE patients and controls ($t = 3.6$, $p < 0.001$; $t = 7.11$, $p < 0.001$ respectively). Also, it was higher in non-renal SLE patients than controls ($t = 4.1$, $p < 0.001$) (Fig. 1).

Correlation between urinary RBP and other parameters

Urinary RBP was positively correlated to SLEDAI score ($r = 0.38$, $p < 0.05$) and to ESR ($r = 0.41$, $p < 0.05$). Significant positive correlations were also found between urinary RBP, total protein and albumin in urine ($r = 0.64$, $p < 0.05$; $r = 0.58$, $p < 0.05$ respectively), whereas a significant negative correlation was found between urinary RBP and the corrected creatinine clearance ($r = -0.55$, $p < 0.05$) (Fig. 2).

Sensitivity, specificity and prognostic value of urinary RBP

The diagnostic sensitivity and specificity were done to determine the studied parameter with the

higher predictive value for renal involvement in our SLE patients. The cut-off value for RBP was 0.66 mg/g Cr, for TP was 0.5 g/g Cr and it was 2.1 mg/g Cr for microalbumin in urine. Urinary RBP had

higher sensitivity than urinary TP and albumin (table 3).

Table 1: Comparison of laboratory parameters between SLE patients and controls.

Parameter	SLE Patients as a whole (n= 22)	Non-renal SLE Patients (n= 7)	Renal SLE Patients (n= 15)	Controls (n= 18)	SLE vs controls		Non renal vs controls		Renal vs controls	
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	t	p	t	p	t	p
CCC (ml/min)	98.9 ±37.2	119.3 ±2.0	89.3 ±40	105 ±11.5	1.4	>0.05	1.65	>0.05	1.96	<0.05
TP in urine (g/g Cr)	2.8 ±3.2	0.076 ±0.05	4.0 ±3.0	0.09 ±0.12	5.6	<0.001	0.76	>0.05	10.5	<0.001
Albumin in urine (g/g Cr)	1.4 ±1.6	0.023 ±0.019	2.0 ±1.6	0.022 ±0.013	6.5	<0.001	0.29	>0.05	39.9	<0.001
Urinary RBP (mg/g Cr)	0.99 ±0.3	0.75 ±0.15	1.1 ±0.32	0.5 ±0.08	6.9	<0.001	4.1	<0.001	7.11	<0.001

CCC: corrected creatinine clearance.

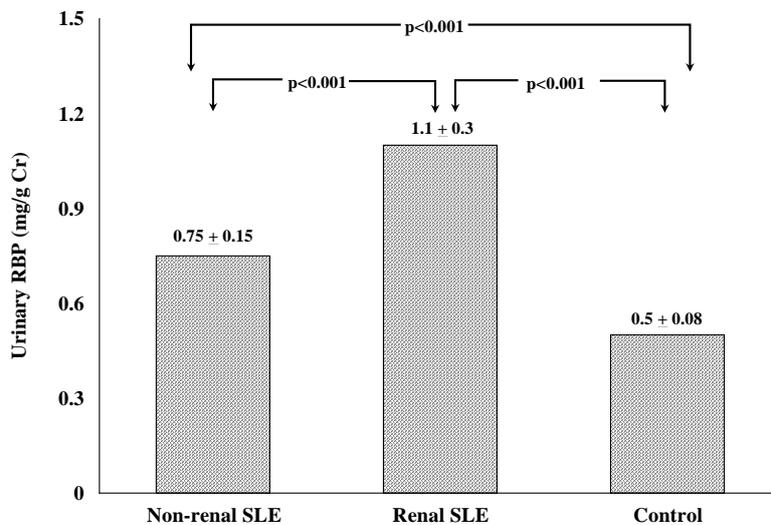


Figure 1: Urinary RBP mean values in the studied groups.

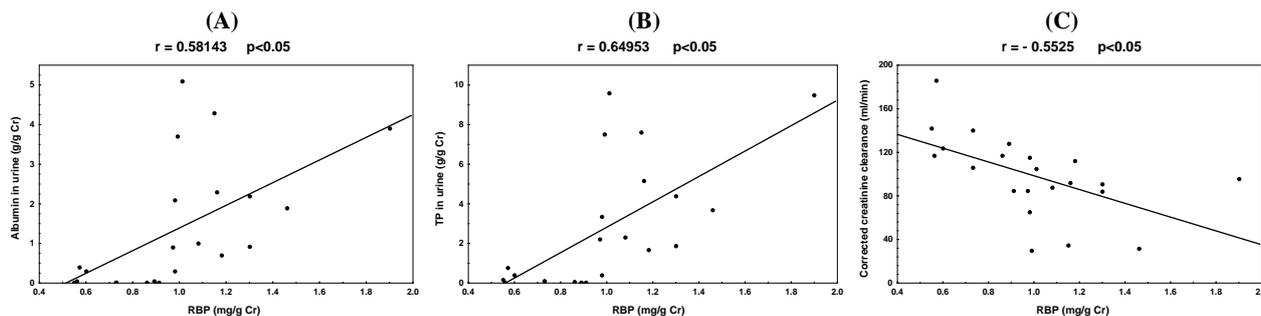


Figure 2: Correlation between RBP and albumin in urine (A), TP in urine (B) and corrected creatinine clearance (C).

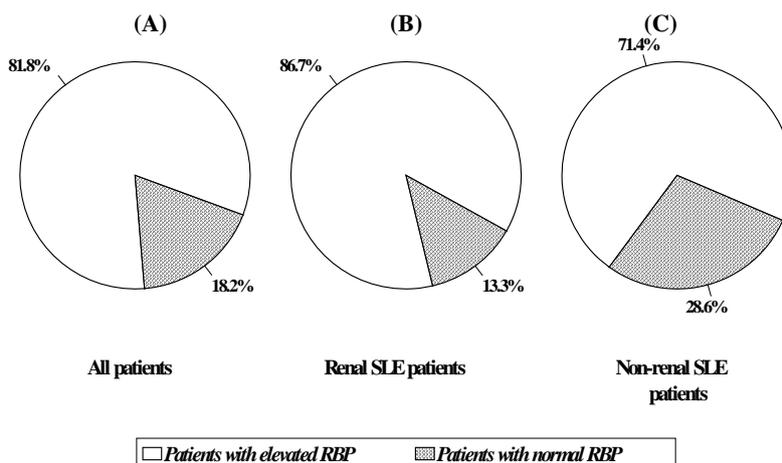


Figure (3): Percentage of patients having elevated RBP among SLE patients compiled as one group (A), renal SLE patients (B) and non-renal SLE patients (C).

Table 2: Comparison of different studied parameters between non-renal and renal SLE patients.

Parameter	Non-renal SLE Patients (n= 7)	Renal SLE Patients (n= 15)	Renal vs non -renal	
	mean ± SD	mean ± SD	t	p
Age (years)	14.7 ±3.9	14.3 ±3.6	0.02	>0.05
Disease duration (years)	3.2 ±2.4	5 ±2.9	0.82	>0.05
SLEDAI	24.6 ±7.0	41.9 ±12.8	4.08	<0.001
CCC (ml/min)	119.3 ±20.0	89.3 ±40	2.6	<0.001
TP in urine (g/g Cr)	0.076 ±0.05	4.0 ±3.0	9.8	<0.001
Albumin in urine (g/g Cr)	0.023 ±0.019	2.0 ±1.6	29.7	<0.001
Urinary RBP (mg/g Cr)	0.75 ±0.15	1.1 ±0.32	3.6	<0.001

Table 4 shows the Z scoring of the studied parameters. In the renal SLE patients, both TP and albumin in urine preceded urinary RBP. While, in non-renal SLE patients urinary RBP lies in the first place and is followed by corrected creatinine clearance and albumin in urine. So, from a prognostic point of view, Z score analysis revealed the importance of RBP in the follow up of non-renal SLE patients.

Table 3: The predicting parameters of renal involvement in SLE patients.

Parameter	Cut off value	Diagnostic sensitivity	Diagnostic specificity
Urinary RBP	0.66 mg/g Cr	82%	100%
TP in urine	0.5 g/g Cr	59%	100%
Albumin in urine	21 mg/g Cr	77%	100%

Table 4: Z scoring of the studied parameters.

Non-renal SLE Patients (n= 7)		Renal SLE Patients (n= 15)	
Parameter	Z score mean± SD	Parameter	Z score mean± SD
Urinary RBP (mg/g Cr)	3.1±1.89	TP in urine (g/g Cr)	31.7±26
CCC (ml/min)	1.2±1.7	Albumin in urine (g/g Cr)	20±15.7
Albumin in urine (g/g Cr)	0.86±0.19	Urinary RBP (mg/g Cr)	7.7±4.1
TP in urine (g/g Cr)	-0.11±0.4	CCC (ml/min)	-1.4±3.5

From the studied 22 SLE patients, there were 18 patients (81.8%) showing increased urinary RBP level. From those with high urinary RBP, 8 patients (44.4%) did not have total proteinuria in excess of 1 gm/24 hrs, and 4 patients (22.2%) had no increase of albumin in urine (Fig. 3A). From the 15 renal SLE patients studied, there were 13 patients (86.7%) with high and 2 patients (13.3%) with normal RBP, while all of them showed increased

level of albumin in urine (Fig. 3B). From the studied 7 non-renal SLE patients, who had total protein <0.2 gm/24 hrs and no increase of albumin in urine, there were 5 patients (71.4%) with increased urinary RBP level (Fig. 3C).

DISCUSSION

Renal disease is a common manifestation of SLE. Although the multiple immunologic abnormalities of lupus can affect virtually any organ system, involvement of the kidneys is often a major source of patient's morbidity and mortality. Renal disease in SLE is extremely diverse, ranging from asymptomatic urinary finding to fulminate renal failure or florid nephrotic syndrome. Pathologic alterations may affect the glomerular, tubular, tubulointerstitial and vascular compartments¹⁷.

Urinary RBP is used to detect the prevalence of proximal tubular dysfunction as RBP is almost completely reabsorbed and catabolized by proximal tubular function. So, the disturbance of tubular function may therefore lead to elevation of urinary RBP excretion^{18,19}.

Our study revealed that proximal tubular dysfunction measured by increased urinary RBP was present in 18 out of 22 SLE patients (81.8%), 13 of 15 (86.7%) renal SLE patients and 5 of 7 (71.4%) non-renal SLE patients. Sesso et al.¹⁹ demonstrated raised urinary RBP in 35 % (25 out of 70) of patients with SLE and 75% of patients with SLE and active nephritis. In their study, 59% (41 out of 70 patients) were classified as having active or probably active nephritis and of the 25 patients having a raised urinary RBP concentration, 19 had proteinuria greater than 1 g/24 hrs. In the study of Guy et al.³, 14 out of 40 (36%) SLE patients had an elevated urinary RBP, and 7/40 (17.5%) of their patients had evidence of renal disease and only 3/40 had a random urine total protein greater than 0.5 g/L. Our results revealed a significantly higher urinary RBP levels in patients with non-renal SLE when compared to controls, whereas differences in corrected creatinine clearance, total proteins in urine, and albumin in urine did not reach statistical significance. Similar data were reported by Guy et al.³. In the absence of any evidence of renal dysfunction, such increase in urinary RBP could indicate subclinical nephropathy¹⁹. In our study, urinary RBP of renal SLE patients was significantly higher than that of the controls. Sesso et al.¹⁹ using the BILAG score, found a significant trend of increasing urinary RBP values for patients classified as having no renal disease, stable renal disease, probably active, and active lupus nephritis respectively. Patients with active nephritis had

significantly greater urinary RBP values than the other groups showing that tubular dysfunction is associated with renal activity. Furthermore, in a subgroup of patients, they observed that urinary RBP returned to normal values after the improvement in the status of the renal disease¹⁹. It is not justifiable to perform a renal biopsy in most patients with mild or stable renal disease and it would not be ethical to do it in those patients without current evidence of renal disease. As a result they used a clinical-laboratory index for the measurement of renal disease activity which is the British Isles Lupus Assessment Group (BILAG)²⁰. The BILAG score and other clinical-laboratory indices of renal activity have not incorporated the evaluation of tubulointerstitial dysfunction. Since this abnormality is common in SLE patients with active renal disease, it is suggested that the measurement of this parameter should be more often used in the assessment of renal involvement. Of particular interest, was their finding of a significant increase of urinary RBP in patients with active nephritis compared to those with stable renal disease. Another possible contribution of urinary RBP testing compared with the BILAG score is that it appears to yield additional evidence to better distinguish patients with active from those with 'probably active' nephritis.

In our series, out of the 7 non-renal SLE patients having urinary total protein concentrations less than 0.2 g/L (i.e. approximating to a negative Albustix test), 5 had elevated urinary RBP and none had increased albumin in urine. In the study of Guy et al.³, 29 patients had urine total protein concentrations less than 0.2 g/L, 14 of them had elevation of RBP/creatinine or albumin/creatinine ratios. Interestingly, only 2 of these 14 patients had elevation of both. Using a lower total protein concentration of 0.15 g/L as the cut-off level made little difference in the interpretation of results. In the absence of any evidence of renal dysfunction, these increases in urinary albumin and RBP could indicate subclinical nephropathy. Furthermore, as RBP and albumin are handled differently by the kidney²¹, isolated increases in their excretion may be reflecting differences in renal pathology e.g. of either a glomerular or tubular origin. It is also interesting to note that in the Guy et al.³ study, whilst all the seven patients with renal disease had elevated albumin/creatinine ratios in urine, only 4 had an increase in urinary RBP/creatinine ratios. This might suggest a change in the pattern of protein excretion in progressing from subclinical to overt renal disease or alternatively in response to treatment. Further studies are required to establish

this. In our study, 15 patients had renal SLE; all of them had elevated urinary albumin and 13 (86.7%) had an elevated urinary RBP.

Renal tubular damage in the absence of any significant glomerular changes has been thought to occur rarely in SLE and has been reported in only a few patients^{22,23}. Our results showed that 4 (22.2%) out of the 18 studied SLE patients demonstrated elevation in urinary RBP, but not in albumin. Guy et al.³ found that approximately one-quarter of their patients demonstrated elevations in urinary RBP but not in albumin. It is possible therefore, that such elevations could represent early changes in tubular function prior to the development of nephropathy. Serial measurements in individual patients would be required to confirm this.

It has been suggested that greater glomerular filtration load of proteins is associated with tubulointerstitial damage in patients with glomerulonephritis^{24,25,26}. We observed a significant correlation between urinary RBP and total proteinuria in SLE patients, and similar results were reported by Sesso et al.¹⁹. Our data showed that there are 8 patients (44.4%) from those 18 with increased urinary RBP, did not have total proteinuria in excess of 1g/24 hrs, which is usually a reflection of glomerular disease. In the study of Sesso et al.¹⁹, 6 of the 25 patients with increased urinary RBP did not have total proteinuria in excess of 1g/24 hrs. This enforces the concept that tubular dysfunction may occur in the absence of significant glomerular proteinuria. Also in the Sesso et al.¹⁹ study, multivariate analysis adjusting for differences in creatinine clearance, total proteinuria, blood pressure and duration of disease, confirmed that increased urinary RBP was significantly associated with active nephritis. Such increased urinary RBP detected when renal disease is active cannot be completely explained by the effects of the above-mentioned factors. It seems probable that the immune mechanisms responsible for the renal aggression affecting simultaneously glomeruli and tubulo-interstitial region secondarily promote tubular dysfunction.

In lupus nephritis, most emphasis has previously been placed on glomerular changes and some earlier reports made little or no mention of tubular lesions²⁷. It is now well established that both tubulointerstitial and glomerular abnormalities occur in SLE^{28,29}. For instance, O'Dell et al.²⁹ reported that 51 % of SLE patients with clinically apparent renal disease showed additional tubulointerstitial lesions. Similarly, Brentjens et al.²⁸ demonstrated interstitial abnormalities in 50-70% of patients with lupus nephritis. Other markers have

reported specific tubular abnormalities in patients with SLE and glomerulonephritis such as impaired ability of the kidney to concentrate and acidify urine³⁰, defects in hydrogen ion and electrolyte handling^{31,32}, and increases in the fractional excretion of beta₂-microglobulin^{30,33}. ter Borg et al.³³ also showed a fall in fractional excretion of beta₂-microglobulin to original levels during treatment suggesting a reversibility of the tubular lesion.

In the present study, evaluation of the diagnostic performance of the studied markers revealed the superiority of the RBP in the prediction of renal involvement in SLE patients. Urinary RBP has been shown to be a sensitive marker of proximal tubular dysfunction in several clinical situations^{18,26}. Compared to other markers of the tubular damage, urinary RBP has been shown to be more sensitive than beta-n-acetyl glucosaminidase, and because it is more stable in acid urine, it is a more practical analyte to measure than beta₂-microglobulin¹⁸. We calculated the Z score for each parameter to find the most prognostic one that can be used in future follow up protocols to predict the renal affection. Urinary RBP followed by corrected creatinine clearance were found to be the best.

In conclusion, urinary RBP seems to be a marker of lupus nephritis activity, that should be monitored in combination with other parameters of renal function in SLE patients. In SLE patients with no clear evidence of active renal disease, the finding of increased urinary RBP suggests subclinical nephropathy and hence the need for a more aggressive treatment, while normal RBP excretion could lead to a more conservative approach. In SLE patients with evidence of active renal disease, measurement of urinary RBP may be useful in monitoring the progress of active nephritis or its response to treatment. Clearly, a prospective study is required to confirm this.

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