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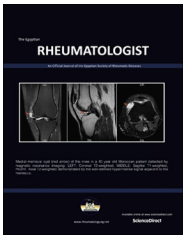

ORIGINAL ARTICLE

Urinary podocalyxin and nephrin levels as biomarkers in lupus nephritis patients: Relation to renal involvement and disease activity

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KEYWORDS

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Abstract *Aim of the work:* To evaluate the impact of systemic lupus erythematosus (SLE) on urinary levels of podocalyxin and nephrin and to determine their relationship to renal biopsy and disease activity in lupus nephritis (LN) patients.

Patients and methods: The study included 50 LN patients with their renal biopsy classified according to the international society of nephrology. Disease activity was determined using the British Isles Lupus Assessment Group (BILAG). All patients underwent clinical and laboratory evaluation. Urine samples were collected for the assessment of urinary podocalyxin (UPx) and nephrin (UN) by ELISA and for the estimation of protein (UP) and creatinine (Cr) concentrations. The UPx:Cr, UN:Cr and UP:Cr ratios were calculated.

Results: Urinary levels of podocalyxin (593.8 ± 282.2 ng/ml), nephrin (304.1 ± 236.8 ng/ml) and protein (2.36 ± 0.56 g/l) were significantly higher, while urinary creatinine levels (101.4 ± 28.7 mg/l) lower in LN patients compared to control (38.1 ± 9 ng/ml, 19.2 ± 4.1 ng/ml, 0.34 ± 0.13 g/l and 155.4 ± 26.7 mg/l; $p = 0.0008$, $p = 0.0003$, $p = 0.00002$ and 0.0009, respectively). Consequently, UNCr, UPxCr and UPCr ratios were significantly higher in patients compared to control. There was a significant correlation of the estimated ratios with the LN class and with the BILAG scores being most significant with UPx:Cr ratio. ROC curve and regression analyses defined UPx:Cr ratio as the specific significant predictor of pathological LN grade.

Conclusion: SLE deleteriously affects fine glomerular structure as reflected by increased urinary levels of podocyte-related proteins; podocalyxin and nephrin. Urinary podocalyxin/creatinine ratio

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significantly predicts the pathological impact of SLE on the kidney and could be used as a non-invasive marker for such effect and its progression.

1. Introduction

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease characterized by the production of antinuclear antibodies [1]. Lupus nephritis (LN) patients present with proteinuria that has generally been associated with immune complex deposition in the glomerular capillary wall and endo-capillary proliferation and inflammation [1]. Many studies focused on the importance of finding potential biomarkers, to estimate the degree of LN in SLE patients, such as serum cystatin C and urinary neutrophil gelatinase-associated lipocalin [2], cytokines [3], markers of oxidative stress [4], matrix metalloproteinase [5], adipokines [6,7] and growth arrest specific protein 6 [8]. The search for reliable easily measured markers is still necessary and ongoing.

Podocytes are highly differentiated glomerular epithelial cells that line the outside of the glomerular capillary with foot processes linked to the glomerular basement membrane with their actin cytoskeleton [9]. The foot processes form a characteristic inter-digitating pattern leaving filtration slits in-between. Integrity of this filtration barrier is important in order to prevent the loss of protein into the urine [10]. Podocyte injury is an important feature of several renal diseases. Regulation of the podocyte actin cytoskeleton is of critical importance for sustained function of the glomerular filter and is mediated by several podocyte proteins such as nephrin and podocin [11,12]. Several markers of podocyte injury include nephrin, synaptopodin, podocalyxin and podocin [13,14]. The relationship between urinary podocyte protein and renal diseases is supported by their detection in patients with immunoglobulin A nephropathy, Henoch–Schönlein purpura nephritis, lupus nephritis, diabetic nephropathy and focal segmental glomerulosclerosis. The detection of urinary markers of podocyte injury would have broad implications for the evaluation of disease activity, the degree of dedifferentiation and the possibility of podocyte regeneration [15].

The aim of the current study was to evaluate the impact of SLE on fine glomerular architecture using podocyte injury related markers; podocalyxin and nephrin in urine and to determine their relationship to pathological classes of renal biopsy and disease activity.

2. Patients and methods

This study was conducted at Rheumatology, Internal Medicine and Clinical Pathology departments, Dallah and Ibn Sina College Hospitals, Kingdom of Saudi Arabia (KSA). The study included 50 lupus nephritis (LN) patients diagnosed by previous renal biopsy. The research protocol was approved by the Ethics Committee of our hospitals and informed written consents were obtained from all participants. Systemic lupus erythematosus (SLE) was diagnosed according to the systemic lupus international collaborating clinics (SLICC) classification criteria for SLE [16]. All patients underwent full clinical examination and laboratory tests were performed including complete blood count (CBC), C-reactive protein (CRP), complement (C3 and C4), anti-nuclear antibodies (ANA) and anti-double stranded deoxyribonucleic acid (anti-dsDNA). Anemia was defined by a hemoglobin (Hb) concentration of $\leqslant$12 g/dl for women and of $\leqslant$13.5 g/dl for men [17]. Proteinuria was measured by a dipstick method. The study also included 20 matched normal subjects free of renal disease as control group for estimated urinary biomarkers.

Disease activity was determined using the British Isles Lupus Assessment Group (BILAG) score which consisted of evaluation of 8 points of interest: general, muco-cutaneous, neurological, musculoskeletal, cardio-respiratory and renal manifestations, vasculitis and hematological findings. To obtain a global score, BILAG component scores are assigned numerical values: $A = 9$ (most active disease), $B = 3$ (intermediate activity), $C = 1$ (mild, stable disease activity), $D = 0$ (inactive disease) and $E = 0$ (no activity), resulting in a potential summed range of 0–72 points with 72 = most active disease affecting the 8 organs [18].

Lupus nephritis was diagnosed depending on the presence of proteinuria and hematuria [19] and renal biopsy histopathology was classified according to the International Society of Nephrology/Renal Pathology Section (ISN/RPS) classification as minimal mesangial (class I), mesangial proliferative LN (class II), focal LN (class III), diffuse LN (class IV), membranous LN (class V) and advanced sclerosis (class VI) [20].

2.1. Urine sample collection

Urine (10 ml) was collected in plastic tubes, without preservative. Samples were clarified by centrifugation at 3.000 rpm for 5 min and supernatant collected in Eppendorf tubes and kept frozen at −80 °C till assayed for:

(1) *Urine protein concentrations* were measured by the Bradford method [21].
(2) *Estimation of urinary markers of podocyte injury*: urinary podocalyxin and urinary nephrin was estimated using commercially available ELISA kits (Exocell Inc., Philadelphia, PA). Urine samples were diluted with dilution buffer provided by the ELISA kits in a ratio of 1:2 for urinary podocalyxin [9] and 1:1 for Urinary nephrin [22]. Each sample was measured in duplicate. The values are expressed as ng/ml.
(3) *Urine creatinine* was measured by the Jaffe reaction on the same aliquot of urine to [27] adjust the ratio of urinary podocalyxin to creatinine (UPx:Cr), urinary nephrin to creatinine (UN:Cr) and urine total protein-to-creatinine ratio (UP:Cr).