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Urinary podocin; is it a valuable disease activity biomarker in patients with lupus nephritis?

Maha A. Behairy[1], Abdelbassit ElShaarawy[1], Somia Bawady[2], Fatma Elsayed[1], Walid A Bichari[1*]

[1]Internal Medicine and Nephrology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2]Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

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ABSTRACT

Introduction: Podocyturia can be considered as a noninvasive marker for evaluation and follow up of glomerular disease progression.

Objectives: In this study, we aimed to assess the clinical utility of urinary podocin as an index of lupus nephritis activity.

Patients and Methods: This cross-sectional study included 45 patients with systemic lupus erythematosus (SLE). Patients were subdivided into three groups: group (I) 10 SLE, patients without clinical or laboratory evidence of lupus nephritis (LN), which were assessed by Systemic Lupus Activity Measure (SLAM) score of the disease activity. Group (II), which included 15 patients with evident active LN before starting the immunosuppressive induction treatment and group (III) which is consisted of 20 patients with LN in partial or complete remission. Urinary podocin assay was conducted by enzyme-linked immunosorbent assay enzyme-linked immunosorbent assay (ELISA) technique.

Results: There was a statistically significant difference between the studied groups regarding urinary podocin levels. The mean of urinary podocin (ng/mL) was (2.29 ± 0.71, 37.20 ± 14.38, 10.5 ± 2.30; $P \leq 0.001$) in the three groups consecutively, with significant decrease of urinary podocin in LN patients after remission versus high level in patients with active LN. Highly significant positive correlations were found between urinary podocin and global SLAM activity (r = 0.852; $P \leq 0.001$), SLAM-Renal score (r= 0.854; $P \leq 0.001$), urine albumin to creatinine ratio, (mg/g) (r=0.895; $P \leq 0.001$). Highly significant negative correlations of urinary podocin and C3 (r=0.803; $P \leq 0.001$), C4 (r= -0.760; $P \leq 0.001$) and eGFR (r = -0.759; $P \leq 0.001$) were detected.

Conclusion: Urinary podocin as non-invasive biomarker is significantly correlated to SLE disease activity and LN activity measured by global SLAM clinical score with both high sensitivity and specificity. Urinary podocin can be also considered as a prognostic marker in the management of LN patients.

Original Article

Implication for health policy/practice/research/medical education:
Our cross-sectional study included 45 patients with systemic lupus erythematosus (SLE), demonstrated the clinical significance of urinary podocin as a non-invasive biomarker to detect the mild activity of lupus nephritis, severity of lupus nephritis and response to treatment..

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Introduction

Lupus nephritis (LN) occurs in nearly about 40% of systemic lupus erythematosus (SLE) cases (1). LN is the most common major sequence of SLE with an elevated risk of death and end-stage renal disease (2). Nearly 35% to 50% of patients with SLE have evidence of renal disease at presentation, which increases later to be more than 60% of cases during the following up process. LN significantly affects African Americans, Afro-Caribbean's, Asians and Hispanics more than white Caucasians (3).

Diagnosis of LN depends on clinical, laboratory and renal biopsy findings. Most cases of LN are diagnosed by persistent proteinuria more than 0.5 g/d, proteinuria more than 3+ by dipstick and /or cellular casts including red

*Corresponding author: Walid A Bichari,
Email: bichari2005@gmail.com

blood cells (RBCs), granular and active urinary sediment. Renal biopsy is a cornerstone procedure and commonly shows immune complex-mediated glomerulonephritis compatible with LN (3).

Estimated glomerular filtration rate (eGFR), proteinuria and regular interval examination of the urine sediment are the main follow up investigations. There are many formulas employed to determine eGFR, the Modification of Diet in Renal Disease (MDRD) equation, Cockcroft-Gault, or CKD-EPI equations and modified Schwartz formula (4). In addition to other laboratory tests for SLE disease activity such as antibodies to double-stranded DNA (dsDNA), complement (C3, C4), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (5).

Management of SLE is a dynamic process. The improvement of disease status or minimally stopping its deterioration are the main goals. Consequently, it is very important to be precise in determining disease activity and exacerbation and state evidence-based and clinically significant response criteria measured with valid and reproducible techniques (6).

The podocyte mass is small compared to the whole kidney mass. It forms a part of the glomerular filtration barrier. Podocytes have multiple foot processes that interdigitate with each other to form the slit diaphragm through which the plasma is filtered (7). Podocytes are separated from the glomerular basement membrane (GBM) and are found in the urine of patients with different glomerular diseases, which lead to a decrease in the number of podocytes at the GBM. Podocyte loss at the GBM is associated with proteinuria, since podocyturia was found to occur earlier in the course of glomerular disease than proteinuria (8). However, there are some disadvantages for assay of podocyturia, while the available methods for detection of podocyturia are time-consuming, expensive and require a specific kind of microscopes, in addition to skilled staff. Molecular biology techniques demand suitable reagents, antibodies and proper techniques.

A novel biological marker is a genetic or a chemical substance that can be easily measured and its level is associated with physiological or pathological events. Recently, significant effort has been put to identify an accurate, easily measurable, non-invasive biomarker that reflects renal disease activity, predicts flares and correlates with renal histology in lupus nephritis to guide therapeutic decisions (9).

One of the major structural proteins of the GBM is called podocin, which contributes to the stability of the slit diaphragm and guarantees the stable anchorage of the membrane complexes to the actin cytoskeleton. Podocin, a 42-kDa membrane protein, is located on the podocyte foot process where it is COOH terminus combined with the transmembrane protein nephrin and CD2-associated protein (10).

Objectives

The present study aimed to evaluate the clinical utility of urinary podocin as a marker of LN activity, its association with global SLE disease activity and its relationship with the response to treatment.

Patients and Methods

Study design

This study was conducted on 45 adult patients recruited from Ain-Shams University hospitals, who were diagnosed with SLE based on four or more criteria of the American College of Rheumatology (ACR). Patients were selected from Ain Shams University hospitals, Cairo, Egypt, outpatient clinics and inpatient wards.

A cross-sectional study was carried out including 45 patients, where they were divided into three groups:

- Group I; ten SLE patients without clinical or laboratory evidence of LN were assessed by Systemic Lupus Activity Measure (SLAM) score of the disease activity.
- Group II; fifteen SLE patients with active LN not on immunosuppressive medications yet.
- Group III; twenty SLE patients with LN were achieved clinical remission, either partial or complete. A complete remission is proved by the absence of active urine sediments (more than 8 to 10 erythrocytes per high power field) or casts, non-nephrotic range proteinuria (less than 1 g/24 h), serum creatinine levels for males less than 1.3 mg/dL and for females less than 1.1 mg/dL. Patients with diabetes mellitus, uncontrolled hypertension, fever or acute infection, with an evident history of congestive heart failure and malignancy were excluded.

According to the World Health Organization (WHO) classification based on renal biopsy in lupus patients, group II; consisted of five patients in class III and 10 patients in class IV of LN. Group III; included seven patients in class III of LN and 12 patients in class IV of LN and one patient in class V of LN.

All the studied patients were subjected to complete history taking and thorough clinical examination. SLE disease activity was measured in all patients, employing the SLAM index, which depends on specific manifestation in nine organs/systems, plus seven laboratory assays [hematocrit (%), white blood cell count (per mm^3), lymphocyte count (per mm^3), platelet count (×1000 per mm^3), Westergren ESR (mm/h), serum creatinine (mg/dL) or creatinine clearance (mL/min/1.73 m^2)]. Organ manifestations are scored 0-3 points if present within the last month (severity takes a higher score per item).

The laboratory category makes a score of a maximum of 21 points. One of its limitations is that many points are subjective because the scoring depends on the reporting of symptoms by the patients rather than examination. For this index, a score of more than seven is considered clinically important because it indicates the probability of starting therapy in 50% of cases (11). The formula is body mass index (BMI) = kg/m^2 where kg is a person's weight in kilograms and m^2 is the height in meters squared (12). Estimated GFR was calculated by MDRD equation for adults as; eGFR (mL/min/1.73 m^2) = 186×(PCR)-1.154×(age)-0.203×(0.742 if female)×(1.210 if African American), where PCR is plasma creatinine (mg/dL).

Five milliliters of venous blood were collected under complete aseptic precautions. It was divided between an ethylenediamine tetraacetic acid (EDTA) tube and a plain test tube without an anticoagulant. EDTA tube was utilized to conduct complete blood count (CBC), after clotting, plain test tubes were centrifuged at 4000 rpm for 10 minutes. The separated serum was employed for assay of blood urea, serum albumin, serum creatinine, anti-nuclear antibodies (ANA), anti-double stranded DNA, C3 and C4. Around 15 ml of morning urine samples were collected from all subjects included in the study, 1 mL was used for urinary albumin/creatinine (A/C) ratio, 10 ml were used for urine analysis and the rest of the sample was centrifuged for five minutes at 1500 rpm then the supernatant was collected into aliquots and stored at -20ºC for podocin levels estimation. Repeated freezing and thawing were avoided.

Urinary podocin levels were measured by enzyme-linked immunosorbent assay (ELISA) using a kit supplied by Sun Long Biotech Co., LTD (Gongzhu District, Hangzhou, Zhejiang, China, Catalogue Number; SL1430Hu). The established standard curve was utilized for the calculation of the final concentration of urinary podocin.

Statistical analysis

Data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) software package version 20.0. Qualitative data were described using numbers and percentages. Quantitative data were described using range (minimum and maximum), mean and standard deviation. The significance of the obtained results was judged at the 5% level, where $P \leq 0.05$ is considered statistically significant. The chi-square test is employed for categorical variables, to compare between different groups. Fisher's exact test or Monte-Carlo correction is utilized for correction for chi-square when more than 20% of the cells have an expected count less than 5. Student t test is used for normally quantitative variables, to compare between two studied groups. F-test (ANOVA) is employed for normally quantitative variables, to compare between more than two groups and post hoc tests (Tukey test) for pairwise comparisons. Kruskal Wallis test was applied for abnormally quantitative variables for comparing more than two studied groups. Pearson's coefficient was applied to correlate between two normally quantitative variables. To estimate the best cut off, receiver operating characteristic curve (ROC) was used.

Results

This study was conducted on 45 adult SLE patients. Females represent 95.6% of cases with the mean age of 27.71 ± 6.77 years. Additionally, the mean of eGFR was 91.29 ± 25.83 mL/min/1.73 m^2 and the mean of serum creatinine levels was 0.84 ± 0.24 mg/dL. Moreover, urinary podocin concentrations mean were 2.29 ± 0.71 ng/mL, 37.20 ± 14.38 ng/mL and 10.58 ± 2.30 ng/mL in patients' groups consecutively with a significant decrease of urinary podocin in LN patients after remission versus high levels in patients with active LN ($P \leq 0.001$; Tables 1, 2; Figure 1). In this study, we found, highly significant positive correlations of urinary podocin concentration with global SLAM activity (r= 0.852; $P \leq 0.001$), SLAM renal score (r=0.854, $P \leq 0.001$), urinary albumin/creatinine ratio (mg/g) (r=0.895, $P \leq 0.001$) and anti-double stranded DNA titer (r=0.736; $P < 0.001$). Moreover, highly significant negative correlations of urinary podocin value with C3 (r=-0.803, $P \leq 0.001$) and C4 levels (r=-0.760, $P \leq 0.001$) and also eGFR (r=-0.759; $P \leq 0.001$) were detected (Tables 3-5). Sensitivity and specificity tests were analyzed for urinary podocin to diagnose different degrees of global SLAM score activity in LN. Table 6 and Figure 2 showed the high sensitivity and specificity to detect mild degree to severe degree of LN activity.

Discussion

Involvement of the kidney in SLE can be of different degrees and occurs in 50%-70% of patients with lupus.

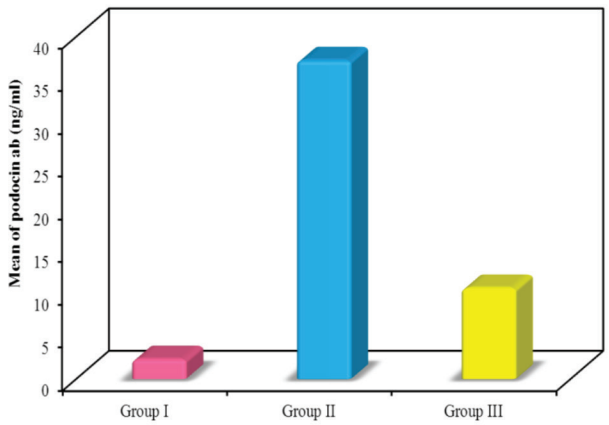

Figure 1. Comparison between the studied groups according to urinary podocin (ng/mL).

Table 1. Comparison between the studied groups according to demographic data

Variables	Group I (n=10)		Group II (n=15)		Group III (n=20)		Significance	*P* value
	No.	%	No.	%	No.	%		
Gender								
Male	0	0.0	0	0.0	2	10.0	χ^2= 2.6	^{MC}P = 0.490
Female	10	100.0	15	100.0	18	90.0		
Age (y)								
Min–Max	18.0 – 44.0		18.0 – 42.0		19.0 – 39.0		F= 0.04	0.954
Mean ± SD	28.30 ± 7.79		27.60 ± 6.95		27.50 ± 6.45			
BMI (kg/m^2)	25.39 ± 1.59		26.68 ± 2.13		25.81 ± 1.85		F=1.5	0.217
Normal (18.5–24.9)	3	30.0	2	13.3	5	25.0		
Overweight (25–29.9)	7	70.0	11	73.3	15	75.0	χ^2= 3.7	^{MC}P = 0.407
Obese (≥ 30)	0	0.0	2	13.3	0	0.0		
Duration of SLE (mon) (Mean ± SD)	8.10 ± 3.07		0.60 ± 0.30		34.50 ± 14.61		F=55.5*	<0.001*
Significance between groups			P_1= 0.071, P_2<0.001*, P_3<0.001*					
Systolic BP (mm Hg)	115.50 ± 11.17		124.0 ± 15.72		121.0 ± 11.19		F=1.3	0.280
Diastolic BP (mm Hg)	75.0 ± 8.50		80.0 ± 6.81		78.50 ± 9.33		F=1.08	0.347
HCV								
Absent	10	100.0	14	93.3	20	100.0	χ^2= 1.9	^{MC}P =0.555
Present	0	0.0	1	6.7	0	0.0		
Hypertension								
Absent	9	90.0	12	80.0	15	75.0	χ2= 0.8	^{MC}P = 0.803
Present	1	10.0	3	20.0	5	25.0		
SLAM renal score activity								
Normal	10	100.0	0	0.0	15	75.0		
Mild	0	0.0	1	6.7	5	25.0	χ^2=44435	^{MC}P<0.001*
Moderate	0	0.0	12	80.0	0	0.0		
Severe	0	0.0	2	13.3	0	0.0		
Significance between groups			P_1<0.001*, P_2= 0.140, P_3<0.001*					
SLAM renal score (Mean ± SD)	0.0–0.0		2.07 ± 0.46		0.25 ± 0.44		F= 114.7*	<0.001*
Significance between groups*			P_1<0.001*, P_2= 0.113, P_3<0.001*					
Global SLAM score activity								
Mild (0–3)	4	40.0	0	0.0	6	30.0	χ^2 = 35.6	^{MC}P<0.001*
Moderate (4–7)	6	60.0	0	0.0	11	55.0		
Severe (>7)	0	0.0	15	100.0	3	15.0		
Significance between groups			*P* 1<0.001*, P_2= 0.639, P_3<0.001*					
Global slam score (Mean ± SD)	4.30 ± 1.70		13.33 ± 2.26		5.0 ± 2.03		F= 88.2*	<0.001*
Significance between groups			P_1<0.001*, P_2= 0.382, P_3<0.001*					

F, *P*: F and *P* values for ANOVA test.
Significance between groups were done using post hoc test (LSD).
χ^2, *P*: χ^2 and *P* values for Chi square test for comparing between the studied groups.
MC: Monte Carlo for Chi-square test for comparing between the studied groups.
P_1: *P* value for comparing between group I and group II. P_2: *P* value for comparing between Group I and Group III. P_3: *P* value for comparing between group II and group III.
* Statistically significant at $P \leq 0.05$.

Table 2. Comparison between the studied groups regarding laboratory investigations

	Group I (n=10)	Group II (n=15)	Group III (n=20)	Test of sig.	*P*-value
ANA (No. of patients)					
Negative	0	0	0	-	-
Positive	10 (100%)	15 (100%)	20 (100%)		
Hemoglobin (g/dl) (Mean ± SD)	10.30 ± 0.81	9.61 ± 1.46	11.47 ± 1.45	F=8.5*	0.001*
Significance between groups		P_1 = 0.212, P_2 = 0.030*, P_3<0.001*			
Creatinine(mg/dL) (Mean ± SD)	0.69 ± 0.07	1.11 ± 0.15	0.72 ± 0.16	F=41.9*	<0.001*
Significance between groups		P_1 <0.001*, P_2 =0.582, P_3 <0.001*			
Albumin (g/dL) (Mean ± SD)	3.69 ± 0.32	2.48 ± 0.41	3.49 ± 0.24	F=56.2*	<0.001*
Significance between groups		P_1<0.001*, p_2 =0.119, P_3 <0.001*			
BUN (mg/dL) (Mean ± SD)	10.10 ± 1.66	37.20 ± 12.14	12.85 ± 3.17	F=59.2*	<0.001*
Significance between groups		P_1<0.001*, P_2 =0.341, P_3<0.001*			
Anti-DNA ds (No. of patients)					
40-60 (Borderline)	3 (30%)	0	6 (30%)	χ^2=6.2*	MCP=0.038*
>60 (Positive)	7 (70%)	15 (100%)	14 (70%)		
Anti-DNA ds titer (Mean ± SD)	66.70 ± 6.91	99.67 ± 27.68	71.40 ± 14.51	F=12.5*	<0.001*
Significance between groups		P_1<0.001*, P_2 = 0.526, P_3<0.001*			
C3 (mg/dL) (Mean ± SD)	97.40 ± 13.33	51.0 ± 20.47	91.75 ± 7.70	F= 44.944*	<0.001*
Significance between groups		P_1 <0.001*, P_2 = 0.314, P_3<0.001*			
C4 (mg/dL) (Mean ± SD)	25.90 ± 5.51	9.49 ± 4.24	25.38 ± 6.80	F= 38.6*	<0.001*
Significance between groups		P_1<0.001*, P_2 = 0.816, P_3 <0.001*			
A/C ratio(mg/g)					
<30 (norm albuminuria)	10 (100%)	0	14 (70%)	χ^2= 50.3*	MCP<0.001*
30–299 (micro albuminuria)	0	0	6 (30%)		
≥ 300 (macro albuminuria)	0	15 (100%)	0		
A/C ratio (mg/g) (Mean ± SD)	8.90 ± 3.18	406.4 ± 43.92	25.60 ± 25.60	H=30.7*	<0.001*
Significance between groups		P_1<0.001*, P_2 = 0.089, P_3<0.001*			
GFR (mL/min/1.73 m^2) (Mean ± SD)	110.1 ± 15.66	63.73 ± 10.66	102.6 ± 20.63	F= 30.8*	<0.001*
Significance between groups		P_1<0.001*, P_2 = 0.255, P_3<0.001*			
CKD stage (No. of patients)					
1	8 (80%)	1 (6.7%)	15 (75.0%)	χ^2=22.1*	MCP<0.00*
2	2 (20%)	9 (60.0%)	5 (25.0 %)		
3a	0 (0.0)	5 (33.3 %)	0 (0.0%)		
Significance between groups		P_1= 0.001*, P_2 = 1.000, P_3<0.001*			
Podocin (ng/mL) (Mean ± SD)	2.29 ± 0.71	37.20 ± 14.38	10.58 ± 2.30	F=63.674	<0.001*
Significance between groups		P_1<0.001*, P_2= 0.015*, P_3<0.001*			
Urine sediment score (Mean ± SD)	0.0 ± 0.0	2.0 ± 0.38	0.30 ± 0.47	H=35.2*	<0.001*
Significance between groups		p_1<0.001*, P_2=0.057, P_3<0.001*			

χ^2, *P*: χ^2 and *P* values for Chi square test.
MC: Monte Carlo for chi square test.
F, *P*: F and *P* values for ANOVA test. Significance between groups were done using post hoc test (LSD).
H, *P*: H and *P* values for Kruskal-Wallis test. Significance between groups using Mann Whitney test.
P_1: *P* value for comparing between group I and group II; P_2: *P* value for comparing between group I and group III; P_3: *P* value for comparing between group II and group III.
*Statistically significant at $P \leq 0.05$

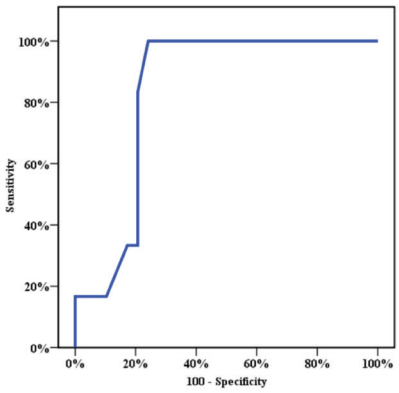

Figure 2. ROC curve for urinary podocin to diagnose mild global SLAM score activity in lupus nephritis..

Morbidity and mortality are still high despite recent regimens of treatment. LN leads to end-stage renal failure in 17%-25% of patients (13).

Podocytes and their slit diaphragms play a crucial role in the integrity of the renal basement membrane, which prevents the loss of urinary proteins. Podocytes were affected from the start of kidney affection and correlated with the histological changes. The loss of podocytes in the urine (podocyturia) was observed in patients with glomerular diseases especially LN, which could be useful in follow up of the activity of the disease (14).

The current study aimed to assess the urinary podocin levels as an early marker of the glomerular lesion in patients with LN. This study was conducted over 45 SLE adult patients, who were collected from Ain- Shams university hospitals, Egypt. Patients were classified into three groups; group I involved ten SLE patients without clinical or laboratory indices evidence of LN activity, group II involved fifteen SLE patients with active LN not on immune-suppressive medications yet and group III involved twenty SLE patients with LN in remission either partial or complete.

Results of our study showed a statistically significant difference between studied groups regarding serum creatinine, albumin, blood urea nitrogen (BUN) and plasma hemoglobin, which is agreed with the study by Sui et al (15). They reported that serum creatinine and BUN were significantly higher in LN patients with nephrotic syndrome (NS) than those in the non-NS group. However, plasma hemoglobin was significantly lower in LN patients with NS than the individuals in the other group (15). The current study revealed a positive significant correlation between urinary podocin level and both serum creatinine and also BUN values. Our study also showed a negative significant correlation between urinary podocin level and both plasma hemoglobin level and serum albumin. These results were in agreement with the study by Zheng et al, which reported that urinary

Table 3. Comparison between podocin and degrees of SLAM scores in total sample (n=45)

	N	Podocin (ng/mL)	F	*P* value
Global SLAM score activity				
Mild	10	6.28 ± 4.04		
Moderate	17	7.80 ± 4.37	31.722*	<0.001*
Severe	18	33.17 ± 16.02		
SLAM renal score activity				
Normal	25	6.69 ± 3.92		
Mild	6	18.25 ± 11.94	39.111*	<0.001*
Moderate	12	33.71 ± 13.22		
Severe	2	55.50 ± 11.31		

F, *P*: F and *P* values for ANOVA test.
*Statistically significant at $P \leq 0.05$.

Table 4. Correlation between urinary podocin and different parameters in total sample (n = 45)

	Urinary Podocin(ng/mL)	
	r	*P* value
Global slam score	0.852*	<0.001*
Global slam score activity	0.694*	<0.001*
Slam renal score	0.854*	<0.001*
Slam renal score activity	0.854*	<0.001*
Demographic data		
Age (year)	-0.066	0.664
BMI (kg/m^2)	0.264	0.079
Laboratory investigations		
Hemoglobin (g/dL)	-0.477*	0.001*
Creatinine (mg/dL)	0.827*	<0.001*
Albumin (g/dL)	-0.810*	<0.001*
BUN (mg/dL)	0.762*	<0.001*
Anti DNA ds titer	0.736*	<0.001*
C3	-0.803*	<0.001*
C4	-0.760*	<0.001*
A/C-ratio(mg/g)	0.895*	<0.001*
GFR (mL/min)	-0.759*	<0.001*
Urine sediment score	0.824*	<0.001*

r: Pearson's coefficient; *Statistically significant at $P \leq 0.05$.

podocin mRNA has a positive and significant correlation with BUN and serum creatinine levels (*P*=0.006) (16).

Furthermore, we found statistically significant differences between the studied groups regarding serum anti-double stranded DNA, C3, C4 and A/C ratio and also eGFR. Our finding was in agreement with the study by Sui et al, who demonstrated that serum anti-dsDNA positivity was more common in LN patients with NS, with a very high specificity value. They also showed a lower level of serum C3 and C4 concentration than those in non-NS patients (15).

In the context of SLAM scores (global SLAM score activity degree, mean values of global SLAM score, SLAM renal score activity degree and mean values of SLAM renal score), we found a statistically significant difference between studied groups. Furthermore, our study showed highly significant positive correlations between urinary podocin levels with SLAM scores and anti-double

Table 5. Correlation between urinary podocin level and different parameters in the studied groups

Variables	Podocin (ng/mL)					
	Group I (n = 10)		Group II (n = 15)		Group III (n = 20)	
	r	*P* value	r	*P* value	r	*P* value
Global slam score	0.462	0.179	0.450	0.092	0.724*	<0.001*
Global slam score activity	0.576	0.081	-	-	0.520*	0.019*
Slam renal score	-	-	0.340	0.215	0.729*	<0.001*
Slam renal score activity	-	-	0.340	0.215	0.729*	<0.001*
Age (year)	0.360	0.308	-0.191	0.494	0.052	0.826
BMI (kg/m^2)	0.516	0.127	0.009	0.975	0.511*	0.021*
Hemoglobin (g/dl)	-0.211	0.559	-0.520*	0.047*	-0.292	L0.212
Creatinine (mg/dL)	0.829*	0.003*	0.568*	0.027*	0.745*	<0.001*
Albumin (g/dl)	-0.791*	0.006*	-0.281	0.310	-0.660*	0.002*
BUN (mg/dL)	0.512	0.130	0.063	0.825	0.617*	0.004*
Anti-DNA ds	0.550	0.100	0.574*	0.025*	0.546*	0.013*
C3 (mg/dL)	-0.432	0.212	-0.349	0.203	-0.545*	0.013*
C4 (mg/dL)	-0.396	0.257	-0.391	0.150	-0.596*	0.006*
A/C-ratio (mg/g)	0.301	0.397	0.737*	0.002*	0.873*	<0.001*
eGFR(ml/min)	-0.824*	0.003*	-0.443	0.098	-0.782*	<0.001*
Urine sediment score	-	-	0.066	0.816	0.807*	<0.001*

r: Pearson's coefficient; *Statistically significant at $P \leq 0.05$.

Table 6. Agreement (sensitivity and specificity) for urinary podocin to diagnose different degrees of global slam score activity in lupus nephritis

Urinary podocin	Cutoff	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Mild	<11	100.0	75.86	46.15	100.0
Moderate	11–14	100.0	88.89	89.47	100.0
Severe	>14	88.89	100.0	100.0	89.47

stranded DNA. We also found negative correlation of podocin levels with C3 and C4 values. Mansur et al also demonstrated the highest urinary podocyte levels in patients with LN, which clinically classified as active or moderately active. They showed a significant association between C3 and C4 levels and higher podocytes counts in urine (17).

Moreover, our study showed a statistically significant difference between studied groups as detected by albuminuria categories, eGFR and chronic kidney disease (CKD) stages. Our results demonstrated that urinary podocin level has a positive significant correlation with the A/C ratio (mg/g), which was in accordance with the study by Pereira et al, who showed the number of podocytes in the urine was positively correlated with the urine A/C ratio (18). On the contrary, Mansur et al detected, podocyturia had no significant correlation with the A/C ratio, which is in contrast to our results (17).

Furthermore, we showed that the podocin level had a significantly negative correlation with eGFR ($P < 0.001$). However, this finding was not in line with the results of the study conducted by Sabino et al, who showed no relationship between levels of eGFR and podocyturia. Around 70% of their patients had eGFR equal to or greater than 60 mL/min. However, podocyturia was not significantly different between the groups (19). Some authors also found that eGFR levels did not significantly correlate with urinary podocin mRNA levels (r=-0.202; P=0.127); however, their studies showed a significantly negative correlation of eGFR levels with podocalyxin expression (r=-0.349; P=0.01) (16).

In this study, we detected a statistically significant difference between the studied groups in terms of urinary podocin level. Urinary podocin concentration appeared even in the normoalbuminuria group with a mean of 2.29±0.71 ng/mL. A higher level of urinary podocin was found in group II with a mean of 37.20±14.38 ng/mL. Urinary podocin level appeared in group III with a mean of 10.58±2.30 ng/mL. Group II had higher levels of proteinuria, RBCs, WBCs and casts than other two groups, since the division of the groups was based on urinary findings in differentiating activity from remission (20). Lioudaki et al also had reported significant correlations of podocyte molecule expression in urine with the degree of proteinuria (21).

In our current study, we found that podocin level did not correlate with A/C ratio (mg/g) in group I. All patients in group I had normoalbuminuria (less than 30 mg/g). These results were in agreement with the study by Maestroni et al, who found in vivo podocyte differentiation is the origin of viable podocyturia in healthy individuals. It is possible that podocyturia in healthy individuals may represent a "side effect" of physiologic podocyte turnover (22). In addition to this finding, Sabino et al found that podocytes

were also seen in the urine of healthy individuals without renal affection; however, their quantities were lower than in patients with LN (19).

Facca et al studied women with preeclampsia and found podocytes in the urine of pregnant women without preeclampsia in the control group (23). However, the podocin level was positively correlated with A/C ratio (mg/g) in group II [whose patients had macroalbuminuria (≥300 mg/g)] and group III [whose 14 patients had normoalbuminuria (<30 mg/g)] since six patients had microalbuminuria (30-299 mg/g).

In agreement with our study, Hernandez et al found that the podocytes isolated from urine and the podocyte-derived mRNA differed in the presence of LN when compared with SLE without nephritis (24).

Likewise, El-Gohary et al stated that albumin/creatinine ratio is a better marker in differentiating between SLE with renal involvement and SLE with no renal involvement [$P = 0.008$, the area under curve (AUC) (95% CI) = 0.803 (0.610–0.995)]. A value of albumin/creatinine ratio 126 mg/g as a cut-off value is best for differentiating renal from non-renal involvement with 90% sensitivity and 75% specificity (25).

In group II (active group), we detected that podocin level has a positive significant correlation with serum creatinine, anti-double stranded DNA, A/C ratio (mg/g). In this group, we also found a negative significant correlation of urinary podocin level with plasma hemoglobin. There was no correlation of urinary podocin level with global SLAM score, global SLAM score activity, SLAM renal score, SLAM renal score activity, age, BMI, albumin, BUN, ANA, C3, C4, eGFR and urine sediment activity score. This finding is in accord with a previous study that showed significant increase in serum creatinine in the macroalbuminuria group compared to the other three groups ($P < 0.001$) (16).

Besides, the same results were also found in the study by El-Gohary et al, which demonstrated that albumin/creatinine ratio also can significantly differentiate between active renal SLE from active non-renal SLE [$P = 0.009$, AUC (95% CI)=0.900 (0.744–1.000)] with a cut off value of 126 mg/g which is the best indicator with 100% sensitivity and 80% specificity (25).

In group III, we detected that urinary podocin level has a positive significant correlation with mean values of global SLAM score, global SLAM scores activity degree, SLAM renal score activity degree, BMI, creatinine, BUN and anti-double stranded DNA. However, A/C-ratio (mg/g) and urine sediment activity score show a significant negative correlation with plasma albumin, C3and C4 levels and also value of eGFR. There was no significant correlation between the urinary podocin level and age and also plasma hemoglobin or ANA.

Recent studies revealed that podocyte loss indicated by podocalyxin immunohistochemical expression in LN renal biopsy reflects the degree of disease pathological activity and severity and the degree of podocyte effacement by electron microscope (26).

Accordingly, we found that sensitivity of urinary podocin as a marker of the mild clinical activity of LN is 100% while specificity is 75.86% (cut off <11, with regards to global SLAM score). In addition, we detected that the sensitivity of urinary podocin as a marker of the moderate activity of LN according to global SLAM score is 100% while specificity is 88.89% (cut off 11-14). Furthermore, urinary podocin as a marker of the severe activity of LN according to global SLAM score is 88.89% while specificity is 100 % (cut off >14). These results demonstrated the clinical significance of urinary podocin as a non-invasive biomarker to detect the mild activity of LN and follow up the severity of LN and also the response to treatment.

Conclusion

Urinary podocin as non-invasive biomarker is significantly correlated to SLE disease activity and LN activity measured by global SLAM clinical score with both high sensitivity and specificity. It considered to be a prognostic marker in the management of LN patients and monitoring the response to treatment.

Limitations of the study

Small sample size as no fund was received.

Authors' contribution

MAB, SB, WAB participated in conceptualization, design of research methodology, investigation, formal analysis, data curation, validation and original draft preparation. FE coordinated the acquisition of data and analysis. AS designed the research plan and supervised the study. MAB and WAB contributed to the review and editing of the manuscript. All authors revised the manuscript, evaluated the intellectual contents, approved the manuscript's content.

Conflicts of interest

The authors claimed no conflicts of interest regarding the research, authorship and publication of this article.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The study was approved by the medical ethics committee of the faculty of medicine, Ain-Shams university (ethical code#000017585) and informed consent was taken from the patients (or their caregivers) before participation in the current study. Additionally,

ethical issues (including plagiarism, data fabrication, double publication) were completely observed by the authors.

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