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Role of urinary podocytes excretion versus renal biopsy in assessment of lupus nephritis patients

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***Abstract*---**Background: Systemic lupus erythematosus often affects the kidneys, and its treatment continues to provide new challenges to clinicians every day. Proteinuria caused by either direct or indirect podocyte damage is diagnostic of lupus nephritis. Aim: The purpose of this study was to evaluate the prognostic usefulness of urinary podocytes in predicting renal complication in individuals with SLE. Subjects and methods: Twenty individuals with SLE participated in this cross-sectional observational research. Complete history collection, clinical examination, and laboratory investigation were performed on all individuals. Podocytes detection in the urine and a kidney biopsy were performed. Results: The relationship between urinary podocytes and histological subtype is quite significant. Also, urinary podocytes significantly positively correlated with all parameters except with albumin significantly negatively correlated. Conclusion: Lupus patients who have renal damage may exhibit urinary podocytes, however this is not a diagnostic indication for lupus nephritis per se. Flares of lupus nephritis may be defined with the use of renal function tests and kidney biopsies.

Keywords---lupus nephritis, podocytes, podocyturia, proteinuria, kidney biopsy.

Introduction

The chronic autoimmune illness systemic lupus erythematosus (SLE) disproportionately affects females. A very serious organ complication of SLE, lupus nephritis (LN) is a medical emergency. Ten percent to thirty percent of these people will develop kidney failure. (Ortega et al.,2010). Accumulation of autoantibodies in the glomerulus, activation of complement and macrophages, and generation of proinflammatory cytokines and chemokines all contribute to the pathogenesis of LN. The precise pathophysiology of LN is, however, still poorly known. (Rekvig and Van der Vlag, 2014). In order to better track the progression of SLE and lupus nephritis, researchers are looking at a number of potential biomarkers. (Juliana et al.,2016).

Kidney biopsy (RB) has been used extensively for many years as the most reliable method for determining the severity of LN and its prognosis. Systemic lupus erythematosus international collaborative clinic criteria (Petri et al., 2012). An effect of SLE may be diagnosed if the patient has both a pathology in the RB suggestive of LN and a positive result for either antinuclear or anti double-stranded DNA antibodies. (Haladyj et al.,2016). The method's intrusive nature, however, means that new noninvasive laboratory assays are required to determine renal involvement without renal biopsy. (Perez Hernandez et al., 2016). Injuries to podocytes have been linked to the development and worsening of SLE. As an urine marker of glomerular illness, podocytes have recently been characterized as playing a key part in the etiology of glomerulosclerosis. (Sun et al., 2012). Actively resorbing LN included podocytes, cells that undergo development in the urinary tract; proteinuria and histological characteristics of LN were shown to be linked with the protein levels of these podocytes. Based on these results, it may serve as a noninvasive diagnostic tool for monitoring glomerular disease in SLE. Podocyte antigen-specific antibodies and indirect immunofluorescence are often used to evaluate podocyturia (Juliana et al., 2016).

Our study set out to determine whether or not podocyte-containing urine (podocyturia) may replace the more invasive renal biopsy in gauging glomerular disease development in individuals with systemic lupus erythematosus (RB). The goals of this research are to (1) determine whether or not podocyturia is a reliable marker of glomerular disease progression in SLE, and (2) determine whether or not podocyturia is correlated with clinical and laboratory indicators of disease severity.

Patients and Methods

We performed a cross-sectional, observational study at the Aswan University School of Medicine Rheumatology clinic from October 2018 to November 2019. All participants gave written agreement, and the Aswan University Local Ethics Committee approved the study.

The current study involved patients with SLE aged from18 to 50 years satisfied the 2015 American College of Rheumatology classification criteria for SLE (Cattran et al., 2012) and proteinuria (> 0.5 g/24h or >1 g/24h with previous proteinuria) or an active urine sediment that included hematuria, especially leukocyturia (in the absence of infection), red and white blood cell casts. Small, shrunken, hyperechogenic kidneys with marginal abnormalities on renal ultrasonography, severe hypertension, and bleeding diathesis were excluding criteria for patients with end-stage renal failure.

The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was used for the clinical evaluation of SLE disease activity by a separate physician (Perez-Hernandez et al., 2016). Kidney failure owing to proteinuria (>0.5g/24hor>1g/24hwith prior proteinuria), active urine sediment with hematuria (leukocyturia in the absence of infection), or red and white blood cell casts in the urine were all diagnostic of active LN.

The whole participant pool had a thorough history taken, clinical examination, and laboratory work performed (including CBC, creatinine, urea, albumin, anti doublestrand DNA antibody, C3, C4 and 24-hour proteinuria). A physician's clinical evaluation of SLE disease activity was made using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Perez-Hernandez et al., 2016). Kidney failure with proteinuria, active urine sediment with hematuria (particularly leukocyturia in the absence of infection), red and white blood cell casts, or a kidney biopsy indicating active glomerulonephritis were all diagnostic of active LN. A kidney biopsy and urine Podocytes were analyzed.

Podocyturia assessment

A mid-stream freshly voided urine sample was collected on-site after a minimum of 3 h without voiding; and 20 ml of urine were centrifuged at 700g for 5 min in a cytospin. The supernatant was discarded and the sediment was stored in 100 µl aliquots at room temperature mixed with a 1.5 ml solution of 40 % formaldehyde diluted in phosphate-buffered saline (PBS) (pH 7.2–7.4) to reach a final 10 % concentration. After a further washing step, the slides will be incubated with a primary antibody specific for podocytes, an anti podocin rabbit antibody (Sigma-Aldrich, St.Louis, MO)at 1:250 dilution, and then with the secondary fluorescein-linked goat anti-rabbit IgG antibody (FITC, Sigma Aldrich, St.Louis, MO) at 1:320 dilution. Podocytes had been measured by counting the number of podocytes in 30 random fields, corrected by urinary creatinine concentration in the same sample. Only one trained observer evaluated podocyte count in urine and the person will be blinded to patient characteristics *(Juliana et al., 2016).*

Renal biopsy

The gold standard for kidney biopsy is percutaneous biopsy. Radiologists and nephrologists are the most usual medical professionals to carry it out. In 95%-98% of PRB, sufficient tissue is recovered, with an unusual yield of 10-20 glomeruli on average when utilizing 14 and 16 gauge needles. If there are no particular areas of interest, the patient is positioned in the prone position, and the biopsy is obtained from the lower pole of the kidney. Ultrasound is generally

used to guide the biopsy needle to the correct spot on the kidney (Kitterer et al.,2015).

Statistical analysis

Data was analyzed using IBM's SPSS (2011 release). IBM's Windows statistics program version 21.0 (Armonk, NY: IBM Corp.) Statistical significance tests depended on whether the data was qualitative (numbers and percentages) or quantitative (means and standard deviations) (X2). Kappa, Pearson, one-way and two-way ANOVA for statistical significance; t-tests for comparing means. When the P value was less than 0.05, results were deemed statistically significant.

Results

This study included twenty patients with SLE, their mean of age was36.83±5.28) years and ranged from 20 to 57 years. The majority of the patients was females (90%) (Table 1). Regarding laboratory results, mean serum creatinine was 2.83±0.75, with range of (1.3-3.4), urea level was 90.21±20.5 with range of (61.2-120.2), serum albumin was 3.07±0.71 with range of (2.5-4), C3 was 95.1±11.9 with range of (21-197), C4 was 22.3±5.6 with range of (8-55) and podocyturia was 15.3±2.1 with range of (10-321) (Table 2). Podocyturia is slightly increased according to disease activity without significant difference among degrees of disease activity (p value=0.911) (Table 3). However, podocyturia is increased according to histological class with significant difference among classes (p value=0.003) (Table 4).

Urinary podoccytes significantly positive correlated with creatinine and blood urea while it significantly negative correlated with albumin (Table 5) Regarding the prognostic value of urinary podocytes in detection of severity of SLE, it has a significant area under curve with cutoff >18 with a Sensitivity of 68.9% and specificity of 71.2%. While according to prognostic value of biopsy in detection of severity of SLE, it has a significant area under curve with cutoff >25 with a Sensitivity of 73.9% and specificity of 75% (figure 1&2).

Table (1): Age and sex distribution of cases

	Patients (N=20)	
Age (year) Mean ±SD Range	36.83±5.28 (20-57)	
	No.	%
Sex Male Female	 2 18	 10.0 90.0

Table (2): Laboratory parameters distribution between studied groups

Laboratory Findings	Patients (N=20)
creatnine Mean ±SD Range	 2.83±0.75 (1.3-3.4)
Blood urea (mg/dl) Mean ±SD Range	 90.21±20.5 (61.2-120.2)
Anti DNA (IU/mL) Mean ±SD Range	 40.07±5.71 (20-140)
Podocyturia (mg urinary creatinine) Mean ±SD Range	 15.3±2.1 (10-321)
Albumin (g/dl) Mean ±SD Range	 3.07±0.71 (2.5-4)
C3 (mg / dl) Mean ±SD Range	 95.1±11.9 (21-197)
C4 (mg/dL) Mean ±SD Range	 22.3±5.6 (8-55)

Table (3): Association between podocyturia and disease activity

	Inactive N=5	Mild N=7	Moderate N=5	Severe N=3	F	P value
Podocyturia (podocytes/mg Urinary creatinine) Mean ±SD	15.1±2.0	15.3±1.9	15.4±2.1	15.9±2.3	0.092	0.911

F is for ANOVA test

Table (4): Association between podocyturia and Histological class

	III	IV	V	F	P value
Podocyturia (podocytes/mg Urinary creatinine) Mean ±SD Range	9.1±3.0	18.3±6.1	10.4±3.4	8.32	0.003 (S)

S: P value is significant

Table (5): Correlations between urinary podocytes and other parameters

		Urinary podocytes
creatinine	*r*	0.886
	P value	0.000**
Blood urea	*r*	0.772
	P value	0.000**
Albumin	*r*	-0.858-
	P value	0.000**

r: correlation coefficient, ** p value is highly significant

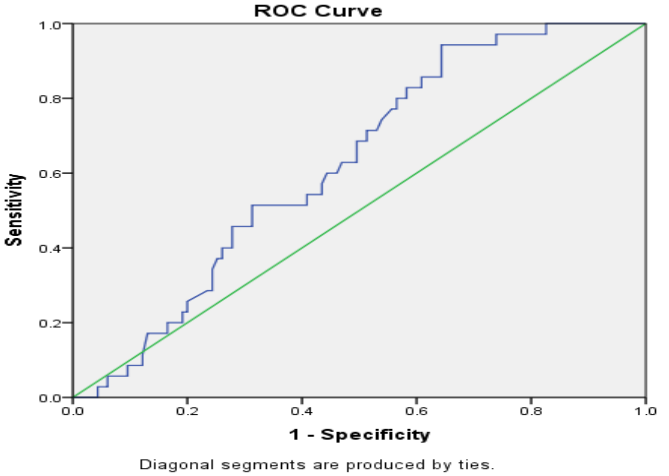

Figure (1): ROC Curve for detection of severity of SLE

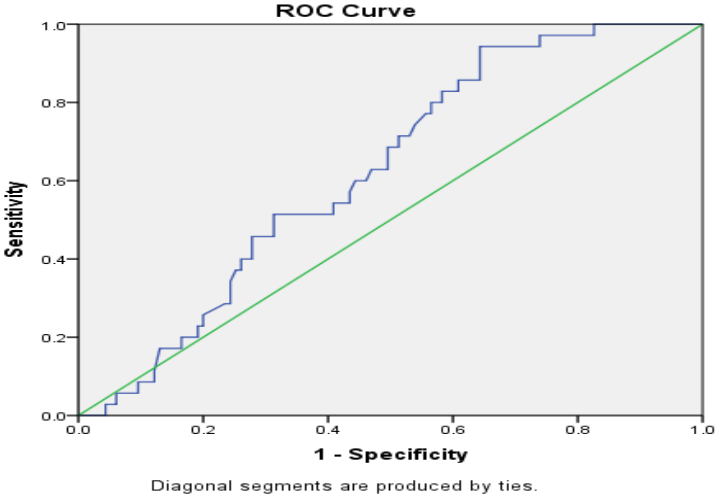

Figure (2): ROC Curve for detection of severity lupus nephritis detected by biopsy

Discussion

Podocytes are visceral epithelial cells in the glomerular membrane that maintain glomerular filtrate moving across intercellular gaps. These cells are the final line of defense in the ultrafiltration barrier, which inhibits protein loss *(Colvin et al., 2019)*. Often the first sign of glomerular illness, this condition is linked to severe cases of lupus nephritis *(Hao, et a.,2019)*. We discovered no evidence of a correlation between podocyturia and illness in this investigation. *Wang et al. (2014)* in their research Patients in full or partial remission showed no difference from nonresponsive individuals in terms of urine podocyte markers, as we also showed. *Wickman et al. (2013)* Podocyturia was also proposed as a potential more reliable indication of disease activity and progression than proteinuria.

In this thesis we found that there is significant relation between podocyturia and Histological class. This was on the same side with *Dos Santos et al. (2015)* who demonstrated that moderate-to-severe lesions, which had higher excretion of podocyte mRNAs in urine. Also, *Mansur et al. (2016)* verified that Patients with LN had substantially increased urine nephrin, podocalyxin, and protein levels compared to controls, suggesting podocyte damage. This study showed that sensitivity of urinary podocytes detection of severity of SLE was 68.9%, specificity 71.2% with area under curve 0.690 and at a cut off >18. While sensitivity of detection of detection of severity lupus nephritis detected by biopsy was 3.9%, specificity 75% with area under curve 0.71 and at a cut off >25. *Mansur et al. (2016)* demonstrated in their ROC curve analysis for podocyclin that area undr curve for podocyclin was 0.63.

We found that urinary podoccytes significantly positive correlated with all parameters except with albumin significantly negative correlated. This was in concordant with *Bollain-Y-Goytia et al. (2011)* who reported that proteinuria is linked to low podocyte counts in lupus glomeruli. *Dos Santos et al. (2015)* confirmed the association between proteinuria and higher urinary podocyte-associated mRNAs, but not found a correlation with mRNA tissue expression. On the other hand, *Sabino et al., (2013)* reported that There was no correlation found between GFR and the presence of podocyturia. Podocyturia was not substantially different between individuals with normal and lower estimated GFR (around 70% of patients). The main limitation of this study was the the small sample size which did not help us to demonstrate if there is a significant relation between disease activity and podocyturia. Another limitation is the variability of glomerular filtration rates in our patients at the time of biopsy, and therefore, the expression of podocyte molecules.

Conclusion

Loss of glomerular podocytes, a cell marker of renal injury, would be a prognostic component of the loss of filtration function in the kidneys of individuals with lupus nephritis. In conclusion, podocyturia does not serve as a particular marker for lupus nephritis but rather reflects renal injury in a lupus patient. Flares of lupus nephritis may be defined with the use of renal function tests and kidney biopsies.

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