

Clinical Significance of Urine Podocyte Messenger Ribonucleic Acids in Idiopathic Nephrotic Syndrome and Lupus Nephritis Patients

Amir Mohamed Elokely¹, Emam Waked², Mohamed A Shemis³, Sameh Abdel Azeem Soliman¹, Malak Nabil^{2*}, Manar Raafat² and Anas Hassan²

¹Internal Medicine and Nephrology Department Faculty of Medicine, Zagazig University, Zagazig, Egypt

²Nephrology Department Theodor Bilharz Research Institute, Cairo, Egypt

³Biochemistry & Molecular Biology department Theodor Bilharz Research Institute, Cairo, Egypt

*Corresponding author: Malak Nabil, Mobile: (00201222158622) E-mail: nabil.malak59@yahoo.com

ABSTRACT

Introduction: Podocytes are specific epithelial cells that surround the outside of the glomeruli's basement membrane (GBM). They play a significant role in glomerular function. This study aimed to detect the presence of podocyte in idiopathic nephrotic syndrome and lupus nephritis patients, and to correlate them with the proteinuria levels.

Methods: Patients were divided to three groups (patients with idiopathic nephrotic syndrome, patients with lupus nephritis and, healthy controls). Quantification of podocyte mRNA proteins in urinary sediment by real time PCR was performed to all groups. **Results:** For those who had nephrotic syndrome showed significant increase in the alpha actin 4 value ($p=0.001$) and significant decrease in the value of synaptopodin and podoclyxin in relation to controls ($p=0.001$). The level of urinary expression of mRNA of podocytes correlated positively to renal function tests and estimated glomerular filtration rate between nephrotic and control groups. The reverse occurred with proteinuria. A significant rise in the alpha actin 4 value, podocin and synaptopodin in comparison with control ($p=0.001$). Podocalyxin was decreased significantly ($p=0.021$). There was a statistically significant difference, when comparing renal function tests and estimated glomerular filtration rate between LN and control group. Also, an increase in the value of podocin in active group relative to control and non-active groups ($p=0.001$ and $p=0.007$, respectively).

Conclusion: Significant increase in level of mRNA of alpha actin in nephrotic syndrome, while mRNA podocin, synaptopodin and alpha actin were increased in LN, with podocin could mark increased activity of the disease.

Keywords: Synaptopodin, Podocalyxin, α actin-4, Podocin, Idiopathic nephrotic syndrome, Lupus nephritis.

INTRODUCTION

Podocytes are specific epithelial cells that surround the outside of the glomeruli's basement membrane (GBM). They play critical role in glomerular functions. Decrease podocytes number and effacement of foot procedures have been documented in various glomerular diseases. This may stimulate a sequence of events, which end in decline of renal function and glomerulosclerosis⁽¹⁾. Podocytopathies are considered essential in various types of human glomerular diseases^(2,3).

Proteinuria with nephrotic syndrome (>3.5 g/day) due to loss of glomerular filtration barrier selectivity is a common complication in each renal disease of this syndrome⁽⁴⁾. Lupus nephritis (LN) is considered one of the most serious organ involvements in systemic lupus erythematosus (SLE)⁽⁵⁾. About 40 to 60% of SLE patients have LN⁽⁶⁾, and about 10–30% of patients develop end-stage kidney disease depending on the disease severity⁽⁷⁾. The immunopathogenesis of LN is complicated and incompletely understood. It shows autoantibodies deposition in the complement, glomerulus, and macrophages activation, leading to release of pro-inflammatory chemokines and cytokines⁽⁸⁾. Recently the crucial role of podocytes has been defined in the glomerulosclerosis pathogenesis⁽²⁾.

Recently, the urinary sediment as emerging modality for studying renal glomerulopathies showed a promising role instead of the renal biopsy which is considered difficult and invasive tool⁽⁹⁾.

The identification of biological markers that can lead to pathogenic mechanisms or histopathological patterns,

and establish prognostic subgroups in every disease type, that predict the reaction to therapy and/or relapses, is one of the pending challenges in the modern nephrology⁽¹⁰⁾. Screening of slit diaphragm proteins as synaptopodin, podocalyxin, α actin-4 and podocin might be a unique technique in examining the glomerulopathy progression⁽¹¹⁾. When glomerular injury arises, the slit diaphragm is damaged and the slit diaphragm proteins in urine could be used as a tool for monitoring the renal disease progression⁽¹²⁾. Screening of podocyte proteins and related molecules in the urine pellet by real time PCR is sensitive and accurate. It has the ability to measure low abundance genes⁽¹³⁾. Urine pellet podocyte mRNAs could be effective in observing the progression of various glomerular diseases and response to therapy⁽³⁾.

The objective of the current study was to detect podocyte presence in idiopathic nephrotic syndrome and lupus nephritis patients, and to correlate them with the proteinuria levels.

PATIENTS AND METHODOLOGY

The work was performed on 30 cases from the Department of Nephrology, Theodor Bilharz Research Institute in addition to 15 healthy control subjects of comparable age and sex.

Inclusion criteria: Patients according to clinical history, investigations and renal biopsy proving the etiology of proteinuria were divided to two groups:

- **Group A:** Idiopathic nephrotic syndrome patients.
- **Group B:** Lupus nephritis patients.

In addition

- **Group C** (15 subjects): the healthy controls.

Exclusion criteria: Patients infected or had other systemic disease. Concerning control group, Hypertension, diabetes, clinical or laboratory evidence of kidney disease.

All patients and control underwent:

- Detailed history and full medical assessment.
- Schedule laboratory analyses as:
 - Kidney function tests: Creatinine & blood urea by colorimetric assay.
 - Beckman Coulter's AU480 analyzer measured 24-hour urine proteins and measured albuminuria using a dipstick (Beckman Coulter, Inc., Brea, California).
 - Glomerular filtration rate (eGFR) and creatinine clearance using the abbreviated modification of diet in renal disease (MDRD) equation.

Nephrotic syndrome group contained 11 patients presented with membranous nephropathy (MN) and 4 patients with focal segmental glomerulosclerosis (FSGS) according to kidney biopsy. All patients were idiopathic MN and FSGS after exclusion of secondary causes by history and examination, imaging studies and negative serology of different secondary causes. Two patients with membranous nephropathy had the antiphospholipase A2 receptor antibody test positive.

All patients in lupus nephritis (LN) group were diagnosed to be SLE cases in line with American college of rheumatology diagnostic standards. They were also diagnosed to have LN by the presence of proteinuria, active urinary sediments and kidney biopsy. LN patients' group was divided to 2 subgroups according to LN activity at the time of the study:

Subgroup (A): Active LN contained 9 patients

Subgroup (B): Non-active LN contained 6 patients

LN activity was determined by clinical manifestations, increase the amount of proteinuria, appearance of active urinary sediment, deterioration of kidney function, increase levels of anti-double strand deoxyribonucleic acid (Anti ds DNA) titre and consumed C3 and C4.

Specific laboratory tests: Podocyte mRNA proteins (α actin-4, podocalyxin, synaptopodin, and podocin) quantification in urinary sediment by real time PCR.

Methods of Realtime PCR:

Real-time PCR

1- Urine sample collection and total RNA extraction:

Urine was centrifuged at 3000 rpm for 30 minutes at 4°C as soon as it was collected. The leftover cell pellet was kept at -80°C until usage after the supernatant was removed. Total RNA was extracted according to the manufacturer's protocol (RNeasy Mini Kit, Qiagen, Germany). RNA concentration and purity were verified using a NanoDrop spectrophotometer (Thermo, Wilmington, USA) using a 260/280 relative absorbance ratio on the Nanodrop 2000.

2- Reverse transcription (RT):

RT reaction was done according to manufacturer's protocol RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, USA). Briefly, 2 μ l of total RNA, 1 μ l oligo (dT) primer, 1 μ l RiboLock RNase inhibitor (20 U/ μ l), 4 μ l (5X) reaction buffer, 2 μ l (10 mM) dNTP mix, and 1 μ l RevertAid M-M ul V RT (200 U/ μ l) solution were mixed and the volume was bring up to 20 μ l with nuclease-free water. The RT reaction was conducted at 42°C for 60 minutes, then a 5-minutes an inactivation phase at 70°C. The produced cDNA was kept at -20 °C until it is needed.

3- Real-time PCR

Relative abundances of synaptopodin, podocalyxin, α -actin 4 and podocin mRNA was determined using the StepOne™ Real-Time PCR System (Applied Biosystems, California, USA). Human β -actin was used as a reference housekeeping gene. The following sequences of oligonucleotide primers were used:

Real-time PCR reaction was performed as follows: 2 μ l cDNA, 10 μ L SYBR Green / ROX qPCR Master Mix (2X Maxima SYBR Green / ROX qPCR Master Mix), 0.4 ml forward and reverse primer (10 mM), 0.4 μ L of ROX reference dye, 6.8 μ L of nuclease-free water was added to obtain a reaction volume of 20 μ L. All samples were tested in duplicate ⁽³⁾. The thermal profile of the real-time PCR Reaction was: 95 °C for 10 min, 40 cycles for 15 s at 95 °C and 60 °C for 30 s. Then, dissociation (DC) and melting temperature (Tm) curves are plotted and determined.

The expression level of each gene was assessed using the target gene abundance /housekeeping gene abundance formula. A negative control containing ddH₂O was included in all runs.

Oligonucleotide	Sequence
Synaptopodin	Forward: 5'-CTTACGGCGGTGACATCTC-3' Reverse: 5'-GGTCCTGAGCCTCGATCC-3'
Podocalyxin	Forward: 5' CTTGAGACACAGACACAGAG-3' Reverse: 5'- CCGTATGCCGCACTTATC-3'
α-actin4	Forward: 5'-CTTGAGACACAGACACAGAG-3' Reverse: 5'- CCGTATGCCGCACTTATC-3'
Podocin	Forward: 5' TGGCTGTGGAGGCTGAAG-3' Reverse" 5'- TGAAGGGTGTGGAGGTATCG-3'
β-actin	Forward: 5'- TGGCACCCAGCACAATGAA-3' Reverse: 5'- CTAAGTCATAGTCCGCCTAGAAGCA-3'

Ethical approval:

The work was permitted by the Ethical Committee, Faculty of Medicine, Zagazig University, which was fully aligned with the World Medical Association Ethics code (Declaration of Helsinki) for studies involving humans. All participants signed a printed informed consent forms.

Statistical Methods

Results are represented as mean ± SD or as a number. Chi square test or Fisher exact test instead if cell count was less than 5 used for comparison between categorical data. Distribution of data was measured by test of normality, Kolmogorov-Smirnov test. Accordingly, data were not normally distributed, hence comparison between variables in the three groups was performed using Kruskal Wallis ANOVA test followed by Mann Whitney test if significant results were recorded. Spearman's Rank correlation coefficient was used for correlation between different variables in each group. Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant.

The mean value of age in nephrotic group was notably higher relative to control (p= 0.010) and LN (p= 0.012) groups, may be due to the fact that membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults and usually affects the elderly rather than the young adults (mean age in nephrotic group is 43.40 ± 13.92 years). While the mean value of age in both control and LN groups was statistically comparable (p= 0.755).

As regards gender distribution, there was no statistically substantial difference between control and both nephrotic and LN groups (p= 0.139 and p= 0.427, respectively). While the number of females was statistically higher in LN than in nephrotic (p= 0.009) that also is compatible with the fact that the incidence of SLE male to female ratio is 10:1.

There was no statistically considerable difference in weight value between the three groups. As regards kidney function tests (creatinine and urea), they were substantially elevated in both nephrotic and LN groups when compared to control group, while they were statistically comparable in both nephrotic and LN groups (Table 1).

RESULTS

Table (1): Comparison between the values of age, gender, weight, renal functions, proteinuria, and eGFR by MDRD equation in the three studied groups

	Control (n= 15)	Nephrotic (n= 15)	LN (n= 15)	p value		
				Control vs nephrotic [§]	Control vs LN [§]	Nephrotic vs LN
Age (yrs.)	30.87 ±8.50	43.40 ± 13.92	31.27 11.68	0.010	0.755	0.012*
Gender (F/M) [#]	9/6	4/11	12/3	0.139	0.427	0.009*
Weight (kg)	74.67 ±11.39	77.10 ± 11.30	73.40 11.59	0.406	0.884	0.205
S. Creatinine (mg %)	0.77 ± 0.10	1.19 ± 0.60	1.43 ± 0.78	0.001*	0.001*	0.110
S. urea (mg %)	24.73 ± 3.26	42.74 ± 25.39	54.17 37.96	0.034*	0.001*	0.319
Proteinuria(gm/day)	0.30 ± 0.00	4.25 ± 2.67	1.73 ± 0.95	----	----	----
EGFR/MDRD(ml/min)	106.95 ±6.97	80.02 ± 29.04	59.15 ± 6.30	0.008*	0.001*	0.033*

Data are expressed as mean ± SD or numbers, [#]= Chi square test or Fisher exact test, [§]= Mann-Whitney test.
*p< 0.05= significant, p> 0.05= Not significant.

The mean value of EGFR/MDRD was significantly decreased in both nephrotic and LN groups when compared to control group. Remarkably decreased in LN relative to nephrotic (Table 1). There was a statistically substantial increase in the value of alpha actin 4 in both nephrotic and LN groups relative to the control (p= 0.001). A statistically considerable increase in the value of podocin in LN group in relation to the controls (p= 0.001). But no statistically remarkable difference in value of podocin between nephrotic and control groups (p= 0.494). The value of synaptopodin was notably lower in nephrotic patients (p= 0.001) and significantly increased in LN patients (p= 0.001) as against the control group (p= 0.001). As regards podocalyxin, there was a statistically substantial decline in its value in both nephrotic and LN groups when compared to controls (p= 0.001 and p= 0.021, respectively) (Table 2 and Figures 1 & 2).

Table (2): Comparison between values of alpha actin 4, podocin, synaptopodin and podocalyxin in the three studied groups

	Control (n= 15)	Nephrotic (n= 15)	LN (n= 15)	p value	
				Control vs nephrotic ^{\$}	Control vs LN ^{\$}
Alpha actin 4	1.01 ± 0.14	3.21 ± 1.34	2.42 ± 0.82	0.001*	0.001*
Podocin	1.02 ± 0.25	1.81 ± 1.71	3.52 ± 2.96	0.494	0.001*
Synaptopodin	1.03 ± 0.24	0.49 ± 0.65	1.85 ± 0.35	0.001*	0.001*
Podocalyxin	1.03 ± 0.25	0.42 ± 0.45	0.87 ± 0.96	0.001*	0.021*

Data are expressed as mean ± SD. \$= Mann-Whitney test, *p< 0.05= Significant, p> 0.05= Not significant

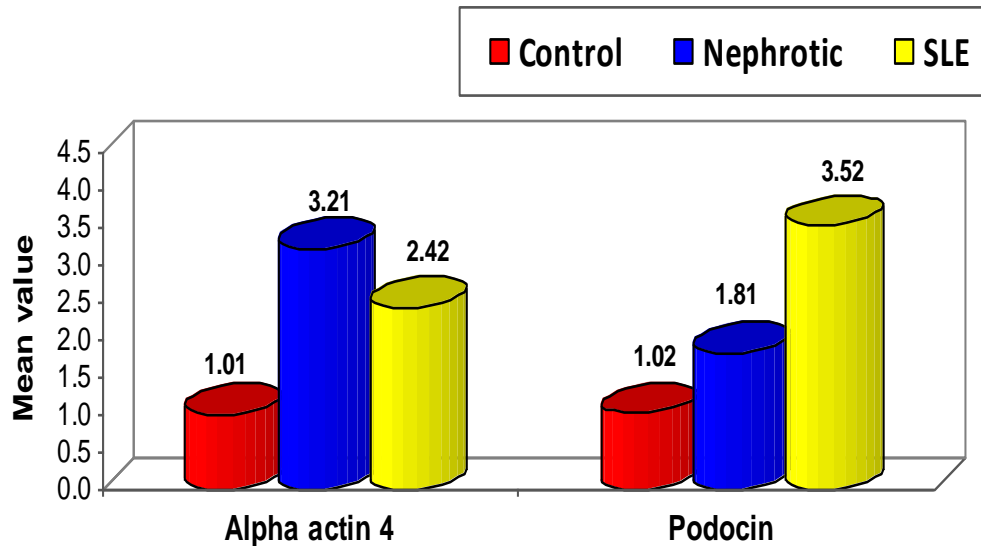


Figure (1): Mean values of alpha actin 4 and podocin in the three groups

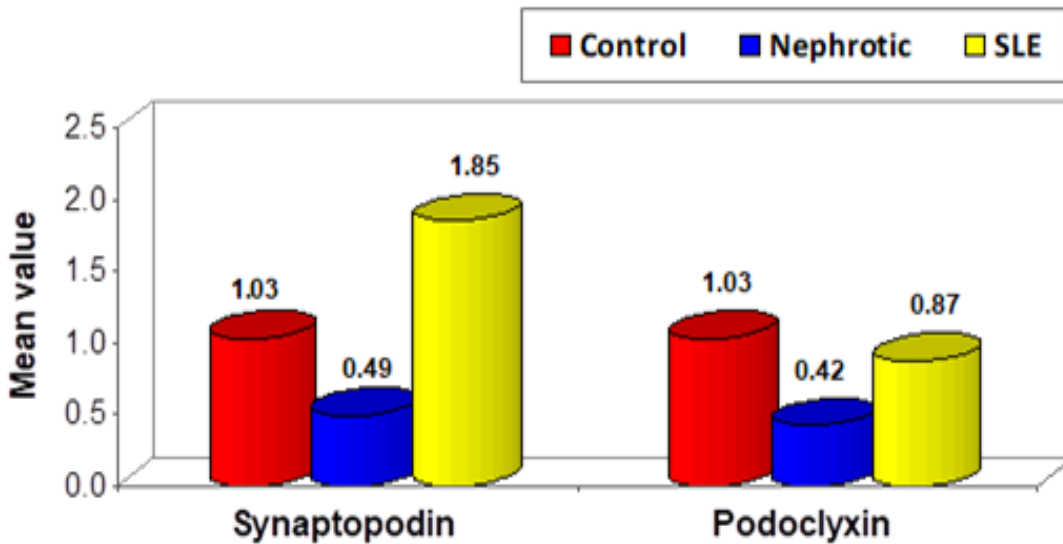


Figure (2): Mean values of synaptopodin and podocalyxin in the three groups

No statistically significant difference when comparing age, weight, renal functions, proteinuria, eGFR by MDRD equation in both active and non active LN subgroups. Mean proteinuria level in non active LN subgroup was 1.04 ± 0.82 g/day and mean proteinuria level in active LN subgroup was 2.19 ± 0.75 g/day (Table 3).

Table (3): Comparison between the values of age, gender, weight, renal functions, proteinuria, eGFR by MDRD equation in both active and non active LN subgroups and control group

	Control (n= 15)	Non-active LN (n= 6)	Active LN (n= 9)	p value		
				Control vs Non-active [§]	Control vs active [§]	Non-active vs active [§]
Age (yrs.)	30.87 ± 8.50	27.67 ±8.41	33.67 ±3.36	0.483	0.929	0.315
Weight (kg.)	74.67 ± 11.39	68.83 ± 7.19	76.44 ±13.30	0.226	0.473	0.175
S. creatinine (mgm%)	0.77 ± 0.10	1.75 ± 0.19	1.22 ± 0.14	0.001*	0.001*	0.157
S. urea(mgm%)	24.73 ± 3.26	62.58 ± 4.59	48.57 ±4.51	0.001*	0.005*	0.239
Proteinuria (gm/day)	0.30 ± 0.01	1.04 ± 0.12	2.19 ± 0.25	----	----	----
EGFR/MDRD (ml/min)	106.95 ± 6.97	46.95 ± 7.97	67.29 ±8.67	0.001*	0.001*	0.195

Data are expressed as mean ± SD, §= Mann-Whitney test, *p< 0.05= Significant, p> 0.05= Not significant

Statistical notable increase in alpha actin 4 value in both non-active and active subgroups relative to control group (p= 0.001), while its value was similar in both active and non-active groups (p= 0.126). The value of podocin in active group was increased significantly relative to control and non-active groups (p= 0.001 and p= 0.007, respectively). Also, there was statistically considerable increase in its value in non-active group relative to control group (p= 0.016). The value of synaptopodin was notably increased in both non-active (p= 0.001) and active patients (p= 0.001) as against control. But no statistically substantial difference between non-active and active patients (p= 0.126). As regards podocalyxin, its value was decreased in non-active group relative to controls (p= 0.019). While no statistically considerable difference between active patients and both control (p= 0.128) and non-active groups (p= 0.157) (Table 4 and Figures 3 & 4).

Table (4): Comparison between values of alpha actin 4, podocin, synaptopodin and podocalyxin in both non active and active LN subgroups and control group

	Control (n= 15)	Non-active LN (n= 6)	Active LN (n= 9)	p value		
				Control vs Non-active [§]	Control vs active [§]	Non-active vs active [§]
Alpha actin 4	1.01 ± 0.14	1.91 ± 0.75	2.76 ± 0.72	0.001*	0.001*	0.126
Podocin	1.02 ± 0.25	1.62 ± 0.82	4.79 ± 3.22	0.016	0.001*	0.007*
Synaptopodin	1.03 ± 0.24	1.71 ± 0.27	1.94 ± 0.38	0.001*	0.001*	0.126
Podoclayxin	1.03 ± 0.25	0.64 ± 0.91	1.03 ± 1.01	0.019*	0.128	0.157

Data are expressed as mean ± SD, §= Mann-Whitney test, *p< 0.05= Significant, p> 0.05= Not significant.

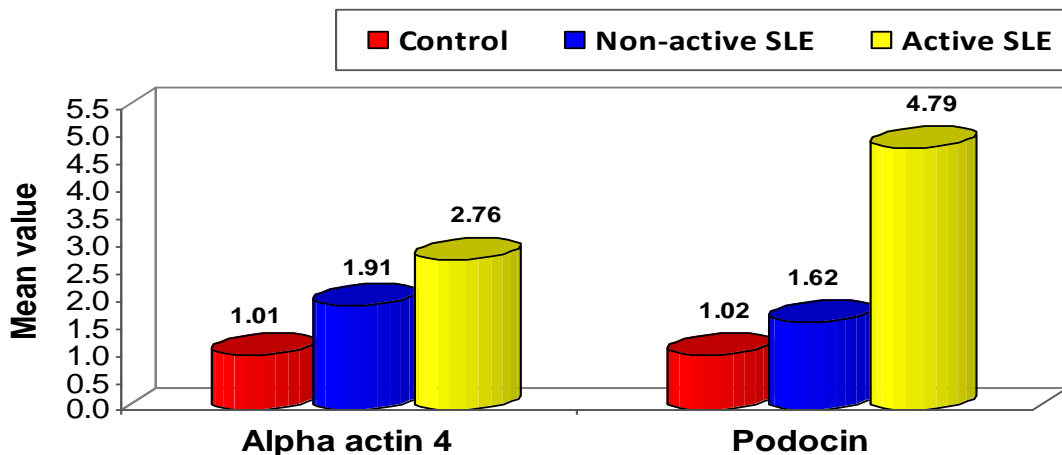


Figure (3): Mean values of alpha actin 4 and podocin in both non active and active LN subgroups and control group.

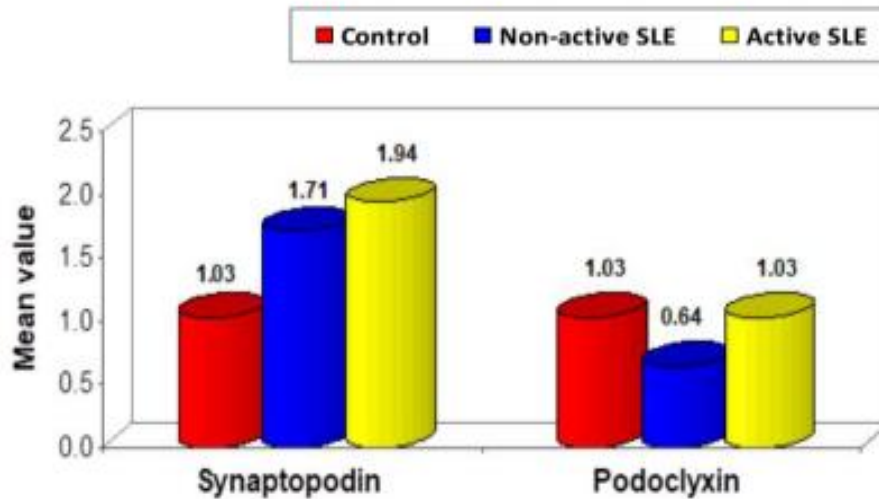


Figure (4): Mean values of urinary synaptopodin and podocalyxin in both non active and active LN subgroups and control group.

In nephrotic group,

Alpha actin 4 level was directly proportional with creatinine level ($r= 0.514$; $p= 0.050$), but not with other different studied parameters. The level of synaptopodin was in positive correlation with age ($r= 0.569$; $p= 0.027$), but not with other different studied parameters. Also, there was no significant correspondence between either podocin and podocalyxin levels or different studied parameters.

In LN group

Alpha actin 4 level was positively correlated with podocin level ($r= 0.596$; $p= 0.019$), but there was no significant correlation with other different studied parameters. Podocin was negatively associated with creatinine level ($r= -0.567$; $p= 0.027$), but there was no other significant correlations. There was no statistical significant correlation between level of both synaptopodin and podocalyxin and different studied parameters.

DISCUSSION

Urinary podocytes loss is a process that can accelerate glomerulosclerosis in the presence of a glomerular injury, which is mostly due to the inability of podocyte division in vivo. Normally daily podocyte loss does not lead to proteinuria as the podocytes pool exceeds the requirement of a glomerulus throughout a lifespan, and the exposed glomerular basement membrane (GBM) is shielded by podocytes. When urinary podocytes loss outnumbers its normal level, urinary protein loss appears, so proteinuria is considered a late event. The search for early noninvasive urinary markers is essential to be found for early diagnosis and treatment plan to prevent the progression of glomerular injury, so urinary podocytes loss could be used as an early urinary marker of glomerular injury and for follow-up after treatment ⁽¹⁴⁾.

Several clinical studies have reported the existence of viable podocytes and proteinuria in patients presented by different glomerular diseases. Proteinuria is usually present during active and chronic forms of glomerulopathy but podocyturia looks to be associated with active disease only. Urinary podocytes are not considered an indicator for proteinuria ⁽¹⁵⁾. Podocyturia can be used as an early marker of glomerular injury ⁽¹⁶⁾.

In our study in patients with nephrotic syndrome we found a statistically noteworthy rise in the value of alpha actin 4 as against the control group. Podocin value was insignificant between nephrotic and control groups ($p= 0.494$). There was significant decrease in the value of synaptopodin and podoclyxin in relation to control group (the levels of $p= 0.001$).

Also, urinary expression of mRNA of podocytes was statistically significant when comparing renal function tests and estimated glomerular filtration rate (by MDRD study equation) between nephrotic and control groups. The obtained results agree with Hara *et al.* ⁽¹⁷⁾ and Fukuda *et al.* ⁽¹⁸⁾ who both documented that renal function measures the effect of the accumulation of loss and damage of podocytes over time, which includes periods of increase and decrease podocyte loss, this may be because of success of therapy. Cumulative podocyturia over time reflects the progression of glomerular disease. So, these results support that urinary mRNAs of podocytes provide disparate and supplementary information, which can be complementary to proteinuria.

The result of this study documented that there was low correlation between proteinuria and urinary mRNA of podocytes especially in patients with membranous nephropathy (most of the patients of the nephrotic group). This is endorsed by the data of Troyanov *et al.* ⁽¹⁹⁾ and Heeringa *et al.* ⁽²⁰⁾ as they proved that various glomerular diseases exhibited different relations between urinary protein loss and podocyte depletion rate, as in membranous nephropathy, there is

no correlation between podocyturia and proteinuria levels. This result is homogeneous with the clinical experience that disease progression in membranous nephropathy does not go hand in hand with proteinuria.

Concerning patients with LN, there was statistically significant difference when comparing renal function tests and estimated glomerular filtration rate (by MDRD study equation) between LN and control group. We found a statistical substantial rise in the value of alpha actin 4, synaptopodin and podocin against the control group ($p=0.001$). As regards podocalyxin, it was considerably declined in relation to the control group ($p=0.021$). Also, our results showed that in the active LN subgroup the mean proteinuria range was 2.19 ± 0.75 g/day, while in the non-active subgroup it was 1.04 ± 0.82 g/day. There was a statistical noteworthy rise in the value of podocin in active group in relation to both control and non-active groups. For the other three markers (Alpha actin 4, synaptopodin and podocalyxin), there were no statistically substantial variance between non-active and active patients.

This indicated the possibility of the use of podocin levels especially as a marker to determine the severity and the activity of LN. Wang *et al.*⁽²¹⁾, documented that urinary mRNAs of podocytes were identified with lupus nephritis (LN) and diabetic nephropathy patients, and they have postulated these results to the disease progression. Bollain *et al.*⁽²²⁾ proved that decrease of podocytes significantly in the kidney, related to the progressive excretion of podocytes in urine and proteinuria in LN patients. Also, urinary podocytes detachment is correlated with the albumin/creatinine ratio and both were correlated to LN activity⁽²³⁾.

As regarding, the observation in this study that podocin is significantly correlated to LN activity, it is also supported by the result of ElShaarawy *et al.*⁽²⁴⁾ as they proved that urinary podocin was highly sensitive and specific in relation to LN activity and may be used clinically as prognostic marker in LN patients. This concept is supported by the data of Sabino *et al.*⁽²³⁾, who studied podocyturia by indirect immunofluorescence technique by utilizing primary antibodies to podocytes (anti podocin, synaptopodin, nephrin). They found that podocin positive cells were remarkably associated with the LN severity. So, they concluded that anti-podocin antibody was the most appropriate biomarker in comparison with anti-synaptopodin and anti-nephrin in monitoring the LN activity. Sir Elkhatim *et al.*⁽²⁵⁾ revealed that urinary podocytes proteins have been clinically used to evaluate various glomerulopathies, and the diagnosis of podocyturia may become a non-invasive method in different glomerular disease evaluation.

CONCLUSION

In nephrotic syndrome, alpha actin mRNA was increased significantly in relation to controls, but there was significant decrease of synaptopodin and podocalyxin mRNA. Levels of mRNA podocyte proteins had a

correlation with renal function and GFR, but not with proteinuria. Concerning lupus nephritis, there was significant increase of alpha actin, synaptopodin and podocin. Contrastingly, Podocalyxin significantly decreased. Podocin was significantly increased in the active disease subgroup, while no statistical difference for the other 3 mRNA proteins. Podocin can be used as marker of activity in L N.

So, as consequence from the previous mentioned studies and data, these all hypothesizes that urinary mRNA podocyte detection could be used as markers for the different glomerular disease, diagnosis and follow up.

Source of funding

This work is based off of Research project supported by Theodor Bilharz Research Institute (TBRI).

Conflicts of interest disclosed: None.

REFERENCES

1. Steffes M, Schmidt D, McCreery R *et al.* (2001): International diabetic nephropathy study group. Glomerular cell number in normal subjects and in type 1 diabetic patients. *Kidney Inter.*, 59 (6): 2104-13.
2. Wiggins R (2007): The spectrum of podocytopathies: a unifying views of glomerular diseases. *Kidney Inter.*, 71: 1205-14.
3. Wickman L, Afshinnia F, Wang S *et al.* (2013): Urine podocyt e mRNAs, proteinuria, and progression in human glomerular diseases. *J Am Soc Nephrol.*, 24: 2081-95.
4. Alcázar R, Egido J, Fisiopatología Y (2004): Tratamiento general. In: Hernando L (ed.): *Nefrología Clínica*. 2nd. Madrid: Panamericana, Pp: 277-89.
5. KDIGO clinical practice guideline for glomerulonephritis, chapter 12 (2012): lupus nephritis. *Kidney International Supple.*, 2: 221-32.
6. Seshan S (2014): Renal Pathology: SY23-1 classification of lupus glomerulone- phritis: an update. *Pathol.*, 46: S40-41.
7. Ortega L, Schultz D, Lenz O (2010): Review: lupus nephritis: pathologic features, epidemiology and a guide to therapeutic decisions. *Lupus*, 19: 557-74.
8. Gigante A, Gasperini M, Afeltra A *et al.* (2011): Cytokines expression in SLE nephritis. *European Rev Med Pharmacol Sci.*, 15: 15-2.
9. Rekvig O, Van der Vlag J (2014): The pathogenesis and diagnosis of systemic lupus erythematosus: still not resolved. *Seminars in Immunopathol.*, 36: 301-11.
10. Segarra-Medrano A, Carnicer-Cáceres C, Arbós-Via M *et al.* (2012): Biological markers of nephrotic syndrome: a few steps forward in the long way. *Nefrologia*, 32 (5): 558-72. doi :10.3265/Nefrologia.pre2012.Jun.11396
11. Carmici M (2007): Urinary detection of podocyte injury. *Biomed Pharmacotherapy*, 61: 245-9.
12. Zheng M, Lv L, Ni J *et al.* (2011): Urinary podocyte-associated mRNA profile in various stages of diabetic nephropathy. *PLoS ONE*, 6 (5): e20431.
13. Liss B (2002): Improved quantitative real-time RT-PCR for expression profiling of individual cells. *Nucleic Acids Res.*, 30: e89.

14. **Trimarchi H (2015):** Podocyturia :What is in a name? *J Translational Internal Med.*, 3 (2): 51-6.
15. **Yu D, Peterman A, Kunter U et al. (2005):** Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J Am Soc Nephrol.*, 16: 1733–41.
16. **Petermann A, Floege J (2007):** Podocyte damage resulting in podocyturia: a potential diagnostic marker to assess glomerular disease activity. *Nephron Clin Pract.*, 106: c61-6. PMID: 17570931.
17. **Hara M, Yanagihara T, Kihara I (2007):** Cumulative excretion of urinary podocytes reflects disease progression in IgA nephropathy and Schönlein–Henoch purpura nephritis. *Clin J Am Soc Nephrol.*, 2: 231–238.
18. **Fukuda A, Sato Y, Iwakiri T et al. (2015):** Urine podocyte mRNAs mark disease activity in IgA nephropathy. *Nephrology Dialysis Transplantation*, 30:1140–50.
19. **Troyanov S, Roasio L, Pandes M et al. (2006):** Renal pathology in idiopathic membranous nephropathy: A new perspective. *Kidney International*, 69: 1641–1648.
20. **Heeringa S, Branten A, Deegens J et al. (2007):** Focal segmental glomerulosclerosis is not a sufficient predictor of renal outcome in patients with membranous nephropathy. *Nephrol Dialysis Transplantat.*, 22: 2201–7.
21. **Wang G, Lai F, Kwan B et al. (2009):** Podocyte loss in human hypertensive nephrosclerosis. *Am J Hypertension*, 22: 300–6.
22. **BollainY, Goytia J, Gonza'lez-Castaneda M et al. (2011):** Biomarkers in lupus nephritis patients 181 et al. Increased excretion of urinary podocytes in lupus nephritis. *Indian J Nephrol.*, 21 (3): 166–71.
23. **Sabino A, Teixeira Vde P, Nishida S et al. (2013):** Detection of podocyturia in patients with lupus nephritis. *Brazilian J Nephrol.*, 35 (4): 252–8.
24. **ElShaarawy A, Behairy M, Bawady S et al. (2017):** MP139 study of urinary podocin as marker of lupus nephritisactivity. *Nephrol Dialysis Transplantat.*, 32 (3): iii479 <https://doi.org/10.1093/ndt/gfx164.MP139>
25. **Sir Elkhatim R, Li J, Yong T et al. (2014):** Dipping your feet in the water: podocytes in urine. *Expert Rev of Molecular Diagnostics*, 14 (4) : 423-37.