Clinical and Experimental Rheumatology 2014; 32: 572-574.

Urinary excretion of nephrin in rheumatoid arthritis patients with proteinuria

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Received on December 23, 2013; accepted in revised form on February 18, 2014.

Key words: rheumatoid arthritis, nephrin, proteinuria, urine

Competing interests: none declared.

ABSTRACT

Objective. *To investigate urine excretion of nephrin in patients with proteinuric nephropathies associated with rheumatoid arthritis (RA).*

Methods. *We enrolled in the study 42 patients with seropositive RA and proteinuria, the control group (20 persons) was formed from healthy blood donors, the comparison group (23 persons) was formed from RA patients without proteinuria. Kidney biopsy was performed in 26 patients (glomerulonephritis was diagnosed in 14 patients, AA-amyloidosis in 7 patients and tubulointerstitial nephritis in 5 patients).*

Results. *Urine nephrin concentration in patients with RA and proteinuria was 6.2 (3.0; 8.8) ng/ml and significantly differed in its levels both in controls – 3.6 (2.4; 5.3) ng/ml (p=0.03) and RA patients without proteinuria – 3.2 (2.1; 5.1) ng/ ml (p=0.015) group.*

In RA patients with proteinuria, we found a positive correlation between urine nephrin and protein concentrations (r=0.4; p=0.04). Urine nephrin levels in patients of the glomerulonephritis – 7.3 (5.9; 9.2) and amyloidosis groups – 6.9 (3.9; 9.8) ng/ml were higher than in the controls (p=0.001; p=0.04) and in the group of patients without proteinuria (p=0.005; p=0.03). In the patients with tubulointerstitial nephritis urine nephrin concentration did not differ significantly with the values in both the control and the RA patients without proteinuria groups.

Conclusion. *According to our data, proteinuria in the overall cohort of patients with seropositive RA is associated with increased levels of urine nephrin excretion, the highest levels of nephrin excretion were registered in patients with glomerulonephritis and amyloidosis.*

Introduction

Podocyte injury is a very important cause of the progression of different renal diseases associated with proteinuria. Podocytes are the cells located in the outside of the glomerular basement membrane. These cells and their pedicles form a tight interdigitating network that controls the filtration of circulating plasma proteins from the capillary lumen into Bowman's space. Nephrin is a protein expressed in the slit diaphragm between the podocyte foot processes. Shedding of nephrin and some other proteins would disrupt the glomerular filtration barrier and result in plasma protein leakage into the urine. Recent studies have demonstrated that different nephropathies, such as, systemic lupus erythematosus, glomerulonephritis, diabetes, preeclampsia are associated with increased urine nephrin concentration (1-5).

Rheumatoid arthritis (RA) is one of the most frequent autoimmune diseases with multiple organ involvement, that may determine poor prognosis (6-8). That is why many researchers investigate different biomarkers for early detection of system injury in RA (9, 10).

It is significant that the prevalence of chronic kidney disease (CKD) in rheumatoid arthritis has been reported as 5–50% (11-13). Moreover, autopsy findings in patients with RA have found renal failure as a major cause of death in 3–20% of cases (14, 15). Patients with RA may have various clinical and morphological variants of renal diseases (different types of glomerulonephritis, tubulointerstitial nephritis, amyloidosis) (16), most of which are associated with proteinuria. Proteinuria is also one of the key mechanisms of CKD progression in various diseases and in RA, particularly (17). There is no data about urine nephrin excretion in patients with nephropathies and proteinuria associated with RA.

The aim of this study was to investigate urine excretion of nephrin in patients with proteinuric nephropathies associated with RA.

Methods

The present study is a cross-sectional randomised pilot study that assesses urine nephrin excretion in patients with rheumatoid arthritis and proteinuria. This study was approved by the Ethics Committee of the Human Studies of Kuban State Medical University (Krasnodar, Russia) and was performed according to principles of the Helsinki Declaration of the World Medical Association (2008 revision). All patients enrolled in the study signed a form of informed consent.

A total of 42 patients (34 women, 8 men) with seropositive RA and proteinuria that exceeded 300 mg/24 hour were enrolled in this study. The control group (20 persons: 4 men and 16 women) was formed from healthy blood donors, the comparison group was formed from RA patients without proteinuria (23 persons: 5 men and 18 women). The research group, control and comparison groups did not differ according to demographic characteristics after randomisation (Table I). Nor did the RA groups differ according to clinical characteristics and medications (Table I). We excluded the patients with concomitant infectious, oncological diseases, purulent conditions of any localization, hepatic or cardiac insufficiency and patients with stage II-V of CKD (National Kidney Foundation classification, 2002).

Kidney biopsy was performed in 26 patients (tubulointerstitial nephritis was diagnosed in 5 patients, AA-amyloidosis in 7, mesangial proliferative glomerulonephritis in 9, membranous nephropathy in 3 and focal segmental glomerulosclerosis in 2 patients).

Proteinuria <3000 mg/24 hour was detected in 27 patients, we determined nephrotic syndrome with proteinuria >3000 mg/24 hour in 15 patients. Serum concentration of creatinine and glomerular filtration rate in most patients were within normal limits. CKD with stage I was registered in 12 patients in the group of RA patients with proteinuria and in 6 patients in the group of RA patients without proteinuria.

Urine samples were collected after patient consent was obtained. Spot urine samples were collected in sterile containers. Freshly obtained urine specimens were centrifuged, aliquoted, and stored at -70°C in a freezer until assay.

Urine nephrin concentrations were measured by ELISA using immune-enzyme analysis (analyzer Statfax 2100, Awareness Technology Inc., Palm City, FL, USA). ELISA kits for nephrin were purchased from Wuhan EIAab Science Co., Ltd (Wuhan, China). The range of the standard curve was 0.15–10 ng/ml. For each assay, all the samples were measured in duplicate on the same assay day. Within-assay variations were 7% for all the assays. Total urine protein concentrations were measured in all the urinary samples by the Bradford method.

Statistical analysis was performed using Statistica 6.0. (Statsoft, Tulsa, OK, USA). Data were presented as medians and ranges considering the asymmetrical type of distribution (Kolmogorov-Smirnov test). Differences between unpaired samples were measured with the non-parametric Mann-Whitney test. We also performed a correlation analysis between all parameters studied in patients with RA. Correlations were determined with the Spearman rank order correlation test.

Table I. Basic demographic and clinical characteristics of the study cohort.

Parameter	Controls (n=20)	Patients with RA and proteinuria (n=42)	Patients with RA without proteinuria (n=23)
n (%) female	16 (80)	34 (81)	18 (78)
Age, years	48.8 (42.3; 60.2)	46.8 (39.9; 61.4)	49.2 (40.5; 59.4)
Current smoking, n (%)	3 (15)	6 (14)	4 (17)
Duration of RA, years	-	7.8 (4.8; 10.3)	6.3 (5; 9.9)
Rheumatoid factor positive, n (%)	-	35 (83)	18 (78)
ACCP positive, n (%)	-	36 (86)	21 (91)
CRP, mg/l	-	20.2 (14.8; 29.3)	25.3 (15.9; 26.9)
ESR, mm/h	8 (2; 10)	32.5 (25; 36.2)	28.5 (13; 39)
Serum creatinine, mkmol/l	73.1 (62.3; 86.2)	78.7 (67.9; 89.3)	71.5 (66.8; 87.6)
Patients with glomerular filtration rate > 90 ml/min., n (%)	-	12 (29)	6 (26)
DAS28	-	7.2 (6.1; 8.1)	7.3 (6.8; 7.9)
Current methotrexate users, n (%)	-	30 (71)	18 (78)
Current NSAID users, n (%)	-	35 (83)	19 (83)
Current anti-TNF users, n (%)	-	10 (24)	5 (22)

Data presented as number (%) or as median (25; 75 percentiles).

Table II. Nephrin urine concentration in patients with rheumatoid arthritis and proteinuria.

Group	Number of patients	Urine nephrin concentration, ng/ml
Controls	20	3.6 (2.4; 5.3)
Patients with RA without proteinuria	23	3.2 (2.1; 5.1)
Patients with RA and proteinuria	42	6.2 (3; 8.8) p (p=0.03) §(p=0.015)

Data presented as median (25; 75 percentiles).
*significant differences with control group (Mann-Whitney test); §significant differences with group of patients with RA without proteinuria (Mann-Whitney test).

Results

The data of urine nephrin excretion in the overall cohort of RA patients with and without proteinuria are shown in Table II. Urine nephrin concentration in patients with RA and proteinuria was 6.2 (3.0; 8.8) ng/ml and was significantly different in its levels both in control group – 3.6 (2.4; 5.3) ng/ml (p=0.03) and RA patients group without proteinuria – 3.2 (2.1; 5.1) (p=0.015). We found a positive correlation between urine nephrin and protein concentrations (r=0.4; p=0.04) in the group of RA patients with proteinuria.

The data of nephrin urine excretion in patients with morphologically confirmed nephropathy are presented in Table III. Urine nephrin levels in patients of the glomerulonephritis group – 7.3 (5.9; 9.2) ng/ml were higher than in the controls (p=0.001) and in the patients without proteinuria group (p=0.005). In the group of patients with amyloidosis urine nephrin concentrations, 6.9 (3.9; 9.8) ng/ml were higher than both in the control group (p=0.04) and in the group of RA patients without proteinuria (p=0.03). In the patients with tubulointerstitial nephritis urine nephrin did not differ significantly with the values both in the control group and in the RA patients group without proteinuria.

Table III. Nephrin urine concentration in patients with various morphological types of nephropathies associated with rheumatoid arthritis.

Group	Number of patients	Urine nephrin concentration, ng/ml
Controls	20	3.6 (2.4; 5.3)
Patients with RA without proteinuria	23	3.2 (2.1; 5.1)
Patients with RA with proteinuria (glomerulonephritis)	14	7.3 (5.9; 9.2)* (p=0.001) §(p=0.005)
Patients with RA with proteinuria (AA-amyloidosis)	7	6.9 (3.9; 9.8) *(p=0.04) §(p=0.03)
Patients with RA with proteinuria (tubulointerstitial nephritis)	5	3 (1.2; 5)

Data presented as median (25; 75 percentiles).
*Significant differences with control group (Mann-Whitney test); §Significant differences with group of patients with RA without proteinuria (Mann-Whitney test).

Discussion

The mechanisms of selective permeability of the glomerular filter for plasma proteins are intensively investigating at present time, and the podocytes and slit diaphragm proteins are crucial for the maintenance of renal selectivity. Podocyte injury is a major pathogenetic component of the proteinuria and the renal disease progression. The violation of the slit diaphragm proteins expression in all the proteinuric nephropathies may be the stereotypical reaction of podocyte damage (via immune-mediated and non-immune mechanisms). Therefore, at the present level of understanding the increased expression of urine nephrin could be regarded as a marker of the slit diaphragm damage.

According to our data, proteinuria in the overall cohort of patients with seropositive RA was associated with increased levels of urine nephrin excretion. Also urine nephrin levels in patients with glomerulonephritis and amyloidosis were higher than those in the control group and in the group of RA patients without proteinuria. In patients with tubulointerstitial nephritis nephrin levels were not elevated.

Thus, we have first found that in RA patients proteinuria was associated with increased concentrations of nephrin in the urine. It is noteworthy that podocyte injury is defined in glomerulonephritis in the experimental and clinical studies (18, 19), but we have not found any data about nephrin urine concentration in patients with amyloidosis. Our study demonstrated that patients with amyloidosis may have increased urine nephrin levels, but additional experimental studies are needed to explore the possible mechanisms of this process. It is interesting that in patients with tubulointerstitial nephritis we have not registered significant differences with controls or RA patients without proteinuria, this fact may demonstrate the less severity of podocyte damage in this disease.

Our data about urinary excretion of nephrin in RA associated nephropathies are preliminary, and it is necessary to continue the clinical and experimental research.

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