Clinical Biochemistry 48 (2015) 546–549

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Short Communication

Increased urinary cystatin C level is associated with interstitial fibrosis and tubular atrophy in kidney allograft recipients

Maria de Fátima Castro Mendes [a], João Victor Salgado [b,*], José de Ribamar Lima [a], Teresa Cristina Alves Ferreira [c], Gyl Eanes Barros Silva [d], Natalino Salgado Filho [e]

[a] Clinical Chemistry Service, University Hospital, Federal University of Maranhão, Brazil
[b] Kidney Disease Prevention Centre and Department of Physiological Sciences, Federal University of Maranhão, Brazil
[c] Kidney Transplant Service, University Hospital, Federal University of Maranhão, Brazil
[d] Department of Pathology, Federal University of Maranhão, Brazil
[e] Kidney Disease Prevention Centre and Department of Medicine I, Federal University of Maranhão, Brazil

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 6 February 2015
Accepted 13 February 2015
Available online 6 March 2015

Keywords:
Interstitial fibrosis tubular atrophy
Kidney allograft
Kidney transplantation
Renal biopsy
Urinary biomarkers
Cystatin C

ABSTRACT

Aim: The objective of this study was to investigate the correlation between the urinary excretion of cystatin C (CysC) and the presence of interstitial fibrosis/tubular atrophy (IF/TA) in renal transplant (RT) recipients.

Methods: This prospective study included 21 adult patients who had undergone renal biopsy and RT ≥6 months prior. According to the renal biopsy reports, the patients were divided into groups with (n = 12) or without (n = 9) IF/TA. Analytical parameters included the following: serum and urinary levels of CysC, creatinine (Cr) and sodium (Na), total urinary protein, urinary CysC/creatinine ratio [u(CysC/Cr)], fractional excretion of sodium (FENa) and estimated glomerular filtration rate (eGFR) based on the Chronic Kidney Disease Epidemiology Collaboration equation.

Results: The values of uCysC, u(CysC/Cr), proteinuria, and FENa were significantly higher in patients with IF/TA than in patients without IF/TA. The values of eGFR were statistically lower in patients with IF/TA ($p = 0.001$). Values of uCysC significantly correlated with those of serum Cr, FENa, and eGFR ($p < 0.001$). Among the patients with IF/TA, 67% presented with glomerulosclerosis (segmental/global).

Conclusion: Elevated levels of urinary CysC are associated with interstitial fibrosis and tubular atrophy in RT recipients and may become a useful tool for monitoring kidney allografts.

Introduction

Renal transplantation (RT) is considered the main alternative to renal replacement therapy in patients with end-stage kidney chronic disease since it ensures longer survival and better quality of life [1–3]. However, interstitial fibrosis/tubular atrophy (IF/TA) and death that occur while the graft is still functional continue to be the main causes of allograft loss beyond the first year after RT. IF/TA is defined as a slow, progressive, irreversible, functional, and morphological deterioration of the kidney allograft [4].

In clinical practice, kidney allograft biopsy is commonly used as the gold standard method to diagnose IF/TA. However, it has obvious limitations, including high cost, sampling errors, and invasiveness that carries the risk of potential morbidity [5]. Although IF/TA may also be suspected by progressive increases in serum creatinine (sCr), there is no current clinical or laboratory tests to identify IF/TA [6]. Therefore, it is very important to seek new non-invasive biomarkers that may quickly and accurately assess short-and long-term kidney allograft function.

In the last decade, low-molecular-weight urinary proteins such as α1-microglobulin, retinol binding protein, and cystatin C (CysC) have been studied as injury or tubular dysfunction markers in RT patients [7,8]. CysC is freely filtered by renal glomeruli and then, almost completely reabsorbed and metabolised by proximal tubular cells in the absence of tubular or extra-renal excretion. Therefore, the level of urinary CysC (uCysC) is usually low [9]. This finding is in agreement with the results published in the literature showing that elevated levels uCysC levels may reflect tubular dysfunction as well as primary or secondary tubulointerstitial disease [10–13].

A recent study assessed uCysC as a marker of early allograft dysfunction during the immediate postoperative period in RT patients [8]. However, no studies have assessed the possible correlation between urinary CysC excretion and chronic tubulointerstitial injury in RT patients. Therefore, this study aimed to investigate whether urinary CysC excretion is associated with the presence of interstitial fibrosis and tubular atrophy in RT recipients.

* Corresponding author at: Rua das Limeiras Quadra D Casa 17, Jardim Renascença I, 65075260 São Luís, Maranhão, Brazil.
E-mail address: joao.salgado@ufma.br (J.V. Salgado).

http://dx.doi.org/10.1016/j.clinbiochem.2015.02.008
0009-9120/

Methods

Patients

A prospective study was carried out in 21 renal transplant patients of the Renal Transplantation Service of the University Hospital of the Federal University of Maranhão (HUUFMA). For these patients, the exclusion criteria were: age <18 years old, period after RT <6 months, non-attendance to the regular outpatient clinic follow-up after RT, no realisation of renal biopsies during the study period, obstructive uropathy in the urinary tract, transplant renal artery stenosis, thyroid disorder, decompensated diabetes mellitus, neoplasms, biopsy containing less than 5 glomeruli, and graft acute rejection. In this study, all patients received prednisone (5–10 mg/day) in association with other immunosuppressive drugs administered in different combinations (tacrolimus, mycophenolate mofetil, mycophenolate sodium, cyclosporine A, azathioprine, sirolimus, and everolimus) at maintenance dosages. The study was approved by the ethics committee of HUUFMA, and those patients who agreed to participate in the study signed a Free and Informed Consent Form.

Histological analyses

Kidney biopsies were performed with the aid of an ultrasound and Pro-Mag™ Ultra automatic instrument with an 18-gauge needle and processed in the Laboratory of Surgical Pathology of HUUFMA. Kidney biopsies were performed upon medical indications and carried out in renal dysfunction or protocol cases (medication conversion). Banff 2007 classification was used for the diagnosis of IF/TA [14].

The samples were fixed in formalin and stained with haematoxylin-eosin, periodic acid–Schiff, Grocott's methenamine silver, and Masson's trichrome stain. The presence and classification of IF/TA were assessed by Masson's trichrome staining and divided into two groups, with (n = 12) and without (n = 9) IF/TA. None of the patients reported recurrence of underlying disease, de novo glomerulonephritis, calcineurin inhibitor nephrotoxicity (morphological or functional), or acute tubular necrosis.

Laboratory analyses

The patients' clinical and demographic data were obtained from medical records on the day that the kidney biopsies were performed. Twenty days after the kidney biopsy, during the morning after an 8-hour fast, the patients were referred to the Clinical Chemistry Service of HUUFMA for laboratory testing. Serum (sCr) and urinary (uCr) levels of creatinine were assessed using the alkaline picrate method according to the Jaffé kinetic reaction with a calibration traceable to isotope dilution mass spectrometry. Serum (sNa) and urinary (uNa) values of sodium were measured with an ion-selective electrode, whereas proteinuria was measured using the turbidimetric method. These analyses were carried out on a chemistry analyser (Cobas c501; Roche Diagnostics, Switzerland). The serum (sCysC) and urinary (uCysC) levels of CysC were measured by immunonephelometry (BN II Analyzer System, Siemens, Germany). The estimated glomerular filtration rate (eGFR) was calculated based on the values of calibration sCr, using the equation Chronic Kidney Disease Epidemiology Collaboration formula [15]. Fractional excretion of sodium (FENa) and urinary cystatin C/creatinine ratio [u(CysC/Cr)] were also assessed. FENa was calculated as follows: $FENa\ (\%) = (uNa \times sCr/sNa \times uCr) \times 100$.

Statistical analyses

The Shapiro–Wilk test was used to assess the normality of the quantitative variables. To compare quantitative variables between study groups, Student's *t* test was used for independent samples, whereas the Mann–Whitney test was used in the absence of normality. Fisher's exact test was used to compare qualitative variables between groups. The correlation between laboratory variables was assessed using the Spearman's rank correlation coefficient. A significance level of 5% was adopted and the data analysis was performed using Stata version 10.0 (Stata, Texas, USA).

Results

In this study, RT patients were predominantly men (57%) with an average age of 35.5 ± 10.2 years, median period of 9 months after RT, and average body mass index of 23.8 ± 4.0 kg/m^2. Overall, 61.9% of the RT patients reported underlying disease due to undetermined cause. A total of 76% of patients received allografts from living donors. The biopsies were performed for increased sCr levels (46%), elevated proteinuria 2 (13%), elevated sCr and proteinuria 4 (27%) and microscopic hematuria 2 (13%). Regarding IF/TA diagnosis, 11 patients presented with mild IF/TA, and only one showed severe IF/TA. The clinical variables among RT patients are shown in Table 1 and compared between groups with and without IF/TA.

Regarding laboratory parameters, the concentrations of sCr ($p = 0.006$) and sCysC ($p = 0.017$) were significantly higher in patients with IF/TA than in patients without IF/TA. Patients with IF/TA presented higher values of proteinuria ($p = 0.019$), uCysC ($p = 0.001$), u(CysC/Cr) ($p = 0.005$), and FENa ($p = 0.044$) than patients without IF/TA. Moreover, values referring to eGFR were significantly lower in patients with IF/TA ($p = 0.001$). The Spearman's rank correlations test was used to analyse the correlations between uCysC and u(CysC/Cr) and

Table 1
Clinical variables of renal transplant patients with and without interstitial fibrosis/tubular atrophy (IF/TA).

Variables	Total (n = 21)	IF/TA		*p* Value
		No (n = 9)	Yes (n = 12)	
Age (years)	35.5 ± 10.2	34.9 ± 9.1	36.1 ± 11.4	0.798
Gender				
Male	12 (57)	7 (78)	5 (42)	0.184
Female	9 (43)	2 (22)	7 (58)	
BMI (kg/m^2)	23.8 ± 4.0	23.6 ± 3.5	24.0 ± 4.7	0.876
Underlying disease				
Undetermined	13 (61.9)	8 (89)	5 (42)	0.315
SAH	2 (9.5)	1 (11)	1 (8)	
CGN	2 (9.5)	0	2 (17)	
FSGS	2 (9.5)	0	2 (17)	
SLE	1 (4.8)	0	1 (8)	
Reflux nephropathy	1 (4.8)	0	1 (8)	
Donor				
Alive	16 (76)	6 (67)	10 (83)	0.611
Dead	5 (24)	3 (33)	2 (17)	
Time after RT (months)	9 (7–27)	7 (6–9)	21 (8–29)	0.068
Biopsy indication				
Renal dysfunction	15 (71)	4 (44)	11 (92)	0.029*
Protocol	6 (29)	5 (66)	1 (8)	
Renal dysfunction				
Elevated sCr	7 (47)	1 (11)	6 (50)	0.054
Elevated proteinuria	2 (13)	1 (11)	1 (8)	
Elevated sCr and proteinuria	4 (27)	0	4 (33)	
Microscopic haematuria	2 (13)	2 (22)	0	
Segmental/global glomerulosclerosis				
Presence	9 (43)	1 (11)	8 (67)	0.016*
Absence	12 (57)	8 (89)	4 (33)	
Co-morbidities				
SAH	18 (86)	9 (100)	9 (75)	0.229
DM	3 (14)	0	3 (25)	0.229
SLE	1 (5)	0	1 (8)	1.000

*Level of significance, $p < 0.05$. BMI, body mass index; post-TR period, post-renal transplantation period; SAH, systemic arterial hypertension; CGN, chronic glomerulonephritis; SLE, systemic lupus erythematosus; FSGS, focal and segmental glomerulosclerosis; DM, diabetes mellitus; sCr, serum creatinine.