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Urinary mitochondrial DNA level in non-diabetic chronic kidney diseases

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ABSTRACT

Background: Mitochondrial dysfunction plays an important role in the pathogenesis and progression of chronic kidney disease (CKD). We study the relation between urinary mitochondrial DNA (mtDNA) levels and renal dysfunction in non-diabetic CKD.

Methods: We recruited 32 CKD patients (20 had hypertensive nephrosclerosis, 12 had IgA nephropathy). Urinary supernatant mtDNA level was measured and compared to baseline clinical and pathological parameters. The patients were followed 57.8 ± 30.5 months for renal function decline.

Results: The average urinary supernatant mtDNA level was 222.0 ± 210.3 copy/μL. There was a modest but significant correlation between urinary mtDNA level and proteinuria (Spearman's $r = 0.387$, $p = 0.035$), but not any other baseline clinical or pathological parameter. Urinary mtDNA level had a significant inverse correlation with the slope of GFR decline ($r = -0.402$, $p = 0.023$). Urinary mtDNA level is a predictor of renal survival even after adjusting for baseline proteinuria with multivariate Cox analysis. In this model, every increase in urinary mtDNA by 100 copy/μL confers a 25.0% increase in risk of doubling of serum creatinine or need of dialysis (95%CI, 0.7% to 55.1%).

Conclusion: Mitochondrial DNA is readily detectable in the urinary supernatant of non-diabetic CKD, and its level correlates with the rate of renal function decline and predicts the risk of doubling of serum creatinine or need of dialysis. Further studies are needed to determine the value of urinary supernatant mtDNA level as a prognostic indicator of non-diabetic CKD

1. Introduction

Chronic kidney disease (CKD) is characterized by long-lasting reduced glomerular filtration rate or increased urinary albumin excretion, and is increasingly recognized as a global public health issue [1]. The prevalence of CKD is estimated to be 8–16% worldwide and varies by ethnicity and socioeconomic indices [2]. A considerable proportion of CKD patients eventually progresses to end stage renal disease (ESRD), which is fatal if not treated by renal replacement therapy (RRT). Given the increasing prevalence of CKD, the burden of the disease is heavy and ever-rising [3,4].

The underlying mechanism of progressive renal function loss in CKD, however, is complicated and remains incompletely understood. Recent studies showed that acquired mitochondrial dysfunction is an important contributing factor to the progression of CKD [5–7]. Notably, proteinuria caused by a primary insult to the kidney induces oxidative stress to renal tubular cells, resulting in mitochondrial dysfunction [8–10], which leads to cellular damage by the generation of reactive oxygen species as well as epithelial-mesenchymal transition (EMT) [11,12]. In experimental models of CKD, aborting mitochondrial dysfunction prevents renal tubular cell EMT and renal fibrosis [8,13].

Previous studies showed that damaged mitochondria release their DNA content into the systemic circulation [14,15]. Cell-free mitochondrial DNA (mtDNA) could easily be detected in plasma, and has been explored as the biomarker of various diseases [16–21]. Notably, Chinese patients with end stage renal disease had higher circulating cell-free mtDNA contents, but less intracellular mtDNA, than healthy controls [18]. In this regard, dysfunctional mitochondria of renal tubular cells are released mtDNA into urine. We have recently showed that cell-free mtDNA is readily detectable in the urinary supernatant of patients with diabetic nephropathy, and urinary mtDNA levels correlate with renal function and scarring in diabetic nephropathy [22]. In the present study, we determine the relation between urinary mtDNA level and kidney damage in non-diabetic kidney diseases.

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2. Patients and methods

2.1. Patient selection

This observational study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. All study procedures were in compliance with the Declaration of Helsinki. We studied 32 CKD patients: 20 patients with biopsy-proved hypertensive nephrosclerosis (HTN), and 12 with IgA nephropathy (IgAN) between January and December 2009. After written informed consent, a whole-stream early morning urine specimen was collected for the measurement of mtDNA level on biopsy day. Clinical, biochemical, and histopathological data were collected by chart review. Glomerular filtration rate (GFR) was estimated by a standard Eq. [23]. CKD was defined and staged according to the standard criteria [24].

2.2. Urine mtDNA quantification

All urine specimens were processed immediately after collection according to methods described previously [25]. Briefly, protease inhibitors were added, and then samples were centrifuged at 1000-g to remove cells and cellular debris. Urinary supernatant specimens were stored at −80 °C until use. Urinary mtDNA was isolated and purified from supernatant by QIAamp Blood DNA Midi Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Urinary mtDNA level was then measured by digital polymerase chain reaction (dPCR) and adjusted for the urinary creatinine level. The chip-based dPCR platform (QuantStudio 3D digital PCR System, Life Technologies, Carlsbad, CA) was used. The method of quantification of urinary mtDNA has been reported previously [26]. Briefly, a maximum of 3 μl of DNA is mixed with 8 μl of digital PCR master mix (2×), 0.8 μl of mtDNA assay (primers, 150 nM each with MGB TaqMan; probe, 250 nM; both from Life Technologies) and make up to 16 μl. The PCR mix was then loaded on the digital PCR 20 K chip using the QuantStudio 3D digital PCR Chip Loader. PCR reaction was performed using flat-block ProFlex PCR system according to manufacturer instructions. Each dPCR run was performed with a blank control by adding water in place of template. The PCR condition is as follows: 96 °C for 10 min and 40 cycles of 60 °C for 2 min and 98 °C for 30 s, finally 60 °C for 2 min. After the thermal cycling, the chips were imaged using the QuantStudio 3D digital PCR Chip Reader and analyzed by the QuantStudio 3D AnalysisSuite software.

2.3. Morphometric study of kidney biopsy

Jones' silver staining was performed on 4 μm thick sections of renal biopsy specimen of each patient. As previously described by others [27,28], computerized image analysis method was used to semi-quantify nephrosclerosis. Briefly, a Leica Twin Pro image analysis system (Leica Microsystems, Wetzlar, Germany) was connected to a Leica DC500 digital camera on a Leica DMRXA2 microscope working with a × 40 objective (final calibration: 0.258 mm/pixel) and to a microcomputer for storage of the morphometric measurements and to perform image analysis by using image-analysing software (MetaMorph 4.0; Universal Imaging Corporation TM, Downingtown, PA, USA). Ten glomeruli and 10 randomly selected tubulointerstitial areas were assessed for each patient, and the average percentage of scarred glomerular and tubulointerstitial areas, as represented by the percentage of the area with positive staining, were computed for each patient.

2.4. Outcome assessment

After renal biopsy, all patients were followed for at least 24 months. Clinical management was decided by individual nephrologist and not affected by the study. Renal function test, including serum creatinine, urea and proteinuria levels, was assessed at least every 4 months.

As mentioned previously, estimated GFR was calculated by a standard Eq. [23]. The rate of GFR decline was calculated by the least squares regression method. The primary end point was the slope of GFR decline. Secondary outcomes is renal survival; events are defined as doubling of baseline serum creatinine and need of dialysis.

2.5. Statistical analysis

Statistical analysis was performed by SPSS for Windows software version 18.0 (SPSS Inc., Chicago, IL). Data were expressed as mean ± SD if normally distributed, or as median and range if not. Data between groups were compared by unpaired Student's *t*-test, or Chi square test as appropriate. Relation between urinary mtDNA level and clinical and histological parameters was explored by Spearman's rank correlation coefficient. For the relation with GFR decline, linear regression analysis was performed. For renal survival, Cox regression analysis was performed. In either case, multivariate analysis was performed to adjust for baseline proteinuria, which was found to be correlated with the urinary mtDNA level. A P value below 0.05 was considered statistically significant. All probabilities were two-tailed.

3. Results

We recruited 32 CKD patients; 20 had biopsy-proved HTN, and 12 had IgAN. Their demographic, baseline clinical and biochemical information are summarized in Table 1. There is no statistical significant difference in any baseline clinical or biochemical characteristics between the HTN and IgAN groups.

3.1. Relation with baseline parameters

Mitochondrial DNA was detectable in the urine supernatant of all patients. The median urinary supernatant mtDNA level was 149.9 (range 0.2–796.9) copy/μL. Urinary mtDNA level had a modest but

Table 1
Baseline demographic and clinical data.

	All patients	HTN	IgAN	P value
No. of patients	32	20	12	
Urinary supernatant mtDNA level (copies/μL)	222.0 ± 210.3 (0.2–796.9)	243.6 ± 244.9 (0.2–796.9)	186.1 ± 137.2 (20.6–482.4)	P = 0.5[b]
Sex (M:F)	13:19	10:10	3:9	p = 0.3[a]
Age (years)	50.4 ± 12.6 (20.8–75.7)	52.9 ± 12.6 (29.4–75.7)	46.3 ± 12.0 (20.8–61.4)	p = 0.16[b]
Serum creatinine (μmol/l)	226.8 ± 179.8 (47.0–892.0)	253.7 ± 196.3 (62.0–892.0)	181.8 ± 144.8 (47.0–548.0)	p = 0.3[b]
Estimated GFR (ml/min/1.73m^2)	43.7 ± 37.4 (3.9–180.6)	35.1 ± 24.2 (3.9–100.0)	58.0 ± 50.7 (7.5–180.6)	p = 0.17[b]
Proteinuria (g/day)	4.0 ± 2.9 (0.2–10.8)	4.7 ± 3.2 (0.2–10.8)	3.0 ± 2.1 (0.3–6.0)	p = 0.11[b]
Histological damage (%)				
Glomerulosclerosis	37.3 ± 28.0 (0.0–100.0)	36.1 ± 23.6 (0.0–90.0)	39.3 ± 35.3 (0.0–100.0)	p = 0.8[b]
Tubulointerstitial fibrosis	39.2 ± 23.0 (0.0–75.0)	43.3 ± 20.5 (0.0–70.0)	32.5 ± 26.3 (0.0–75.0)	p = 0.2[b]

GFR, glomerular filtration rate; HTN, hypertensive nephrosclerosis; IgAN, IgA nephropathy.
Data are presented as mean ± standard deviation (range), and compared by [a]Fisher's exact-test or [b]Student's t-test.