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Increased total cytokeratin-18 serum and urine levels in chronic kidney disease

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ABSTRACT

Background: Increased cell death in chronic kidney disease (CKD) by either necrosis or apoptosis has been confirmed by a variety of studies. Possible sources are an inadequate persistent inflammation and ischemia as a consequence of CKD or caused by the underlying renal disease. Detection of total or caspase cleaved cytokeratin 18 (CK-18) is a novel and elegant method to determine necrosis or apoptosis of epithelial cells in the patients' sera and urine.
Methods: 120 patients with CKD stages 1 to 5 were included in the study. Twenty healthy volunteers served as controls. Total and caspase cleaved CK-18 urine and serum concentrations were determined by ELISA.
Results: The concentration of serum total CK-18 was significantly higher in CKD stages 3–5 as compared to the healthy controls. Urinary total CK-18 excretion was increased in patients with CKD 5 compared to controls. A significant correlation between urine total CK18 and urine protein and albumin levels was found. Moreover, ROC curve analysis showed the potential of serum and especially urine total CK-18 levels to predict various CKD stages.
Conclusions: We provide evidence for increased total CK-18 serum and urine levels in CKD patients, possibly indicating that epithelial cell necrosis is prevalent in CKD.

1. Introduction

Chronic kidney disease (CKD), defined by either an estimated glomerular filtration rate (eGFR) of less than 60 ml/min/1.73 m^2 of body-surface area or the presence of kidney damage for three or more months, is an increasing public health problem. Currently, approximately 19 million adults are suffering from chronic kidney disease in the United States; similar figures apply also for Europe [1,2].

Worsening of kidney function leads to endocrine and metabolic derangements, and perturbation of the patients' immune system, such as the cell-mediated immunity [3]. Clinical evidence of impaired lymphocyte, granulocyte and monocyte functions, which progress during the development of uremic retention, has been reported [4–6]. Another hallmark of CKD is increased systemic inflammation, indicated by the upregulation of pro-inflammatory cytokines like interleukin-6 and tumor necrosis factor alpha, which contributes to the high rates of cardiovascular morbidity and mortality in patients with CKD [7].

The impaired immune system in CKD patients leads to increased cell death by either necrosis or apoptosis. This notion is confirmed by increased concentrations of soluble histones as well as death inducing receptors in the sera of patients suffering from end stage renal disease (ESRD) [5,8]. Increased apoptosis of polymorphonuclear leukocytes as well as lymphocytes contributes to the diminished immune response to various pathogens in ESRD [4,5,8,9]. In addition the inadequate constant inflammation present in CKD can cause tissue injury and atherosclerosis. Metabolic acidosis and pro-inflammatory cytokines also lead to the activation of the ubiquitin–proteasome pathway, causing protein catabolism [7,10]. In various necropsy studies in ESRD patients, apoptosis and necrosis could also be found in the kidney itself. Possible causes are local tissue destruction by inflammation or ischemia in the context of the underlying renal disease [11,12].

Measurement of total or caspase cleaved cytokeratin 18 (CK-18) is a novel and elegant serologic measure to determine necrosis or apoptosis. It is based on the M30 antibody, and has been successfully used as a diagnostic tool in acute myocardial infarction and septicaemia [13–15]. In this study we investigated the relevance of

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total and caspase cleaved CK-18 in patients with different stages of chronic renal failure.

2. Methods

2.1. Patients

The study has been approved by the institutional ethics committee and is in accordance with the Helsinki Declaration of 1975. 120 patients with CKD stages 1–5 (without dialysis) were included in the study. All patients were screened and followed up in the outpatient clinic of the Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna. CKD was defined as the presence of kidney damage and/or decreased glomerular filtration rate according to the K/DOQI criteria [16]. Twenty healthy volunteers served as controls. The patients' diagnoses, baseline demographics and laboratory values are shown in Table 1. Patients with known liver disease were excluded from the study. In a subset of 34 patients a 24 hour urine specimen was collected.

2.2. Laboratory data

Blood and urine samples were obtained from each patient and control. Immediately after collection, blood was allowed to clot for up to 60 min at room temperature (22 °C). Next the blood was centrifuged at 2000 RCF for 10 min at 4 °C in a refrigerated centrifuge. Serum and urine aliquots were then transferred in tubes and immediately placed on dry ice. The samples were stored at −70 °C until further analyzed.

For the quantitative determination of total CK-18 in serum we used the M65 enzyme-linked immunosorbent assay (ELISA) kit (Peviva AB, Bromma, Sweden) according to the manufacturer's instructions. The M65 ELISA consists of two monoclonal antibodies (clones M5 and M6) specific for conventional epitopes on CK-18, present on both intact/uncleaved and cleaved CK-18. Serum samples react with solid phasecatcher M6 antibody directed against CK-18 and horseradish peroxidase-conjugated M5 antibody directed against another epitope on CK-18.

Circulating soluble caspase cleaved CK-18 was measured by the commercially available M30 ELISA kit from Peviva AB. This ELISA uses a monoclonal antibody recognizing an epitope on the 238–396 fragment of CK-18 as catcher and horseradish peroxidase-conjugated M30 as detector. The fractional CK-18 excretion was calculated with the formula: ((urine CK-18 × serum creatinine) / (serum CK-18 × urine creatinine)) × 100. For validation testing three different known concentrations of uncleaved or cleaved CK-18 were added to respectively 30 urine samples of five healthy probands, whereof half of those samples underwent a freeze–thaw cycle to determine the proteins' stability. Furthermore, urine samples of five healthy individuals have been multiply tested to ascertain the reproducibility of the used M30 and M65 ELISA kits for urine. Results of M65 ELISA kit showed an intra-assay variability of 15% and an inter-assay variability of 17% for urine samples. For the detection of cleaved CK-18, the variability within assay was <10% and between assays 11%. After freeze–thawing, protein concentration showed a median decrease of 2% for caspase cleaved CK-18 and 1% for total CK-18. Furthermore, total CK-18 concentrations two times higher than in patients with CKD could be properly detected. For cleaved CK-18 the upper tested detection range was about three times higher than in patients with CKD.

2.3. Statistical analysis

Total and caspase cleaved CK-18 serum and urine levels were compared between CKD stages 1–5 and the control group with the non-parametric Mann–Whitney-test. Under the Bonferroni adjustment for multiple comparisons, an individual $p<0.01$ is necessary to achieve statistical significance at the 5% level. Correlation between total and caspase cleaved CK-18 serum and urine levels and various clinical parameters were assessed in all patients and controls by Spearman's correlation coefficient. Unless otherwise stated data are given as median and range.

3. Results

3.1. Total and caspase cleaved CK18 serum and urine levels

Total CK18 serum levels were significantly higher in patients with CKD stages 3 to 5 as compared to the control group (Fig. 1A). The serum levels of total CK-18 did not differ between patients with CKD 1 or 2 and controls. Meanwhile absolute total CK-18 excretion was only increased in CKD 5 (Fig. 1B), the fractional excretion was increased in CKD 4 and 5 (Fig. 1C).

Caspase cleaved CK-18 serum levels were not significantly increased in CKD stage 1 (441 (107–1804) U/L), stage 2 (292 (79–4096) U/L), stage 3 (163 (68–910) U/L), stage 4 (172 (87–931) U/L)

Table 1
Underlying kidney disease, baseline demographic data and laboratory variables.

	All patients	CKD 1	CKD 2	CKD 3	CKD 4	CKD 5	Controls
	120	10 (8.3%)	23 (19.2%)	41 (34.2%)	27 (22.5%)	19 (15.8%)	23
Age (years)	59 (19–88)	36 (19–61)	50 (19–80)	63 (23–78)	61 (29–88)	65 (20–81)	30 (21–67)
Gender (male/female)	67/53	7/3	9/14	27/14	15/12	9/10	15/8
Kidney disease							
Glomerulonephritis	30	4	7	13	7	3	
Polycystic kidney disease	16	2	3	8	2	1	
Vascular nephropathy	26	2	5	7	8	4	
Interstitial nephropathy	5		1	1	1	2	
Bilateral nephrectomy	2		1			1	
Urine stasis	5		1	1	1	2	
Unknown	36	2	5	11	8	6	
Serum creatinine (mg dL^{-1})	1.84 (0.72–6.88)	0.90 (0.72–1.03)	0.99 (0.77–1.52)	1.62 (1.02–2.34)	2.73 (2.04–3.89)	5.00 (3.47–6.88)	0.99 (0.77–1.20)
Blood urea nitrogen (mg dL^{-1})	31.9 (7.1–91.2)	12.6 (7.5–17.6)	13.9 (7.1–33.4)	30.5 (11.6–64.1)	51.2 (23.8–91.2)	63.3 (31.9–87.3)	13.1 (8.2–20)
Urine creatinine (mg dL^{-1})	69.2 (12.7–294.5)	69.1 (22.7–252.9)	77.3 (14.8–243.5)	78 (12.7–294.5)	60.9 (29.3–172.1)	41.65 (17.7–108.2)	
Urine urea (mg dL^{-1})	844 (247–2557)	948 (336–1814)	976 (247–2557)	923 (291–2464)	854 (267–1370)	647 (273–1381)	
Urine protein (g L^{-1})	0.2 (<0.05–6.94)	0.06 (<0.05–0.21)	0.1 (<0.05–4.45)	0.1 (<0.05–2.79)	0.29 (<0.05–3.46)	1.07 (0.05–6.94)	
GOT (U L^{-1})	22 (9–88)	24.5 (15–88)	22 (12–62)	26 (14–57)	22 (9–60)	18 (10–69)	
GPT (U L^{-1})	21 (<3–98)	29.5 (11–75)	20 (11–72)	25 (9–63)	17 (6–51)	15 (<3–98)	
Gamma-GT (U L^{-1})	28.5 (9–561)	38.5 (13–561)	25 (10–134)	36 (9–472)	27 (10–303)	24 (13–346)	
LDH (U L^{-1})	183.5 (81–333)	157.5 (147–289)	172 (129–297)	195 (92–333)	197 (81–285)	185 (109–259)	

Date are given as median with range; CKD, chronic kidney disease.