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Urinary inflammatory cytokines as indicators of kidney damage in type 2 diabetic patients

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ABSTRACT

Background: The aim of this study was to investigate whether urinary levels of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) are altered in normoalbuminuric patients with type 2 diabetes *mellitus* (T2DM), and whether these cytokines are able to identify diabetic kidney disease (DKD) among these patients.
Methods: This study included 125 T2DM patients classified into 3 groups according to urinary albumin/creatinine ratio (uACR): uACR <10 mg/g creatinine, uACR 10–30 mg/g creatinine and uACR >30 mg/g creatinine. Urinary inflammatory cytokines were measured.
Results: The urinary IL-6 concentrations increased from uACR <10 (97.2 ± 26.4 pg/ml) to uACR 10–30 (113.6 ± 28.0 pg/ml) and to uACR >30 mg/g creatinine (163.5 ± 25.6 pg/ml) ($P < 0.05$ and $P < 0.001$, respectively) patients. The urinary IL-10 concentrations decreased in these uACR ranges [100.0 (58.0–141.0) pg/ml vs. 62.0 (54.5–71.5) pg/ml vs. 42.0 (32.0–48.0) pg/ml] ($P < 0.05$ and $P < 0.001$, respectively). All urinary cytokines demonstrated good ability to identify DKD (areas under curves >0.9).
Conclusions: Urinary inflammatory cytokines, especially IL-6 and IL-10, may assist in the identification of DKD in T2DM patients, even in the absence of micro- and macroalbuminuria.

1. Introduction

Diabetic kidney disease (DKD) is defined as functional, structural and clinical alterations of the kidneys caused by diabetes [1]. This is the foremost microvascular complication of diabetes and the leading cause of end-stage renal disease. Epidemiological data have shown that type 2 diabetes mellitus (T2DM) accounts for a great proportion of the patients on renal replacement therapy programs [2,3]. Therefore, DKD contributes considerably to morbidity and mortality in the diabetic population [4], underscoring the importance of prevention, early identification, and adequate treatment [5]. Although urinary albumin (uAlb) is a classical DKD marker, some patients with diabetes have renal pathological lesions even in the normoalbuminuric range. Moreover, the predictive power of albuminuria is limited [6,7]. In this context, it is extremely important to search for new biomarkers that can predict and identify DKD in the early stages and provide information about the pathophysiology of the disease. Toward this end, a number of new and important biomarkers present in urine have been identified according to the major pathways involved in the development and progression of DKD [8].

Besides the traditional and hemodynamic risk factors, accumulating evidence now indicates that inflammation and more specifically inflammatory cytokines play a significant role in the development and progression of kidney damage in diabetes [9]. Inflammatory response is

Abbreviations: DKD, diabetic kidney disease; T2DM, type 2 diabetes mellitus; IL, interleukin; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; uAlb, urinary albumin excretion; uACR, urinary albumin/creatinine ratio; HbA_{1c}, glycated hemoglobin; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

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activated by metabolic and hemodynamic derangements in the diabetic kidney. This may occur at a very early stage of diabetes [10] and result in several damaging effects involving the dysregulation of inflammatory cytokines [8,10,11]. Proinflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) have been recognized as pathogenic mediators that contribute to organ damage [12]. The effects of these cytokines include modifications of the renal structure, intrarenal hemodynamic alterations, modification in the permeability of glomerular endothelium, changes in the expression of diverse molecules, cellular necrosis and apoptosis, and increment in the production of reactive oxygen species [9]. Among proinflammatory cytokines, IL-6 affects extracellular matrix dynamics at both mesangial and podocyte concentrations, stimulates proliferation of mesangial cells, increases fibronectin expression, and enhances endothelial permeability [9,13]. Otherwise, interleukin-10 (IL-10) exerts predominantly anti-inflammatory and immunosuppresive effects [14]. Data from humans linking IL-10 and type 2 DM are limited to a few studies evaluating the serum concentrations of this cytokine and the available contributions are not sufficient to clarify the causative or protective role of IL-10 in the renal damage secondary to DM [15,16].

Importantly, it has been demonstrated that patients with diabetes who progress to DKD display features of inflammation years prior the onset of the disease [11,17,18]. Additionally, inflammation in the kidney contributes to the worsening of the pathology [17,19,20]. These observations indicate that the inflammatory cytokines are causally linked to kidney damage in patients with diabetes and may be useful as early biomarkers for the identification of DKD. However, modifications in urinary concentrations of inflammatory cytokines in patients with normal or mildly increased albuminuria are uncertain and the diagnostic properties of these markers were not assessed in the aforementioned studies. Therefore, the aim of this study was to investigate whether the urinary levels of the inflammatory cytokines IL-1, IL-6, IL-10, TNF-α and IFN-γ are altered in T2DM patients with normal or mildly increased albuminuria, and whether urinary cytokines are able to identify DKD among patients with T2DM.

2. Materials and methods

2.1. Study population

The study was conducted between June 2013 and October 2014. Data from 196 previously diagnosed T2DM patients from the University Hospital of Santa Maria, Rio Grande do Sul, Brazil were screened. To avoid potential confounding factors, patients who had urinary tract diseases, prior renal disease other than DKD, malignancy, infectious and liver diseases, acute or chronic inflammatory diseases, pregnancy, renal transplantation and the use nephrotoxic drugs were excluded from the study. Thus, a total of 125 T2DM patients were eligible for the present study. The subjects were divided into 2 main groups: without DKD (n = 101) and with DKD (n = 24). The clinical diagnosis of DKD was defined based on the detection of uAlb in a random urine sample (albumin excretion of >30 mg/g of creatinine in 2 out of 3 random urine samples collected within a 6 month period) [21]. With the objective of investigating the potential of urinary cytokines for early detection of DKD, patients were stratified into the following 3 subgroups according to the urinary albumin/creatinine ratio (uACR) [22,23]: normal (uACR <10 mg/g creatinine, n = 67), mildly increased (uACR 10–30 mg/g creatinine, n = 34) and moderately/severely increased (uACR >30 mg/g creatinine, n = 24). Clinical study characteristics (age, gender, weight, height, diabetes duration, hypertension, treatment) and medical history were recorded by reviewing the hospital's medical registry and clinical and epidemiological evaluation questionnaire answered by the patients. Written informed consent was obtained from all subjects, and the study was carried out in accordance with guidelines approved by the Institutional Ethics Review Board for Human Studies (12303113.0.0000.5346).

2.2. Sample collection and biochemical analysis

Blood samples were collected from all patients after an overnight fast (for a minimum period of 8 h) by venous puncture technique into the Vacutainer® (BD Diagnostics) tubes with EDTA, with sodium fluoride plus EDTA, or no anticoagulants. Blood samples were routinely centrifuged at 2500 × *g* for 15 min. Whole blood in EDTA was used to assess glycated hemoglobin concentrations (HbA_{1c}) using the chromatographic method in a D10® automated analyzer (BioRad). Plasma with fluoride plus EDTA was used for measurement of fasting glucose and serum was used to determine the concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, high-sensitivity C-reactive protein (hsCRP) and creatinine. Measurements of these variables were performed using standard methods on a Dimension RXL MAX® (Siemens Healthcare Diagnostics Inc.). Creatinine concentrations were measured by a rate-blanked compensated Jaffe method [24]. Morning urine samples were collected from each patient for biochemical analysis. The urine specimens were centrifuged at 1000 × *g* for 5 min and the supernatant was utilized for analysis. uAlb and creatinine were determined using a Dimension RXL MAX® (Siemens Healthcare Diagnostics Inc.). uAlb results were expressed as milligrams of albumin per gram of creatinine as a tool to match the albumin concentrations in accordance with the concentration of urine [25]. Urinary inflammatory cytokine quantification was assessed by ELISA using commercial kits for human IL-1, IL-6, IL-10, TNF-α and IFN-γ (R&D Systems Inc.®). The detection range of the IL-1 assay was 3.9–250.0 pg/ml and its sensitivity was 1 pg/ml. Intra-assay CV was 2.2%, and inter-assay CV was 3.4%. The detection range of the IL-6 assay was 0.2–10.0 pg/ml and its sensitivity was 0.11 pg/ml. Intra-assay and inter-assay CV were 5.5%. The detection range of IL-10 assay was 0.8–50.0 pg/ml and its sensitivity was 0.17 pg/ml. Intra-assay CV was 4.6%, and inter-assay CV was 8.5%. The detection range of TNF-α assay was 0.5–32 pg/ml and its sensitivity was 0.19 pg/ml. Intra-assay CV was 3.1%, and inter-assay CV was 7.4%. The detection range of IFN-γ assay was 15.6–1000.0 pg/ml and its sensitivity was 8.0 pg/ml. Intra-assay CV was 2.8%, and inter-assay CV was 3.7%. All assays were carried out according to the manufacturer's instructions. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [26].

Table 1
Baseline clinical characteristics of study participants.

	Without DKD	With DKD	*P* value
Age (y)	60.0 ± 12.3	57.1 ± 12.5	NS
Male (%)	35	29	NS
BMI (kg/m^2)	29.6 (26.6–35.5)	32.3 (26.7–43.4)	NS
Hypertension (%)	73	86	NS
Smokers (%)	9	12	NS
Diabetes duration (years)	12.0 (6.0–20.0)	10.0 (7.0–20.0)	NS
Hypoglycemic agents (%)	94	87	NS
Antihypertensive agents (%)	78	75	NS
Statins use (%)	83	70	NS
Fasting glucose (mmol/l)	6.5 (5.7–8.5)	6.9 (5.6–13.4)	NS
HbA_{1c} (%)	6.9 (5.9–8.2)	7.1 (5.9–9.7)	NS
HbA_{1c} (mmol/mol)	52.0 (41.0–66.0)	54.0 (41.0–82.0)	NS
Total cholesterol (mmol/l)	4.6 (4.0–4.9)	4.5 (4.0–5.4)	NS
LDL cholesterol (mmol/l)	2.5 ± 0.7	2.7 ± 1.0	NS
HDL cholesterol (mmol/l)	1.3 ± 0.5	1.2 ± 0.4	NS
Triglycerides (mmol/l)	1.4 (1.0–2.1)	1.6 (1.0–2.3)	NS
hsCRP (mg/dl)	0.3 (0.2–0.5)	0.5 (0.2–0.6)	NS
Serum creatinine (μmol/l)	79.6 (70.7–97.2)	79.6 (70.7–97.2)	NS
eGFR (ml/min/1.73 m^2)	76.3 ± 21.1	77.5 ± 24.4	NS
uACR (mg/g creatinine)	7.3 (4.7–11.5)	104.8 (94.2–299.3)	<0.001

Data are expressed as percentages, mean ± SD or median and interquartile range. T2DM, type 2 diabetes *mellitus*; DKD, diabetic kidney disease; uACR, urinary albumin/creatinine ratio.