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Noninvasive Analyses of Kidney Injury Molecule-1 Messenger RNA in Kidney Transplant Recipients With Graft Dysfunction

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ABSTRACT

Background. Kidney graft fibrosis is a major factor related to chronic loss of kidney function. At present, the finding of fibrosis depends on the analysis of tissue in the renal biopsy, which has important limitations. In this study, we evaluated the messenger mRNA transcription and gene expression of kidney injury molecule-1 (KIM-1) in kidney tissue and in urinary sediment cells of kidney transplant patients with graft dysfunction aiming at the development of techniques that may allow the noninvasive diagnosis of interstitral fibrosis/tubular atrophy (IF/TA).

Patients and methods. RNA extracted from cells in tissue and urine of 77 renal transplant patients whose biopsies were classified according to the Banff scheme-2007. Four diagnostic groups were established: (1) acute tubular necrosis ($n = 9$); (2) acute rejection ($n = 49$); (3) acute calcineurin inhibitors nephrotoxicity ($n = 10$); and (4) interstitial fibrosis and tubular atrophy (IFTA, $n = 29$). Tissue and urine cell RNA was amplified and quantification were made by real-time polymerase chain reactron. Data from the quantification of gene expression are presented as median and 25th to 75th percentiles.

Results. Messenger RNA levels of the KIM-1 gene were higher in the biopsies (26.17; 3.38–294.53) and urinary sediment cells (0.09; 0–5.81) of the patients classified as having IF/TA as compared with all others groups. A significant correlation between gene expression in samples of urine and tissue cells was found ($P < .01$).

Conclusion. These initial data suggests that KIM-1 gene mRNA quantification can be used as a noninvasive biomarker of IF/TA.

KIDNEY FIBROSIS is a major factor related to chronic graft loss. Currently the diagnosis of interstitial fibrosis and tubular atrophy (IF/TA) depends on the analysis of graft biopsies.[1] The development of noninvasive biomarkers would be important for better graft monitoring. Kidney injury molecule-1 (KIM-1) is a protein present in toxic and ischemic acute renal injury and in chronic kidney diseases.[2–4] In this study, we evaluated the messenger (mRNA) transcription and gene expression of KIM-1 in kidney tissue and in urinary sediment cells (USC) of kidney transplant patients with graft dysfunction.

PATIENTS AND METHODS

Seventy-seven kidney transplant recipients who undertook an indication graft biopsy between January 2008 and December 2009 were included. At each biopsy one-third of a core was immediately frozen in liquid nitrogen for later molecular analyses. Urine samples were obtained immediately before biopsy: USC were isolated and frozen until RNA extraction. Pathological analyzes were performed according to the Banff 2007 classification.[1] The biopsies were classified in four diagnostic groups: (1) acute tubular necrosis (ATN, $n = 9$); (2) acute rejection (AR, $n = 49$); (3) acute calcineurin inhibitors nephrotoxicity (CIN, $n = 10$); and (4) IFTA,

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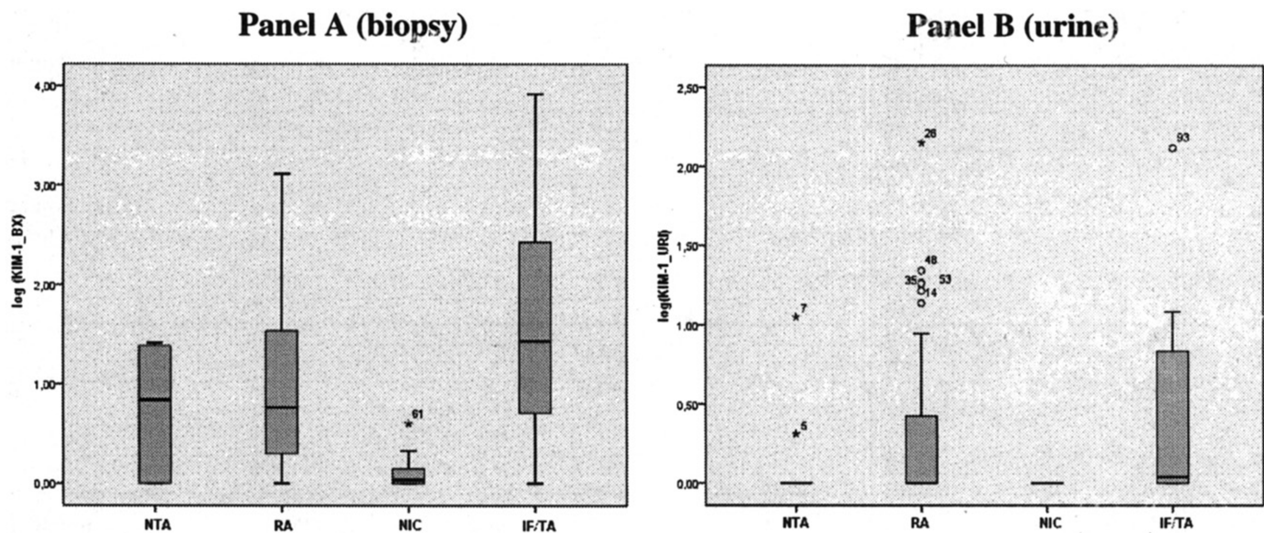

Fig 1. The box plot representation graphics showing the logarithmic transformed medians and 25th to P75th percentile values if the quantification levels of normalized messenger RNA ($2^{-\Delta\Delta CT}$). KIM-1 messenger RNA expression levels in kidney tissue and in urinary sediment cells in the diagnostic groups NTA, RA, NIC, and IF/TA are presented. KIM-1 gene mRNA levels were significantly higher in IF/TA as compared with CIN ($P < .01$; **A)** and ($P < .05$; **B)**. NTA, acute tubular necrosis; RA, acute rejection; NIC, calcineurin inhibitor-induced nephrotoxicity; IF/TA, interstitial fibrosis and tubular atrophy; KIM-1, kidney injury molecule-1.

($n = 29$). Tissue and USC RNA was extracted, reversed transcribed, and amplified by real-time polymerase chain reaction polymerase (TaqMan, ABI-PRISM 7000 SDS, Applied Biosystems) using specific primers (HAVCR-1, Applied Biosystems) for the KIM-1 gene and 18s rRNA as an endogenous control. The analysis of the amplified products were performed by the relative quantification method $2^{-\Delta\Delta CT}$. Data are presented as means ± standard deviations and medians and percertiles (P25–75) values. Multiple comparisons where made by Tukey test, and nonparametric data was analyzed by Kruskal-Wallis test. P levels lower than .05 were considered statistically significant.

RESULTS

Patients were predominantly males (79%), Caucasians (87%), and 78% received grafts from deceased donors. A significantly higher serum creatinine, at the day of the biopsy, was observed in the ATN group (6.4 ± 1.9 mg/dL) as compared to the patients classified in the CIN (2.0 ± 0.71 mg/dL) and IF/TA groups (3.5 ± 1.6 mg/dL; $P < .05$). The group with AR (4.5 ± 2.3 mg/dL) also presented higher serum creatinine as compared to the CIN group ($P < .05$). Cold ischemia time was significantly higher in the ATN group (23.6 ± 5.7 hours) as compared to the CIN group (13.3 ± 9.6 hours; $P < .05$). Time interval between the transplant surgery and graft biopsy was higher in the IF/TA group (672; 242–1752 days) as compared to other groups ($P < .05$).

Figure 1 shows KIM-1 mRNA quantifications from tissue and UCS. KIM-1 mRNA levels were higher in the biopsies with IF/TA (26.17; 3.38–294.53) as compared with all others groups ($P < .01$). Also the AR (4.79; 0.92–34.07) had a higher expression than the CIN group (0.09; 0–0.58; $P <$ .05). Likewise KIM-1 mRNA levels were higher in the UCS of IF/TA biopsies (0.09; 0–5.81) as compared with ATN (0; 0–0.52), AR (0; 0–2.0), and CIN (0–0; $P < .05$). In addition, a significant correlation between gene expression USC and biopsies was found ($P < .01$).

DISCUSSION

KIM-1 is a type I transmembrane gl coprotein with immunoglobulin and by mucin domains the extra-cellular portion. The cytoplasmic domain is relatively short and has a potentially phosphorilated site, indicating that KIM-1 is perhaps involved in molecular signaling. The ectodomain is cleaved by a metalloproteinase and released in urine.[5,6] KIM-1 expression in dedifferentiated tubular epithelium suggests that this protein is involved in the processes of tubular fibrosis and may also play a role as a biomarker for proliferation in regenerative mechanisms.[7] We have previously shown increased KIM-1 mRNA transcription in biopsies of kidney transplant recipients with IF/TA and chronic CIN. In this study, we confirmed our previous findings in another cohort and expanded by showing it is also significantly increased in UCS.[8] These data suggest that USC KIM-1 mRNA quantification may be used as a noninvasive biomarker of fibrosis in kidney grafts with IF/TA.

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