ORIGINAL ARTICLES

Clinical evaluation of urinary excretion of liver-type fatty acid–binding protein as a marker for the monitoring of chronic kidney disease: A multicenter trial

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To confirm the clinical usefulness of the measurement of urinary liver-type fatty acid–binding protein (L-FABP) in chronic kidney disease (CKD), we carried out a multicenter trial. Clinical markers were measured in patients with nondiabetic CKD (n = 48) every 1 to 2 months for a year. We divided patients retrospectively into progression (n = 32) and nonprogression (n = 16) groups on the basis of the rate of disease progression, then assessed several clinical markers. Initially creatinine clearance (Ccr) was similar in the 2 groups; however, the urinary L-FABP level was significantly higher in the former group than in the latter (111.5 vs 53 μg/g creatinine, $P < .001$). For the monitoring CKD, we set the cutoff values for urinary L-FABP and urinary protein at 17.4 μg/g creatinine and 1.0 g/g creatinine, respectively. Urinary L-FABP was more sensitive than urinary protein in predicting the progression of CKD (93.8% and 68.8%, respectively). However, urinary protein showed greater specificity than did urinary L-FABP (93.8% and 62.5%, respectively). Over time, the progression of CKD tended to correlate with changes in urinary L-FABP ($r = -.32$, $P < .05$), but not in urinary protein ($r = .18$, not significant). The dynamics of urinary protein differed from that of urinary L-FABP, which increased as Ccr declined. Urinary L-FABP is more sensitive than urinary protein in predicting the progression of CKD. Urinary excretion of L-FABP increases with the deterioration of kidney function. Urinary L-FABP is therefore a useful clinical marker in the monitoring of CKD. (J Lab Clin Med 2005;145:125–33)

Abbreviations: BMI = body-mass index; Ccr = creatinine clearance; CKD = chronic kidney disease; cr. = creatinine; FABP = fatty acid–binding protein; FFA = free fatty acid; H-FABP = heart-type fatty acid–binding protein; L-FABP = liver-type fatty acid–binding protein; ELISA = enzyme-linked immunosorbent assay; NAG = *N*-acetyl-β-D-glucosaminidase; NS = not significant; ROC = receiver-operator characteristic

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Tubulointerstitial injury has been noted to play an important role in the progression of CKD.[1–8] Urinary protein excretion is widely accepted as the most important aggravator of tubulointerstitial damage and the best independent predictor of progression to end-stage kidney failure.[1,2,9–12]

FFAs are bound to albumin,[13] filtered through glomeruli, and reabsorbed into the proximal tubules. It is therefore assumed that FFAs bound to albumin may play a role in the pathogenesis of the tubulointerstitial damage observed in massive proteinuria.[14–20] FFAs loaded to the proximal tubule are bound to cytoplasmic FABP and transported to mitochondria or peroxisomes,[21,22] where they are metabolized by way of β-oxidization. In the human kidney, 2 types of FABPs have been identified[23]: L-FABP, which is expressed in the proximal tubule; and a heart-type, H-FABP, that is expressed in the distal tubule. Expression of the L-FABP gene is induced by FFAs[24]; L-FABP may regulate the metabolism of fatty acids and may be a key regulator of fatty-acid homeostasis in the cytoplasm.[25,26]

In an experimental study of protein overload–induced nephropathy, L-FABP gene expression in the kidney was up-regulated and urinary excretion of L-FABP was increased by the stress which causes the tubulointerstitial damage.[27] In the clinical study, urinary excretion of L-FABP was correlated with the severity of the tubulointerstitial damage.[27] Furthermore, the level of urinary L-FABP was significantly higher in patients whose kidney function deteriorated than in those whose kidney function was stable, and therefore urinary L-FABP may be a new and unique clinical marker for predicting the progression of CKD.[28]

A recent study of the American Heart Association suggests that CKD is an independent risk factor for cardiovascular disease, warns of the increasing number of patients with CKD, and emphasizes the importance of measuring clinical parameters in serum or urine.[29] However, few clinical markers exist with which to predict or monitor the progression of CKD. As a means of confirming the clinical usefulness of urinary L-FABP as a marker of CKD, we therefore carried out a multicenter trial in patients with nondiabetic CKD.

METHODS

ELISA for the measurement of urinary L-FABP. We used a human L-FABP ELISA kit from CMIC (Tokyo, Japan).[27,28]

Reference values of urinary L-FABP. To determine control reference values of urinary L-FABP, we examined 550 subjects who underwent checkups at the Health Center of the Dokkyo University School of Medicine (Tochigi, Japan) for general physical health and clinical parameters in serum and urine. The inclusion criteria for control subjects were as follows: absence of a history of diabetic mellitus, kidney disease, liver disease or coronary heart disease; absence of abnormal findings on urinalysis, glucose-tolerance testing, tests of kidney or liver function; absence of hyperlipemia, hypertension, anemia, or inflammation; and a BMI of 18 to 25. From the 550 subjects, we selected 150 who fulfilled these criteria.

Table I. Clinical background of all patients (mean ± SD)

Variable	Value
No. of patients	48
Age (yr)*	55 ± 11 (29–77)
Male (n = 36)	54 ± 2 (29–77)
Female (n = 12)	57 ± 3 (39–69)
No. of patients with renal biopsy (%)	15 (31%)
Mesangial proliferative glomerulonephritis	10
Membranous nephropathy	1
Focal segmental glomerulosclerosis	1
Membranoproliferative glomerulonephritis	1
Nephrosclerosis	2

*Data expressed as mean ± SD (range).

Clinical significance of urinary L-FABP in CKD. This multicenter trial, carried out between March 2001 and May 2002, included 48 adult patients from outpatient clinics. Patients were monitored for periods ranging from 7 to 13 months (11 ± 2 months, mean ± SD). The diagnosis of CKD was made on the basis of kidney-biopsy findings (n = 15) or clinical history (n = 33) without underlying systemic disease. The inclusion criteria for the patients were as follows: no history of liver disease, diabetic nephropathy, cancer or collagen disease; serum creatinine concentration ranging from 1.2 mg/dL (106.1 μmol/L) to 3 mg/dL (265.2 μmol/L) in men and 0.9 mg/dL (79.6 μmol/L) to 2.3 mg/dL (203.3 μmol/L) in women; and age between 20 to 80 years. This research was carried out in accordance with the principles of the Declaration of Helsinki, informed consent was obtained from each participant, and our institutional review board approved the study. All serum and urine samples were stored at −70°C until they could be analzyed. Relevant clinical parameters were checked every month or second month without a change in medication. Tables I and II summarize clinical and laboratory findings in the subjects.

We measured the serum levels of creatinine and total cholesterol, as well as the urine concentrations of L-FABP, creatinine, protein, and NAG . Serum and urine creatinine and serum cholesterol were measured with the use of enzymatic methods, urinary protein was assessed with the use of the pyrogallol red–molybdate complex method,[30] and urinary NAG was measured with the use of chlorophenol red-*N*-acetylglucosaminide as a substrate. The levels of urinary parameters are expressed as a ratio of the level of urinary creatinine. We calculated Ccr using the