Clinica Chimica Acta 413 (2012) 187–191

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Urinary excretion of liver-type fatty acid-binding protein as a marker of progressive kidney function deterioration in patients with chronic glomerulonephritis

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ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form 9 September 2011
Accepted 13 September 2011
Available online 17 September 2011

Keywords:
L-FABP
Chronic glomerulonephritis
Progression of renal disease

ABSTRACT

Background: To evaluate the value of basal urinary L-FABP (uL-FABP) excretion as a prognostic indicator of the progression of kidney function impairment in patients with chronic glomerulonephritis (CGN).
Methods: One hundred twenty-three patients with newly diagnosed, biopsy-proven primary CGN were included. In all patients, and in 28 healthy subjects, uL-FABP was measured using an ELISA. Risk factors of the progression of kidney function were evaluated. The patients were in follow-up for at least 5 years.
Results: uL-FABP in the patients with CGN (76.58 ± 17.3 μg/g.cr) was greater than in the healthy subjects. A significant positive correlation between uL-FABP and proteinuria ($R = 0.501$, $P < 0.01$), serum creatinine ($R = 0.601$, $P < 0.01$) were found. Kaplan–Meier analysis revealed that uL-FABP >76.58 μg/g.cr predicts progression of renal function. The cut off values for L-FABP at 119.8 μg/g.cr was found to be more sensitive, area under the curve (AUC) was 0.95.
Conclusion: Urinary L-FABP may be a useful clinical biomarker for monitoring chronic glomerular disease. Urinary L-FABP can help predict the progression of chronic glomerular disease.

1. Introduction

Chronic kidney disease (CKD) is a worldwide common disease and progresses to end stage renal disease (ESRD). So many studies suggest that CKD is an independent risk factor for developing cardiovascular disease, and emphasizes the importance of measuring clinical parameters in serum or urine [1]. However, there are few clinical markers to predict and monitor the progression of CKD.

Increasing clinical and experimental evidence shows that the progressive nature of chronic glomerular disease depends significantly on tubulointerstitial involvement [2]. Although there are many factors causing tubulointerstitial injury, recent studies have shown that urinary protein has renal toxicity and is the particularly noteworthy factor to play a deleterious role in the progression of renal damage [3–5].

Free fatty acids (FFAs) bound to albumin [6] may play a role in the generation of tubulointerstitial disease. In massive proteinuria, FFAs are overloaded in the proximal tubule and induce inflammatory factors such as monocyte chemotactic protein-1 [7], which in turn aggravate urinary protein-related tubulointerstitial damage [8]. Researchers showed that FFAs may be responsible for mechanism leading to tubulointerstitial damage seen in massive proteinuria [9]. FFAs loaded to the proximal tubules are bound to cytoplasmic fatty acid binding protein (FABP) and transported to mitochondria or peroxisomes [10]. In the human kidney, 2 types of FABPs have been identified [11]: L-FABP, which is expressed in the proximal tubule; and a heart-type, H-FABP, that is expressed in the distal tubule. L-FABP may regulate the metabolism of FFAs and may be a key regulator of FFA homeostasis in the cytoplasm.

Furthermore the researchers had found that L-FABP is expressed in the human proximal tubules [12]. Because renal L-FABP has not yet been investigated in patients with chronic kidney disease, we hope to clarify the clinical relevance of urinary L-FABP excretion in chronic glomerular disease. In the present study, the urinary excretion of L-FABP (uL-FABP) was evaluated to identify its potential relationship with clinical markers of kidney injury in the patients with newly diagnosed chronic glomerulonephritis. The value of basal urinary L-FABP excretion as prognostic indicator of the progression of kidney function impairment was also assessed.

2. Patients and methods

This study carried out between January 2004 and December 2005. 123 Chinese patients (48 men and 75 women, with a mean age of 51.59 ± 7.52 y) with newly diagnosed, biopsy-proven primary chronic glomerulonephritis (CGN) were included in the study. Patients with any concomitant disease and the symptoms or sign of acute and chronic inflammation were excluded from the study. All specimens of kidney tissue were obtained by percutaneous kidney biopsy, and standard examination of the cortical tissue by light microscopy and immunofluorescence was performed by an experienced morphologist. IgA nephropathy (IgAN) was diagnosed in 36 patients,

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doi:10.1016/j.cca.2011.09.018

minimal change disease had 24 patients, glomerulosclerosis had 33 patients, followed by mesangial proliferative glomerulonephritis (non-IgA nephropathy, MesPGN) (12 patients), focal segmental glomerulosclerosis (FSGS) (9 patients) and membranous nephropathy (9 patients).

Urinary protein excretion (UPE) on the basis of 24-h urine collection was measured, and microscopic analysis of urinary sediment was performed. Serum and urine creatinine concentrations were measured using standard laboratory procedure, and estimated glomerular filtration rate (eGFR) using the MDRD formula was calculated and corrected for a standard body surface area of 1.73 m^2. The mean (±SD) of eGFR was 61.17 ± 21.96 ml/min/1.73 m^2.

Normal renal function was defined as eGFR ≥ 90 ml/min/1.73 m^2. 30 patients were in stage 1 of CKD; 36 patients presented stage 2; 21 patients presented stage 3; 6 patients presented stage 4 and 30 stage 5 of CKD. The characteristics of the study group are presented in Table 1. In all patients, fresh urine samples were collected in the morning prior to renal biopsy and before the administration of therapy. The sample was centrifuged at 1000 *g* for 5 min, and supernatant specimens were kept at −80 °C until tested.

Enzyme-linked immunosorbent assay (ELISAs R$D Systems, Minneapolis, MN) was used to measure urinary L-FABP concentration. The L-FABP excretion was expressed as micograms per milligram creatinine. L-FABP excretion evaluated in 28 healthy volunteers, matched for sex and age with patients, served as control values. None of the volunteers had a history of renal disease or abnormal finding on urinalysis.

The levels of lipid profile, blood cell test, albumine as well as the urine concentrations of creatinine, protein, and N-acetyl-β-D-glucosaminidase (NAG) are measured. Arterial hypertension (blood pressure ≥ 140/90 mm Hg or the use of anti-hypertensive medications) was present in 74 patients. Twenty-five patients received statins. Other anti-hypertensive drugs (diuretics, calcium channel blockers) were also used to reach the recommended blood pressure target (<130/80 mm Hg or <125/75 mm Hg, when proteinuria >1.0 g/24 h was detected).

Patients were followed up in the out-patient clinic at 3-month intervals during the 5-y follow-up. At the end of follow-up, the values of eGFR were analyzed, and the patients were classified as progressors when the eGFR value decreased (below normal range) ≥ 5 ml/min/1.73 m^2/y or received renal replacement therapy during the follow-up period. The patients demonstrating stabilization or a slower decrease of eGFR were considered as non-progressors. The initial values of L-FABP were compared between progressors and non-progressors [13]. The additional risk factors of progression were age, gender, initial values of eGFR, lipid profile, hemoglobine, ferrtin protein and CRP were compared between the progressor and non-progressor groups. As predicting the progression of CGN, we calculated the cutoff points for urinary L-FABP using a receiver operating characteristic (ROC) curve and determined the sensitivity, specificity.

2.1. Statistical analysis

The data are given as the mean ± SD and 95% confidence intervals. The correlations between 2 continuous variables were calculated using Spearman's bivariate correlations. Differences in the patients' demographic, clinical and laboratory parameters between the 2 groups were evaluated by a Student's *t*-test. Logistic regression was performed to determine risk factors for faster eGFR decline, identified at initial examination of the patients. Receiver operating characteristic (ROC) curves were constructed for clinical features and progression of CGN by plotting sensitivity versus 1-specificity, and the areas under the ROC curves (AUC) were calculated. We make a comparison of traditional risk factors for progression in CGN (such as age, sex, serum creatinine etc.) and then to assess the AUC of u-LFABP. Patient survival were analyzed further using Kaplan–Meier life survival analysis. Statistical analysis was performed using Statistical Package for the Social Sciences (Chicago, IL) for Windows software, ver. 13.0. A 2- tailed *P*-value <0.05 was considered statistically significant.

3. Results

In the whole group of patients with CGN, the mean urinary L-FABP was significantly higher than in healthy subjects (20.12 ± 3.69 vs 76.58 ± 17.3 μg/g.cr, respectively, P<0.00002). A significant positive correlation between L-FABP and creatinine was found (Fig. 1). A significant positive correlation between L-FABP and proteinuria in patients with CGN was also detected (Fig. 2).

At the end of follow-up, 36 patients were classified as progressors. The comparison of the initial values of the parameters considered as risk factors for faster renal function decline in the groups of patients qualified after follow-up as progressors and non-progressors is presented in Table 2. Gender, secum creatinine, uric acid, proteinuria, hemoglobin, triglyceride, eGFR values and urine L-FABP were found to be different when comparing the two groups. The differences between the means of L-FABP in the progressors, non-progressors and healthy subjects are presented in Fig. 3. Neither the value of urinary NAG nor age or CRP value was different in the progressors when compared with the non-progressors.

Table 1
Characteristics of the study group and healthy subjects.[a]

Characteristics	CGN patients	Healthy subjects
Gender (n; men/women)	123 (48/75)	28 (11/17)
Age (y)	51.59 ± 7.52	50.77 ± 2.25
Creatinine (μmol/l)	200.18 ± 138.69[b]	71.96 ± 12.93
Uric acid (mmol/l)	413.48 ± 65.41	408.42 ± 18.45
Hemoglobine (g/dl)	113.19 ± 27.18[b]	130.69 ± 13.29
Albumin (g/l)	34.51 ± 3.63[b]	42.03 ± 3.62
TG (mmol/l)	2.08 ± 0.79	1.77 ± 0.22
TCh (mmol/l)	5.57 ± 1.04	5.60 ± 1.96
HDL (mmol/l)	1.35 ± 0.19	1.66 ± 0.51
LDL (mmol/l)	3.57 ± 0.78	3.21 ± 1.23
Ferrtin protein (μg/l)	226.47 ± 90.22[b]	111.43 ± 85.09
Urinary protein excretion (g/24 h)	2.24 ± 2.71[b]	0.01 ± 0.01
CRP (mg/l)	13.16 ± 6.71[b]	1.03 ± 0.42
eGFR (ml/min/1.73 m^2)	61.17 ± 21.96[b]	96.18 ± 13.76
L-FABP (μg/g.cr)	76.58 ± 17.30[b]	20.12 ± 3.69
NAG (IU/g.cr)	4.2 ± 1.7	2.1 ± 0.5

[a] The values are given as number of patients (n) or as mean ± SD.
[b] P<0.01, compared with healthy subjects.

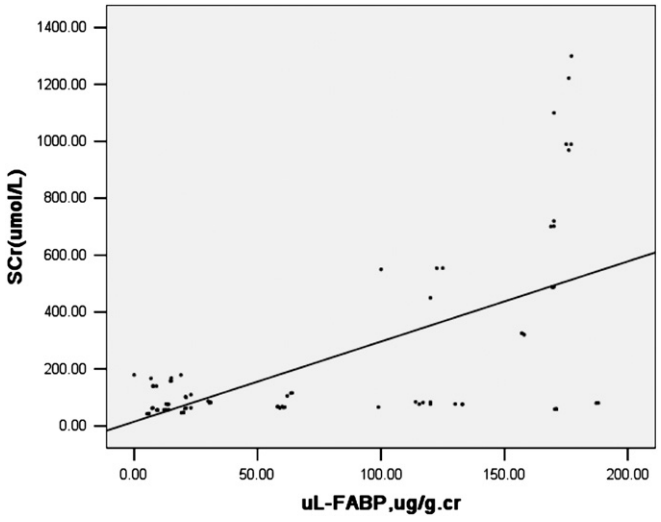

Fig. 1. The correlation between uL-FABP and serum creatinine in patients with primary chronic GN. R = 0.601 and P = 0.001.