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Invited critical review

Urinary liver type fatty acid binding protein in diabetic nephropathy ☆

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ABSTRACT

Deterioration of diabetic nephropathy (DN) is largely determined by the degree of tubulointerstitial changes rather than the extent of histological changes in the glomeruli. Therefore, a tubular marker that accurately reflects tubulointerstitial damage may be an excellent biomarker for early detection or prediction of DN. Liver-type fatty-acid binding protein (L-FABP) is a 14 kDa small molecule that is expressed in the cytoplasm of human proximal tubules. *In vivo* experimental studies revealed that renal L-FABP gene expression was up-regulated by various stresses that cause tubulointerstitial damage, such as massive proteinuria, hyperglycemia, hypertension, ischemia and toxins, and that urinary excretion of L-FABP was increased. Recent clinical studies of patients with type 1 or type 2 diabetes demonstrated that urinary excretion of L-FABP derived from proximal tubules is a suitable biomarker for predicting and monitoring deterioration of renal function in DN. Moreover, therapeutic interventions with renoprotective effects reduced urinary L-FABP concentrations. Therefore, urinary L-FABP measured using the Human L-FABP ELISA Kit developed by CMIC Co., Ltd. (Tokyo, Japan) was confirmed as a newly established tubular biomarker by the Ministry of Health, Labour and Welfare in Japan in 2010. This review article summarizes the clinical significance of urinary L-FABP in DN.

Contents

1. Introduction

Diabetic nephropathy (DN) is the leading cause of chronic kidney disease (CKD), which ultimately progresses to end-stage renal failure and increases the risk of cardiovascular disease. Therefore, early diagnostic markers for predicting and monitoring the progression of DN are needed to enable the timely administration of the most appropriate protective treatments.

Tubulointerstitial injury has been suggested to have an important impact on the progression of DN [1]. Liver-type fatty-acid binding

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protein (L-FABP) is expressed in the proximal tubules of the human kidney and participates in fatty acid metabolism [2]. Results of animal studies of kidney disease showed that human *L-FABP* gene expression in the kidney was up-regulated and that urinary excretion of human L-FABP was increased by stress (e.g., urinary protein overload [3], tubular ischemia [4], tubular stretch [5], hyperglycemia [6], toxins [7,8] and hypertension [9] that causes tubulointerstitial damage. In a clinical study of patients with non-diabetic CKD, urinary excretion of L-FABP was found to correlate with the severity of tubulointerstitial damage [3] and with the rate of CKD progression [10]. Urinary L-FABP thus offers potential as a clinical marker to screen for kidney dysfunction and thereby to identify patients who are likely to experience deterioration of renal function in the future.

With respect to the relationship between urinary L-FABP and DN, increased urinary L-FABP is widely known to be associated with the severity of DN [11–15] and the progression of DN in future [11,14,16,17]. Furthermore, there have been numerous reports of intervention studies in which urinary L-FABP possesses response to renoprotective treatment [12,13,18–24]. This review summarizes recent findings on the clinical significance of urinary L-FABP in DN. Those findings showed that urinary L-FABP concentrations increased in parallel with the progression of DN and that high concentrations of urinary L-FABP presented a risk of progression of DN, of cardiovascular events and anemia.

2. Measurements of urinary L-FABP

To avoid variations that occur through differences in the ELISA kit used for measuring urinary L-FABP concentrations, only studies pertaining to the clinical significance of urinary L-FABP measured using the Human L-FABP ELISA Kit developed by CMIC Co., Ltd. (Tokyo, Japan) were reviewed [10]. In 2010, the Ministry of Health, Labour and Welfare approved the use of only this kit for diagnosis in clinical practice in Japan. The urinary L-FABP concentration was expressed as the ratio of the urinary L-FABP concentration to the urinary creatinine concentration in all studies.

To determine control reference values for urinary L-FABP in spot urine, 412 healthy volunteers were examined [11]. The mean value of urinary L-FABP in spot urine, determined from the logarithmic-transformed data (log L-FABP), was 1.6 μg/g creatinine, with individual values ranging from 0.3 μg/g creatinine (mean − 2 SD) to 8.4 μg/g creatinine (mean + 2 SD).

3. Dynamics of renal L-FABP in DN from an experimental study

Because renal L-FABP is not endogenously expressed in the kidneys of mice, we generated human L-FABP chromosomal transgenic (Tg) mice and evaluated the dynamics and pathophysiological role of renal L-FABP [25]. With regard to DN, a streptozotocin (STZ)-induced diabetic model, which has type 1 diabetes, was used and tubulointerstitial damage was provoked [6]. Our findings revealed that renal human L-FABP gene expression was up-regulated (around 9-fold increase) and that urinary excretion of human L-FABP increased (around 9-fold increase) in the STZ-induced diabetic Tg mice compared with control mice at 8 weeks after STZ injection. From the observation of lipid accumulation in human proximal tubules in DN [26], it could be suggested that lipid or peroxidation product generated in the proximal tubules of DN might promote the up-regulation of renal L-FABP expression. Our Tg mice were generated by microinjections of the genomic DNA of human L-FABP including its promoter region; therefore, it is possible for the transcription of the human L-FABP gene in the Tg mice to be regulated in the same mode as in humans. The dynamics of human L-FABP in the experimental diabetic model might mimic those under pathological conditions in humans as reported in the clinical cross-sectional studies described below.

4. Clinical significance of urinary L-FABP in diabetic nephropathy

4.1. Cross-sectional studies (Table 1)

Two studies of type 1 diabetes [13,14] and three studies of type 2 diabetes [11,12,15] reported on the relationship between urinary L-FABP concentrations and the severity of DN. In type 1 diabetes, urinary L-FABP concentrations increased with the progression of DN and were higher in normoalbuminuric patients than in control subjects [13,14]. These results indicated that urinary L-FABP accurately reflected DN severity and may be a suitable biomarker for early detection of DN.

Why urinary L-FABP concentrations increased in patients with normoalbuminuria in comparison with control subjects is not yet known. It is possible that in the normoalbuminuric phase chronic hyperglycemia may provoke microvascular damage [27], leading to tubular hypoxia and finally tubulointerstitial damage. Tubular hypoxia activates hypoxia inducible factor-1 (HIF-1) [28], which binds the hypoxia responsive element in the promoter region of the L-FABP gene, up-regulates the gene expression of L-FABP and promotes urinary excretion of L-FABP [4]. Therefore, chronic hypoxia could have induced an increase in urinary L-FABP in the normoalbuminuric phase.

In type 2 diabetes, urinary L-FABP concentrations increased with the progression of DN [11,12,15] and reflected DN severity. In one report, urinary L-FABP levels were significantly higher in patients with normoalbuminuria than in control subjects [11]. However, among clinical studies of type 2 diabetes, urinary L-FABP concentrations were comparable between patients with normoalbuminuria and those with microalbuminuria [15] and between patients with normoalbuminuria and control subjects [12]. In the study of Suzuki et al. [15], the frequency of measuring urinary albumin was not described and the urinary L-FABP concentration in patients with microalbuminuria (mean value, 5.2 μg/g creatinine [15]) was lower than that reported in other studies (mean value, 8.6 μg/g creatinine [11] or 19.6 μg/g creatinine [12]). Possibly the frequency of urinary albumin measurements was insufficient and the diagnosis of DN severity according to the urinary albumin concentration led to over-diagnosis. Therefore, patients with normoalbuminuria might have been entered by mistake into the microalbuminuria group, resulting in a lower median or average urinary L-FABP concentration in the microalbuminuria group. In the study of Nakamura et al. [12], urinary L-FABP in the control subjects (mean value, 5.8 μg/g creatinine) was higher than that reported in another study (mean value, 1.6 μg/g creatinine [11]) and was almost the same as that of microalbuminuric patients in another study (mean value, 5.2 μg/g creatinine [15]). It is possible that the criteria for the selection of control subjects was not sufficiently strict and that the number of control subjects was too small [12]. Because DN developing from type 2 diabetes is known to be a multifactorial disorder that can deteriorate through the presence of various factors such as hyperglycemia, hypertension, obesity and hyperlipidemia and since type 2 diabetes patients are more heterogeneous than type 1 diabetes patients, identical results might not be obtainable in clinical studies of type 2 diabetes. Furthermore, cut-off values for urinary L-FABP for detection of normo- or microalbuminuria have not been investigated yet. These points should be considered in future clinical studies.

Anemia induces tubular hypoxia and leads to the progression of DN [29,30], and is frequently observed even in diabetic patients in the early stage of DN. These patients not only experience a fast decline in renal function, but also increased mortality and morbidity [31,32]. The correlation between urinary L-FABP and anemia has been studied [33]. The superiority of urinary L-FABP in comparison with urinary kidney injury molecule (KIM-1) [34] and urinary N-acetyl-b-glucosaminidase (NAG), known as another tubular damage marker, was emphasized. That research involved 130 type 2 diabetes patients with albuminuria and creatinine clearance more than 60 mL/min and 40 healthy control subjects [33]. Only urinary L-FABP was correlated with the hematocrit ($r = -0.188$, $p = 0.032$), hemoglobin ($r = -0.190$, $p = 0.030$) and anemia based on the WHO definition ($r = -0.266$, $p = 0.002$), but not