Clinical significance of urinary liver-type fatty acid binding protein at various stages of nephropathy

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ABSTRACT

This cross-sectional study was to evaluate the levels of urinary liver-type fatty acid binding protein (u-LFABP pg/mg urine creatinine ratio) at different stages of diabetic nephropathy and to see its correlation with other clinical parameters in South Indian patients with type 2 diabetes mellitus (T2DM). A total of 65 (M: F; 42:23) T2DM subjects were divided into three groups, and were compared with 13 (M: F; 3:10) nondiabetic controls. The study groups were as follows: normoalbuminuric (n = 22), microalbuminuric (n = 22) and macroalbuminuric (n = 21). Estimated glomerular filtration rate (eGFR) was calculated using Cockcroft and Gault formula. u-LFABP levels in spot urine samples were measured with a solid phase enzyme linked immunosorbent assay. This study showed that u-LFABP levels were undetectable in healthy controls and was very low in the normoalbuminuric subjects. Elevated levels of u-LFABP are evident from the microalbuminuric stage indicating tubular damage. The levels of u-LFABP increased gradually with declining renal function. Geometric mean (95% confidence interval) for normoalbuminuria was 0.65 (0.47–0.97), microalbuminuria was 0.99 (0.55–1.97) and macroalbuminuria was 5.16 (1.8–14.5), (P = 0.005). In conclusion, u-LFABP levels were elevated in patients with reduced eGFR and showed a positive correlation with systolic blood pressure and protein to creatinine ratio in the total study subjects.

Key words: Diabetic nephropathy, type 2 diabetes, urinary liver-type fatty acid binding protein, urinary marker

Introduction

The burden of diabetes is huge, especially in the low- and middle-income countries (International Diabetes Federation Atlas 6th edition accessed on April 18, 2014). The Indian Council of Medical Research–India Diabetes national study conducted in India, reported that there are 62.4 million people with type 2 diabetes and 77 million people with pre-diabetes in India.[1] Approximately, 30–40% of all patients with diabetes develop diabetic nephropathy (DN)[2] and this is the leading cause of end-stage renal disease. In addition, DN is associated with a higher risk of other complications with an increase in all-cause mortality.[3] The expenditure on hospital admission for treating chronic kidney disease was considerably higher in India.[4]

It is known that tubulointerstitial and glomerular damage play an important role in DN.[5,6] Albuminuria serves as a marker of glomerular damage, and it is important to explore new predictive markers of tubular damage for better understanding of kidney damage. This contributes to more accurately predict the patients at risk of developing DN, to understand the clinical course or prognosis that may help in early diagnosis and to plan appropriate intervention.

The fatty acid-binding proteins (FABP) are small cytoplasmic proteins abundantly expressed in tissues with active fatty acid metabolism. Liver-type FABP (LFABP), an intracellular carrier protein of free fatty acids, is expressed in the liver and kidney.[7-9] In the kidney, the expression of LFABP is predominantly located in the proximal tubules. Urinary LFABP (u-LFABP) is mainly regarded as a urinary tubular biomarker associated with kidney damage.[10] The

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increased levels of u-LFABP were previously suggested to be associated with renal tubulointerstitial damage due to the excessive reabsorption of free fatty acids into the proximal tubules that induces tubulointerstitial damage.[7,11,12] In a previous report, clinical significance of u-LFABP has been proved thereby indicating that this clinical marker can identify patients who are likely to develop DN in future.[13] u-LFABP levels have not yet been assessed in Indian population with type 2 diabetes. Hence, we aimed to evaluate u-LFABP levels at different stages of DN and to see its correlation with other clinical parameters in patients with type 2 diabetes.

Materials and Methods

Patients

This cross-sectional study comprised of 65 (M: F; 42:23) subjects with type 2 diabetes and 13 (M: F; 3:10) non-diabetic control subjects recruited from the Outpatient Department of a Tertiary Care Center in India. The control subjects were the attenders of the patients who had participated in the study. History of diabetic ketoacidosis or hypoglycemic coma in the past 3months preceding the study, presence of urinary tract infection, hepatic, other renal disease, rheumatological, neoplastic, other endocrine diseases (except diabetes) was the exclusion criteria. Subjects on antihypertensives, statins or using immunomodulatory medications were also excluded from the study. Ethics Committee of the institution approved the study, and all the subjects provided the written informed consent.

Group 1 consisted of non-diabetic control subjects (n = 13, M: F; 3:10). Type 2 diabetic subjects were divided into three groups based on their renal status. The study groups were as follows: Group 2 (n = 22, M: F; 10:12) were the normoalbuminuric subjects having urinary albumin to creatinine ratio (ACR) of <30 μg/mg creatinine estimated by immunoturbidimetric method. Group 3 (n = 22; M: F; 18:4) were the microalbuminuric subjects having ACR 30–300 μg/mg creatinine and Group 4 (n = 21; M: F; 12:9) were the macroalbuminuric subjects having massive proteinuria of expected protein excretion rate of >500 mg/day with the presence of diabetic retinopathy. Estimated Glomerular filtration rate (eGFR) was calculated using Cockcroft and Gault formula[14] and was normalized per 1.73 m^2 of body surface area.

Methods

Demographic and anthropometric details like age, weight, height, duration of diabetes, duration of DN were recorded for all the study subjects. Family history of diabetes and smoking and alcohol consumption habits were obtained from the medical records of the study subjects. Body mass index (BMI) (kg/m^2) was calculated using the standard formula. Blood pressure was measured using a standard mercury sphygmomanometer. Blood samples were collected for the biochemical estimations. Fasting and post-prandial samples were collected from the known cases of diabetes and other subjects underwent a standard oral glucose tolerance test. All the biochemical investigations were done by standard enzymatic procedures using BS 400 auto analyzer. Plasma glucose was measured by glucose oxidase peroxidase method. The diagnosis of diabetes was based on previous history of diabetes or on the criteria of World Health Organization for the classification of glucose intolerance.[15] Glycosylated hemoglobin (Glycated hemoglobin A1c% [HbA1c%]) was measured by high-performance liquid chromatography method using Bio-Rad variant turbo equipment (Hercules, CA). Fasting serum sample was used for the estimation of lipid profile, urea and creatinine.

Urine liver-type-fatty acid-binding protein assay

Freshly voided urine samples were collected and centrifuged at 2000 rpm/min for 10 min. Two ml of supernatant were aliquoted for the estimation of u-LFABP levels and stored at −20°C until tested. u-LFABP levels were measured with a solid phase enzyme linked immunosorbent assay (ELISA) using the human u-LFABP kit (HK404, Hycult Biotechnology, Uden, The Netherlands). The coefficient of mean variations in the samples were (<10%). The minimum detectable L-FABP level with this kit was <5pg/ml. No significant cross-reactivity or interference was observed with this assay kit. u-LFABP levels were expressed as values adjusted for the urinary creatinine concentration (mg of creatinine/dl).

Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 16.0 Version software (SPSS Inc., IL, USA). Mean and standard deviation for continuous variables and percentages for categorical variables are reported as relevant. u-LFABP showed a skewed distribution and are reported as a geometric mean (95% confidence interval) Significant differences between groups were tested using the χ^2-test and Analysis of variance where ever appropriate. The relationship between u-LFABP and the other variables of study subjects was tested by Pearson correlation test. A $P<0.05$ was considered as statistically significant.

Results

The study group subjects with normoalbuminuria, microalbuminuria and macroalbuminuria were older

(>50 years) than the subjects in the control group (<50 years). BMI was similar in all the groups (control: 26.7 ± 2.3, normoalbuminuria: 26.8 ± 3.7, microalbuminuria: 27.9 ± 4.1 and macroalbuminuria: 27.1 ± 4.0). Both systolic and diastolic blood pressure values were significantly higher in macroalbuminuria group (142.9 ± 20.0/88.6 ± 10.8 mmHg) when compared to normo (124.7 ± 17.4/80.5 ± 8.1 mmHg) and microalbuminuric groups (128.6 ± 17.0/83.6 ± 9.5 mmHg). Few subjects in the micro and macroalbuminuria groups chewed tobacco and consumed alcohol. A higher percentage of subjects in the macroalbuminuria group (57.1%) were on combination of oral hypoglycemic agent (OHA) and insulin therapy, whereas higher percentage of subjects in the normoalbuminuric (54.5%) and microalbuminuric group (50.0%) were on OHA.

Table 1 shows the biochemical details of the study subjects. Fasting and postprandial glucose levels increased gradually with declining renal status, whereas HbA1c% was higher in subjects with normoalbuminuria, compared to micro and macroalbuminuria groups. Urea and creatinine levels were similar in all the groups. The lipid profile was also similar in all the groups. The mean eGFR values decreased with the progression of DN ($P = 0.001$).

Urinary liver-type fatty acid binding protein levels in healthy controls and study groups

Urinary liver-type fatty acid binding protein was undetectable in healthy control urine samples by solid phase ELISA. The levels of u-LFABP were higher in subjects with macroalbuminuria as compared with normoalbuminuric and microalbuminuric subjects. The levels of u-LFABP in subjects with type 2 diabetes, increased gradually with declining renal status ($P = 0.005$).

Among all the total subjects, u-LFABP positively correlated with systolic blood pressure ($R = 0.344$, $P = 0.005$) and protein to creatinine ratio ($R = 0.441$, $P = 0.04$). u-LFABP negatively correlated with eGFR and high-density lipoprotein (HDL) cholesterol but was found to be nonsignificant. No significant correlation was found between u-LFABP and age, BMI, diastolic blood pressure, duration of diabetes, fasting and postprandial glucose levels, HbA1c, total cholesterol, triglycerides, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, urea, creatinine and ACR.

No correlation was observed between u-LFABP and all the other parameters in the normoalbuminuric group. In the microalbuminuric group, u-LFABP significantly correlated with systolic blood pressure ($R = 0.640$, $P = 0.001$), fasting plasma glucose ($R = 0.569$, $P = 0.006$) and HbA1c ($R = 0.651$, $P = 0.001$). In the macroalbuminuric group, u-LFABP correlated with duration of diabetes ($R = 0.510$, $P = 0.018$) and negatively correlated with HDL cholesterol ($R = -0.464$, $P = 0.034$).

Figure 1 shows the scatter plot of all urine LFABP measurements by ELISA comparing healthy controls and u-LFABP values at different stages of eGFR. u-LFABP levels were significantly elevated in patients with lower eGFR values.

Discussion

The evidence suggests that the tubulointerstitial damage as well as glomerular damage contributes to a decline in renal function.[4] u-LFABP has been demonstrated to be a marker of tubular damage.[10] Several studies have shown that u-LFABP could be a useful marker for the detection

Table 1: Biochemical details of the study group

Parameters	Study group *n* (male:female)				
	Control 13 (3:10)	Normoalbuminuria 22 (10:12)	Microalbuminuria 22 (18:4)	Macroalbuminuria 21 (12:9)	*P*
Plasma glucose (mg/dl)					
Fasting	93±8.4	170±82.2	174±74.5	207±111.7	0.003
Postprandial	120±33.6*	256±121.7	269±76.8	312±117.9	<0.0001
HbA1c (%)	5.8±0.4	14.8±18.8	9.1±1.4	9.7±1.6	0.072
Urea (mg/dl)	24.2±4.5	20.7±5.6	26.6±8.6	34.2±21.3	0.007
Creatinine (mg/dl)	0.9±0.1	0.8±0.1	1.0±0.19	1.03±0.23	0.192
Total cholesterol (mg/dl)	164±16.2	168.3±57.2	172.8±39.1	173.3±48.5	0.928
Triglycerides (mg/dl)	119±42.0	142.1±52.7	159.4±52.5	171±99.3	0.147
HDL cholesterol (mg/dl)	43.6±7.8	43.1±8.8	39.7±6.3	41.6±10.6	0.507
LDL cholesterol (mg/dl)	100.9±19.1	97.6±31.6	97.1±29.7	94.1±34.9	0.938
VLDL cholesterol (mg/dl)	27.9±13.2	39.1±19.6	30.1±13.8	27.8±9.4	0.047
Estimated GFR (ml/min/1.73 m^2)	124.3±31.3	96.3±21.0	88.0±25.5	75.8±24.2	0.001
L-FABP/Ucr** (pg/mg)	-	0.65 (0.47-0.97)	0.99 (0.55-1.97)	5.16 (1.8-14.5)	0.005

*2 h post glucose, **Geometric mean. Values are geometric mean (95% CI). CI: Confidence interval, HbA1c: Hemoglobin A1c, HDL: High-density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, GFR: Glomerular filtration rate, L-FABP: Liver-type fatty acid binding protein, Ucr: Urine creatinine

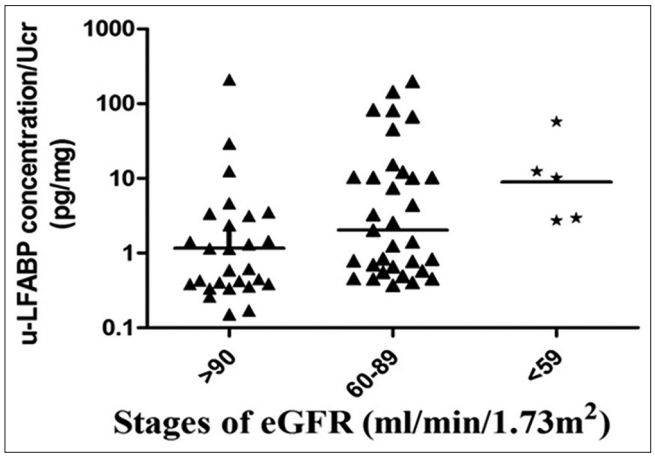

Figure 1: Urinary liver-type fatty acid binding protein/urine creatinine ratio concentrations at different stages of estimated glomerular filtration rate (ml/min/1.73 m²)

of early stage of DN.[16-18] But the levels of u-LFABP at different stages of DN among Indian subjects with type 2 diabetes are unknown.

The present study results demonstrated that u-LFABP levels are increased gradually with increasing severity of DN in subjects with type 2 diabetes indicating tubular damage. Macroalbuminuric subjects had higher levels of u-LFABP as compared to normoalbuminuric and microalbuminuric subjects. The levels of u-LFABP were too low when compared to other studies.[17-19] This difference may be due to the difference in the sensitivity of the kit used. In our study, u-LFABP was undetectable in healthy control urine samples by sandwich ELISA. Supporting to our findings, Ferguson *et al*. also shown the u-LFABP was undetectable in healthy control urine samples.[19]

The levels of u-LFABP were significantly higher in subjects with lower eGFR values. The association between u-LFABP and eGFR define the use of u-LFABP as a marker that reflects the degree of kidney damage as estimated by GFR. u-LFABP level was significantly correlated with proteinuria and systolic blood pressure in total subjects, but did not correlate with microalbuminuria. Therefore, u-LFABP may reflect the condition associated with progression of DN that is not possible with urinary albumin levels and a combination of urinary albumin and LFABP could be a better marker for early diagnosis of DN. The above findings indicate that in addition to albuminuria, tubular markers also should be added to assess the risk of development of DN.

Urinary liver-type fatty acid binding protein was reported to be an independent predictor of microalbuminuria and death in patients with type 1 diabetes.[16] Similarly, u-LFABP appeared to be a useful marker for the detection of early stage of DN and also for the prediction of the progression of DN in patients with type 2 diabetes.[17,18] We could not conclude whether glomerular damage occurred first or tubular damage at an early stage of development of DN since our study was a cross sectional design. Large well-designed prospective studies may demonstrate whether high levels of u-LFABP are evident even before the development of microalbuminuria in Indian patients with type 2 diabetes. Another limitation of this study was smaller sample size.

Conclusion

The current study indicated that high levels of u-LFABP were associated with declining renal function. The results suggest the importance of tubular damage in the development of renal dysfunction among patients with type 2 diabetes in South India.

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