Internal Medicine

Prognostic Value of Liver Fatty Acid-Binding Protein for Impairment of Renal Function in Type2 Diabetic Patients

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ABSTRACT

Background: Diabetic microvascular disease is mainly manifest as retinopathy, nephropathy, polylineeuropathy and vascular anomalies of the lower limbs, leading to visual impairment, renal failure, stroke, lower limb diabetic cardiomyopathy, disrespect and dysfunction. Fatty acid-binding proteins (FABPs) are members of the intracellular lipid-binding protein (ILBP) family which plays an important role in the pathogenesis of diabetic nephropathy and chronic kidney diseases.

Aim of the work: To evaluate Urinary liver-type fatty acid-binding protein (u-LFABP) biomarker for the occurrence and development of nephropathy in diabetic patients with and without renal disease.

Patients and Methods: In this cross sectional study 100 patients were enrolled divided into three groups; Control Group; included 40 normal healthy individuals; Early Diabetic Nephropathy Group (Early-DN); included 30 patients of Type II diabetes and Diabetic Kidney Disease Group (DKD) 30 patients of type II diabetes, all groups evaluated u-LFABP biomarker using ELISA technique.

Result: our results revealed that u-LFABP-levele were significantly increased in early DN and DKD groups in comparison to control group shich indicates tubular damage, U-LFABP level increasing gradually with denying of renal function and e-GFR.

Conclusion: u-LFABP tubular markers are good predictive factors for DN and DKD characterized with high diagnostic performance. Considering the urinary protein, creatinin, eGFR and provided the high diagnostic information for diagnosing DN. u-LFABP considered as tubular factor that determine kidney state of diabetic patients.

Keywords: *Diabetes kidney disease; Diabetic nephropathy; Fatty acid-binding proteins; Type 2 diabetes mellitus; Urinary liver-type fatty acid-binding protein.*

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic disease in which the main finding is chronic hyperglycemia.[1] The cause of insulin secretion is reduced: or reduced insulin action. In diabetes, chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels.[2]

Diabetic nephropathy (DN), a recurrent complication occurring in approximately 30% of diabetics and leading to renal failure, is a common microvascular complication of T2D characterized by albuminuria (> 300 mg/g creatinine (Cr), permanent and irreversible decrease in Glomerular filtration rate (GFR) and arterial hypertension were confirmed at least twice, 3 to 6 months apart.[3]

Early diagnosis of DN is important to avoid the negative effects of renal failure in diabetics. Although microaluminuria has been recognized as an important predictive marker for the diagnosis of archaic DN. Some diabetic DNs can develop without overt microalbuminuria. The mean incidence of diabetes mellitus due to DN is high (3% per year) in the first 10 to 20 years after onset of diabetes. It is estimated that more than 20% to 40% of diabetics are affected. develop chronic kidney disease.[3&4]

Liver fatty acid protein (L-TFABP), also known as FABP1, is an intracellular fatty acid expressed in the liver and kidneys. In the kidney, L-FABP expression is mainly localized in the proximal tubules. We have previously proposed that the elevated L-FABP level is associated with interstitial damage in the renal tubules, such that excess free fatty acid uptake into the proximal tubules causes interstitial damage.[4]

But if L-FABP is more sensitive than DN tags. Albumin secretion (AER) is not yet known if its predicted role is limited to disease progression.[4,5]

Hyperglycemia is the main factor causing organ damage, the extent of damage from proteinuria and GFR estimated during use.[5]

This study purposes to estimate prognostic value-of urinary liver fatty-acids binding protien in type2 diabetes mellitus (T2DM) with diabetic nephropathy and renal impairment.

PATIENTS AND METHODS

This study were conducted a cross sectional on 100 patients with type2 diabetes at Al Azhar University hospitals for follow up renal functions, other complications of diabetes and controlled of blood sugar. The study was performed in the period between July-2020 to June-2021.

In this study all subjects were classified into three groups as follow: Control Group; it included 40 normal healthy individuals. Early Diabetic Nephropathy Group (Early-DN): It included 30 patients of Type II diabetes with Micro albuminuria and with normal blood pressure. Diabetic Kidney Disease Group (DKD): It included 30 patients of type II diabetes with microalbuminuria and with systolic/ diastolic blood pressure value of. 129-151mm/Hg / 90.0 -95mm/Hg. Informed consent were approved was taken from Al-Azhar Assuit Hospital.

Age of diabetic patient more than 30 years, Diagnosis of diabetes from 5 to 10 years were included; Type 1 diabetes and liver disease, Active infection or inflammation, History of drug taking that affecting on immune system like corticosteroid, Had severe physical inactivity were excluded.

Full history taking, including: Age, Sex, Exclusion other diseases which may affect on kidneys with diabetes, Symptoms related to diabetic-nephropathy in diabetic patients, Duration of diabetes and Symptoms related to complications.

Thorough clinical examination including; . Abdominal examination including; Inspection, Palpation and Percussion. Body mass index (BMI) was calculated as body weight in kg divided by height in meter-squared. Waist circumference (WT) was measured midway between the lower chest and the iliac crest.

Laboratory investigations: CBC, Fasting blood sugar, c- Serum creatinin and Albumin in urine or urinary albumin create ratio c. Lipid profiles, Measurement of U-LFABP and HBA1C.

Early venous blood samples of 10 ml were collected from healthy subjects and controls who had fasted overnight. Each blood sample was divided into the following aliquots: Whole blood: 2 ml of unclogged venous blood was collected into EDTA tubes (1.2 mg EDTA/ml) and mixed thoroughly by gently shaking the tubes up and down several times. . Serum: The remaining blood sample was stored in a clean glass tube without any coagulation additives at 37°C for 20 min, then centrifuged at 3000 rpm for 10 min. The serum was then separated, aliquoted and stored at -20°C, thawed once if necessary. Urine Samples: A 24-hour urine sample was collected from each subject in a clean container for protein and creatinine quantification. Fresh urine samples were collected for the quantitative determination of u-LFABP.

Total Hemoglobin Assay: Five ml of deionized water were pipette into tubes labeled standard (s), unknown (u) and control. 20 μl of hemolysate was pipette into labeled tubes. Absorbance (Atot) of standard, control and unknown were measured at 415nm within an hour protein restriction and strict blood pressure control on the progression of renal disease.

The final MDRD Study prediction equation for GFR is as follows with Pcr being serum or plasma creatinine in mg/dL: GFR (mL/min/1.73 m2) = 186 x $(Pcr)^{-1.154}$ x $(age)^{-0.203}$ x (0.742 if female) x (1.210 if African American) The GFR is expressed in mL/min/1.73m^2

Quantitative Determination of blood urea nitrogen; was determined according to the enzymatic method described by Kaplan [6] using a commercial assay kit.

Quantitative Determination of Urinary Protein; was determined by Watanabe et al. [7] using a commercial assay kit.

Quantitative determination of Creatinine in Urine; were determined using a Randox kit, Antrim, United Kingdom based on Jafe kinetic method Quantitative Determination of serum creatinine; was determined according to the colorimetric kinetic method using a commercial assay kit.

Quantitative Determination of Total Cholesterol; was determined according to the colorimetric enzymatic method described by Allian et al., [8] using a commercial assay kit.

Quantitative Determination of Triacylglycerol (TAG); was determined according to the colorimetric enzymatic method described by Young et al., [9] using a commercial assay kit.

Quantitative Determination of High-Density-Lipoprotein Choles-terol (HDL-c); was estimated by the cholesterol PAP method.

Quantitative Determination of Low Density Lipoprotein Cholesterol (LDL-c); calculated if triacylglycerol concentration is less than 400 mg/dl. LDL-C (mg/dl) = Total cholesterol- (TG / 5) - HDL-C.

Quantitative Determination of Very Low Density Lipoprotein Cholesterol (VLDL-c); was calculated formula: $VLDL-c \frac{mg}{dl} = \frac{Triacylglycerol}{5}$.

Determination of U-LFABP Principle; was carried out by ElISA technique according to the method of Yokoyama et al. [10]

Statistical analysis was performed using a digital computer using prior art version 23 Excel and SPSS programs using the following statistical analysis: arithmetic mean (X) standard deviation (SD). The significance of the results of this work was evaluated with p <0.05 = significant. p <0.01 = high significance. p> 0.05 = not significant (NS). A one-way analysis of variance (ANOVA) was used to measure the statistical significance of the differences between the means of more than 2 groups. The Receiver Operating Characteristic (ROC) curve is a

graphical method used to measure the ability of a test to distinguish between patients with and without disease. The predicted values of the parameters studied for the patient group were matched to the control group data by ROC curve analysis, the data were in the area under the curve (AUC).

RESULTS

Signs	DN N=30		DKD N=30		P-Value
	N	%	N	%	
Neuropathy	21	70.0	25	83.3	0.62 NS
Retinopathy	13	43.3	19	63.3	<0.001 S

Table 1: Neuropathy and retinopathy among DN and DKD.

Parameters	Control N=40	DN N=30	DKD N=30
Plasma glucose (mm/L) (Mean±SD)	5.31±0.32	14.96 ± 1.2	15.2±1.28
P-Value versus control group	---------	<0.001	<0.001
HbA1C%	4.94±.0.62	14.3±1.2	14.5 ±1.0
P-Value versus control group	---------	<0.001	<0.001

Table 2: Plasma glucose, and HbA1C% of the studied groups.

Parameters	Control N=40	DN N=30	P-Value versus control group	DKD N=30	P-Value versus control group
BUN (mg/dl)	25.60 ± 5.6	48.7 ±6.0*	0.045	104.8±34.5*	<0.001
s-Cr (mg/dl)	0.7±0.20	1.2±0.2*	0.042	2.1 ±0.4**	<0.001
u-Cr (g/l)	122.5±20.4	87.5±12.7*	0.036	71.8±13.6*	<0.001
UEAR(mg/dl)	10.7±2.7	256.8±43.9*	<0.001	293.2±40.0*	<0.001
eGFR (ml/min/1.73m2)	106.65±15.8	65.4 ±12.9*	<0.001	53.1±10.9*	<0.001

Table 3: Descriptive analysis of kidney function tests in all studied groups.

Parameters	Control N=40	DN N=30	P-Value versus control group	DKD N=30	P-Value versus control group
TC (mg/dl)	134.82±8.5	223.3 ±12.3	<0.001	269 ±35.8*	<0.001
TAG (mg/dl)	95.8±9.3	184.3±9.25	<0.001	230±2.56*	<0.001
HDL-c (mg/dl)	49.5±9.6	32.2 ±5.2*	0.036	34.7 ±5.8*	0.042
LDL-c (mg/dl)	78.1±14.6	140.4±20.4*	<0.001	165.7±34.6*	<0.001
VLDL-C (mg/dl)	26.9 ±3.1	42.3 ± 11.2*	0.048	56.2 ± 12.3*	0.028

**Table 4:** Descriptive analysis of lipid profile in all studied groups.

Parameters	Control N=40	DN N=30	DKD N=30
u-LFABP (Pg/ml)	80.5±5.35	143±9.25[*1]	223.32±12.42[*2]
P-Value[1]		P-Value[2]	P-Value[3]

Table 5: Levels of u-LFABP of all the studied groups.

Groups Parameters	DN		DKD	
	r	P value	r	P value
HbA1C	0.25	NS	0.59 *	<0.001
s-Cr	0.5*	<0.05	0.72*	<0.001
UAER	0.30	NS	0.70*	<0.001
eGFR	-0.59 *	<0.001	-0.68*	<0.001

Table 6: Pearson's correlation coefficients (r) between u-LFABP level and biochemical parameters

In DN group: A significant positive correlation was shown between s–CR.On the other hand, there was a significant negative correlation between e-GFR.
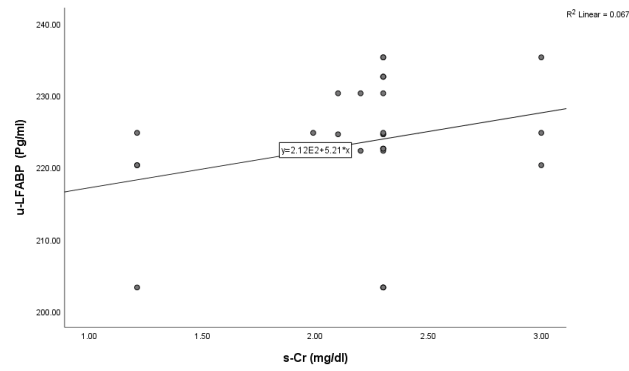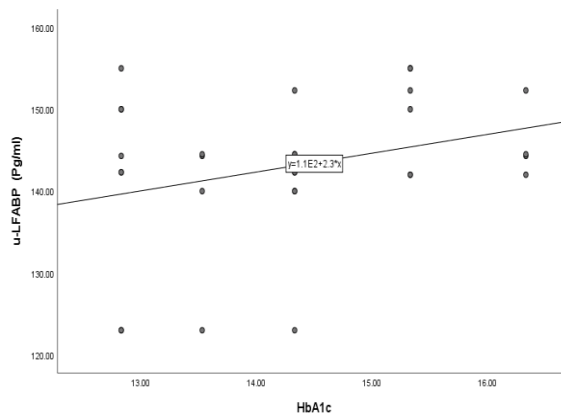
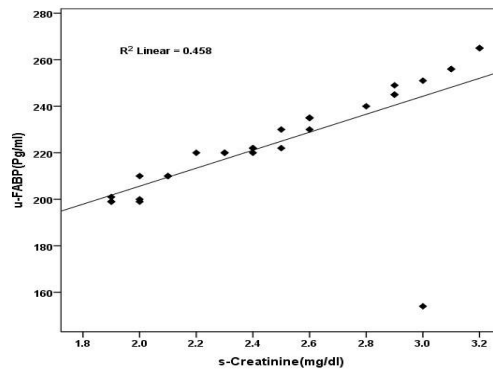**Fig. 1:** Positive correlation between u-LFABP and s-Cr in DN group.
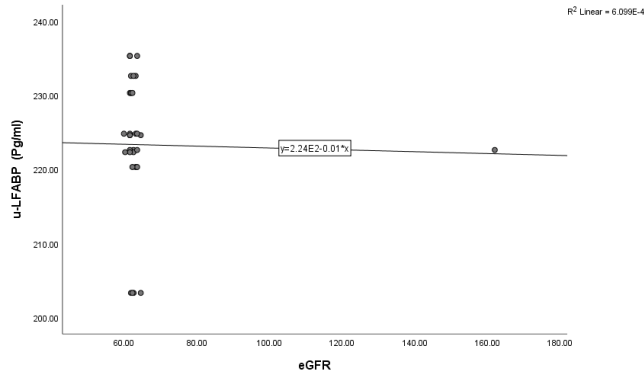
Fig. 2: Positive correlation between u-LFABP and eGFR in DN group.
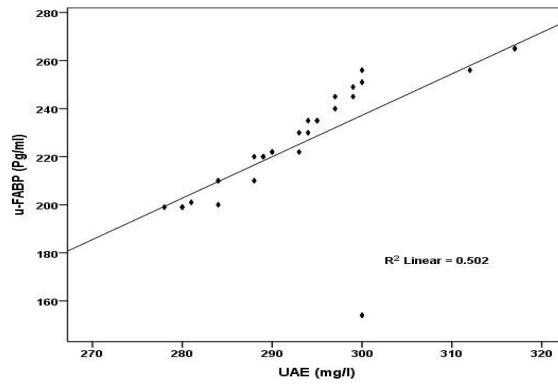
In DKD Group: As shown in figures (3); A positive correlation between HbA1C, s-Cr, and UAER. On the other hand, there was a significant negative correlation and eGFR.
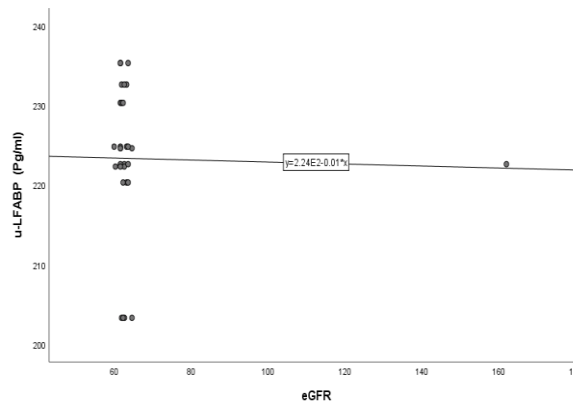

Fig. 3: Positive correlation between u-LFABP and HbA1c level in DKD group.

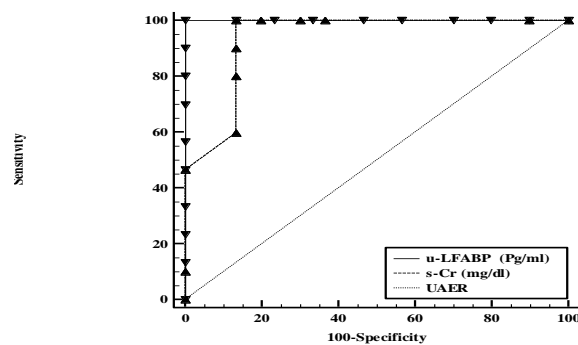**Fig. 4:** Positive correlation between u-LFABP and s-creatinine in DKD group.

Fig. 5: Positive correlation between u-LFABP and UAE in DKD group.

Fig. 6: Negative correlation between u-LFABP and e-GFR in DKD group.

Variable	AUC	Cutoff	P Value
LFAB	0.56	126.6	<0.001 S
S_Cr	0.938	1.33	<0.001 S
UAER	0.82	9.64	<0.001 S

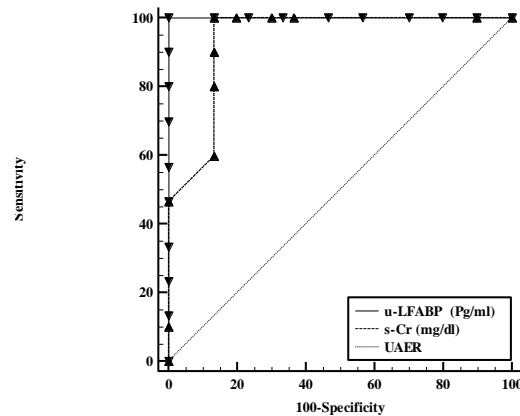**Table 7:** Area under the curve and cutoff value of uLFABP, and in DN group.

Fig. 7: ROC curve for, u-LFABP and UAER in DN group.

Variable	AUC	Cutoff	P Value
LFAB	1	199.5	<0.001 S
S_Cr	0.92	1.42	<0.001 S
UAER	0.99	9.1	<0.001 S

Table 8: Area under the curve and cutoff value of u-LFABP, S_Cr and UAER in DKD group.

Fig. 8: ROC curve for, u-LFABP, S_Cr and UAER in DKD group.

DISCUSSION

The current study revealed that prevalence of retinopathy and neuropathy associated with decreased eGFR so retinopathy and neuropathy highly significant among diabetic kidney diseased compared to diabetic nephropathy and these findings were in line with Dash et al [11] that provided that prevalence of retinopathy and neuropathy related to decrease eGFR and increase proteinuria.

Our findings determine the occurrence of diabetic nephropathy and variabilities ofHBA1C and this in agreement with Lincc et al. [12] who confirm that relationship between annual variation of HBA1C and incident of diabetic nephropathy.

Determination of plasma and especially urinary L-FABP have been reported in recent studies as potential biomarkers for early diagnosis of acute kidney injury (AKI) caused by various factors such as after cardiopulmonary bypass surgery Kamijo-Ikemori et al.[13] after cardiac surgery or in critically patients Cho et al.[14]

Burkhardt et al.[15] detect a significant increase of plasma creatinine level accompanied by high significant increase of BUN in diabetic patients with high level of microalbumin in urine. The role of serum creatinine and BUN as a marker for early nephropathy were reviewed by Mussap et al.[16] who reported that, they are altered after the development of diabetic nephropathy, they are also diagnostic and follow up parameters, but their predictive value is not evident.

The results of this study are consistent with Ayodele et al.[17], who reported that in DN there are changes in glomerular filtration rate (GFR) with early hyperfiltration, development of proteinuria, arterial hypertension, and subsequent loss of renal function with decreased GFR.

Renal failure has been reported to be associated with the degree of tubulointerstitial injury.

In the current study showing u-LFABP in early PN patients, u-FABP was very significantly elevated. This increase was more pronounced in the DKD patient group. The present results were consistent with Tsukasa et al.[18] , who reported that elevated u-LFABP decreased e-GFR in patients with type 2 diabetes mellitus.

In addition, Tramonti and Kanwar [19] clarified that the increase in proteins filtered through the glomerular barrier exerts enormous pressure on the proximal tubules and causes an accelerated secretion of uLFABP from the tubular space in the urine.

The results of this study showed a significant increase in CT, TAG, LDL-C and VLDL-C, while a significant decrease in HDL-C was recorded at baseline in ND and DKD in patient groups compared to controls.

The results of the current study are also consistent with Kaysen [20], who reported that in patients with proteinuria and mild reduction in GFR, LDL synthesis is increased, HDL-c particle maturation is reduced, and chylomicron clearance is reduced. , VLDL and triglycerides are reduced due to the decreased activity of the lipoprotein lipase. These findings are consistent with the results of Zhang et al.[21], which indicated that high glucose levels increase lipid accumulation in podocytes. These changes, together with other risk factors, can contribute to the development of DN.

The results of the ROC curves describing the diagnostic performance of different biomarkers for the early DN and ND patient groups revealed that u-LFABP appeared to be a more sensitive marker for detecting early DN and also predicting its progression of that with type 2 diabetes.

CONCLUSION

2D-STE is a quick and dependable approach for U-LFABP levels represents as one of the tubular factors, detrmine renal status in diabetic patients. U-LFABP represents as sensitive marker to predict DN progression than proteinuria in patients with type 2 diabetes mellitus.

Recommendation

Automated analyzers for routine applicability of L-FABP to improve sensitivity and specificity for early

diagnosis of renal failure. U-FABP marker for monitoring treatment effect to prevent progression of glomerulosclerosis in this patient group.

Conflict of interest : none

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