FABP1 as an Early Marker of Diabetic Nephropathy in Type 2 Diabetic Patients

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KEYWORDS

Diabetetic nephropathy ,FABP1,,metabolic syndrome

ABSTRACT:
Background: The prevalence of diabetes mellitus, a worldwide burden, is expected to affect more than 350 million people by 2035, About one-third of diabetic patients have microalbuminuria after 15 years of disease duration, and nearly half of them develop real kidney disease, Early detection and intervention are essential in the prevention and treatment of diabetic nephropathy (DN). LFABP(u-LFABP) has been demonstrated to be a marker of tubular damage., Several studies have shown that u-LFABP could be a useful marker for the detection of early stage of DN. **Aim**: Assess if FABP1 is an early marker of diabetic nephropathy in type 2 diabetic patients.
Methods: This is a cross sectional study 120 diabetic patients (40 with norm albuminuria, 40 micro albuminuria and 40 macroalbuminuric was conducted at El-Kasr Aini Cairo University Hospital. The patients were recruited from the outpatient clinics of endocrinology and internal medicine. Detailed medical history, complete physical examination for type II diabetic patients aged (35-65) years old., Laboratory investigations including Fasting blood glucose, blood urea, serum creatinine, HbA1c and lipid profile for all participants. Estimated GFR will be measured by Modification of Diet in Renal Disease (MDRD) equation, The relation between morning spot urine sample ACR and urinary FABP1 will be studied, also FABP1 urinary was correlated to (eGFR, HbA1c, lipid profile).
Results: Urinary L-FABP1 levels were elevated in the microalbuminuric and macroalbuminuric groups compared to the normoalbuminuric group, but this difference was not statistically significant. The highest concentration was noted in the microalbuminuric group.
Conclusion: urinary L-FABP1 may serve as a marker of tubular damage in diabetic nephropathy. However, its predictive and diagnostic value remains uncertain due to the lack of statistically significant differences observed in our study.. Larger-scale studies with longer follow-up periods may help clarify whether urinary L-FABP1 can be a reliable biomarker for DN progression in diabetic patients.

1. Introduction

The rising disease burden of diabetes mellitus globally is a major public health priority, placing unsustainable demands on individuals, health systems, and society. The latest estimates show that there was a global prevalence of 425 million people with diabetes in 2017, which is expected to rise to 629 million by 2045 [1].

Renal disease in diabetic patients had been clinically characterized by increasing rates of urinary albumin excretion and decreasing renal function, with at-risk patients marching through the stages of normoalbuminuria, microalbuminuria, overt proteinuria, and finally ESRD [2].

The screening and early diagnosis of DKD is based on the measurement of urinary albumin excretion and the detection of microalbuminuria, the first clinical sign of DKD. The management of DKD is based on the general recommendations in the treatment of patients with diabetes, including optimal

glycemic and blood pressure control, adequate lipid management, and abolishing smoking, in addition to the lowering of albuminuria [3].

Early detection and intervention are essential in the prevention and treatment of diabetic nephropathy (DN). So far, albuminuria or the urinary albumin-to-creatinine ratio (ACR) has been the standard marker for early detecting DN as recommended by various guidelines and reports. However, there have been controversies over its diagnostic and prognostic significance since, in several studies, macroalbuminuria accompanied rather than preceded progression to advanced chronic kidney disease (CKD). Furthermore, patients with persistent microalbuminuria still progress to CKD stages 3-5. Therefore, it is necessary to find novel biomarkers with better specificity and sensitivity to effectively detect and intervene in DN at the onset for better prevention of CKD progression [4].

L-FABP being a promising candidate; it is recognized as the most useful alternative biomarker of kidney injury. Matsui et al. described urinary L-FABP as an early predictor of AKI. In addition, Kamijo et al. showed urinary L-FABP to be an excellent biomarker for clinical prediction and monitoring of renal disease. Several studies have shown the usefulness of urinary L-FABP for the detection of kidney injury [5].

This study aimed to assess if FABP1 is an early marker of diabetic nephropathy in type 2 diabetic patients.

Patients and Methods:

Patients for this study were selected from the Internal Medicine Department and the Endocrinology Clinic at Kasr Al-Aini Hospital. The total sample size included 120 diabetic patients, categorized into three equal groups: 40 patients with normoalbuminuria, 40 with microalbuminuria, and 40 with macroalbuminuria. All subjects met the inclusion criteria and were free from any of the exclusion criteria.

The inclusion criteria for the study required participants to be type 2 diabetic patients between the ages of 35 and 65 years. Exclusion criteria included individuals with liver disease, pregnancy, severe infections within the last three months, current acute infections, type 1 diabetes, cerebrovascular accidents, cardiovascular disease, end-stage renal disease, malignancy, or those undergoing hormonal therapy.

Approval for this study was granted by the institutional ethical committee in July 2021. Informed consent was obtained from all participants or their legal representatives after they were given a full explanation of the study. They were also informed of their right to withdraw at any point.

Participants were recruited from the endocrinology and internal medicine outpatient clinics at Kasr Al-Aini Hospital between March 2023 and October 2023. Each participant underwent a thorough medical history evaluation and a complete physical examination. Laboratory investigations included fasting blood glucose, blood urea, serum creatinine, HbA1c, and lipid profile assessments. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation, with a correction factor of 0.85 for female participants.

To assess kidney function, fresh early morning urine samples were collected for urinary creatinine and albumin measurements. Patients were classified into three groups based on their albumin-to-creatinine ratio (ACR): normoalbuminuric (ACR <30 mg/g), microalbuminuric (ACR 30-300 mg/g), and macroalbuminuric (ACR >300 mg/g). Urinary fatty acid-binding protein 1 (FABP1) levels were analyzed in spot urine samples. The collected samples were centrifuged, and the supernatant was used for FABP1 quantification via enzyme-linked immunosorbent assay (ELISA). FABP1 levels were adjusted for urinary creatinine. Correlations between urinary FABP1 levels and ACR, eGFR, HbA1c, and lipid profile were assessed.

The total sample size of 120 diabetic patients was determined using the PASS 11 sample size calculator, with an alpha error of 0.05, a confidence interval of 95%, and a study power of 80%. The sensitivity and specificity of FABP1 in diagnosing diabetic nephropathy were reported as 98.1% and 90%, respectively. According to previous studies, FABP1 levels were observed to be 6.30 (SD 2.30) in normoalbuminuric patients, 6.77 (SD 4.79) in microalbuminuric patients, and 18.07 (SD 38.69) in macroalbuminuric patients, as Additionally, FABP1 levels were found to be 2.51 (SD 0.28) in normal-weight individuals and 2.76 (SD 0.12) in obese individuals, as per Shi et al. (2012).

A convenient sampling technique was employed, ensuring that patients meeting the inclusion and exclusion criteria were enrolled until the required sample size was achieved. Statistical analysis of the collected data was conducted using SPSS version 21. This study was designed as a cross-sectional study. Patients were selected from the Internal Medicine Department and Endocrinology Clinic at Kasr Al-Aini Hospital and were divided into three groups according to their albuminuria status.

Results

Our study included 75 diabetic female and 45 diabetic male patients from Internal Medicine Department and endocrinology clinic at Kasr Al-Aini Hospital, 37.5 % and 62.5 % male and female respectively for assessment of urinary LFAB1 as an early marker of diabetic nephropathy. No statistical significant difference between participants in age, and weight distributions

Table 1: Descriptive Analysis Of Patient Characteristics

Category	Frequency	Percent	Valid N	Missing N	Mean	Std. Deviation	Minimum	Maximum
Male	45.0	37.5						
Female	75.0	62.5						
Total	120.0	100.0						
No BP	42.0	35.0						
Yes BP	78.0	65.0						
Total	120.0	100.0						
Age			120.0	0.0	50.95	6.7809	37.0	65.0
Weight			120.0	0.0	90.35	15.3626	60.0	140.0

The mean LDL-C level among all participants was 140.358 ± 34.06, while the mean HDL was 45.76 ± 9.4. The mean triglyceride (TG) level was recorded at 131.39 ± 34.51, and the mean total cholesterol was 212.372 ± 39.08. The estimated glomerular filtration rate (eGFR) had a mean value of 88.146 ± 19.374, whereas the mean creatinine (CREAT) level was 1.19 ± 0.36. Additionally, the mean HbA1C was 6.8 ± 1.13, and the mean fasting blood sugar (FBS) was 149.45 ± 31.6. Notably, there was no significant difference in these parameters across the study groups.

Table (2): Description of laboratory parameters among participants

Statistics										
		LDL-C	**HDL**	**TG**	**Cholesterol. mg.dl**	**urea.mg.dl**	**eGFR**	**CREAT. mg.dl**	**HbA1C**	**FBS**
N	**Valid**	120	120	120	120	120	120	120	120	120
	Missing	0	0	0	0	0	0	0	0	0
Mean		140.358	45.767	131.392	212.325	27.39	88.146	1.1983	6.801	149.450
Std. Deviation		34.068	9.402	34.5120	39.08	7.570	19.374	.36429	1.138	31.682
Minimum		75.0	32.0	73.0	138.0	1.12	37.698	.78	5.1	109.0
Maximum		266.0	68.0	225.0	345.0	48.00	129.479	2.80	10.2	249.0

The mean age of participants in the Normo, Micro, and Macro groups was 49.075 ± 5.6631, 51.125 ± 7.984, and 52.65 ± 6.1542, respectively, with no significant difference observed across the groups. Similarly, the mean weight was 89.579 ± 12.4032 in the Normo group, 89.8 ± 14.9464 in the Micro group, and 91.675 ± 18.443 in the Macro group, showing no significant variation. Overall, there was no statistically significant difference in the distribution of age,or weight, among the three groups.

Table (3): Descriptive analysis between 3 studied groups in age and anthropometric measures.

Descriptives										
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.	P value
						Lower Bound	Upper Bound			
Age	normo	40	49.075	5.6631	.8954	47.264	50.886	39.0	60.0	0.060
	micro	40	51.125	7.9846	1.2625	48.571	53.679	37.0	65.0	
	macro	40	52.650	6.1542	.9731	50.682	54.618	43.0	65.0	
	Total	120	50.950	6.7809	.6190	49.724	52.176	37.0	65.0	
Weight	normo	40	89.575	12.4032	1.9611	85.608	93.542	62.0	112.0	0.801
	micro	40	89.800	14.9464	2.3632	85.020	94.580	64.0	118.0	
	macro	40	91.675	18.4438	2.9162	85.776	97.574	60.0	140.0	
	Total	120	90.350	15.3626	1.4024	87.573	93.127	60.0	140.0	

No statistical significant difference in HTN distributions among the 3 studied groups. No statistical significant difference between sex distributions and level of albuminuria

Table 3: Comparison of Hypertension and Gender Across Albuminuria Groups

Group	BP No (Count)	BP Yes (Count)	Total	BP No (%)	BP Yes (%)	P Value	Group	Male (Count)	Female (Count)	Total	Male (%)	Female (%)	P Value
Normo	16	24	40	40.0	60.0	0.463	Normo	9	31	40	22.5	77.5	0.059
Micro	15	25	40	37.5	62.5		Micro	17	23	40	42.5	57.5	
Macro	11	29	40	27.5	72.5		Macro	19	21	40	47.5	52.5	
Total	42	78	120	35.0	65.0		Total	45	75	120	37.5	62.5	

The mean urinary level of FABP1 was 50.47 ± 54.4 in the Normo group, 93.7 ± 107.76 in the Micro group, and 67.222 ± 79.69 in the Macro group. Although there was no statistically significant

difference in urinary FABP1 levels among the three groups, it was observed that concentrations were higher in the Micro and Macro groups compared to the Normo group. Notably, the highest urinary FABP1 concentration was recorded in the Micro group, though this difference was not statistically significant.

Table (4): Relation of urinary FABP1 to albuminurea

ANOVA									
LFABP.ng.L									
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Min.**	**Max.**	**P value**
					Lower Bound	**Upper Bound**			
Normo	40	50.472	54.42062	8.60466	33.0679	67.8771	1.50	237.50	0.070
Micro	40	93.700	107.76534	17.03920	59.2350	128.1650	1.50	390.50	
Macro	40	67.222	79.69222	12.60045	41.7357	92.7093	5.50	333.00	
Total	120	70.465	84.71952	7.73380	55.1513	85.7787	1.50	390.50	

There is no statistical significant change in LFABP levels to HbA1c, LDL-C, cholesterol, TG eGFR and others.

Table (5): Relation between FABP1 levels to other laboratory parameters

Pearson Correlations		
		LFABP.ng.L
LFABP.ng.L	Pearson Correlation	1
	N	120
HbA1C	Pearson Correlation	.076
	Sig. (2-tailed)	.409
	N	120
LDL-C	Pearson Correlation	.099
	Sig. (2-tailed)	.283
	N	120
HDL	Pearson Correlation	-.071
	Sig. (2-tailed)	.438
	N	120
TG	Pearson Correlation	.038
	Sig. (2-tailed)	.679
	N	120
Cholesterol.mg.dl	Pearson Correlation	-.051
	Sig. (2-tailed)	.583
	N	120
eGFR	Pearson Correlation	.062
	Sig. (2-tailed)	.500
	N	120
urea.mg.dl	Pearson Correlation	.032
	Sig. (2-tailed)	.725
	N	120
CREAT.mg.dl	Pearson Correlation	-.096
	Sig. (2-tailed)	.299
	N	120
FBS	Pearson Correlation	.072
	Sig. (2-tailed)	.432
	N	120
Correlation		**LFABP.ng.L**

Discussion:

The prevalence of diabetes mellitus, a worldwide burden, is expected to affect more than 350 million people by 2035. About one-third of diabetic patients have microalbuminuria after 15 years of disease duration, and nearly half of them develop real kidney disease, a serious complication with negative impacts on health, quality of life, and even life expectancy [8].

Early detection and intervention are essential in the prevention and treatment of diabetic nephropathy. So far, albuminuria or the urinary albumin creatinine ratio has been the standard marker for early detecting DN as recommended by various guidelines and reports. However, there have been controversies over its diagnostic and prognostic significance since, in several studies, macroalbuminuria accompanied rather than preceded progression to advanced chronic kidney disease. Furthermore, patients with persistent microalbuminuria still progress to CKD stages 3-5. Therefore, it is necessary to find novel biomarkers with better specificity and sensitivity to effectively detect and intervene in DN at the onset for better prevention of CKD progression [9].

The LFABP has been demonstrated to be a marker of tubular damage. Several studies have shown that u-LFABP could be a useful marker for the detection of early-stage DN. But the levels of u-LFABP at different stages of DN among type 2 diabetic patients are unknown. Therefore, u-LFABP may reflect the condition associated with the progression of DN that is not possible with urinary albumin levels [10].

In this cross-sectional study, we divided type 2 diabetic patients into three groups: normoalbuminuric, microalbuminuric, and macroalbuminuric. We aimed to study L-FABP as an early marker for diabetic nephropathy. We compared our results to previous similar studies. The present study didn't show a statistically significant correlation between different groups in gender and age. The current study showed that FABP1 urinary level was higher in micro and macroalbuminuria groups compared to the normoalbuminuric group, although not statistically significant. This result was in accordance with Kubota et al., as their study showed that L-FABP was higher in patients with micro and macroalbuminuria compared to persons without albuminuria [11].

We also noted that FABP1 urinary level was higher in the microalbuminuric group compared to the normoalbuminuric group, though not statistically significant. That was also reported by Suzuki et al., who mentioned that urinary L-FABP also tended to be greater in microalbuminuric than in normoalbuminuric patients, although this difference did not reach statistical significance [12].

These findings are in line with many previous studies. It was reported by Kamijo-Ikemori et al. in their study, which included over 100 type 2 diabetic patients, that high urinary L-FABP levels were associated with an increase in albuminuria and progression. The same finding was reported by Viswanathan et al. in their study that showed u-LFABP levels were undetectable in healthy controls and were very low in normoalbuminuric subjects. Elevated levels of u-LFABP are evident in the microalbuminuric and macroalbuminuric stages, indicating tubular damage [13].

Many previous studies showed that urinary FABP1 was an early marker for the prediction of diabetic nephropathy and a promising early marker for the prediction of diabetic nephropathy progression. We

aimed to evaluate u-LFABP levels at different stages of DN and to see its correlation with other laboratory parameters in patients with type 2 diabetes.

Our study showed no significant difference in the relation between urinary L-FABP and eGFR. This was also reported by Tomohito Gohda et al., as their study aimed to investigate the association between various biomarker levels and estimated glomerular filtration rate in patients with type 2 diabetes mellitus, and none of the biomarkers, including FABP1, were associated with reduced eGFR [14]. The FABP1 urinary level wasn't correlated to BP, lipid profile, HbA1c, or FBS, and that was consistent with Zhang et al., as their study showed that urinary L-FABP levels were not significantly correlated with HbA1c or FPG [15].

Furthermore, because the progression of DN was defined as an increase in urinary albumin concentrations until progression to end-stage renal failure, it may be difficult to determine that the potency of urinary L-FABP for diagnosis or prediction of progression of DN is higher than that of urinary albumin. Therefore, follow-up clinical studies on large populations are needed to confirm that high L-FABP levels reflect tubulointerstitial stress, predict glomerular impairment, and ultimately lead to a decline in kidney function in diabetic patients [11].

HbA1c represents the average glucose concentration over 2-3 months and is accepted as a useful index of mean blood glucose [20]. One of the purposes of the study was to investigate the relationship between HbA1c and urinary albuminuria in patients with type 2 diabetes mellitus. The current study didn't show a significant relation between the degree of proteinuria and HbA1c, in agreement with findings reported by Afkhami-Ardekani et al., as in their study, there was no statistically significant correlation found between the prevalence of albuminuria and HbA1c [21]. This was consistent with many other studies that didn't find a statistically significant correlation between HbA1c and albuminuria [22, 23].

Conclusion

Urinary L-FABP1 may serve as a marker of tubular damage in diabetic nephropathy. However, its predictive and diagnostic value remains uncertain due to the lack of statistically significant differences observed in our study.. Larger-scale studies with longer follow-up periods may help clarify whether urinary L-FABP1 can be a reliable biomarker for DN progression in diabetic patients.

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