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"Much more than proteinuria in diabetic kidney disease"

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Abstract

Background: One of the most common long term complications of type 2 diabetes is Diabetic nephropathy (DN). Classically, DN progresses from the normo-albuminuric stage to microalbuminuria to end in overt proteinuria. Recently it has been shown that there might be rapid decline of glomerular filtration rate (GFR) in diabetic normo-albuminuric patients.

Aim: This work aimed to compare data referring to both proteinuric and non-proteinuric type 2 diabetic patients and to study the significance of urinary liver-type fatty acid–binding protein in both groups.

Material and methods: 200 patients were studied and divided into 2 groups, Group I: 100 diabetic patients with eGFR < 75 mL/min, without albuminuria and Group II: 100 diabetic patients with eGFR < 75 mL/min with albuminuria. History taking, clinical examination and laboratory tests including GFR, serum creatinine, HbA1c and L-FAB were assessed.

Results: No clinically significant difference was found between the 2 groups regarding BMI, duration of diabetes, BP, S.creatinine, eGFR and retinopathy. Urinary L-FAB levels were significantly greater in non- albuminuric compared to the other group.

Conclusion and Recommendations: non-albuminuric DKD is increasing and clinicians should pay much attention to it, larger studies are to assess this phenotype's regarding pathogensesis and potential biomarkers.

Urinary LFABP may be a useful biomarker for non-albuminuric DKD.

Key words: type 2 DM, Diabetic kidney disease, non -albuminuria, urinary L-FAB.

Introduction

Diabetes mellitus is a rapidly increasing health problem. There were 366 million people with diabetes in 2011 according to the International Diabetes Federation, and this number is expected to largely increase in 2030 [(1)]

Diabetic nephropathy (DN) is defined as diabetes with albuminuria, reduced glomerular filtration rate, or both. [(2)]

Patients with DN are classically thought to develop albuminuria before renal function deterioration. This classical scenario has been recently changed by growing evidence that some patients either with DM type 1 or 2 have progressive decline in renal functions in the absence proteinuria, which is known as non-proteinuric diabetic kidney disease (DKD). [(3)]

The prevalence of non-proteinuric DKD is about 20% and 40% among diabetic patients type 1 and 2 respectively in different studies suggesting that DKD is clinically heterogeneous. [(4)]

In spite of increasing knowledge about non-proteinuric DKD, more studies are needed to identify clinical pictures, pathological findings, renal prognosis, and mortality.

Female sex, smoking, hyperglycemia, elevated blood pressure, and the presence of diabetic retinopathy all were found to be predictors associated with non-proteinuric DKD. [5]

DN is characterized by pathological changes in the kidney including glomerular and tubular hypertrophy , together with excessive accumulation of extracellular matrix components and thickening of glomerular basement membrane (GBM) finally leading to proteinuria and renal impairment. [6]

Tubulointerstitial injury contributes largely in the pathogenesis of diabetic nephropathy. So as albuminuria, is a marker of glomerular damage, finding a marker for tubular damage would give a clearer picture about kidney injury in DM.

Therefore, it is important to study molecules linked to tubular dysfunction serving as new markers for DN. [7]

Liver-type fatty acid-binding protein (LFABP) is an intracellular carrier protein, expressed in the proximal tubules in the kidney and participates in fatty acid metabolism.

Studies found urinary excretion of L-FABP to be a useful biomarker for screening for renal affection and identifying patients at risk of deterioration of renal function later on [8], where it's considered to be a useful tubular biomarker associated with structural and functional kidney damage. [9].

This biomarker levels are usually elevated early in diabetes, they also predicts poorer prognosis in acute kidney injury and chronic kidney disease progression other than DKD. [10]

Aim of the work

- As the characteristics of non-proteinuric DKD patients are still poorly defined, we aimed to compare proteinuric and non-proteinuric diabetic patients and also to assess the role of LFABP in both groups.

Material and methods

Across sectional study on 200 patients applied in diabetes outpatient clinics of Alexandria University Hospitals.Patients were divided into two groups:

Group I (non –proteinuric) : 100 diabetic patients with eGFR < 75 mL/min, without albuminuria

Group II (proteinuric) : 100 diabetic patients with eGFR < 75 mL/min patients with albuminuria

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Inclusion criteria:

- Type 2 DM
- patients aged more than 18 years .

Exclusion

- Type 1 diabetic patients and those with Secondary diabetes
- Other possible causes for CKD
- Nephrotic proteinuria
- eGFR ≥ 75 mL/min .

Methods

Patients were subjected to:

- History taking including demographic data, duration of DM, use of anti hyperglycemic and antihypertensive drugs.
- History of diabetic retinopathy diagnosed with fundus examination was reported.
- Physical examination measuring Weight, height for calculation BMI and measures blood pressure.
- Laboratory test (CBC, Serum creatinine , Blood urea , HbA1c spot urinary albumin/creatinine ratio (ACR) , eGFR using the standard (CKD-EPI) [11]
- Urinary L-FAB by ELISA[12]

In accordance with the Declaration of Helsinki, written informed consent was signed by each patient.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. Chi-square test was applied to investigate the association between the categorical variables.

For continuous data, they were expressed as range, mean, standard deviation and median Student t-test was used to compare two groups for normally distributed quantitative variables and Pearson coefficient used to correlate between two normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

The study was performed among 200 patients.

Group I 100 patients with DKD, with eGFR < 75ml/min without albuminuria

Group II 100 patients with DKD, with eGFR < 75ml/min with albuminuria

Table 1 compared the two groups regarding BMI, duration of DM, blood pressure, S.cr, HbA1c, eGFR, presence of retinopathy and urinary L.FABP levels.

There was no clinically significant difference between the two groups regarding BMI, duration of diabetes, BP, S.cr, eGFR or retinopathy.

As for HbA1c levels, in group I, it ranged from 6.50 – 11.0 % with a mean of 8.63 ± 1.23, whereas in group II it ranged from 7.0 – 11.0 % with a mean of 8.07 ± 0.93 , HbA1c level was significantly higher in group I compared to group II.

As for urinary L.FABP levels, in group I ranged 40.0 – 100.0 ng/ml with a mean of 81.27 ± 18.29, while in group II ranged 40.0 – 75.0 ng/ml with a mean of 49.24 ± 9.31. Urinary L-FABP levels were significantly higher in group I compared to group II.

Table (1): Comparison between both groups regarding different factors

	Group I (n = 100)	**Group II (n = 100)**	**Test of Sig.**	**p**
BMI (kg/m^2)				
Mean ± SD.	22.70 ± 2.88	23.14 ± 3.81	t= 0.922	0358
Median (Min. – Max.)	23.0 (18.0 – 29.0)	22.0 (18.0 – 33.0)		
Duration DM (years)				
Mean ± SD.	11.10 ± 3.23	11.66 ±3.79	t= 1.125	0.262
Median (Min. – Max.)	11.0 (4.0 – 18.0)	11.0 (6.0 –22.0)		
SBP (mmHg)				
Mean ± SD.	147.9 ± 11.68	153.5 ± 15.05	t= 2.939*	0.004*
Median (Min. – Max.)	150.0 (120.0 – 170.0)	155.0 (120.0 – 180.0)		
DBP (mmHg)				
Mean ± SD.	89.30 ± 8.99	90.80 ± 9.01	t= 1.179	0.240
Median (Min. – Max.)	90.0 (70.0 – 110.0)	90.0 (70.0 – 110.0)		
Serum creatinine (mg/dl)				
Mean ± SD.	1.69 ± 0.39	1.67 ± 0.37	t= 0.447	0.656
Median (Min. – Max.)	1.60 (1.20 – 3.0)	1.60 (1.20 – 3.0)		
HbA1c (%)				
Mean ± SD.	8.63 ± 1.23	8.07 ± 0.93	t= 3.621*	<0.001*
Median (Min. – Max.)	8.50 (6.50 – 11.0)	8.0 (7.0 – 11.0)		
eGFR (ml/min/1.73^2)				
Mean ± SD.	40.38 ± 13.64	43.72 ± 10.18	t= 1.962	0.051
Median (Min. – Max.)	45.0 (15.0 – 60.0)	45.0 (19.0 – 65.0)		
Retinopathy				
Absent	62 (62.0%)	49 (49.0%)	χ^2= 3.421	0.064
Present	38 (38.0%)	51 (51.0%)		
L.FABP (ng/ml)				

Mean ± SD.	81.27 ± 18.29	49.24 ± 9.31	t= 15.605*	<0.001*
Median (Min. – Max.)	89.0 (40.0 – 100.0)	48.0 (40.0 – 75.0)		

SD: **Standard deviation** **t: Student t-test** χ^2: **Chi square test**

p: p value for comparing between the studied groups

*: Statistically significant at p ≤ 0.05

Table (2) Showed correlation between urinary L-FABP levels and retinopathy among each group.

There was no clinically significant correlation between urinary L-FABP levels and presence of retinopathy among patients in group I, while urinary L-FABP level was significantly correlated with retinopathy in group II patients.

Table (2): Relation between retinopathy and urinary L.FABP (ng/ml) in each group

L.FABP (ng/ml)	Retinopathy		t	p
	Absent	Present		
Group I (n = 100)	**(n = 62)**	**(n = 38)**		
Mean ± SD.	79.48 ± 18.34	84.18 ± 18.08	1.251	0.214
Median (Min. – Max.)	89.0 (40.0 – 100.0)	97.0 (49.0 – 100.0)		
Group II (n = 100)	**(n = 46)**	**(n = 54)**		
Mean ± SD.	52.39 ± 10.30	46.56 ± 7.47	3.193*	0.002*
Median (Min. – Max.)	50.0 (40.0 – 75.0)	43.0 (40.0 – 70.0)		

SD: **Standard deviation** **t: Student t-test**

p: p value for association between different categories

*: Statistically significant at p ≤ 0.05

Table (3) Showed significant correlation between urinary L-FABP levels and HbA1c, BMI, S.cr, duration of DM and eGFR among group I patients, while urinary L-FABP level was significantly correlated only with HbA1c among group II patients.

Table (3) **Correlation between urinary L.FABP and different parameters in each group**

L.FABP (ng/ml) vs.	Group I (n = 100)		Group II (n = 100)	
	r	p	r	p
BMI (kg/m^2)	0.382	<0.001*	-0.104	0.305
Duration DM (years)	0.432	<0.001*	-0.179	0.074
SBP (mmHg)	-0.017	0.865	-0.054	0.596
DBP (mmHg)	-0.154	0.125	-0.122	0.228
Serum creatinin (mg/dl)	0.370	<0.001*	-0.064	0.526
HbA1c (%)	0.448	<0.001*	-0.205*	0.041*
eGFR (ml/min/1.73^2)	-0.482	<0.001*	0.159	0.114

r: Pearson coefficient

*: Statistically significant at p ≤ 0.05

Discussion

The hallmark of DKD was classically thought to be proteinuria and was considered to precede renal function decline.

However it has become clear now that a good proportion of patients with type 2 DM have renal function loss without proteinuria, known as nonproteinuric DKD. Unfortunately data regarding this type of DKD is limited.

In the present study there was no significant difference between the 2 groups regarding BMI, duration of diabetes ,blood pressure ,serum creatinine, retinopathy and estimated GFR as shown in table 1 .

Similar results were found by Kamijo et al [17] and Nielsen et al **[18]**. These findings also matches Masayuki Yamanouchi et al [13] , who conducted a study on groups of 82 non- proteinuric DKD and 164 proteinuric DKD patients, and they did not find significant difference regarding age , gender, duration of diabetes, BMI and retinopathy, but BP was better controlled among the non-proteinuric group.

Similar to the finding of Yamanouchi et al, the levels of HbA1c in the present study was higher significantly in non proteinuric diabetic patient than in proteinuric diabetic patients [13] but in their study the level of HbA1c was higher in non proteinuric diabetic patients but in their study it wasn't statistically significant.

According to guidelines, diagnosis of DN should be made by 2 out of 3 abnormal Urinary ACR measures in a morning urine sample or abnormal albumin level in 24-hour urine collection. [14] However, CKD can occur at normo-albuminuric levels. [15] Several studies suggest that microalbuminuria is less sensitive or specific than previously thought.

Tubulointerstitial injury contributes largely in DKD, so an available tubular injury marker will be useful in addition to albuminuria which is mainly considered as a marker of glomerular injury. LFABP is a protein expressed in the proximal tubules in the kidney. In this study our results revealed that urinary L-FABP levels were higher in group I (non-proteinuric patients) than in group II (proteinuric patients) with statistical significance.

Also in group 1 (non proteinuric) there was significant correlation between urinary L-FABP and BMI, serum creatinine, HbA1c and GFR.

Levels of urinary L-FABP were found to be suggestive of tubular injury in type 2 diabetics by study of Nakamura et al [16] . Urinary L-FABP levels were increased in patients with reduced eGFR and showed a significant correlation with protein to creatinine ratio [16]. While some studies explained the presence of non-proteinuric diabetic nephropathy with insignificant level of urinary L-FABP by well-preserved tubule leads to albumin reabsorption, thus reducing its excretion. And so, the presence of both glomerular and tubular injury is needed for proteinuria to be evident.[21] .

Both retinopathy and nephropathy are microvascular diabetic complications.

In the present study we looked for the relation between diabetic nephropathy (non- proteinuric ,proteinuric)and retinopathy, we found that presence of retinopathy in group II (proteinuric) was 51% and in group I (non- proteinuric) was 38% . However this difference was not statistically significant. Further study on large sample size is needed.

There was no clinically significant correlation between urinary L-FAB levels and presence of retinopathy among patients in group I (non proteinuric), while L-FAB level was significantly correlated with retinopathy in grouop II (protinuric patients) as shown in table 2. In agreement with these findings Penno,et al [19] found that a nonalbuminuric renal impairment was described in Type 2 diabetic patients, and are less well correlated with retinopathy and elevated blood pressure [19] . Conversely Rigalleau et al. [20] followed up 89 patients with diabetes and an estimated GFR < 60 mL/min for 38 ± 11 months. Of the subjects, 15 (17%) were normo-albuminuric. They were less affected by diabetic retinopathy.

The current study revealed significant correlation between urinary L-FABP levels and HBA1C ., BMI, S.cr, duration of DM and eGFR among group I patients (non proteinuric), while urinary L-FABP level was significantly negatively correlated only with HBA1C among group II patients (proteinuric) as seen in figure 1and 2 Our explanation to this finding is more glomerular affection in group II (presented by proteinurea) than tubular affection, further research including pathological study is needed to confirm these finding . This finding was in agreement with a prospective observational study of diabetic patients,[22] which demonstrated that urinary L-FABP levels increased with the advancement of diabetic nephropathy and the levels were higher in patients with normo-albuminuria than in the control subjects.

Conclusions

From these results, urinary L-FABP may be a marker of non- proteinuric DKD denoting the presence of tubular damage. Its role in early detection and predicting progression needs further studies.

Figures

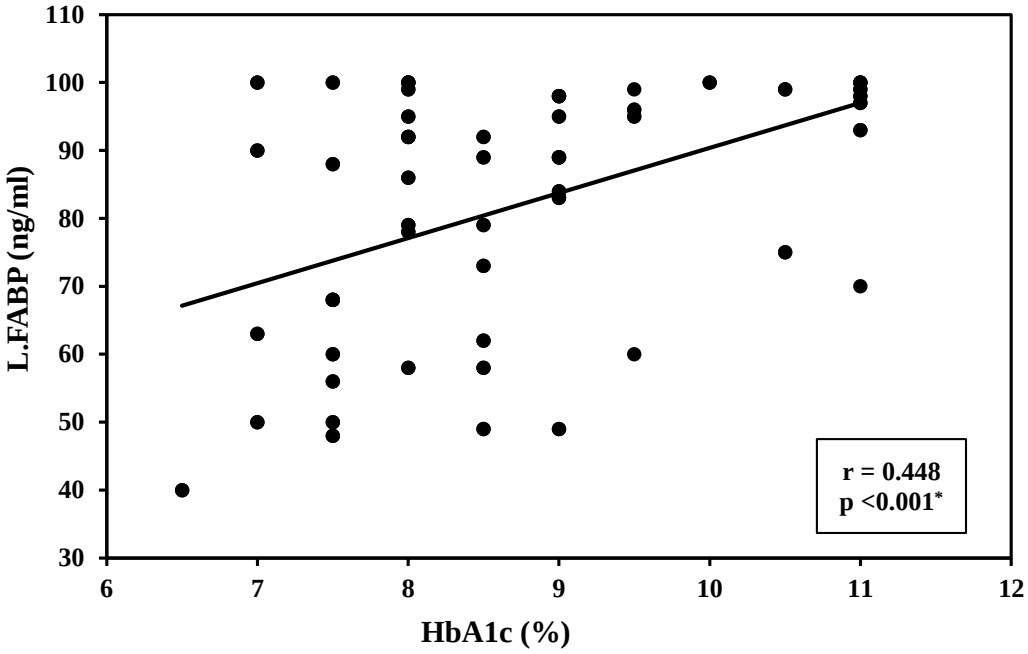

Figure (1): Correlation between L.FABP and HbA1c in group I

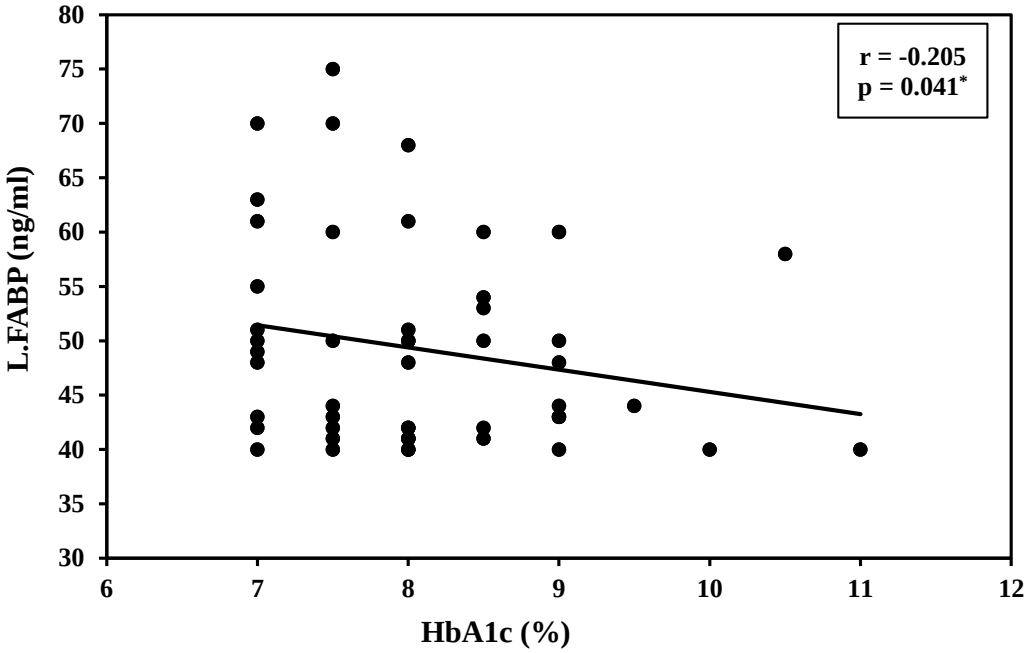

Figure (2): Correlation between L.FABP and HbA1c in group II

The present study also find a statistical negative correlation between urinary L-FABP and eGFR as seen in figure 3

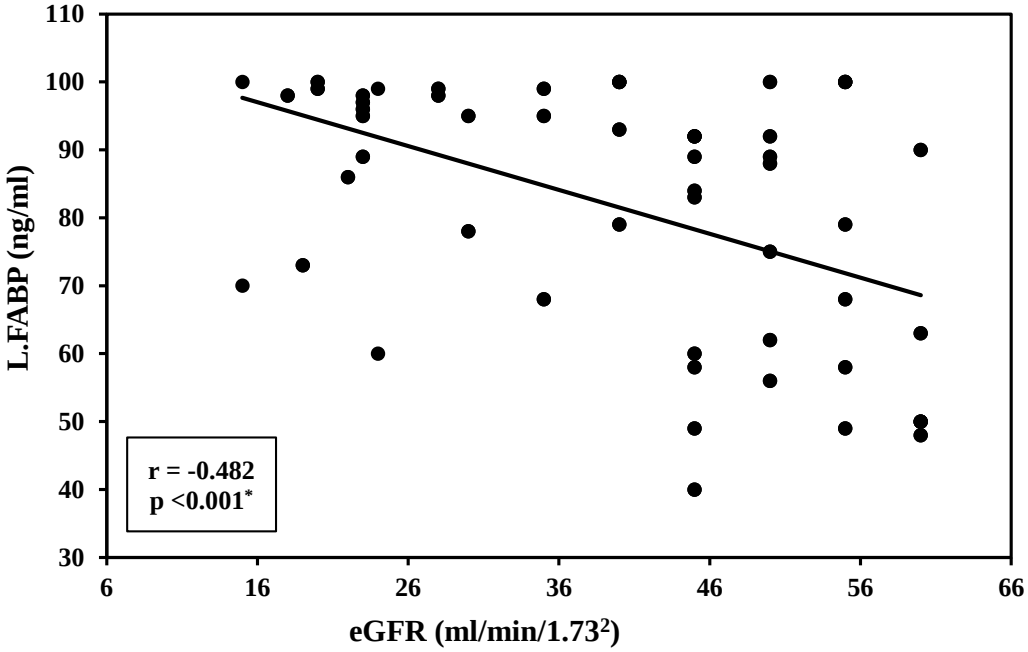

Figure (3): Correlation between L.FABP and eGFR in group I

Conclusion and recommendations

-Cumulative evidence has shown that renal insufficiency in type 2 diabetic patients may co-exist within a normal range of protein excretion.

-This new phenotype is quite frequent, and have distinct pathological pathway.

- Albuminuria is believed to be the best marker of diabetic nephropathy till now

- However, tubular markers such as urinary L–FABP seem promising markers for early detection of renal disease.

- Further studies are needed to confirm the possible use of these markers either alone or in association with albuminuria.

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