Evaluation of Liver Type Fatty Acid Binding Protein (L-FABP) as a Biomarker in Patients with Diabetic Nephropathy

El-Saeid, M. E. El-Bawab[1], Abdelzaher M.H[2], Ashraf Abo Kebir[3], Hussein M. Eldeeb[4a,b]

[1,2,4a,] Department of Biochemistry, Faculty of Medicine, Al-Azhar University, Assuit, Egypt
[3]Department of Internal Medicine, Faculty of Medicine, Al-Azhar University, Assuit, Egypt
[4b] Medical Biochemistry Department Collage of pharmacy, Qassim university, Buraidah Saudi Arabia
Corresponding author. E-mail address: eldeebhussein40@yahoomail.com (Hussein M. Eldeeb).
Current work: Collage of pharmacy, Qassim university, P.O. Box 6880, 51452 Buraidah, Saudi Arabia.
husseineldeeb@qumed.edu.sa, Cell number: +966547387065

ABSTRACT: Background: Liver-type fatty-acid binding protein (L-FABP) is a 14 kDa small molecule that is expressed in the cytoplasm of human proximal tubules. 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 diabetic nephropathy. The aim of present study is to investigate the urinary level of L-FABP as biochemical marker that can determine the severity of tissue injury resulting from diabetic nephropathy. **Material and methods:** This study was carried out on 86 patients and classified into four groups; Control group, Normal albuminuria, Micro-albuminuria and Macro-albuminuria. We assessed urinary levels of LFABP, urinary albumin, urinary creatinine, Albumin/creatinine ratio, serum creatinine, urea, HbA1c and eGFR. **Results:** We found an increase in the level of urinary L-FABP and positive correlation with the progression of kidney disease. Also we found that significant positive correlation between L-FABP and both HbA1c ($P < 0.05$) and urinary albumin. The results of the present study showed that by ROC curve, urinary L-FABP is a significant marker in diagnosing of microalbuminuric group (AUC=0.62), ($P<0.001$) and macroalbuminuric group (AUC=0.80), ($P<0.001$). **Conclusion:** Measurement of L-FABP in urine provides a marker for the severity of diabetic nephropathy but not considered as an early marker of diabetic nephropathy.
[El-Saeid, M. E. El-Bawab, Abdelzaher M.H, Ashraf Abo Kebir, Hussein M. Eldeeb. **Evaluation of Liver Type Fatty Acid Binding Protein (L-FABP) as a Biomarker in Patients with Diabetic Nephropathy.** *Researcher* 2017;9(9):44-50]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). http://www.sciencepub.net/researcher. 7. doi:10.7537/marsrsj090917.07.

Keywords: Liver-type fatty-acid binding protein (L-FABP) - Diabetic nephropathy

1-Introduction

Fatty acid-binding proteins (FABPs) are a class of cytoplasmic proteins that bind long chain fatty acids. FABPs are small intracellular proteins (~13-14 kDa) with a high degree of tissue specificity. They are abundantly present in various cell types and play an important role in the intracellular utilization of fatty acids, transport and metabolism [1]. 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. 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 [2,3]. High levels of the tubular inflammation marker u-LFABP predict the initiation and progression to diabetic nephropathy. Also, L-FABP is an independent predictor of progression of DN irrespective of disease stage [4,5].

The aim of the current study is to evaluate urinary Liver FABP as a biomarker in Diabetic nephropathy.

2. Materials and methods

This study was conducted at the internal medicine and Gastroentrology departments of Alazhar Assuit University Hospitals. It carried out on 66 type 2 diabetic patients and 20 apparently healthy volunteers working as controls from March to October 2015 after approval of the ethical committee of Al-Azhar University Hospitals. **Subjects under study were classified into the following groups**: **Control Group (n=20):** It included 20 apparently healthy volunteers selected from those working in auxillary jobs in Alazhar Assuit University Hospital, (10 males and 10 females) with age range from 39-60 years. **Patients' Group (n-66):** This group included 66 patients with type 2 DM under control (35males and 31 females).

The diagnosis was based on the ADA criteria for diagnosis of DM [6]**.** Patients were further classified according to their albumin in urine into three groups: group I; normoalbuminuric diabetic group, group II; microalbuminuric diabetic group and group III; macroalbuminuric diabetic group. **Group I: (Normoalbuminuric diabetic group):** It included 6 type 2 diabetic patients with normoalbuminuria (4males and 2 females), with age range from 44-61years. The albumin in urine in these patients was less than 30 mg/L. **Group II; (Microalbuminuric diabetic group):** It included 53 type 2 diabetic patients with microalbuminuria (26males and 27 females), with age range from 55-70 years. The albumin in urine in these patients was 30 – 300 mg/L. **Group III; (Macroalbuminuric diabetic group**): It included7 type 2 diabetic patients with macro albuminuria (5males and 2 females), with age range from 53-77 years. The albumin in urine in these patients was more than 300 mg/L

Inclusion criteria: All patients were Type 2 diabetes.

Exclusion criteria: Hypertensive patients under treatment with ACEI or ARBS, patients with cancer, infection, patients with any inflammatory conditions, patients with liver cirrhosis or impairment and patients with primary kidney disease

The patients were subjected to: Full history (age, sex and duration of the disease), Clinical examination and samples were collected and prepared as follow; Seven milliliters (7 mL) of venous blood were withdrawn under complete aseptic conditions from each of controls and patients, divided into two parts, two milliliters (2 mL) of venous blood were collected into an EDTA tube for glycated hemoglobin. The other part (5 ml) were collected in plain tube and left to clot. Serum was separated by centrifugation at 1200 rpm for 10 minutes. The separated serum was used for immediate estimation of, blood urea (colorimetric method), creatinine (kinetic method) and HbA1C (Ion Exchange Resin method). Ten milliliters (10 mL) of first morning urine sample without effort were collected. Centrifuged urine divided into two parts one for immediate estimation of creatinine and microalbumin (ELISA method). The other part was stored at -20 ºC for subsequent assay of (L-FABP) Liver type fatty acid binding protein (ELISA method). Estimation of GFR was done by The CKD-EPI Creatinine Equation.

Statistical analysis

Data were expressed as Mean ± SD, for normally distributed data, comparison between two independent population were done using independent t-test while more than two population were analyzed F-test (ANOVA) to be used. Comparison between different groups regarding categorical variables was tested using Chi-square test. Correlations were performed using person correlation. P.value considered insignificant if it > 0.05, significant if it ≤ 0.05, highly significant if it ≤0.01 and very highly significant if it ≤0.001. The statistical analysis were done using SPSS version 20.0. program.

Results

The level of urinary L-FABP is significantly increased in group II and III in comparison with control group and groupI (*p<0.05*)

Table (1): Comparison between patients subgroup regarding L-FABP.

	Control group	Group I Normalbuminuria	Group II Microalbuminuria	Group III Macroalbuminuria
L-FABP Range Mean S.D.	 429.6 - 925.4 741.04 142.07	 673.7-820.0 751.6 72.2	 701-925 824.6 70.6	 902.2-1047.2 980.6 62.4
F test p	16.65 0.011*			
P1 P2 P3		0.103	0.002* 0.046*	0.001* 0.002* 0.038*

P1 comparison between control group and other groups., P2 comparison between group I and both II and III, P3 comparison between group II and III.

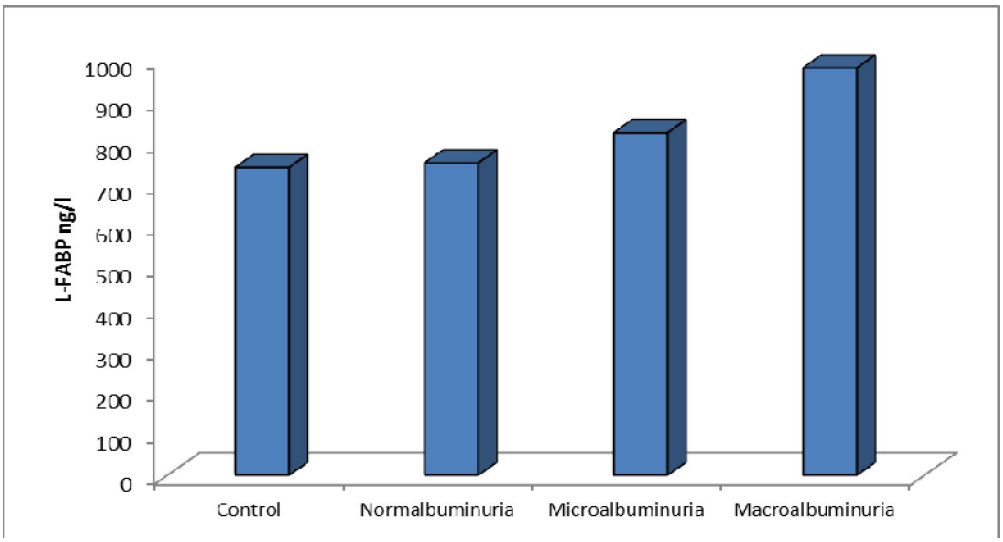

Figure (1): Comparison between patients subgroup regarding L-FABP.

Albumin/creatinine ratio is significantly increased in group II and III in comparison with either control group or group I *(p<0.05)*

Table (2): Comparison between patients subgroup regarding albumin/creatinine ratio.

	Group I Normalbuminuria	Group II Microalbuminuria	Group III Macroalbuminuria
alb/creatinine ratio Mean S.D.	27.07 8.325	105.07 52.76	199.77 85.11
F test P	22.52 0.0001*		
P1 P2		0.0001*	0.0016* 0.003*

P1 comparison between group I and both II and III - P2 comparison between group II and III.

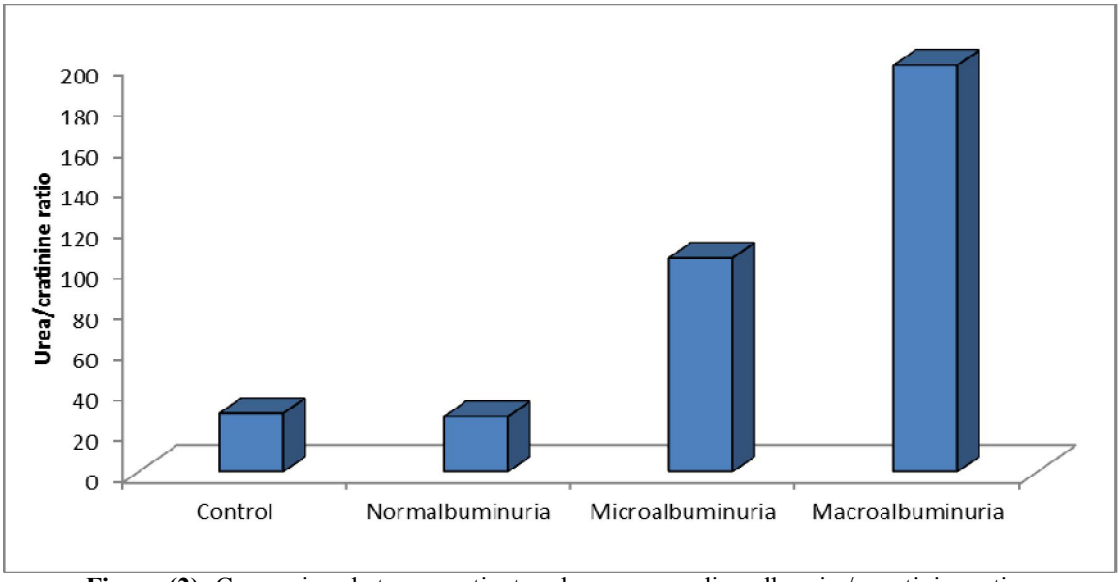

Figure (2): Comparison between patients subgroup regarding albumin / creatinine ratio.

L-FABP showed a positive correlation with the level of microalbumin in urine *(r: 0.460, P value: 0.0042)*. Also, there was a positive correlation between L-FABP and the level of HbA1C *(r: 0.425, P value: 0.0001)*. On the other hand, there was no significant correlation between L-FABP and both of eGFR and ACR.

Table (3): Correlation between L-FABP and other variables.

L-FABP	r	p
Urea	-0.019-	0.859
Creat	-0.114-	0.297
HbA1c	0.425	0.0001**
u. Albumin	0.460	0.0042**
A.C.R (Albumin/creatinine ratio)	0.167	0.125
e.GFR	0.085	0.437

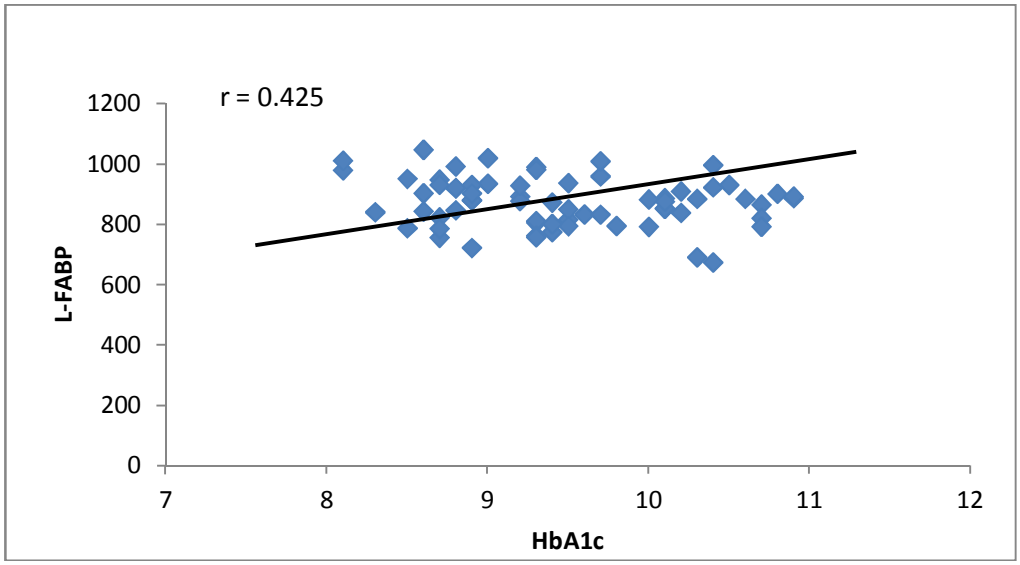

Figure (3): Correlation between L-FABP and HbA1c.

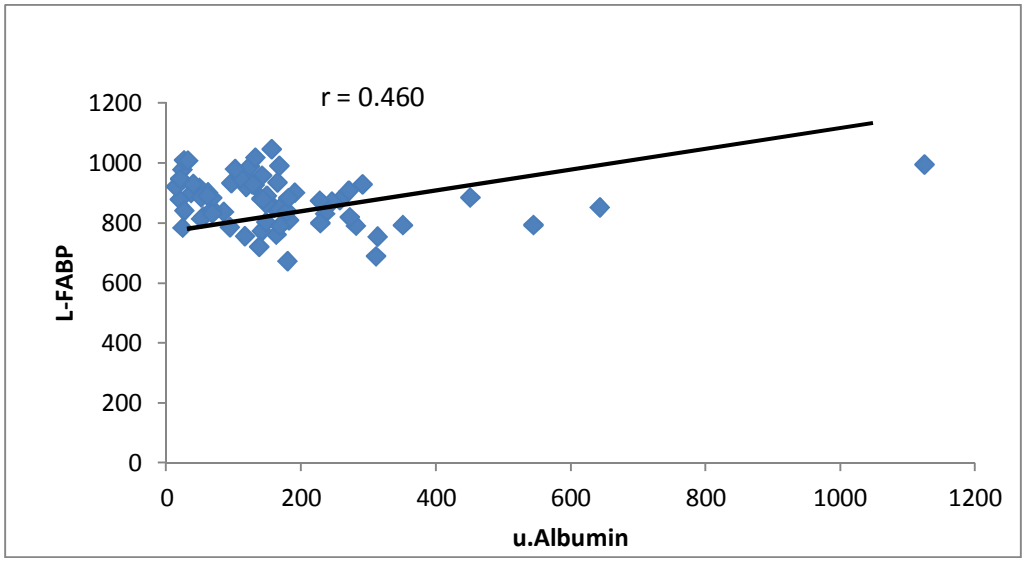

Figure (4): Correlation between L-FABP and u. Albumin.

ROC curve to determine the cut off value, sensitivity and specificity of L-FABP in detection of the disease.

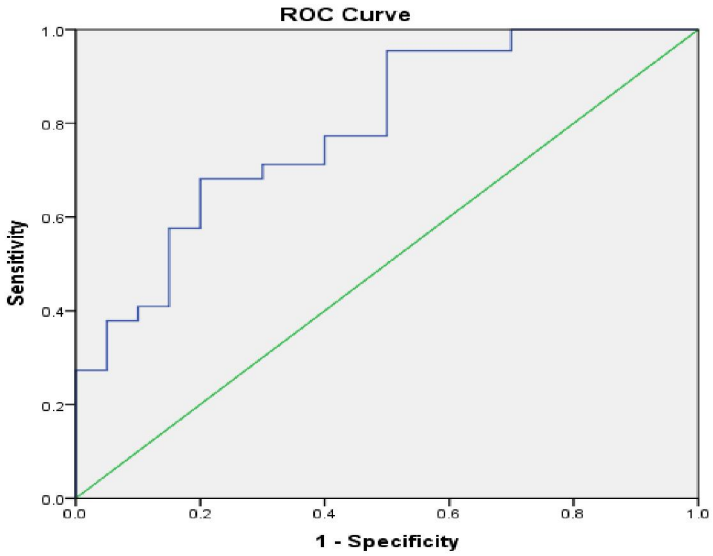

Figure (5): ROC curve to determine the cut off value, sensitivity and specificity of L-FABP in detection of the disease.

As shown in table 4 it was found that the cut off value of L-FABP to diagnose the disease was 802.65, the sensitivity at this point was 80.0% and specificity was 62.0%.

Table.4: Area Under ROC curve to determine the cut off value, sensitivity and specificity of L-FABP in detection of the disease.

Area Under the Curve

Test Result Variable (s): L-FABP

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.789	0.058	0.000	0.677	0.902

Coordinates of the Curve

Test Result Variable (s): L-FABP

Cut off value	Sensitivity	1 - Specificity
802.6500	80.0	62.0

Discussion

Diabetic nephropathy (DN) is one of the important microvascular complications of diabetes mellitus. It is responsible for 25% of cases of uremia [7]. Microalbuminuria is a predictive factor for cardiovascular events and nephropathy in type II diabetics [8]. It develops over a series of phases from microalbuminuria and macroalbuminuria to progressive decline in glomerular filtration rate. This ultimately results in renal failure. Duration of diabetes mellitus, poor glycemic control and hypertension are the main risk factors for DN [9]. Urinary L-FABP may be a useful clinical biomarker for monitoring chronic glomerular disease and reflect the clinical prognosis of chronic kidney disease [10]. The progression from micro-to macroalbuminuria (overt nephropathy) is associated with several risk factors including: elevation of urinary albumin excretion, poor glycemic control, genetic factors, and long duration of diabetes [11]. Microalbuminuria is an early clinical marker for diabetic nephropathy, which is associated with disease progression to end-stage renal disease and cardiovascular events [12].

The present study found that there was increase in the level of urinary L-FABP (ng/l.) in macroalbuminuric diabetic patients group compared to microalbuminuric diabetic patients group, normoalbuminuric diabetic patients group and in control group. There was increase in the level of urinary L-FABP (ng/l.) in microalbuminuric diabetic patients group compared to normoalbuminuric diabetic patients group and control group. These results were in consistent with the results obtained from previous studies [13,14,15]. Another study on 356 patients with type 2 diabetes reported that in patients with clinical albuminuria and renal failure, urinary L-FABP was significantly increased compared with patients with normo- and microalbuminuria [16]. In agreement with

our study, another study found that urinary L-FABP level was significantly correlated with urinary albumin level in all of the patients [17]. It has been concluded that L-FABP have been established as a promising biomarker in chronic kidney disease [18]. Moreover, another study noticed that L-FABP may be a suitable biomarker of the progression of chronic kidney disease and tubular ischemia [19]. Sasaki et al. (2009) [20] found that urinary levels of L-FABP (expressed in μg/g creatinine) were significantly higher in diabetic nephropathy. Yamamoto et al. (2007) [21] reported that the urinary levels of L-FABP increased in proteinuric and/or tubular ischemic human kidney diseases. Najafian et al. (2011) [22] demonstrated that urinary tubular damage markers, such as KIM-1, NGAL and L-FABP, may have the potential to be clinical markers for identifying the development or progression of diabetic nephropathy. However, Kim et al. (2012) [23] showed that the urinary tubular marker L-FABP, were not significantly increased in the normoalbuminuria and microalbuminuria groups, compared to the normal control group.

The results of the present study showed that by ROC curve, urinary L-FABP is a highly significant marker in diagnosing of microalbuminuric group (AUC=0.62), (P<0.001) and macroalbuminuric group (AUC=0.80), (P<0.001). So it is suggested that urinary L-FABP is a prognostic and a predictive marker for overt diabetic nephropathy.

Our results showed that there was a positive correlation between L-FABP and the level of microalbumin in urine (r: 0.460, P value: 0.0042). Also, there was a positive correlation between L-FABP and the level of HbA1C (r: 0.425, P value: 0.0001). On the other hand, there was no significant correlation between L-FABP and both of eGFR and ACR. While Kamijo et al. (2011) [17] reported that higher urinary L-FABP levels were associated with the progression of GFR to <60 mL/min/1.73 m2. Nielsen et al. (2011) [24] reported that urinary L-FABP levels are not related to a rapid decline in GFR in a 3-year intervention study on 63 type 1diabetic patients with overt proteinuria.

Finally, our results clearly demonstrate that, urinary L-FABP is a marker of the severity of diabetic nephropathy but **not** considered as an early marker of diabetic nephropathy.

4. Conclusion

The current study found that the level of urinary L-FABP reflected the severity of diabetic nephropathy. In addition to urinary albumin, the measurement of L-FABP in urine provides a biomarker for detection of diabetic nephropathy but it is not suitable for early detection.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

This study was carried out at Medical Biochemistry and Internal Medicine Departments, Alazhar University and during the summer months (May, June and July) of 2016. Written informed consent was taken from each participants and this study was approved from the ethics committee of Faculty of Medicine, Alazhar University, Assuit, Egypt.

References

1. Rosman J, Kavala G, Obunai K and Bergmann SR, (2009) The role of heart-type fatty acid-binding protein in the diagnosis of acute coronary syndrome. Int J Angiol. 2009 Summer;18(2):79-81.
2. Kamijo-Ikemori A1, Sugaya T, Ichikawa D, Hoshino S, Matsui K, Yokoyama T, Yasuda T, Hirata K and, Kimura K, (2013). Urinary liver type fatty acid binding protein in diabetic nephropathy. Clin Chim Acta. 2013 Sep 23;424:104-8. doi: 10.1016/j.cca.2013.05.020. Epub 2013 May 30.
3. Kim SS, Song SH, Kim IJ, Kim WJ, Jeon YK, Kim BH, Kwak IS, Lee EK and Kim YK, (2014). Nonalbuminuric proteinuria as a biomarker for tubular damage in early development of nephropathy with type 2 diabetic patients. Diabetes Metab Res Rev. 2014 Nov;30(8):736-41. doi: 10.1002/dmrr.2546.
4. Nielsen SE, Sugaya T, Hovind P, Baba T, Parving HH and Rossing P, (2010). Urinary liver-type fatty acid-binding protein predicts progression to nephropathy in type 1 diabetic patients. Diabetes Care. 2010 Jun;33(6):1320-4. doi: 10.2337/dc09-2242. Epub 2010 Feb 25.
5. Nicolae M. Panduru, Carol Forsblom, Markku Saraheimo, Lena Thorn, Angelika Bierhaus, Per M. Humpert, and Per-Henrik Groop, (2013). Urinary Liver-Type Fatty Acid–Binding Protein and Progression of Diabetic Nephropathy in Type 1 Diabetes. Diabetes Care. 2013 Jul; 36(7): 2077–2083.
6. American Diabetes Association (ADA), (2012): Standards of medical care in diabetes. Diabetes Care. 35: 11–63.
7. Alam I, (2008). Diabetic nephropathy. J Postgrad Med Inst; 11(2): 124-128.
8. De-Zeeuw D, Remuzi G, Parving HH, et al., (2008). Albuminuria, a therapeutic target for cardiovascular protection in type II diabetic

patients with nephropathy. Circulation;110: 921-3.
9. Muzafar R, Qasmi R, Tahir Z, et al., (2010). Microalbuminuria and early diagnosis of insulin dependent and non-insulin dependent diabetes mellitus. Biomedica: 19:60-7.
10. Kamijo A, Sugaya T, Kimura K, et al., (2006). Urinary fatty acid binding protein in renal disease. Clinica Chimica Acta; 374: 1–7.
11. Defronzo RA, (1997). Diabetic nephropathy: etiologic and therapeutic consideration. Diabetes Rev.;3:510.
12. Ninomiya T, Perkovic V, de Galan BE, et al., (2009). Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol 20: 1813–1821.
13. Nakamura T, Sugaya T, Kawagoe Y, et al., (2005). Effect of pitavastatin on urinary liver-type fatty acid-binding protein levels in patients with early diabetic nephropathy. Diabetes Care;28:2728–2732.
14. Araki S, Haneda M, Koya D, et al., (2013). Predictive effects of urinary liver-type fatty acid binding protein for deteriorating renal function and incidence of cardiovascular disease in type 2 diabetic patients without advanced nephropathy. Diabetes Care;36:1248–53.
15. Kamijo A, Sugaya T, Ichikawa D, et al., (2013). Urinary liver type fatty acid binding protein in diabetic nephropathy, Clinica Chimica Acta 424, 104–108.
16. Suzuki K, Babazono T, Murata H, et al., (2005). Clinical significance of urinary liver-type fatty acid-binding protein in patients with diabetic nephropathy. Diabetes Care X; 28: 2038–2039.
17. Kamijo A, Sugaya T, Yasuda T, et al., (2011). Clinical significance of urinary liver-type fatty acid-binding protein in diabetic nephropathy of type 2 diabetic patients. Diabetes Care;34:691–696.
18. Nakamura T, Inoue T, Sugaya T, et al., (2007). Beneficial effects of olmesartan and temocapril on urinary liver-type fatty acidbinding protein levels in normotensive patients with immunoglobin A nephropathy. Am J Hypertens 20: 1195–1201.
19. Ferguson MA, Vaidya VS, Waikar SS, et al., (2010). Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. Kidney Int; 77:708–714pmid:19940842.
20. Sasaki H, Kamijo A, Sugaya T, et al., (2009). Urinary fatty acids and liver-type fatty acid binding protein in diabetic nephropathy. Nephron Clin Pract;112: c148–c156.
21. Yamamoto T, Noiri E, Ono Y, et al., (2007). Renal L-type fatty acid—binding protein in acute ischemic injury. J Am Soc Nephrol; 18:2894–2902.
22. Najafian B, Alpers CE, Fogo AB, et al., (2011). Pathology of human diabetic nephropathy. Contrib Nephrol 170: 36–47.
23. Kim SS, Song SH, Kim IJ, et al., (2012). Clinical implication ofurinary tubular markers in the early stage of nephropathy with type 2 diabetic patients. Diabetes Res Clin Pract 97: 251–257.
24. Nielsen SE, Andersen S, Zdunek D, et al., (2011). Tubular markers do not predict the decline in glomerular filtration rate in type 1 diabetic patients with overt nephropathy. Kidney Int 79: 1113–1118.

9/25/2017