

Research Article

Evaluation of Urinary Liver-Type Fatty Acid Binding Protein As a Marker for Monitoring Type 2 Diabetic Nephropathy

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Abstract: Background: Diabetic nephropathy (DN) is one of the most important microvascular complications associated with type 2 diabetic patients. It is known that tubulointerstitial damage plays an important role in diabetic nephropathy. Therefore; it would potentially be beneficial if albuminuria, as a marker of glomerular damage, could be supplemented by a marker of tubular damage to provide a more complete status of the kidney injury. Liver-type fatty acid-binding protein (LFABP) is an intracellular carrier protein that is expressed in the proximal tubules in the human kidney and participates in fatty acid metabolism.

The present study aimed to evaluate the relation between the u-LFABP and some biochemical parameters to find their association with the occurrence of diabetic nephropathy. The study included 60 patients were divided into 3 groups diagnosed with type 2 diabetes ($n=20/\text{group}$): group I: normoalbuminuria (ACR <30), group II: microalbuminuria (ACR <30-300), group III: macroalbuminuria (ACR >300) and control subjects comprised 20 non-diabetic healthy, age-matched subjects as the control group. All of the patients underwent a standardized laboratory evaluation including fasting and postprandial serum levels of glucose, glycated hemoglobin (HbA1c), kidney function, lipid profile, non-esterified fatty acids (NEFA) and serum and urinary levels of Liver-type Fatty Acid Binding Protein (L-FABP).

Our results revealed that UL-FABP levels were significantly higher in group I versus the control group and significantly increased according to the severity of diabetic nephropathy. Urinary L-FABP levels showed positive significant correlation with triglycerides and HDL-C, N-EFA and ACR and a negative significant correlation with age, duration of diabetes, HbA1c, serum creatinine, LDL-C, serum total cholesterol and systolic and diastolic blood. Urinary L-FABP provides a suitable biomarker for the early detection and monitoring of progression of diabetic nephropathy in clinical practice.

Keywords: Liver-type fatty acid-binding protein (LFABP), non-esterified fatty acids (NEFA), glycated hemoglobin (HbA1c), albumin creatinine ratio (ACR).

Introduction

Diabetes mellitus is a metabolic disorder of multiple causes which is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism. Long-term hyperglycemia can lead to severe complications later in life. These complications of diabetes are divided into macrovascular and microvascular complications.⁽¹⁾ Diabetic nephropathy (DN) is one of the

most important microvascular complications associated with type 2 diabetic patients and has emerged as a leading cause of the end-stage renal disease in developed countries. DN is characterized by structural abnormalities of kidney including hypertrophy of both glomerular and tubular elements, increase in the thickness of glomerular basement membranes, and progressive accumulation of extracellular matrix components, eventually leading to proteinuria and renal failure.^(2,3)

It is known that tubulointerstitial damage plays an important role in diabetic nephropathy. Therefore, it would potentially be beneficial if albuminuria, as a marker of glomerular damage, could be supplemented by a marker of tubular damage to provide a more complete status of the kidney injury. This could help us first to more accurately predict the patients at risk of developing diabetic nephropathy and second to provide a better and possibly different treatment for diabetic nephropathy.⁽⁴⁾

Liver-type fatty acid-binding protein (LFABP) is an intracellular carrier protein that is expressed in the proximal tubules in the human kidney and participates in fatty acid metabolism. In clinical study, urinary excretion of L-FABP was reported to offer potential as a clinical marker to screen for kidney dysfunction and thereby to identify patients who are likely to experience deterioration of renal function in the future^(5,6). The interest for the use of biomarkers for early DN derives from the observation that patients with type 2 diabetes pass through a period of pre-diabetes and may experience renal impairment at the time of diagnosis. Although microalbuminuria has been considered as the earliest marker of DN in clinical practice, 29.1–61.6 % of individuals with type 2 diabetes could have renal impairment even before the onset of microalbuminuria, the gold standard for early diagnosis⁽⁷⁾. Therefore, it is important to implement different strategies for earlier detection of DN aiming to prevent the long term devastating outcomes of renal loss in diabetic.

The present study aimed to evaluate the relation between the urinary Liver- Type Fatty Acid-Binding Protein (u-LFABP) and some biochemical parameters in a trial to find their association with the occurrence of diabetic nephropathy.

Subjects and Methods

The present study were conducted on 60 diabetic subjects categorized as follows: Group I: 20 diabetic patients with normoalbuminuria (ACR <30). **Group II:** 20 diabetic patients with microalbuminuria (ACR <30-300). **Group III:** 20 diabetic patients with macroalbuminuria (ACR >300). and 20 non-diabetic healthy age-matched subjects as the control group. Full clinical examination with special stress on history of the onset of diabetes mellitus and blood pressure measurement was done to all the enrolled subjects.

Sampling:

Blood Samples

After an overnight fast, blood samples were taken, centrifuged (15,000 rpm for 15 minutes) and Serum was separated. 2-h post prandial blood samples were also collected for glucose estimation.

Urine Samples

After an overnight fast, early morning first void sterile urine samples were obtained from all subjects, centrifuged to remove cellular components. The supernatant was aliquoted in 1.8 ml eppendorf tubes and frozen within 2 h of collection at -20°C .

Methods

I. Biochemical Parameters:

1. Determination of Renal Functions: serum level of urea,⁽⁸⁾ serum and urinary creatinine,⁽⁹⁾ urinary albumin,⁽¹⁰⁾ serum uric acid⁽¹¹⁾
2. Determination of Glycemic Status: serum glucose level,⁽¹²⁻¹⁵⁾ glycosylated hemoglobin A1c.⁽¹³⁾
3. Estimation of serum nonesterified fatty acid concentration (NEFA)⁽¹⁴⁾
4. Determination of serum lipid profile: total cholesterol,⁽¹⁵⁾ triglycerides⁽¹⁶⁾ high density lipoprotein (HDL-Cholesterol),⁽¹⁷⁾ low density lipoprotein (LDL-Cholesterol).⁽¹⁸⁾
5. Estimation of Serum and urinary L-FABP :⁽¹⁹⁾ by using ELISA kit (HK404, Hycult Biotechnology, Uden, The Netherlands). Urinary L-FABP levels were normalized to urinary creatinine.

Results

The mean values of all studied biochemical parameters \pm SD are illustrated in table I. Statistical analysis revealed

significant increase in mean glucose levels among different groups ($P < 0.05$) as compared to normal control group.

Similarly, studied groups showed a significant elevated glycated haemoglobin levels along with fasting and post prandial glucose values ($p < 0.05$) as compared to normal control group. The mean values of NEFA displayed a pronounced elevation in all studied groups. Statistical analysis revealed significant increase in mean NEFA levels among different groups as compared to normal control group ($F = 12.88$, $P = 0.001$). There were gradual increase in the mean levels of cholesterol, triglycerides and LDL-C. The mean differences among different group were found to be statistically significant ($P = 0.0012$, $P = 0.001$ and $P = 0.001$ for cholesterol, triglycerides and LDL-C respectively). In contrast, the mean levels of HDL-C showed gradual decrease in groups I, II and III, as compared with control group.

Statistical analysis of cholesterol showed no significant differences between controls and groups I, however there was significant differences between controls and groups II and III, moreover there was significant differences between groups II and III ($P < 0.05$). Both triglycerides and LDL-C showed significant difference among controls and groups II and III ($P < 0.05$), HDL-C showed significant differences between controls and groups I, II, and III. ($P = 0.033$).

Statistical analysis showed significant increase in the means values of ACR in studied group I, II and III as compared to normal control group ($P < 0.05$).

Statistical analysis showed statistically significant increase for both urea and creatinine among different groups as compared to normal control group ($P = 0.0001$).

Also, serum uric acid exhibited similar trend showing significant increase in mean levels among different groups as compared to normal control group ($P = 0.0013$). Serum L-FABP was progressively increased in controls, groups I, II and III, respectively. Statistical analysis showed statistically significant difference in patient groups as compared to normal control ($P < 0.05$). Urinary L-FABP was progressively increased with the progression of diabetic nephropathy in controls, groups I, II and III, respectively.

Statistical analysis revealed that Urinary levels of L-FABP in patients with normoalbuminuria were significantly higher as compared to normal control subjects ($P < 0.05$). Moreover, the levels of urinary L-FABP in each diabetic nephropathy group were significantly different from the levels in all of the other groups and significantly increased according to the severity of diabetic nephropathy ($P < 0.05$).

The results also indicated significant positive correlation between serum and urinary L-FABP (figure 1), between urinary L-FABP and Non Esterified Free Fatty acids ($r = 0.704$, $p = 0.0001$) (figure 2). There was a significant positive correlation between HbA1c and ACR (figure 3). There were a significant positive correlation between Non Esterified Free Fatty acids and lipid profile ($r = 0.539$ $p = 0.001$, $r = 0.485$ $p = 0.001$, $r = 0.477$ $p = 0.001$) for T Ch (figure 4), TG, LDL respectively on the other hand Non

Esterified Free Fatty acids was negatively correlated with HDL ($r = -0.32$, $p = 0.003$).

Discussion

In massive proteinuria, there is an overload of free fatty acids (FFAs) bound to albumin in the proximal tubules. Following accumulation of FFAs in the proximal tubules, they become bound to cytoplasmic fatty acid-binding protein (FABP) and are then metabolized. Thus, L-FABP could potentially be useful in preventing FFA-induced tubulointerstitial cell damage⁽²⁰⁾ L-FABP can also transport FFAs from the cytosol to the nucleus and then interacts with the nuclear protein peroxisome proliferator-activated receptors (PPAR), which is a nuclear target of FFAs and initiates the gene expression of enzymes involved in lipid metabolism.⁽²¹⁾

In the present study significant elevation ($p > 0.05$) of Serum L-FABP (SL-FABP) was noticed in nephropathy groups, as compared to control and normoalbuminuric diabetic patients. Moreover, U-LFABP levels were significantly higher in the normoalbuminuric group versus the control group and increased with increasing levels of albuminuria (microalbuminuric group and macroalbuminuric) ($p < 0.05$).

This finding was in agreement with early published study⁽²²⁾, which demonstrated that urinary L-FABP levels increased with the progression of diabetic nephropathy in patients with type 1 diabetes and the levels were higher in patients with normoalbuminuria than in the control subjects. Moreover, in a prospective observational study of patients with diabetes, urinary L-FABP was reported to be an independent predictor for the progression of diabetic nephropathy. From these results, urinary L-FABP may be useful for early detection and may be a predictor for the progression of diabetic nephropathy.⁽²²⁾

Urinary L-FABP was shown to reflect the progression rate of chronic kidney disease including minimal change nephrotic syndrome, nephrosclerosis and lupus nephritis, as determined by significantly higher L-FABP levels in patients with deteriorating renal function as opposed to low levels in those with stable renal function. In the present study, urinary L-FABP levels increased in relation to the deterioration of kidney function, a phenomenon that was not observed in urinary protein levels. Urinary L-FABP was demonstrated to be more sensitive than urinary protein in predicting the progression of chronic kidney disease (CKD).⁽²³⁾

Our findings were in alignment with previous studies⁽²⁴⁾ regarding the relationship between tubulointerstitial damage and urinary L-FABP, the degree of tubulointerstitial damage quantified in renal tissue obtained from renal biopsies was shown to correlate with urinary excretion of L-FABP.⁽²⁴⁾ In a recent experimental study in transgenic mice, it has been shown that u-LFABP accurately reflects the degree of tubulointerstitial damage and is dynamic as a measure.⁽²⁵⁾ These results highlight the usefulness of

urinary L-FABP as a more appropriate clinical marker in the monitoring of CKD progression than urinary protein, thus, endorsing urinary L-FABP as a candidate target in the management of CKD.⁽²⁵⁾ With respect to acute kidney disease (AKI), urinary L-FABP facilitates the early detection of AKI before an increase in serum creatinine⁽²⁶⁾

In the present study, correlation analysis demonstrated that Serum L-FABP was positively correlated with urinary L-FABP in all studied groups. This was motivated by a report in an experimental model which showed that, under normal physiological conditions, L-FABP derived from liver is released into the circulation, filtered through glomeruli and reabsorbed into the proximal tubule. In renal disease however, tubulointerstitial damage reduces proximal tubular re-absorption of L-FABP, and leads to an increased level of urinary L-FABP. These results suggested that serum L-FABP levels do not influence urinary L-FABP levels. Moreover these observations support the concept that the human urinary L-FABP level is mostly determined by proximal tubule injury.⁽²⁷⁾ Recent study suggested that elevated levels of urinary L-FABP are evident from the microalbuminuric stage indicating tubular damage in type 2 diabetic patients. Urinary L-FABP levels were elevated in patients with reduced eGFR and showed a positive correlation with protein to creatinine ratio⁽²⁸⁾.

Concerning the relation of u-LFABP to age and duration of diabetes. As indicated in the present study, no significant correlation was found between urinary L-FABP and age of the study population. Similar result were found by Kamijo et al (2011)⁽²⁹⁾ and Nielsen et al (2010).⁽³⁰⁾ The reason for these results might stem from the variability in experimental design and age difference.

Urinary L-FABP levels showed significant positive correlation with NEFA and ACR, such finding was in agreement with that reported by X G Lu,⁽³¹⁾ And Nakamura et al,⁽³²⁾ who reported a significant positive correlation between urinary L-FABP and N-EFA and ACR.

The positive relationship between urinary L-FABP and N-EFA and ACR could be attributed to "lipotoxicity" and cell dysfunction due to oxidative stress.⁽³³⁾ Adipocytes have the unique ability to store excess FFAs in lipid droplets, a plasticity not seen in other cells. In nonadipose tissues, excess cytosolic FFAs lead to cell dysfunction and death by promoting endoplasmic reticulum stress and excess production of reactive oxygen species, processes collectively designated as "lipotoxicity." This mechanism is the foundation of a growing spectrum of diseases currently known to encompass obesity, type 2 Diabetes, cardiomyopathy, atherosclerosis, and T2D-associated renal injury.⁽³³⁾

Concerning the general characteristics of the studied groups, the present study showed that fasting and post prandial glucose levels in normo, micro and macroalbuminuria were significantly higher as compared to control group ($p < 0.05$), however, no significant

differences were found between the three diabetic groups. More over glycated hemoglobin values were significantly higher in overt proteinuric group followed by microalbuminuric and normoalbuminuric group. Increase serum glucose levels might be explained by an increase in risk of development of diabetic nephropathy. These findings supported the suggestion that hyperglycemia is a contributing factor to diabetic complications, including diabetic nephropathy. Similar result was obtained by Narashima et al.⁽³⁴⁾ who reported that, the increase in HbA1c could be due to increase in non-enzymatic glycation of hemoglobin in conditions of sustained hyperglycemia. This glycosylation is the result of post translational modification of HbA1c molecules.

Our results demonstrated that serum NEFAs were significantly higher in patients with microalbuminuria and macroalbuminuria than in those with normoalbuminuria. These results were in consistence with Ataru et al.,⁽³⁵⁾ who confirmed the presence of elevated NEFAs in type 2 diabetic patients with microalbuminuria. In this respect, the report of Michaud et al.⁽³⁶⁾ that fatty acids enhance macrophage LPL production is very interesting. LPL secreted from macrophage is known to contribute to the development and progression of atherosclerosis. Moreover, Lumeng CN et al.⁽³⁷⁾ demonstrated action of free fatty acids on adipose tissue macrophages dynamically regulates the expression of plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor that suppresses the breakdown of blood clots, in the blood of type 2 diabetic patients. Enhanced coagulation cascade sequence in conjunction with decreased fibrinolytic capacity due to overexpression of PAI-1 might explain the reason why serum NEFA level is associated with the early stage of atherosclerosis and microalbuminuria in type 2 diabetic patients.

In the current study, Lipid profile parameters were altered in the three diabetic groups reaching a significant increase in macroalbuminuria. The cluster of lipid abnormalities associated with type 2 diabetes is manifested by an increase in Cholesterol, triglycerides (TG), small and dense low density lipoprotein Cholesterol (LDL-C) concentration and decrease in high density lipoprotein Cholesterol (HDL-C). These finding are in agreement with other studies,^(38, 39) which confirmed dyslipidemia in type 2 diabetic patients. The prevalence of hypercholesteremia could be due to factors such as increased age of subjects or increased consumption of saturated fats and cholesterol in the diet which was not assessed in our study and the presence of genetic lipid disorders. Hypertriglyceridemia is common in diabetes and is due to overproduction of VLDL in the liver and to a disposal defect in the periphery, the latter being the consequence of a deficiency of lipoprotein lipase, an insulin dependent enzyme. LDL oxidation might decrease catabolism of LDL-C thus causing increase in LDL-C levels. This elevation of LDL-C was explained by decreased activity of Cholesterol ester transfer protein and lipoprotein lipase activity.^(40, 41)

As expected complication with diabetes, renal functions was altered in this study, revealed by elevated albumin creatinine ratio (ACR), significant increase in urea and creatinine in micro-albuminuric as well as overt proteinuric groups in comparison to patients without proteinurea. Our study was in the favour of the study by Jha et al.,⁽³⁸⁾ who showed that, serum urea and creatinine were significantly increased in micro and macroalbuminuria compared to normoalbuminuria. This is because diabetes mellitus is one of the systemic diseases affecting the kidneys. Once microalbuminuria is present, creatinine clearance declines and the rate that varies widely from patient to patient on an average reduction of 10-12ml/min. Basement membrane thickening in diabetic nephropathy due to various factors may be other cause for the raised urea and creatinine.⁽³⁸⁾

In the present study, albumin creatinine ratio (ACR) correlated positively with HbA1c in patients with incipient nephropathy (figure 40). This finding was also supported by an evidenced based study which showed a graded relationship between HbA1c and the risk of nephropathy in type 1 and type 2 DM patients.⁽⁴²⁾ Persistent increase in glycated hemoglobin may be an indicator of worsening albumin creatinine ratio and diabetic nephropathy.⁽⁴³⁾ Renal Parameters like Blood urea and serum creatinine were higher in patients with positive microalbuminuria. Hence, microalbuminuria can be used as rapid screening test for early detection of diabetic nephropathy. A high prevalence of dyslipidemia was present in all diabetic patients. This needs to call for a strict lipid control in diabetic patients to prevent complications.⁽⁴⁴⁾

Conclusions

The measurement of urinary L-FABP in urine provides a suitable biomarker for the early detection and monitoring of progression of diabetic nephropathy in clinical practice. The, u-LFABP may be used as an indicator of tubular damage early in the course of diabetes and therefore may find a place as a new tool in the prediction of diabetic nephropathy.

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Table I: Statistical Analysis of different studied biochemical parameters in normal control and patient groups (values represented as mean \pm SD)

	CONTROL n=20	Patient group n=20			Test of significant	
		Albuminuria			F	P
		Group I Normo	Group II Micro	Group III Macro		
Age (years)	53.95 \pm 6.72	56.3 \pm 5.71	61.2 \pm 6.29	62.5 \pm 5.13	3.05	0.069
Diabetes duration (years)	----	9.4 \pm 3.817	10.05 \pm 3.348	12.05 \pm 3.97	2.98	0.077
Systolic blood pressure	127.00 \pm 8.25	130.20 \pm 18.20	139.10 \pm 17.20	142.50 \pm 10.6@	6.25	0.01*
Diastolic blood pressure	74.50 \pm 8.25	81.50 \pm 7.65	81.00 \pm 6.25	83.20 \pm 8.22@	4.65	0.025*
Fasting plasma glucose (mg/dl)	97.15 \pm 6.74	155.45 \pm 27.01	209.75 \pm 52.49	277.6 \pm 77.38	28.65	0.001*
Post prandial glucose (mg/dl)	114 \pm 7.13	178.25 \pm 26.43	235.05 \pm 50.47	305.75 \pm 83.01	20.1	0.001*
HbA1c (%)	4.34 \pm 0.6	5.63 \pm 0.70	6.81 \pm 0.662	8.54 \pm 0.97	16.25	0.005*
Serum NEFA (mmol/L)	0.34 \pm 0.09	0.59 \pm 0.07@	0.69 \pm 0.06@\$	0.93 \pm 0.58@\$#	12.88	0.001*
Total cholesterol (mg/dl)	154.8 \pm 19.62	178.2 \pm 22.43	208.2 \pm 31.73	231.55 \pm 36.46	10.74	0.0012*
Triglycerides (mg/dl)	82.55 \pm 19.43	107.85 \pm 18.80	125.25 \pm 35.55	171.8 \pm 50.39	11.33	0.001*
LDL-Cholesterol (mg/dl)	91.15 \pm 21.9	114.7 \pm 25.82	145.35 \pm 34.49	164.7 \pm 40.16	12.25	0.001*
HDL-Cholesterol (mg/dl)	51.05 \pm 6.7	45.48 \pm 5.18	44.735 \pm 5.48	40.62 \pm 8.41	5.01	0.033*
Serum urea (mg/dl)	26.2 \pm 4.84	34.2 \pm 5.39	43.35 \pm 4.75	94.95 \pm 49.40	12.65	0.0001*
Serum creatinine (mg/dl)	0.62 \pm 0.14	0.77 \pm 0.10	0.94 \pm 0.15	2.41 \pm 0.70	14.58	0.0001*
Serum uric acid Levels (mg/dl)	3.345 \pm 0.94	4.84 \pm 1.01	6.03 \pm 1.14	8.495 \pm 1.38	9.85	0.0013*
Serum L-FABP (ng/ml) levels	7.66	8.95@	9.92@\$	10.1@\$#	4.75	0.05*
Urinary L-FABP (ng/ml) levels	13.68	16.72@	22.17@\$	38.41@\$#	15.59	0.001*

F for ANOVA test

@: compares control versus patient groups.

\$: compares group I versus DN patient groups

#: compares group II versus Group III

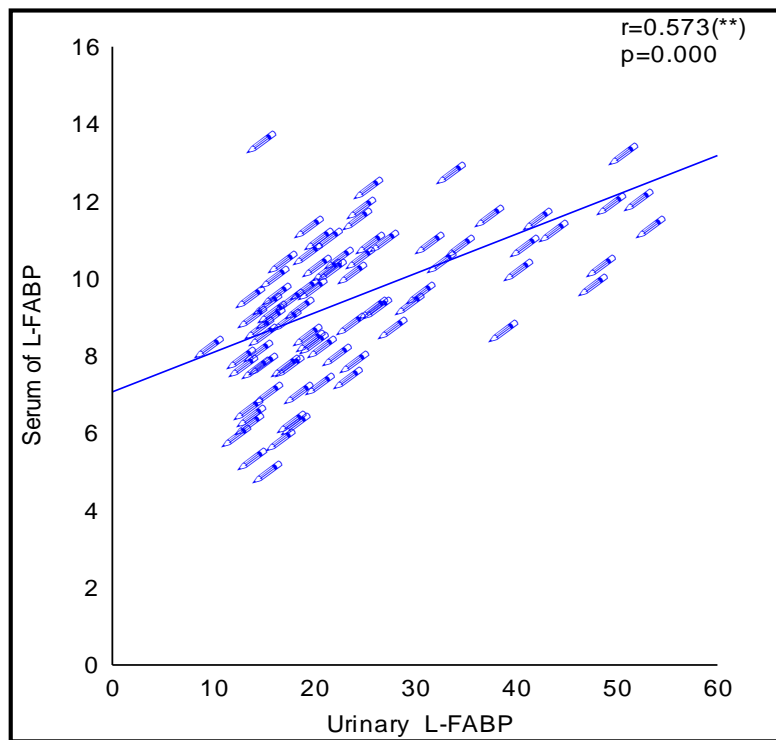


Fig. (1): Correlation between serum and u- L- FAB(ng/ml)

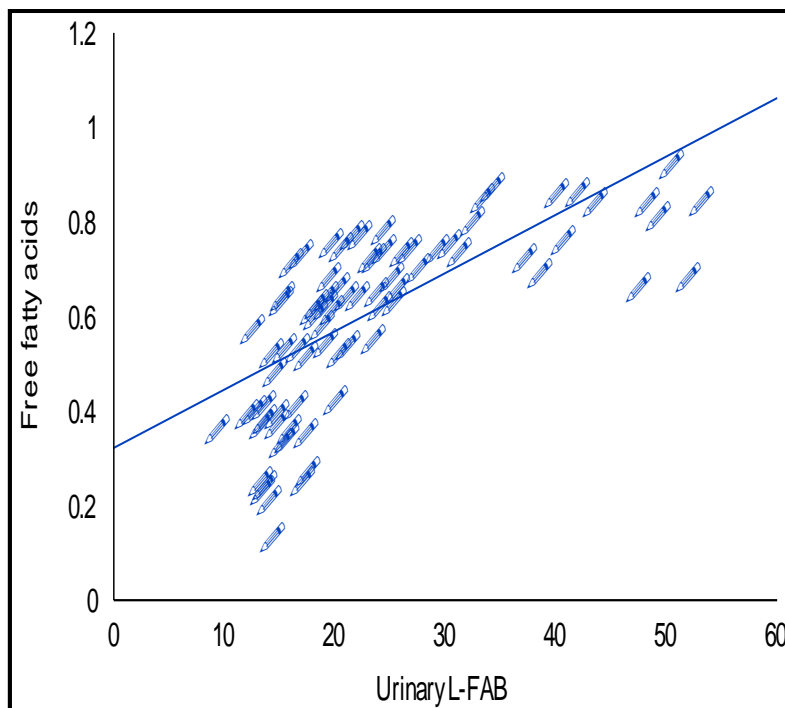


Fig. (2): Correlation between U- L-FAB(ng/ml) and free fatty acids

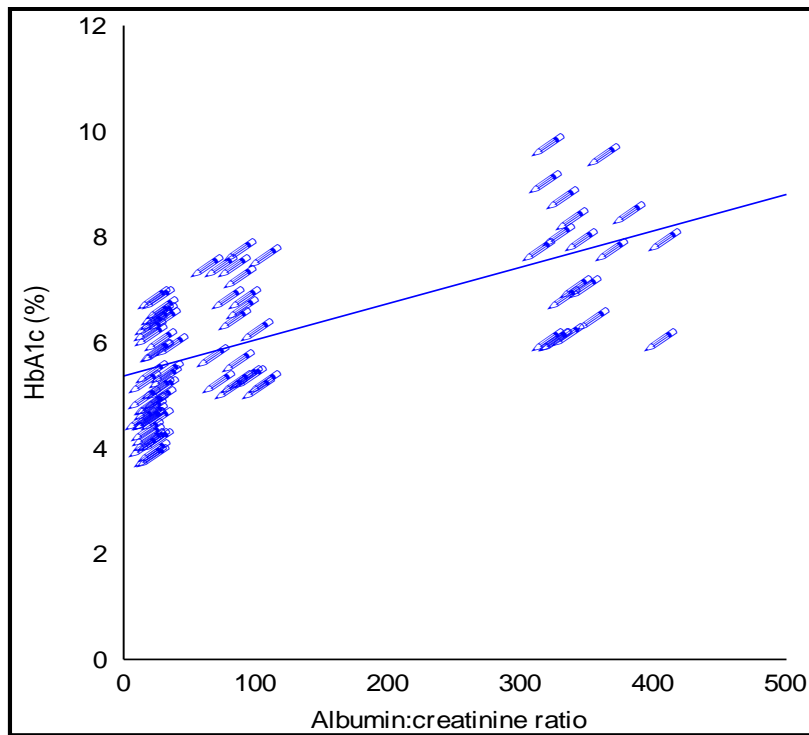


Fig (3): Correlation between albumin creatinine ratio (ACR) (mg/g) and HbA1c (%).

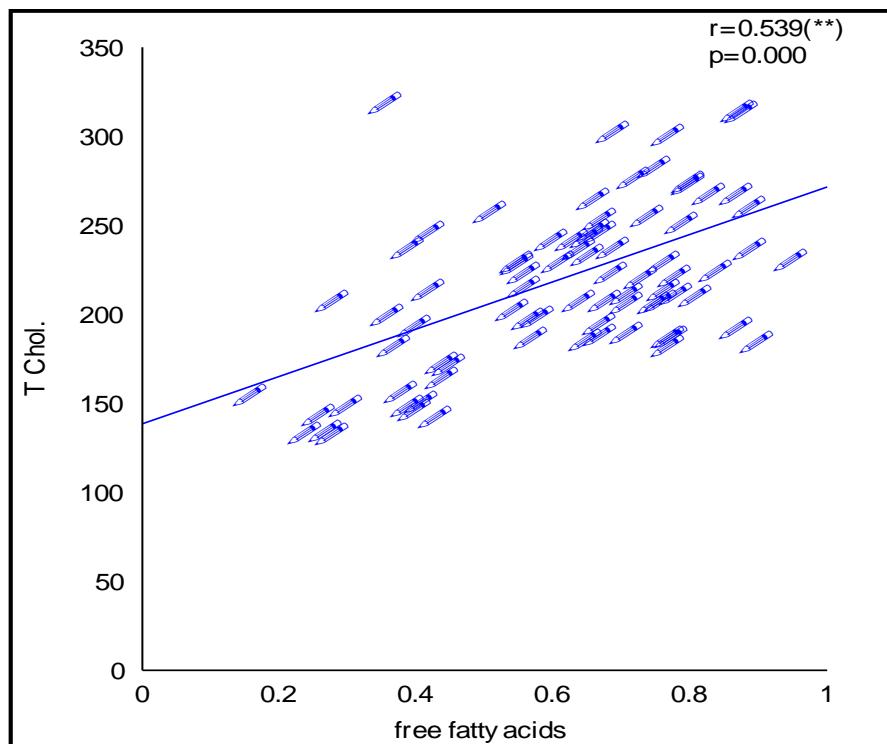


Fig (4): Correlation between Non Esterified Free Fatty acids (mmol/L) and Total cholesterol (mg/dl)