Scientific Research Publishing

Open Journal of Gastroenterology, 2019, 9, 113-124
http://www.scirp.org/journal/ojgas
ISSN Online: 2163-9469
ISSN Print: 2163-9450

Assessment of Liver Fatty Acid Binding Protein (L-FABP) as a Diagnostic Marker in Non-Alcoholic Fatty Liver Disease

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How to cite this paper: Abdulaziz, B.A., Abdu, S.A., Amin, A.M., El Menyawi, A.K.A.H., Ahmed, A., Khalil, M.A. and Halim, W.A.A. (2019) Assessment of Liver Fatty Acid Binding Protein (L-FABP) as a Diagnostic Marker in Non-Alcoholic Fatty Liver Disease. *Open Journal of Gastroenterology*, **9**, 113-124.
https://doi.org/10.4236/ojgas.2019.96014

Received: May 9, 2019
Accepted: June 25, 2019
Published: June 28, 2019

Abstract

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common liver disease worldwide, it causes chronic hepatitis, which leads to cirrhosis and hepatocellular carcinoma. We aimed to assess the value of liver fatty acid binding protein (L-FABP) in the diagnosis of non-alcoholic fatty liver disease in comparison to ultrasonography. **Patients and Methods:** Ninty subjects were enrolled in this study who attended the Hepatology, Gastroenterology and Internal medicine clinics in Benha University Hospitals between January 2017 and January 2018 and divided into group I included 70 consecutive patients with non-alcoholic fatty liver disease who were diagnosed by ultrasound with or without elevated liver enzymes and group Π included 20 healthy control subjects without NAFLD (by ultrasound) with normal liver enzymes. Serum levels of L-FABP were determined by enzyme-linked immunosorbent assay. **Results:** NAFLD patients were slightly older than healthy subjects as mean age in group I was (37.74 ± 11.7) while in group Π was (36.5 ± 11.31). There was a slight increase in NAFLD in males, there was a high prevalence of NAFLD in the urban population. L-FABP levels in NAFLD patients were higher than in the control group (levels were 188.6 ± 34.94 and 137.7 ± 13.05 ng/l respectively). A strong correlation was found between L-FABP and ALT, AST, BMI and glucose levels. Analysis of ROC curve revealed that at a level 151.1 ng/sensitivity, specificity, PPV, NPV and accuracy were 83.3%, 71.8%, 31.3%, 96.6% and 73.3% respectively with AUC 0.839 and at a level 189.5 ng/sensitivity, specificity, PPV, NPV and accuracy were 90%,

90%, 95.4%, 95.4%, 88.9% with AUC was 0.950. **Conclusion:** Serum L-FABP could be used as a new diagnostic biomarker for detecting NAFLD.

Keywords

NAFLD, L-FABP, Chronic Hepatitis, Cirrhosis, Hepatocellular Carcinoma

1. Introduction

NAFLD is one of the most important causes of liver disease worldwide and will probably emerge as the leading cause of end-stage liver disease in the coming decade [1]. It encompasses a wide spectrum of histological and clinical manifestations, ranging from simple steatosis to steatohepatitis, fibrosis and cirrhosis [2].

The global prevalence of NAFLD is currently estimated to be 24 [3]. But the highest rates are reported from South America (31%) and the Middle East (32%), followed by Asia (27%), the USA (24%) and Europe (23%), whereas NAFLD is less common in Africa (14%) [3]. Fatty acid-binding proteins (FABPs) are a family of small and highly conserved lipid chaperone molecules with highly varied functions [1]. Different members of the FABP family exhibit unique patterns of tissue expression and are expressed most abundantly in tissues involved in active lipid metabolism in hepatocytes, adipocytes and cardiac myocytes, where fatty acids are prominent substrates for lipid biosynthesis, storage or breakdown, the respective FABPs make up between 1% and 5% of all soluble cytosolic proteins [4]. In the hepatic lobule, L-FABP is expressed in hepatocytes in a declining gradient from portal to central location [5]. In addition, epithelial cells of the proximal tubules in kidneys express L-FABP mRNA [6]. A previous study has shown L-FABP to be a promising biochemical marker for the early detection of hepatocellular injury in a small group of liver transplant patients [7]. It was reported that urinary excretions of L-FABP are increased in patients with chronic hepatitis [8]. Liver type FABP (L-FABP), significantly expressed in hepatocytes, enterocytes and to a lesser degree in renal tubular cells [9]. L-FABP is a protein involved in multiple biologic functions, such as intracellular fatty acid transport, cholesterol and phospholopid metabolism, which plays an important facilitative role in hepatic fatty acid oxidation [10].

2. Material and Methods

1) This observational case control study was conducted on 90 subjects attended the clinics of Hepatology, Gastroenterology and Infectious Diseases, and Internal medicine in Benha University Hospitals during the period from January 2017 to January 2018 and a written informed consent was obtained from all participants prior to recruitment and divided into group I included 70 consecutive patients with non-alcoholic fatty liver disease who were diagnosed by ultrasound

with or without elevated liver enzymes and group II included 20 healthy control subjects without NAFLD (by ultrasound) with normal liver enzymes. Subjects with any amount of alcohol consumption or history of alcohol consumption, steatogenic medications (amiodarone, valproic acid, corticosteroids and tetracyclines).

2) HCV antibody positive, Hepatitis B surface antigen positive, chronic kidney disease, polycystic kidney disease, Other Causes of chronic liver disease other than HCV and HBsAg positive patients (by history and examination): hemochromatosis, Wilson disease, autoimmune hepatitis and drug abusers were excluded. Full history taking, clinical examination with stress on weight and height which measured in light clothing without shoes and body mass index (BMI) was calculated by dividing the weight by the square of the height (kg/m^2) and laboratory tests including CBC, blood sugar, ALT, AST, Serum creatinine, viral markers (HBsAg, anti-HCV Ab)were assayed using an enzyme immunoassay (EIA) Kit (Abbott, Axyam USA), lipid profile and serum liver fatty acid binding protein (L-FABP ng/L): L-FABP was measured with a sandwich enzyme-linked immunosorbent assay developed in collaboration with sunshine Biotechnology. The detection limit of the assay was 151.1 ng/L.

2.1. Assay Procedure for FABP

Add L-FABP to a monoclonal antibody enzyme well which is precoated with human L-FABP monoclonal antibody, incubation; then, add (L-FABP) antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution A, B, the color of the liquid changes into blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human substance L-FABP of the sample were positively correlated.

Also, Ultrasonography TOSHIBA SSA-700A (Apilo 5) was performed to assess the liver condition including NAFLD according to [11]. as follows:

Grade 1: The echogenicity of the liver is just increased.

Grade 2: When the echogenic liver obscures the echogenic walls of portal vein branches.

Grade 3: When the echogenic liver obscures the diaphragmatic outline.

2.2. Statistical Analysis

The analysis of the data was carried out using SPSS (SPSS Inc., Chicago, ILL Company) 16 software. Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation Median, IQR and range. Fisher-exact test (FET) was used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at $P > 0.05$. Student "t" test was used to analyze normally distributed variables among 2 independent groups. While non-parametric va-

riables were analyzed using Man Whitney U test (Z MWU). Difference among 3 independent means was analyzed using ANOVA for parametric variables or Kruskal Wallis test (KW) for non-parametric ones. Significant ANOVA and KW tests were followed by post hoc multiple comparisons using Bonferroni tests to detect the significant pairs. Correlations between L-FABP and the studied variables were assessed by Spearman's correlation coefficient (rho). ROC curves were constructed to determine cutoff values of L-FABP in the prediction of fatty liver and its grades. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant).

P value > 0.05 is non-significant (NS)

$P < 0.05$ is significant (S)

$P \leq 0.001$ is highly significant (HS)

Positive predictive value is the probability of patients with positive test results who are correctly diagnosed. Negative predictive value is the proportion of patients with negative test results who are correctly diagnosed.

3. Results

This study included 70 consecutive patients with NAFLD and 20 apparently healthy subjects. The epidemiologic characteristics and laboratory parameters of the two groups were summarized in Table 1. The mean age of NAFLD patients was 37.74 ± 11.7 years while in healthy control subjects was 36.5 ± 11.31 with no statistical significant difference between the two groups. There was female predominance among both groups with no statistical significance difference. Concerning the residence, either rural or urban, 64.29% of group (I) cases had urban residence in comparison with a group (Π) which was 35% urban residence and this was statistically significant ($p < 0.01$).

As regard BMI, there was a significant statistical difference between group I and group II concerning height, weight and BMI with mean value (171.5 ± 8.3), (84.5 ± 9.5), (28.76 ± 4.3) in group I and (177.6 ± 6.67), (74.65 ± 7.44), (23.72 ± 3.04) in group II respectively (Table 1).

As regard biochemical and molecular parameters: There was a highly statistically significant difference between studied groups as regard total cholesterol, TG, HDL and LDL ($p < 0.001$). Also there was a statistical significant difference between studied groups as regard AST, ALT and FBS ($p < 0.04$, $p < 0.03$ and $P < 0.03$ respectively). Also, there was a highly statistically significant difference between studied groups as regard L-FABP ($P < 0.001$) that was increased in group I more than in group II (Table 1). There was increase in levels of L-FABP in serum of NAFLD patients in relation to grades of fatty liver by ultrasound as mean levels of L-FABP (177.6 ± 26.0) corresponds to grade I fatty liver, mean levels of (189.4 ± 29.2) corresponds to grade Π fatty liver and mean levels of (220.5 ± 48.5) corresponds to grade Ш fatty liver and this was clinically and statistically significant ($p < 0.001$) (Table 2 and Figure 1). Also, there was a good positive correlation between grades of fatty liver by ultrasound and serum levels of L-FABP and this was statistically significant ($p = 0.002$) (Table 3 and Figure 2).

Table 1. Sociodemographic features of the studied groups.

	Group I n = 70	Group II n = 20	Test of sig.	p
Sex				
Male	32 (45.7)	8 (40%)	0.206	0.650
Female	38 (54.3)	12 (60%)		
Residence				
Urban	45 (64.29)	7 (35%)	5.46	<0.01
Rural	25 (35.71)	13 (65%)		
DM	15 (21.4%)	0 (0%)	-	<0.02
Age				
mean ± SD	37.74 ± 11.7	36.5 ± 11.31	0.49	0.625
Hepatomegaly	18 (25.71%)	1 (5%)	4.08	<0.04
Splenomegally	6 (8.57%)	2 (10%)	0.03	0.84
Height	171.5 ± 8.3	177.6 ± 6.67	2.86	<0.01
Weight	84.5 ± 9.5	74.65 ± 7.44	4.26	<0.01
BMI	28.76 ± 4.3	23.72 ± 3.04	4.7	<0.01
Total cholesterol	220.09 ± 22.01	203.20 ± 15.81	3.22	<0.01
TGs	219.09 ± 62.92	173.50 ± 29.43	3.13	<0.01
HDL	40.61 ± 7.49	62.10 ± 6.58	11.59	<0.00
LDL	151.76 ± 19.53	120.50 ± 21.49	6.17	<0.00
S. creatinine (mg/dl)	0.97 ± 0.23	1.01 ± 0.18	0.71	0.47
Bilirubin (mg/dl)	0.99 ± 0.16	0.956 ± 0.16	0.86	0.38
Salbumin (g/dl)	3.73 ± 0.22	3.72 ± 0.18	0.17	0.86
INR	1.17 ± 0.14	1.2 ± 0.18	0.78	0.43
AST (IU/L)	36.7 ± 13.46	33.8 ± 11.4	2.08	<0.04
ALT (IU/L)	40.31 ± 14.94	34.35 ± 12.01	2.21	<0.03
ALP (U/L)	38.2 ± 13.28	30.95 ± 7.85	2.32	<0.02
FBS	118.7 ±	96.8 ± 11.9	2.17	<0.03
Serum L-FABP (ng/ml)	188.6 ± 34.94	137.7 ± 13.05	6.11	<0.001

Table 2. Serum L-FABP level according to US grade of fatty liver.

Group	n.	Serum L-FAB			KW test a7 P	Sig pairs
		Mean	± SD	Range		
Normal	20	137.7	13.0	120 - 160	45.08 & <0.001 (HS)	N ≠ I N ≠ II N ≠ III I ≠ III II ≠ III
Grade I	36	177.6	26.0	130 - 230		
Grade II	22	189.4	29.2	148 - 261		
Grade III	12	220.5	48.5	177 - 334		

Table 3. Correlation between the grade of fatty liver and Serum L-FABP.

With	L.FABP	
	Rho	P
Grade of fatty liver	0.362	0.002 (S)

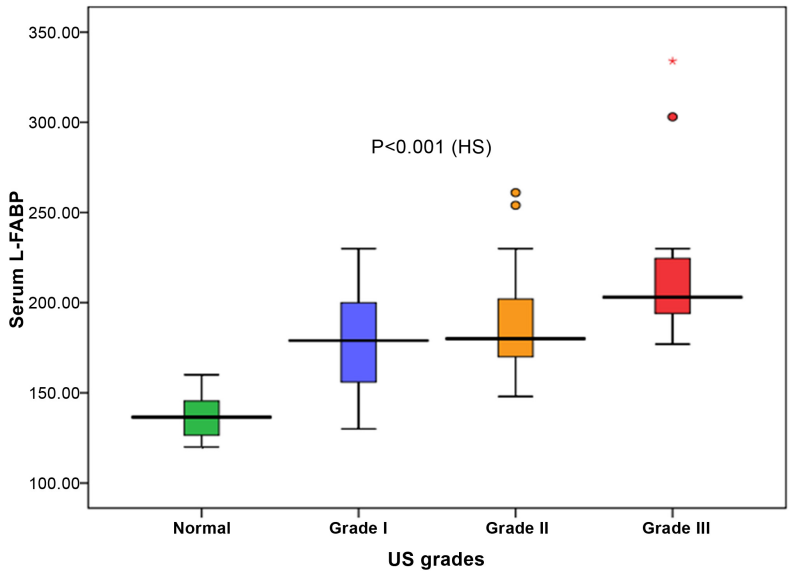

Figure 1. Box plot showing median and IQR of S. L-FABP according to US grades, with an increase of U/S grades of fatty liver there was an increase in s.L-FABPn and this was statisticaly significant ($p < 0.001$).

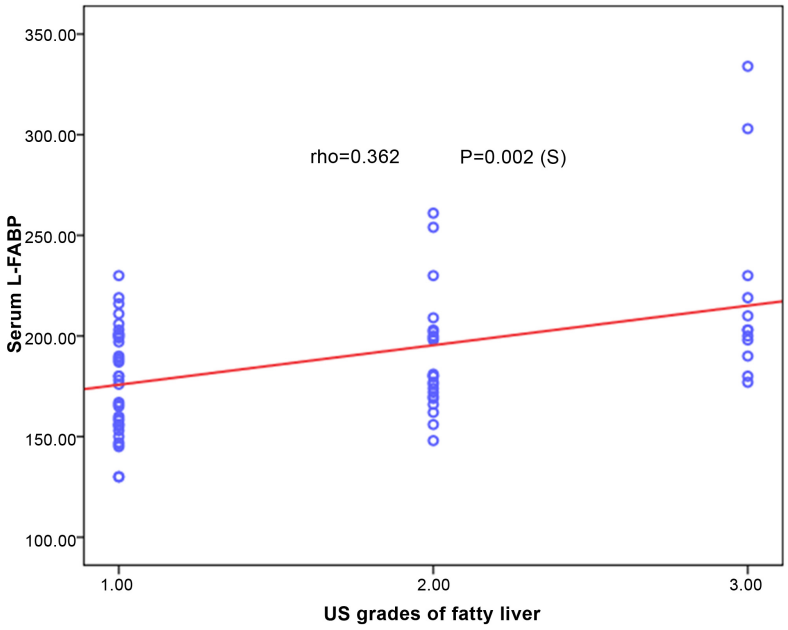

Figure 2. Scatter graph showing significant positive correlation between grades of fatty liver and L-FABP and this was statistically significant ($p = 0.002$).

In the receiver operating curve (ROC), the area under curve (AUC) for L-FABP was 0.839 at a cutoff point 151.1 ng/L with sensitivity, specificity, Positive and negative predictive values were 83.3%, 71.8%, 31.3% and 96.6% respectively while at a cutoff point 189.5 ng/L the AUC was 0.950 with sensitivity, specificity, positive and negative predictive values were 90%, 85%, 95.4% and 70.8% respectively and this was highly statistically significant ($p < 0.001$) (**Table 4** and **Figure 3** & **Figure 4**). As regard the degree of agreement between U/S and serum

Table 4. Shows the performance of Serum L-FABP in the prediction of Group I (fatty liver).

Score	Sens%	Spec%	PPV%	NPV%	Accuracy%	AUC	95% CI	P
LFAB ≥ 151.1	83.3%	71.8%	31.3%	96.6%	73.3%	0.839	0.75 - 0.93	<0.001 (HS)
LFAB ≥189.5 ng/L	90%	85%	95.4%	70.8%	88.9%	0.950	0.91 - 0.99	<0.001 (HS)

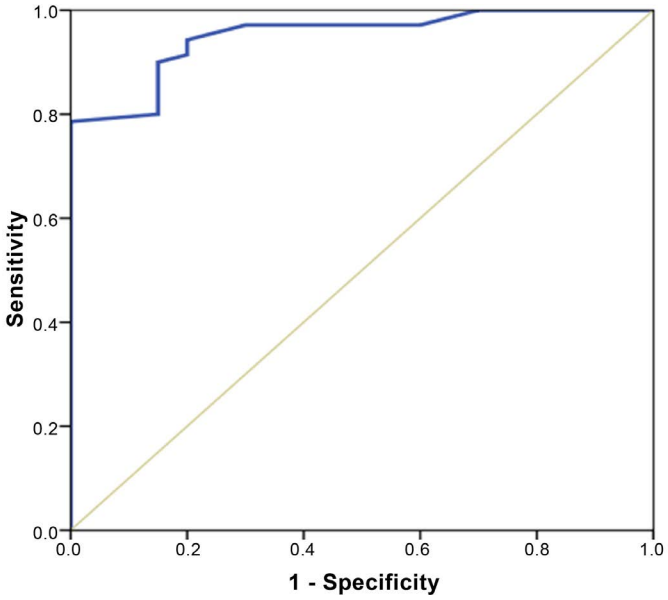

Figure 3. ROC curve for the performance of Serum L-FABP in the prediction of Group I (fatty liver) that at level ≥ 151.1 sensitivity, specificity and AUC were (83.3%, 71.8% and 0.839 respectively).

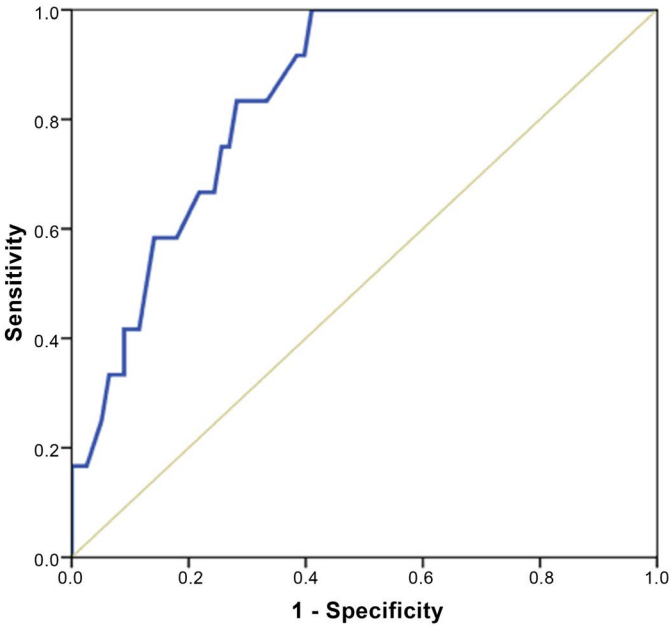

Figure 4. ROC curve for the performance of Serum L-FABP in the prediction of grade III fatty liver among Group I that at level ≥ 189.5. sensitivity, specificity and AUC were (90%, 85% and 0.950 respectively).

L-FAB in the detection of NAFLD, the degree of agreement was 88.9% (Cohen Kappa test = 0.70) and this was statistically significant ($p < 0.05$) (Table 5). Also, the degree of agreement between U/S and serum L-FABP in the detection of grade III NAFLD was 72.8% (Cohen Kappa test = 0.461) and this was statistically significant ($p < 0.05$) (Table 6).

4. Discussion

NAFLD is one of the most prevalent causes of chronic liver disease worldwide [3]. And represents a spectrum of diseases with some patients developing cirrhosis and hepatocellular carcinoma (HCC)) [2]. A large number of patients with NAFLD with potential for progressive liver disease creates challenges for screening, as the diagnosis of NASH necessitates invasive liver biopsy [3]. L-FABP has a low molecular weight and is present in liver cells [7]. The properties of L-FABP lead to its elevation even with a small amount of cell injury. Since hepatocytes are in direct contact with the blood and have no interstitial barrier, small proteins appear sooner in the circulation than large proteins. For these reasons, L-FABP could be a promising biochemical marker for early detection of liver cell injury [7]. The aim of this study was to assess the diagnostic value of L-FABP in

Table 5. Degree of agreement between U/S and serum L-FAB in detection of NAFLD.

			NAFLD by sonar		Total
			Yes	No	
NAFLD by marker (≥151.1)	Yes	Count	63	3	66
		%	90.0%	15.0%	73.3%
	No	Count	7	17	24
		%	10.0%	85.0%	26.7%
Total		Count	70	20	90
		%	100.0%	100.0%	100.0%

Cohen Kappa test = 0.70, P < 0.001 (HS), Degree of agreement = 88.9%.

Table 6. Degree of agreement between U/S and serum L-FABP in detection of grade III NAFLD.

			NAFLD grade III by sonar		Total
			Yes	No	
NAFLD by marker (≥189.5)	Yes	Count	10	17	27
		% within SonarIII	83.3%	29.3%	38.6%
	No	Count	2	41	43
		% within SonarIII	16.7%	70.7%	61.4%
Total		Count	12	58	70
		% within SonarIII	100.0%	100.0%	100.0%

Cohen Kappa test = 0.461, P < 0.001 (HS), Degree of agreement = 72.8%.

detection of NAFLD in comparison to ultrasound. In the current study, NAFLD patients were slightly older than healthy subjects as mean age in group I was (37.74 ± 11.7) while in group Π was (36.5 ± 11.31), but this was statistically non-significant. In the present study, there was a slight increase of NAFLD in males (n = 32, 45.7%) in group I than group Π (n = 8, 40%). While females were (n = 38, 54.3%) in group I and (n = 12, 60%) in group Π. *Sayiner et al.,* (2016) reported that several studies provide data to suggest a higher prevalence in males while others proposed the opposite [12]. In the current study, there was a high prevalence of NAFLD in urban population (n = 45, 64.29%) than rural ones (n = 25, 35.71%). This matches the study of *Niriella et al.,* (2017) who reported that the urban cohort, when followed up for 7 years and subjected to ultrasonography of the liver again, showed their prevalence of NAFLD had increased dramatically to nearly 66% in that age (42 - 71 years) population. The annual incidence of NAFLD in this population was 6.6% [13]. In the present study, there was a strong relationship between NAFLD and body mass index. BMI was elevated markedly in group I (28.76 ± 4.3) than group Π (23.74 ± 3.04). This matched the study of *Williams et al.,* (2011) who stated that NAFLD is strongly linked to obesity, with a reported prevalence as high as 80% in obese patients and only 16% in individuals with a normal BMI and without metabolic risk factors [14]. In the current study serum triglycerides (219.09 ± 62.92), total cholesterol (220.24 ± 22.01) and LDL (151.76 ± 19.53) were higher in group I than group Π and this finding was in agreement with Agrawl *et al.* (2009) who reported hypertriglyceridemia in 63.7%, hypercholesterolemia in 50% - 80%, elevated LDL in 25% in patients with NAFLD [15]. Also, this finding was in agreement with [16] Nseir *et al.* (2011) who reported that dyslipidemia in patients with NAFLD is atherogenic in nature and it is characterized by increased levels of serum triglycerides and decreased levels of HDL cholesterol [16]. In this study, AST and ALT levels were elevated in NAFLD group more than control group and this finding was in agreement with *Armstrong et al.* (2012) who reported that NAFLD detected by ultrasonography was the most common cause of abnormal liver biochemistry [17]. In a study done by Debmalya *et al.* (2015) who concluded that NAFLD was significantly associated with higher ALT and GGT. Also, he reported that diabetic subjects with NAFLD had significantly higher ALT, AST and GGT and significantly lower AST: ALT ratio in comparison with diabetic subjects without NAFLD, but there was no significant difference in ALP levels [18]. In the present study, there was a relation between NAFLD patients and elevated blood sugar levels. Diabetes mellitus was observed in 15 patients in group I (21.4%) and 0% in group Π. Mean levels of fasting blood sugar was higher in group I (118.7 ± 42.9) than group Π (96.8 ± 11.9). This matched the opinion of several authors as Leite *et al.*, (2009) who stated that the prevalence of ultrasonographic NAFLD was 69.4% in 180 patients with T2DM [18]. Also, this was in agreement with *Nascimbeni et al.* (2013) who reported that NAFLD is strictly associated with metabolic risk factors especially obesity and type 2 diabetes mellitus (T2DM) [19]. In the current study, L-FABP levels were positively

correlated with BMI (r = 0.289, p = 0.015), AST (r = 0.350, p = 0.003), ALT (r = 0.291, p = 0.015), total cholesterol (r = 0.334, p = 0.005), triglycerides (r = 0.244, p = 0.042), and LDL (r = 0.301, p = 0.011). These findings matched with *Akbal et al.* (2014) who said in a similar study that L-FABP levels were positively correlated with BMI, glucose, AST, ALT and GGT levels [20]. The current study revealed statistically significant elevation in serum concentration of L-FABP levels in NAFLD group (group I) which was (188.6 ± 34.94) more than control group (group Π) which was (137.7 ± 13.05). This was in agreement with Akbal *et al.*, (2014) who reported that serum L-FABP levels were elevated in NAFLD patients [20]. In the present study, Roc curve analysis of L-FABP as a diagnostic test of NAFLD suggested that at the cut off value 151.1 ng/L (that differentiate NAFLD from healthy group) the sensitivity, specificity, Positive and negative predictive values were 83.3%, 71.8%, 31.3% and 96.6% respectively with AUC was 0.834, but at the cut off value 189.5ng/L the sensitivity, specificity, positive and negative predictive values were 90%, 85%, 95.4% and 70.8% respectively with AUC was 0.950. So, in NAFLD pateints, increased serum levels of L-FABP increases the diagnostic accuracy(at the cut off value 151.1 ng/L diagnostic accuracy was 73.3% and at the cut off value 189.5 ng/L diagnostic accuracy was 88.9%). This was in agreement with Akbal *et al.*, (2014) who reported that to differentiate NAFLD from healthy controls, the cut-off value was 222.54 ng/mL for L-FABP (80% sensitivity and 80% specificity), positive and negative predictive values of L-FABP were 82% and 81%, respectively and when the cut-off value was 284 ng/mL, L-FABP had 73% sensitivity and 100% specificity, Positive and negative predictive values for L-FABP were 100% and 79%, respectively [20]. In the current study, there was a relation between levels of L-FABP in serum of NAFLD patients and grades of fatty liver by ultrasound as mean levels of L-FABP (177.6 ± 26.0) corresponded to grade I fatty liver, mean levels of (189.4 ± 29.2) corresponded to grade Π fatty liver, mean levels of (220.5 ± 48.5) corresponded to grade III fatty liver and this was clinically and statistically significant (p < 0.001). Also, there was a good positive correlation between grades of fatty liver by ultrasound and serum levels of L-FABP and this was statistically significant (p = 0.002). As regards to concordance correlation coefficient between serum L-FABP and ultrasound in detection of NAFLD patients was 88.9% (by cohen kappa test = 0.70) and this was statistically significant (p < 0.001 HS). This means that there is a good degree of agreement between U/S and L-FABP in detection of NAFLD patients. Also, the concordance correlation coefficient between serum L-FABP and ultrasound in detection of grade III of NAFLD was 72.8% (by cohen kappa test = 0.461) and this was statistically significant (p < 0.001).

5. Conclusion

Liver fatty acid binding protein is a promising biomarker for early detection of liver injury in NAFLD when suspected by ultrasound and its levels correspond to the degree of fatty infiltration in liver tissue.

Acknowledgements

The authors would like to thank the staff of Hepatology, Gastroenterology and Infectious Diseases Department in Benha University Hospital.

Ethics Approval and Consent

The Benha University Hospital Ethics Committee approved the study. All patients enrolled for the validation of this study gave a written informed consent.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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