Original Article

Potential Role of Circulating Adipocyte Fatty Acid Binding Protein 4 (A-FABP /FABP-4) Level in Chronic Hepatitis C - Associated Insulin Resistance: a Case - Control Study

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Abstract

The Aim of the Work : was to evaluate the role of A-FABP in development of IR in CHC patients with early stage liver disease **Patients & Methods:** The study included 85 Egyptian males categorized as 70 chronic hepatitis C (CHC) patients and 15 healthy controls. Oxidized low density lipoproteins (oxLDL) and A-FABP were determined in serum using enzyme-linked immunosorbent assay (ELISA).**Results:** According to HOMA-IR results,49 CHC patients (70%) were found to be insulin resistant while 21 patients (30%) were non- insulin resistant. A-FABP showed a significant increase in CHC with IR. It showed significant positive correlation with the duration of HCV infection and viral load in CHC with IR. At cutoff value of 9.4ng/ml, A-FABP had higher sensitivity compared to serum insulin in CHC with an area under curve (AUC) of 0.998 versus 0.966 respectively.

Conclusion: A direct relation between HCV infection and increased circulating A-FABP level, through elevation of oxLDL level, may induce insulin resistance in CHC. Serum A-FABP surpassed fasting serum insulin in determination of insulin resistance and it could be a more sensitive biomarker in CHC patients with early stage liver disease.

Key words: Chronic hepatitis C, Insulin resistance, A-FABP, oxLDL

Introduction

Chronic HCV infection is considered a systemic disease as it affects not only the liver, but other organs as well leading to several extrahepatic complications.[1] Insulin resistance (IR) is a well known complication associated with chronic HCV infection [2] It plays the main role in the development of Type 2 diabetes mellitus (T2DM).[3] HCV associated IR increases the risk of hepatocellular carcinoma [4], cardiovascular events.[5] and associated with low response rates to antiviral treatment. [6] Thus, HCV associated IR is a therapeutic target at any stage of HCV infection. It has been reported that IR occurs in early stages during HCV infection and is not exclusively related to cirrhosis. [7] HCV associated IR has been found to be genotype-dependent (1 and 4), [8] to be related to HCV viral load [9] and to be improved in patients with HCV clearance following antiviral therapy. [10] Therefore, it was found that the underlying mechanism of HCV associated IR is not completely understood [11]

Adipocyte fatty acid binding protein (A-FABP) also called FABP-4 or adipocyte P2 (aP2), which belongs to fatty acid binding proteins (FABP) family of intracellular chaperones, is highly expressed in adipose tissue and activated macrophages. [12] It has a low molecular mass (15 kDa) and it is the major cytosolic protein of mature adipocytes, accounting for about 6% of the total cellular protein. [12] The expression of A-FABP is regulated by insulin and fatty acids, [13] with higher circulating levels in females than males owing to the higher subcutaneous fat content.[14] Several proinflammatory stimuli have been reported to upregulate FABP-4 expression in macrophages, including peroxisome proliferator–activated receptor- (PPARγ) agonists, oxidized low-density lipoproteins (oxLDL) and toll-like receptor (TLR) agonists [15, 16] Clinical studies showed that A-FABP is associated with metabolic disorders including IR, diabetes, obesity, non-alcoholic liver

disease (NAFLD) and cardiovascular diseases [13, 17] Studies on A-FABP revolve around the hypothesis that inhibition or eradication of this protein could help the treatment and prevention of diseases like diabetes, atherosclerosis and other problems in association with adipose tissue. [18, 19]The aim of this work was to evaluate the potential role of circulating AFABP level in the development of chronic hepatitis C (CHC)-associated IR.

Subjects and methods:

Eighty five Egyptian males were selected for the study. They were categorized as 70 patients diagnosed with chronic HCV infection (CHC group) recruited from the Experimental and Internal Medicine Department of the Medical Research Institute during the period from February 2015 to May 2016 and they were not receiving any antiviral treatment. The second category was 15 age matched apparently healthy male controls (group I). Informed oral and written consents were taken from all studied subjects and after the approval of the Ethical Committee of the Medical Research Institute, the study was started. Patients with diabetes, obesity (BMI ≥30), hypertension, cardiovascular or renal disease were excluded. Liver diseases other than CHC or advanced liver cirrhosis (Chid-Pugh class more than A) were ruled out.

To all the studied subjects, full history, clinical examination and abdominal ultrasound were done. Anthropometric measures were done and the body mass index (BMI) was calculated using the following formula: (weight/height2 in kg/m2).

Fatty liver condition was evaluated by abdominal ultrasonography and confirmed by calculating the validated Fatty Liver Index (FLI) to quantify the degree of liver steatosis using the following formula : e y / (1 + e y) × 100, Where y = 0.953 × log (triglycerides, mg/dL) + 0.139 × BMI (Body mass index), kg/m2 + 0.718 × log (gamma gluatmyl transferase activity, U/L) + 0.053 × waist circumference, cm – 15.745[20]

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. Logistic regression analysis was done for the statistically significant factors where anxiety and depression were the dependant variables. The used tests were: Mann Whitney test which was used to compare between two studied groups for abnormally quantitative variables, and Spearman coefficient to correlate between two abnormally quantitative variables .

Laboratory Workup:

To all studied subjects, 10 ml venous whole blood samples were withdrawn after 12 hours fasting and the following laboratory investigations were done:

1. Routine biochemical laboratory investigations including liver function tests and glucose parameters were performed on the auto-analyzer OLYMPUS AU400 Germany.

2. Non-estrified Free Fatty acids (NEFA) were determined in serum by titration (Dole's method) [21]

3. Fasting serum insulin was measured using chemiluminescent enzyme immunometric assay (CLIA) on Immulite 1000 Automated Analyzer (Diagnostic Products Corporation) then Homeostasis Model for Assessment of insulin resistance (HOMA-IR = fasting insulin (μIU/ml) * fasting glucose (mmol/L)/22.5) [22] was calculated and any patient with a result of ≥ 2.5 was considered to be insulin resistant.

4. Hepatitis viral markers; HBsAg and anti-HCV Ab were measured by enzyme-linked immunosorbent assay (ELISA) while HCV RNA was detected by quantitative PCR.

5. Serum oxLDL concentration was determined using a commercial ELISA kit (Mercodia, Uppsala, Sweden)

6. Serum level of adipocyte fatty acid binding protein (A-FABP) was determined by ELISA technique from commercially available kit, (BioVendor Human Adipocyte FABP (FABP4), Laboratorni medicina a.s.).

Statistical method:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov test was used to verify the normality of distribution of variables. Student t-test was used to compare two groups for normally distributed quantitative variables while ANOVA was used for comparing between more than two groups and followed by Post Hoc test (Tukey) for pairwise comparison. Mann Whitney test was used to compare between two groups for not-normally distributed quantitative variables, while Kruskal Wallis test was used to compare different groups and followed by Post Hoc test (Dunn's) for pairwise comparison. Spearman coefficient was calculated to assess the association between A-FABP and other risk factors for IR. Univariate and multivariate logistic regression was assessed for the parameters found to be significantly associated with HOMA-IR. Receiver operator characteristic (ROC) curves for A-FABP and insulin against HOMA-IR were constructed. Youden's indices were calculated to get their best cut off values. Significance of the obtained results was judged at the 5% level..

Results:

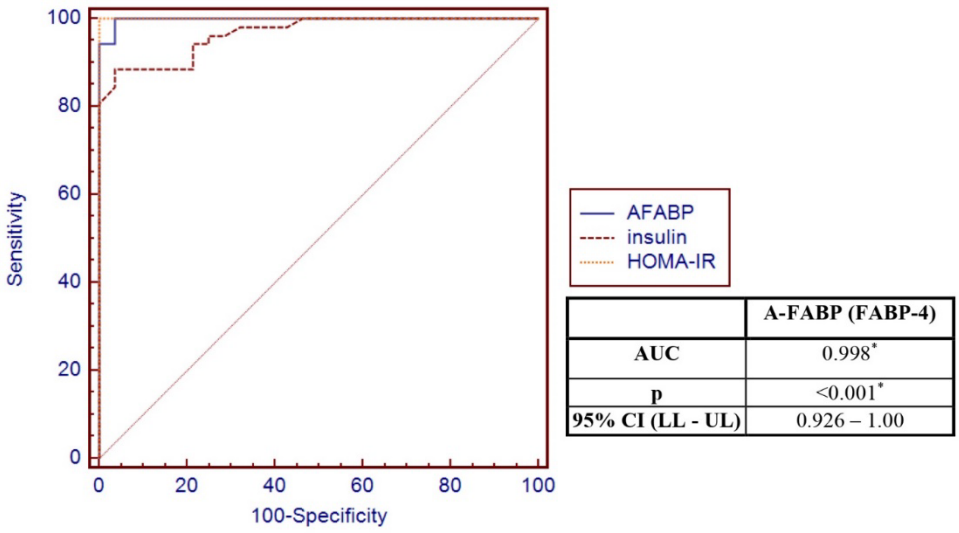
HOMA-IR was found to be high in most CHC (70%) patients. Accordingly, CHC were classified into insulin resistant group (group II-b) (n=49, 70%) (HOMA-IR ≥ 2.5) and non- insulin resistant group (group II-a), (n=21, 30%) (HOMA-IR <2.5) as shown in Table (I).The median serum A-FABP values showed a significant increase in group II-b (with IR) more than group II-a (without IR) as well as group I (controls), (39.25, 12.1 and 8.5 ng/ml, respectively), (p <0.001), but A-FABP level in group (II-a) was not significantly different from group (I). Regarding the fatty acid status and steatosis markers including FLI, serum oxLDL as well as NEFA in the three studied groups are shown in Table (I). Significant correlations of A-FABP are shown in Table (II).

Diagnostic performance of A-FABP (FABP-4):

Figure (1) shows the characteristics of Receiver operator characteristic (ROC) curves for A-FABP and insulin against HOMA-IR, the most widely used indicator of insulin sensitivity to compare their diagnostic performance.

Determinants of insulin resistance in CHC patients:

Univariate logistic regression analysis revealed that duration of HCV infection, viral load (HCV-PCR) and serum A-FABP were positively associated with the occurrence of IR in CHC patients, whereas multivariate regression analysis showed that serum A-FABP level remained the independent determinant of IR in CHC patients after adjusting for all confounding factors, (standardized β = 0.146, P =0.009), Table (III).



	A-FABP (FABP-4)
AUC	0.998*
p	<0.001*
95% CI (LL - UL)	0.926 – 1.00

Figure (1): ROC curves of A-FABP and insulin to determine insulin resistance in CHC.

Table (I): Comparison between the studied groups according to different clinical and biochemical parameters

	Group I (n=15)	Group II-a(n=21)	Group II-b (n=49)	Test of sig.	P
Age (years)	49.4±5.99	46.15±5.72	45.85±7.25	F=1.499	0.233
BMI (Kg/m2)	25.53±1.22	26.47±1.54	26.75±1.83	F=2.795	0.070
Waist circumference (cm)	92.5± 6.6	96.5 ± 6.1	94.5±7.6	F=1.133	0.330
Duration of HCV(years)	-	1(1–1.5)	3(2–5)	U=0.0*	<0.001*
Liver function tests:					
AST (U/L)	19b(15–30)	33a(19–123)	40.5a(18–329)	H=21.282*	<0.001*
ALT(U/L)	14b(10 – 27)	42a(10 – 97)	27a(10 – 124)	H=18.290*	<0.001*
GGT (U/L)	14b(13–20)	17b(15–23)	29ab(22–40)	H=9.262*	0.010*
Total bilirubin (mg/dl)	0.7b(0.3–0.9)	0.9a(0.4–3.1)	0.85a(0.4–4.9)	H=12.108*	0.002*
Direct bilirubin (mg/dl)	0.2b(0.1–0.3)	0.3a(0.1–1.7)	0.3a(0.1–3.4)	H=9.995*	0.007*
Indirect bilirubin (mg/dl)	0.4b(0.2–0.6)	0.5a(0.3–1.4)	0.5a(0.3–2.3)	H=9.377*	0.009*
HCV RNA (IU/ml)	-	113490±69901	671062±344426	t=7.934*	<0.001*
NEFA (mmol/L)	0.6b(0.4–0.9)	1a(0.5–1.4)	0.85a(0.5–1.8)	H=11.179*	0.004*
Cholesterol (mg/dl)	155±12.87	167.77±33.36	159.04±23.64	F=1.018	0.368
Triglycerides (mg/dl)	80(61–100)	97(37–155)	90(47–274)	H=0.946	0.623
HDL-cholesterol (mg/dl)	49.6a±6.3	33.46b±11.05	37.38ab±10.09	F=11.812*	<0.001*
LDL-cholesterol (mg/dl)	88.85b±9.55	115.12a±31.62	101.83ab±23.11	F=4.609*	0.014*
FSG (mg/dl)	83.6b ± 6.4	86.4b± 7.2	106.1a ± 12.3	F=8.062*	0.001*
PPG2h (mg/dl)	95.93b±17.42	102.62b±13.2	121.96a±12.75	F=18.138*	<0.001*
Fasting insulin (µIU/mL)	2.52b(2.0–6.25)	6.51b(3.37–10.0)	14.0a(9.29–35.30)	H=42.671*	<0.001*
HOMA-IR	0.56b(0.35–1.37)	1.39b(0.76–1.97)	3.16a(2.6–7.66)	H=43.470*	<0.001*
FLI	22.3b±6.4	47.8a±13.3	56.7ab±15.1	F=34.496*	<0.001*
A-FABP (ng/ml)	8.5b(5.0–14.5)	12.1b(10–22.5)	39.3a(19.8–104.9)	H=42.317*	<0.001*
oxLDL(U/L)	11.4b±2.4	24.9a±4.3	31.5ab±11.5	F=27.349*	<0.001*

F: **F for ANOVA test**, Pairwise comparison between each 2 groups was done using **Post Hoc Test (Tukey)**

H: H for **Kruskal Wallis test,** Pairwise comparison between each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the studied groups Group I:controls, group II-a: CHC without IR

Group II-b: CHC patients with IR *: Statistically significant at $p \leq 0.05$

Common letters are not significant (i.e. **Different letters** are significant)

BMI: body mass index.

FLI: fatty liver index.

OxLDL: oxidized low density lipoproteins.

NEFA: non-estrified fatty acids.

FSG: fasting serum glucose.

PPG2hr: two hours postprandial glucose.

A-FABP (FABP-4): adipocyte fatty acid binding protein-4.

HOMA-IR: homeostasis model of assessment for insulin resistance.

HDL-cholesterol: high density lipoprotein-cholesterol.

LDL-cholesterol: low density lipoprotein-cholesterol.

AST: aspartate transaminase.

ALT: alanine transaminase.

GGT: gamma gluatmyl transferase.

HCV-RNA: ribonucleic acid of hepatitis C virus

Table (II): Correlations between A-FABP and other parameters in each studied group

Groups	Parameter	A-FABP (FABP-4)	
		r_s	p-value
Group I (n=15)	Insulin(µIU/mL)	0.567	0.028*
	FLI	0.060	0.832
	oxLDL (U/L)	0.055	0.847
Group II-a (n=21)	Age (years)	0.694	0.008*
	AST(U/L)	0.694	0.008*
	FLI	0.070	0.821
	D.bilirubin (mg/dl)	0.655	0.015*
Group II-b (n=49)	HCV –RNA	0.800	<0.001*
	FPG (mg/dl)	0.426	0.030*
	Insulin(µIU/mL)	0.620	0.001*
	HOMA-IR	0.526	0.006*
	FLI	0.327	0.103
	Duration of HCV (years)	0.747	<0.001*
	oxLDL (U/L)	0.526	0.006*
	NEFA (mmol/L)	0.381	0.001*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Table (III): Univariate and multivariate analysis showing factors independently associated with HOMA-IR (n = 70)

	Univariate				#Multivariate			
	B	P	95% CI.		B	P	95% CI.	
			LL	UL			LL	UL
Age (years)	0.018	0.646	-0.062	0.099				
BMI (Kg/m2)	0.141	0.365	-0.170	0.452				
Waist circumference	0.004	0.913	-0.072	0.080				
Duration of HCV (years)	0.791	<0.001*	0.456	1.125	0.105	0.528	0.229	0.439
NEFA(mmol/L)	-0.880	0.296	-2.563	0.802				
CHOL (mg/dl)	0.0	0.980	-0.020	0.020				
TG (mg/dl)	-0.002	0.787	-0.013	0.010				
HDL-C (mg/dl)	0.018	0.491	-0.034	0.069				
LDL-C (mg/dl)	-0.002	0.839	-0.022	0.018				
HCV–RNA (IU/ml)	0.267	<0.001*	0.0	0.0	0.128	0.645	0.237	0.259
A-FABP (ng/ml)	0.112	<0.001*	0.036	0.069	0.146	0.009*	1.003	4.175
Fatty liver by US	1.069	0.042	0.041	2.097	0.033	0.922	0.706	0.641
Fatty liver index (FLI)	0.034	0.057	-0.001	0.068				
oxLDL (U/L)	0.039	0.160	-0.016	0.094				

B: Unstandardized coefficient LL: Lower limit UL: Upper Limit # All variables with $p \leq 0.05$ were included in the multivariate analysis CI: Confidence interval *: Statistically significant at $p \leq 0.05$

Discussion

The prevalence of insulin resistance is higher in patients with HCV infection compared to that in the general population and patients with other hepatic disorders. [23] In accordance to these findings, the present work revealed that 70%of the studied CHC patients were insulin resistant, even after exclusion of obesity, diabetes and advanced cirrhosis. This was in agreement with Ahmed et al [24] who found that (69%) of the studied Egyptian CHC patients had become insulin resistant according to their HOMA-IR.

Since both CHC infection and A-FABP are related to the development of insulin resistance and NAFLD, [17, 25] so this study aimed to investigate whether there is an association between A-FABP and CHC associated insulin resistance.

In the present study, the difference in A-FABP level found between CHC with IR (group II-b) and CHC without IR (group II-a) compared to controls could be attributed to HCV infection itself, as CHC patients with metabolic diseases that could contribute to IR were excluded. This link between A-FABP level and HCV infection is also supported by the significant high correlation found between A-FABP and the viral load (HCV-RNA), (r=0.8, $p<0.001$), Table (II).The later correlation also explains the absence of significant difference in A-FABP level between CHC patients without IR (group II-a) and the control group, as group (II-a) had lower viral load than group (II-b),($p<0.001$) Table (I). Up to our knowledge in the present work it was the first time to measure A-FABP in CHC patients.

The observed significant elevation of serum A-FABP levels in CHC patients with IR, may be explained as HCV is known to cause NAFLD [25]. Moreover, NAFLD was found to increase A-FABP expression in macrophages.[17]Similar increase in serum A-FABP level due to NAFLD was described by Jeon et al.[26] This finding could be confirmed in our study by the higher value of FLI found in CHC patients with IR compared to those without IR as well as the controls, confirming possible A-FABP role in lipid accumulation in the liver of CHC patients adding to the risk of development insulin resistance in such patients

In addition, the significant positive correlation found between A-FABP and serum NEFA in CHC patients with IR may be attributed to previous finding that A-FABP enhances the hydrolytic activity of hormone-sensitive lipase (HSL) and could increase the risk of IR. [27]

Furthermore, elevation of serum A-FABP could be explained by the previous finding that HCV core protein induces elevation of PPARγ transcriptional activity, [28] which in turn increases the expression of A-FABP leading to insulin resistance [15] This suggestion is supported by the significant high positive correlation between A-FABP with HOMA-IR, insulin, FBS, PP2h in CHC patients, Table (II).

Another possible mechanism for elevated A-FABP concentration in CHC patients that we suggest in our study is the elevated oxLDL found in CHC patients compared to control group, Table (I). Solbach et al [29] showed that serum oxLDL level was increased in HCV patients to inhibit HCV cell entry and inhibit its infectivity.

Remarkably, our results showed significant elevation of serum oxLDL level in CHC patients especially in group II-b with IR more than group II-a and controls, Table (I). On the other hand, as previously mentioned, oxLDL increases A-FABP expression in activated macrophages.[16] From these findings, it became evident that elevated oxLDL added to the risk of IR development in CHC through stimulation of A-FABP expression.

Moreover, the present study revealed that serum A-FABP levels were independently and positively correlated with insulin resistance after adjusting for confounding factors among CHC patients, Table (III). In addition, our results showed that at the best cut off value of 19.4 ng/ml, serum A-FABP (FABP-4) had higher diagnostic sensitivity for determination of IR compared to serum insulin, (100% versus 88.46%; respectively) with similar specificity of 92.86% for both and an AUC of (0.998 versus 0.966, respectively) Figure (1).

From our previously mentioned results and as the role of A-FABP in the development of insulin resistance in CHC has been illuminated, it is hoped that this relation will allow the internist to use the available A-FABP blockers to protect CHC patients from the serious complications of insulin resistance. However, larger scale studies including female subjects are warranted.

In conclusion, insulin resistance was highly prevalent in early stage CHC even after all other possible causes were excluded. CHC male patients with IR had higher serum A-FABP levels than those without IR and the control group. A-FABP level showed high positive correlation with HCV-RNA. In addition, elevation of serum oxLDL level in CHC could be the possible explanation of the direct relation between HCV infection and increased A-FABP level leading to development of insulin resistance. Serum A-FABP is an

independent determinant of IR in CHC and its sensitivity surpassed serum insulin in detection of insulin resistance at early stage CHC.

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