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Assessment of Insulin Resistance, Serum Adiponectin and Ferritin Levels in HCC Patients Before and After Radiofrequency Ablation

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Abstract
Hepatitis C virus (HCV) is a major cause of chronic hepatitis, hepatocellular carcinoma (HCC), and recently HCV has been identified as a cause of insulin resistance (IR). Recent studies have found that HCV-associated IR causes hepato-carcinogenesis and proliferation of HCC where IR may synergize with viral hepatitis in HCC development. The aim of the present work was to study IR, serum alpha-fetoprotein (AFP); adiponectin and ferritin levels in HCV-positive cirrhotic patients with and without HCC. Also, these markers were assessed in early stage HCC patients after successful treatment with radiofrequency ablation (RFA). Moreover, liver stiffness was measured using acoustic radiation force impulse imaging (ARFI) in all studied patients.
Key Words: Hepatitis C virus, Hepatocellular carcinoma, Insulin resistance, Alpha fetoprotein, Adiponectin, Ferritin, Acoustic radiation forse impulse imaging

INTRODUCTION

Hepatitis C virus (HCV) infection is a worldwide problem; it continues to be a major disease burden on the world. Estimates indicate that 3-4 million persons are newly infected each year, 170 million people are chronically infected and at risk of developing liver diseases including cirrhosis and cancer with 350,000 deaths annually due to HCV-

related causes. [1] The highest HCV prevalence in the world occurs in Egypt, where the prevalence rates reached up to 15%. [2]

Globally, over half a million people develop hepatocellular carcinoma (HCC) annually and almost equal number die of it. [3, 4] Hospital-based studies from Egypt have reported an overall increase in the relative frequency of all liver-related cancers (> 95% as HCC), from approximately 4% in 1993 to 7.3% in 2003, with increasing importance of HCV infection estimated to account for 40–50% of HCC cases. [5, 6]

Percutaneous radiofrequency ablation (RFA) is among therapies that offer a high rate of complete response and a potential for cure. [7] RFA induces tumor destruction by heating the tumor tissue to a temperature that exceeds 60°C. This heating is generated from a high-frequency alternating current that is delivered through an electrode placed in the center of the tumor. The generated heat results in coagulative necrosis of the tumor tissue with denaturation of the intra-cellular proteins and dissolution of the cell membrane lipid bilayers. [8]

The primary risk factors for HCC include hepatitis B virus (HBV) infection; HCV; dietary aflatoxin exposure and chronic alcohol consumption. [9]

Recent studies showed that Insulin resistance (IR) is a key factor that plays a crucial role in the progression of liver cancer in HCV-infected patients. [10]

IR is a condition where body cells fail to respond to the normal actions of insulin leading to hyperglycemia, pancreatic beta cells subsequently increase their production of insulin with further hyperinsulinemia. [11] IR represents interplay between genetic (inherited) and environmental (acquired) factors. Genetic factors include abnormal insulin, abnormal insulin receptors and abnormal signaling proteins. While, acquired factors include abdominal obesity and HCV infection. [12]

Obesity and hepatic steatosis are associated with IR through inducing down regulation of insulin receptors with impairment of post-receptor signaling where overflow of free fatty acids (FFAs) from adipose tissue interferes with intrahepatic insulin signaling pathways via increased levels of tumor necrosis factor-α (TNF-α), reactive oxygen species (ROS) together with proteosomal degradation of the insulin receptor substrates (IRS) 1 and 2. [13]

HCV, both directly and indirectly, affects insulin signaling pathways and promotes IR. HCV core protein is known as the inducer of hepatic steatosis, oxidative stress and HCC, where core protein is able to up regulate ROS production by induced nitric oxide synthetase (iNOS) which activates cyclo-oxygenase-2 (COX-2) expression in hepatocytes, providing a potential mechanism for oxidative stress and HCC. [14] Also, HCV core protein induces aggregation of triglycerides (low-density lipoprotein) through activation of fatty acid synthase, down regulation of microsomal triglyceride transport protein, reduction of PPARγ, and upregulation of sterol regulatory element-binding protein-1c, producing molecular and cellular changes that result in excess accumulation

of triglycerides in the hepatocytes. [15] Thus, liver steatosis is frequently observed in patients with chronic HCV infection with a rate of approximately twice than its rate in people with other common liver disorders as HBV infection. [16]

Moreover, HCV related chronic liver disease involves low grade of inflammation and oxidative stress leading to decreased insulin sensitivity in hepatocytes, also, hyperinsulinemia could be a "secondary hit" to propagate proliferation of pre-malignant cells in the cirrhotic liver promoting the development of HCC. [17]

IR with HCV infection is detected at early stages of liver disease even without liver fibrosis, [18] moreover, the relationship between HCV infection and IR development has been demonstrated by the improvement of IR after successful standard antiviral therapy with pegylated interferon and ribavirin, while no improvement was observed in non responders. [19]

IR with its variable causes is responsible for excessive production of FFAs with increased liver adiposity and reduced serum adiponectin level. [20] Adiponectin is an insulin-sensitizing, vascular-protective and anti-inflammatory protein, [21] where hypo-adiponectinaemia is associated with obesity, insulin resistance, type 2 diabetes, and cardiovascular diseases. [22, 23] Recent studies showed that circulating serum adiponectin level is inversely associated with the risk of HCC where patients with low serum adiponectin levels are likely to have a more aggressive tumor phenotype being associated with larger tumor size, vascular invasion, and metastatic spread. [24, 25]

Ferritin is the primary intracellular iron-storage protein, its concentration increases drastically in the presence of an infection or cancer. [26] Iron is considered as a pro-inflammatory and pro-carcinogenic agent in patients with or without chronic viral infection. Iron mediated oxidative stress plays a role in the initiation and promotion of hepatic carcinogenesis where excess hepatic iron along with cirrhosis, is a risk factor for HCC. Depletion of iron reduces hepatic inflammation and retard HCC development in animal and human studies. [27]

Acoustic radiation force impulse imaging (ARFI) is a new promising ultrasound based diagnostic technique that evaluates the wave propagation speed in the tissues and allows the assessment of tissue stiffness. [28] ARFI is implemented in the ultrasound scanner and it evaluates deep tissues stiffness providing complementary information useful for the diagnosis. [29] ARFI is able to identify the presence of cirrhosis with good accuracy and it can differentiate between different stages of fibrosis. [30, 31] Moreover, many studies proved the ability of ARFI to differentiate between benign and malignant hepatic lesions. [32-34]

AIM OF THE WORK

The aim of the present work was to study insulin resistance (IR), serum alpha-fetoprotein (AFP); adiponectin and ferritin levels in HCV-positive cirrhotic patients with and without HCC. Also,

these markers were assessed in early stage HCC patients after successful treatment with radiofrequency ablation (RFA). Moreover, liver stiffness was measured using acoustic radiation force impulse imaging (ARFI) in all studied patients.

SUBJECTS AND METHODS

The present study was conducted on 60 HCV-related cirrhotic patients; the diagnosis was based on positive anti-HCV antibodies, positive HCV RNA, clinical and ultrasonographic findings. Patients were divided into 3 groups; **Group I**: 20 cirrhotic patients with early stage HCC according to The Barcelona Clinic Liver Cancer (BCLC) staging classification [35] (single lesion ≤ 5 cm, or 2-3 nodules ≤ 3 cm) who were fit for RFA, **Group II**: 20 cirrhotic patients with intermediate/advanced stage HCC (single lesion > 5 cm, or multinodular lesions, ± portal vein thrombosis) who were unfit for RFA, **Group III**: 20 cirrhotic patients without HCC.

Recent guidelines for diagnosis of HCC recommended triphasic CT scan or dynamic contrast enhanced MRI, if there are radiologic hallmarks of HCC (arterial enhancement and venous/late phase wash out), then the diagnosis of HCC is made. If the radiologic hallmarks of HCC are not seen then one of two strategies is acceptable, either a second study (CT or MRI, whichever was not performed) or a biopsy. [36]

Exclusion criteria included diabetes mellitus or glucose intolerance, Obesity (BMI ≥30), active alcohol consumption, treatment with corticosteroids or any medications known to affect glucose tolerance or insulin secretion, also, any other concomitant diseases or conditions such as HBV infection, human immunodeficiency virus (HIV) infection, chronic pancreatitis, renal failure or other serious medical problems. Informed written consent was obtained from all participants.

All patients were subjected to the followings:

*Proper history taking and full clinical evaluation focusing on diagnosis of HCV-related liver cirrhosis and HCC.

*Assessment of IR [37] by calculation of homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated by the use of the following equation: HOMA-IR = fasting insulin (μu/ml) × fasting glucose (mmol/L) / 22.5.

*Measurement of serum AFP, [38] adiponectin [39] and ferritin [40] levels using sandwich enzyme-linked immunosorbant assay.

*Radiological Investigations; including:
a- Abdominal ultrasonography (US) [41] to assess liver cirrhosis, hepatic focal lesion(s) as regards site and size, and for needle guidance during percutaneous RFA.
b- Triphasic CT scan [42] to confirm the diagnosis of HCC where the nodules showed the radiologic hallmarks of HCC, also to confirm complete

ablation of the tumor after RFA and to detect tumor recurrence in the follow up period. c- ARFI is a noninvasive; rapid bedside method to assess liver fibrosis by measuring liver stiffness. Liver was examined using B-mode standard ultrasonography with ARFI technology (Siemens Acuson S2000 with 4C1 transducer). [43] ARFI involves mechanical excitation of the tissues using short-duration (~262 microseconds) acoustic pulses with a fixed transmission frequency of 2.67 MHz to generate localized tissue displacements, these displacements result in shear waves, whose velocity (SWV) of propagation can be assessed in a region of interest in (m/s) where the higher the tissue stiffness, the higher the SWV. [44] In the present study, ARFI was performed to assess liver stiffness in cirrhotic patients without HCC **Group III**, also, it was performed in HCC patients **Group I** and **Group II** both intra and extra lesionally. Moreover, in **Group I** patients with early stage HCC who were fit for RFA, it was done both intra and extra lesionally one month after completed RFA.

*RFA for **Group I** patients with early stage HCC was performed percutaneously under US guidance and deep sedation in the presence of an anesthetist and with continuous non-invasive hemodynamic monitoring. A 15 cm long, 17 Gauge electrode was used to deliver radiofrequency energy into the tumor from a mono-polar radiofrequency generator (RITA Medical Systems, Inc. USA); also, a standard grounding pad was placed on each of the patient's thighs. [45]

*Triphasic CT was performed 2 weeks after the last session of RFA to confirm complete response. HOMA-IR, serum AFP; adiponectin; and ferritin levels were measured at 1, 3, 6, 9 and 12 months after initial complete RFA of HCC lesion. Also, triphasic CT was done at the same intervals during the follow up period to confirm complete tumor ablation or recurrence.

*In case of tumor recurrence, if the patient still met the inclusion criteria, RFA was repeated, while in case of multicentric HCC, either TACE or only symptomatic treatment was performed according to BCLC algorithm. [35]

RESULT

HOMA-IR, serum AFP; adiponectin and ferritin levels: (Table 1)

The mean HOMA-IR was 2.56±1.76 in **Group I**, 6.52±7.06 in **Group II** and 1.74±1.48 in **Group III**. It was significantly higher (P = 0.005) in **Group II** in comparison to **Groups I** and **III**.

The mean serum AFP level was significantly higher (P = 0.001) in **Group II** (824.45±727.2 ng/ml) compared to **Group I** (79.04±62.85 ng/ml) and **Group III** (3.85±2.5 ng/ml).

The mean serum adiponectin level was 0.47±0.39 ng/ml in **Group I**, 0.40±0.22 ng/ml in **Group II** and 1.17±1.1 ng/ml in **Group III**, it was significantly higher (P = 0.002) in **Group III** compared to HCC patients of **Groups I** and **II**.

Moreover, the mean serum ferritin level was 170.9±155.8 ng/ml in **Group I**, 518.9±402.93

ng/ml in **Group II** and 319.9±301.9 ng/ml in **Group III**; it was significantly higher in HCC patients compared to cirrhotic non-HCC patients. Also, it was significantly higher (P = 0.005) in intermediate/advanced stage HCC **Group II** compared to early stage HCC **Group I**.

Table (1): Comparison between different studied groups as regards HOMA-IR, serum AFP; adiponectin and ferritin levels.

	Group I (n = 20)	Group II (n = 20)	Group III (n = 20)	Anova-Test
HOMA-IR				
▪ **Range**	0.88-6.38	1.26-30.47	1.2-8.07	F= 5.729
▪ **Mean ± SD**	2.56±1.76	6.52±7.06	1.74±1.48	P = 0.005*
Serum AFP (ng/ml)				
▪ **Range**	3.5-312	2.4-372	0.8-9.1	F= 8.355
▪ **Mean ± SD**	79.04±62.85	824.45 ±727.2	3.85±2.5	P = 0.001*
Serum adiponectin (ng/ml)				
▪ **Range**	0.08-1.57	0.15-0.90	0.18-3.8	F= 6.974
▪ **Mean ± SD**	0.47±0.39	0.40±0.22	1.17±1.1	P = 0.002*
Serum ferritin (ng/ml)				
▪ **Range**	12-580	42-1150	18-1170	F= 5.852
▪ **Mean ± SD**	170.9±155.8	518.9±402.93	319.9±301.9	P = 0.005*

ARFI reading values in different studied groups: (Table 2, Figure 1, 2)

By comparing ARFI reading values in HCC groups intra-lesionally, the SWV was significantly higher (p= 0.000) in intermediate/advanced stage HCC patients **Group II** (3.9±0.74 m/s) compared to early stage HCC patients **Group I** (2.66±0.70 m/s).

While, comparing ARFI reading values in diffuse liver tissue between the 3 studied groups revealed that the SWV was significantly higher (p= 0.046) in **Group II** (2.8±0.45) compared to **Group I** (2.51±0.34) and **Group III** (2.45±0.59).

Table (2): ARFI reading values among different studied groups

	ARFI				
	Intra-lesional		Extra-lesional		
	Group I	Group II	Group I	Group II	Group III
Min-Max	1.42-4.18	1.01-4.51	1.55-3.05	2.12-4.11	1.27-3.44
Mean±SD	2.66±0.70	3.9±0.74	2.51±0.34	2.8±0.45	2.45±0.59
	t = 14.26		F= 3.261		
	P = 0.000*		P = 0.046*		

P* is significant if < 0.05

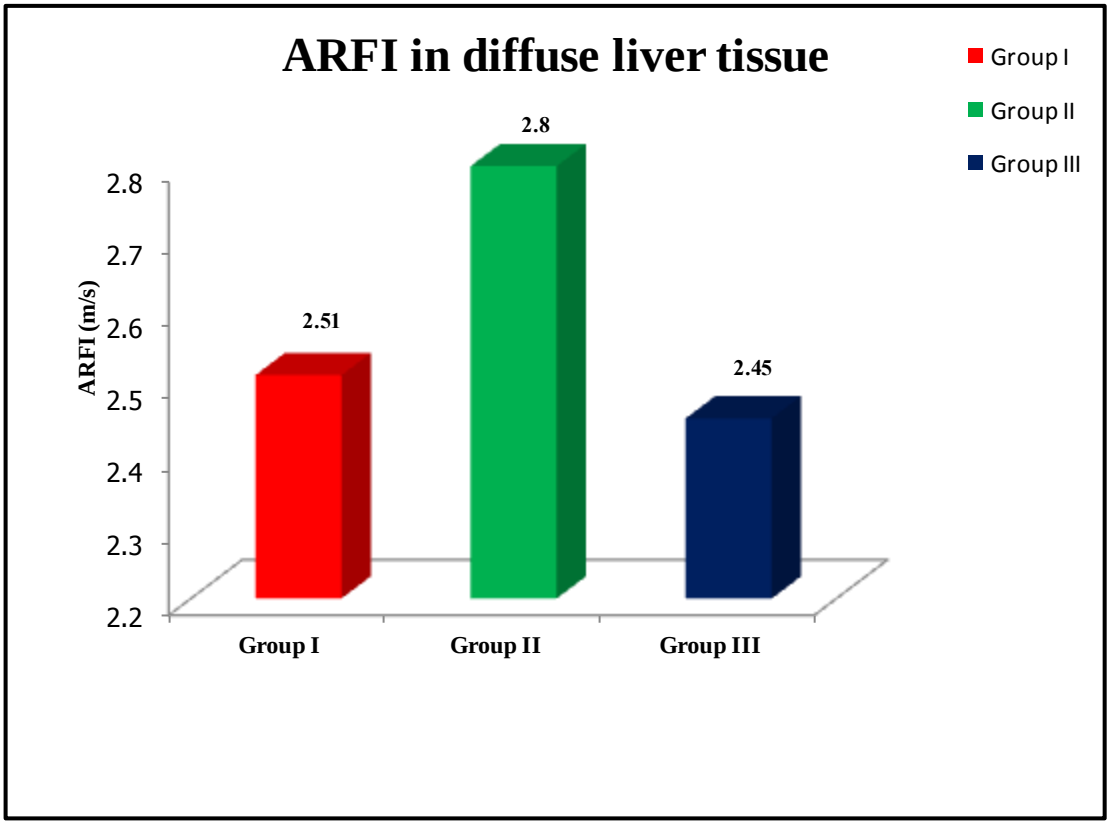

Figure (1): ARFI reading values inside HCC lesions in groups I & II.

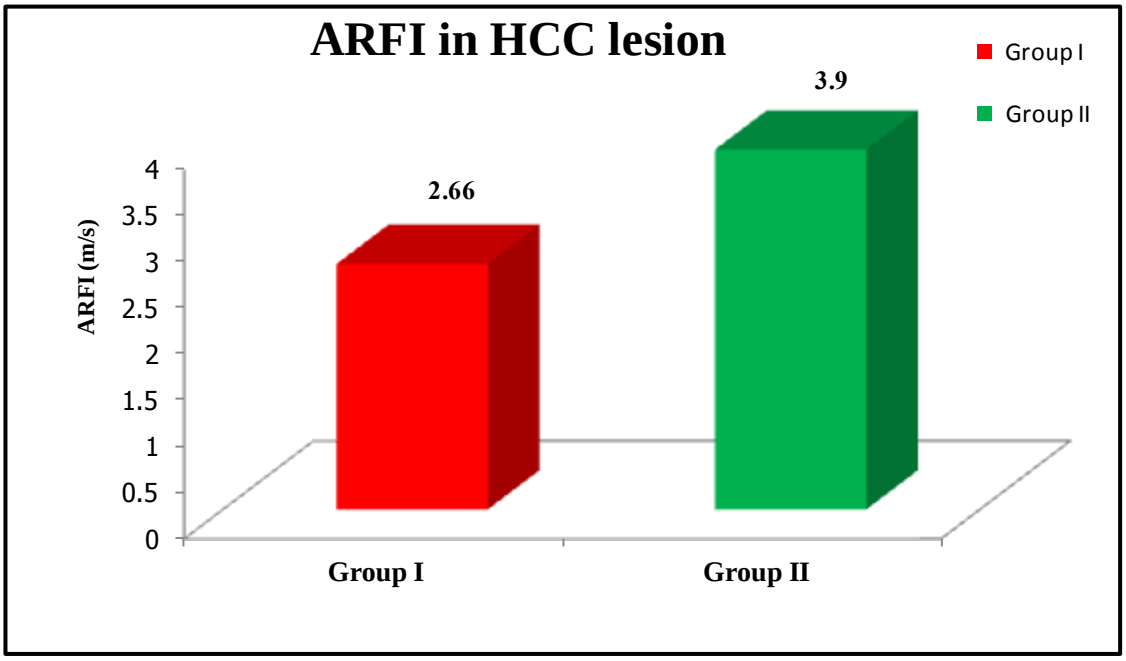

Figure (2): ARFI reading values in diffuse liver tissue in different studied groups.

Follow up changes in HOMA-IR level in cured HCC patients of Group I:

Before doing RFA for early stage HCCs in **Group I** patients, the mean basal HOMA-IR was 2.56±1.76. One month later, all patients (n=20) were cured; the mean HOMA-IR decreased significantly to 2.09±1.31. After three months, only 17 patients showed complete response with no recurrence or denovo lesions and the mean HOMA-IR (2.04 ±1.06) was significantly lower than the basal value (2.77±1.78). After six months, the mean HOMA-IR (1.18±0.57) was significantly lower than the basal value (2.15±1.87) in cured patients (11 patients). After nine months, the mean HOMA-IR (1.56±0.82) was significantly lower than the basal value (2.57±1.96) in cured patients (10 patients). Lastly, after 12 months; the mean HOMA-IR (1.68± 1.07) was significantly lower than the basal value (2.33±1.66) in cured patients (9 patients). **(Table 3)**

Table (3): Follow up changes in HOMA-IR in cured HCC patients of Group I.

	HOMA-IR									
	n = 20		n = 17		n = 11		n = 10		n = 9	
	Basal	After 1 month	Basal	After 3 months	Basal	After 6 months	Basal	After 9 months	Basal	After 12 months
Mean	2.56	2.09	2.77	2.04	2.15	1.18	2.57	1.56	2.33	1.68
SD	1.76	1.31	1.78	1.06	1.87	0.57	1.96	0.82	1.66	1.07
T		**2.134**		**2.141**		**2.237**		**2.339**		**2.551**
P		**0.046***		**0.048***		**0.049***		**0.044***		**0.031***

Follow up changes in serum AFP level in cured HCC patients of Group I:

Before doing RFA for early stage HCCs in **Group I** patients, the mean basal serum AFP level was 79.04±62.85 ng/ml. After one month, all patients were cured and the mean serum AFP level decreased significantly to 16.12±15.81 ng/ml. After three months, the mean serum AFP level(6.71±3.57 ng/ml) was significantly lower than the basal level (83.62±67.38 ng/ml) in cured patients (17 patients). After six months, the mean serum AFP level (6.4±6.54 ng/ml) was significantly lower than the basal level (69.43±53.89 ng/ml) in cured patients (11 patients). After nine months, the mean serum AFP level (3.55±1.86 ng/ml) was significantly lower than the basal level (67.12±55.48) in cured patients (10 patients). Lastly, after 12 months; the mean serum AFP level (7.38±6.34 ng/ml) was significantly lower than the basal level (70.63±56.95) in cured patients (9 patients). **(Table 4)**

Table (4): Follow up changes in serum AFP level in cured HCC patients of Group I.

	AFP (ng/ml)									
	n = 20		n = 17		n = 11		n = 10		n = 9	
	Basal	After 1 month	Basal	After 3 months	Basal	After 6 months	Basal	After 9 months	Basal	After 12 months
Mean	79.04	16.12	83.62	6.71	69.43	6.40	67.12	3.55	70.63	7.38
SD	62.85	15.81	67.38	3.57	53.89	6.54	55.48	1.86	56.95	6.34
T		**5.198**		**4.780**		**3.853**		**3.572**		**3.445**
P		**0.000***		**0.000***		**0.003***		**0.006***		**0.007***

Follow up changes in serum adiponectin level in cured HCC patients of Group I:

Before doing RFA for early stage HCCs in **Group I** patients, the mean basal serum adiponectin level was 0.47±0.39 ng/ml. After one month, all patients were cured and the mean serum adiponectin level increased significantly to 0.57±0.38 ng/ml. After three months, the mean serum adiponectin level (0.49±0.25 ng/ml) was significantly higher than basal adiponectin level(0.39±0.29 ng/ml) in cured patients (17 patients). After six months, the mean serum adiponectin level (0.59±0.47 ng/ml) was significantly higher than basal serum adiponectin level (0.46±0.44 ng/ml) in cured patients (11 patients). After nine months, the mean serum adiponectin level (0.62± 0.44 ng/ml) was significantly higher than the basal level (0.47±0.44 ng/ml) in cured patients (10 patients). Finally, after 12 months the mean serum adiponectin level (0.62 ±0.38 ng/ml) was significantly higher than its basal level (0.49±0.45 ng/ml) in cured patients (9 patients). **(Table 5)**

Table (5): Follow up changes in serum adiponectin level in cured HCC patients of Group I.

	Serum adiponectin (ng/ml)									
	n = 20		n = 17		n = 11		n = 10		n = 9	
	Basal	After 1 month	Basal	After 3 months	Basal	After 6 months	Basal	After 9 months	Basal	After 12 months
Mean	0.47	0.57	0.39	0.49	0.46	0.59	0.47	0.62	0.49	0.62
SD	0.39	0.38	0.29	0.25	0.44	0.47	0.44	0.44	0.45	0.38
T		**2.306**		**2.148**		**2.261**		**2.356**		**3.680**
P		**0.033***		**0.047***		**0.047***		**0.043***		**0.005***

Follow up changes in serum ferritin level in cured HCC patients of Group I:

There were no statistical significant changes in serum ferritin level through all follow up periods after RFA (at 1, 3, 6, 9, and 12 months).

Follow up changes in ARFI reading values in cured HCC patients of Group I:

Before RFA, the mean intra-lesional ARFI reading value was 2.66±0.70 m/s; it was decreased significantly one month after RFA to 0.73±0.14 m/s. On the other hand, the mean extra-lesional ARFI reading value was 2.51±0.34 m/s before RFA, and it was 2.63±0.42 m/s after ablation with no statistical significant changes. **(Table 6)**

Table (6): ARFI reading values before and after RFA in early stage HCC patients of Group I.

	ARFI			
	Intra-lesional		Extra-lesional	
	Before Ablation	After Ablation	Before Ablation	After Ablation
Min – Max	1.42 – 4.18	0.49 -1.07	1.55 -3.05	1.52 – 3.35
Mean ± SD	2.66 ± 0.70	0.73 ± 0.14	2.51 ± 0.34	2.63 ± 0.42
T		**13.029**		**1.601**
P		**0.000***		**0.126**

Multivariate analysis of markers of cure in HCC patients of Group I:

Multivariate analysis of markers of cure in early stage HCC patients of **Group I** showed that the best one is serum AFP level (P = 0.000), followed by serum adiponectin level (P=0.010) then HOMA-IR (P = 0.032). On the other hand, serum ferritin level was not a statistically significant marker. **(Table 7)**

Table (7): Multivariate analysis of markers of cure in HCC patients of Group I.

	Regression coefficient	P
AFP (ng/ml)	0.281	0.000*
Serum adiponectin (ng/ml)	0.188	0.010*
HOMA-IR	0.140	0.032*
Serum ferritin (ng/ml)	0.000	0.885

DISCUSSION

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, hepatocellular carcinoma (HCC), and recently HCV has been identified as a cause of metabolic syndrome which includes dyslipidemia, diabetes and insulin resistance (IR). IR is a key feature of this syndrome and a variety of potential molecular pathways by which HCV may contribute to IR have been suggested. (46)

Recent studies have found that HCV-associated IR causes hepatic steatosis, resistance to anti-viral treatment, hepatic fibrosis, esophageal varices, hepato-carcinogenesis and proliferation of HCC where IR may synergize with viral hepatitis in HCC development. (47)

In the present study, HOMA-IR was significantly higher in intermediate/advanced stage HCC patients **Group II** compared to early stage HCC and HCV-positive cirrhotic patients **Groups I** and **III** respectively. In consistent with our results, Donadon V et al, (48) found that the mean levels of HOMA-IR increase progressively among chronic hepatitis C (CHC), liver cirrhosis and HCC patients.

Our results were also in agreement with those reported by Mohamed AA et al, (49) who investigated the effects of HCV genotype-4 on the prevalence of IR in CHC and HCC Egyptian patients. They reported that HCC patients showed higher IR frequency; moreover, both moderate and high viral loads were significantly associated with higher values of HOMA-IR compared to those with lower viral loads ($p < 0.001$). Similarly, Hung CH et al, (50) had demonstrated that IR is associated with high risk of HCC development in patients with chronic HCV, thus, HOMA-IR measurement could represent a novel marker to identify hepatic patients at risk for the progression of liver carcinogenesis. (51)

Similar results were proved by Nkontchouemail G et al, (52) who reported that in patients with compensated HCV cirrhosis, high HOMA-IR index was associated with HCC occurrence ($p=0.026$), and it was a strong predictor of liver related death or transplantation ($p<0.0001$).

Our data revealed that the mean serum AFP level was significantly higher ($P = 0.001$) in intermediate/advanced stage HCC patients **Group II** compared to early stage HCC patients **Group I** and HCV-positive cirrhotic patients **Group III**. This goes with Pawlik TM et al, (53) who reported a strong association between high serum AFP level, tumor size and vascular invasion. Also, Arrieta O et al, (54) stated that AFP level was significantly higher in patients with HCC compared to patients with liver cirrhosis.

As regards serum adiponectin level, our results showed that it was significantly higher ($P = 0.002$) in HCV-positive cirrhotic patients **Group III** (1.17±1.1 ng/ml) compared to HCC patients **Group I** and **Group II** (0.47±0.39 ng/ml and 0.40±0.22 ng/ml respectively).

In agreement with our results, Ebrahim M et al, (55) concluded that decreased circulating adiponectin levels may play a role in the

development and progression of HCC. Also, Saxena NK et al, [56] concluded that adiponectin protects against liver tumorigenesis and its reduced expression is associated with poor prognosis in obese patients with HCC.

Against our results, a study done by Wang SN et al, [57] suggested that increased adiponectin level may contribute to HCC through its activation of AKT signaling. Also, Arano T et al, [58] concluded that patients who had CHC with high serum adiponectin levels had a higher risk of HCC development.

As regards the mean serum ferritin level, our research reported that it was significantly higher in HCC patients compared to non-HCC cirrhotic patients, also it was significantly higher (P = 0.005) in intermediate/advanced stage HCC patients **Group II** compared to early stage HCC patients **Group I**. A recent study by Patil PS et al, [59] reported that the mean serum ferritin values were higher in HCC cases as compared to controls (425.8 vs. 135.6 ng/mL, p = 0.000). They hypothesized that long-term iron deficiency could attenuate the hepatic inflammation and lead to a lower incidence of HCC.

By following-up early stage HCC patients **Group I** who performed RFA, our data showed that the mean HOMA-IR level was significantly decreased from the basal level (2.56±1.76) before RFA to (2.09±1.31, 2.04±1.06, 1.56±0.82 and 1.68±1.07) at 1, 3, 6, 9 and 12 months respectively. Our results were in line with Imai K et al, [60] who proved that IR raises the risk of HCC.

Also, our data showed that the mean serum AFP level was significantly decreased from the basal level (79.04±62.85 ng/ml) before RFA to (16.12±15.81 ng/ml, 6.71±3.57 ng/ml, 6.40±6.14 ng/ml, 3.55±1.86 ng/ml & 7.38±6.34 ng/ml) at 1, 3, 6, 9, 12 months respectively in all cured HCC patients after RFA. Similarly, Berry K et al, [61] concluded that the absolute serum AFP level and the changes in its level strongly predict survival independently of the tumor burden.

Moreover, the mean serum adiponectin level was significantly increased from the basal level (0.47±0.39 ng/ml) before RFA to (0.57±0.38 ng/ml, 0.49±0.25 ng/ml, 0.59±0.47 ng/ml, 0.62±0.44 ng/ml and 0.62±0.38 ng/ml) at 1, 3, 6, 9 and 12 months respectively in all cured patients after RFA. This was in agreement with Saxena NK et al, [56] who concluded that adiponectin protects against liver tumorigenesis and its reduced expression is associated with poor prognosis in obese patients with HCC.

As regards serum ferritin level, there were no statistical significant changes in its level through all follow up periods after RFA (at 1, 3, 6, 9, and 12 months).

In the present study, intra-lesional ARFI reading values were significantly higher (p= 0.000) in intermediate/advanced HCC patients **Group II** (3.9±0.74 m/s) compared to early stage HCC patients **Group I** (2.66±0.70 m/s). Also,

comparing extra-lesional ARFI reading values between the 3 studied groups revealed significantly higher values (p= 0.046) in intermediate/advanced HCC patients **Group II** (2.8±0.45 m/s) compared to early stage HCC patients **Group I** (2.51± 0.34 m/s) and cirrhotic non-HCC patients **Group III** (2.45± 0.59 m/s).

By comparing the intra-lesional ARFI reading values in early stage HCC patients **Group I** before and one month after RFA, our study showed that before ablation, ARFI value was 2.66±0.70 m/s, and it was decreased significantly one month after ablation to 0.73±0.14 m/s. While, the extra-lesional ARFI value was 2.51±0.34 m/s, and it was 2.63±0.42 m/s after ablation with no statistical significant changes.

In agreement with our data, Kwon HJ et al, [62] evaluated the potential usefulness of ARFI imaging to detect recurred HCCs where the brightness of the tumor was checked and the SWV was measured for the quantification of stiffness.

CONCLUSION

HOMA-IR, serum AFP and adiponectin levels can be used as predictors for high risk cirrhotic patients who may develop HCC. Moreover, these markers can be used to follow up cured HCC patients to confirm treatment response and detect de novo lesions.

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