Role of Insulin Resistance and Cytokeratin 18 on the Recurrence of Hepatocellular Carcinoma after Radiofrequency Ablation

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Background and study aim: Many studies have reported that insulin resistance raises the risk of primary hepatocellular carcinoma (HCC). Caspase-generated cytokeratin-18 (CK-18) fragments are produced during apoptosis of hepatic cells. The present study aims to evaluate the value of serum CK-18 and the impact of insulin resistance on recurrence of HCV related HCC in patients treated with radiofrequency ablation (RFA).

Patients and Methods: The present study was conducted on 60 HCV patients. Group I: 30 patients with HCC treated by RFA and group II: 30 non HCC cirrhotic patients. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) and CK-18 level was measured for all patients using ELISA.

Results: HOMA-IR and CK-18 levels were significantly elevated in HCC patients when compared to non HCC cirrhotic patients. There was a significant increase in CK-18 and HOMA-IR levels in patients with local recurrence versus those with no recurrence ($p<0.001$). Univariate analysis revealed that HOMA-IR ($p=<0.001$) and CK-18 ($p=<0.001$) were significant predictors of recurrence of HCC.

Conclusion: CK-18 and HOMA-IR are potential prognostic markers as they can predict the recurrence of HCC.

INTRODUCTION

Hepatoceullar carcinoma (HCC) is a common cause of cancer-related death and its incidence is increasing worldwide **[1]**. Egypt has a rising rate of HCC. The currently increasing incidence of HCC in Egyptians may be due to the increased prevalence of hepatitis C virus (HCV) as a primary risk factor **[2]**. Diabetes mellitus (DM) increased the risk of chronic non-alcoholic liver disease and HCC in male patients without concomitant liver disease **[3]**. Insulin resistance (IR) is a condition where body cells fail to respond to the normal actions of insulin leading to hyperglycemia **[4]**.

Steatohepatitis is associated with IR and characterized by inflammation, apoptosis of liver cells, fat and fibrotic tissue accumulation in the liver, which may progress to cirrhosis and HCC. IR seems to play a fundamental role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) **[5]**.

Current data suggest that an alteration in the regulation of hepatocyte apoptosis could play an important role in hepatic damage, steatohepatisis and HCC progression **[6]**.

The HCV core protein induces insulin resistance by increasing tumor necrosis factor α which disturb tyrosine phosphorylation of insulin receptor substrate-1 thus, diabetes mellitus including insulin resistance seems to be closely associated with various liver diseases that can lead to HCC **[7]**.

Liver cell damage in chronic HCV infection is mediated by the induction of apoptosis. During apoptosis, there is an activation of specific intraceullar protesases (caspases) that able to cleave

different substrates, including cytokeratin-18 (CK-18), a major inter-mediate filament protein in the liver. The action of these caspases shows a neoepitope in CK-18(M_{30}) which is not detectable in alive or necrotic cells **[8]**.

When surgery is not possible for HCC, there are several minimally invasive options for tumor ablation either chemical or thermal **[9-11]**. The most frequent event observed during the follow up of curatively treated HCC patients is intrahepatic recurrence **[12-14]**. Which may be either local tumor progression or intrahepatic distant recurrence **[15]**.

Radiofrequency ablation (RFA) is among therapies that offer a high rate of complete response and potential cure . RFA induces tumor destruction by heating the tumor tissue to a temperature that exceeds 60^{o}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 result in coagulative necrosis of the tumor tissue with denaturation of the intra-cellular proteins and dissolution of the cell membrane lipid bilayers **[16]**.

The objective of this study is to evaluate the impact of insulin resistance and cytokeratin 18 on HCC recurrence after initial treatment with RFA.

PATIENTS AND METHODS

The present study was carried out in Tropical Medicine, Radiotherapy, Clinical Pathology and Family Medicine Departments, Zagazig University. The study included 60 chronic HCV patients; the diagnosis of HCV was based on positive anti-HCV antibodies. Patients were divided into 2 groups: group I: 30 patients with HCV related HCC, group II: 30 patients with HCV related cirrhosis without HCC.

Exclusion criteria included diabetes mellitus, treatment with any medication known to affect glucose tolerance or insulin secretion, alcoholics, pregnant and breast feeding women.

This study was approved by the local Ethical Committee of Faculty of Medicine, Zagazig University and an informed written consent was obtained from each participant in the study.

All patients were subjected to the following:

- Full history taking and clinical examination.
- Venous blood samples were taken from all patients, serum was separated from the cellular fraction via centrifugation at 5,000 RPM for 3 minutes. Serum samples were kept at-80°C till the time of assay. Serum CK-18 assay was performed using CK-18 M30 apoptosis ELISA assay (PEVIVA, Alexis, Günwald, Germany).
- HCVAb in serum was detected by ELISA third generation, using kit from ORTHO. Catalogue number; 631300942.
- Insulin resistance using homoestasis model assessment of insulin resistance (HOMA-IR) which was calculated on the basis of fasting values of plasma glucose and insulin according to the HOMA model formula; HOMA-IR= fasting insulin (mIU/L) x fasting glucose (mmol/L)/ 22.5 **[17]**.
- AFP level was measured on Cobas E411 immunoassay (Roche Diagnostics, USA) **[18]**.
- Routine laboratory tests include liver function tests: Albumin, total bilirubin, ALT, AST; were analyzed using Cobas 501 (Roche diagnostics, Switzerland) and platelet count was done by automated cell counter Cell Dyne (APOTT, USA).

Radiological investigations including:

a) Abdominal ultrasonography (US) to assess liver cirrhosis, hepatic focal lesions as regard its number, size and for needle guidance during percutaneous RFA**[19]**.

b) Triphasic CT scan to confirm the diagnosis of HCC, diagnosis of HCC based on the typical hypervascular tumor stain on angiography and typical dynamic-study findings of enhanced staining in the early phase and attenuation in the delayed phase**[20]**.

Tumor ablation:

The technique was started by a small puncture into the skin using a scalpel (No 11). We used a common, commercially available RFA technique and system (RITA 1500X RF generator and RITA StarBurst XL, RITA Medical Systems, Mountain View, California). Grounding was achieved by attaching 2 pads to the patient's thighs. After administration of analgesia as well as local anesthesia, the electrode needles were introduced into the tumor under ultrasonographic guidance, then a gradual unfolding of the electrodes was obtained, and the generator was

activated to achieve RF energy and maintain an average temperature of 105°C. At first, the electrodes were moved by 2 cm, then the electrode needles were pushed forward and unfolded gradually to 3 cm, 4 cm and 5 cm until they reached or crossed the borders of the tumor according to the ablation range, delivering RF energy for 5 minutes for every intermediate step and for 7 to 10 minutes in the final step of the procedure. The ablation area was intended to cover the tumor as well as at least 0.5 to 1.0 cm of the surrounding tissue **[21]**. In case of tumor recurrence, RFA was repeated while in cases of multiple new focal lesions or metastases other treatment options were performed **[22]**.

Follow up of therapy

Triphasic CT, HOMA-IR, serum AFP and CK-18 levels were performed at 1, 3 and 6 months after the last session of RFA to evaluate the response to treatment and its effect on these parameters.

Statistical Analysis

Data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Quantitative variables were expressed as mean ± SD and median (range). Mann Whitney U test was used to compare between two groups of independent non-normally distributed data. Friedman test was used to compare more than two groups of dependent non-normally distributed data. The Spearman's rank correlation coefficient (r) was calculated to assess the relationship between CK-18 & selected study parameters. Receiver Operating Characteristic (ROC) curves were obtained to calculate the optimized cutoff point for CK-18, AFP, and HOMA-IR to reach the best compromise in the prediction of recurrence. The cutoff point with maximum sensitivity and specificity (validity) is used as the recommended cutoff point and also Area Under Curve (AUC) was calculated. Survival (S) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). Stratification of S was done according to CK-18 & HOMA-IR subgroups. These time-to-event distributions were estimated using the method of Kaplan-Meier plot, and compared using two-sided exact log-rank test. All tests were two sided with $p<0.05$ was considered statistically significant (S), $p<0.01$ was considered highly statistically significant (HS), and $p>0.05$ was considered non statistically significant (NS).

RESULTS

This study was conducted on 30 HCV related HCC patients and 30 HCV related cirrhotic patients. Their demographic, clinical and biochemical characteristics are shown in Table 1. There was a statistically significant difference in the studied markers (AFP, CK-18 and HOMA-IR) among subgroups of Child classes as well as tumor size in HCC patients (Table 2). There was positive significant correlation between CK-18 and maximum tumor diameter, AFP and HOMA-IR. Also, there was positive significant correlation between HOMA-IR and maximum tumor diameter and AFP (Table 3 & Figure 1).

After treatment with RFA, the level of HOMA-IR, CK18 and AFP showed significant reduction in the responded patients [1 month 26 patient, 3 months 21 patient and lastly, after 6 months 18 patients] (Table 4).

Most patients with recurrent tumor were in Child class B and all patients with recurrence had maximum tumor diameter (3-5cm) ($P<0.001$). CK18 and HOMA-IR level were significantly increased in patients with recurrent HCC versus those without recurrence [324.8(317.9-340.9)] versus 308.5 (287.8-317.2) ng/ml, $P<0.001$ and 3-4(2.9-6.4) versus 2.3(1.29), $P<0.001$) respectively (Table 5).

According to receiver operating characteristic curve (ROC) the most accurate predictor of recurrence in HCC patients was CK-18 (Table 6 & Figure 2). Six months recurrence-free survival in total was 60% (Table 7). Analysis of the data using Kaplan-Meier estimates of survival probability for patients with serum CK-18 ≤318.9 ng/mL and HOMA-IR ≤2.7 showed significant longer free survival and higher overall survival probability when compared to CK-18> 318.9 and HOMA-IR>2.7 (p value=<0.001) (Tables 7 and 8, Figures 3 and 4).

Table (1): Demographic, clinical and biochemical characteristics of studied groups

Demographic, clinical and biochemical characteristics	HCC patients (N=30)	Cirrhotic patients (N=30)	*t*	p-value
Demographic characteristics				
Age(year)	53 (25 – 68)	50 (34 – 57)	-1.672‡	0.095
Sex				
Male	27 (86.7%)	25 (83.3%)	0.180*	0.672
Female	3 (13.3%)	5 (16.7%)		
Clinical characteristics				
Splenomegaly	8 (26.7%)	0 (0%)	10.479*	0.001
Lower limb edema	5 (16.7%)	2 (6.7%)	1.731*	0.188
Hepatic Encephalopathy	1 (3.3%)	0 (0%)	1.552*	0.213
Child-Pugh classification				
Child A	21 (70%)	-	-	
Child B	9 (30%)	-	-	
Diameter of focal lesion (cm)	3.5 (1.9 – 5)			
Biochemical characteristics				
AST (IU/L)	83.5 (43 – 203)	17 (12 – 23)	-7.705‡	<0.001
ALT (IU/L)	77.5 (41 – 216)	16 (11 – 21)	-7.706‡	<0.001
Total bilirubin (mg/dL)	1.4 (0.7 – 3.8)	0.6 (0.2 – 1.5)	-6.531‡	<0.001
Serum albumin (g/dl)	2.8 (2.2 – 3.4)	2.8 (1.1 – 5.1)	-0.360‡	0.719
Platelet count ($x10^3/mm^3$)	160 (96 – 210)	273.5 (233 – 298)	-7.705‡	<0.001
AFP	215.5 (2 – 398)	2.7 (0.8 – 8.7)	-7.579‡	<0.001
CK-18 (ng/mL)	431.4 (287.8 – 557)	92.6 (81.4 – 100)	-5.797‡	<0.001
HOMA-IR	3 (1 – 24)	1.3 (1 – 2.2)	-5.250‡	<0.001

Continuous variables were expressed as the median (range); Categorical variables were expressed as number (percentage); ‡ Mann Whitney U test;* Chi-square test; p< 0.05 is significant.

Table (2): AFP, CK-18 and HOMA-IR difference among different demographic and clinical subgroups of HCC patients (N=30)

	AFP	CK18	HOMA-IR
Sex			
Male (n=27)	62 (2 – 321)	314.4 ± 13.4	2.6 (1 – 6.3)
Female (n=3)	49 (12 – 63)	308.8 ± 10.7	2.5 (2 – 2.7)
Test	-0.899‡	0.702•	-0.729‡
p-value	0.369	0.489	0.466
Child-Pugh classification			
Child A (n=21)	41.7 ± 25.1	310.9 (287.8 – 317.9)	2.3 ± 0.5
Child B (n=9)	187.9± 65.1	328.2 (318.9 – 340.9)	4.2 ± 1.2
t	-6.532•	-4.279‡	-4.381•
p-value	<0.001	<0.001	<0.001
Diameter of focal lesion			
< 3 cm (n=4)	11.5 (2 – 12)	292.2 ± 3.76	1.6 (1 – 2)
3 – 5 cm (n=26)	65.5(23 – 321)	317.2 ± 10.55	2.8 (2 – 6.3)
t	-3.173‡	-4.639•	-3.065‡
p-value	0.002	<0.001	0.002

Continuous variables were expressed as the mean ± SD for normally distributed data or median (range) for non-normally distributed data; ‡ Mann Whitney U test; • Independent samples Student-t test;* Chi-square test; p< 0.05 is significant.

Table (3): Correlation of CK-18 level and HOMA-IR with other studied parameters in HCC patients (N=30)

Studied parameters	CK-18 level		HOMA-IR	
	R	P	r	P
Mean diameter of focal lesion	+ 0.986	<0.001	+ 0.980	<0.001
AFP	+ 0.999	<0.001	+ 0.995	<0.001
CK-18	---	---	+ 0.994	<0.001
HOMA-IR	+ 0.994	<0.001	---	---

r Spearman rank correlation coefficient; p< 0.05 is significant.

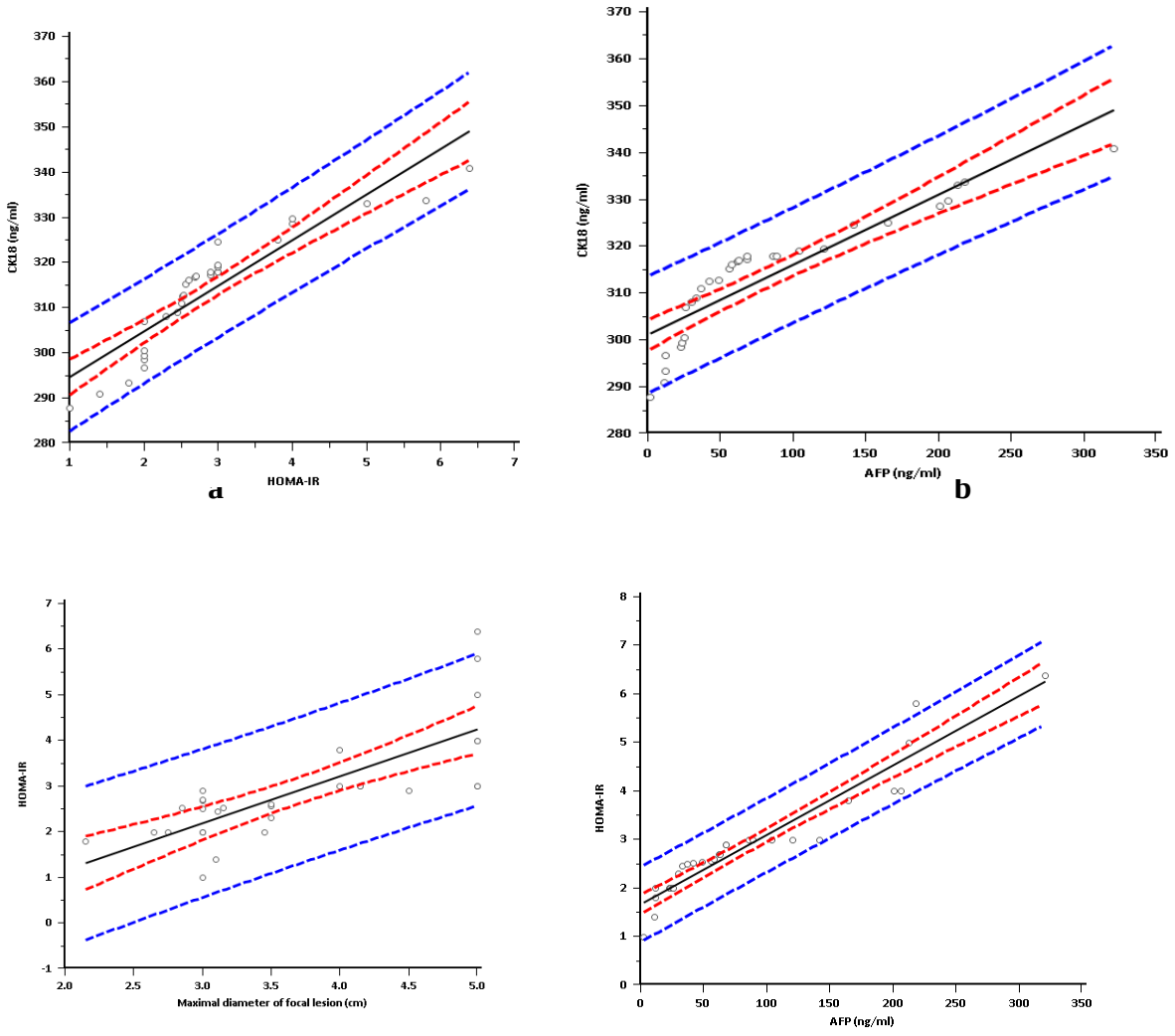


Figure (1): Scatter plot with regression line shows correleation between the studied markers in HCC patients (N=30). Blue lines representing the 95% confidence interval (CI) & red lines representing the 95% prediction interval of regression line. (a) CK-18 & HOMA-IR, (Spearman's rank correleation coefficient r= +0.994, p<0.001); (b) CK-18 & alpha-fetoprotein level (Spearman's rank correleation coefficient r= +0.999, p<0.001); (c) HOMA-IR & maximal diameter of focal lesion, (Spearman's rank correleation coefficient r=0.980, p<0.001); (d) HOMA-IR & alpha-fetoprotein level, (Spearman's rank correleation coefficient r=0.995, p<0.001).

Table (4): Analysis of AFP, CK-18 and HOMA-IR in HCC patients after treatment

	AFP	CK18	HOMA-IR
Before treatment (n=30)	42 (2 – 121)	312.6 (287.8 – 319.4)	2.5 (1 – 3)
1 month after treatment (n=26)	21.6 (10.5 – 33.5)	247.8 (220 – 257.5)	1.4 (0.2 – 2.01)
3 month after treatment (n=21)	19 (11.9 – 33.5)	216.4 (203.6 – 228)	1.1 (1 – 1.8)
6 month after treatment (n=18)	15 (10.8 – 66.8)	192 (146.2 – 202)	1.02 (0.3 – 1.3)
X^2	38.053	69.000	55.339
p-value	<0.001	<0.001	<0.001

Continuous variables were expressed as the median (range); χ^2 Friedman test; p< 0.05 is significant.

Table (5): Univariate analysis difference in characteristics of patients with recurrent HCC versus those without recurrence

Parameter	No recurrence (N=18)	Recurrence (N=12)	Test	p-value
Age	40.2 ± 9.4	50.7 ± 4.3	3.598•	0.003
Sex				
Male	15 (83.3%)	12 (100%)	2.222*	0.136
Female	3 (16.7%)	0 (0%)		
Clinical characteristics				
Child-Pugh classification				
Child A	18 (100%)	3 (25.0%)	19.286*	<0.001
Child B	0 (0%)	9 (75.0%)		
Diameter of focal lesion				
< 3 cm	4 (22.2%)	0 (0%		
3 – 5 cm	14 (77.8%)	12 (100%)	-4.633‡	<0.001
Biochemical characteristics				
AST (IU/L)	74.5 (59 – 122)	70.5 (43 – 99)	-1.673‡	0.076
ALT (IU/L)	64 (45 – 109)	45 (41 – 90)	-2.375‡	0.034
Total bilirubin (mg/dL)	1.7 ± 0.8	1.6 ± 0.6	0.275•	0.785
Serum albumin (g/dl)	2.8 ± 0.3	2.9 ± 0.3	-0.639•	0.528
Platelet count ($x10^3/mm^3$)	149.8 ± 32.1	153.2 ± 33.4	-0.281•	0.781
AFP	35.1 ± 20.3	161.2 ± 73.8	-5.772•	<0.001
CK18	308.5 (287.8 – 317.2)	324.8 (317.9 – 340.9)	-4.574‡	<0.001
HOMA-IR	2.3 (1 – 2.9)	3.4 (2.9 – 6.4)	-4.572‡	<0.001

Continuous variables were expressed as the mean ± SD for normally distributed data or median (range) for non-normally distributed data; Categorical variables were expressed as number (percentage); ‡ Mann Whitney U test; • Independent samples Student-t test;* Chi-square test; p< 0.05 is significant.

Table (6): Diagnostic performance of AFP, CK-18 and HOMA-IR in prediction of recurrence in HCC patients (N=30).

Cutoff value	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	LR+ (95%CI)	LR- (95%CI)	Accuracy (95%CI)	AUC (95% CI)
AFP > 63	100% (73.2-100)	94.4% (72.7-99.9)	92.3% (64-99.8%)	100% (80.5-100)	18 (16.1-20.1)	0	96.6% (72.9-999)	0.998‡ (0.880-1.000)
CK-18 > 317.2	100% (73.5-100)	100% (81.5-100)	100% (73.5-100)	100% (81.5-100)	18 (16.1-20.1)	0	100% (78.3-100)	1.000§ (0.884-1.000)
HOMA-IR > 2.7	100% (73.5-100)	94.4% (72.7-99.9)	92.3% (64-99.8%)	100% (80.5-100)	18 (16.1-20.1)	0	96.6% (72.9-999)	0.998* (0.880-1.000)

‡p<0.001 (HS); § p<0.001 (HS);*p<0.001 (HS)

ROC curve: Receiver Operating Characteristic curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value; LR+: positive Likelihood Ratio; LR-: negative Likelihood Ratio; AUC: Area Under Curve; 95%CI: 95% Confidence Interval; p< 0.05 is significant.

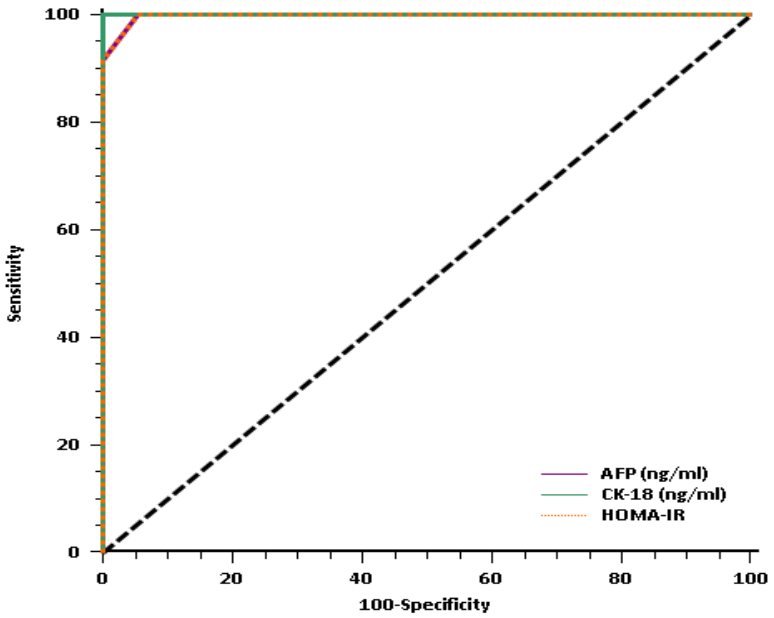


Figure (2): Receiver Operating Characteristic (ROC) curves of AFP, CK18 and HOMA-IR in prediction of recurrence in HCC patients (N=30).

Table (7): Recurrence free survival (RFS) in HCC patients (N=30).

Characteristics	All (N=30)	AFP			CK-18			HOMA-IR		
		≤63 (n=17)	>63 (n=13)	p-value	≤317.2 (n=18)	>317.2 (n=12)	p-value	≤2.7 (n=17)	>2.7 (n=13)	p-value
1 month RFS (%)	86.7%	100%	69.2%	<0.001§	100%	66.7%	<0.001§	100%	69.2%	<0.001§
3 month RFS (%)	70%	100%	30.8%		100%	25%		100%	30.8%	
6 month RFS (%)	60%	100%	7.3%		100%	0%		100%	7.3%	
Recurrent	12 (40%)	0 (0%)	12 (92.3%)	<0.001*	0 (0%)	12 (100%)	<0.001*	0 (0%)	12 (92.3%)	<0.001*
Non-recurrent	18 (60%)	17 (100%)	1 (7.7%)		18 (100%)	0 (0%)		17 (100%)	1 (7.7%)	
Max. tumor diameter < 3 cm	4 (13.3%)	4 (23.5%)	0 (0%)	0.060*	4 (22.2%)	0 (0%)	0.079*	4 (23.5%)	0 (0%)	0.060*
Max. tumor diameter 3 – 5 cm	26 (86.7%)	13 (76.5%)	13 (100%)		14 (77.8%)	12 (100%)		13 (76.5%)	13 (100%)	

Qualitative data are presented as number(%);*Chi-square test.; § Log rank test; p< 0.05 is significant.

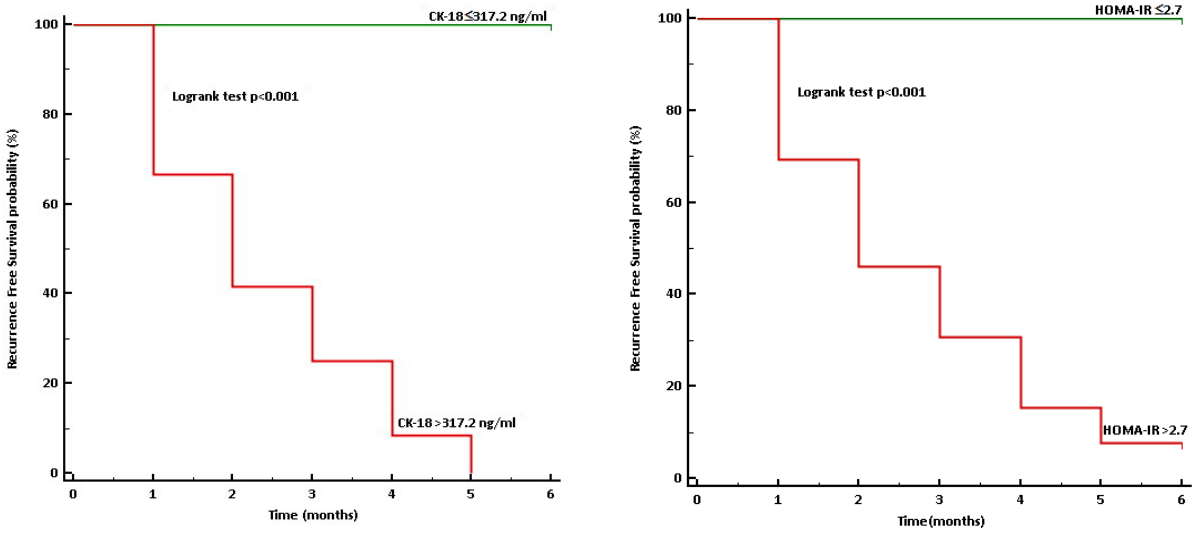
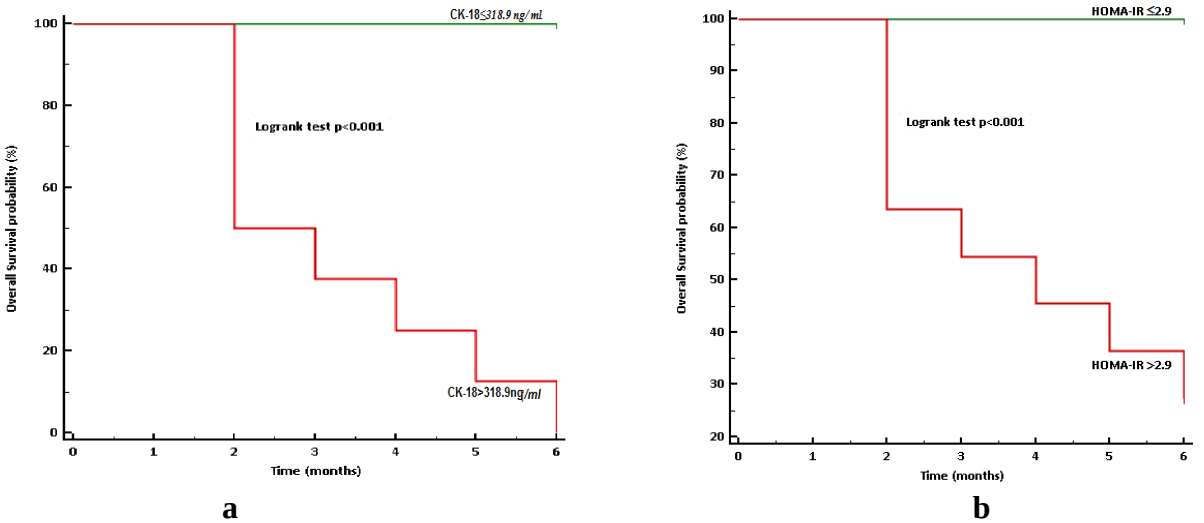


Figure (3): Kaplan-Meier estimates recurrence free survival probability in relation to time in HCC patients (N=30). (a) as regard CK-18 subgroups. (b) as regard HOMA-IR subgroups.

Table (8): Overall survival (OS) in HCC patients (N=30).

Characteristics	All (N=30)	AFP			CK-18			HOMA-IR		
		≤104 (n=22)	>104 (n=8)	p-value	≤318.9 (n=22)	>318.9 (n=8)	p-value	≤2.9 (n=19)	>2.9 (n=11)	p-value
Median OS (months)	NR	NR	2.5	---	NR	2.5	---	NR	4	---
1 month OS (%)	100%	100%	100%	<0.001§	100%	100%	<0.001§	100%	100%	<0.001§
3 month OS (%)	83.3%	100%	37.5%		100%	37.5%		100%	54.5%	
6 month OS (%)	73.3%	100%	0%		100%	0%		100%	27.3%	
Dead	8 (26.7%)	0 (0%)	8 (100%)	<0.001*	0 (0%)	8 (100%)	<0.001*	0 (0%)	8 (72.7%)	<0.001*
Alive	22 (73.3%)	22 (100%)	0 (0%)		22 (100%)	0 (0%)		19 (100%)	3 (27.3%	
Max. tumor diameter < 3 cm	4 (13.3%)	4 (18.2%)	0 (0%)	0.195*	4 (18.2%)	0 (0%)	0.195*	4 (21.1%)	0 (0%)	0.102*
Max. tumor diameter 3 – 5 cm	26 (86.7)	18 (81.8%)	8 (100%)		18 (81.8%)	8 (100%)		15 (78.9%)	11 (100%)	

Qualitative data are presented as number(%);*Chi-square test.; § Log rank test; p< 0.05 is significant.



a **b**

Figure (4): Kaplan-Meier estimates survival probability in relation to time in HCC patients (N=30). (a) as regard CK-18 subgroups. (b) as regard HOMA-IR subgroups.

DISCUSSION

Hepatocellular carcinoma is a worldwide malignancy, and the incidence rate has increased significantly over the past few decades **[23]**. The reason for this increase has not yet been explained clearly, although more than 50 % of this increase has been attributed to hepatitis virus or alcoholic liver disease **[24]**. Insulin resistance is frequently seen in patients with HCV **[25]**. Although in the general population, lack of exercise and overeating are major causes of insulin resistance, in patients with HCV infection, hepatic inflammation, activated inflammatory cytokines, and HCV-induced impairment of insulin and lipid signaling molecules are also important factors for the development of insulin resistance **[26]**.Therefore, the prevalence of insulin resistance is higher in patients with HCV infection compared to that in the general population and patients with other hepatobiliary disorders **[27-28]**.

In the present study HOMA-IR was significantly higher in HCC patients compared to cirrhotic patients. In consistence with our results, Donadon et al. **[29]** found that the mean levels of HOMA-IR increases progressively among chronic hepatitis C, liver cirrhosis and HCC patients. There is association of IR with cancer in various organs **[30]**. The mechanisms by which insulin acts as carcinogenic factor are: First; insulin functions as a growth factor by phosphorylating insulin receptor substrate 1 and second; hyperinsulinemia increases peripheral lipolysis and hepatic accumulation of free fatty acids. The excess B-oxidation in mitochondria and microsome leads to the production of reactive oxygen species **[31]** that play a significant role in carcinogenesis **[32-33]**.

The results of the present study demonstrated highly significant increase in HOMA-IR level in patients with recurrent lesions versus those without recurrence. Also, HOMA-IR had significant correlation with age, tumor diameter, AFP and CK-18.

The strong relation of recurrent HCC to HOMA-IR may be due to the possibility of the presence of microvascular invasion and angiogensis. This finding not only basically agree with previous studies that suggested an association between insulin resistance and carcinogenesis **[34-35]**, but also, suggests that insulin resistance is a significant risk factor for early recurrence of HCC and thus might be a critical target to prevent the recurrence. Muto and his colleagues described that oral supplementation with Branched-chain amino acid (BCAA) granules inhibited liver carcinogenesis in HCV-related liver cirrhosis with DM and obesity **[34]**.

Current data reveled that there was correlation between HOMA-IR and CK-18 (P=<0.001). These finding could be explained by the importance of IR in the induction of hepatocytes apoptosis such that HCV replication and production in infected hepatocytes can induce apoptosis and a release of inflammatory cytokines/chemokines, such as tumor necrosis factor alpha (TNFα), transforming growth factor-Beta (TGFβ), interferon-gamma (IFNγ), interleukin 10 (IL-10), IL-12, IL-22, chemokine receptor (CCR5) ligands, and regulated on activation, normal T cell expressed and secreted (RANTES) **[36-37].** These inflammatory cytokines/ chemokines can stimulate hepatocyte apoptosis through different pathways and during apoptosis there is an activation of specific intracellular proteases that able to cleave different substrates, including cytokeratin-18(CK-18) **[38]**.

By following up HCC patients who performed RFA, this study revealed that the median HOMA-IR level was significantly decreased at 1, 3 and 6 months progressively in responding patients; Our results were in line with Kenji Imai et al. **[39]** who proved that IR raises the risk of HCC development in patients with chronic HCV.

Our data revealed that the median serum AFP level was significantly higher in HCC group compared to HCV related cirrhotic patients. This finding was in agreement with Arrieta et al. **[40]** who said that AFP level was significantly higher in patients with HCC compared to patients with liver cirrhosis.

After follow up of patients in HCC group who were treated by RFA, our data showed that the median serum AFP level before treatment was significantly decreased at 1, 3 and 6 months progressively in all cured HCC patients. Similarly Berry et al. **[41]** concluded that the serum AFP level and changes in its level strongly predict survival independently of the tumor burden.

Liver cell damage in chronic hepatitis C (CHC) virus infection is mediated by the induction of apoptosis. The key morphological alteration of apoptosis is mediated by family of intracellular

cysteine protease, called caspases **[42]** which cleave a number of different substrates inside the cells including (CK-18) the major intermediate filament protein in the liver **[43]**. Kawai et al. **[44]** revealed that CK-18 plays an important role in tumor genesis of hepatocellular carcinoma

In the present study, there is a significant increase in CK18 level in HCC patients compared to cirrhotic patients. Moreover CK-18 was significantly higher in patients with recurrent tumor versus non recurrent. These could be explained by increase in the spontaneous apoptosis of HCC cells which released into blood vessels.

We have also observed significant increase of CK-18 level in large tumor size. This could be explained by the crosstalk between angiogenesis, cytokeratin-18, and insulin resistance **[45]**. McHugh and co-workers found that microvascular invasion is strongly associated with the tumor size and AFP >100 ng/ml and greatly increase the risk of recurrence after transplantation for HCC **[46]**.

It is vital to assess patients' prognosis to determine the suitable treatment and improve the outcome thus we investigated risk factors of recurrence which could help in its prevention and follow up of patients. The present study revealed that Child class B, large tumor size (3-5cm), increased serum CK-18, AFP and HOMA-IR levels were risk factors that predict HCC recurrence Also, Lai et al. **[47]** found a strong correlation between AFP level, tumor dimensions and microvascular invasion which all are predictors of HCC recurrence. Carcinogenesis may develop when the homeostatic balance between cell survival and apoptosis is disturbed in our study; we found that the CK-18 and HOMA-IR response independently predicted the recurrence free survival (together with Child-Pugh score and tumor size). We can expect more favorable prognosis in patients with CK-18 <317.2 and HOMA-IR had response than that of those with CK-18 >317 and HOMA-IR >2.7. The close observation of the non-response patients might be required to detect disease progression.

The main limitations of our study included small sample size in limited observation period and there was no correlation between tissue and serum CK-18 levels.

Finally, we can conclude that CK-18 correlates with HOMA-IR and is related to HCC size which makes them potential prognostic markers for follow up of patients after therapeutic strategies to predict recurrence of HCC.

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