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RESEARCH ARTICLE

Role of Intercellular Adhesion Molecules as tumor marker for detection of Hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide which accounts for 80%-90% of primary liver cancer. It is characterized by a very poor prognosis. Outcome of Hepatocellular carcinoma (HCC) depends mainly on its early diagnosis. Serum α-fetoprotein (AFP) is the marker that has been widely used for screening and diagnosis of HCCs. However, development of false-negative or false-positive rates with (AFP) was as high as 30%-40% for patients with small hepatocellular carcinomas. Thus the identification of novel biochemical markers for HCC remains an important goal for many laboratories around the world. Intercellular Adhesion Molecule–1(ICAM-1) is one of the positive prognostic markers secreted abundantly in several human tumors including HCC. This study was aimed to evaluate the clinical significance of sICAM-1 as complementary marker for early diagnostic and predictive occurrence of HCC in patients with chronic Hepatitis C induces liver cirrhosis. This study was conducted on a total number of 120 patients. The patients were subdivided as follows. Group(I): included 20 normal healthy subjects (as controls), while Group (2): included 50 patients with chronic hepatitis C genotype 4 developed to cirrhosis and Group(3)included 50 hepatocellular carcinoma patients proven to be infected with chronic HCV genotype -4 diagnosed by HCV PCR, ultrasound assessment, abdominal triphasic CT and serum AFP. Serums AFP, ICAM-1 were measured for all groups by ELISA technique. Serum ICAM-1 levels ranged from (419 – 554, 540 -1800 and 1135- 2514 ng/mL) in the control, chronic hepatitis C and HCC groups respectively. Serum ICAM-1 levels were highly significantly elevated in HCC group than that of chronic hepatitis C and the control groups ($p<0.001$). Also, this study indicates that, the level of Serum ICAM-1 increase with the degree of liver function impairment in patients groups. Moreover, there was a statistically significant positive correlation was found between the level of Serum ICAM-1 and the tumor size in HCC group ($p<o.oo1$ $r=0.899$). The mean serum AFP levels were elevated in all patients groups in comparison to control group, this elevation was statistically highly significant ($p<0.001$). In HCC patients a highly significant correlation was found between levels of ICAM-1 and AFP ($r= 0.747$, $p<0.001$). By using a cut off value of AFP> 15.56 ng/ml, we found that specificity and sensitivity of AFP were 68.6% and 86.0 % respectively.

While the cut off value of ICAM-1 was > 1285.15 ng/ml with specificity 98.6 % and its sensitivity was 88%. By combination the two markers together the sensitivity enhanced up to 98% and diagnostic accuracy up to 97.5% for HCC detection compared to their individual values.

In conclusion, serum ICAM-1 has the potential to be complementary biomarker combined to AFP for early detection of Hepatocellular carcinoma with highly sensitivity and specificity. Also it can be used to assess the severity of underlying liver disease and reflect well the degree of hepatic dysfunction.

Introduction

Hepatocellular carcinoma (HCC) is the third most common cancer in the world and accounts for more than 90% of human liver cancers. Hundreds of thousands of deaths result from HCC worldwide every year, and as many as 90% of these cancer-associated deaths are related to metastasis [1]. HBV and HCV account for 80% to 90% of all cases of HCC worldwide [2], [3].

In Egypt, up to 90% of HCC cases were attributed to hepatitis C viral (HCV) infection [4]. Over the course of 20 years or more, 10%–30% of HCV carriers develop cirrhosis; patients with cirrhosis have an annual risk of 1%–2% for developing HCC [5] Serum α-fetoprotein (AFP) is the marker that has been widely used for screening and diagnosis of HCCs. However, in some cases AFP has poor specificity in detection of HCC [6]. Recently rapid progress in studies on expression of (Intercellular Adhesion Molecule 1) ICAM-1 in patients with hepatocellular carcinoma (HCC) has been achieved, including clinical and experimental researches. Intercellular adhesion molecule 1 (ICAM-1; CD54), a 90-kilodalton cell surface glycoprotein of the immunoglobulin super family. The expression of ICAM-1 has been reported to mediate adhesion-dependent cell-cell interactions and facilitate the movement (or retention) of cells through the extracellular matrix **w**hich plays an important role in cells differentiation, movement, and immunity [7] In normal liver tissue, hepatocytes don't express ICAM-1,but in various kinds of hepatitis, the expression of ICAM-1 is enhanced in liver sinusoidal endothelial cells and vascular endothelial cells, and it will be positive on liver cells, bile duct epithelial cells, lymphocytes, and fibroblasts [8] . A variety of inflammatory cytokines and stimulus affect its expression through the nuclear factor-kappa B (NF-κB) signal transduction pathway [9]. In malignancies the expression ICAM-1 may strongly express in two forms membranous one on the surface of tumor cells (membrane-bound ICAM-1) and soluble one in circulation (soluble ICAM-1, sICAM-1)[10,11]. Previous studies had shown that ICAM-1 is expressed on hepatocytes in cancerous areas but not on hepatocytes in noncancerous areas [12] therefore, the studies on detecting serum sICAM-1 in patients with hepatocellular carcinoma (HCC) have revealed that serum levels of sICAM-1 were well correlated to progression and prognosis of the disease [13,14]. It has been shown to be positively correlated with tumor size and poor prognosis in HCC [15]. Previous study has been considered that sICAM-1 may be useful for monitoring the response to treatment [13]. Moreover Kam et al. and his colleagues [16] reported that ICAM-1 may be a critical factor in the process of blood borne metastasis and recurrence of cancer. However, whether sICAM-1 is a diagnostic marker in HCC is still controversial. The aim of this work was to evaluate the clinical, significance of measuring sICAM-1 for early diagnosis of HCC.

Materials and methods

Patients

This study was carried out on a total number of 120 participants, they were subdivided as follows: Group I: included 20 normal healthy subjects (as controls) were matched to the patient group by sex (male = 9, female =11) and mean age 54.1±9.74years. Group II: included 50 patients with chronic hepatitis C viral infection with cirrhosis (male =29 and female = 21) their mean age was 55.04 ±9.58 years and Group III: included 50 hepatocellular carcinoma patients (male = 31, female=19) with mean age 58.8 ±9.66 years. The patients were admitted to Endemic Medicine and Internal Medicine Departments in Faculty of medicine, Kasr EL- Aini Hospital and Ain Shams University Hospitals. Subjects were excluded from the study if they were known to have any disease other than liver cancer or

cirrhosis. Patients with a history of alcohol abuse, renal insufficiency, proteinuria, suspected infections, clinically overt diabetes mellitus, thyroid dysfunction, or any other endocrine disorder were also excluded from the study. No hormone or thyroid-regulatory medication was administered. Liver cirrhosis and Hepatocellular carcinoma were proven through physical examination and imaging. All the patients with HCC had cirrhosis on top of HCV, They were newly diagnosed and none had received any form of anti-cancer therapy. We selected our patient with solitary nodule Tumor size was less than 3 cm in diameter in 9 patients, from 3-5 cm in 20 patients and more than 5 cm in 21 patients. None of our patients have intravascular thrombosis or intra or extra hepatic metastasis. Informed consent was obtained from all participating subjects before the study.

Blood sampling and biochemical assays

Fasting venous blood samples (5 ml) were collected by trained laboratory technicians. A portion of blood was allowed to clot and then centrifuged at 3500g for 5 min to separate the serum used for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, g-glutamyltranspeptidase (GGT) and Albumin. Serum aliquots were stored at -80^{o}C until assayed and thawed immediately before the measurements of AFP, ICAM-1 levels. Another portion of blood was collected in vacutainer tubes containing citrate for complete blood picture. AST, ALT, total bilirubin, direct bilirubin, g-glutamyltranspeptidase (GGT) and albumin were assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). AFP was measured using Abbott, Axyam (USA, Supplied by al kamal company Cairo, Egypt).

Serum level of ICAM-1 was determined with commercially available Assay Human ELISA kit (IBL international, code: BE59011). This assay employs a quantitative sandwich enzyme immunoassay technique. EASIA is a solidphase enzyme-amplified sensitivity immunoassay performed on microliter plate. ICAM-1 (ng/ml) was calculated by interpolation from a reference curve generated in the same assay with reference standards of known concentrations. All assays were performed in duplicate according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 11.0; SPSS, Chicago, IL, USA). Receiver operating characteristics (ROC) curves were drawn in order to determine the best cut-off value of serum ICAM and AFP to compare their sensitivity, specificity while the Tukey-Kramer test was used to compare each pair of groups. A probability of less < 0.05 was considered significant.

Results

Our healthy normal control mean age was (54.1±9.74 yrs) and that of (G2) was (55.04±9.58 yrs), while the mean age of (G3) was (58.8±9.66 yrs). Preponderance of males/ females ratio was observed among (G2) and (G3) 1: 1.5 and 1:1.6 respectively. There was no significant difference between (G2) and (G3) patients regarding age and sex ($p=0.14$ and $p=0.838$, respectively (as shown in table 1). The biochemical parameters for all groups were summarized in (Table 2). Patients groups (G2 and G3) showed significantly higher in mean values of ALT, AST, T Bil, INR, GGT, when compared to healthy group ($p<0.001$), while (G3) showed significant lower mean values of albumin and platelets than (G1) and (G2) (P value equal to 0.04,0.01 respectively). The mean serum AFP levels were elevated in group s (G2) and (G3) in comparison to (G1) [15.95 ±.7.2 ng/ml and 132.80 ± 51.46 ng/mL] respectively vs [5.24±1.244 ng/ml] in (G1). This elevation was statistically highly significant $p<0.001$ (Table 3).

Serum ICAM-1 levels ranged from (419 – 554, 540 -1800 and 1135- 2514 ng/mL) in G1, G2, G3 respectively (Table 3). Serum ICAM-1 levels were highly significantly elevated in (G2) and (G3) than of (G1) $p<0.001$.

The mean levels of AFP in patients with HCC with different sizes of tumor showed highly significant difference. As regard the mean serum level of AFP in HCC patients it was 12.52±3.59 ng/ml belong to the tumor size < 3cm and 47.71±17.2ng/ml in 3-5cm ng/ml ,while in tumor size>5cm, it was 265.39±127.11 ng/ml , ($p<0.001$ as shown in table 4). Circulating levels of sICAM-1 were highly significant difference with different sizes of tumor. Its level was elevated in tumor size more than 5cm , it was 2153.99±201.82 ng/ml while in tumor size from 3-5cm, it was 1584.86 ± 161.64 and in tumor size less than 3cm it was 1229.15±78.78 ng/ml, ($p <0.001$ as shown in table 4).

Serum levels of ICAM-1 was found to be correlated with AST level in (G3), it has highly positive significant correlation regarding AST ($r= 0.642$, $p<0.001$ table 5). Also this table showed a highly positive significant correlation was found between levels of ICAM-1 and AFP ($r= 0.747$, $p<0.001$). By using a cut off value of AFP> 15.56 ng/ml, we found that specificity and sensitivity of AFP were 68.6 % and 86.0 % respectively. While the cut

off value of ICAM-1 was > 1285.15 ng/ml had specificity and sensitivity 98.6 %, 88% respectively. By combination the two markers together the sensitivity enhanced up to 98% and diagnostic accuracy up to 97.5% for detection of HCC compared to their individual values (Table 6 and fig 1).

Table 1: Comparison between all groups regarding demographic data

Variables	(G1) N=20	(G2) N=50	(G3) N=50	P-value
Age(Mean± SD)	54.1±9.74	55.04±9.58	58.8±9.66	0.14
Sex Male	9(45%)	29(58%)	31(62%)	0.838
Female	11(55%)	21(42%)	19(38%)	

P < 0.05 was considered significant

Table 2: laboratory Findings among all studied groups

Variables	(G1) N=20	(G2) N=50	(G3) N=50	ANOVA		Tukey's test		
				F	P-value	P1	P2	P3
ALT (U/L)	30±1.25	51±4.54	64±2.476	597.65	<0.001*	<0.001*	<0.001*	<0.001*
AST (U/L)	32±3.41	57±4.10	149±5.14	7396.97	<0.001*	<0.001*	<0.001*	<0.001*
T. Bil (mg/dl)	0.7±0.05	1.4±0.101	2.7±0.21	1581.18	<0.001*	<0.001*	<0.001*	<0.001*
Albumin (g/dl)	3.8±0.11	2.7±0.15	2.15±0.17	520.09	<0.001*	<0.001*	<0.001*	0.04*
INR	1.12±0.02	1.9±0.024	2.0±0.08	1880.08	<0.001*	<0.001*	<0.001*	<0.001*
GGT (IU/L)	34±2.4	46±4.02	224±15.54	4178.76	<0.001*	<0.001*	<0.001*	<0.001*
Platelets×10^3/ml	426±200.05	190±30.45	122±12.05	37.37	<0.001*	<0.001*	<0.001*	0.01*

P1 :Correlation between(G1) and (G2) , P2 :Correlation between(G1) and (G3) , P3: Correlation between(G2) and (G3), ALT: alanine aminotransferase; AST; aspartate aminotransferase; T.Bil :Total bilirubin, INR: International normalization ratio; GGT: Gammaglutamyltransferase.

Table 3: Serum level of ICAM-1 and AFP in all studied groups

Variables		(G1) N=20	(G2) N=50	(G3) N=50	ANOVA		Tukey's test (P-value)		
					F	P-value	P1	P2	P3
ICAM-1 (ng/ml)	Range	419 – 554	540 -1800	1135- 2514	137.08	<0.001*	<0.001*	<0.001*	<0.001*
	Mean ± SD	487.10±143.85	997.90±335.42	1759.863±398.213					
AFP (ng/ml)	Range	4.9 – 6.8	5.12– 35.56	7.00 – 386.8	21.45	<0.001*	<0.930.	<0.001*	<0.001*
	Mean ± SD	5.24±1.244	15.95±7.21	132.80±51.46					

AFP: alpha fetoprotein, ICAM-1: Intercellular Adhesion Molecule-1.

Table 4: Serum ICAM-1 and AFP levels in different tumor size in G3

Parameters	Tumor sizes	N	Mean±SD	ANOVA	
				F	P-value
ICAM -1 ng/ml	<3	9	1229.15±78.78	110.69	<0.001*
	3-5	20	1584.86±161.64		
	>5	21	2153.99±201.82		
AFP ng/ml	<3	9	12.52±3.59	31.07	<0.001*
	3-5	20	47.71±17.2		
	>5	21	265.39±127.11		

AFP: alpha fetoprotein, ICAM-1: Intercellular Adhesion Molecule-1.

Table 5: The correlation between ICAM-1 level and different parameters in G3

ICAM-1 (ng/ml)		
	r	P-value
ALT (U/L)	0.327	0.013
AST (U/L)	0.642	0.001
T. Bil (mg/dl)	0.714	0.001
Albumin (g/dl)	0.010	0.922
INR	0.089	0.379
GGT (IU/L)	0.158	0.117
Platelets×103/ml	-0.029	0.776
AFP (ng/ml)	0.747	0.001
Tumor Size	0.899	0.001

ALT: alanine aminotransferase; AST; aspartate aminotransferase; T.Bil : Total bilirubin, INR: International normalization ratio; GGT: Gammaglutamyltransferase, AFP: alpha fetoprotein.

Table 6: ROC analysis to study the HCC diagnostic ability of sICAM-1 and AFP

	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
ICAM-1	> 1285.15	88.0	98.6	97.8	92.0	96.8
AFP	> 15.56	86.0	68.6	66.2	87.3	82.9
Both		98.00	97.14	96.08	98.55	97.50

ROC, receiver-operating characteristic, NPV: negative predictive value, PPV: positive predictive value; AFP: alpha fetoprotein, ICAM-1: intercellular adhesion molecule -1

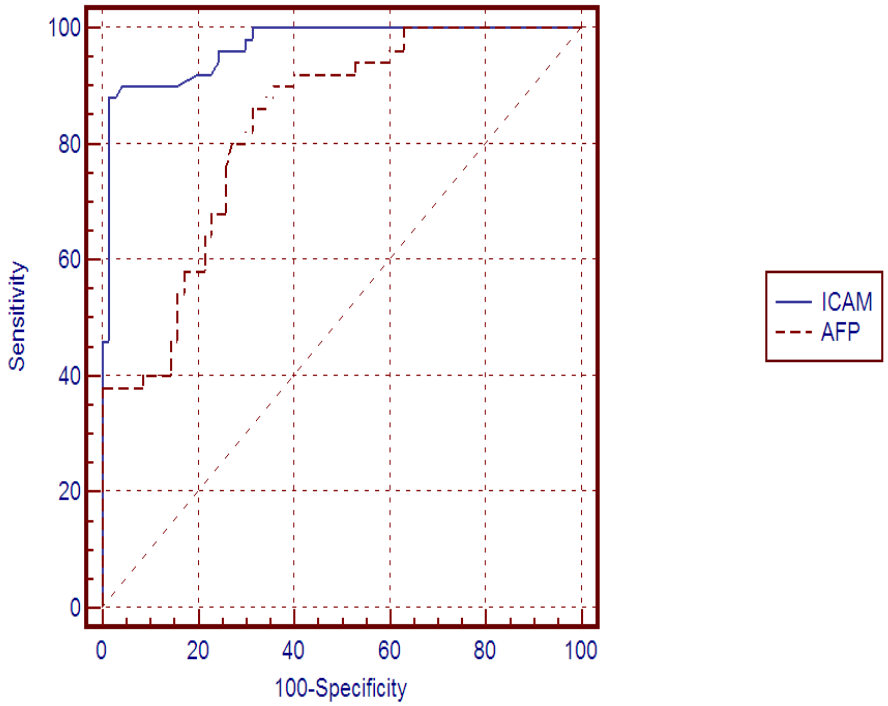

Figure 1: Receiver Operating Characteristic (ROC) of ICAM-1 and AFP

Discussion

Intercellular adhesion molecule-1(ICAM-1) is a member of the immunoglobulin super family, which belongs to Cell adhesion molecule (CAM). ICAM-1, one of the most important molecule in the tumor-associated mechanism, often be used for an index of tumors arises, metastasis, recurrence and prognosis [10]. Previous studies approved that, Intercellular Adhesion Molecule–1 (ICAM-1) is one of the positive prognostic markers secreted, abundantly in several human tumors including HCC [11]. It has been demonstrated that measurement of sICAM might be of clinical value for early diagnosis and monitoring recurrence of HCC [17,18] .

In the present study, HCC patients were more common in males than females; that males represented 60% of all patients, these results are similar to Zakhary et al. [19]who reported that males represented 70.8% of all patients in HCC group, with 83.3% of patients over 50 years. In our study we found that the mean value of AST, ALT , Bilirubin , INR and GGT were higher in HCC patients than that of both the chronic hepatitis C and control group ,however albumin was lower in HCC group than that of other groups .These findings are consistent with Sun et al.[15] who reported that the previous parameters usually indicate the type of liver injury, whether hepatocellular or cholestasic but cannot be expected to differentiate one form of hepatitis from another or to determine whether cholestasis is intra or extra hepatic.

In the present study, patients with HCC had significantly higher in mean serum level of ICAM-1 than chronic hepatitis C and control groups $p<0.001$ this concordance with previous studies Momomsak et al., and Lee goldman et al., [12,20] who reported that a significantly higher serum levels of sICAM-1 in patients with HCC than those with LC and healthy controls. On the other hand, the mean serum levels of ICAM-1 were highly significant elevated in chronic hepatitis C than control group $p <0.001$. In agreement with many studies have shown high levels of sICAM-1 in patients with HCV compared to controls Capra et al., and peng et al., [21,22] this could be explained by the increased expiration from endothelial cells activated by several cytokines during the inflammatory process. Against to our results as the study results by Hyodo et al., [13] showed that there was no difference in serum levels of sICAM-1 between their patients with HCC and liver cirrhosis. Based on this they declared that sICAM-1 is only a marker for progression and prognosis of the disease, but not a diagnostic marker for HCC. Moreover, Rosanna et al., [23] reported that Serum levels of sICAM are not useful as prognostic factors for HCC in clinical practice.

Serum levels of ICAM were found to correlated with laboratory makers of disease activity in patients group but this correlation was more with AST and T. Bil ($r=0.642$ and 0.714 respectively, $p<0.001$). Our results are in accordance

with Shimizu et al.,[14] who found that liver necroinflammation was more closely related to ICAM and patients with normal transaminases had reduced ICAM. Similar Bruno et al., [24]who suggest that the determination of serum ICAM-1 can be considered as an additional useful marker of hepatocellular necrosis and inflammatory activity in chronic hepatitis, Thus, measurement of serum ICAM-1 may be useful for monitoring progression or regression of liver inflammation in chronic HCV infection .

The mean levels of ICAM-1 have a positive significant correlation with tumor size in G3 (r =0.899- P< 0.001), Large tumor > 5 cm had higher level than the smaller size, and tumor from 3- 5 cm had higher level than that < 3 cm. This goes with Sun et al., [15] who reported that ICAM was positively correlated with tumor size(r= 0.5,p<0.05). Moreover, Shimizu et al.,[14] reported that, in patients with HCC, circulating sICAM-1 levels were significantly (P < 0.001) correlated with tumor volume (r =0.50), Furthermore, ICAM was significantly elevated in patients with multiple HCC (tumor number > 3) or HCC with tumor embolus in the first branch or trunk of portal vein. In this study we found thatsICAM-1 can predict HCC, with sensitivity 88%, and specificity 98.6 %, the best cut-off value of ICAM-1>1285.15ng/ml. By comparing this result with AFP, the sensitivity and specificity of AFP in HCC diagnosis depends on the cut off level used. We used the cut off level of AFP in our study >15.56 ng/ml the sensitivity was 86.0% whereas the specificity was 68.6 %. Tao and his colleagues, [25] reported that if the cut off level of AFP increases from 20 ng/ml to 200 ng/ml, the sensitivity falls from 78.9% to 52.6% whereas the specificity increases. Similar results were obtained by Toyoda et al. [26], who reported that AFP plays a limited role in detection and diagnosis of HCC. Therefore, use of AFP alone for hepatocellular carcinoma surveillance is not recommended [27].

From our study, one of the interesting results by analysis of ranges of sICAM-1 levels in patients with HCC demonstrated that the patients who was positive had a high serum level of sICAM-1 exceeding1000 ng/ml according to the study by Mei et al, [17] who concluded that the diagnosis of HCC should be strongly suspected when a patient with an uncertain intrahepatic lesion had a serum level of sICAM-1 higher than 1000 ug/L[17]. The results of the present study further confirm their previous conclusion.

Moreover, In the current work, a highly significant positive correlation was found between ICAM-1 and AFP in HCC group (P<0.001, r=0. 899) this observation in accordance with Ghada et al., [28]. Therefore, in our work we combined two markers for detection of HCC enhanced the sensitivity up to 98% compared to their individual sensitivities also, these combinations improved the diagnostic accuracy up to 97.50% compared to their individual values this goes with Ghada et al., [28] who concluded that the combined detection using AFP and ICAM -1 produced enhanced sensitivity a maximum sensitivity of 100% was achieved compared to their individual sensitivities.

In conclusion,
Serum ICAM-1 has the potential to be complementary biomarker combined to AFP for early detection of Hepatocellular carcinoma with highly sensitivity and accuracy; also it can be assess the severity of underlying liver disease and reflect well the degree of hepatic dysfunction.

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Competing interests
All The authors declare that they have no competing interests.
All authors have contributed to the work, all authors have agreed to submit the manuscript for publication, and all human studies have been reviewed by the appropriate ethics committees.

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