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Comparison between multiple serum biomarkers in diagnosis of hepatocellular carcinoma in egyptian patients

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Abstract
Background: Hepatocellular carcinoma (HCC) is the most well-known essential primary tumor of the liver. It is the second leading cause of cancer-related deaths worldwide, with a very poor prognosis. Early diagnosis and effective treatment of HCC remain a challenging in worldwide. If detected very early, HCC can be cured with an excellent long-term prognosis. HCC frequently develops in the setting of underlying chronic liver disease. Viral hepatitis (infection with either hepatitis C virus [HCV] or hepatitis B virus [HBV]) is thought to be the most common etiology of HCC where estimated 78% of global HCC cases. As markers of HCC occurrence are still very scare and early detection of HCC increases the chance of treatment. Analysis of late researches demonstrated that AFP assessment lacks satisfactory sensitivity and specificity for good surveillance and diagnosis**.** As early detection of HCC is essential, new markers with enough sensitivity and specificity are needed.
Aim: This work aims to compare between serum level of AFL-3, ICAM-1, HGF 19 and SCCA levels as diagnostic markers for HCC.
Patients and Methods: The present study was conducted on a total number of 180 subjects divided in to 3 groups: 60 healthy individuals as control group,60 patients with liver cirrhosis and without any evidence of HCC as LC group and 60 patients with HCC as HCC group. Patients with cancers other than HCC or metastatic liver cancer were excluded. Clinical data were collected from patients file. Alpha Fetoprotein (AFP), AFL-3, ICAM-1, HGF, SCCA levels, serum HBsAg and anti-HCV were determined using ELISA method. Abdominal ultrasound scan was also done.
Results: The four biomarkers (AFP-L3, ICAM-1, HGF19 & SCCA) plus AFP were elevated in HCC in comparison with LC patients and in both HCC and LC groups in comparison with control group.
Conclusion Three of our serum biomarkers (SCCA, HGF19 and AFP-L3) were better than the golden standard serum AFP in both sensitivity and specificity. Serum level of ICAM-1had a higher specificity but lower sensitivity in comparison with AFP serum level.

Keywords: Hepatocellular carcinoma, squamous cell carcinoma antigen, AFL-3, ICAM, HGF19, Alpha-fetoprotein

Introduction

Hepatocellular carcinoma (HCC) is the most widely recognized essential threat of the liver. It is the second leading cause of cancer-related deaths worldwide, with a very poor prognosis [1]. Early diagnosis and effective treatment of HCC remains a challenge worldwide [2]**.** If detected very early, HCC can actually be cured with an excellent long-term prognosis [3]. HCC frequently develops in the setting of underlying chronic liver disease. Viral hepatitis (infection with either hepatitis C virus [HCV] or hepatitis B virus [HBV]) is thought to be the most common etiology of HCC where estimated 78% of global HCC cases [4].

As markers of HCC occurrence are still very scare, and early detection of HCC increases the chance of treatment. Analysis of recent studies showed that AFP assessment lacks adequate sensitivity and specificity for effective surveillance and diagnosis [5]. As early detection of HCC is essential, new markers with sufficient sensitivity and specificity are needed [6].

The most widely used HCC biomarker is the serum α-Fetoprotein (AFP) measurement. However, it has been excluded from the present guidelines of the American Association for the Study of

Liver Disease and the European Association for the Study of the Liver for the diagnosis of HCC [5], because it has low sensitivity and poor diagnostic yield at the early stage of HCC [7].

This complication highlights the need to discover characteristic biomarkers for the diagnosis of HCC to increase the number of patients who are suitable for curative treatment.

AFP-L3 is the lectin focal point agglutinin-affinitive AFP glycoform, with an extra α1–6 fucose build-up joined at the diminishing end of N-acetylglucosamine. In patients with AFP less than 20 ng/ml, measurements of AFP-L3% by the highly sensitive method before treatment was more useful for diagnosis and prognosis of HCC than by the conventional method [8, 9].

Leukocyte adhesion molecules, among them intercellular adhesion molecule 1 (ICAM-1) assume an indispensable job in invasion, actuation and official of effectors cells to tissues. Levels of flowing ICAM-1 are expanded in patients with hemochromatosis, liver cirrhosis and hepatocellular carcinoma, and have been demonstrated to be helpful in deciding the seriousness of liver ailment and the level of fibrosis [10, 11].

Squamous cell carcinoma antigen (SCCA) is a member of the high molecular weight family of serine protease inhibitors named serpins that physiologically found in the granular layers of normal squamous epithelium [12]. Many recent literatures have reported that the squamous cell carcinoma antigen (SCCA) is over expressed in HCC tissues with a higher concentration of this antigen in the serum of HCC patients than cirrhotic patients [13, 14].

Human growth factor (HGF) was involved in enhancing the proliferation of HCC cells as it was reported that the tendency of HGF production was consistent with that of tumour volume growth, which led to the hypothesis that HGF played a mediator role in HCC proliferation facilitated by hepatocellular carcinoma Cancer-associated fibroblasts (H-CAFs) [15].

So, this work aims to investigate the diagnostic value of serum AFL-3, ICAM-1, HGF and SCCA levels for HCC among high risk patients and assessment of the sensitivity of each biomarker to determine which one of them can be considered the more sensitive marker for HCC.

Patients and Methods

The present study was done on a total number 180 included 60 healthy individuals as control group while Serum samples were obtained from 120 patients with chronic liver disease, divided into two groups: **LC** group included sixty patients with patients with liver cirrhosis and without any evidence of HCC in addition to sixty healthy adults were recruited as controls. **HCC** group was included sixty HCC patients. The exclusion criteria were for Patients with cancers other than HCC or metastatic liver cancer.

HCC cases were diagnosed by abdominal US and serum AFP, with or without triphasic CT scan and/or liver histopathology. The clinical/pathological data of the patients were recorded, including age, sex, viral infections {Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV)}, biochemical liver function test results, and AFP levels. Tumor characteristics were identified by Abdominal US with or without CT scan (including tumor size, number, site, halo sign and neovascularization). Tumor staging was done using Tokyo staging systems.

Blood sampling and biochemical assays

Fasting venous blood tests (5 ml) were gathered via prepared research center technicians. The blood was allowed to clot and then centrifuged at 10000 rpm for 15 min to separate the serum used for the biochemical tests: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct Bilirubin, Albumin, creatinine and glucose concentrations were assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). Viral infection status (HCV Ab and HBS Ag) were measured using Abbott, Ax yam (USA, Supplied by al Kamal company). Serum AFP, AFL-3, ICAM-1, HGF19 and SCCA levels were determined using an enzyme-linked binding protein assay kit determines in all serum groups using ELISA Kit according to the manufacture's protocol. All measures were performed in copy as indicated by the maker's directions. Serum aliquots were stored at -20◦C until assayed and thawed immediately before the measurement levels.

Statistical analysis

The SPSS version 15 is used in data analysis. Data are expressed as mean ± standard deviation. Analysis of data was done by one-way ANOVA test followed by Tukey's multiple comparison tests. Student's t-test was used to compare two quantitative variables. Correlation between the variables is calculated using Pearson's correlation coefficient. Receiver operating characteristic (ROC) curves are plotted in order to determine the best cut-off values of the studied markers.

Results

Radiological examination, Sonar and Computed tomography of the studied group are shown in table (1) while the biochemical parameters are shown in table (2).There were a statistically significant increase in the serum level of liver function test (ALT, AST, Albumin &Total Bilirubin), glucose and creatinine in both LC and HCC groups compared to control group and also in HCC group compared to LC group.

Regarding our serum markers, there were a statistically significant elevation in the serum level of AFP and all four biomarkers (AFP-L3, ICAM-1, HGF19& SCCA) in both LC and HCC patients in comparison to healthy control and in HCC patients compared to the LC group.

As for the analysis of the ROC curve: three of the biomarkers revealed a higher sensitivity and specificity {SCCA (85%Sensitivity, 82%Specificity & AUC=0.87 at a cut-off 7.85 ng/ml), HGF19(80%Sensitivity, 75%Specificity & AUC=0.83 at a cut-off 27.5 ng/ml) & AFP-L3(76%Sensitivity, 77%Specificity & AUC=0.79 at a cut-off 33.6ng/ml)} than AFP (70%Sensitivity, 73%Specificity & AUC=0.65 at a cut-off 18.9ng/ml). On the other hand, ICAM-1 at a cut-off >97.5 ng/ml showed a higher Specificity (85%) than AFP (73%) but lower sensitivity (60%) than AFP (70%) with AUC=0.71.

Table 1: Radiological examination of studied groups (Control, LC and HCC patients) Sonar and Computed tomography.

Parameters	Control group N (%)	LC group N (%)	HCC group N (%)
Liver			
-Normal liver	60(100%)	0(0%)	0(0%)
-Bright liver	0(0%)	0(0%)	0(0%)
-Coarse liver	0(0%)	60(100%)	60(100%)
Focal lesion	0(0%)	0(0%)	60(100%)
Ascites:			
No	60(100%)	36(60%)	48(80%)
Mild	0(0%)	14(23.3%)	4(6.7%)
Mod	0(0%)	6(10%)	6(10%)
Severe	0(0%)	4(6.7%)	2(3.3%)
PVT:			
Yes	0(0%)	6(10%)	6(10%)
No	60(100%)	54(90%)	54(90%)
Splenomegaly			
Yes	0(0%)	6(10%)	14(23.3%)
No	60(100%)	54(90%)	46(76.7%)
Hepatomegaly: 0(0%)			
Yes No	60(100%)	8(13.3%) 52(86.7%)	6(10%) 54(90%)
Hypertension			
Yes	0(0%)	6(10%)	0(0%)
No	60(100%)	54(90%)	60(100%)

*p-value < 0.05 significant, PVT (portal vein thrombosis).

Table 2: Biochemical parameters of the three studied groups

Parameter	Control group Mean ±SD	LC group Mean ±SD	HCC group Mean ±SD	P-value
ALT(IU/L)	20.33 ± 7.11	57.11 ± 12.71	67.22 ± 13.44	0 .005 [a, b]
AST(IU/L)	23.00± 9.66	87.33 ± 17.11	99.55 ± 16.00	0.000 [a, b]
Albumin(g/dl)	3.9 ± 0.5	2.77±0.4	2.55 ± 0.56	0.000 [a, b]
T.BIL (mg/dl)	0.66 ± 0.17	5.55 ± 2.22	6.33 ± 1.22	0.003 [a, b]
Glucose(mg/dl)	90.2 ± 13.2	133.3 ± 11.23	111.7 ± 6.03	0.000 [a, b]
Creatinine(mg/dl)	0.66 ± 0.17	1.76 ± 0.22	2.17 ± 0.55	0.005 [a, b]
AFP (ng/dl)	4.7 ± 2.55	177.1± 25.0	586.2 ± 33.4	0.000 [a, b]
AFP-L3 (ng/ml)	4.5± 1.6	9.7±2.7	17.2±3.5	0.000 [a, b]
ICAM-1 (ng/ml)	78±13	123±44	217±77	0.004 [a, b]
HGF19 (ng/ml)	15.6±33	31.7±43	67.8±12	0.0005 [a, b]
SCCA (ng/ml)	2.54 ± 0.56	3.62 ± 1.2	6.42 ± 3.18	0.002 [a, b]

ALT: alanine aminotransferase, AST: aspartate aminotransferase, T.BIL: total bilirubin, AFP: alpha-fetoprotein, SCCA: Squamous cell carcinoma antigen. P-value <0.05 considered significant. a Significant difference from control group. b Significant difference from LC group

Table 3: Sensitivity and specificity of diagnostic values of AFP, AFP-L3, ICAM-1, HGF 19 and SCCA levels detection of HCC in different subjected cases.

Biomarker	Cut-off value	AUC	sensitivity	Specificity
AFP	> 18.9 ng/ml	0.65	70%	73%
AFP-L3	> 33.6 ng/ml	0.79	76%	77%
ICAM-1	> 97.5 ng/ml	0.71	60 %	85%
HGF19	> 27.5 ng/ml	0.83	80%	75%
SCCA	>7.85 ng/ml	0.87	85%	82%

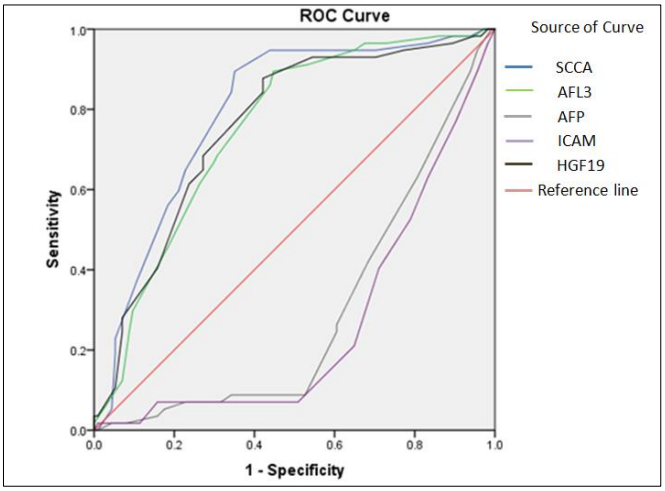

Fig 1: ROC curve of the different biomarkers

Discussion

HCC is an extremely difficult to treat cancer, which generally involves multiple pathologic complications including hepatitis, metabolic (NASH and diabetic), fibrotic and cirrhotic diseased conditions in addition to the notorious tumor burden[16], [17]

The current clinical diagnosis of early HCC is mainly based on medical imaging, including ultrasonography, computed tomography (CT) and magnetic resonance imaging[18], [19]**.**

AFP is the most well studied biomarker for HCC and is also the first biomarker approved for HCC detection in liquid biopsy. AFP had a limited ability to detect early HCC as it results in high false positive rate that is not only because only 61% of HCC expresses AFP, but also the fact that AFP expression is detected in other liver abnormalities such as cirrhosis and acute hepatitis and other tumors, including endodermal sinus tumor and gastrointestinal malignancies [16], [20]**.**

There have been several attempts to increase the sensitivity by combining imaging with AFP biomarker but with limited success so far, indicating the urgent need for new potential biomarkers[7]. Our study illustrated the increase in the serum level of AFP and the four biomarkers (AFP-L3, ICAM-1, HGF19& SCCA) in patients of HCC when compared to LC patients.

The uprising of serum level of AFP-L3 over AFP may be explained by the possibility of production of AFP-L3 only by cancer cells [21]**.** Moreover, ***Davis et al.,2008*** suggested that AFP-L3 was specifically produced by liver malignancy cells.

The hepatocyte growth factor and its tyrosine kinase receptor (HGF–cMET) signaling pathway represents a promising drug target for novel anticancer strategies. Targeting the HGF ligand or the cMET receptor are two possible ways to influence the activation of this pathway[22], [23]. In our research we aim to use the serum level of HGF19 as a diagnostic tool for HCC and our results achieved that goal as it was elevated in HCC patient's serum level comparing to LC.

This work was in consistence with another study that reported that in HCC patients, the SCCA antigen could represent a useful marker for large-scale screening of serum in patients at risk [24]**.**

By using ROC curve analysis our study indicated that 3 of the serum markers (AFP-L3, HGF19& SCCA) possessed a higher sensitivity and specificity than the HCC gold standard AFP (70%Sensitivity, 73%Specificity) with the SCCA coming first with (85%Sensitivity, 82%Specificity) then HGF19 with (80%Sensitivity, 75%Specificity) and finally AFP-L3 with (76%Sensitivity, 77%Specificity). On the other hand, ICAM-1 higher Specificity (85%) but failed to exceed the sensitivity (60%).

Our results disagreed with [25] who reported that serum level of SCCA in HCC patients had a sensitivity of only 60% using ROC curve analysis. They explained their results regarding the low sensitivity and specificity of SCCA may be because SCCA was also increased in chronic liver diseases as liver cirrhosis and chronic hepatitis and that the increased SCCA was a risk factor for HCC in patients with chronic hepatitis.

Our findings was in line with [24] who found that in HCC patients, the SCCA antigen could represent a useful marker for the detection of micro-metastasis in the tissues and for enormous scale screening of serum in patients in danger. This research was agreeing with [26] who showed that AFP-L3 had a higher sensitivity (97.5%) than AFP (75%) with the same specificity for both. The diversity of AFP-L3 sensitivity may be explained by that the sensitivity and specificity of AFP-L3 may vary according to the stage of the HCC and the tumor size [26]**.**

The association between serum ICAM-1 as a biomarker with HCC incidence in chronic liver disease was examined as serum ICAM-1 was found to be produced by pre-malignant HCC stem cells [11], [27] . Our investigation emphasized the finding by the previous study as we illustrated the raise in the serum level of ICAM-1 in HCC patients in comparison with LC patients.

Conclusion

The four investigated serum biomarkers are good diagnostic tools for HCC among high risk patients (as LC). Regarding the sensitivity of the studied biomarkers only ICAM-1 was less than the standard AFP while the other 3 biomarkers were higher and in the following order: SCCA, HGF19 and finally AFP-L3. According to the previous findings, serum SCCA is considered the superior diagnostic marker for HCC detection among the studied ones.

Competing Interests

Authors have declared that no competing interests exist. The authors alone are answerable for the substance and composing of the paper. The authors did not receive any funds from any source.

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