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Original Research Article

Serum levels of Arginase Isoenzyme Activity, Alpha-Fetoprotein-L3 and Endostatin as Biomarkers for Hepatocellular Carcinoma in Egyptian Patients

Azza Faried[1]*, Lamiaa Mobarak[1], Khadiga K. El Gohary[2], Hala H. El-Deeb[3], Said El-Feky[4], Abeer Ahmed Abdelmonem[5], Nashwa Ahmed Zaki[6] and Ayman Abdel-Hady Alkhalegy[7]

[1]Tropical Medicine Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.
[2]Biochemistry Department, El Sahel Teaching Hospital, Egypt.
[3]Clinical Pathology Department, El Sahel Teaching Hospital, Egypt.
[4]Biochemistry Department, Damanhur National Medical Institute, Egypt.
[5]Microbiology and Immunology, Beni-Suef faculty of medicine.
[6]Clinical pathology, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.
[7]Department of General Surgery, Al Sahel Teaching Hospital, Cairo, Egypt.

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Background: **Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. It commonly develops on cirrhotic livers, and surveillance programs have therefore been suggested to identify early HCC, at a stage when it remains suitable for surgical therapy and has a better clinical outcome. The biomarkers alpha-fetoprotein (AFP)-L3, arginase and endostatin can be measured in the routine clinical setting, it may be useful or useless for hepatocellular carcinoma.** ***Aim:*** **The current study introduced a new serum tumor biomarker levels in HCC detection and investigate their benefit as predictors and follow-up of liver cancer.** ***Methods:*** **This study was conducted on a total number of 110 participants admitted to Hepatology and Gastroenterology Department in NHTMRI (National Hepatology & Tropical Medicine Research Institute). The participants of this study were divided into three groups as follows; Group I: 30 cases LCD, Group II 30 patients LC group and Group III as HCC individuals, in addition to 20 healthy subjects (as controls).** ***Results:*** **The obtained increasing specificity of AFP, the AFP-L3 can be used as a measure of cancerous changes in the HCC. The high specificity of arginase (85%) and endostatin (84%) can reflect the usefulness of these markers in HCC follow-up especially endostatin which has a marked sensitivity (90%) in HCC cases under this study.** ***Conclusion:*** **These results demonstrate the beneficial role of Endostatin as it is considered superior to other markers such as AFP, (AFP)-L3, arginase in the diagnosis of HCC.**

Keywords: Alpha-fetoprotein-L3, Arginase, Endostatin and Hepatocellular carcinoma.

INTRODUCTION

Liver cirrhosis is considered as a premalignant state, as about 80% of HCC is associated with liver cirrhosis (Ikai et al., 1998). Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and a leading cause of death in Africa and Asia. The rate of HCC is increasing in Egypt where the major risk factors are chronic infections with hepatitis B and C viruses (HBV & HCV), other risk factors involve aflatoxin B1 exposure, pesticides, alcohol consumption, and genetic defects (But et al., 2008). Although the mortality of HCC has significantly decreased with the development of surgical techniques, about 60% - 100% of patients suffered from HCC recurrence ultimately even after curative resection, and it has become the most important factor that limits the long-term survival of HCC patients. Thus the most urgent needs are to find sensitive markers for early diagnosis and monitoring of postoperative recurrence of HCC, and to give adequate treatment for HCC patients (Yao et al., 2007). HCC is a highly malignant tumor with a very poor prognosis, so early detection and treatment are required and effective (Nomura et al, 1993). In Europe and North America, it commonly develops on cirrhotic livers, and surveillance programs have therefore been suggested to identify early HCC, at a stage when it remains suitable for surgical therapy and has a better clinical outcome (Giannelli et al, 2005). Ramsey and Wu-Gy (1995) stated that alpha-fetoprotein (AFP) level and abdominal ultrasonography remain the cornerstones of screening for HCC. Recently American

**Corresponding Author: Dr. Azza Faried. Tropical Medicine department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt. Tel: 01224847367*

Association for the Study of Liver Diseases (AASLD) practice guideline (2005) recommended that surveillance for HCC should be performed using ultrasonography and AFP for screening at 6 to12 month intervals. AFP is abundantly expressed on activated endothelial cells and remodeling (Duff et al., 2003). AFP is a glycoprotein synthesized by the fetal liver and is a prominent component of serum proteins in early embryonic life. The AFP level begins to fall after birth and it is undetectable or low in healthy adults.

Serum AFP level can rise again in adults with HCC, germ cell tumors and liver disease (Debruyne & Delanghe., 2008). It has been suggested that circulating endostatin levels may reflect the tumor burden rather than the anti-angiogenic activity in the tumor (Feldman, Alexander et al., 2001). The higher serum endostatin levels observed in patients with large tumors in the current study may also reflect a relationship between serum endostatin levels and tumor burden. It has been suggested that AFP-L3, appears to be produced only by cancer cells also, it has been suggested that AFP-L3 is a sensitive and specific marker for HCC (Li et al., 2001).

Arginase is an enzyme highly expressed in the liver that converts L-arginine to L-ornithine plus urea (Whealby, 2004). L-ornithine is the precursor for polyamines, which promote cell growth (Witte & Barbul, 2003). Given a possible role for elevated arginase expression in HCV associated with HCC, experiments were designed to test the hypothesis that arginase was up-regulated by HCV, and if so, that is contributed importantly to the mechanism whereby HCV stimulated hepatocellular growth and tumorigenesis. So the aim of this study was to estimate the accuracy of different recent oncobiomarkers as useful for the HCC diagnosis and follow-up.

SUBJECT AND METHODS

This was a cross-section prospective study. An informed written consent was obtained from all patients. The ethical consideration was informed consent taken from every patient. This study was conducted for one year on 90 patients with liver disease and 20 healthy subjects as control admitted to Hepatology and Gastroenterology Department, National Hepatology and Tropical Medicine Research Institute (NHTMRI). The age of these patients ranged from 30-75 years. These patients were selected for this study according to the following inclusion and exclusion criteria.

Exclusion criteria were cases with abnormal renal function. The inclusion criteria were adult patients with liver disease as classified in table 4. The patients under this study were classified into three groups; twenty healthy subjects (control) group, Group I include 30 patients with chronic liver disease (LCD), Group II include 30 patients with liver cirrhosis (LC) and the Group III include 30 patients with hepatocellular carcinoma (HCC).

All patients were subjected to the following category; firstly, thorough history taking with particular attention to manifestations of liver disease especially abdominal ultrasonography and abdominal spiral CT scanning to patients with hepatic focal lesion (table 4) and/or elevated AFP. Secondly, full general and local examination looking for signs of liver disease will be recorded. Finally full investigations of renal function tests (serum creatinine and blood urea) and liver function tests, transaminases (ALT& AST), serum total, serum albumin and prothrombin time (PT) in addition to serum alpha-fetoprotein (AFP). AFP-L3, arginase and endostatin were detected by Elisa procedures according to manufacturer's instructions.

RESULTS

This study was conducted on four groups of patients without no significant difference of mean age between subjected cases (table 1). These classifications were in accordance to the observation of our obtained data in table 4. Transaminases, total bilirubin and prothrombin time observation indicate significant elevation levels in CLD, LC and HCC cases compared with control healthy individuals (table 1).

The albumin, platelets, hemoglobin, and hematocrit recorded significant decreasing levels in hepatitis groups (table 1). As regards AFP induce a significant difference between HCC group and healthy control (P=0.03), as well as CLD group and liver cirrhosis group (P=0.01), while there was a highly significant difference increasing between HCC group compared with CLD cases (P=0.001) as in table 2. AFP has a specificity 77% and sensitivity 70% with a cut-off point less than 20 ng/ml in HCC compared with different subjects (table 3).

AFPL3 recorded no significant difference between HCC group and control (P=0.05), while there was a significant difference between CLD group and healthy subjects (P=0.02), as well as between HCC group and LCD group (P=0.01). For arginase, there was no significant difference between group I and control (P= 0.06), as well as between group II and control (P=0.09), also there was no significant difference between group III and control (P= 0.055), while there was a significant difference between group I and group II (P=0.01).

As regards endostatin, there was a significance difference between group I and control (P=0.01), as well as a significance difference between group I and group II (P= 0.02) and between group III and control (P=0.006) and a highly significant difference between group II and control (P=0.003).

DISCUSSION

Total serum AFP more than 200 ng/ml was highly suggestive of a diagnosis in HCC patients with liver disease with about 100% predictive for HCC; in addition, AFP-L3 is associated with a7-fold increasing risk of HCC developing (Chrzanowsk et al., 2008). Based on retrospective observations for patients with total AFP level less than 200 ng/ml, the AFP-L3 biomarker specificity approaches 100% for HCC when its percentage exceeds 35% of the total AFP (Leerapun et al., 2007).

Our observation of elevated serum arginase has been detected among patients with cirrhosis and chronic liver diseases agree with Chrzanowsk et al., (2008) but these two suggested biomarker (AFP & AFP-L3) in HCC agree with Ikemoto et al., (2001) studies. This was consistent with microarray analysis, showing the arginase was most highly up-regulated in non-tumor liver compared to tumor among HCV-infected HCC bearing patients (Yokoyama et al., 2004).

In our study, AFP sensitivity was 70% and specificity was 77% at cut-off value more than 20 ng/ml (table 3), while in a previous recent study the best cut-off was 10 ng ml with sensitivity 66.3% and specificity 80.6% when used as a screening test (Biselli et al., 2015). Usually, patients with a higher AFP level were associated with more severe cirrhosis, more frequent vascular invasion, higher tumor burden and poorer performance status. Patients with AFP less than 20 ng/mL had significantly better long-term survival than patients with AFP more than 20 ng/mL and patients with AFP less than 400 ng/mL had significantly better overall outcome than patients with AFP more than 400 ng/mL (Hsu et al., 2015).

Table 1: Biochemical findings in different subjected cases (mean ±SD).

Test		Control group (N= 20)	CLD group (I) (N=30)	LC group (II) (N=30)	HCC group (III) (N=30)	Test (U)	P value
Age (years)	mean	51.50 ±22.2	55.05 ±20.3	55.60±22.4	60.15± 34.9	0.023[a]	0.083
	range	33 – 77	33 - 71	32 - 78	35 - 76	<0.0001[b] <0.0001[c]	
AIT (U/L)	mean	17.35± 9.6	92.90 ±33.5	75.25±28.4	64.65± 33.9	<0.0001[a]	0.0001
	range	11 – 31	32 – 176	12 - 255	34 – 103	0.007[b] 0.85[c]	
AST (U/L)	mean	22.05 ±4.1	69.80± 30	96.45± 33.5	133.35± 77.4	<0.0001[a]	0.0001
	range	13 – 34	30 - 108	31 - 402	80 - 300	0.003[b] 0.02[c]	
Albumin (gram %)	mean	4.01± 1.5	2.76± 1.0	2.53± 1.1	2.79 ± 0.69	0.04[a]	0.0001
	range	2.80 – 5.10	1.90 – 3.60	1.30 – 3.90	1.70 – 3.40	0.005[b] 0.99[c]	
T. bilirubin (mg %)	mean	0.70± 0.14	2.65 ±1.09	5.74± 2.08	3.72 ± 1.9	0.001[a]	0.003
	range	0.40 – 0.90	1.00 – 5.40	1.10 – 13.40	1.50 – 6.01	0.001[b] 0.001[c]	
P T (sec.)	mean	14.05± 1.0	16.20± 2.0	18.95± 3.1	15.80± 3	<0.0001[a]	0.124
	range	13.0 – 16.0	14.0 – 19.0	11.7 – 16.7	14.2 -19.6	<0.0001[b] <0.0001[c]	
TLC ($x10^3$/cmm)	mean	6.97± 3.4	8.40± 3.0	10.37± 3.2	10.37± 5.9	0.002[a]	0.168
	range	4.0 – 10.2	3.1 – 16.2	3.0 - 16.2	3.0 – 16.1	0.008[b] <0.0001[c]	
Hb (g/dl)	mean	13.72± 2.1	9.90 ± 2.0	10.09 ±2.2	11.80± 3.1	0.008[a]	0.0001
	range	12.1 – 15.7	6.1 – 13.0	4.1 – 16.0	9.0 – 15.0	0.004[b] <0.0001[c]	
Hct (%)	mean	42.0± 11.3	31.0± 12	28.97± 9.8	28.97± 8.8	0.006[a]	0.0001
	range	40 – 50	20 - 40	13 – 44.4	13.3 – 44.4	0.001[b] 0.001[c]	
Plt ($x10^3$cmm)	mean	262.10±80	233.95± 76	161.60± 67	123.85 ±34	0.034[a]	0.0001
	range	150 – 450	73 – 431	48 - 331	60 - 160	0.005[b] 0.09[c]	
U (Mann-Whitney test), SD (standard deviation), T (total) PT (prothrombin time), TLC (total leucocytes count), Hb (hemoglobin), Hct (hematocrit) and Plt (platelets count). [a]=Comparing control & HCC group (III). [b]=Comparing control & CLD group (I). [c]=Comparing CLD group (I) & HCC group (III). ([a], [b], [C]) p-value < 0.05 was significant.							

Table 2: Biomarker findings for HCC in different subjected cases (mean ± SD).

Test		**Control group (N= 20)**	**CLD group (I) (N=30)**	**LC group (II) (N=30)**	**HCC group (III) (N=30)**	**Test (U)**	**P value**
AFP (ng/ml)	mean	7.7 ± 2.1	28.9 ± 8.7	99.1 ± 32	609 ± 101.5	0.003[a]	0.0001
	range	0.6 – 8.0	2.4 – 45.7	2.7 – 120.0	12.0 – 1000.0	0.001[b] 0.01[c]	
AFPL3 (ng/ml)	mean	3.6 ± 2.1	9.2 ± 3.3	10.3 ± 3.2	29.1 ± 10.3	0.005[a]	0.0001
	range	0.9 – 7.5	1.4 – 14.9	3.0 – 11.2	3.9 – 34.6	0.02[b] 0.001[c]	
Arginase (ng/ml)	mean	87.0 ± 19.8	100 ± 29.6	120 ± 28.3	130±40.2	0.006[a]	0.0001
	range	67.0 – 99.0	88.1 – 106.0	94.2 – 123.0	98.1 – 137.2	0.09[b] 0.01[c]	
Endostatin (ng/ml)	mean	3.2 ±1.1	13.5 ± 4.1	30.2± 11.2	33 ± 13.2	0.001[a]	0.0001
	range	0.8 – 4.6	2.5 – 19.0	7.0 – 37.0	12.0 – 46.0	0.003[b] 0.002[c]	
U (Mann-Whitney test) & SD (standard deviation). [a]=Comparing control & HCC group (III). [b]=Comparing control & CLD group (I). [c]=Comparing CLD group (I) & HCC group (III). ([a], [b], [C]) p-value < 0.05 was significant.							

Table 3: Sensitivity and specificity of diagnostic values of AFP, AFPL3, arginase and endostatin detection of HCC in different subjected cases.

Test	Cut-off value	AUC	Sensitivity	Specificity
AFP	> 20 ng/ml	0.67	70%	77%
AFPL3	> 29 ng/ml	0.76	80%	80%
Arginase	> 106 ng/ml	0.71	55.77%	85%
Endostatin	> 11.5 ng/ml	0.95	90%	84%

Table 4: Radiological examination of studied groups (Control, CLD, LC and HCC patients) Sonar and Computed tomography (CT).

Parameters	Control N (%)	CLD N (%)	LC N (%)	HCC N (%)	P=value
Liver:					P<0.001*
-Normal liver	20(100%)	10(40%)	0(0%)	0(0%)	
-Bright liver	0(0%)	15(60%)	0(0%)	0(0%)	
-Coarse liver	0(0%)	0(0%)	30(100%)	30(100%)	
Focal lesion	0(0%)	0(0%)	0(0%)	30(100%)	
Ascitis:					P=0.008*
No	20(100%)	25(100%)	18(60%)	24(80%)	
Mild	0(0%)	0(0%)	7(23.3%)	2(6.7%)	
Mod	0(0%)	0(0%)	3(10%)	3(10%)	
Severe	0(0%)	0(0%)	2(6.7%)	1(3.3%)	
PVT:					P=0.19
Yes	0(0%)	0(0%)	3(10%)	3(10%)	
No	20(100%)	25(100%)	37(90%)	27(90%)	
Splenomegaly:					P=0.01*
Yes	0(0%)	0(0%)	3(10%)	7(23.3%)	
No	20(100%)	25(100%)	27(90%)	23(76.7%)	
Hepatomegaly:					P=0.12
Yes	0(0%)	0(0%)	4(13.3%)	3(10%)	
No	20(100%)	25(100%)	26(86.7%)	27(90%)	
Hypertension:					P=0.052
Yes	0(0%)	0(0%)	3(10%)	0(0%)	
No	20(100%)	25(100%)	27(90%)	30(100%)	
*p-value < 0.05 significant, PVT (portal vein thrombosis).					

It is important to emphasize that the American Association for the Study of Liver Disease (AASLD) guidelines remove AFP as a primary screening and surveillance modality for HCC due to the lack of sensitivity and specificity. In the present suggestion, AFP-L3 with a cut-off value more than 29 ng/ml obtained the sensitivity (80%) and specificity 80% (table 3) in agreement with Choi et al., (2013) foundation that observed the AFP-L3 were higher in HCC than in benign liver disease among patients with low AFP levels (>20 ng/ml) while the sensitivity was 71.1% and specificity 83.8% with a cut-off value 5% in patients with HCC. The AFP-L3 has been reported to be more sensitive than AFP for small sized or early stage HCC (Tamura et al., 2010 and Shirake et al., 1995). In accordance with our obtained data, the AFP-L3 is although known to be highly specific for HCC and reflected tumor characteristics including poor differentiation or malignant invasion (Khian et al., 2001).

In our study, there was a significant difference between HCC group and normal control in arginase isoenzyme activity (table 2 & 3), this agrees with a previous study that showed changes in arginase isoenzyme pattern in hepatocellular carcinoma and a significant decrease in activity in tumor tissue when compared to normal liver (Chrzanowska et al., 2008). They also found that arginase activity in the cirrhotic liver, which was used as one of the controls, was lower than in normal liver again in agreement with our results that showed no significant difference between cirrhotic liver group and control. Again, our results disagreed with a later study which was performed on a higher number of cirrhotic patients and showed a significantly lower arginase activity in those cirrhotic livers than in control livers (Chrzanowska et al., 2009).

Our results showed a significant association between serum endostatin level and HCC group as compared to control. This result was in accordance with a previous study that showed a significant decrease in serum endostatin level in postoperative samples when compared to preoperative ones (Dhar et al., 2002). On the contrary, our results were different from other studies that did not show significant association between serum endostatin level and HCC staging (Ming- Yen Hsieh et al., 2011). In another two previous studies, there was no significant association between serum endostatin level and HCC staging and developing (Poon et al., 2004 and Yamagata et al., 2000). From the former suggestion there were no significant influences of serum endostatin levels on overall or disease-free survival; although in our observation there was a trend towards worse survival results in LC and HCC patients with high serum endostatin levels.

CONCLUSION

Arginase, endostatin and AFP-L3, in particular, its high sensitivity measurement, is extremely useful as an index of prognostication and for the degree of hepatocellular carcinoma in Egypt. According to our finding in table 3 the endostatin recorded highly sensitivity (90%) and more provoke in specificity (84%) in HCC subjected cases especially comparing with the other biomarker under investigation in this study. Consequently, endostatin is highly expected and will become more popular worldwide, and not just in the country of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The authors alone are responsible for the content and writing of the paper.

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