

Evaluation of Human Epidermal Growth Factor as a Tumor Marker in Patients with Hepatocellular Carcinoma Related to Hepatitis C Virus

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Abstract

Background: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third leading cause of cancer-related deaths. The primary marker for HCC is Alpha fetoprotein (AFP), however, AFP is not secreted in all cases of HCC and may be normal in as many as 40% of patients with early HCC. Therefore, it was necessary to identify new HCC markers that have a sufficient sensitivity and specificity for the diagnosis of HCC patients. Epidermal growth factor (EGF) is a mitogen for hepatocytes, and mounting evidence supports a role for EGF in malignant transformation, tumor growth and progression.

Aim: The objective of this study was to detect the diagnostic ability of hEGF concentration in serum of cirrhosis and HCC patients as a Tumor marker.

Methods: This study was carried out on 150 individuals divided into 3 groups; 50 control, 50 Cirrhotic and 50 HCC, all three groups were investigated for liver function tests, and markers of liver injury, HCC patients were screened by Triphasic Computed Tomography (CT), also determination of EGF and AFP in serum of all individuals were performed using Enzyme Linked Immuno Sorbent Assay (ELISA).

Results: The serum level of EGF was significantly increased in HCC group as compared to liver cirrhosis and healthy control group. The best cut off value was 299pg/ml of EGF which had sensitivity and specificity of 82% and 88% respectively in comparison to AFP which have sensitivity of 70% and specificity of 62% at cut off 21ng/ml. As far as we know this is the first study on serum EGF as a tumour marker for HCC.

Conclusion: EGF has higher sensitivity and specificity than AFP in HCC patients, so it can be used as a useful HCC marker.

Keywords: EGF, HCC, Tumor Marker, AFP.

Introduction

Hepatocellular carcinoma (HCC) is a common malignancy worldwide and is the main cause of mortality in patients with chronic liver diseases [1]. HCC affects approximately one million individuals annually worldwide with the incidence equal to the mortality rate [2]. In Egypt, HCC was reported to account for about 4.7% of chronic liver disease patients, with a doubling in the incidence rate in the past 10 years [3]. The major risk factors

include chronic HBV and HCV infection, which are represented in 70–95% of HCC patients [4], both HCV and HBV infection are the most common risk factors of HCC among Egyptian patients [5]. About 10–20% of the general Egyptian population is infected with HCV [6]. Cirrhosis is in turn the leading cause of HCC [7], which present in 80% to 90% of patients [8,9], as a process of necrosis and regeneration seen in cirrhosis predisposes hepatocytes to the development of neoplasia and dysplasia [10].

It was reported that Alcohol consumption increases the risk of HCC primarily through the development of cirrhosis [11]. Diagnosis is usually made by history, physical examination, imaging (ultrasound, MRI or CT scan showing a liver mass consistent with HCC) and optionally elevated serum AFP (>400 ng/ml), However AFP is elevated in only 50%–75% of cases [12]. Generally, AFP shows acceptable sensitivity; however, AFP is not secreted in all cases of HCC and may be normal in as many as 40% of patients with early HCC [13,14]. Also, AFP is elevated during pregnancy [15]. Additionally, AFP elevation has also been recognized in the presence of acute and chronic viral hepatitis as well as in patients with cirrhosis caused by hepatitis C [16]. More than 90% of Contrast-enhanced ultrasonography has been shown to be highly accurate in diagnosing HCC in cirrhotic livers [17]; however, Contrast-enhanced ultrasound may produce false-positive findings for HCC in patients with intrahepatic cholangiocarcinoma [18]. Human epidermal growth factor (hEGF), consisting of 53 amino acid residues, is a single chain polypeptide with a molecular weight of about 6,200 Dalton [19]. EGF was isolated in 1962 and has been shown to stimulate the proliferation and differentiation of epidermal and epithelial tissues, via binding to the EGF receptor (EGFR) [20-22], Mounting evidence supports a role for EGF in malignant transformation, tumor growth and progression [23]. Over-expression of a secreted human EGF fusion protein enhances the transformation of fibroblasts to fibrosarcomas and induces the development of HCC in transgenic mice [24,25]. EGF concentrations were found lowered in patients with non-small cell lung cancer and head and neck carcinoma [26]. However, an increase in EGF was found in pancreatic Cancer [27], and papillary thyroid carcinoma [28], So this study aimed to detect and identify diagnostic ability of hEGF concentration in serum of cirrhosis and HCC patients as a Tumor marker for HCC.

Materials and Methods

Subjects

This Study was performed on 150 individuals, who were divided into 3 groups; 50 control, 50 cirrhotic and 50 HCC. All were clinically examined with taken history; these samples of the groups were collected from El Sahel Teaching Hospital and Misr University for Science and Technology in the period from September 2013 to December 2014.

Radiological study: Triphasic computed tomography was performed for all HCC patients.

Histo pathological study: the liver biopsy specimens were collected intra operative from HCC patients and Cirrhotic patients. Specimens were fixed in formalin embedded then sectioned and stained by Haematoxylin and Eosin for routine histological examination to detect the fibrosis score. Histo

pathological grading and staging were performed according to Modified Kondell's Score [29].

Laboratory investigations: Venous blood samples were taken from the individuals in the morning. Blood picture, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Albumin (Alb) and total bilirubin (T.bil) were tested for all groups. ELISA technique was used to measure Epidermal growth factor(EGF) (Quantikine, R&D Systems, Inc. USA) levels and Alpha fetoprotein (AFP) (CanAg AFP EIA Kit, Germany) levels.

Statistical analysis: The statistical package for social science (SPSS version 21) was used for data analysis. Bivariate relationship was displayed in cross tabulations and Comparison of proportions was performed using the chi-square test. T-Independent. P-value was significant at (≤ 0.05) level. Sensitivity and specificity were determined.

Results

The mean age (\pm SD) of control group was 33.76(\pm 8.36) with male frequency of 29 (58%) and female frequency 21 (42%). The mean age for Cirrhotic group was 62.02(\pm 9.69) with male and female frequencies of 29 (58%) and 21 (42%) respectively, while the mean age of HCC group was 59.28(\pm 8.76) with male and female frequencies of 30(60%) and 20(40%) respectively. Regarding to the laboratory parameters was measured in the three studied groups which summarized in Table1. There were very high significance between Cirrhotic and HCC groups at $P=0.0001$ for both EGF and AFP, there was also significance between the two groups at $P=0.01$ for ALT, while there were no significance differences between the two groups regarding other parameters. Fibrosis score measured for cirrhotic and HCC patients which classified into three grades; grade 3, 4 and 5 in our patients. There were 14 (28%) with grade 3 in cirrhotic group while in HCC group there were 13 (26%), for grade 4 cirrhotic and HCC groups were 10 (20%) and 7 (14%) respectively, grade 5 was the dominant grade in both groups with frequency of 26 (52%) and 30 (60%) for cirrhotic and HCC patients respectively and significant at $P=0.0001$. The tumor size in HCC patients was classified into 3 groups; <3cm was detected in only two patients, 3-5cm in 6 patients and >5 cm in 42 patients Table 2. The serum levels of AFP and EGF was significantly increased in HCC as compared to cirrhotic patients and control group ($P<0.0001$) for each, there were significant increase in both AFP and EGF in HCC compared to Cirrhosis ($P<0.0001$) Table1. Regarding to fibrosis score, there were no significant differences in EGF concentration between the three grades, in both Cirrhotic and HCC groups. Also there was no significant differences between AFP and fibrosis score in cirrhotic and HCC groups. At cut-off 21ng/ml serum AFP has Sensitivity of 70% and Specificity of 62% for HCC diagnosis where EGF at cut-off 299pg/ml has Sensitivity of 82% and Specificity of 88% (Table3 and Figure 1).

Table 1: Comparison between the studied groups regarding to Biochemical Laboratory parameters.

Variable	Control group n=50	Cirrhosis group n=50	HCC Group n=50	*P-value
EGF (pg/ml)	117.08 ± 39.58	231.68 ± 58.13	327.60 ± 91.94	0.0001
AFP (ng/ml)	5.94 ± 1.95	19.58±11.29	295.9 ± 277.79	0.0001
AST (U/L)	33.62 ± 10.28	134.84 ± 50.69	150.18 ± 71.21	0.2
ALT (U/L)	30.98 ± 6.14	70.28 ± 17.64	61.28 ± 19.17	0.01
Total bilirubin (mg/dl)	0.756 ± 0.195	2.54 ± 1.34	2.82 ± 0.914	0.3
Total Albumin (g/ml)	3.84 ± 0.2	2.8 ± 0.54	2.64 ± 0.56	0.2
INR	1 ± 0.07	1.25 ± 0.177	1.31 ± 0.22	0.1
platelets count ×10 ³ /ml	295.12 ± 82.705 ×10 ³	122.36±28.276 ×10 ³	123.6 ± 31.8 ×10 ³	0.9

*P-value: comparison between Cirrhotic and HCC patients. Liver function tests. ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase; INR: International Normalization Ratio (for blood clotting); EGF: Epidermal Growth Factor; AFP: Alpha Feto Protein.

Table 2: The mean concentration of serum EGF and tumor size.

Parameter	Tumor size (cm)	n	Mean± SD
EGF	<3	2	313.5±16.26
	3-5	6	272±132.71
	>5	42	336.21±86.09

EGF: Epidermal Growth Factor; N: number of patients.

Table 3: Comparison between AFP and EGF regarding cut off value.

Variables	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
AFP (ng/ml)	21	70%	62%	64.81%	67.39%	66
EGF (pg/ml)	299	82%	88%	87.23%	83.02%	85

PPV: Positive Predictive Value; NPV: Negative Predictive Value; AFP: Alpha Feto Protein; EGF: Epidermal Growth Factor.

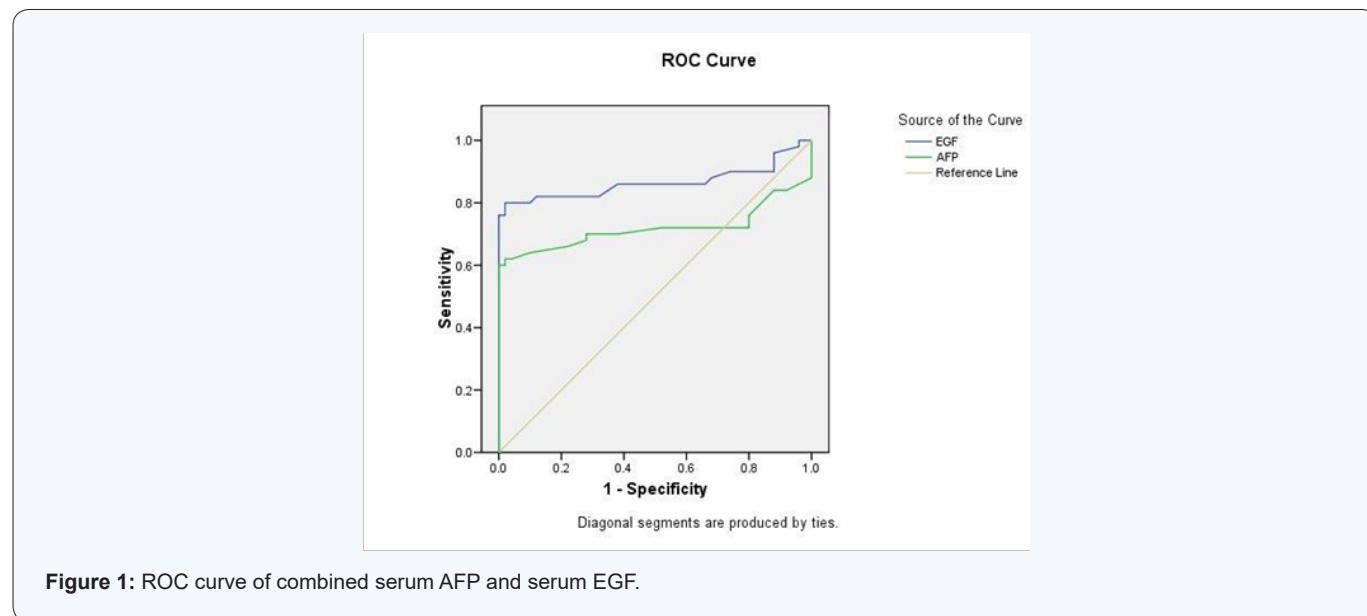


Figure 1: ROC curve of combined serum AFP and serum EGF.

Discussion

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh in women and the third most common cause of death from cancer worldwide [30]. In Egypt,

HCC is the second most common cancer in men and the 6th most common cancers in women [11]. Tumor markers are potential screening tools that are widely used for early diagnosis of tumors [1]. A marker for early diagnosis would meet the following requirements: first, it should achieve high accuracy,

which would increase the probability of a diagnosis being made prior to spread and thus increase the cure rate; second, specimen collection for detecting the marker should be easily operable and non-invasive; and third, the cost-effectiveness should be considered [31]. So in this study we try to investigate the role of EGF as a tumor marker.

In the present study, HCC patients were more common in males than females; that males represented 60 % of HCC patients while female represented 40 % of HCC patients, these results are similar to Zakhary et al (2011) [32]. Who reported that males represented 70.8% of all patients in HCC group, with 83.3% of patients over 50 years [32]. Sun et al. (1998) [33], reported that AST, ALT, Bilirubin and INR usually indicate the type of liver injury [33], in our study there were no significant differences between HCC and cirrhotic groups regarding AST, total bilirubin, albumin, INR and platelet count, so they are not enough to discriminate between HCC and Cirrhotic patients. The only significance difference observed was in the EGF and AFP. In a study by Taketa K (1990) [34], elevated serum AFP is observed in only 60% to 70% of HCC patients and, to a lesser extent (33-65%) in patients with smaller HCCs [34]. In the present study, serum level of EGF in HCC and Cirrhotic patients increased in comparison to healthy control at highly significance-value $P < 0.0001$, this was in agreement with Jo YH et al. (1997) [35], where Serum EGF concentration in hepatocellular carcinoma was significantly higher than that in liver cirrhosis where (P value=0.021695) [35]. In a study by El-Bendary M et al. [36] they found that a significantly elevated EGF serum level in HCC group was found when compared with both cirrhotic and normal control groups ($P < 0.001$), where the mean level of EGF in HCC patients was 527.4 ± 130.6 , they also found that the value of 375 ng/ml was a cutoff point level of developing HCC among cirrhotic patients at 85.6% sensitivity and 87.6% specificity. This was in agreement with the present study where there was a very high significance difference between HCC and cirrhotic patients at $P = 0.0001$, also the sensitivity and specificity of EGF (82% and 88% respectively) was close to that of El-Bendary [36] study but with difference in the cutoff value, this may be due to difference in population number and mean of EGF serum level.

By comparing EGF and AFP it was found that high sensitivity and specificity observed for EGF 82% for sensitivity and 88% for specificity at cut off 299pg/ml, while sensitivity and specificity of AFP were 70% and 62% respectively at cut-off 21ng/ml. In a study carried out by Daniele et al [37].

They found that 60% sensitivity of AFP with 20 ng/ml as cutoff value for diagnosis of HCC [37]. In study by Shehata et al. on HCC and chronic Hepatitis C patients, they reported that a significant difference was found in comparing EGF level of patients with CHC to its corresponding level of HCC group

($P < 0.01$), and Sensitivity was 63.3% while Specificity was 87.5% for EGF [38]. In this study We found that the accuracy of EGF was higher than that of AFP, where the accuracy of EGF was 85 while the accuracy of AFP was only 66, so we can consider that EGF had achieved the three requirements of marker for early diagnosis which were reported by Mc Shane et al. (2005) [31], the high accuracy, cost effectiveness and non-invasive [31]. In our study a positive weak correlation was found between EGF and AFP where; $r = +0.436$ and $P = 0.0001$, as well as EGF and ALT where; $r = +0.442$ while $P = 0.001$. Also in the present study there was significant difference in EGF concentration regarding to tumor size at $P = 0.0001$. The mean concentration of EGF increased by increasing tumor size where in tumor less than 3cm its mean concentration was 313.5 pg/ml, where as in tumor sized from 3-5 its mean concentration was 272 pg/ml while in tumor larger than 5 cm its mean concentration was 336.21 pg/ml. It should be noted that in case of tumor size less than 3cm, concentration of EGF was higher than tumor sized 3-5 cm, this is due to multi focality in few cases with tumor size less than 3cm.

Conclusion

In conclusion, EGF can be used as a biochemical tumor marker for early diagnosis of HCC.

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