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

Amal Ahmed Mohamed1, Ehab Aly Drees2, Abdelmoneim A Makhloul3, Seham Mohmoud4, Hassan Shalaby5 and Asmaa Mohamed Mansour2

1Department of Biochemistry, National Hepatology and Tropical Medicine Institute, Egypt
2Division of Biochemistry, Department of Chemistry, Faculty of Science, Fayoum University, Egypt
3Department of Chemistry, Faculty of Science, Fayoum University, Egypt
4Department of Tropical Medicine, El Sahel Teaching Hospital, Egypt
5Department of Internal Medicine, Misr University for Science and Technology, Egypt

Submission: January 06, 2016; Published: February 01, 2016

*Corresponding author: Amal Ahmed Mohamed, Department of Biochemistry, National Hepatology and Tropical Medicine Institute, Egypt, Tel: +201224947367, +201094918168; email: amalahmedhcp@yahoo.com

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 (Tbil) were tested for all groups. ELISA technique was used to measure Epidermal growth factor (EGF) (Quantikine, R&D Systems,Inc. USA) levels and Alpha feto protein (AFP) (CanAg AFP ELA 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 (±SD) of control group was 33.76(±8.36) with male frequency of 29 (58%) and female frequency 21 (42%). The mean age for Cirrhotic group was 62.02(±9.69) with male and female frequencies of 29 (58%) and 21 (42%) respectively, while the mean age of HCC group was 59.28(±7.76) 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=0.001. 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) Table 1. 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 | 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 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.

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

**References**

1. El-Houseini M, Mohammed M, Ekshemey WM, Hussein TD, Desouky OS, et al. (2005) Enhanced Detection of Hepatocellular Carcinoma. Cancer Control 12(4): 248-253.
2. Yi Z, Ju Q, Li GC (2013) Tumor markers for hepatocellular carcinoma. Mol Clin Oncol 1(4): 593-598.
3. Saad Y, El-Serafy M, Eldin MS, Abdellatif Z, Khatab H, et al. (2013) New Genetic Markers for Diagnosis of Hepatitis C Related Hepatocellular Carcinoma in Egyptian Patients. J Gastrointestin Liver Dis 22(4): 419-425.
4. Lok AS, McMahon BJ (2001) Chronic hepatitis B. Hepatology 34(6):1225-1241.
5. Zakhary N, El-Merzabani M, El-Sawi N, Saleh M, Moneer M, et al. (2011) Impact of different biochemical markers in serum of patients with benign and malignant liver diseases. Journal of Advanced Research 2(1): 49-55.
6. Habib M, Mohamed M, Abdel-Aziz F, Magder LS, Abdel-Hamid M, et al. (2001) Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. Hepatology 33(1): 248-253.
7. Sarwar S, Khan A, Tarique S (2014) Validity of Alpha Fetoprotein for Diagnosis of Hepatocellular Carcinoma in Cirrhosis. J Coll Physicians Surg Pak 24(1): 18-22.
8. El-Serag HB (2012) Epidemiology of Viral Hepatitis and Hepatocellular Carcinoma. Gastroenterology 142(6): 1264-1273.
9. Forner A, Llovet J, Bruix J (2012) Hepatocellular Carcinoma. Lancet 379(9828): 1245-1255.
10. Fattovich G, Strolloinli T, Zagnil, Donato F (2004) Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 127(5 Suppl): S35-S50.
11. Omar A, Abou-Alfa GK, Khairy A, Omar H (2013) Risk factors for developing hepatocellular carcinoma in Egypt. Clin Clin Oncol 2(4): 43.

12. Jelic S, Sotiropoulos GC (2010) Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 21 Suppl 5: v59-v64.

13. Nakatsura T, Yoshitake Y, Senju S, Monji M, Komori H, et al (2003) Glypican-3 over expressed specifically in human hepatocellular carcinoma, is a novel tumor marker. Biochem Biophys Res Commun 306(1): 16-25.

14. El-Housini M, Elsherbiny M, Eldinawad M, Amer M, SaadEldein A, et al. (2001) Serum alpha-L-fucosidase enzyme activity as a marker for hepatocellular carcinoma: comparison with APP using ROC analysis. J Egypt Natl Cancer Inst 13(4): 277-283.

15. Bredaki FE, Wright D, Akolekar R, Cruz G and Nicolaides KH (2011) Maternal serum alpha-fetoprotein in normal pregnancy at 11-13 weeks’ gestation. Fetal Diagn Ther; 30(4): 274-279.

16. Wu JT (1990) Serum alpha-fetoprotein and its lectin reactivity in liver diseases: a review. Ann Clin Lab Sci 20(2): 98-105.

17. Jang HJ, Kim TK and Wilson SR (2009) Small nodules (1–2 cm) in liver cirrhosis: characterization with contrast-enhanced ultrasound. Eur J Radiol 72(3): 418-424.

18. Ferenci P, Fried M, Labrecque D, Brux J, Sherman M, et al. (2009) Hepatocellular carcinoma (HCC): a global perspective, World Gastroenterology Organization Global Guideline. J Gastrointestin Liver Dis 19(3): 311-317.

19. Abdull Razis A, Ismail E, Hambali Z, Abdullah M, Ali A, et al. (2006) The Periplasmic Expression of Recombinant Human Epidermal Growth Factor (hEGF) in Escherichia coli. As Pac J Mol Biol Biotechnol 30: 673. DOI 10.1007/s12032-013-0673-x

20. Cohen S (1962) Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelin opening in the new-born animal. J Biol Chem 237(5): 1555-1562.

21. Carpenter G, Cohen S (1979) Epidermal growth factor. Annu Rev Biochem 48: 193-216.

22. Fisher DA, Lakshmanan J (1990) Metabolism and effects of epidermal growth factor and related growth factors in mammals. Endocrinol Rev 11(3): 418-442.

23. Singletary SE, Baker FL, Spitzer G, Tucker SL, Tomasovic B, et al. (1987) Biological effect of epidermal growth factor on the in vitro growth of human tumors. Cancer Res 47(2): 403-406.

24. Tonjes RR, Lohler J, O’ Sullivan JF, Kay GF, Schmidt GH, et al. (1995) Autocrine mitogen IgEGF cooperates with c-myc or with the Hes locus during hepatocarcinogenesis in transgenic mice. Oncogene 10(4): 765-768.

25. Borlak J, Meier T, Halter R, Spanel R, Spanel-Borowski K (2005) Epidermal growth factor-induced hepatocellular carcinoma: gene expression profiles in precursor lesions, early stage and solitary tumours. Oncogene 24(11): 1809-1819.

26. Nedvídčová J, Nemec J, Stolba P, Vavřejnová V, Bednar J (1992) Epidermal growth factor (EGF) in serum of patients with differentiated carcinoma of thyroids. Neoplasma 39(1): 11-14.

27. Meggati T, Plebani M, Bass effects of epidermal growth factor in development of papillary thyroid cancer. Langenbecks Arch Surg 396: 216-221.

28. Konturek A, Barczynskyz M, Cichon S, Pituch-Noworolska A, Jonkisz J, et al. (2005) Significance of vascular endothelial growth factor and epidermal growth factor in patients with pancreatic cancer. J Hepatol 20(2): 65-71.

29. Ishak K, Baptista A, Bianchi L, Callea F, Groote J, et al. (1995) Histopathological grading staging and of chronic hepatitis. J Hepatol 22(6): 696-699.

30. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, et al. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127(12): 2893-2917.

31. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, et al. (2005) Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 97(16): 1180-1184.

32. Zakhary N, El-Merzabani M, El-Sawi N, Saleh M, Moneer M, et al. (2011) Impact of different biochemical markers in serum of patients with benign and malignant liver diseases. Journal of Advanced Research 2(1): 49-55.

33. Sun JH, Zhou XD, Zhou G, Liu YK (1998) Expression of intercellular adhesive molecule-1 in liver cancer tissues and liver cancer metastasis. World J Gastroenterol 4(3): 202-205.

34. Taketa K (1990) Alpha Feto protein: reevaluation in hepatology. Hepatology 12(6): 1420-1432.

35. Jo YH, Kim BH, Kim HJ, Cho YJ, Lee J, et al. (1997) Changes of Epidermal Growth Factor in Sera among the Patients with Chronic Hepatitis , Cirrhosis and Hepatocellular Carcinoma. Clin Mol Hepatol 3(1): 29-39.

36. El-Bendary N, Neamatallah M, Abd El-Maksoud M, El-Gendy A, El-Wehedy A, et al. (2015) Epidermal Growth Factor Genetic Polymorphism and Its Circulating Serum Level Predict the Risk of Hepatocellular Carcinoma in Egyptian Patients With HCV (Genotype-4)-Related Cirrhosis. Int J Advanced Research 3(1): 697-705.

37. Daniele B, Bencivenga A, Megna AS, Tinessa V (2004) Alphafetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterology 127(5 Suppl 1): 108-112.

38. Shehata F, Abdel Monem N, Sakr M, Kasem S, Balbaa M (2013) Epidermal growth factor, its receptor and transforming growth factor-b1 in the diagnosis of HCV-induced hepatocellular carcinoma. Med Oncol 30: 673. DOI 10.1007/s12032-013-0675-x

How to cite this article: Mohamed AA, Drees EA, Abdelmonem AM, Mohmoud S, Shalaby H, et al. Evaluation of Human Epidermal Growth Factor as a Tumor Marker in Patients with Hepatocellular Carcinoma Related to Hepatitis C Virus. Adv Res Gastroentero Hepatol. 2016; 1(3) : 555565. DOI: 10.19080/ARGH.2016.01.555565