

Association of serum levels of epidermal growth factor with disease severity in patients with unresectable hepatocellular carcinoma

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ABSTRACT

Aim: Epidermal growth factor (EGF) is a mitogen for hepatocyte grown *in vitro*, and its expression is up-regulated during liver regeneration. EGF also plays an important role in tumor initiation and progression. The goal of this study is to assess whether EGF is associated with advanced hepatocellular carcinoma (HCC) and also whether it is a predictive factor of shortened survival. **Methods:** Serum EGF levels were evaluated in a total of 151 subjects: 51 patients with unresectable HCC, (21 of them were eligible for transarterial chemoembolization (TACE) and serum EGF levels were measured before and 1 week after TACE), 40 patients with chronic hepatitis without cirrhosis, 40 patients with cirrhosis, and 20 healthy controls. Patient demographic and laboratory variables were evaluated as predictive factors of survival in a Cox regression multivariate analysis using SPSS software. **Results:** The mean serum level of EGF in patients with HCC was 784.49 pg/mL, which was significantly higher ($P < 0.05$) than all other groups. Mean EGF level in cirrhotic patients was 144.69 pg/mL; in those with chronic hepatitis C without cirrhosis, it was 338.64 pg/mL; and in healthy controls, it was 297.15 pg/mL. In group Ia patients who underwent TACE, the mean serum level of EGF was 759.76 ± 287.88 pg/mL before TACE, and 801.14 ± 276.12 pg/mL 1 week after treatment ($P = 0.34$). On multivariate Cox regression analysis only age ($P = 0.03$) and higher serum EGF level ($P = 0.005$), were inversely correlated with overall survival. **Conclusion:** EGF levels were found to be significantly higher in HCC patients and together with age were the only predictors of poor survival in these patients. There was an increase in EGF levels 1 week after TACE in response to hypoxia; however, this increase was not statistically significant.

Key words: Cirrhosis; epidermal growth factor; hepatocellular carcinoma; survival; transarterial chemoembolization



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Received: 18-02-2015, **Accepted:** 14-10-2015

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How to cite this article: Samy Kohla MA, Al-Haddad OK, Nada A, Al-Warraky M, Obada M, Amer M, Ezzat S, Gabal AA. Association of serum levels of epidermal growth factor with disease severity in patients with unresectable hepatocellular carcinoma. *Hepatoma Res* 2016;2:18-25.

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DOI: 10.4103/2394-5079.168959	

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer deaths all over the world according to the World Health Organization data.^[1,2] In Egypt, incidences of liver cancer have risen dramatically over the last two decades, and now it is the most common cancer in men and the second most common cancer in women,^[3] with an annual rate of HCC development of 1-4%, when hepatitis C virus (HCV)-related cirrhosis is established.^[1] Patients suffering from HCC unlike most solid tumors are confronted with the coexistence of two life-threatening conditions, malignancy and cirrhosis, which makes their prognostic assessments difficult. Despite the usefulness of clinical staging systems for HCC in routine clinical decision making [e.g., Barcelona-Clinic Liver Cancer (BCLC) algorithm], there is still a need to refine and complement outcome predictions.^[4]

There is an obvious lack of minimally invasive, cost-effective, highly sensitive, and specific biomarkers for accurate diagnosis of HCC independent of the cirrhosis status. Alpha-fetoprotein (AFP) is not a reliable marker in early HCC diagnosis due to its low specificity and sensitivity, which renders it unsatisfactory and suggests an urgent need for novel biomarkers for early stage HCC detection.^[5] Measurement of circulating levels of angiogenic factors in patients with cancerous tumors have several advantages over the direct assessment of tumor angiogenesis, it does not require a tumor specimen, thus they are theoretically applicable to every cancer patient for their technical simplicity and the availability of repeated measurements during (i) initial diagnosis, (ii) course of various anticancer treatments, and (iii) long after the treatment is over.^[6,7] Vascular endothelial growth factor (VEGF) is well known to play a crucial role in tumor angiogenesis by inducing new vessel formation and promoting tumor invasion and metastasis, also VEGF levels are higher in HCC patients. VEGF is used as a biomarker of lymph node metastasis in HCC. In addition, the expression of VEGF is closely correlated with tumor recurrence and prognosis. Of note, VEGF receptor expression levels have also been found to correlate with the development of tumor.^[8] Epidermal growth factor (EGF), another key regulator of cell survival and proliferation, is another biomarker identified in the pathogenesis and progression of different types of cancer.^[9] During 1980s, several reports described the overexpression of EGF and EGF receptor (EGFR) in a variety of epithelial tumors, which may have a critical role in the etiology of human cancers.^[10,11]

EGF is also speculated to enhance the transformation of fibroblasts to fibrosarcomas by inducing the development of HCC in transgenic mice.^[12] Additionally, a functional polymorphism in the EGF gene is reported to be associated with the risk of development of HCC.^[13] Kannangai *et al.*^[14] reported overexpression of EGFR associated with late-stage HCC, increased cell proliferation, and degree of tumor differentiation. All these reports support our hypothesis that EGF is a viable candidate for screening for different cirrhotic populations for early detection of HCC. Transarterial chemoembolization (TACE) being the standard of care treatment for patients with intermediate stage HCC, the best candidates are patients with Child A^[1,2] cirrhosis. And, multifocal non-invasive HCC was also included in the study as an arm to determine the EGF levels in response to the treatment. Most importantly, we tried to identify whether circulating EGF levels were altered in cirrhotic livers with and without HCC. The results from the studies showed that EGF was indeed a sensitive biomarker indicative of poor survival outcome, and it was positively correlated with age in the older population.

METHODS

This case-control study was conducted on 151 patients with chronic liver disease, presented to the Hepatology Clinic, from June 2010 to June 2011. Four groups of patients were studied: HCC, chronic hepatitis C with or without liver cirrhosis, in addition to a fourth group of healthy control subjects with well-matched age and sex. Group I comprised 51 patients with unresectable HCC (intermediate, advanced, and terminal stages), lesions were assessed regarding the number, size, vascular invasion, and distant metastasis. Patients in this group were subdivided according to eligibility for TACE into two subgroups. Subgroup Ia comprised 21 patients with an intermediate stage HCC, who were eligible for TACE (BCLC stage B). Their EGF levels were assessed before and 1 week after TACE. Subgroup Ib comprised 30 HCC patients who were not eligible for TACE, in advanced and terminal stages (BCLC stages C and D). Group II comprised 40 chronic hepatitis C patients without cirrhosis. Group III comprised 40 patients with HCV-related cirrhosis with no evidence of HCC. Group IV comprised 20 apparently healthy subjects as a control group with no evidence of liver disease and/or neoplasm. They were all with well-matched age and sex.

All patients were subjected to the following history taking, complete physical examination, and routine laboratory biochemical and hematological tests.

Laboratory investigations

Five milliliter venous blood samples were collected from patients and controls, centrifuged, the serum was separated and divided into two aliquots. The first aliquot was used for routine laboratory investigations including liver function tests (aspartate transaminase, alanine transaminase, bilirubin, and albumin) using fully automated auto analyzer SYNCHRON CX9ALX (Beckman Coulter Inc., CA, USA). Serum AFP concentration was measured using the Automated Chemiluminescence System ACS: 180 provided by Siemens Medical Solutions Diagnostics Corporation, USA. The second aliquot was stored in the deep freezer (-70 °C) for detection of EGF^[15]

Serum EGF enzyme-linked immunosorbent assay

Estimation of serum EGF using Human EGF enzyme-linked immunosorbent assay (ELISA) kit (sandwich ELISA), Anogen, catalogue number EL10010 Mississauga, Ontario, Canada (up to 336 pg/mL) following manufacturer's protocol.

Radiological examination

Abdominal ultrasonography, triphasic computed tomography, and dynamic magnetic resonance imaging was performed on patients when required.

TACE

Chemoembolization was performed percutaneously at the angiography unit of the National Liver Institute with the patient under conscious sedation. After infiltration of local analgesic, the Seldinger technique was used to gain access to the common femoral artery through femoral artery puncture. A 5-french vascular sheath was placed into the common femoral artery over a 0.035-inch guide-wire. Under fluoroscopic guidance, a 5-french glide Cobra catheter (Cordis Corporation, Miami Lakes, Florida, USA) was advanced into the aorta. Angiographic study of the superior mesenteric artery, celiac trunk, and the common hepatic artery was performed to identify all of the vessels feeding the HCC nodule, and to assess patency of the portal vein. In some patients, selective angiography of the phrenic or intercostal arterial branches was required. The arterial branches feeding the tumor were selectively cannulated by microcatheters to proceed with TACE and to ensure better preservation of the surrounding non-tumoral liver tissue. Injection was done using an emulsion of lipiodol-doxorubicin (50 mg of doxorubicin mixed with 6-20 mL of lipiodol according to tumor size, number, and vascularity to form the emulsion); injection was performed far from the origin of the gastroduodenal, right gastric, and cystic arteries; the amount injected into the tumor was adjusted according to the size and uptake of the tumor. Gel foam was the

embolic material injected in all patients.

Follow-up of HCC patients

Follow-up was conducted for a minimum of 1 year to assess their survival and mortality rates.

Statistical analysis

Statistically analysis was conducted using SPSS program version 13 for windows (SPSS Inc., Chicago, Illinois, USA) and for all the analysis. A $P < 0.05$ was considered statistically significant.

RESULTS

Most of our patients were males (92.1% male, 7.9% female in group I; 87.5% male, 12.5% female in group II; and 85% male, 15% female in group III). Mean age for groups I, II, III, and IV was 58.2 ± 8.7 [standard deviation(SD)] years, 48.47 ± 11.51 years, 49.47 ± 6.94 years, and 47.50 ± 6.15 years, respectively, with statistically non-significant difference ($P > 0.05$).

Liver function tests in different patient groups are shown in Table 1. Child-Pugh score and BCLC staging for patients are shown in Tables 2 and 3, respectively.

Radiological criteria of hepatocellular carcinoma

Twenty-three patients (45.1%) had a single focal lesion, 9

Table 1: Liver function tests in the three patient groups

	Group I (n = 51)	Group II (n = 40)	Group III (n = 40)	P
Bilirubin (mg/dL)	1.76 ± 1.2	0.83 ± 0.24	1.72 ± 1.59	< 0.05
Albumin (g/dL)	3.16 ± 0.65	4.35 ± 0.53	3.06 ± 0.84	< 0.05
ALT (U/L)	67.34 ± 38.4	61.80 ± 41.13	55.82 ± 30.17	0.31
AST (U/L)	90.03 ± 55.8	48.55 ± 25.20	67.30 ± 32.03	< 0.05
Hb (g/dL)	11.53 ± 1.86	14.04 ± 1.78	10.76 ± 2.31	< 0.05
Platelet (/mm ³)	114.58 ± 55.0	192.62 ± 47.9	104.37 ± 62.9	< 0.05

Data shown as mean ± SD. Group I: HCC patients; Group II: chronic hepatitis; Group III: cirrhotic patients; ALT: alanine transferase; AST: aspartate aminotransferase; Hb: hemoglobin; SD: standard deviation; HCC: hepatocellular carcinoma

Table 2: Study of CPS in groups I and II

	CPS (A) (%)	CPS (B) (%)	CPS (C) (%)	P
Group I	24 (47)	16 (31.3)	11 (21.7)	< 0.05
Group II	19 (47.5)	7 (17.5)	14 (35)	

CPS: Child-Pugh score; (A): score 5-6; (B): score 7-9; (C): score 10-15; Group I: HCC patients; Group II: chronic hepatitis; HCC: hepatocellular carcinoma

Table 3: BCLC staging in HCC studied patients

Groups	BCLC (B) (%)	BCLC (C) (%)	BCLC (D) (%)	Total	P
Group Ia	18 (85.8)	3 (14.3)	0	21	< 0.05
Group Ib	0	13 (44)	17 (56)	30	
Total	18	16	17	51	

Group Ia: HCC, underwent TACE; Group Ib: HCC, did not undergo TACE. BCLC: Barcelona-Clinic Liver Cancer; (B): intermediate stage; (C): advanced stage; (D): end stage; HCC: hepatocellular carcinoma; TACE: transarterial chemoembolization

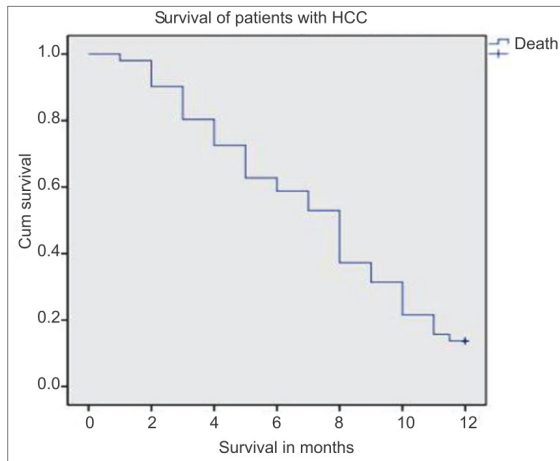


Figure 1: Overall mortality in patients with HCC. HCC: hepatocellular carcinoma

patients (17.6%) had 2 focal lesions, and 19 (37.3%) had ≥ 3 focal lesions. Portal vein thrombosis was present in 12 patients (23.5%), while metastasis was detected in 10 patients (19.6%) [Table 4].

Survival in studied subjects

Overall mortality in patients with HCC was 86% at 12 months. Median survival time was 8 months [Figure 1]. Figure 2 is the Kaplan-Meier cumulative survival curve showing the role of serum EGF in patients' disease-related mortality and cumulative survival. Out of 51 patients with HCC, 44 were deceased after 1 year of follow-up, 9 of them died from upper gastrointestinal bleeding, 15 died from sepsis and spontaneous bacterial peritonitis, 5 died of hepatorenal syndrome, 6 patients died at the intensive care unit after an attack of hepatic encephalopathy, 1 patient died from diabetic hypoglycemic, and the exact cause of death could not be identified in 8 patients.

The majority of patients (85.8%) of group Ia were categorized as BCLC stage B, and 14.3% were in BCLC stage C; while 44% of group Ib were categorized in BCLC stage C and 17% were in BCLC stage D. In Cox regression analysis, age, and serum EGF level were the only factors significantly predicting poor survival in HCC patients ($P < 0.05$) [Table 5].

EGF studies in our subjects

Group I levels were 784.49 ± 313.25 , group II levels were 338.64 ± 224.68 , group III levels were 144.69 ± 124.30 , and for group IV, they were 297.15 ± 175.36 pic/mL. The values are also summarized in Table 6. In pair-wise comparison among individual groups, we found that EGF serum levels were significantly higher in HCC patients compared with the other groups. Statistically significant differences were observed in pair-wise comparison between groups I and II, I and III, I and IV, II and III, and II and IV with $P < 0.05$. Groups III and IV

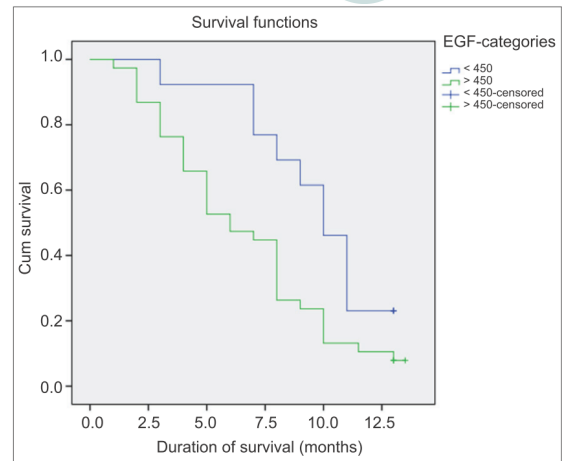


Figure 2: Kaplan-Meier cumulative survival curve showing the role of serum EGF in patients' disease-related mortality and cumulative survival. EGF: epidermal growth factor

Table 4: Radiological criteria of the tumors in HCC patients

HCC criteria	Patients, n (%)
Lesion	
Single lesion	23 (45.1)
2 lesions	9 (17.6)
> 3 lesions	19 (37.3)
PVT	
With PVT	12 (23.5)
Without PVT	39 (76.5)
Metastasis	
With metastasis	10 (19.6)
Without metastasis	41 (80.4)
Lesion size	
Mean \pm SD in Group Ia (cm)	6.17 \pm 3.055
Mean \pm SD in Group Ib (cm)	5.67 \pm 1.683

HCC: hepatocellular carcinoma; PVT: portal vein thrombosis; SD: standard deviation

Table 5: Factors affecting survival in patients with HCC

	HR (95% CI)	P
Age	1.031 (0.984-1.079)	0.03
Sex	1.324 (0.353-4.965)	0.850
Smoking	1.014 (0.893-1.151)	0.490
Pesticide	1.046 (0.851-1.286)	0.206
AFP	1.000 (1.000-1.000)	0.600
EGF	1.003 (1.001-1.004)	0.005
Number of lesions	0.709 (0.478-1.053)	0.705
Lesions size	1.026 (0.890-1.184)	0.066

AFP: alpha-fetoprotein; EGF: epidermal growth factor; HR: hazard ratio; CI: confidential interval; HCC: hepatocellular carcinoma

showed no significant difference in the EGF levels ($P > 0.05$) [Table 7]. EGF levels were 766.05 ± 299.64 pg/mL in BCLC stage B patients, 738.06 ± 320.707 pg/mL in BCLC stage C, and 847.705 ± 328.70 pg/mL in BCLC stage D with no significant difference ($P > 0.05$) [Table 8]. Non-significant difference was found between EGF serum levels in patients with metastatic HCC (mean \pm SD of EGF 847.5 ± 245.4 pic/mL) and in patients with no metastasis (mean \pm SD of EGF 769.1 ± 328.4 pic/mL) ($P > 0.05$) [Table 9]. EGF levels in patients with portal vein thrombosis (mean \pm SD 825.5 ± 318.04 pic/mL) and those without portal vein thrombosis (772.02 ± 314.89 pic/mL) ($P > 0.05$) [Table 9] were similar. Mean

EGF level in HCC patients with one focal lesion was 757.1 ± 327.8 pic/mL, in those with 2 focal lesions was 873.8 ± 334.7 pic/mL, and in those with multiple focal lesions was 775.2 ± 293.9 pic/mL ($P < 0.05$) [Table 9]. Serum EGF levels were strongly correlated to the tumor size and serum AFP levels (using Spearman correlation test, with $P < 0.05$).

Analysis of the receiver operating characteristic (ROC) curve of EGF in HCC prediction [Figure 3] revealed that the area under the curve was 0.93 with 95% confidential interval (CI): 0.89-0.97. Cut-off value of 450 had 74.5% sensitivity, and specificity of 84%, while cut-off value of 700 pg/mL had sensitivity of 60.78% and specificity of 97%, and cut-off value of 900 had sensitivity 39.22% and specificity 98% [Table 10].

Regarding EGF levels in HCC group who underwent TACE (Ia) although EGF levels were higher after TACE than before, no statistically significant difference was found, mean \pm SD 759.76 ± 287.88 pic/mL before TACE, 801.14 ± 276.12 pic/mL after TACE with $P < 0.05$.

DISCUSSION

This case-control study was designed to assess the role of EGF as a predictor factor of progression of HCC in terms of correlation with tumor criteria: size, number, vascular

Table 6: Epidermal growth factor serum levels in the four studied groups

	Number of subjects	EGF serum levels (mean \pm SD, pg/mL)	P
Group I	51	784.49 ± 313.25	< 0.05
Group II	40	338.64 ± 224.68	
Group III	40	144.69 ± 124.30	
Group IV	20	297.15 ± 175.36	
Total	151	432.35 ± 350.35	

Group I: HCC patients; Group II: chronic hepatitis; Group III: cirrhotic patients; Group IV: healthy control; SD: standard deviation; EGF: epidermal growth factor; HCC: hepatocellular carcinoma

Table 7: Pair-wise comparison of epidermal growth factor between individual groups

Groups	P
Group I vs. II	< 0.05
Group I vs. III	< 0.05
Group I vs. IV	< 0.05
Group II vs. III	< 0.05
Group II vs. IV	< 0.05
Group III vs. IV	0.65

Group I: hepatocellular carcinoma patients; Group II: chronic hepatitis; Group III: cirrhotic patients; Group IV: healthy control

Table 8: Epidermal growth factor levels in HCC patients according to different stages of BCLC classification

	n	EGF serum levels (mean \pm SD, pg/mL)	P
BCLC (B)	18	766.05 ± 299.64	0.66
BCLC (C)	16	738.06 ± 320.707	
BCLC (D)	17	847.705 ± 328.70	
Total	51	784.49 ± 313.25	

BCLC: Barcelona-Clinic Liver Cancer; (B): intermediate stage; (C): advanced stage; (D): end stage; SD: standard deviation; EGF: epidermal growth factor; HCC: hepatocellular carcinoma

invasions, and patient survival. Subjects of our study were selected from the Hepatology Clinics, National Liver Institute, Menoufia University in the period from June 2010 to June 2011. Four groups of patients were studied: Group I comprised 51 patients with unresectable HCC (which were further subdivided according to the eligibility for TACE into subgroups Ia and Ib), group II comprised 40 chronic hepatitis C patients, and group III comprised 40 cirrhosis patients. A fourth group of 20 healthy control subjects (age and sex-matched), was also included in the study. HCC patients were followed up for 1 year for evaluation of their 1-year survival rates.

In this study, 45% of our patients had a single tumor, while 17.6% had 2 lesions, and 37.3% had > 3 lesions. Similar results were presented by Shaker *et al.*^[16] who showed that 38.6% of their cohort had more than one hepatic focal lesion. Vascular invasion was found in 23.5% in our HCC patients. These results are not congruous

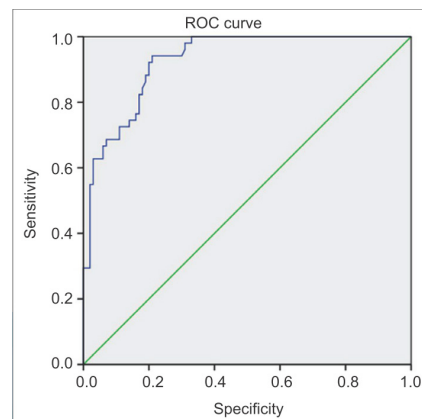


Figure 3: ROC curve of epidermal growth factor value in prediction of hepatocellular carcinoma development. Diagonal segments are produced by ties. ROC: receiver operating characteristic

Table 9: Epidermal growth factor level in HCC patients according to tumor metastases, portal vein thrombosis and number of lesions

Serum level of EGF	EGF serum levels (mean \pm SD, pg/mL)	P
Non-metastatic tumors	769.1 ± 328.4	0.39
Metastatic tumors	847.5 ± 245.4	
No PVT	772.02 ± 314.89	0.60
PVT	825.5 ± 318.04	
One lesion (n = 23)	757.1 ± 327.8	0.64
Two lesions (n = 9)	873.8 ± 334.7	
≥ 3 lesions (n = 19)	775.2 ± 293.9	

EGF: epidermal growth factor; PVT: portal vein thrombosis; SD: standard deviation; EGF: epidermal growth factor; HCC: hepatocellular carcinoma

Table 10: Sensitivity and specificity of epidermal growth factor in HCC group

Studied variable	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
EGF at the cut-off value of 450	74.5	84	70.37	86.60
Cut-off level 700	60.78	97	91.98	82.91
Cut-off level 900	39.22	98	90.91	75.97

EGF: epidermal growth factor; PPV: positive predictive value; NPV: negative predictive value; HCC: hepatocellular carcinoma

with the results of Pirisi *et al.*^[17] which showed that the portal vein thrombosis represented 44% in an autopsied HCC specimen. Another study done by Abdel-Wahab *et al.*^[18] documented that only 15.9% had portal vein thrombosis. This wide range of discrepancy is attributed to the heterogeneity of the studies (some studies evaluated vascular invasion based on histology while others evaluated it based only on imaging). Follow-up of our HCC patients for 1 year revealed that the overall 1-year mortality was 86% with a median survival time of 8 months. Altekruze *et al.*^[19] reported a median survival of < 5 months although a study in Italy found median survival in an untreated group as 10 months,^[20] this could be explained by the fact that the majority of HCC patients had more advanced liver disease.

Evaluation of serum levels of EGF in the four groups revealed significantly higher levels of EGF in HCC patients (784.49 ± 313.25 pg/mL) compared to the other three non-HCC groups. These results signified the role of EGF in tumor growth and progression. Shehata *et al.*^[21] showed higher EGF and transforming growth factor beta 1 levels in patients with HCC compared to the non-HCC counterparts with HCV viral infection and the control subjects. In our study, age and serum EGF levels were the only factors that significantly predicted survival in our HCC patients; higher EGF levels may be associated with tumor aggressiveness and shortened survival. This hypothesis is supported by the *in vitro* findings of Klocke *et al.*^[22] who demonstrated that the Ig EGF (secreted variant of human EGF) imparts immortality to hepatocyte *in vitro*. This also was reported by Inoue *et al.*^[23] who studied vandetanib, an inhibitor of VEGF receptor-2 and EGF receptor, in liver cancer in mice and found that it suppressed tumor development and improved prognosis of liver cancer; improved survival, and reduced number of intrahepatic metastases. Yoneda *et al.*^[24] found that higher levels of EGF were associated with activation of EGF-EGFR pathway associated with the development of CK19-positive HCC, and the EGF-induced increase in growth abilities of HCC may account for the poor prognosis of those patients. DeCicco *et al.*^[25] reported overexpression of EGF receptors (EGFR) in hepatoma cells of rats, suggesting that EGFR may be useful as a dynamic marker for the development of hepatoma. This was confirmed by Sung *et al.*^[26] who concluded that serum EGFR level was a potential biomarker of liver cancer. Kannangai *et al.*^[14] added that EGFR can be considered as a marker for predicting the metastasis and recurrence of HCC. Wu *et al.*^[27] found that EGF was a promoting factor for hepatoma cells stressing on the critical role in EGF-induced proliferation. Wu *et al.*^[28] demonstrated that overexpression of epidermal growth factor-like domain

7 was found predominantly in hepatoma cells and closely correlated with poor prognosis.

ROC curve analysis of EGF in HCC showed that the area under the ROC curve of EGF for the prediction of HCC progression was 0.93 with 95% CI: 0.89-0.97. Cut-off value of 450 had 80% sensitivity while cut-off value of 700 had sensitivity 60.78% and specificity 97% while cut-off value of 900 had sensitivity 39.22% and specificity 98%. Shehata *et al.*^[21] showed that significant higher serum levels of EGF in patients with HCC compared to their levels in patients with HCV infection and control subjects with cut-off value of 914 pg/mL, EGF shows 63.3% sensitivity, and 87.5% specificity for HCC patients.

Our results revealed that the EGF serum level increased slightly in chronic hepatitis activity than levels in established cirrhotic group, reflecting potential role of EGF in fibrosis process as described by other reports such as Iagoda *et al.*,^[29] who studied the growth factors and the histological picture of the liver in chronic viral hepatitis and hepatic cirrhosis and found that EGF levels decreased with increase in histological activity and the degree of hepatic fibrosis to cirrhosis. Predictive factors for progressive HCC in our patients were analyzed by binary logistic regression, serum EGF level was found to be a predictive factor of HCC progression. These results agree with the results of a meta-analysis of eight studies concluding that EGF polymorphism is a risk factor in hepatocarcinogenesis.^[30] Tanabe *et al.*^[31] stated that in a dose-dependent fashion EGF measurements in serum and in liver tissue were presumed to be most relevant to hepatocyte transformation in cirrhosis and concluded that the EGF gene polymorphism was associated with development of HCC in liver cirrhosis through modulation of EGF levels. Regarding factors affecting patients' survival using Cox regression analysis, older age and higher serum EGF levels were the only factors significantly affecting survival ($P < 0.05$).

Overall, there was a strong correlation ($P < 0.05$) between EGF level and tumor size, signifying its potential role in tumor proliferation and its use as a predictive factor of HCC progression. A major limitation of our study is the relatively small number of patients who underwent TACE, heterogeneity of the study cohort is a limitation in many of the TACE studies because of the wide spectrum of HCC patients eligible for TACE compared with the other modalities of treatment of HCC, this can be overcome by conducting future prospective studies on larger number of patients with similar disease. Interestingly, serum EGF levels were higher post-TACE, although this difference was not statistically significant. The explanation of

this marginal increase is not yet known, TACE-induced hypoxia (and angiogenesis) might be a contributing factor which needs further studies. The time point to measure serum EGF (1 week after TACE) was chosen at random as an initial evaluation to also address the effect of TACE on EGF, future studies focusing on including 2 additional time points at 1 and 3 months are warranted. Philip *et al.*^[32] tested five EGFR inhibitors: Erlotinib, gefitinib, cetuximab, lapatinib, and vandetanib. Erlotinib showed activity in a phase II study with mixed HCC populations with a median survival of 13 months, and it was being tested in combination with sorafenib in phase III. The other drugs either have not shown meaningful signals of efficacy in phase II, such as gefitinib and lapatinib, or are still in early stages of investigation.^[33] Gefitinib, a selective EGFR tyrosine kinase inhibitor, is reported to successfully treat lung cancer. When investigating the effects of gefitinib on tumor-induced angiogenesis, it was found that production of both VEGF and chemokine factor by EGF-stimulated HCC was more markedly inhibited by gefitinib. Sogawa *et al.*^[34] in their study used a novel human monoclonal antibody against EGFR as an imaging probe for HCC concluded that the radiolabeled human anti-EGFR monoclonal antibody 048-006 has the potential to be a safer imaging probe for predicting tumor uptake of anti-EGFR antibody therapeutic agents in HCC. Studying EGF and its receptors: pathway, therapies, and pipeline concluded that the exploitation of EGFR-directed therapies offered an improvement in survival and quality of life in non-small cell lung cancer and colorectal carcinoma.^[35] Additional efforts should be exerted directing further studies on EGFR-directed therapies to the poorly treated HCC patients.

In conclusion, serum EGF levels were found to be significantly higher in HCC group in comparison with cirrhosis, chronic hepatitis, and control groups. A serum level of EGF is a predictor factor of HCC progression and together with older age were the only two predictive factors for poor survival in patients with HCC after 1 year of follow-up. There was an increase of serum EGF levels in response to TACE without significant difference. Future studies should be conducted to focus on EGFR and their inhibitors as new promising therapeutic agents for HCC with the inclusion of more patients with respectable tumors amenable to resection, ablation, and/or liver transplantation who are expected to survive long enough to study any potential prognostic importance of EGF.

Financial support and sponsorship

This study was supported by the Hepatology and Interventional Radiology Departments at the National

Liver Institute, Menoufiya University, Egypt.

Conflicts of interest

There are no conflicts of interest.

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