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Internal Medicine

Epidermal Growth Factor as a biomarker for early detection of Hepatocellular Carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the sixth greatest frequent disease in the world and the second biggest cause of cancer mortality in men in developing nations, with over 782,000 deaths globally each year, with China accounting for almost half of them.

Aim of the study: The goal of this research was to see whether epidermal growth factor might be used as an indicator for early detection of hepatocellular cancer.

Patients and Methods: One hundred patients were selected from the Internal Medicine department's outpatient clinic and ward at Sayed Galal University Hospital for this case control research. The study's patients were placed into three groups: Group I : 40 patients with HCC, Group II : 40 cirrhotic patients without HCC, and Group III : 20 healthy people who were devoid of hepatic diseases stigmata.

Result: Sensitivity, specificity and accuracy of AFP in differentiate between HCC and cirrhosis. Sensitivity was (92%), specificity (90%) and accuracy (91%) with cut off value 5.8. Sensitivity, specificity and accuracy of H. EGF in differentiate between HCC and cirrhosis. Sensitivity was (99%), specificity (100%) and accuracy (99%) with cut off value 350. There was statistically substantial connection between AFP, H. EGF and other laboratory findings ($P < 0.05$).

Conclusion: T In the detection of early HCC, epidermal growth factor as an indicator is a helpful biomarker that might supplement the effectiveness of AFP.

Keywords: *Epidermal; Growth Factor; Hepatocellular Carcinoma.*

INTRODUCTION

Hepatocellular carcinoma is a prevalent cancer that is the leading reason of mortality in persons with chronic liver problems across the globe. HCC affects roughly one million people globally each year, with a prevalence rate equal to the fatality rate.[1]

Liver cancer has become the greatest prevalent tumor in males and the 2nd greatest commonly carcinoma in women in Egypt during the previous two decades, with an annual risk of HCC formation of 1-4 percent when Cirrhosis caused by the hepatitis C virus (HCV) has been identified.[2]

The more frequent risk indicators for HCC in Egyptian patients are persistent HBV and HCV infections, which are seen in 70-95 percent of HCC patients.[3]

History, clinical exams, imaging (ultrasound, MRI, or CT scan indicating a liver tumor compatible with HCC) and possibly increased blood AFP (>400 ng/ml) are used to diagnose HCC. However, AFP is only raised in 50-75 percent of patients. AFP has good sensitivity in most instances of HCC; although, it is not generated in all instances of HCC and may be typical in up to 40% of cases of early HCC.[1]

Due to its poor specificity and sensitivity, alpha-fetoprotein (AFP) is an unreliable indicator in initial HCC identification, necessitating the development of new biomarkers for initial stage HCC identification.[4]

Another biomarker implicated in the tumor development and progression is epidermal growth factor (EGF), which is a crucial regulation of cell survival and multiplication. Several publications from the 1980s revealed the elevated expression of EGF and EGF receptor (EGFR) in a range of epithelial malignancies, suggesting that EGF and EGF receptor (EGFR) may play a key role in the genesis of human cancers.[4]

Human epidermal growth factor (EGF) is a single-chain polypeptide with a molecular weight of

roughly 6,200 Dalton and 53 amino acid residues. EGF was discovered in 1962 and has been demonstrated to increase epidermal and epithelial tissue proliferating and differentiating by binding to the EGF receptor (EGFR), EGF seems to have a role in malignant transformation, tumor development, and progression, according to growing data. In transgenic mice, overexpression of a released human EGF fusion protein promotes the conversion of fibroblasts into fibrosarcomas and causes the developing of HCC. In individuals with non-small cell lung tumor and head and neck carcer, EGF levels were shown to be lower.[5]

A number of recent mechanistic investigations have shown a link between HCV and EGF. HCV cellular penetration is aided by an EGFR-mediated mechanism. All of the studies back up the idea that EGF might be a good option for early detection of HCC in various cirrhotic populations. Patients with Child A cirrhosis are the greatest candidates for transarterial chemoembolization (TACE), which is the guideline of care therapy for patients with middle phase HCC.[6]

The goal of this research was to see whether epidermal growth factor might be used as a hepatocellular carcinoma initial detecting indicator.

PATIENTS AND METHODS

One hundred patients were selected from the Internal Medicine department's outpatient clinic and ward at Sayed Galal University Hospital for this case control research.

Inclusion criteria: All patients with HCC irrespective to the etiology who were previously diagnosed based on demonstration of radiologically proven mass lesion of HCC nature according to triphasic abdominal CT examination were eligible for inclusion in group I. All patients with hepatic cirrhosis irrespective to the etiology without radiological evidence of HCC mass lesions were eligible for inclusion in group II, and all healthy subjects without any stigmata of Chronic hepatitis were eligible for inclusion in group III (control group).

Exclusion criteria: All patients with active malignancies (other than HCC in group I of patients). - HCC patients who underwent any therapeutic interventions including; surgical resection, TACE or radio frequency ablations. - Patients with hepatorenal syndrome. All patients with chronic illnesses (other than chronic liver disease in group II) including; CKD , heart failure , Diabetes Mellitus , Hypertension, etc..., and all patients with Acute and chronic inflammatory conditions

All patients of the study was classified into three groups: Group I: 40 patients with HCC, Group II: 40 cirrhotic patients without HCC, and Group III: 20 healthy persons free from stigmata of liver disease as a healthy control group.

All included patients underwent the following procedures:

Taking a complete medical history: focusing on history stigmata of chronic liver.

Clinical Examinations: focusing on clinical manifestations of liver diseases.

Investigations included:

Laboratory Tests: (i) full blood count. (ii) Tests of liver function including liver enzymes, serum bilirubin and albumin. (iii) Viral markers to detect the underlying causes of chronic hepatitis illness including; HBVsAg and HCV antibodies by 3rd generation ELISA technique. (iv) Alfa Feto-Protein as a cancer Indicator for HCC. E. EGF {epidermal growth factor} by ELISA Technique

Radiological Investigations: (i) Abdominal ultrasonography to exclude HCC in subjects of group II & III. (ii) Triphasic Abdominal CT scans to diagnose and stage patients included in group I.

Statistical analysis:

The IBM SPSS software program version 24.0 was utilized to feed the data into the computer. Number and percent were utilized to describe qualitative data. The Chi-square test was employed to see how various groups compared on category variables. The mean± standard deviation (SD) was employed to convey quantitative data (Standard deviation). To compare two independently groups of parameters with a normal distribution, the independent samples t-test was utilized (parametric data). For regularly distributed data, mean and SD were used, whereas abnormally distributed data was reported using median, minimum, and maximum. For normally distributed data, independent t-tests were used to compare two independent populations, whereas F-tests (ANOVA) were used to compare more than two populations. The results of significance tests are expressed as two-tailed probability. The significance of the acquired findings was assessed at a 5% level. P values of < 0.05 were deemed substantial.

RESULTS

	Group I (HCC group) "n=40"		Group II (Cirrhotic group) "n=40"		Group III (Control group) "n=20"	
Age (years)						
Range	45-80		34-70		38-70	
Mean±S.D.	57.7±8.5		57.9±7.0		54.5±10.6	
ANOVA			3.52			
P value			0.107 N.S.			
P1			0.460			
P2			0.1001			
P3			0.0677			
Sex						
Male	23	57.5	19	47.5	12	60.0
Female	17	42.5	21	52.5	8	40.0
X^2			2.01			
P value			0.136 N.S			
P1			0.107			
P2			0.269			
P3			0.09			

P1 Comparing of group I and II. P2 Comparing ofgroup I and III. P3 Comparing of group II and III. X^2 = Chi square test. P was substantial if < 0.05. N.S. Not substantial

Table 1: Comparing the demographic information of the three investigated groups.

Table (1) shows Comparing the demographic information of the three investigated groups. Age in group I varied from 45-80 with median value 57.7±8.5, in group II ranged from 34-70 with mean

value 57.9±7.0 and in group III varied from 38-70 with median value 54.5±10.6. In group I, males were 23(57.5%) and females were 17(42.5%), in group II were 19(47.5%) and 21(52.5%) respectively, in group III were 12(60%) and 8(40%) respectively. In terms of age and sex, there was no statistically substantial variation in the three groups tested (P > 0.05).

	Group I (HCC group) "n=40"	Group II (Cirrhotic group) "n=40"	Group III (Control group) "n=20"
AST U/L Range Mean±S.D.	23-105 49.0±18.1	21-106 37.2±13.8	14.0-37.0 24.4±6.5
ANOVA P value		36.5 0.001*	
P1 P2 P3		0.007* 0.001* 0.001*	
ALT U/L Range Mean±S.D.	15-83 36.3±16.2	16-48 27.4±7.7	10.0-35.0 21.5±6.4
ANOVA P value		28.65 0.002*	
P1 P2 P3		0.0012* 0.001* 0.0025*	
Alb g/dl Range Mean±S.D.	2.60-4.10 3.4±0.4	3.00-4.00 3.5±0.3	3.5-5.0 4.3±0.4
ANOVA P value		16.85 0.002*	
P1 P2 P3		0.0736 N.S. 0.01* 0.01*	
Bil (T) Mg/dl Range Mean±S.D.	0.69-2.10 1.2±0.4	0.38-1.15 0.7±0.2	0.3-0.7 0.5±0.1
ANOVA P value		27.51 0.001*	
P1 P2 P3		0.001* 0.001* 0.001*	

P1 Comparing of group I and II. P2 Comparing ofgroup I and III. P3 Comparing of group II and III. X^2 = Chi square test. P was substantial if < 0.05. N.S. Not substantial

Table 2: Comparing the AST, ALT, and AIB, Bil (T) of the three examined groups

Table (2) shows In terms of AST U/L, there was a statistically substantial variation in the three groups (P <0.05). regarding ALT U/L, there was a statistically substantial variation in the three groups (P > 0.05). Also, there was a statistically substantial variation in group I and group II with respect to III (P2, P3 <0.05), but no statistically substantial variation between group I and II with respect to AIB (P1> 0.05). In terms of Bil (T), there was a statistically substantial variation between the three groups (P< 0.05).

AFP Ng/ml	Group I (HCC group) "n=40"	Group II (Cirrhotic group) "n=40"	Group III (Control group) "n=20"
Range Mean±S.D.	1.2-1234.0 359.8±415.4	3.2-11.6 6.3±2.3	0.8-9.3 3.5±2.0
ANOVA P value		56.5 0.001*	
P1 P2 P3		0.001* 0.002* 0.001*	
H.EGF pg/ml			
Range Mean±S.D.	269-572 404.6±74.6	178-269 219.7±22.5	179.0-241.0 209.9±19.6
ANOVA P value		26.11 0.002*	
P1 P2 P3		0.0019* 0.005* 0.236	

P1 Comparing of group I and II. P2 Comparing ofgroup I and III. P3 Comparing of group II and III. X^2 = Chi square test. P was substantial if < 0.05. N.S. Not substantial

Table 3: Comparison of AFP and H.EGF between the three examined groups

Table (3) shows In terms of AFP, there was a statistically substantial variation between the three groups (P < 0.05). In terms of H.EGF, there was a statistically substantial variation between groups I and II (P1, P2< 0.05), but there was no substantial variation in groups II and III (P3 > 0.05).

	Group I (HCC group) "n=40"		Group II (Cirrhotic group) "n=40"		Group III (Control group) "n=20"		P value
	No.	%	No.	%	No.	%	
HBsAg No	400	100.0	391	97.5	200	100.0	
Yes		0.0		2.5		0.0	0.698
HCV.Abs No	6	15.0	21	52.5	200	100.0	
Yes	34	85.0	19	47.5		0.0	0.001*

Table 4: HBsAg and HCV. Abs comparison between the three examined groups

Table (4) shows In terms of HCV.Abs, there was a statistically substantial variation between the three patient groups (P <0.05), however there was no statistically substantial variation in terms of HBsAg (P > 0.05).

Area	P value	Cut off value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.892	0.001*	5.8	.818	.966
Sensitivity		92.0		
Specificity		90.0		
Accuracy		91.0		

Table 5: Sensitivity, specificity and accuracy of AFP in differentiate between HCC and cirrhosis.

Table (5) and Figure (1) shows sensitivity, specificity and accuracy of AFP in differentiate between HCC

and cirrhosis. Sensitivity was (92%), specificity (90%) and accuracy (91%) with cut off value 5.8.

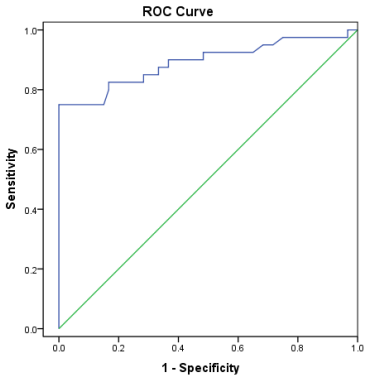

Fig. 1: ROC curve to predict the sensitivity, specificity and accuracy of AFP in differentiate between HCC and cirrhosis.

Area	P value	Cut off value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.99	.00001	350.0	.999	1.000
Sensitivity	99.0			
Specificity	100.0			
Accuracy	99.0			

Table 6: Sensitivity, specificity and accuracy of H. EGF in differentiate between HCC and cirrhosis.

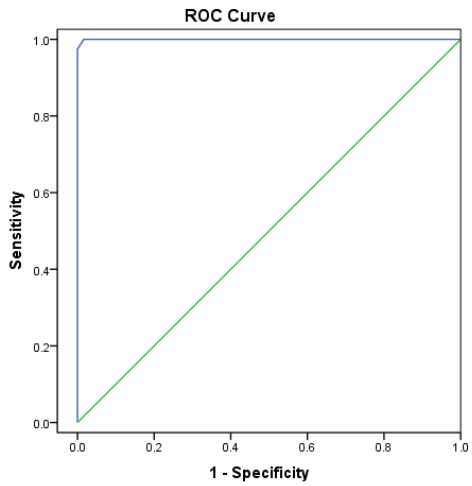

Fig. 2: ROC curve to predict the sensitivity, specificity and accuracy of H. EGF in differentiate between HCC and cirrhosis.

Table (5) and Figure (2) shows sensitivity, specificity and accuracy of H. EGF in differentiate between HCC and cirrhosis. Sensitivity was (99%), specificity (100%) and accuracy (99%) with cut off value 350.

		AFP	H.EGF
H.EGF	Pearson Correlation	.803*	
	Sig. (2-tailed)	.0001	
AST	Pearson Correlation	.489*	.605*
	Sig. (2-tailed)	.0001	.0001
ALT	Pearson Correlation	.359*	.501*
	Sig. (2-tailed)	.0001	.0001
Alb	Pearson Correlation	-.532*	-.543*
	Sig. (2-tailed)	.0001	.0001
Bil(T)	Pearson Correlation	.734*	.799*
	Sig. (2-tailed)	.0001	.0001

Table 7: Correlation between AFP, H.EGF and other laboratory findings.

Table (7) shows correlation between AFP, H.EGF and other laboratory findings. AFP, H. EGF, and other laboratory data showed a statistically substantial relationship ($P < 0.05$).

DISCUSSION

Our findings revealed that the patients in groups I and II, as well as the control group, had symmetrical demographic data, including age and sex, the three groups show insignificant difference regarding age and sex. Also, the history stigmata of chronic liver and clinical signs of chronic liver in two patients groups was matched without significant difference, this results was important to eliminate the effect of different basic demographic and clinical data on the net results of the patients.

Yang et al.[1] The importance of erythropoietin was explored in 67 individuals with varied degrees of cirrhosis, and it was shown that cirrhotic patients had considerably greater plasma erythropoietin levels than controls. They also discovered that levels were greater in anemic patients. They discovered a positive relationship between hepatic venous pressure gradient (HVPG) and erythropoietin, as well as a negative relationship between hepatic blood flow and erythropoietin.

The liver function showed that The AST U/L in group I varied from 23 to 105, with a median of 49.0±18.3, in group II from 21 to 106, with a median of 37.2±13.8, and in group III from 14 to 37, with a median of 24.4±6.5. In terms of AST U/L, there was a statistically substantial variation across the three groups ($P < 0.05$). ALT U/L in groups I and II varied from 15 to 83 with an average value of 36.3±16.2, 16 to 48 with a mean value of 27.4±7.7, and 10-35 with an average value of 21.5±6.4. In terms of ALT U/L,

there was a statistically substantial variation between the three groups (P > 0.05).

In accordance with our findings, Baghdady et al.[7] They discovered that the blood AST level in the HCC group was higher than that in the non-HCC group, which is statistically substantial (P<0.05) in the article Serum indicators for the early detection of hepatocellular cancer in chronic viral hepatitis C patients, and this is in accordance with Durazo et al.[8] and Lopez et al.[9] (The average AST value in HCC was 3.5 times higher than normal), and also with Okonkwo et al.[10], They discovered that the blood AST level in HCC was 1.39 times higher than normal; the serum ALT level in HCC and non-HCC patients exhibited a statistically substantial variation.

The AFP in group I varied from 1.2-1234 with an average value of 359.8±415.4, group II from 3.2-11.6 with an average value of 6.3±2.3, and group III from 0.8-9.3 with an average value of 3.5±2.0, according to our findings. In terms of AFP, there was a statistically substantial variation between the three groups (P 0.05).Sensitivity, specificity and accuracy of AFP in differentiate between HCC and cirrhosis. Sensitivity was (92%), specificity (90%) and accuracy (91%) with cut off value 5.8.

In accordance with our findings, Abd-elfatah et al.[11] studied In persistent viral hepatitis C patients without hepatocellular cancer, the impact of alpha-fetoprotein (AFP) values was examined, and it was discovered that serum AFP levels are a routine screening tool for HCC in patients with chronic liver diseases, as raised AFP concentrations are an indicator of enhanced HCC risk. Serum AFP levels more than 20 µg/L were seen in individuals with HCV-related cirrhosis but not HCC, with a frequency from 10% to 43%.

In Jasirwan et al.[12] study, They found that In the research on the alpha-fetoprotein serum remains reliable as a biomarker for the scanning of hepatocellular tumors in Indonesia, the sensitivity, specificity, positive predictive value, negative predictive value, and probability proportion for positive test results of AFP in the monitoring of HCC of AFP (with a cut-off of 10 ng/ml) were 82.6, 71.2, 65.6, 85.9%, and 2.87, respectively.

Surprisingly, this finding matched that of Chan SL, et al., who found that the AFP sensitivity 82.6 percent and specificity 70.4 percent (with a comparable cut-off of 10 ng/ml), with positive and negative predictive values of 86.6 and 63.6 percent, respectively.

H.EGF in group I varied from 269-572 with median value 404.6±74.6, in group II varied from 178-269 with median value 219.7±22.5 and in group III varied from 179.0-241.0 with median value 209.9±19.6.

There was statistical substantial variation between groups I with II and in group I with III (P1, P2 < 0.05) while there was no statistical substantial variation in group II with III (P3 > 0.05) regarding H.EGF. Sensitivity, specificity and accuracy of H. EGF in differentiate between HCC and cirrhosis. Sensitivity was (99%), specificity (100%) and accuracy (99%) with cut off value 350.

In accordance with our findings, Shehata et al.[13], They discovered that the level of p-EGFR in patients with HCC is considerably higher than the equivalent level in patients with CHC in a study of epidermal growing factor, its receptor, and transformed growing factor-b1 in the identification of HCV-induced HCC . When the serum p-EGFR levels of patients with CHC (19.5 ±8.1 U/ mg protein) are compared to those of patients with HCC at an intial phase (25.1± 9.6 U/mg protein), no differences are identified.

The level of p-EGFR differed significantly between the initial and late stages of HCC. It was recently discovered that EGFR is activated by numerous heterologous ligands and its kinase activities. Others have determined that EGFR is overexpressed in individuals with liver cancer, and that blood EGFR levels might be used as a diagnostic for this illness.[14]

In earlier research, immunohistochemical examination revealed that two-thirds of typical HCCs had surface EGFR expression, but no substantial connection was established with other clinicopathological characteristics. This is consistent with our findings for p-EGFR in the present investigation.[15]

TGF-b1 regulates cell proliferation and transformation, angiogenesis, extracellular matrix synthesis, immunosuppression, and tumor progression, among other things. The rate of HCC differentiating is linked to irregular TGF-b1 expression in the liver.[16]

Furthermore, in line with our findings, a greater amount of TGF-b1 was found in the plasma of patients with HCC than in the plasma of patients with persistent hepatitis and cirrhosis, suggesting that TGF-b1 might be a candidate for a new HCC indicator.[17]

CONCLUSION

In the identification of early HCC, epidermal growth factor as an indicator is a helpful biomarker that might supplement the effectiveness of AFP.

Conflict of interest : none

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