Research Article

Role of TGFB1 as a biomarker in early HCC

Naglaa M. Mohamed, Yehia Z. Mahmoud and Mahmoud H. Khedr
Department Internal medicine, Minia University, Egypt

Abstract
Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. **Aim of the study** to assess the value of transforming beta binding protein-1 in plasma as a biomarker for early detection of hepatocellular carcinoma in chronic hepatitis C infection. **Patients and methods:** This cross-sectional prospective, case-control hospital based study was conducted in Internal Medicine Department, Minia University Hospital in collaboration of our Radiology department and South Egypt Cancer Institute from June 2018 to June 2019. In this study included 90 subjects who were recruited from in patient and out -patient clinic and divided into three groups. **Results:** Simple logistic regression analysis of AFP and TGF-B1 for prediction of HCC among cirrhotic patients revealed that; increase TGF-B1by one unit will increase the risk of HCC by 3.7% (OR=1.037, 95%CI= 1.01-1.05, P value = 0.002), while; increase AFP by one unit will increase the risk of HCC by 2.7% (OR=1.027, 95%CI= 1.02-1.06, P value <0.001). **Conclusion:** We evaluated the serum levels of TGF-β1 in HCC patients, cirrhotic patient and normal subjects. Its aim was to determine the early prediction of hepatocellular carcinoma associated chronic hepatitis c virus.
Keywords: Hepatocellular carcinoma, liver malignancy, hepatitis C

Introduction
Liver cancer is the second leading cause of cancer-related deaths globally after lung cancer (El Khodiry et al., 2018). HCC represents approximately 95% of all cases of primary liver cancer that is doubled over the past 20 years by high prevalence of HBV and HCV and accounting for 9.1% of all cancer deaths worldwide (Wong et al., 2017), approximately 850,000 new cases per year (Ding et al., 2017), despite efforts for prevention, screening and development of new technologies for HCC diagnosis and treatment (Lopez-Valdes and Crus, 2017). Liver cancer is the second leading cause of cancer-related deaths globally after lung cancer El Khodiry et al., 2018.)

Liver cancer is the fifth-leading cause of cancer diagnosed in men worldwide and the ninth cause of cancer in women, representing about 8.6% of the total number of cancer diagnosis (Ferlay et al., 2014). In Egypt, HCC annually affects 5-7 cases per 100.000 populations with a nearly equivalent mortality rate (6 per 100.000) reflecting its high disease fatality (El-Zanaty and Way, 2014) The first serologic assay for detection and clinical follow up of patients with hepatocellular carcinoma was alpha-fetoprotein (AFP) which has been the gold standard and the most commonly used tumor biomarker for HCC for many years.

This cross-sectional prospective, case-control hospital based study was conducted in Internal Medicine Department, Minia University Hospital in collaboration of our Radiology department and South Egypt Cancer Institute from June 2018 to June 2019. In this study included 90 subjects who were recruited from in patient and out-patient clinic and divided into three groups:
First group: patients with HCV-related liver cirrhosis complicated with HCC

It will include 30 patients with HCV-related liver cirrhosis complicated with HCC (20 men and 10 women), their ages ranged from 47 to 71 years old. Diagnosis of HCV will be based on positive anti-HCV antibodies by enzyme-linked immune sorbent assay (ELISA). Diagnosis of liver cirrhosis is based on abdominal ultrasound & laboratory data (Dufour et al., 2000).

Diagnosis of HCC is based on Barcelona Clinic Liver Cancer (BCLC) staging system, which defines early stage of HCC as child A, or B

classification (single or 3 nodules <3cm) (Bruix et al., 2016) diagnosed by Tri phasic-CT (park., 2004).

Second group: patients with chronic HCV-related liver cirrhosis. It will include 30 patients with chronic HCV-related liver cirrhosis (18 men and 12 women), their ages ranged from 40 to 75years old. Diagnosis of HCV will be based on positive anti-HCV antibodies by enzyme-linked immune sorbent assay (ELISA). Diagnosis of liver cirrhosis is based on abdominal ultrasound & laboratory data (Dufour et al., 2000).

Third group: healthy volunteers
It will include 30 healthy volunteers as control group (16 men and 14 women), their ages ranged from 50 to 69 years old.
Subjects of group 2 and group 3 will be age and gender matched to group 1.

Ethical aspects:
The study protocol was approved by the Institutional Ethics Committee. All patients and controls gave informed consent before participating in this study.

Exclusion criteria:
Subject has any one of the following criteria will be excluded from study:
Co-infection with HBV and/or Human immunodeficiency virus (HIV).
Alcohol intake more than or equal 80gm/day for more than 10 years.
Chronic kidney disease.
Any malignancies elsewhere.
Previous anticancer therapy or liver surgery.

All patients and controls were subjected to the following:-

I) Thorough History Taking:-
Each subject answered a standard questionnaire that included:
Personal history with special attention to name, age, sex, residence, marital status, occupation and special habits of medical importance as cigarette smoking and daily alcohol intake.
Current history of symptoms suggestive of hepatic decompensation as fatigue, fever, bleeding tendency, itching, yellowish discoloration of sclera, change of color of urine and stools, abdominal distension, swelling of lower limbs, hematemesis, melena and disturbed level of consciousness.

II) Thorough Clinical Examination:-
Measurement of vital signs (body temperature, pulse, blood pressure and respiratory rate).
General examination with special emphasis on stigmata of chronic liver disease as jaundice, fetor hepaticus, palmar erythema, clubbing of fingers, flappy tremors, spider nevi, scratching marks, evidence of subcutaneous hemorrhage, gynecomastia, lower limb edema, and conscious level.
Careful abdominal examination with special reference to the status of the liver and spleen as regards size, surface, edge, consistency and tenderness on examination. The presence of ascites was evaluated.

Simple logistic regression analysis of AFP and TGF-β1 for prediction of HCC in cirrhotic patients

	OR	95% CI	P value
AFP	1.027	1.01-1.05	0.002*
TGF-β1	1.037	1.02-1.06	<0.001*

OR: Odds Ratio
CI: Confidence Interval
*: Significant level at P value < 0.05

Simple logistic regression analysis of AFP and TGF-B1 for prediction of HCC among cirrhotic patients revealed that; increase TGF-B1by one unit will increase the risk of HCC by 3.7% (OR=1.037, 95% CI= 1.01-1.05, P value = 0.002), while; increase AFP by one unit will increase the risk of HCC by 2.7% (OR=1.027, 95% CI= 1.02-1.06, P value <0.001)
ROC curve analysis of AFP and TGF-β1 for prediction of HCC in cirrhotic patients

	AFP	TGF-β1	AFP & TGF-β1
Optimal cutoff point	>38	>290	
AUC	0.773	0.980	0.833
95% CI	0.646-0.871	0.905-0.999	0.715-0.917
P value	<0.001*	<0.001*	<0.001*
Sensitivity	73.33	93.33	100
Specificity	70	96.67	66.67
PPV	71	96.6	75
NPV	72.4	93.5	100
Accuracy	71.7	95	83.3

AUC: Area Under Curve
CI: Confidence Interval
PPV: Positive Predictive Value
NPV: Negative Predictive Value
*: Significant level at P value < 0.05

ROC curve analysis of AFP & TGF-B1 for prediction of HCC among cirrhotic patients revealed that, AFP ROC curve has AUC=0.773, p <0.001, with optimal cutoff point >38 with sensitivity 70%, specificity 71% and accuracy 71.7%; while TGF-B1 ROC curve showed AUC=0.980, p <0.001, with optimal cutoff point >290 with sensitivity 93.33%, specificity 96.67% and accuracy 95%; combination of both showed AUC=0.773, p <0.001 the sensitivity increased to 100% but specificity was 66.67% and accuracy 83.3%.

Discussion

Liver cancer is the second leading cause of cancer-related deaths globally after lung cancer (El Khodiry et al., 2018 Liver cancer is the fifth-leading cause of cancer diagnosed in men worldwide and the ninth cause of cancer in women, representing about 8.6% of the total number of cancer diagnosis) Ferlay et al., 2014. In Egypt, HCC annually affects 5-7 cases per 100.000 populations with a nearly equivalent mortality rate (6 per 100.000) reflecting its high disease fatality) El-Zanaty and Way, 2014

The first serologic assay for detection and clinical follow up of patients with hepato-cellular carcinoma was alpha-fetoprotein (AFP) which has been the gold standard and the most commonly used tumor biomarker for HCC for many years

However, about 32–59% of patients with HCC have normal AFP levels and inversely non-tumor-related AFP elevations may occur in patients with cirrhosis or chronic hepatitis making it inadequate as a surveillance test (Lok et al., 2010)

AP levels may be elevated initially in the early stages of HCC and then drop or even normalize before rising again as disease progression occurs however, such high values are observed only in a small percentage of patients with HCC (Farinati et al., 2006)

Another potential biomarker for HCC is TGF-β, new diagnostic marker for HCC as it plays an important role in the microenvironment of tumor cells and promotes hepatocellular progr-ession (Giannelli et al., 2014). And it also has a dual nature–it acts as tumor suppressor at early stage carcinogenesis but promotes invasion and metastasis at later stages) Lin et al., 2015. TGF-β1 acts as a growth inhibitor in normal cells, whereas in tumor cells, it loses the ability to mediate growth inhibition and instead promotes tumor progression by enhancing mig-ration, invasion, and survival of tumor cells Mohamed Ahmed Samy Kohla et al., 2017

Our study evaluated the serum levels of TGF-β1 in HCC patients, cirrhotic patient and normal subjects. Its aim was to determine the early prediction of hepatocellular carcinoma-associated chronic hepatitis c virus. This study included three groups Group (I) consists of 30 patients with HCC, consisted of 20 males and 10 females, While Group (2) include 30 patients with chronic HCV-related liver cirrhosis 18 males and 12 females as males are more subje-cted to carcinogenic risk factors than females,

While Group (3) include 30 healthy volunteers as control group.

In Egypt, a study of prevalence and epidemiological features of HCC was done and revealed that male patients were forming 82.55% while female patients were forming 17.45% among 321 studied HCC patients) EL-Zayadi et al., 2001 and Mubarak et al., 2010) that agrees with this study that the percentage of males was higher than that of females but no significant difference was found between the III groups and also (Yeh & Chenb, 2010) reported that men have a higher incidence of hepatocellular carcinoma suggesting that it might be due to the stimulatory effects of androgen and the protective effects of estrogen (can protect hepatocytes from malignant transformation via down regulation of IL-6 release from Kupffer cells.

Regarding the Age among the patients in the groups, it was found that the incidence of HCC is higher in the older patients (Mean age is 58.6 years), in comparison to the mean age in group II patient (the mean age was 57.4 years) but the difference was statistically insignificant

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