

ROLE OF SERUM GLYPICAN-3 (GPC-3) IN EARLY DETECTION OF HEPATOCELLULAR CARCENOMA

By

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ABSTRACT

Background The false negative rate of alpha-fetoprotein (AFP) level alone may be as high as 40% for patients with early stage of hepatocellular carcinoma (HCC) and may be elevated in non-malignant chronic liver diseases and other malignancies. Glypican-3 (GPC-3) is a new tumor marker for HCC.

Objective: Evaluation of the role of serum GPC-3 in the early diagnosis of HCC.

Results: There was a highly significant difference between control and HCC group as regard AFP and serum GPC-3. There was a highly significant difference between liver cirrhosis and HCC group as regard AFP and serum GPC-3. No significant difference between control and liver cirrhosis groups as regard AFP and GPC-3. AFP showed sensitivity (75%), specificity (63%), positive predictive value (PPV) (62.8%) and negative predictive value (NPV) (69.9%) at cut-off value 195ng/ml for HCC group. Serum GPC-3 showed sensitivity of (87%), specificity of (95%), PPV of (93.8%) and NPV of (91.2%) at cut-off 5.1ng/ml for HCC group. Combined serum GPC-3 and AFP showed sensitivity (85.9%), specificity (88%) PPV (85.4%) and NPV (84.1%) at cut of value 5.1 ng/ml and 195 ng/ml respectively for HCC group. No significant correlation of serum GPC- 3 or AFP to the tumor size, number or vascular invasion in HCC group.

Conclusion: GPC-3 is a promising diagnostic marker with high sensitivity and specificity for HCC than AFP in detection, screening HCC and follow up treatment of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality (*Lai. et al., 2002 and El-Serag & Rudolph, 2007*). AFP has been used as a serum marker for HCC in humans for many years and it has a sensitivity of 39%- 65%, a specificity of 76%-94% and a positive predictive value of 9% - 50% according to the cutoff level used (*Daniele et al., 2004*).

Not all HCC secrete AFP. Only a small proportion of tumors at an early stage (10-20%) present with abnormal AFP serum level (*Yamashita et al., 2008*). Serum AFP level may be elevated in non-malignant chronic liver diseases including chronic hepatitis and cirrhosis, as well as in other primary and secondary liver cancers. Therefore, the identification of alternative serum markers of HCC is needed (*Spangenberg et al., 2006*).

Glypican-3 (GPC-3) belongs to the glypican family of glycosyl-phosphatidy-

linositol (GPI)-anchored heparan sulfate proteoglycans, which plays an important role in cellular growth, differentiation and migration (*Filmus and Selleck, 2001*). GPC-3 as a potential tumor marker for HCC was first suggested by *Hsu et al., (1997)*. GPC3 has been reported to be increased in HCC in comparison with pre-neoplastic lesions and cirrhotic tissues at the mRNA and protein levels (*Liovet et al., 2006*).

In contrast, GPC-3 has been shown to be down-regulated in various cancers including breast cancer, ovarian cancer, lung adenocarcinoma and cholangiocarcinoma (*Man et al., 2006*). Given the detection of circulating GPC-3, it appears that this oncofetal protein can serve as a potential serum marker for the detection of HCC. However, data on its clinical correlations in patients with HCC are currently unknown (*Hippo et al., 2004*).

The present work aimed to evaluate the role of serum GPC-3 in the early detection of HCC.

PATIENTS AND METHODS

In this prospective study, we randomly selected 70 patient 40 of them have proved HCC (29 males, 11 females), and 30 patient with liver cirrhosis (21 males and 9 females). This in addition to 20 healthy subjects (15 males and 5 females). Age ranged between 55.7 ± 11.9 (26 and 76). They were selected from Outpatient clinic and Inpatient Department of Tropical Medicine in Al-Hussein and Sayed Galal University Hospitals, Hepatology Department - National Institute - Monufiya University and Mabara Insurance Hospital (Assiut). The study was done from January 2011 to May

2014. The study was approved by the local ethical committee in Al-Azhar University Hospitals and informed consent was obtained from the patients.

I. HCC group: The patients were diagnosed as HCC according to American Association for study of liver disease (*Bruix and Sherman, 2011*).

None of the patients had received local or systemic therapy for HCC before. Any patient with cancer other than HCC was excluded.

II. Liver cirrhosis group: Patients were selected according to clinical examination, abdominal U/S, laboratory investigation and histopathological examination in liver biopsy samples. Patients were diagnosed to have liver cirrhosis if they have the clinical splenomegaly, ascites, thrombocytopenia, decreased S. albumin, increased bilirubin, prolonged prothrombin time and endoscopic examination (varices).

III. Control group: Subjects were selected according to clinical examination, abdominal U/S, and laboratory investigation.

Each patients and control were subjected to Careful history and thorough clinical examination, liver function tests, PT, PC, INR, HCV Ab and HBsAg, CBC, AFP and GPC-3, abdominal U/S: All patients in the HCC group were diagnosed by triphasic spiral CT abdomen and/or dynamic MRI. One patient of HCC group was diagnosed with liver biopsy.

Statistical analysis of data was carried out using the SPSS computer package version 17.0 (SPSS Inc. Chicago. IL, USA). The mean \pm SD was used for quantitative variables while number and%

were used for qualitative variables. Independent samples t-test was applied. In order to assess the differences in means of quantitative variables within the patients group. One-Way ANOVA test was used. Correlation analysis (using Pearson's correlation coefficient) was used to assess the strength of association between various quantitative variables within the patients' group. The statistical methods were verified, assuming a significance level of $p < 0.05$. Receiver operating

characteristic curve (ROC) was performed on SPSS-16 to determine the sensitivity and specificity of serum GPC-3 and AFP.

RESULTS

No significant difference between control and liver cirrhosis as regard AFP and serum GPC-3, while there was significant difference between control and HCC. A highly significant difference was found between liver cirrhosis and HCC groups (Table 1).

Table (1): Comparison between control, liver cirrhosis and HCC groups regarding AFP, GPC-3..

Parameters \ Groups	Control (20 case)	Liver cirrhosis (30 case)	HCC (40 case)	P. value
AFP (ng/ml)	10.19 ± 5.7	13.8 ± 9.9	146.4 ^a ± 153	0.147*
S. GPC-3 (ng/ml)	1.58 ± 0.87	1.93 ± 1.36	9.04 ^a ± 3.1	0.313*

Fifteen cases (37.5%) of HCC group have tumor size ≤ 5cm or 3 lesions each ≤ 3 cm. with no significant difference between groups. Twenty nine cases of HCC group

(72.5%) have a single lesion, with no significant differences between tumor number and GPC-3 or AFP (Table 2).

Table (2): Correlation of tumor size and number in HCC group to GPC-3 and AFP.

Variables	Tumor size		P. Value	Tumor number		P. Value
	≤3 lesion in number and ≤3cm in size or single lesion < 5cm (15 cases)	≥5cm (25 cases)		Single (29 cases)	Multiple (11 cases)	
Parameters						
GPC-3	8.82 ± 3.65	9.11 ± 2.89	0.788	9.38 ± 3.18	7.84 ± 2.82	0.184
AFP	107.2 ± 149	177.1 ± 159	0.182	156.6 ± 164	115.4 ± 130	0.478

Twelve cases (30%) of HCC group showed vascular invasion. No significant correlation was found between serum

GPC-3 or AFP levels and vascular invasion (Table 3).

Table (3):Correlation of vascular invasion and Serum GPC-3 in HCC group.

Parameters	Vascular invasion	Absent (28 cases)	Present (12 cases)	P. value	Sig
S. GPC-3 (ng/ml)		8.95 ± 3.95	9.26 ± 2.68	0.728	NS
AFP (ng/ml)		149.6 ± 54.7	163.4 ± 63.8	0.526	NS

On differentiating HCC from cirrhosis and normal controls, AFP showed sensitivity 75%, specificity 63%, Positive Predictive Value (PPV) 62.8% and Negative Predictive Value (NPV) 69.9% at cut-off value 195ng/ml. Serum GPC-3 showed sensitivity of 87%, specificity of

95%, PPV 93.8% and NPV 91.2% at cut-off 5.1ng/ml. Combined serum GPC-3 and AFP showed sensitivity 85.9% specificity 88% PPV 85.4% and NPV 84.1% at cut of value 5.1 ng/ml and 195 ng/ml respectively (Table 4).

Table (4): Sensitivity, specificity, PPV and NPV of serum GPC-3 and AFP in prediction of HCC.

Parameters	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AFP (ng/ml)	195	75	63	62.8	69.9
S. GPC-3 (ng/ml)	5.1	87	95	93.8	91.2
Combined GPC-3 and AFP	5.1 and 195	85.9	88	85.4	84.1

DISCUSSION

HCC is the most common primary malignant tumor of the liver, accounts for >80% of liver cancer cases, and affects men 3 to 4 times more frequently than women. The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years (*Mizokami and Tanaka, 2005*).

AFP is an oncofetal proteins produced during fetal life, disappear after birth and reappear in cancer patients (*Marrero et al., 2009*). AFP levels at value of 20 ng/ml showed good sensitivity but low specificity, whereas, at higher cut-offs of 200 ng/ml sensitivity drops to 22% with high specificity (*Trevisani et al., 2001*). Furthermore, only a small proportion of

tumors at an early stage (10-20%) present with abnormal AFP serum level (*Yamashita et al., 2008*). The AASLD recommended cut-off level for diagnosis of HCC is 200ng/ml. Although lower levels, particularly if rising, should be followed very carefully (*Bruix and Sherman, 2011*).

The sensitivity of AFP is not good enough for it to be used alone for detection of HCC as over a third of cancers will be missed (*Bruix and Sherman, 2005*).

Normally, GPC-3 plays a role in regulating cell proliferation and survival during embryonic development by modulating the activity of various growth factors. Under normal physiological conditions, the tissue GPC-3 protein resides in the endoplasmic reticulum and is transported into the nucleus under stress (*Pang et al., 2008 and Abd El Gawad, et al., 2014*). GPC-3 is only detected in HCC cells, not in benign liver tissues and can thus be used as a potential biomarker for the screening and diagnosis of early HCC. Serum GPC3 level was higher than 300 ng/l in 50% of early HCC patients, although their serum AFP level was below 100 ?g/l (*Lee et al., 2014*).

Our study aimed to determine serum level of GPC-3 in patients with HCC, liver cirrhosis and healthy control to find its value in relation to serum AFP for early HCC detection. As regard correlation of AFP to serum GPC-3 no significant difference between normal control and liver cirrhosis, but in HCC group a significant positive correlation was detected between AFP and GPC-3 in the HCC group. Our results were in agreement with *El-Shenawy et al. (2012)*.

Contrarily, no correlation was found between serum levels of AFP and GPC-3 (*Jackbovic and Jothy, 2007*).

As regard relation of serum GPC-3 and AFP to tumor size, number and vascular invasion, our study showed that 37.5% of HCC patients have a single lesion ≤ 5 cm or three lesions each less each ≤ 3 cm. Also, 30% of HCC group have vascular invasion at the time of detection with no significant correlation of serum GPC-3 or AFP to the tumor size, number or vascular invasion. *Ozkan et al. (2011)* found no correlation between GPC-3 levels and tumor size, number and portal vein invasion in patients with HCC. A positive correlation was found between serum levels of each of AFP and GPC-3 with both tumor size and portal vein invasion by *El-Shenawy et al., (2012)*, while *Youssef et al. (2010)* reported statistically significant results between GPC-3 and the staging of HCC. This discrepancy of results may be due to the different sample size or the different underlying etiology of HCC.

Serum GPC-3, in our study, showed a cut-off level of 5.1ng/ml, gave sensitivity of 87%, specificity of 95%, PPV 93.8% and NPV 91.2% for early detection of HCC and AUROC of 0.198. *Nakatsura et al.(2003)* stated that GPC-3 could be detected in 40–53% of HCC patients and 33% of AFP sero-negative HCC patients, while *Shafizadeh et al. (2008)* found GPC3 positive cells in 90% of patients with their serum AFP level < 400 ng/ml.

As regard AFP, in our study, gave a sensitivity of 75% and specificity of 63%, PPV 62.8% and NPV69.9% at cut-off level of 195 ng/ml and AUROC of 0.266. The combined AFP and serum GPC-3

gave sensitivity of 85.9%, specificity of 88%, PPV 85.4% and NPV 84.1% which is much better than AFP alone. Combination of GPC-3 and AFP showed that the sensitivities were between 84–92% and specificities between 90–95% (*Gomaa et al., 2012*). Serum GPC-3 levels in patients with small-sized HCC tumors (<2 cm) provided a sensitivity of >60%, while the sensitivity of AFP was extremely low <10% (*Lee et al., 2014*).

Hippo et al. (2004) demonstrated that during the follow-up of their cirrhotic patients having detectable GPC-3 levels, HCC developed within 6 months among considerable number of patients with neither significant change of serum AFP levels nor in abdominal U/S.

AFP proteins do not seem to play a critical role in tumor progression, but have been used as tumor markers or as targets for immunotherapy. Evaluation of GPC-3 as a diagnostic and immunotherapeutic target may be used in the future for treatment of HCC *Sung et al. (2003)*.

In conclusion, serum GPC-3 is a sensitive and specific tumor marker for early detection and for follow up after treatment of HCC.

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خلفية البحث: يُستخدم الألفا فيتو بروتين بالدم في تشخيص سرطان الكبد بالرغم من أن دقة هذا التحليل في تشخيص المرض تتراوح بين 39% - 65% فقط.

الهدف من البحث: معرفة دور الجليبيكان-3 بالدم في التشخيص المبكر لسرطان الكبد.

المرضى وطرق البحث: أجري البحث علي 90 حالة مقسمة إلى أربعين مريض مصابون بسرطان الكبد تم تشخيصهم بناءً على توصيات الجمعية الأمريكية لدراسة أمراض الكبد لسنة 2011 وثلاثين مريضاً مصابون بتليف الكبد مع عدم وجود سرطان بالكبد وعشرين شخصاً طبيعياً .

وتم أخذ التاريخ المرضي مع الفحص الإكلينيكي الكامل، مع عمل وظائف كبد كاملة، و دلالات فيروسات، وصورة دم كاملة، و زمن و تركيز بروثرومين، والألفا فيتو بروتين، وحساب نسبة الجليبيكان 3 في الدم، وموجات صوتية علي البطن، وأشعة مقطعية ثلاثية المراحل علي البطن، وأشعة رنين مغناطيسي علي البطن في الحالات المصابة بسرطان الكبد، وفحص باثولوجي للأنسجة (حالة واحدة).

النتائج: توصل البحث إلى أن حساسية الألفا فيتو بروتين بروتين في تشخيص حالات سرطان الكبد 75% ودقته في التشخيص 63% عند مستوي 195 نانوجرام /ملييلتر، أما الجليبيكان-3 فكانت حساسيته في تشخيص حالات سرطان الكبد 87% ودقته في التشخيص 95% عند مستوي 5,1 نانوجرام /ملييلتر، مما يدل علي مدي حساسية ودقة الجليبيكان-3 علي الألفا فيتو بروتين في التشخيص المبكر لسرطان الكبد، كما أن نسبة الجليبيكان-3 بالدم تكون مرتفعه في مرضي سرطان الكبد عندما تكون نسبة الالف فيتو بروتين طبيعية.

الاستنتاج: يمكن القول بأن قياس نسبة الجليبيكان-3 في الدم يعتبر من الإختبارات الدقيقة والتي يمكن إستخدامها في التشخيص المبكر لسرطان الكبد ويمكن إستخدامها في تشخيص سرطان الكبد بدلا من الألفا فيتو بروتين.