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Diagnostic Value of Serum Glypican-3 Level among Patients with Hepatocellular Carcinoma: A Cross-Sectional Study in BSMMU, Bangladesh

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Abstract **Original Research Article**

This cross-sectional study was performed in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University. The purpose of this study was to assess the diagnostic value of serum Glypican-3 in detecting hepatocellular carcinoma. It was conducted during January 2015 to December 2016. Through convenience sampling technique, 30 diagnosed patients of hepatocellular carcinoma were enrolled in this study. Mean age was 50.26 ± 14.7 years. Males were the predominant gender in the study, which was 86.7%. Among the 30 respondents, 76.7% had HBsAg positive, 10% had anti HCV antibodies present. Mean serum glypican 3 level was 378.00 ± 168.42 ng/ml. Significant association of Alfa-fetoprotein and serum Glypican 3 in hepatocellular carcinoma patients was found. Serum Alfa-fetoprotein and serum Glypican 3 both were significantly detected in hepatocellular carcinoma but Glypican 3 was more sensitive and specific to detect hepatocellular carcinoma.

Keywords: Glypican-3, Hepatocellular carcinoma, Biomarker, Diagnosis, liver disease.

INTRODUCTION

Hepatocellular carcinoma is being ranked the sixth most common cancer as well as the third most prominent cause of cancer-related deaths across the world. The incidence of this disease is rising rapidly among both Asian and Western regions [1].

Chronic liver disease and cirrhosis of the liver are significant risk factors for development of this fatality and for these risk factors; the etiologies mainly responsible are viral hepatitis and excessive consumption of alcohol. Certain chronic morbidities such as diabetes mellitus and obesity increase the risk for developing hepatocellular carcinoma. It is seen that, males are more vulnerable to this type of cancer in comparison to females [2].

Many individuals exhibit no symptoms until the disease has reached an advanced stage. Among the symptoms, patients commonly present with abdominal pain, any swelling or mass, fatigue, malaise, weight loss and obstructive symptoms such as ascites and jaundice [3].

As far as diagnostic modules are concerned, imaging studies play a major role. For instance, ultrasonography, CT scan and MRI are one of the significant tests which can be conducted to diagnose hepatocellular carcinoma [4]. Various biomarkers are used to detect the disease as well. Namely, Alfa-fetoprotein, Glypican-3, Squamous Cell Carcinoma Antigen, Golgi Protein-73, Osteopontin are amongst the many tumor markers used for both diagnostic and prognostic purposes of HCC as well as numerous other conditions. They not only assist in early diagnosis, but also guide therapeutic measures and embody different strategies [5].

GPC-3, a protein, is one of the members of heparin sulfate proteoglycans. It plays a role to regulate proliferation of cells as well as survival during development of embryo and works as a tumor suppressor. GPC-3 is not present in liver cells of healthy individuals and patients with nonmalignant ailments of the liver, and can be found in approximately 50% of HCC patients and 33% of HCC patients seronegative for AFP [5].

Intervention guidelines include resection, adjuvant therapy, loco-regional therapy, bridging therapy, down-staging and transplantation, trans arterial chemoembolization, systemic therapy and so on [6].

The study summarizes the diagnostic value of GPC-3 as a biochemical marker in HCC. The aim of this study is to determine whether GPC-3 plays a vital role in the diagnosis of HCC.

MATERIALS AND METHODS

This cross-sectional study took place in the Department of Hepatology, BSMMU during the period of January 2015 to December 2016. The study population consisted of confirmed hepatocellular carcinoma patients attending Hepatology OPD or admitted in inpatient department during the study period who fulfill the inclusion criteria. A total of 30 patients were included in the study by convenience sampling technique after taking written informed consent and doing all relevant examinations and investigations for confirmation of diagnosis. Structured questionnaire was used to collect the necessary information.

Serum GPC-3 and serum AFP tests were performed of these 30 patients. Serum GPC-3 and AFP were done from the Microbiology department of BSMMU.

GPC-3 was done by ELISA. This assay employs an antibody specific for human GPC-3 coated on a 96-well plate. Standards and samples were pipetted into the wells and GPC-3 was present in a sample bound to the wells by immobilized antibody. The wells were washed and biotinylated anti-human GPC-3 antibody is added. After washing away unbound biotinylated antibody, Horseradish Peroxidase-conjugated streptavidin was pipetted to the wells. The wells were re-washed and a Tetramethylbenzidine substrate solution was added to the wells. Then, color developed in proportion to the amount GPC-3 was bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 40 nm.

All continuous data were presented as mean ± standard deviation (SD). Qualitative data were analyzed by Chi- square test & quantitative data were analyzed by student's t-test. The sensitivity and specificity was evaluated by multivariate logistic regression analysis after adjusting for the potential confounding variables. Risk analysis was performed by calculating the odds ratio (OR) and the 95% confidence interval (CI). All tests were two tailed, with a significant p value defined as < 0.05. All statistical analyses were performed by using SPSS version 20.

Inclusion Criteria

- HCC (irrespective of etiology) which was diagnosed by positive cytology of fine needle aspirate from hepatic space occupying lesion.

Exclusion Criteria

- Patients who were on treatment or previously treated for HCC.
- Patient with co-morbid conditions; not physically fit for liver FNAC.
- Patients with dysplastic nodules or inconclusive result in FNAC.
- Presence of clinical or biomarkers suggestive of other malignancies.

RESULTS

Table 1: Socio-Demographic characteristics of the respondents (n=30).

Characteristics	Frequency (N)	Percentage (%)
Age (in years)		
16-30	3	10.0
31-45	8	26.7
46-60	11	36.7
>60	8	26.7
Mean ± Standard Deviation	50.26 ± 14.7	
Sex		
Male	26	86.7
Female	4	13.3
Education		
Illiterate	14	46.7
Primary	8	26.7
SSC	4	13.3

Characteristics	Frequency (N)	Percentage (%)
HSC	3	10.0
Bachelor and above	1	3.3
Occupation		
Farmer	16	53.3
Business	7	23.3
Housewife	4	13.3
Service	2	6.7
Others	1	3.3

*SSC= Secondary School Certificate; HSC=Higher secondary School Certificate.

Table 1 above shows the socio-demographic characteristics of the respondents. Majority of the respondents (36.7%) belonged to age group 46-60 years. Males were frequently affected (86.7%) compared to females (13.3%). In case of education, illiterate people (46.7%) are more prone to HCC than educated people. Same thing reflected on occupation, those people who are service holder, are less affected (6.7%) compared to other occupations.

Figure 1 below, which is a pie chart, showing HBsAg test results among the participants. It is evident that, 76.7%, that is maximum respondents were HBsAg positive.

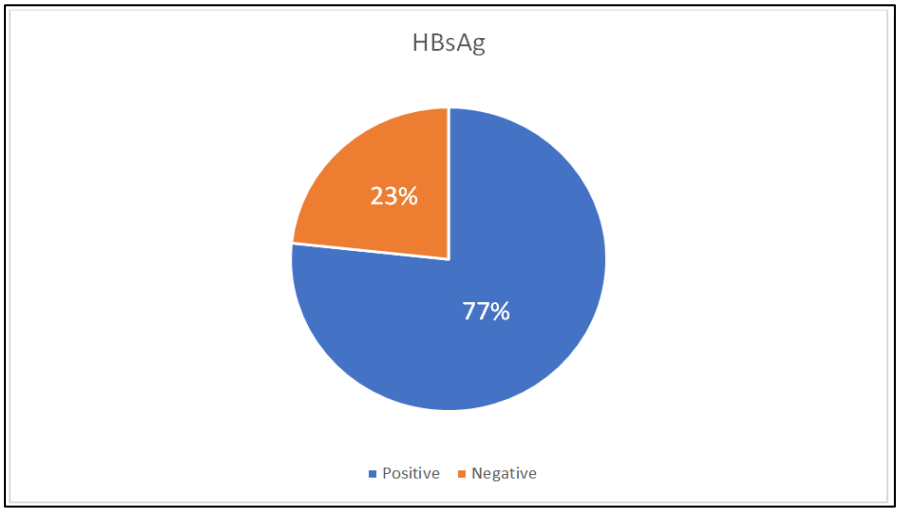

Figure 1: Demonstration of HBsAg test results among the respondents (n=30)

Figure 2 below contains a pie chart showing test results of Anti HCV among the participants. It can be seen that, majority of the respondents which is 90.0% respondents were negative.

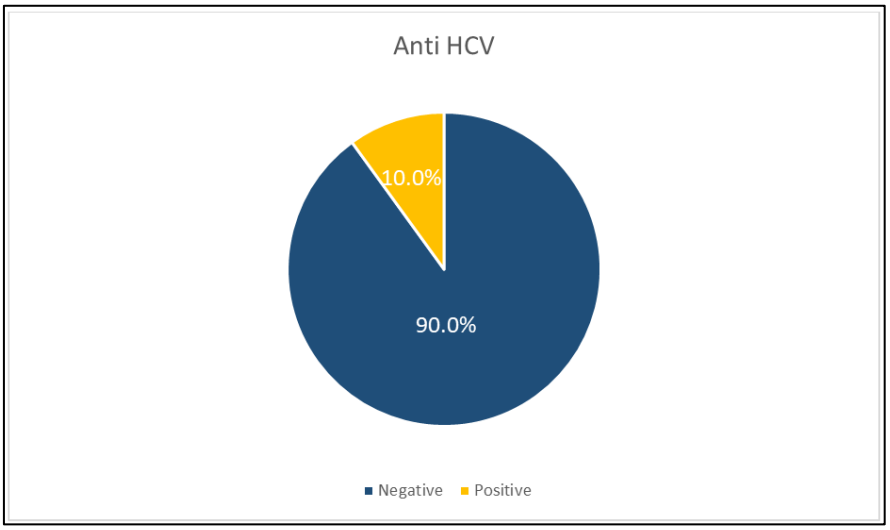

Figure 2: Illustration of Anti HCV test results among the respondents (n=30)

Figure 3 below shows a bar chart about Child Pugh score among the HCC patients. Stage B held the majority of the respondents that is 53.3%. The least number of participants belonged to Stage C (6.7%).

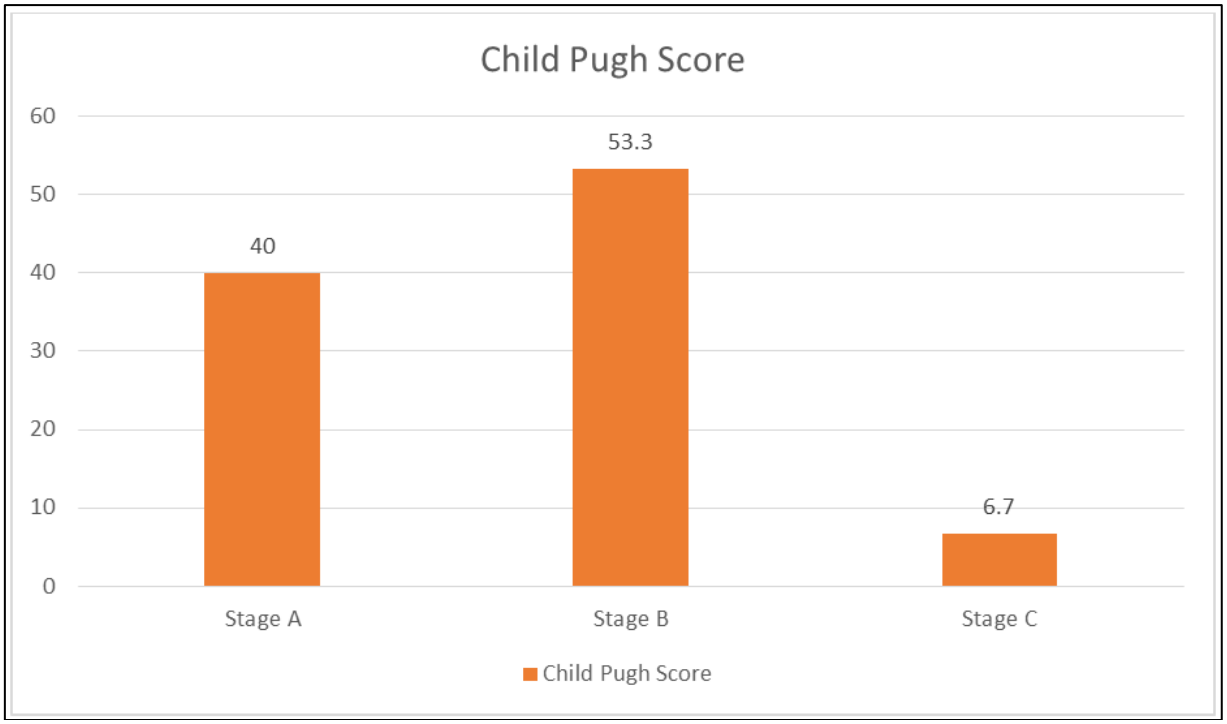

Figure 3: Demonstration of Child Pugh score among the respondents (n=30)

Table 2: Mean distribution of AFP and Serum Glypican-3 (n=30)

Trait	Hepatocellular Carcinoma(n=30) (Mean ± Standard Deviation)
Alfa Fetoprotein (ng/ml)	816.17± 560.96
Serum Glypican-3 (ng/ml)	378.00 ± 168.42

Table 2 above demonstrates the mean level of AFP which was 816.17 ± 560.96 ng/ml in HCC group of patients. On the other hand, mean value of serum Glypican-3 level was 378.00 ± 168.42 ng/ml.

Table 3: Association of Serum GPC-3 according to clinical characteristics in HCC patients (n=30)

Clinical characteristics	Serum GPC-3 (ng/ml) Mean ± Standard Deviation	P-value
HBsAg		
Positive	383.50 ± 153.85	0.001*
Negative	359.92 ± 223.20	
Anti HCV		
Positive	471.00 ± 50.76	0.044
Negative	367.66 ± 174.16	
Child Pugh score		
Stage A	363.00 ± 194.951	0.002*
Stage B	386.44 ± 163.16	
Stage C	400.50 ± 20.50	

*Statistically significant

Table 3 above shows the association of Serum Glypican-3 according to clinical characteristics among the respondents. It was clearly evident that, higher levels of Serum GPC-3 were found in both HBsAg and anti HCV positive HCC patients, which was 383.50 ± 153.85 ng/ml and 471.00 ± 50.76 ng/ml respectively. It was also observed that, higher level of Serum Glypican-3, that is 400.50 ± 20.50 ng/ml was found in HCC patients who belonged to Child Pugh C class. Regarding level of significance, association of HBsAg positive and Child Pugh score was found highly significant.

Table 4: Association of AFP according to clinical characteristics in HCC patients (n=30)

Clinical characteristics	Alfa fetoprotein (ng/ml) Mean ± SD	P value
HBsAG		
Positive	22074 ± 62669	0.001*
Negative	9717 ± 11334	
Anti HCV		
Positive	110523 ± 164706	
Negative	9043 ± 15106	0.001*
Child Pugh score		
Stage A	7928 ± 11675	
Stage B	11224 ± 17839	0.001*
Stage C	150500 ± 211424	

*Statistically significant

Table 4 demonstrates higher levels of serum Alfa-fetoprotein 3 were significant in both HBsAg and anti HCV positive HCC patients. It was also evident that, higher levels of AFP found in HCC patients of Child Pugh C class. All the associations were highly statistically significant.

Table 5: Association of AFP and serum glypican 3 in HCC patients (n=30)

AFP (ng/ml)	Number of patients	Serum Glypican-3 (ng/ml)	P value
<200	5	324.77 ± 120.24	
200-5000	18	402.82 ± 145.27	0.001*
>5000	7	425.60 ± 55.01	

*Statistically significant

Table 5 reveals significant association of AFP and serum Glypican 3 in HCC patients (P<0.05). Even Glypican-3 was found significantly raised among 5 HCC patients (324.77 ± 120.24) ng/ml who had Alfa-fetoprotein below cut of value range.

Figure 4 below indicates Receiver-operator characteristic (ROC) curve of Serum Glypican-3 levels for prediction of significant HCC patients whereas sensitivity was 0.8.

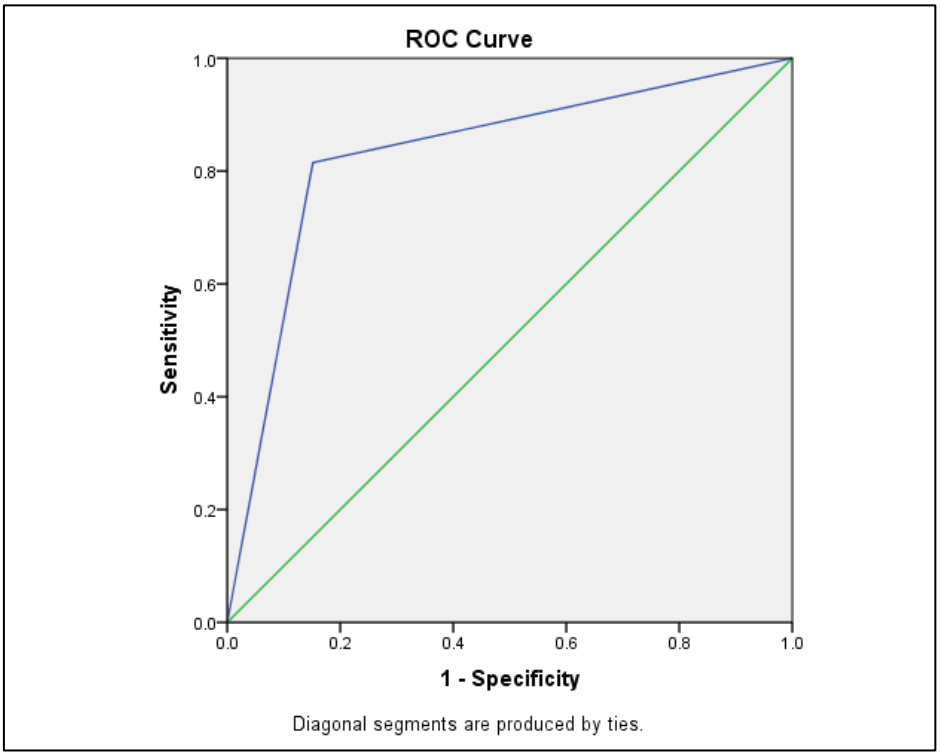

Fig. 4: Receiver-operator characteristic (ROC) curve of Serum Glypican-3 level for prediction of significant HCC patients (n=30)

DISCUSSION

A total of 30 patients were included in this cross-sectional study which was done with the goal of assessing of the diagnostic value of serum Glypican-3 for detection of hepatocellular carcinoma in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka between March, 2015 to August, 2016.

Liver cancer is one of the most dangerous contributors to the worldwide cancer burden. Increasing incidence rate of this disease has been a threat in many countries in the recent years [7]. The use of biomarkers in early diagnosis has played an important role nowadays. GPC-3 was recognized as an established serologic biomarker with one of the best diagnostic performances than AFP with the means of its ability to accurately differ between patients with small, well-differentiated HCC tumors [8].

In this study, among the 30 respondents, mean age was found to be 50.26 ± 14.7 years. Maximum participants (86.7%) were males. In a similar study done in Egypt in 2018, mean age was 59 ± 9 years with 77.5% males [9]. It is also seen that, 76.7% respondents were HBsAg positive and 10% were anti-HCV positive. In another study back in 2018, 82.2% and 5.7% cases were found to be HBsAg positive and anti-HCV positive respectively [10].

In this study it was found that, mean value of serum Glypican-3 level was found to 378.00 ± 168.42 ng/ml. In addition, mean value of serum Alfa-Fetoprotein was 816.17 ± 560.96 ng/ml. In a previous study in Egypt, mean levels of GPC-3 and AFP were found to be 551.47 ± 185.25 ng/ml and 703.43 ± 744.69 ng/ml respectively [9].

We found that, higher levels of Serum GPC-3 were found in both HBsAg and anti HCV positive HCC patients. It was also seen that, higher levels of serum Alfa-fetoprotein 3 were significant in both HBsAg and anti HCV positive HCC patients. Significant association of AFP and serum Glypican 3 in HCC patients was demonstrated. Our findings were consistent with another study done in 2012. The receiver-operator curve in our study showed area under the curve to be around 0.8. This study also showed, the area under the curve (AUC) for GPC3 was 0.996 [11].

CONCLUSION

Serum GPC-3 could be used as a highly potential serum biomarker for early detection of HCC. Combined use of both GPC-3 and AFP for this purpose may enhance the diagnosis of this fatality.

Abbreviations:

AFP: Alfa-fetoprotein
BSMMU: Bangabandhu Sheikh Mujib Medical University
CT Scan: Computed Tomography Scan
FNAC: Fine Needle Aspiration Cytology
GPC-3: Glypican-3
HBsAg: Hepatitis B surface antigen
HCC: Hepatocellular Carcinoma
HCV: Hepatitis C Virus
MRI: Magnetic Resonance Imaging
OPD: Out-Patient Department
SCCA: Squamous Cell Carcinoma Antigen

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