The diagnostic value of serum glypican 3 in patients with hepatocellular carcinoma and its role in evaluation of treatment efficacy after loco-regional therapy

Sherif Monier Mohamed [(1),] Mohamed Abd Elmoghny [(1),] Samir Abd El-Hameed Ghait [(1)], Khaled Mahmoud Makboul [(1)], Eslam Safwat [(1)], Dalia Abd Elhamid Hosny [(2)]

Internal Medicine Department [(1)], Clinical Pathology Department [(2)] Faculty of Medicine - Ain Shams University-Cairo –Egypt
Sherifmonier@yahoo.com

Abstract: **Background and Aims:** Hepatocellular carcinoma (HCC) is one of the commonest cancers worldwide and most patients are diagnosed at advanced stages and thus the prognosis is generally poor. It was reported that glypican3 is only detected in HCC cells, and can thus be used as a potential bio marker for the diagnosis of early HCC. The aims of this study was to study the diagnostic value of serum glypican 3 in patients with hepatocellular carcinoma and its role in evaluation of treatment efficacy after loco regional therapy. **Patients and Methods**: Three groups were studied which included 20 healthy subjects as a control, 40 patients with liver cirrhosis, and 40 patients with HCC. Serum α fetoprotein (AFP) and glypican 3 levels were measured. Patients in HCC group who fulfill criteria for local regional therapy (n=13) were followed up after 1month of therapy and serum AFP and glypican-3 level were evaluated post-treatment. **Results:** The serum levels of glypican 3 were significantly increased in HCC patients (18.1+16 ng/ml) as compared with patients with liver cirrhosis (3.5+1.2 ng/ml) and controls (3+1.3 ng/ml) with statistically significant difference in between ((P<0.001). Elevated glypican 3 values correlate with serum bilirubin, AFP, number of nodules and vascular invasion. At a cutoff level of 15 ng/ml glypican 3 yielded a sensitivity of (91%), Specificity of (70%) for diagnosis of HCC.However AFP gave sensitivity of (80%), Specificity of (70%) at cutoff level of 200 ng/ml. The combined glypican 3and AFP improve the sensitivity and Specificity to 95 and 80 % respectively. The level of serum glypican 3 was declined markedly from (32.8+13 to 18+11 ng/ml) after loco-regional therapy with statistically significant difference in between (p<0.001) **Conclusion:** Serum glypican3 is highly sensitive and specific for detecting HCC specially if combined with AFP and can be used in screening programs and may be used in evaluation of loco-regional treatment efficiency.

[Sherif Monier Mohamed,Mohamed Abd Elmoghny,Samir Abd El-Hameed Ghait, Khaled Mahmoud Makboul, Eslam Safwat, Dalia Abd Elhamid Hosny. **The diagnostic value of serum glypican 3 in patients with hepatocellular carcinoma and its role in evaluation of treatment efficacy after loco-regional therapy.** *Life Sci J* 2013; 10(3): 1938-1945]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 287

Keywords: Hepatocelluler carcinoma, cirrhosis, Glypican 3, a-fetoprotein, loco regional therapy.

1.Introduction

Hepatocellular carcinoma is one of the commonest cancers worldwide. It is a major health problem and its incidence is increasing. The presence of cirrhosis of the liver is the major risk factor and worldwide this is largely due to chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infection.[1, 2]

Most patients with HCC are diagnosed at advanced stages and thus the prognosis is generally poor. The diagnosis of HCC could be achieved at an earlier stage by regular screening programs among high-risk populations by using imaging studies and serum tumor markers.[3]

Currently, AFP, a fetal-specific glycoprotein, has undoubtedly been the most widely used tumor marker for the detection and monitoring of HCC. However, serum AFP is not always elevated to a diagnostic level in all patients, particularly in small HCC, and considerable numbers of patients with more advanced stages would be missed unless another diagnostic tool is used.[4, 5] Moreover, its 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.[6] Therefore, the identification of alternative serum markers of HCC is needed.

Glypican 3 is a heparin sulfate proteoglycan that is bound to the cell surface by glycosylphosphatidylinositol anchors (GPI) and highly expressed in fetal, but not in adult liver. It has been shown that glypican 3 is closely related to HCC.[7, 8] It was recently reported that Glypican 3 is only detected in HCC cells,but not in benign liver tissues, and can thus be used as a potential biomarker for the diagnosis of early HCC.[9, 10, 11]

Aim of the work

To study the diagnostic value of serum glypican 3 in patients with hepatocellular carcinoma and its role in evaluation of treatment efficacy after loco-regional therapy.

2. Patients and Methods

This study was carried out on one hundred subjects admitted to Ain shams university hospitals as in-patient or in the out-patient clinic. The subjects were included in this study after obtaining their consent and were divided into three groups.
Group A: include 40 patients with HCC.
Group B: include 40 patients with liver cirrhosis.
Group C: include 20 healthy subjects as a control.

Inclusion criteria:

1 Age >18 years old.
2 Liver cirrhosis was diagnosed based on physical examination, laboratory tests, abdominal ultrasonography or computed tomography (CT) scan, and liver biopsy or fibroscan when possible.
3 Hepatocelluler carcinoma was diagnosed based on at least one of the following criteria in the guidelines of clinical diagnosis and staging for hepatocellular carcinoma[12]:

i. Hepatic space-occupying lesion with a serum AFP level ≥ 400 ng/ml.
ii. Hepatic space occupying lesions with arterial phase enhancement and rapid washout in portovenous phase in triphasic CT or magnetic resonance imaging.
iii. Liver biopsy in some patients.

Liver was staged according to the Barcelona Clinic Liver Cancer (BCLC) staging system.[13]

Exclusion criteria: all patients who had a prior loco-regional therapy, systemic therapy and/or any surgical intervention (liver resection or transplantation) were excluded from the analysis**.**

All Subjects included in the study were subjected to the following:

1. Full history taking and complete physical examination.
2. Routine laboratory investigations include liver function tests, renal function tests, complete blood count, blood sugar and viral markers.
3. Measurement of serum AFP and glypican-3 levels
   - Measurement of serum AFP levels was determined using a commercially available ELISA kit (Cobus Core; Roche Diagnostics, Basel, Switzerland).
   - Measurement of serum glypican-3 level by Human Glypican-3(GPC3) ELISA Kit a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Glypican-3(GPC3) in samples(Glory Science Co., Ltd USA)
4. Radiological investigations include abdominal ultrasonography and Triphasic CT or magnetic resonance imaging.
5. Liver biopsy in some patients.

Patients with HCC who fulfill criteria for loco-regional therapy (n=13) [Radiofrequency ablation (RFA) in 3 patients & Transcatheter arterial chemoembolization (TACE) in 9 patients and surgical resection in 1 patient] according to Barcelona clinic liver cancer staging classification and treatment.[14] were followed up after 1month of therapy and serum AFP and glypican-3 levels were evaluated post treatment.

Statistical Methodology:

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12) as follows: Description of quantitative variables as mean, SD and range, description of qualitative variables as number and percentage.Chi-square test was used to compare qualitative variables between groups. Unpaired t-test was used to compare two groups as regard quantitative variable.Mann Whitney test was used instead of unpaired t-test in non parametric data. One way ANOVA test was used to compare more than two groups as regard quantitative variable.Fisher exact test was used when one expected cell or more are less than 5.Spearman Correlation coefficient test was used to rank different variables positively or inversely versus each other. Willcoxon test was used to compare quantitative non parametric variables among the same group before and after.ROC (receiver operator characteristic curve) was constructed to evaluate the diagnostic performance of glypican 3 and AFP in discriminating HCC. Best cut off, Sensitivity, specificity, positive and negative predictive values and diagnostic accuracy were calculated in accordance with standard methods.

P value >0.05 insignificant, *P*<0.05 significant and *P*<0.01 highly significant.

3. Results:

Comparison between the studied groups as regard general data showed statistically no significant difference between the studied groups as regard age, gender and co-morbidities as diabetes mellitus (DM) or hypertension (HTN). On the other hand, there is a significant difference between the studied groups as regard smoking. **(Table 1)**

Comparison between the studied groups as regard laboratory data showed statistically highly significant difference between the studied groups as regard different laboratory data apart from serum sodium. **(Table 2)**

Comparison between patients with HCC and patients with cirrhosis showed statistically significant difference in between as regard child classification and history of encephalopathy.On the other hand no significant difference as regard ascites, viral etiology and performance status. **(Table 3)**

In HCC 21 patients (52.5%) had single hepatic focal lesion (HFL),12 patients (30%) had two HFLs and 7 patients (17.5%) had multiple HFLs.The right lobe of the liver was affected in 28 patients(70%), the left lobe was affected in 9 patients (22.5%) and both lobes were affected in 3 patients (7.5%). The average total size of HFLs was 70±40 mm and vascular invasion affect 8 patients (20%). **(Table 4)**

HCC group had the highest level of AFP (266.5±200 ng/ml) compared to cirrhotic group (49.6±50ng/ml) and control group (14.6±10ng/ml) with statistically highly significant difference in between (P<0.001). (**Table 5)**

Figure (1) showed that HCC group had the highest level of glypican 3 (18.1+16 ng/ml) compared to cirrhotic group (3.5+1.2 ng/ml) and control group (3+1.3 ng/ml) with statistically highly significant difference in between (P<0.001).

Correlation between serum glypican 3 and prognostic parameters in HCC showed statistically significant positive correlation between glypican 3 versus serum bilirubin, AFP, number of nodules and vascular invasion (P< 0.05). On the other hand, there is no significant correlation between glypican3 and other variables. **(Table 6 & figure 2)**

At the cutoff value of 15 ng/ml the sensitivity and specificity of serum glypican3 for prediction of HCC were 91% and 70% respectively. On the other hand**,** at the cut of value of 200 ng /ml the sensitivity and specificity of serum for prediction of HCC were 80% and 70% respectively. When combination of both serum glypican 3 and AFP were used the sensitivity and specificity were increased to 95% and 80 % respectively. **(Table 7&Figure 3)**

In Patients with HCC who full fill criteria for loco-regional therapy (n=13) the level of serum glypican 3 was declined markedly from (32.8+13 to 18+11 ng/ml) after loco-regional therapy with statistically highly significant difference in between (P <0.001). Also, the level of serum AFP was declined markedly from (449+300 to 90+55 ng/ml) with statistically highly significant difference in between (P <0.001). **(Figure 4)**

Table (1): Comparison between the studied groups as regard general data

Variables	HCC (n= 40)	Cirrhotic (n= 40)	Controls (n=20)	*P* Value
Mean age (in years)	58.5±8	57±7.6	59.2±6	>0.05 NS
Gender				
Male	29 (72.5%)	34 (85%)	17 (85%)	>0.05 NS
Female	11 (27.5%)	6 (15%)	3 (15%)	
Smoking				
No	19 (47.5%)	18 (45%)	9 (45%)	**<0.05 S**
Yes	5 (12.5%)	14 (35%)	1 (5%)	
Ex-smoker	16 (40%)	8 (20%)	10 (50%)	
co morbidities				
HTN	4 (10%)	3 (7.5%)	1 (5%)	>0.05 NS
DM	7 (17.5%)	2 (5%)	2 (10%)	
Both	1 (2.5%)	0	0	

Table (2): Comparison between the studied groups as regard laboratory data

Variables	HCC (n=40)	Cirrhotic (n=40)	Controls (n=20)	*P* Value
Hemoglobin (g / dl)	10.5±1.9	11.4±1.8	12.5±1.4	**<0.001 HS**
Platelet count (10^3/ml)	87±50	106.5±45	265±38	**<0.001 HS**
Bilirubin (mg/dL)	2.3±1.2	2.2±1.1	0.8±0.2	**<0.001 HS**
INR	1.6±0.3	1.4±0.3	1.1±0.5	**<0.001 HS**
Albumin (g/dl)	2.6±0.5	3.1±0.6	4.2±0.6	**<0.001 HS**
AST (U/ l)	67.7±31	66.8±33	20.6±9	**<0.001 HS**
ALT (U/ l)	52.6±26	49.3+20	22±9	**<0.001 HS**
Urea (mg/dl)	28.6±11	27.5±5	23.4±6	**<0.001 HS**
Creatinine (mg/dl)	1.4±0.4	0.90±0.4	0.8±0.2	**<0.001 HS**
Na (mEq / L)	124±4.9	127.5±5	137.5±5	>0.05 NS

Table (3): Comparison between patients with HCC and liver cirrhosis as regard child pugh classification, ascitis, encephalopathy, viral etiology and performance status

Variables	HCC (n= 40)	Cirrhotic (n= 40)	*P* Value
Child pugh classification			
A	3 (7.5%)	12 (30%)	**<0.001**
B	13 (32.5%)	8 (20%)	**HS**
C	24 (60%)	20 (50%)	
Ascites			
No	11 (27.5%)	14 (35%)	>0.05
Mild	23 (57.5%)	24 (60%)	NS
Moderate to severe	6 (15%)	2 (5%)	
History of Encephalopathy	29 (72.5%)	18 (45%)	**<0.05** **S**
Viral infection			
HBV	5 (12.5%)	4 (10%)	>0.05
HCV	35 (87.5%)	35 (87.5%)	NS
Both	0	1(2.5%)	
Performance Status			
0	1 (2.5%)	0	>0.05
1	32 (80%)	34 (85%)	NS
2	5 (12.5%)	6 (15%)	
3	2 (5%)	0	

Table (4): Criteria of hepatic focal lesions in HCC group

Variables	No	%
Number of nodules		
1	21	52.5%
2	12	30%
Multiple	7	17.5%
Site		
Right lobe	28	70%
Left lobe	9	22.5%
Bilateral	3	7.5%
Total size (mm)	70±40	
Vascular invasion		
Present	8	20%
Absent	32	80%

Table (5): Comparison between the studied groups as regard serum AFP

AFP	HCC n=40	Cirrhotic n=40	Controls n=20	*P* value
Mean ± SD ng/ml	266.5±200	49.6±50	14.6±10	**<0.001** **HS**

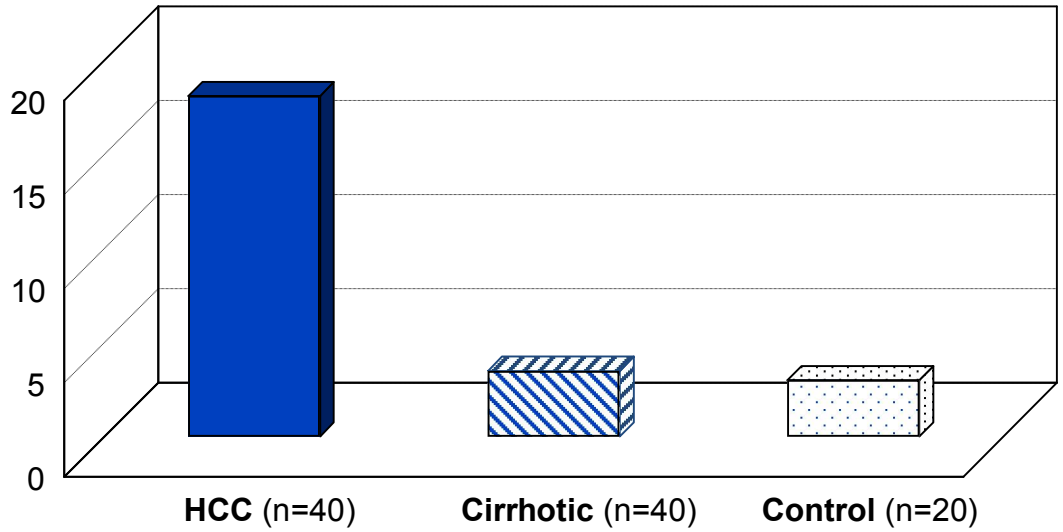

Figure (1): Comparison between the studied groups as regard glypican 3

Table (6): Correlation between glypican 3 versus prognostic parameters in HCC group

Variables	Glypican-3	
	r	***P* value**
Age (years)	0.02	>0.05
Bilirubin (mg/dl)	0.27	**<0.05S**
INR	0.09	>0.05
Albumin(g/dl)	-0.02	>0.05
AST (U/ l)	-0.14	>0.05
ALT (U/ l)	0.15	>0.05
AFP (ng /ml)	0.23	**<0.05 S**
Tumor size (mm)	0.21	>0.05
Number of nodules	0.28	**<0.05 S**
Vascular invasion	0.48	**<0.05 S**
Child Pugh class	0.09	>0.05
Performance status	-0.04	>0.05

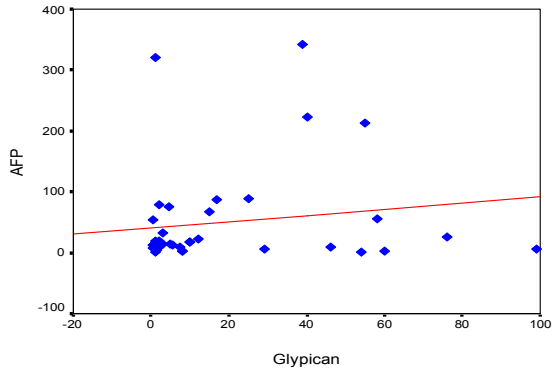

Figure (2): Correlation between glypican 3 versus AFP among HCC group

Table (7):Validity of glypican3 versus AFP in prediction of HCC

Variables	**Glypican-3** (ng/ml)	**AFP** (ng/ml)	**Both**
Best cut off	15	200	
AUC	0.90	0.75	
Sensitivity	91%	80%	95%
Specificity	70%	70%	80%
PPV	78%	76%	85%
NPV	94%	84%	96%
Accuracy	80%	71%	85%

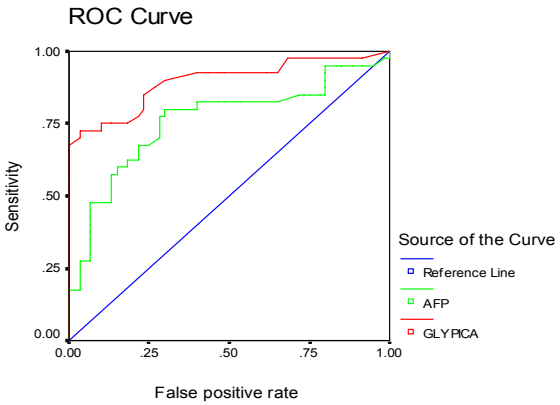

Figure (3): ROC curve for glypican3 and AFP in prediction of HCC

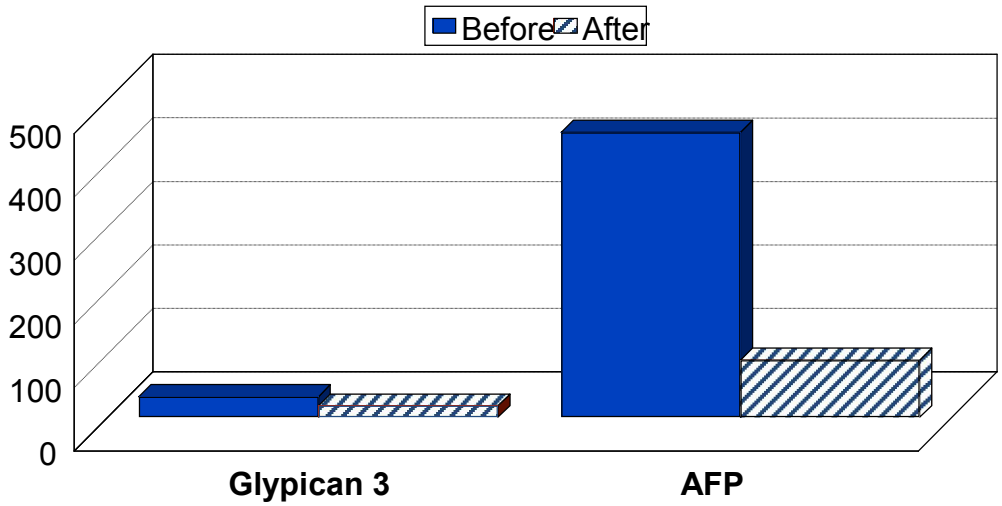

Figure (4): Comparison between glypican 3 and AFP before and after loco- regional therapy (n=13)

4. Discussions

Hepatocellular carcinoma was found in this study to be more prevalent in men 29 (72.5%) than in women 11 (27.5%). This was in conformity with that found by **El-Zayadi *et al.,*** [15] in their study. This may at least be explained in part by the differences in exposure to risk factors, sex hormones and other X-linked genetic factors. It has been speculated that estrogen and androgen could modulate hepatocarcionogenesis and explain the higher incidence of HCC in men.[16]

The right lobe was affected in 28 patients (70%), the left lobe in 9 patients (22.5%), and both lobes in 3 patients (7.5%) of the HCC group. **El-Zayadi *et al.,*** [15] found that 65% of HCC affecting the right lobe, 13.4% the left lobe and 21.6% affecting both lobes**.** This was contrary to the findings in the study of **Ajayi *et al.,*** [16] who found that in the majority (62.3%) of the patients the two lobes were affected, while 15.1% had the lesions confined to the left lobe and 22.6% in the right lobe at the time of presentation at the hospital.

Hepatitis C and B infections are considered, the major risk factors that contribute to the development of HCC worldwide. The main etiological factor for primary HCC in our study is HCV infection (87.5%) and HBV (12.5%). It has been shown that HCV infection plays a role in pathogenesis of primary HCC [17]. However, in China the main etiological factor for primary HCC is HBV infection [18, 19]

In our study, we found that serum levels of AFP were significantly higher in HCC group (266.5±200ng /ml), compared to cirrhotic group (49.6±50 ng/ml) and control group (14.6±10ng /ml). The cutoff value of AFP was set at 200 ng /ml by (ROC) curve analysis. At this cutoff value, the sensitivity and specificity of serum AFP for prediction of HCC was 80% and 70%, respectively. The AASLD recommended cutoff level for diagnosis of HCC at 200 ng /ml, although lower levels, particularly if rising should be followed very carefully.[20]

In our study, we found that serum levels of glypican-3 were significantly higher in HCC group (18.1+16 ng /ml), compared to cirrhotic group (3.5+1.2 ng/ml) and control group (3+1.3 ng /ml). The cutoff value of glypican 3 was set at 15 ng /ml by (ROC) curve analysis. At this cutoff value, the sensitivity and specificity of serum glypican 3 for prediction of HCC was 91% and 70%, respectively. A similar finding was reported in another study by **Hippo *et al.,*** [21]**.** However, **Liu *et al.,*** [9] in their study showed lower sensitivity 46.7% but higher specificity 93.5%.

Nakatsura *et al.,* [22] found that serum glypican 3 levels were increased in 53% of patients with HCC,but was increased in only one patient with non-malignant chronic liver disease. And found that the sensitivity and the specificity of this serum maker for differentiating HCC from benign liver disease were 53% and 99%.

In our study glypican 3 was considered more accurate than AFP (80% versus71%) in diagnosis of HCC. The sensitivity of glypican 3 was (91%) compared to AFP (80%) in prediction of HCC. The glypican 3 have better positive predictive value than AFP (78%versus76%) and also better negative predictive value (94%versus84%) but with similar specificity (70%). The combined glypican 3 and AFP gave a sensitivity of 95%, specificity of 80 % and increase accurace to85%. our results were in agreement with other studies [23, 24, 25, 26]

Gomaa *et al.,* [27] in their study showed that at the cutoff 5.41 ng /ml, serum glypican 3 gave a sensitivity of 90.3%, specificity of 98 % for HCC diagnosis. However AFP gave a sensitivity of 77.4%, specificity of 60 % at cut off 42.3 ng /ml. The combined glypican 3 and AFP gave a sensitivity of 84%, specificity of 90 %.

Glypican3 had been reported to be increased in HCC in comparison with pre-neoplastic lesions and cirrhotic tissues at the mRNA and protein levels.[26] On the other hand; the AFP level can reach 250 µg/L in around 20%-25% of patients with chronic hepatitis, liver cirrhosis and other liver diseases. [28, 29]

Another important observation in a study by **Capurro *et al.,*** [30] was that glypican 3 would be a better marker for the detection of small HCC less than 3 cc in size than AFP, As it had been demonstrated that the expression of glypican 3 in small HCC was significantly greater than that of AFP. Also, reported that glypican3 mRNA levels are more frequently elevated than those of AFP, with the difference even greater in small HCC.

It was reported that the frequency of glypican3 expression in AFP-negative HCC patients is as high as 90%, suggesting that it can be used in diagnosis of HCC.[21, 31, 32]

In our study significant positive correlations were detected between serum glypican3 and each of bilirubin, AFP, number of nodules and vascular invasion in HCC group (*P* value <0.05). **Gomaa *et al.,***[27] reported positive correlation between glypican3 and AST, albumin, prothrombin concentration, tumor size &number and blood vessel invasions, but no correlation was found between glypican3 and AFP levels**.** Also**, Song *et al.,*** [33] reported in their study that no correlation was found between glypican3 and AFP levels in patients with HCC, they have also found that, due to the lack of correlation between AFP and glypican 3 in patients with HCC the simultaneous use of both markers significantly improve the sensitivity of AFP alone.

The combination of both markers in our study improves overall accuracy (85%), sensitivity (95%), specificity (80%), PPV (85%), and NPV (96%) in prediction of HCC**.** A similar finding reported in other studies which reported that the combination of both markers improved overall sensitivity from 50% to 72%.[20, 23]Also **Liu *et al.,*** [9] reported that the sensitivity of combined serum glypican 3 and AFP was increased for the diagnosis of HCC at all stages.

Capurro *et al.,* [30] and **Nakatsura *et al.,*** [22] showed that the simultaneous measurement of glypican 3 and AFP significantly increased the sensitivity and accuracy for HCC diagnosis. The combination of these markers yielded an improved sensitivity for detecting small HCC to 75**%.**[25]

In Patients with HCC who full fill criteria for loco-regional therapy (RFA, TACE or resection) (n =13) the levels of serum AFP and serum glypican 3 were declined markedly from (449±300 & 32.8±13 ng/ml respectively) to (90±55&18±11 ng/ ml respectively) after loco-regional therapy with statistically highly significant difference in between (p<0.001).

To the best of our knowledge this was the first study that uses glypican 3 to follow up of HCC after loco-regional therapy. **Suriawinata *et al.,*** [34] found that serum glypican 3was detectable only in HCC patients and confirmed that glypican 3 had disappeared after surgical treatment for HCC

Conclusion and recommendation

Glypican 3 is highly sensitive and specific tumor marker for detecting HCC specially if combined with AFP and can be used in screening programs for HCC and may be used as the tool for the evaluation of loco-regional treatment efficiency. Furthermore, it may be used for the prediction of tumor recurrence.

References:

1. **Kawano Y, Sasaki A, Kai S, *et al.,*** Short- and long-term outcomes after hepatic resection for hepatocellular carcinoma with concomitant esophageal varices in patients with cirrhosis. Ann Surg Oncol 2008; 15: 1670-1676.
2. **Verslype C, Rosmorduc O and Rougier P**. Hepatocellular carcinoma: ESMO–ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology, 2012; 23 (7): 41–48
3. **Spangenberg HC, Thimme R and Blum HE.** Serum markers of hepatocellular carcinoma. Semin. Liver Dis. 2006; 26: 385–90.
4. **Stefaniuk P, Cianciara J and Wiercinska-Drapalo A.** Present and future possibilities for early diagnosis of hepatocellular carcinoma. World J Gastroenterology 2010; 16: 418-424.
5. **Choi JY, Jung SW, Kim HY, *et al.,*** Diagnostic value of AFP-L3 and PIVKA-II in hepatocellular carcinoma according to total-AFP. World J Gastroenterology. 2013;19(3):339-46
6. **Yuen MF and Lai CL.** Serological markers of liver cancer. Best Pract Res Clin Gastroenterology 2005; 19(1): 91-99.
7. **Zinkin NT, Grall F, Bhaskar K, *et al.,*** Serum proteomics and biomarkers in hepatocellular carcinoma and chronic liver disease. Clin Cancer Res 2008; 14: 470-477
8. **Block TM, Marrero J, Gish RG, *et al.,*** The degree of readiness of selected biomarkers for the early detection of hepatocellular carcinoma: notes from a recent workshop. Cancer Biomarker 2008; 4:19-33

9. **Liu H, Li P, Zhai Y, *et al.,*** Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. World J Gastroenterology 2010; 16(35): 4410-4415.
10. **Shafizadeh N, Ferrell LD, Kakar S**. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. Mod Pathol 2008; 21: 1011-1018
11. **Shirakawa H, Kuronuma T, Nishimura Y, *et al.,*** Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. Int J Oncology 2009; 34(3):649-56
12. **Zhang BH, Yang BH, Tang ZY:** Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol 2004; 130: 417-422.
13. **Kim KA, Lee JS, Jung ES, *et al.,*** Usefulness of serum alpha-fetoprotein (AFP) as a marker for hepatocellular carcinoma (HCC) in hepatitis C virus related cirrhosis (analysis of the factors influencing AFP elevation without HCC development): Korean J Gastroenterology 2006; 48: 321-326.
14. **Rampone B, Schiavone B and Confuorto G**. Current management of hepatocellular cancer. Curr Oncol Rep 2010; 12(3):186-192.
15. **El-Zayadi AR, Badran HM, Barakat EMF, *et al.,*:** Hepatocellular carcinoma in Egypt: A single centre study over a decade. World J. Gastroenterology 2005; 11(33): 5193-5198.
16. **Ajayi AO, Ajayi EA and Komolafe OA.** Hepatocellular carcinoma: Risk factors, pattern of presentation and outcome in a tertiary health facility. International Journal of Medicine and Medical Sciences 2009; 1: 84-87.
17. **Madhoun MF, Fazili J, Bright BC *et al.,*** Hepatitis C prevalence in patients with hepatocellular carcinoma without cirrhosis. Am J Med Sci 2010; 339:169-173.
18. **Ming L, Thorgeirsson SS, Gail MH, *et al.,*** Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology 2002; 36: 1214-1220.
19. **Gao JD, Shao YF, Xu Y, *et al.,*** Tight association of hepatocellular carcinoma with HBV infection in North China. Hepatobiliary Pancreat Dis Int 2005; 4: 46-49.
20. **Bruix J and Sherman M.** Management of hepatocelluler carcinoma. Hepatology 2005;42:1208-1236
21. **Hippo Y, Watanabe K, Watanabe A, *et al.,*** Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early stage hepatocellular carcinoma: Cancer Res 2004; 64: 2418-2423.
22. **Nakatsura T, Yoshitake Y, Senju S, *et al.,*** Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. Biochem Biophys Res Commun 2003; 306: 16–25.
23. **Zhou L, Liu J and Luo F:** Serum tumor markers for detection of hepatocellular carcinoma. World J Gastroenterology 2006; 12: 1175-1181.
24. **Coston WM, Loera S, Lau SK, *et al.,*** Distinction of hepatocellularcarcinoma from benign hepatic mimickers using glypican-3 and CD34 immuno histochemistry. Am J Surg Pathol 2008; 32: 433-44.
25. **Tangkijvanich P, Chanmee T, Komtong S, *et al.,*** Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. J Gastroenterology Hepatology 2010; 25(1): 129–137.
26. **Wang XY, Degos F, Dubois S, *et al.,*** Glypican-3 expression in hepatocellular tumors: diagnostic value for pre neoplastic lesions and hepatocellular carcinomas. Hum Pathol 2006; 37: 1435-41.
27. **Gomaa AI, Hendy OM, Abou Raia GY *et al.,***.The diagnostic value of peripheral blood glypican 3in patients with hepatocellular carcinoma. World. J. Med. Sci 2012; 7(2):105-112.
28. **Libbrecht L, Severi T, Cassiman D, *et al.,*** Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. Am J Surg Pathol 2006; 30: 1405–11.
29. **Zinkin NT, Grall F, Bhaskar K, *et al.,*** Serum proteomics and biomarkers in hepatocellular carcinoma and chronic liver disease. Clin Cancer Res 2008; 14(2): 470-477.
30. **Capurro M, Wanless IR, Sherman M, *et al.,*** Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 2003; 125: 89–97.
31. **Zhou XP, Wang HY, Yang GS, *et al.,*** Cloning and expression of MXR7 gene in human HCC tissue. World J Gastroenterology 2000; 6: 57-60.
32. **Shafizadeh N, Ferrell LD and Kakar S** Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. Mod Pathol 2008; 21: 1011-1018.
33. **Song HH, Shi W and Filmus J** OCI-5/rat glypican-3 binds to fibroblast growth factor-2 but not to insulin-like growth factor-2. J Biol Chem 1997; 272: 7574-7577.
34. **Suriawinata A, SN Thung.**Molecular signature of early hepatocelluar carcinoma. Oncology 2010;3:7836-39.

8/12/2013