The Egyptian Journal of Hospital Medicine (October 2021) Vol. 85 (1), Page 3236-3243

# Value of Glypican-3 as a Diagnostic and Prognostic Biomarker for Hepatocellular Carcinoma before and after Treatment. A Prospective Study

**Amira Isaac*, Hanan Mahmoud Mohamed Badawy, Inas Elkhedr Mohamed, Al Saeed Ezzat Ibrahim**
Department of Internal Medicine, Gastroenterology and Hepatology Unit,
Faculty of Medicine, Ain Shams University, Egypt.
***Corresponding Author:** Amira Isaac Samaan, **Phone No.:** (+2) 01222216927, **E-mail:** dr.amira@med.asu.edu.eg

## ABSTRACT
**Background:** Hepatocellular carcinoma is a highly prevalent tumor globally and the world's second leading cause of cancer-related deaths. Glypican-3, a heparan sulfate proteoglycan expressed on the surface of HCC cells, has emerged as a new molecule with a strong link to occurrence and progression of HCC. **Objective:** This study was done to determine the role of Glypican-3 in diagnosis of HCC and its prognostic value following different treatment modalities. **Patients and methods:** The study included thirty patients with liver cirrhosis and HCC on top, and thirty patients with liver cirrhosis without HCC. Standard laboratory investigations, abdominal ultrasound and triphasic computed tomography were done for all patients. Serum alpha fetoprotein and Glypican-3 were measured in all patients before and one month after different treatment modalities. **Results:** Glypican-3 was significantly higher in HCC group (2.28 ± 0.97) in comparison to cirrhosis group (0.56 ± 0.31) with P-value <0.001. Glypican-3 level was higher in larger sized lesions with p value 0.023, one month after treatment with different modalities, Glypican-3 declined significantly (from 2.28 ± 0.97 to 1.44 ± 0.93) with p value <0.001. At a cutoff point of > 1.1 ng/ml Glypican-3 has 93.3% sensitivity, 96.67% specificity, 96.6% PPV and 93.5% NPV for detection of HCC. **Conclusion:** Glypican-3 can be a valuable diagnostic marker for HCC diagnosis and prognosis after various treatment modalities and may be complementary to alpha fetoprotein increasing overall sensitivity of HCC detection.
**Keywords:** Glypican-3, Hepatocellular carcinoma.

## INTRODUCTION
The most frequent type of liver cancer is hepatocellular carcinoma (HCC). In the United States, the incidence of HCC has increased by 80% in the last two decades. This was also seen in several developed countries [(1)]. In Egypt, the burden of HCC is growing, with the incidence rate doubling in the last ten years. The high frequency of hepatitis C virus (HCV) in Egypt is implicated for the high incidence of HCC [(2)].

Although Egyptian HCC patients are diagnosed according to international guidelines, there are few Egyptian reports on HCC relapse and survival. In comparison to other countries, both basic and clinical HCC research is still scarce in Egypt [(3)]. Alpha-fetoprotein (AFP) is the commonest marker for detection of HCC, with false negative rate of 40% when used alone for early-stage HCC. Its value may still be normal in 15% to 30% of patients, even those with late-stage HCC [(4)].

Glypican-3 (GPC3) is a glycosyl-phosphatidyl inositol anchored cell surface heparan sulphate proteoglycan that belongs to the glypican family. Because GPC3 is not seen in healthy liver tissue and increases significantly in patients with HCC, it has been discovered as a useful tumor marker for HCC detection [(5)]. Several studies on the utility of serum GPC3 as a marker for the initial diagnosis of HCC and detection of recurrence following liver transplantation have demonstrated its sensitivity and specificity [(6)].

**The Aim of this work was** to determine the role of Glypican 3 in HCC diagnosis and its prognostic value following different treatment modalities.

## PATIENTS AND METHODS
This study was conducted on 60 patients including 30 patients with liver cirrhosis and HCC on top, HCC was diagnosed based on development of characteristic vascular enhancement in triphasic abdominal CT scan according to 2011 AASLD guidelines [(7)], and 30 patients with liver cirrhosis with no evidence of HCC based on absence of any hepatic focal lesions by triphasic CT. The patients were recruited from hepatology outpatient clinics of Ain Shams University Hospitals and Ahmed Maher teaching hospital, during the period From February 2019 to December 2020.

### All patients underwent:
Detailed history taking, thorough clinical examination, laboratory investigation including complete blood count, full hepatic profile, serum creatinine, assessment of Barcelona clinic liver cancer (BCLC) staging.

Determination of serum AFP level was assayed using human AFP EIA kit lot, Sweden, and serum GPC3 levels was measured using ELISA kit (INTRON)





Received: 1/6 /2021
Accepted: 27/7 /2021

according to the manufacturer's instruction. Both were measured at baseline and one month after treatment. Abdominal ultrasonography and triphasic CT abdomen were done, with special comment on size and number of lesions and portal vein invasion. For patients with HCC, triphasic abdominal CT was repeated one month after treatment.

Patients with any malignancies other than HCC, chronic kidney disease, diabetes, autoimmune diseases, severe burns were excluded from the study.

Radiofrequency ablation (RF) was offered to patients with early (BCLC-0) or (BCLC-A). Transarterial Chemo Embolization (TACE) was offered to intermediate BCLC-B patients unsuitable for resection or RF. Systemic treatment was offered to BCLC-C staged patients using Sorafenib or Lenvatinib.

**Ethical approval:**

**This study was in accordance with the ethical principles of the 1975 Helsinki Declaration that was granted by the local Ethics Committee of Ain Shams University Faculty of Medicine (FWA 000017585) December 2018. Participants gave their informed written consent before being enrolled in the trial, after being informed about the study's goal and required interventions.**

***Statistical analysis***

Data were analyzed using the IBM SPSS V23. Descriptive statistics were computed for quantitative data as mean and standard deviation and for qualitative data as frequency and percentage. For qualitative data Chi square test and Fisher's exact test were used. Independent t-test, Mann-Whitney U, Wilcoxon rank and Kruskal-Wallis tests were used for quantitative data. P-value < 0.05 was considered significant and < 0.01 was considered highly significant (HS).

**RESULTS**

This study was conducted on 60 HCV related liver cirrhosis; 30 patients with HCC on top, and 30 patients without HCC. Demographic and laboratory data were shown in (Table 1).

**Table (1):** Demographic and laboratory profile of the two studied groups

| | | Cirrhosis group No. = 30 | HCC group No. = 30 | P-value |
|---|---|---|---|---|
| Age (years) | Mean ± SD<br>Range | 58.20 ± 9.05<br>37 – 76 | 58.63 ± 9.40<br>45 – 82 | 0.856 |
| Sex (n, %) | Female<br>Male | 9 (30.0%)<br>21 (70.0%) | 5 (16.7%)<br>25 (83.3%) | 0.222 |
| Hemoglobin (gm/dL) | Mean ± SD | 10.68 ± 1.96 | 10.75 ± 1.80 | 0.891 |
| TLC ($x10^3$/uL) | Mean ± SD | 6.39 ± 3.04 | 7.44 ± 3.73 | 0.236 |
| PLT ($x10^3$/uL) | Mean ± SD | 144.53 ± 86.64 | 158.73 ± 60.06 | 0.464 |
| ALT (IU/L) | Mean ± SD | 45.69 ± 35.55 | 50.03 ± 47.98 | 0.657 |
| AST (IU/L) | Mean ± SD | 48.33 ± 37.97 | 81.30 ± 86.07 | 0.041 |
| T. Bilirubin (mg/dL) | Mean ± SD | 3.79 ± 4.38 | 4.71 ± 4.66 | 0.195 |
| Direct Bilirubin (mg/dL) | Mean ± SD | 2.57 ± 3.30 | 3.31 ± 4.23 | 0.211 |
| GGT (IU/L) | Mean ± SD | 69.77 ± 77.32 | 101.07 ± 96.08 | 0.119 |
| Alkaline phosphatase (mg/dL) | Mean ± SD | 145.97 ± 103.45 | 254.47 ± 300.05 | 0.011 |
| Albumin (g/dL) | Mean ± SD | 2.97 ± 0.65 | 2.95 ± 0.53 | 0.914 |
| PC (%) | Mean ± SD | 63.13 ± 15.73 | 65.47 ± 17.69 | 0.591 |

Baseline serum GPC3 was significantly higher among HCC group in comparison to cirrhosis group and also for AFP as shown in table 2.

**Table (2):** Comparison between the two studied groups regarding Baseline levels of AFP and Glypican 3

| | | Cirrhosis group No. = 30 | HCC group No. = 30 | P-value |
|---|---|---|---|---|
| AFP Baseline (ng/mL) | Mean ± SD | 26.69 ± 45.26 | 5748.17 ± 15963.37 | <0.001 |
| Glypican-3 Baseline (ng/mL) | Mean ± SD | 0.56 ± 0.31 | 2.28 ± 0.97 | <0.001 |

Among HCC patients, serum GPC3 showed insignificant difference in relation to different CT findings or type of intervention (Table 3). Patients underwent different treatment modalities according to the approved selection criteria, where 8 patients were candidate for RF, 8 patients for TACE and 14 patients were candidates for systemic therapy (Sorafenib, Lenvatinib).

**Table (3):** Comparison between baseline Glypican 3 serum level and other studied parameters among the HCC group

| | | Glypican 3 at baseline (ng/mL) | P-value |
|---|---|---|---|
| | | Mean ± SD | |
| Ascites | No | 2.06 ± 1.09 | 0.425 |
| | Yes | 2.37 ± 0.93 | |
| Number of hepatic focal lesions | Single focal lesion | 2.14 ± 1.11 | 0.519 |
| | Multiple focal lesions | 2.43 ± 0.8 | |
| Portal vein thrombosis | No | 2.26 ± 1.06 | 0.917 |
| | Yes | 2.29 ± 0.88 | |
| Lesion size | < 3 | 2.08 ± 0.48 | 0.715 |
| | (3-5) | 2.2 ± 1.15 | |
| | >5 | 2.46 ± 0.93 | |
| BCLC staging | A | 1.76 ± 0.86 | 0.473 |
| | B | 2.56 ± 0.98 | |
| | C | 2.62 ± 1.06 | |
| Intervention | RF | 1.76 ± 0.86 | 0.339 |
| | TACE | 2.56 ± 0.98 | |
| | Systemic (Sorafenib, Lenvatinib) | 2.41 ± 0.98 | |

One month after different treatment modalities, serum GPC3 levels significantly declined in comparison to its levels before treatment. Also follow up AFP showed the same significant reduction as shown in table 4.

**Table (4):** Comparison between serum Glypican 3 and AFP levels before and one month after intervention among HCC group

| | | HCC group | | P- value |
|---|---|---|---|---|
| | | Before treatment | One month after treatment | |
| **AFP (ng/mL)** | Mean ± SD | 5748.17±15963.37 | 4553.42 ± 14056.05 | <0.001 |
| **Glypican 3 (ng/mL)** | Mean ± SD | 2.28 ± 0.97 | 1.44 ± 0.93 | <0.001 |

On sorting patients according to different offered treatment modalities, there was a significant decline in AFP after RF and after TACE. On the other hand, the decline in AFP after systemic treatment (Sorafenib, Lenvatinib) was insignificant. while regarding tumor size, baseline and one month AFP levels showed insignificant difference except for tumors 3-5 cm in diameter (Table 5 and 6). While, for GPC3, its levels decreased following RF as well as following TACE. On the other hand GPC3 levels were not affected after systemic treatment. Also, its level one month after treatment was significantly higher among those on systemic treatment than those on TACE than those on RF (Table 7)

**Table (5):** Comparison between serum AFP (ng/mL) level before and one month after different treatment modalities among HCC group

| | | Treatment Modality | | | P-value |
|---|---|---|---|---|---|
| | | RF | TACE | Systemic (Sorafenib, Lenvatinib) | |
| | | No. = 8 | No. = 8 | No. = 14 | |
| **AFP before treatment** | Mean ± SD | 543.38 ±501.32 | 3623.50 ± 3791.05 | 9936.44 ± 22852.82 | 0.323 |
| **AFP one month after treatment** | Mean ± SD | 179.38 ±169.01 | 1503.88 ± 2050.75 | 8795.47 ± 20039.53 | 0.295 |
| **Wilcoxon Rank test** | **P-value** | 0.025 | 0.012 | 0.198 | |

**Table (6):** Comparison between AFP levels at baseline and one month after intervention in different sizes of hepatic focal lesions

| | | Lesion size | | | P-value |
|---|---|---|---|---|---|
| | | < 3 | (3-5) | >5 | |
| | | No. = 5 | No. = 14 | No. = 11 | |
| **AFP before treatment** | Mean ± SD | 649.00 ± 531.57 | 7113.66 ± 18038.05 | 6328.09 ± 17318.44 | 0.996 |
| **AFP one month after treatment** | Mean ± SD | 230.20 ± 190.13 | 5017.19 ± 14569.42 | 5928.27 ± 16889.99 | 0.833 |
| **Wilcoxon Rank test** | **P-value** | 0.080 | 0.006 | 0.091 | |

**Table (7):** Comparison between serum Glypican 3 level before and one month after different treatment modalities among HCC group

| | | Treatment Modality | | | P-value |
|---|---|---|---|---|---|
| | | RF | TACE | Systemic (Sorafenib, Lenvatinib) | |
| | | No. = 8 | No. = 8 | No. = 14 | |
| **Glypican-3 before treatment** | Mean ± SD | 1.76 ± 0.86 | 2.56 ± 0.98 | 2.41 ± 0.98 | 0.339 |
| **Glypican-3 one month after treatment** | Mean ± SD | 0.59 ± 0.46 | 1.13 ± 0.78 | 2.10 ± 0.73 | <0.001 |
| **Wilcoxon Rank test** | **P-value** | 0.017 | 0.012 | 0.218 | |

Baseline GPC3 levels showed no significant difference between patients with variable tumor sizes. However, its follow up levels showed significant rise in larger tumor sizes (Table 8).

**Table (8):** Comparison between GLP3 levels at baseline and one month after intervention in different sizes of hepatic focal lesions

| | | Lesion size | | | P-value |
|---|---|---|---|---|---|
| | | < 3 | (3-5) | >5 | |
| | | No. = 5 | No. = 14 | No. = 11 | |
| **Glypican-3 before treatment** | Mean ± SD | 2.08 ± 0.48 | 2.20 ± 1.15 | 2.46 ± 0.93 | 0.715 |
| **Glypican-3 one month After treatment** | Mean ± SD | 0.64 ± 0.43 | 1.33 ± 0.88 | 1.94 ± 0.91 | 0.023 |
| **Wilcoxon Rank test** | **P-value** | 0.042 | 0.006 | 0.040 | |

Correlations between baseline GPC3 level and different demographic, laboratory and sonographic parameters are shown in table 9.

**Table (9):** Correlations between baseline Glypican 3 and other parameters among studied groups

| | Baseline Glypican 3 | | | |
|---|---|---|---|---|
| | Cirrhosis group | | HCC group | |
| | r | P-value | r | P-value |
| AFP at baseline (ng/mL) | -0.108 | 0.571 | 0.245 | 0.192 |
| Age (years) | -0.061 | 0.749 | 0.029 | 0.878 |
| Hemoglobin (gm/dL) | -0.048 | 0.802 | -0.287 | 0.124 |
| TLC ($x10^3$/uL) | -0.101 | 0.597 | -0.126 | 0.508 |
| Platelets ($x10^3$/uL) | -0.105 | 0.582 | 0.012 | 0.948 |
| ALT (IU/L) | 0.148 | 0.436 | 0.102 | 0.592 |
| AST (IU/L) | -0.039 | 0.839 | 0.067 | 0.726 |
| Total Bilirubin (mg/dL) | 0.052 | 0.784 | -0.237 | 0.208 |
| Direct Bilirubin (mg/dL) | 0.114 | 0.547 | -0.249 | 0.185 |
| Alkaline phosphatase (mg/dL) | -0.174 | 0.359 | 0.013 | 0.946 |
| Albumin (g/dL) | -0.040 | 0.832 | -0.129 | 0.496 |
| Prothrombin concentration (%) | -0.003 | 0.989 | 0.003 | 0.989 |
| Creatinine (mg/dl) | -0.308 | 0.098 | -0.054 | 0.777 |
| Liver size (cm) | 0.085 | 0.654 | 0.010 | 0.958 |
| Splenic size (cm) | -0.008 | 0.965 | 0.163 | 0.388 |

On applying ROC curve, the best cutoff value of GPC3 in diagnosis of HCC was > 1.1 ng/mL (Table 10, Figure 1).

**Table (10):** Diagnostic performance of Glypican 3 in detection of HCC

| Variable | Cutoff | AUC | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| **Glypican 3** | >1.1 | 0.959 | 93.33 | 96.67 | 96.6 | 93.5 |
| **AFP** | >100 | 0.864 | 70.00 | 93.33 | 91.3 | 75.7 |

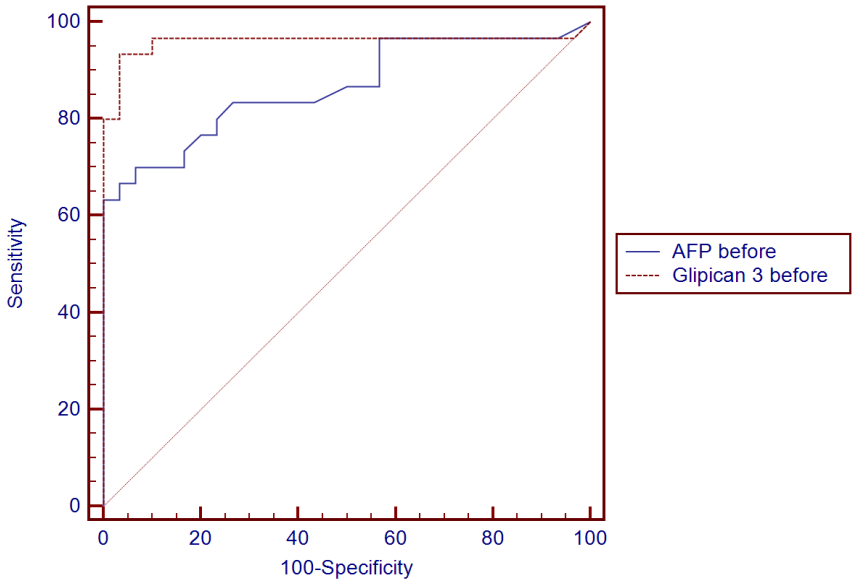


**Figure (1):** ROC curve for baseline Glypican 3 and AFP in prediction of HCC.

## DISCUSSION

HCC is viewed as the fifth most common cause of malignancy-related morbidity. Also, HCC incidence is in alarming rising rate, and it has become a major health problem world-wide [(8)]. In the past few years, screening for HCC in Egypt markedly improved, going in parallel with international recommendations. However, there are insufficient Egyptian reports about HCC survival and recurrence [(3)].

AFP has been widely used for HCC diagnosis and follow-up. However, it is not always elevated to a diagnostic level, particularly in small HCC [(9)]. Also, it may be elevated in patients with chronic HCV without evidence of HCC [(10)]. Therefore, a novel biomarker with superior diagnostic accuracy than AFP is greatly desired [(11)]. Glypican-3 (GPC3) is one of the glypican family of glycosyl-phosphatidylinositol-anchored cell-surface heparan-sulfate proteoglycans. The levels of which are significantly elevated in HCC patients [(5)]. Trials showed that serum GPC3 is an early sensitive and specific biomarker for initial laboratory diagnosis of HCC and for detection of recurrence [(12)].

The aim of this study was to evaluate the role of glypican 3 as a possible diagnostic and prognostic marker of HCC after different treatment modalities.

Our study was conducted on 30 patients with liver cirrhosis and HCC on top, and 30 patients with liver cirrhosis with no evidence of HCC recruited from hepatology outpatient clinics of Ain Shams University Hospitals and Ahmed Maher teaching hospital, during the period From February 2019 to December 2020.

In the current study the mean age in cirrhotic group was (58.20 ± 9.05) and the mean age in HCC group was (58.63 ± 9.40), with male predominance in both groups with percentage of 70% in cirrhosis group and 83.3% in HCC group. These results agree with the study conducted by **Keddeas and Abo-shady** [(13)] who found that the mean age of HCC patients ranged from 40 to 72 years and, in accordance with **El-Zayadi *et al.*** [(14)] who found that HCC was much prevalent in males (85.8%) than females (14.2%). This could be explained by what **Salama and Chen** [(15)] stated that these results are due to more exposure of males to risk factors. Moreover, sex hormones and other x-linked genetic factors may also, be considered.

In the current study we found that AST and ALP were statistically significantly higher in HCC group when compared to cirrhosis group. These results agreed with **Mahmoud and Mahgoub** [(16)] who reported a statistically significant higher ALT, AST and ALP levels in HCC patients than in cirrhotic patients and healthy controls. They also stated that AST levels were more elevated than ALT and attributed that to release of mitochondrial AST that is primarily related to further disease progression. Another study performed by **Hagag *et al.*** [(17)] demonstrated higher serum levels of ALT, AST, TBIL, DBIL, and INR in HCC versus HCV cirrhosis and control groups ($P < 0.001$).

Glypican 3 was found to be significantly higher in HCC patients (2.28± 0.97 ng/ml) in comparison to cirrhosis patients (0.56±0.31 ng/ml). These results are in conformity with **El-Saadany *et al.*** [18] who revealed that GPC3 was markedly elevated in HCC patients than in healthy controls. Another recent study by **Hagag *et al.*** [17] stated that serum GPC3 was significantly elevated in patients with HCC compared to those with liver cirrhosis and both have higher GPC3 levels than healthy controls. Also, **Sun *et al.*** [19] discovered that combining both AFP and GPC3 in HCC screening can have better diagnostic value [20]. On the contrary, **Beale *et al.*** [21] stated that GPC3 has no value at all in screening of HCC in patients with steatohepatitis-related cirrhosis. Also, **Wang *et al.*** [22] **and Nault *et al.*** [23] found that serum GPC3 in patients with cirrhosis regardless its underling etiologies like HCV, HBV and alcoholism is higher than in patients with HCC.

In response to this controversy**, Yang *et al.*** [11] conducted a meta-analysis of 22 studies about the role of GPC3 in HCC detection; 18 studies found that serum GPC3 was a reliable biomarker for detection of HCC, with a sensitivity and specificity when combined with other markers of 69% and 93% respectively. This confliction in results may be attributed to variable patients' characteristics, presence of HCV-related cirrhosis as a single etiology of HCC or simply considering different cutoff values for GPC3 [24].

In the present study, mean values of AFP were significantly higher in HCC patients (5748.17±15963.37 ng/ml) than cirrhosis patients (26.69±45.26 ng/ml). These results go along with **El-Saadany *et al.*** [18] who found that HCC patients had markedly elevated serum AFP when compared to normal controls.

Several studies have reported the ability of AFP in predicting response to therapy and survival outcomes. However, there is no consensus yet regarding the magnitude of the decrease in AFP that defines AFP response [25]. Studies had reported that the change in AFP values at baseline and after treatment better predicts surgical outcomes [26]. **Memon *et al.*** [25] followed 629 HCC patients undergoing TACE and concluded that the AFP decrease could be described as the decline in AFP level more than 50% compared to baseline level and stated that those patients usually had better prognosis.

In the current study we found that AFP levels declined significantly in all patients one month after treatment of HCC, while on sorting patients according to different treatment modalities they were subjected to, there was a significant decrease in AFP after RF (from 543.38±501.32 to 179.38±169.01 ng/ml) and after TACE (from 3623.50±3791.05 to 1503.88±2050.75 ng/ml). On the other hand, the decline in AFP after systemic treatment (Sorafenib, Lenvatinib) was insignificant. This could be attributed to the advanced disease stages in those patients and the need of longer time for their follow up. These results go along with **Toro *et al.*** [27] who stated that AFP decreased significantly in HCC patients who underwent either TACE or RF.

In current study AFP levels at baseline and one month after treatment showed no significant difference regarding tumor size. These results agreed with **Mahmoud and Mahgoub** [16] and **Toro *et al.*** [27] who revealed that no association was found between AFP serum level and tumor size before treatment or even in recurrent tumors. On the contrary **El-Saadany *et al.*** [18] discovered a significant association between AFP and the tumor size.

GPC3 serum level may act as a prognostic marker for HCC. Although, data about its potential as a recurrence predictor is contradictory [28]. However, **Guo *et al.*** [29] found that GPC3 may be useful to predict tumor recurrence, assess survival rates, and help formulate the plan of management.

Regarding GPC3 our study demonstrated that in all patients GPC3 levels declined significantly one month after HCC treatment while on dividing patients according to different treatment modalities they received, GPC3 levels decreased following RF (From 1.76 ± 0.86 to 0.59 ± 0.46 ng/ml) and following TACE (from 2.56 ± 0.98 to 1.13 ± 0.78 ng/ml). On the other hand GPC3 levels were not affected after systemic treatment.

Also, its level one month after treatment was significantly higher among those on systemic treatment (2.10±0.73 ng/ml) than those on TACE (1.13±0.78 ng /ml) than those on RF (0.59±0.46 ng/ml). These results could be attributed to the curative effect of RF in comparison to the palliative effect of both TACE and systemic therapies that don't guarantee HCC cure. These results are in conformity with **Guo *et al.*** [29] who conducted a study on 162 patients with advanced HCC undergoing TACE and stated that serum GPC3 levels after intervention decreased notably than before intervention levels.

Our study revealed that baseline GPC3 levels showed no significant difference between patients with variable tumor sizes. These results are in conformity with **Mahmoud and Mahgoub** [16] who discovered insignificant association between GPC3 or AFP levels and tumor size, Also **Badr *et al.*** [12] and **Zakhary *et al.*** [30] found that serum GPC3 levels were not associated with tumor size.

On the other hand, our study revealed that there was significant association between GLP3 one month after treatment and tumor size. Where GLP3 levels increased with advancement in tumor size being lower in smaller ones. Yet, no previous studies had dealt with the relation between GPC3 after treatment and focal lesion size.

Current study also demonstrated that there was no statistically significant correlation between baseline GPC3 and age, gender, biochemical tests, lesion size and number, ascites, BCLC staging and type of intervention, these results are in conformity with **Jia *et al.*** [31] who found no correlation between serum GPC3 and age, gender, HBV, Child score, number or size of tumors.

Also, **El-Saadany *et al.*** [18] found no relevant correlation between GPC3 expression and the tumor size or Child score. Similarly, **Lee *et al.*** [32] reported a non-significant correlation between serum GPC3 levels and tumor size or tumor stage. This means that, GPC3 expression was not affected by the HCC size suggesting its role as a potential biomarker for the diagnosis of early stage and small sized HCC [24].

In the current study ROC curve showed that the best cutoff value of GPC3 in diagnosis of HCC is > 1.1 ng/mL with sensitivity of 93.33 % and specificity of 96.67%. These findings are in accordance with **Mahmoud and Mahgoub** [16] who revealed that serum GPC3 has sensitivity (93%) and specificity (94 %) in HCC detection and also, with **Ibrahim and Abdel-Raouf** [33] who found the sensitivity and specificity of GPC-3 in HCC diagnosis to be 96.7% and 100% respectively. While for AFP cutoff value of >100 ng/mL had sensitivity of 70% and specificity of 93.33%. These results are in conformity with previous studies conducted by **Zhang *et al.*** [34] who concluded that GPC3 was much more sensitive (91.7%) than AFP (41.7%) and specific (100%) than AFP (80.4%), indicating that GPC3 is more sensitive and specific than AFP in detection of HCC.

These results go along with **Rojas *et al.*** [5] who demonstrated that AFP and GPC3 combination carried higher sensitivity and specificity (98.5% and 97.8%, respectively). Moreover, GPC3 was detected in around one third of confirmed HCC patients with normal serum AFP**.** Where **Omata *et al.*** [35] found that a significant fraction of small HCC (< 3 cm) does not have a detectable AFP level. These results are in conformity with **Xu *et al.*** [20] meta-analysis which pointed that for HCC detection, GPC3 alone had 55% sensitivity and 58% specificity, while on combining GPC3 to AFP, sensitivity became 85% and specificity became 79%. Consequently, serum GPC3 may be complementary to AFP in detection of HCC, and it is obviously stated that GPC3 could be integral to AFP and provides higher sensitivity in HCC detection [17].

Finally, more high-quality research on different subgroups regarding tumor etiology, size and stages together with longer follow up intervals are needed to evaluate the value of GPC3 for the detection of de novo and recurrence of HCC**.**

## CONCLUSIONS

Glypican-3 can be a valuable diagnostic marker for HCC diagnosis and prognosis after various treatment modalities and may be complementary to alpha fetoprotein increasing overall sensitivity of HCC detection.

## ACKNOWLEDGMENTS

The authors are grateful to the staff members of Ain Shams University's Clinical Pathology, Interventional Radiology, and Gastroenterology and Hepatology Departments.

**Financial support and sponsorship:** Nil.
**Conflict of interest:** Nil.

## REFERENCES

1. **El Shazly Y, Abd Elmoghny M, Al swaff R *et al.* (2018):** Assessment of serum retinol binding protein 4 in patients with hepatocellular carcinoma. Journal of Clinical and Diagnostic Research, 12(10): 27-31.
2. **Mohamed I, Rasmy H, Aly W (2018):** Evaluation of serum resistin levels in patients with hepatocellular carcinoma before and after treatment. Egyptian Liver Journal, 8(2):61-67.
3. **Rashed W, Kandeil M, Mahmoud M *et al.* (2020):** Hepatocellular carcinoma (HCC) in Egypt: A comprehensive overview. J Egypt Natl Canc Inst., 32: 5-9.
4. **Zhang Z, Meng H, Wang N (2014):** Serum microRNA 143 and microRNA 215 as potential biomarkers for the diagnosis of chronic hepatitis and hepatocellular carcinoma. Diagn Patho., 9: 135-139.
5. **Rojas A, Sánchez-Torrijos Y, Gil-Gómez A *et al.* (2018):** Performance of different biomarkers for the management of hepatocellular carcinoma. Hepatoma Res., 4: 31-34.
6. **Yu J, Xu X, Ma R *et al.* (2015):** Development of a clinical chemiluminescent immunoassay for serum GPC3 and simultaneous measurements alone with AFP and CK19 in diagnosis of hepatocellular carcinoma. Journal of Clinical Laboratory Analysis, 29: 85-93.
7. **Bruix J, Sherman M (2011):** Management of hepatocellular carcinoma: an update. Hepatology, 53(3):1020-1022.
8. **Kimhofer T, Fye H, Taylor-Robinson S (2015):** Proteomic and metabonomic biomarkers for hepatocellular carcinoma: a comprehensive review. British Journal of Cancer, 112: 1141-1156.
9. **Stefaniuk P, Cianciara J, Wiercinska-Drapalo A (2010):** Present and future possibilities for early diagnosis of hepatocellular carcinoma. World Journal of Gastroenterology, 16(4):418-424.
10. **Yoshida K (2015):** New biomarkers for early detection of hepatocellular carcinoma. EBio Medicine, 5: 370-371.
11. **Yang S, Fang X, Huang Z *et al.* (2014):** Can serum glypican-3 be a biomarker for effective diagnosis of

hepatocellular carcinoma? A meta-analysis of the literature. Dis Markers, 14:127831.

12. **Badr E, Korah T, Ghani A *et al.* (2014):** Role of serum glypican-3 in the diagnosis and differentiation of small hepatocellular carcinoma from hepatitis-C virus cirrhosis. Alexandria Journal of Medicine, 50: 221-226.
13. **Keddeas M, Abo-shady R (2011):** Evaluation of plasma osteopontin level as a biomarker for hepatocellular carcinoma in Egyptian patients. Egyptian Liver Journal, 1: 38-42.
14. **El-Zayadi A, Badran H, Barakat E *et al.* (2005):** Hepatocellular carcinoma in Egypt: a single center study over a decade. World Journal of Gastroenterology, 11(33): 5193-5198.
15. **Salama H, Chen Y (2010):** Gender disparity of hepatocellular carcinoma: the roles of sex hormones. Oncology, 78: 172-179.
16. **Mahmoud S, Mahgoub E (2020):** Role of serum Glypican 3 as a predictive tool for early diagnosis of hepatocellular carcinoma (HCC). Al-Azhar International Medical Journal, 1(1): 21-30.
17. **Hagag N, Ali Y, Elsharawy A *et al.* (2020):** Clinical impact of circulated miR-1291 in plasma of patients with liver cirrhosis (LC) and hepatocellular carcinoma (HCC): Implication on Glypican-3 Expression. J Gastrointest Canc., 51: 234-241.
18. **El-Saadany S, El-Demerdash T, Helmy A *et al.* (2018):** Diagnostic value of Glypican-3 for hepatocellular carcinomas. Asian Pac J Cancer Prev., 19(3): 811-817.
19. **Sun B, Huang Z, Wang B *et al.* (2017):** Significance of Glypican-3 (GPC3) expression in hepatocellular cancer diagnosis. Medical science monitor. International Medical Journal of Experimental and Clinical Research, 23: 850-855.
20. **Xu D, Su C, Sun L *et al.* (2019):** Performance of serum Glypican3 in diagnosis of hepatocellular carcinoma: A meta-analysis. Annals of Hepatology, 18(1): 58-67.
21. **Beale G, Chattopadhyay D, Gray J *et al.* (2008):** AFP, PIVKA II, GP3, SCCA-1 and follisatin as surveillance biomarkers for hepatocellular cancer in non-alcoholic and alcoholic fatty liver disease. BMC Cancer, 8: 200-204.
22. **Wang Y, Yang H, Xu H *et al.* (2014):** Golgi protein 73, not Glypican- 3, may be a tumor marker complementary to α- fetoprotein for hepatocellular carcinoma diagnosis. Journal of Gastroenterology and Hepatology, 29: 597-602.
23. **Nault J, Guyot E, Laguillier C *et al.* (2013):** Serum proteoglycans as prognostic biomarkers of hepatocellular carcinoma in patients with alcoholic cirrhosis. Cancer Epidemiology and Prevention Biomarkers, 22(8):1343-1352.
24. **Tsuchiya N, Sawada Y, Endo I *et al.* (2015):** Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol., 21: 10573-10583.
25. **Memon K, Kulik L, Lewandowski R *et al.* (2012):** Alpha–fetoprotein response correlates with EASL response and survival in solitary hepatocellular carcinoma treated with transarterial therapies: A subgroup analysis. J Hepatol., 56(5):1112-1120.
26. **Rungsakulkij N, Suragul W, Mingphruedhi S *et al.* (2018):** Prognostic role of alpha-fetoprotein response after hepatocellular carcinoma resection. World J Clin Cases, 6(6): 110-120.
27. **Toro A, Ardiri A, Mannino M *et al.* (2014):** Effect of pre- and post-treatment α-fetoprotein levels and tumor size on survival of patients with hepatocellular carcinoma treated by resection, trans-arterial chemoembolization or radiofrequency ablation: a retrospective study. BMC Surg., 14: 40-45.
28. **Xiao W, Qi C, Chen D *et al.* (2014):** Prognostic significance of glypican-3 in hepatocellular carcinoma: a meta-analysis. BMC Cancer, 14:104-108.
29. **Guo Z, Wang J, Li L *et al.* (2020):** Value of miR-1271 and glypican-3 in evaluating the prognosis of patients with hepatocellular carcinoma after transcatheter arterial chemoembolization. World J Clin Cases, 8(16):3493-3502.
30. **Zakhary N, Mohamed M, Khorshid O *et al.* (2012):** Role of glypican-3 in the early diagnosis of hepatocellular carcinoma among Egyptian patients. J Genetic Engineer Biotechnol., 10: 73-79.
31. **Jia X, Gao Y, Zhai D *et al.* (2016):** Assessment of the clinical utility of Glypican 3 as a serum marker for the diagnosis of hepatocellular carcinoma. Technol Cancer Res Treat., 15(6):780-786.
32. **Lee H, Yeon J, Suh S *et al.* (2014):** Clinical utility of plasma glypican-3 and osteopontin as biomarkers of hepatocellular carcinoma. Gut Liver, 8: 177-185.
33. **Ibrahim T, Abdel-Raouf S (2015):** Immunohistochemical study of Glypican-3 and HepPar-1 in differentiating hepatocellular carcinoma from metastatic carcinomas in FNA of the liver. Pathol Oncol Res., 21: 379-387.
34. **Zhang Q, Xiao Q, Lin Z *et al.* (2010):** Development of a competitive radioimmunoassay for glypican-3 and the clinical application in diagnosis of hepatocellular carcinoma. Clin Biochem., 43: 1003-1008.
35. **Omata M, Cheng A, Kokudo N *et al.* (2017):** Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int., 11: 317-370.