# Serum Golgi protein 73 and glypican-3: early diagnostic biomarkers for hepatocellular carcinoma

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**Objectives**
The prognosis of hepatocellular carcinoma (HCC) is extremely worse, and chronic hepatitis C virus infection is one of the most important causes of HCC. The use of diagnostic serological markers in following up the high-risk individuals for developing HCC may help in its early detection and therapy. This study aimed to assess the effectiveness of serum Golgi protein 73 (GP73) and glypican-3 (GPC3) as new tumor biomarkers for early detection of HCC.
**Patients and methods**
A case–control study included 125 patients infected with hepatitis C virus, comprising 60 with cirrhosis and 65 with HCC, in addition to 60 healthy individuals considered as the normal control group. Serum levels of GP73, GPC3, and alpha-fetoprotein (AFP) were assessed by enzyme-linked immunosorbent assay technique.
**Result**
Serum GP73 and AFP levels were significantly higher in patients with HCC as compared with cirrhosis and control groups; however, their levels were significantly increased in patients with cirrhosis as compared with the healthy group (both $P<0.001$). However, GP73 was more sensitive than AFP in the diagnosis of HCC as the area under the receiver operating characteristic curve (area under the curve) with 95% confidence interval was 0.98 (0.95–1.0) for GP73 and 0.82 (0.74–0.90) for AFP. However, the area under the curve with 95% confidence interval for GPC3 was 0.44 (0.36–0.53). There was a significant association between AFP level and Barcelona clinic liver cancer staging of HCC ($P=0.02$). In addition, the serum level of GPC3 was higher in cirrhotic patients than other groups ($P=0.007$).
**Conclusion**
Serum GP73 and not GPC3 can be used as early potential tumor biomarker for HCC diagnosis and also to differentiate HCC from cirrhosis.

**Keywords:**
alpha-fetoprotein, cirrhosis, glypican-3, Golgi protein 73, hepatocellular carcinoma, hepatitis C virus

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## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common reason for cancer-induced mortality in the world [1]. It was noticed that hepatitis C virus (HCV) infection presents in ~40–80% of patients with HCC [2]. HCC is characterized by high mortality with decreased 5-year survival rate as most of the cases are diagnosed in advanced stage, a problem making therapy ineffective [3]. Early diagnosis of HCC makes effective treatment by either surgical resection of the tumor or liver transplantation [4]. Early detection and intervention of HCC improve its prognosis; hence, the use of early detection serological markers in high susceptible patients decreases HCC mortality [4]. The routine investigations used for early discovery of HCC were serum alpha-fetoprotein (AFP) level and abdominal ultrasound. During the past four decades, AFP was the only used marker for HCC with 80–94% specificity and 41â 65% sensitivity [5].

Many HCC cases were diagnosed without increased AFP level. However, other cases showed elevation only when tumor size is more than 3–5 cm [6]. This controversy makes AFP unsuitable marker for HCC. Therefore, the identification of highly sensitive and more specific marker than AFP is urgently needed for early detection of HCC [7,8]. Many noninvasive serum

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markers such as Golgi protein 73 (GP73) and glypican-3 (GPC3) have been specified for HCC [9].

GP73, named additionally as Golgi phosphoprotein 2, is a 73-kDa protein composed of 400 amino acids. Type II Golgi membrane protein contains a transmembrane domain, a short N-terminal cytoplasmic domain, and a larger luminal C-terminal domain [10]. The C-terminal ectodomain is the main functional domain of GP73 and secreted and can be detected in the blood [11]. The hepatic expression of GP73 is very low in normal adults, and it is mainly produced by the biliary cells not hepatic cells [12]. The hepatic expression of GP73 is highly upregulated in patients with liver diseases [13]. HCV infection causes an increase in the GP73 level, which in turn upregulates apoprotein E, which enhances and stimulates HCV secretion [14]. The hepatic expression of GP73 is gradually increased from healthy liver and hepatitis progressing to liver cancer [15].

GPC3 is a cell-surface 60-kDa protein, related to heparin-sulfate proteoglycans family [16], which contains GPC1 to GPC6. GPC3 is cut at Arg358 and Ser359 to produce amino (N) terminal 40-kDa protein and carboxyl (C) terminal 30-kDa membrane-bound protein. The N-terminal protein of GPC3, named also soluble GPC3, is released in the serum and can be used as a marker in diagnosis of patients with HCC [17]. GPC3 has an integral role in cell proliferation and tumor suppression [18]. GPC3 is expressed normally in fetal hepatic tissues and placenta with very minimal expression in hepatic tissues of adults [16]. Patients with HCC had high level of serum GPC3 in comparison with healthy individuals and chronic hepatitis patients, a result making serum GPC3 a possible specific biomarker for HCC [19]. The aim of the current study was to assess the efficiency of GP73 and GPC3 as tumor biomarkers for HCC detection.

## Patients and methods

A case–control study was conducted on 125 patients infected with HCV, comprising 60 patients with cirrhosis and 65 patients with HCC. Patients were recruited from specialized medical hospital, Mansoura University from February 2018 to February 2019. The AQ6 patients had positiveHCV antibodies (Access Bio-RadCo., France,3 BOULEVARD RAYMOND POINCARE 92430) and were confirmed by quantitative viral RNA detection using Taq Man HCV quantitative test version 2.0 (Roche Molecular Diagnostic, Branchburg, New Jersey, USA) according to guidance of manufacturer. Exclusion criteria included individuals with HBV and with other cancers. All patients were diagnosed by clinical, laboratory, and radiological examinations including abdominal ultrasound and computed tomography. Patients were further classified according to histopathological grading, tumor size, Child–Pugh class system, and Barcelona clinic liver cancer (BCLC) staging system [20]. In addition to the patients, the study included 60 healthy individuals as a control group. The research was approved by Mansoura University ethical committee (Code: R.19.05.498), and approval consent was obtained from each participant.

Ten milliliters of blood was withdrawn from each participant and divided into two parts; the first part was collected into citrated tube for complete blood count and the third part into plain tube, and sera were separated and subjected to the analysis for complete liver functions and the determination of AFP by enzyme-linked immunosorbent assay (Catalog #: EIA-1468; ELISA-DRG International Inc., USA). The DRG AFP ELISA kit is a solid-phase ELISA based on the sandwich principle. Moreover, sera were subjected to the determination of GP73 and GPC3 by ELISA technique.

### Serum Golgi protein 73 by sandwich-based enzyme-linked immunosorbent assay method

Serum GP73 level was assayed by sandwich-based ELISA method (RayBiotech GP73 Quantitative Detection Kit, Catalog #: ELISA-GP73-96; RayBiotech, Norcross, Georgia, USA) according to instructions of the manufacturer. A specific anti-human GP73 monoclonal antibody is immobilized on the surface of a 96-well plate. Standards and samples were added into the wells of the plate. After incubation, a secondary detection antibody was added. A second incubation was done, then an enzymatic conjugate was added that can bind antibody bound to the plate to create a complex of solid-phase antibody–antigen–antibody. After addition of a coloring substrate, this enzyme reacts to produce a color relative to GP73 concentration in the sample. Finally, the OD of the wells at 450 nm was read on a spectrophotometric plate reader. The concentration was expressed as ng/ml.

### Glypican-3 by quantitative sandwich enzyme immunoassay

This assay employs the quantitative sandwich enzyme immunoassay technique (Catalog Number DGLY30; RD-Quantikine 614 McKinley Place NE, Minneapolis, Minnesota, USA) according to the manufacturer's

instructions. Human GPC3-specific monoclonal antibody had been precoated onto the surface of a 96-well microplate. Samples as well as standards were added to the wells, and any GPC3 substrate was bound to the fixed antibody. Washing of any free substrates was done, and then human GPC3-specific enzyme-linked polyclonal antibody was added to the wells. Washing was done again to remove any free antibody enzyme reagent, and then substrate was added to the wells to give color, which is directly proportional to GPC3 concentration. The color development was stopped, and the intensity of the color was measured. Finally, the OD of the wells at 450 nm was read on a spectrophotometric plate reader. A total of 27 assays were evaluated and the minimum detectable dose of human GPC3 ranged from 3.07 to 20.6 pg/ml. The mean minimum detectable dose was 9.18 pg/ml.

### Statistical analysis

Data were analyzed using (SPSSsoftware,version22.0. Chicago, IL, USA). Quantitative nonparametric data were analyzed by Kruskal–Wallis test to compare more than two independent groups. Quantitative parametric data were assessed by one-way analysis of variance test to compare more than two independent groups. Receiver operating characteristic (ROC) curve analysis was test to discriminate diseased cases from nondiseased cases. Significance of the obtained results was judged at the (0.05) level.

## Results

Our study found that HCC occurred at a relatively younger age in comparison with the cirrhotic group (50±5.2 and 55.6±9.4, respectively) and more in males than females as compared with other groups. There were significant elevations of alanine transferase, aspartate transferase, and total bilirubin; however, there was a significant decrease in white blood cells and platelets levels in HCC group as compared with other groups. Regarding tumor markers, there was a significant elevation of AFP and GP73 in HCC group in comparison with the other groups. Moreover, there was a significant elevation of AFP and GP73 in cirrhosis group in comparison with the controls. However, there is notable elevation of GPC3 in cirrhotic group in comparison with the other groups. The cancer grading in the HCC group revealed that most of patients with HCC were grade 2 (38%) and BCLC stage A (35%), tumor size more than 5 cm (43%) and most of them were Child–Pugh classes B and C (42 and 46%). Data are shown in Table 1.

Table 2 shows the relationship between the levels of tumor markers and the tumor grading among HCC group. There was no association between tumor markers and any of the tumor grade among HCC group, except that there was a significant association between AFP level and BCLC staging ($P$=0.01). In addition, GPC3 was increased in Child–Pugh class C than other groups ($P$=0.009).

ROC curve of the efficiency of serum AFP, GP73, and GPC3 in the diagnosis of HCC from the whole study population showed that GP73 was more sensitive and specific than other tumor markers in the diagnosis of HCC, as shown in Fig. 1. The area under the receiver operating characteristic (AUROC) with 95% confidence interval (CI) for GP73 was 0.98 (0.95–1.0), with 98% sensitivity and 98% specificity at an optimal cutoff value of 265 ng/ml. The AUROC for AFP with 95% CI was 0.82 (0.74–0.90), with 76% sensitivity and 87% specificity at a cutoff of 9.4 ng/ml. The AUROC with 95% CI for GPC3 was 0.45 (0.36–0.53), with 63% sensitivity and 34% specificity at a cutoff value of 5.8 pg/ml.

ROC curve of the efficiency of serum AFP, GP73, and GPC3 in diagnosis of HCC from cirrhosis group revealed that GP73 has higher sensitivity and specificity than AFP, as shown in Fig. 2. The AUROC with 95% CI for GP73 was 0.96 (0.91–1.0), with 97% sensitivity and 95% specificity at an optimal cutoff value of 273.5 ng/ml. The AUROC with 95% CI for AFP was 0.80 (0.71–0.88), with sensitivity of 73% and specificity of 84% at a cutoff value of 10.3 ng/ml. The AUROC with 95% CI for GPC3 was 0.41 (0.31–0.52), with 44% sensitivity and 47%specificity at a cut-off value of 6.9 pg/ml.

## Discussion

Chronic HCV infection is considered a fundamental cause of HCC, which is the second most prevalent cause of cancer mortality [21]. It was reported that GP73 was first discovered in biliary cells in patients with acute hepatitis, cirrhosis, and HCC [22]. GP73 is considered a possible biomarker for early HCC diagnosis as it can differentiate HCC cases from cirrhosis [23]. Our findings demonstrated that the serum level of GP73 in HCC group was higher (288±7.8 ng/ml) than those in cirrhosis (230 ±27.8 ng/ml) and normal control group (135±9.7 ng/ml), a result explained by the secretion of GP73 protein by hepatoma cells into the circulation in first stage, resulting in the significantly elevated serum GP73 in

**Table 1 Demographic and laboratory data in studied groups**

| | HCC group (*N*=65) | Cirrhosis group (*N*=60) | Control group (*N*=60) | Test of significance |
|---|---|---|---|---|
| Age (years) | 50±5.2 | 55.6±9.4 | 50.0±4.9 | *P*=0.007 |
| Sex: M/F | 48 /17 | 34/26 | 28 /32 | *P*=0.006 |
| Albumin (g/dl) | 3.70±0.51 | 3.9±0.5 | 3.9±0.5 | *P*=0.6 |
| Bilirubin (mg/dl) | 1.2 (0.7–2.6) | 0.7 (0.5–0.9) | 0.8 (0.7–0.9) | *P*=0.001 |
| ALT (U/l) | 46 (37–75) | 38 (25–71) | 28 (25–33) | *P*=0.001 |
| AST (U/l) | 61 (47–96) | 41 (26–77) | 28 (25–32) | *P*=0.001 |
| WBCs ($\times10^3$/cmm) | 5.81 (1.43–27.2) | 6.75 (3.0–52.0) | 7.2 (4.2–13.5) | *P*=0.04 |
| HB (g/dl) | 13.05±2.54 | 12.89±1.77 | 13.11±1.79 | *P*=0.86 |
| PLT ($\times10^3$/cmm) | 155.39±84.44 | 177.68±66.89 | 234.68±88.17 | *P*<0.001 |
| AFP (ng/ml) | 80 (26–285) | 7.0 (5–10) | 6.0 (4–8) | *P*=0.001 |
| GP73 (ng/ml) | 287.0±9.6 | 230.5±27.8 | 136±9.7 | *P*=0.001 |
| GPC3 (pg/ml) | 6.8±2.4 | 8.3±4.2 | 6.8±2.1 | *P*=0.007 |
| BCLC staging | | | | |
| A | 23 (35) | NA | NA | |
| B | 19 (29) | | | |
| C | 14 (22) | | | |
| D | 9 () | | | |
| Child–Pugh | | | | |
| A | 8 (22) | 6 (10) | | *P*=0.55 |
| B | 27 (42) | 29 (48) | NA | |
| C | 30 (46) | 25 (42) | | |
| Tumor grade | | | | |
| 1 | 16 (25) | NA | NA | |
| 2 | 25 (38) | | | |
| 3 | 15 (23) | | | |
| 4 | 9 (14) | | | |
| Tumor size (cm) | | | | |
| <2 | 20 (31) | NA | NA | |
| 2–5 | 17 (26) | | | |
| >5 | 28 (43) | | | |

Data are represented in the form of median (lower-upper quartiles), mean±SEM, *n* (%). AFP, alpha-fetoprotein; ALT, alanine transferase; AST, aspartate transferase; BCLC, Barcelona clinic liver cancer staging; F, female; GP73, Golgi protein 73; GPC3, glypican-3; HB, hemoglobin; HCC, hepatocellular carcinoma; M, male; NA, not applicable; PLT, platelets; WBC, white blood cell.

HCC [24]. Results were in agreement with Anand *et al*. [25], who found that increased hepatic expression of GP73 in chronic liver disease, including hepatitis, cirrhosis, and HCC.

Comparing GP73 and AFP as tumor markers in diagnosis of HCC and in differentiating HCC from cirrhosis, our study has shown that GP73 is a good specific marker than AFP in HCC diagnosis, as AUROC for GP73 was 0.98 whereas for AFP was 0.82. This result is in agreement with Hu *et al*. [26], Tian *et al*. [27], and Mariam *et al*. [28] who reported that serum level of GP73 is highly increased in patients with HCC as compared with either patients with nonmalignant liver diseases or the healthy individuals, and has a good diagnostic value than AFP for diagnosis of HCC, but not in agreement with Ozkan *et al*. [29], who reported that GP73 has a low value for the diagnosis and prognosis of early HCC, whereas AFP was better. These differences might be owing to different population characteristics, including the causes of cancer, ethnicity, income level, and detection methods used. However, the use of GP73 to replace AFP or the combination of both parameters in HCC detection should be further evaluated. Previous studies examined the relation between both GP73 and AFP markers and severity of HCC, but results are still controversial. Our study demonstrated that there is no association between tumor markers and any of the tumor characteristics among HCC group, except that, there was a significant association between AFP level and BCLC staging. Hu *et al*. [26] showed that GP73 had a good ability to differentiate cirrhosis from stages I and II PHC. In addition, serum GP73 and AFP levels were significantly higher in patients with stages III and IV cancer compared with stages I and II [30].

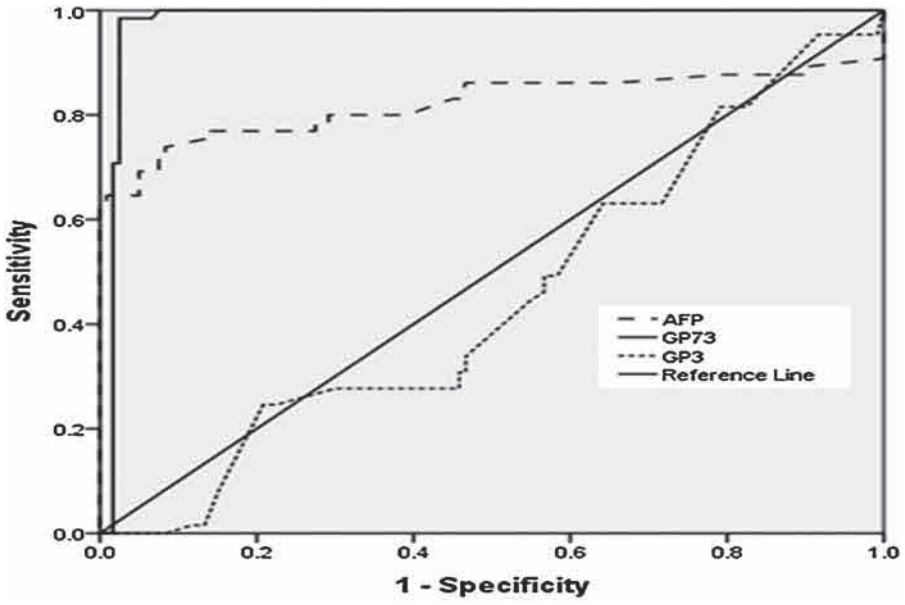
GPC3, one of glypican family, is a second protein marker that inhibits cell proliferation and causes apoptosis and is found to be variable in different types of cancers [18]. Previous studies have proposed

**Table 2 Serum levels of alpha-fetoprotein, Golgi protein 73, and glypican-3 in different hepatocellular carcinoma grades**

| Tumor characteristics | AFP (ng/ml) | GP73 (ng/ml) | GPC3 (pg/ml) |
|---|---|---|---|
| BCLC stage | | | |
| Grade A (23) | 13.8 (7–1175) | 288±6.7 | 7.5±2.6 |
| Grade B (19) | 25 (6.4–100) | 290±9.1 | 6.2±27 |
| Grade C (14) | 736 (89–2000) | 289±9.5 | 6.3±1.7 |
| Grade D (9) | 210 (80–230) | 288±18 | 6.5±1.4 |
| *P* value | 0.01 | 0.83 | 0.2 |
| Child–Pugh class | | | |
| Class A (10) | 107(80–201) | 291±18 | 7.1±0.31 |
| Class B (27) | 24 (8.8–246) | 286±7.8 | 5.7±2.1 |
| Class C (28) | 80 (7–443) | 291±7.6 | 7.6±2.6 |
| *P* value | 0.7 | 0.2 | **0.009** |
| Tumor grade | | | |
| Grade 1 (16) | 31 (8.6–100) | 286±14 | 7.1±2.8 |
| Grade 2 (25) | 112 (7.2–1032) | 290±7.6 | 7.1±2.4 |
| Grade 3 (15) | 177 (20–2000) | 289±7.6 | 5.4±1.8 |
| Grade 4 (9) | 25 (7–230) | 291±7.9 | 7.6±2.3 |
| *P* value | 0.6 | 0.9 | 0.1 |
| Tumor size (cm) | | | |
| <2 (20) | 59 (13–188) | 286±18.0 | 7.5±2.7 |
| 2–5 (17) | 115 (47–238) | 286±8.0 | 5.8±1.4 |
| >5 (28) | 24 (6.5–2000) | 291±7.6 | 6.8±2.6 |
| *P* value | 0.6 | 0.9 | 0.16 |

Data are represented in the form of median (lower-upper quartiles), mean±SEM. AFP, alpha fetoprotein; BCLC, Barcelona clinic liver cancer staging; GP73, Golgi protein 73; GPC3, glypican-3.

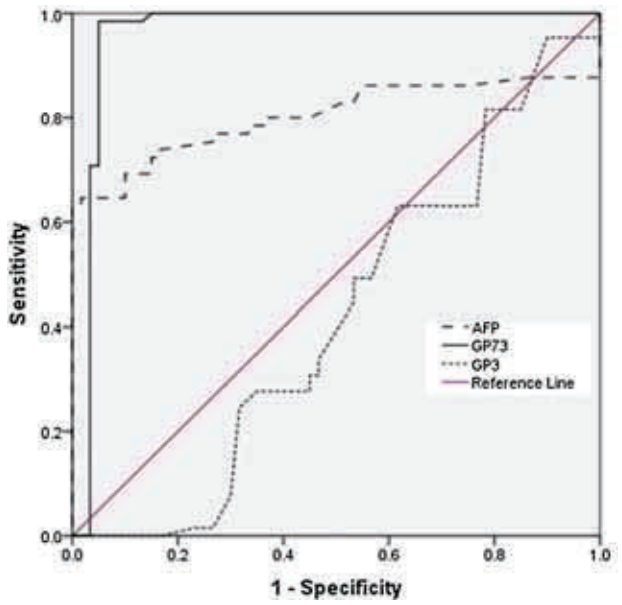
**Figure 1**




Receiver operating characteristic curve of the efficiency of serum AFP, GP73, and GPC3 in diagnosis of HCC from the whole study population. AFP, alpha-fetoprotein; GP73, Golgi protein 73; GPC3, glypican-3; HCC, hepatocellular carcinoma.

that serum GPC3 is a specific marker for HCC [19]. Our results revealed that there was a significant elevation of GPC3 in cirrhotic group in comparison with HCC and normal control groups (8.43, 6.75, and 6.98 ng/ml, respectively), and there was no differences between HCC and normal group. In addition, the AUROC for GPC3 was 0.43 (95% CI, 0.33–0.52), with a sensitivity of 58% and a specificity of 34% at a cut-off value of 5.8 ng/ml, indicating that GPC3 has no role in HCC diagnosis. Some studies have found lower or equivalent serum levels of GPC3 in patients with HCC compared with patients with liver cirrhosis [29,31]. Another study showed that serum GPC3 is elevated in patients with HCC as compared with

**Figure 2**




Receiver operating characteristic curve of the efficiency of serum AFP, GP73, and GPC3 in differentiating HCC from cirrhosis. AFP, alpha-fetoprotein; GP73, Golgi protein 73; GPC3, glypican-3; HCC, hepatocellular carcinoma.

healthy individuals and hepatitis or liver cirrhotic patients [32]. Liu *et al*. [33] showed that GPC3 has higher diagnostic value in patients with HCC and liver cirrhosis, with sensitivity of 56% and specificity of 89%, and the AUROC curve was 0.88, indicating that GPC3 has a good diagnostic value. The differences in the results owing to different characteristics of HCC with many underlying risk factors make serum GPC3 a possible suitable marker for HCC in one population but not in the other.

## Conclusion

In conclusion, our study strongly observed that GP73, not GPC3, is a potential tumor biomarker for HCC. The GP73 is superior to AFP in HCC diagnosis, and to establish the use of GP73 as a tumor marker for HCC diagnosis, future studies on a large sample should be done.

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### Conflicts of interest

Authors declare no conflicts of interest

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