Golgi Protein 73 (GP73) as a Novel Serum Marker for Early Detection of Hepatocellular Carcinoma in Egyptian Patients

Mona A El Shafie[1], Amal M Fawzy[2], Elhamy Abd Al Monem[3], Sherif Abbass[4], Doaa M Zakaria[5], Sherif El Baz[6]

[1]Departments of Clinical Pathology, Menoufyia Faculty of Medicine, Egypt
[2]Clinical Pathology, National Cancer Institute- Cairo University, Egypt
3Clinical Pathology, National Liver Institute-Menoufyia University, Egypt
[4]Hepatology, National Liver Institute-Menoufyia University
[5]Internal Medicine -Al Azhar Faculty of Medicine for Girls, Egypt
amalfawzy69@hotmail.com

Abstract: Background: Serum Golgi protein 73 (sGP73) is a novel and promising biomarker for detection of hepatocellular carcinoma (HCC). However, there are few reports on the predictive values levels of GP73 in diagnosis of liver cirrhosis (LC), HCC and the relationship of this level to clinicopathologic features of patients. **Methods:** This study included 66 patients, 31 of them were proved HCC and 35 patients have LC, additionally, 20 apparently healthy subjects were included as a control group. Clinical examination, abdominal ultrasonogrphy, Triphasic C.T to patients with focal lesion. Liver function tests, complete blood cell count and serum AFP were measured. Des-γ-carboxyprothrombin (DCP) and Golgi Protein 73 (GP73) were determined by an ELISA technique. Correlations with clinical parameters were done. **Results:** The serum levels of AFP, DCP and GP73 were significantly elevated in LC and more elevated in HCC cases as compared to controls. The sensitivity and specificity of GP73 for HCC were superior to those of AFP and DCP especially in early detection of HCC, GP73 had a sensitivity of 87% and a specificity of 95% at the optimal cut-off value of 7.62 ng/ml. The area under the receiver-operating characteristic curve (AUROC) was 0.87. DCP give a sensitivity of 80.6% and specificity of 85% at a cut-off 32.64 ng/ml, while, AFP had a sensitivity of 77.4% and a specificity of 60% at a cut-off 28.51 ng/ml. However, when GP73 used in combination with AFP, they lead to an enhanced the sensitivity of HCC detection up to 90.3% and the area under receiver-operating characteristic curve (AUROC) was 0.83. A significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and child score) and more aggressive tumor characters (tumor size and vascular invasion).
Conclusion: the serum level of GP73 may be implicated in development of LC and disease progression to HCC. In combination with AFP, it had an overall performance that was better than AFP alone in early detection of HCC. Future study was needed to be confirmed in larger cohorts of patients to determine if these markers are true indicators of early HCC.

[Mona A El Shafie, Amal M Fawzy, Elhamy Abd Al Monem, Sherif Abbass, Doaa M Zakaria, Sherif El Baz. Golgi Protein 73 (GP73) as a Novel Serum Marker for Early Detection of Hepatocellular Carcinoma in Egyptian Patients. Life Sci J 2012; 9(2):823-830]. (ISSN: 1097-8135). http://www.lifesciencesite.com.122

Key words: HCC, Golgi protein 73, DCP, AFP, ELISA.

List of abbreviations:
sGP73: Serum Golgi protein 73, DCP: Des-γ-carboxyprothrombin, AFP: α-fetoprotein.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer type, and is the third leading cause of cancer mortality worldwide **[1,2].** Recent reports show that HCC is becoming more wide-spread and has dramatically increased in North America Western Europe and Japan **[3,4**].Additionally, there is an increasing incidence of the disease among younger age groups that warrants Further investigations **[5, 6**].

The progression of liver disease into liver cancer is primarily monitored by serum levels of the oncofetal glycoprotein, α-fetoprotein (AFP), or the core fucosylated glycoform of AFP (AFP- L3). However, AFP can be produced under many circumstances, including other liver diseases **[7],** and is not present in all those with HCC. Therefore, the use of AFP as a primary screen for HCC has been questioned **[8**] and more sensitive serum biomarkers for HCC are desired.

Des-γ-carboxyprothrombin (DCP), also known as PIVKA-II (protein induced by vitamin K absence or antagonist), is an abnormal, inactive prothrombin, lacking carboxylation of the 10 glutamic acid residues in the N-terminus, which is the result of an acquired post-translational defect of the prothrombin precursor in HCC cell lines. DCP was discovered in serum of patients during their anticoagulant therapy with a vitamin K antagonist. In **1984 Liebman et al. [9]** first described a higher DCP level both in patients with HCC and in cases of HCC recurrence after surgical resection, suggesting the usefulness of DCP as an HCC biomarker **[9]**. It has been proved that significant concentrations of serum DCP are present in 50%-60% of all HCC patients, but in only 15%-30% of early HCC case **[10**]. HCC is usually

diagnosed at an advanced stage resulting in limited therapeutic options and poor prognosis. The identification of prognostic biomarkers is an important issue since such markers could facilitate detection of HCC. Furthermore, such biomarkers could display potential therapeutic targets for HCC **[2]**.

In the search for serum markers of hepatocellular cancer, several investigators have recently focused on Golgi protein 73 (GP73); also known as Golgi membrane protein 1 (Golm1) or Golph2]. GP73 is a 400 amino acid, 73 kDa transmembrane glycoprotein that normally resides within the cis- Golgi complex. Its mRNA was first identified in a search for upregulated hepatic genes in a patient with syncytial giant cell hepatitis **[11]**. Subsequent studies revealed minimal GP73 expression in normal hepatocytes but marked expression in patients with acute and chronic hepatitis and liver cirrhosis **[11,12]**, regardless of the specific disease aetiology. Resolution of hepatitis is paralleled by a reduction and normalization of GP73 expression, indicating that GP73 may be triggered by the hepatic injury response **[13,14]** .In addition to hepatocytes, GP73 was consistently expressed by normal biliary epithelial cells as well as hepatic stellate cells in injured livers **[12]**. Further studies demonstrated constitutive expression in cells of the epithelial lineage, especially in the prostate, gut, breast, and thyroid, and within the central nervous system **[11]**. A circulating form of GP73 is found in the serum of patients with hepatocellular cancer (HCC**) [15].**These data indicate that serum GP73 is a promising diagnostic serum marker for liver cancer **[15,16,17].**

Aim:

The present study aimed to evaluate the serum level of GP73 as an early marker for HCC diagnosis as comparing to DCP and AFP serum levels.

2. Patient and Methods:

This study included 66 patients, selected from the hepatology department of National Liver Institute-Menoufyia University, National Cancer Institute-Cairo University, Internal Medicine-Al Zahraa University Hospital and Hepatology Centre- National Medical Centre; 31 of them were diagnosed as HCC according to clinical examination, radiological investigations including abdominal ultrasonography, triphasic C.T and laboratory investigations. All patients were newly diagnosed cases and did not receive prior chemotherapy. They were 26 males and 5 females, their age ranged from 42-71 with a mean of 59.27±9.14 years. The remaining 35 patients have post HBV or HCV liver cirrhosis, 29 males and 6 females, their age ranged from 34-

68 with a mean of 54.71±7.12 years, they were diagnosed by clinical examination, abdominal ultrasound, laboratory investigations and liver biopsy.

Additionally, 20 apparently healthy subjects (15 females and 5 males); their ages ranged from 28 to 53 years with a mean of 51.65±5.23 and all were matched for age and sex with patients were included as a control group, they had normal values of serum alanine aminotransferase (ALT) and were seronegative for hepatitis B surface markers (HBs Ag,HBeAg and HBc-Ab) and HCV antibodies. The study was approved by the local ethical committee in university hospitals and informed consent was obtained from all participated.

All patient and control groups subjected to the following:

- Full history taking, thorough clinical examination.
- Abdominal ultrasonogrphy, and ultrasound guided liver biopsy was performed by true-cut needle or liver biopsy gun for the cirrhotic patients when possible.
- Triphasic C.T. to patients with focal lesion.
- The following investigations: liver function tests including: ALT, AST, serum albumin and total bilirubin was done on Cobas Integra-400 (Roche-Germany). Prothrombin concentration was done on fibrintimer (Dade Behring-Germany). Complete blood cell counts was measured by Sysmix K-21 automatic cell counter (Japan). Serum AFP was measured using automated eleceyes (Roche- Diagnostic, Branchburg, NJ- Germany).
- Hepatitis markers (HBsAg, anti-HBc and HCV antibody) were done by EIA (COBAS- amplicore, Roche- Germany).
- HCV-RNA levels were analyzed by reverse Transcriptase polymerase chain reaction (RT-PCR) using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.
- Measurement of des-γ-carboxy prothrombin (DCP): It was measured using a commercially available Enzyme-linked Immunosorbent Assay (ELISA) kit (Asserachrom PIVKA-II kit, Stago, France), according to the manufacturer's instructions.
- Determination of Golgi Protein 73 (GP73): It was determined by ELISA Kit For Golgi Protein 73 (GP73) provided by Uscn, Life Science (Inc-USA), the catalogue no E91668Hu. The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of GP73 in human serum, plasma and other biological fluids. Briefly, the microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to GP73. Standards or serum samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for GP73. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. Only those wells that contain GP73, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a

change in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of GP73 in the samples was then determined by comparing the O.D. of the samples to the standard curve [14].

Statistical analysis:

Data are expressed as mean ± SD. The SPSS computer program version 12.0 was used fostatistical analysis. Kruskal-Wallis test was done to compare three or more of non normally distributed variables and Tamhane test is a Post Hoc test done to variables of significant difference of more than two groups of not normally distributed data after Kruskal-Wallis test to detect the significant difference between either groups. Correlation coefficients (r) were calculated using the Pearson's correlation analysis. p value was significant at <0.05 level. Sensitivity, specificity and the area under the receiver-operating characteristic curve (AUROC) were determined.

3. Results:

Patients characteristic are shown in (Table 1). The comparison between HCC, liver cirrhosis and controls revealed that, However the age in significantly not differed, a significant increase in AST, ALT, Alkaline phosphatase, GGT, total bilirubin in both patient groups compared to control group with the higher levels in HCC group. In contrast, serum albumin level, prothrombin concentration, HB concentration and platelet counts were significantly decreased as compared in both patient groups as compared to controls (Table 2).

The serum levels of AFP, DCP and GP73 were significantly elevated in LC and HCC patient groups as compared to control group and more elevated in HCC cases than in LC cases and control group as shown in (p<0.001) for each (Table 3).

At a cut-off 28.51 ng/ml. AFP had a sensitivity of 77.4%, a specificity of 60%, positive predictive value (PPV) of 75% and negative predictive value (NPV) of 63.2% for early HCC diagnosis. DCP give a sensitivity of 80.6%, specificity of 85%, PPV of 89.3% and NPV of 73.7% at a cut-off 32.64 ng/ml. The GP73 had a sensitivity of 87%, a specificity of 95%, PPV 96.4% and NPV of 82.6% at the optimal cut-off value of 7.62 ng/ml. The area under receiver-operating characteristic curve (AUROC) was 0.87 (Figure 1). However, when GP73 used in combination with AFP for early detection of HCC, they increased sensitivity up to 90.3%, whereas, specificity was 90%, PPV was 93.3%, NPV was 85.7% and AUROC was 0.83 (Figure 2) and (Table 4).

A significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and Child score) (p<0.5) for each, and more aggressive tumor characters (tumor size and vascular invasion), (p<0.05 and p<0.01) respectively. Also, a significant correlation was detected between serum GP73 level and DCP levels (p<0.05), while AFP serum levels show no significant correlation (p>0.05) (Table 5).

Table (1) Patients demographic data

Variables	HCC (n = 31)	Cirrhosis (n = 35)
Gender (Male/Female)	**26/5**	**29/6**
Jaundice	15 (48.4%)	16 (45.7%)
Hepatomegaly	26 (83.9%)	29 (82.9%)
Splenomegaly	16 (51.6%)	21 (60.0%)
Hematemesis	19 (61.2%)	13 (37.1%)
Melena	12 (34.3%)	10 (28.6%)
Lower limb oedema	14 (45.2%)	11 (31.4%)
Ascites	17 (54.8%)	14 (40.0%)
Child Score:		
A	3 (9.7%)	9 (25.7%)
B	11 (35.5%)	12 (34.3%)
C	17 (54.8%)	14 (40.0%)
Tumor Characters:		
Single	14 (45.2%)	------
<3 cm	10 (32.3%)	
Encapsulated	18 (58.1%)	
Blood vessel invasion	4 (12.9%)	

Table (2): Comparison between HCC, cirrhotic and control groups as regards biochemical data.

Variables	HCC (n = 31)	Cirrhosis (n = 35)	Controls (n = 20)	P value
Age (years)	59.27±9.14	54.71±7.12	51.65±5.23	>0.05
AST (U/L)	121.3 ± 85.2	76.5±39.4	15.9±4.7	<0.01
ALT (U/L)	126.2 ± 89.8	57.1±29.7	17.2±5.6	<0.01
ALP (U/L)	127.5 ± 65.3	90.4±27.8	40.5±10.7	<0.01
GGT (U/L)	79.5±34.6	34.1±12.1	18.2±5.3	<0.001
TB (mg/dl)	6.2 ± 3.7	4.5±2.6	0.7±0.13	<0.001
Albumin (g/dl)	2.76±0.54	2.85±0.61	4.61±0.53	<0.001
Proth. Conc %	58.6±19.1	61.2± 7.5	95.4±4.8	<0.001
HB (g/dL)	11.3±2.4	11.06±1.6	13.5±0.49	<0.01
Platelets (/mm)	156.8± 61.7	109.3±45.2	261.4±36.2	<0.01

P<0.05 is statistically significant, p>0.05 is not statistically significant.

Table (3): Comparison between HCC, cirrhotic and control groups as regards Tumor markers

Variables	HCC (n = 31)	Cirrhosis (n = 35)	Controls (n = 20)	P value
AFP (ng/ml)	508 ±314	16.08±7.15	1.95±0.23	<0.001
DCP (ng/ml)	27.36±5.35	12.42±6.24	4.27±1.25	<0.001
GP73 (ng/ml)	10.32±2.46	3.79±2.18	1.65±0.79	<0.001

P<0.05 is statistically significant, p>0.05 is not statistically significant.

Table (4): Sensitivity, specificity, positive and negative predictive value of tumor markers for early diagnosis of HCC (n=31).

Variables	Cut-Off	Sensitivity	Specificity	PPV	NPV
AFP (ng/ml)	28.51	77.4%	60 %	75%	63.2%
DCP (ng/ml)	32.64	80.6 %	85 %	89.3%	73.7%
GP73 (ng/ml)	7.62	87%	95%	96.4%	82.6%
Combined AFP & GP73	AFP= 28.51 ng/ml & GP73= 7.62 ng/ml	90.3%	90%	93.3%	85.7%

PPV=Positive Predictive Value NPV=Negative Predictive Value

Table (5): Correlation between GP73 serum levels and prognostic parameters in HCC group (n=31)

Parameters	GP73 serum levels	
	r-value	p-value
ALT	0.48	<0.05
AST	0.52	<0.05
Serum albumin	0.49	<0.05
AFP levels	0.23	>0.05
DCP levels	0.51	<0.05
Child score	0.46	<0.05
Tumor size	0.56	<0.05
Tumor number	0.24	>0.05
Blood vessel invasion	0.67	<0.01

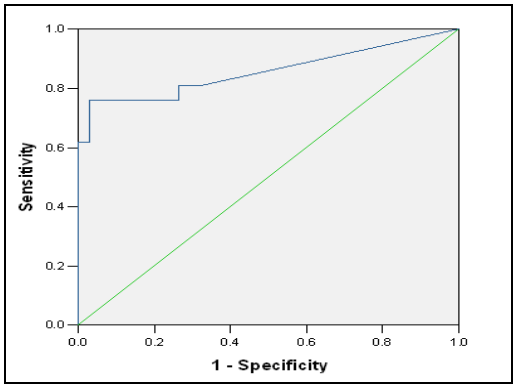

Figure (1) shows the ROC curve of GP73 in early HCC diagnosis

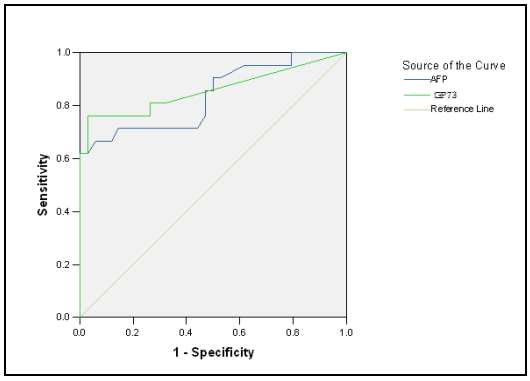

Figure (2) shows the ROC curve of combined GP73 and AFP in early HCC diagnosis

4. Discussion:

Since HCC is among the cancers with the worst prognosis, early diagnosis and treatment are the keys for effective treatment of patients with HCC. The use of serological markers in patients at the highest risk for developing HCC may thus decrease HCC mortality and reduce medical costs **[18].**

AFP has been used as a serum marker for HCC for many years, but it lack of high sensitivity and Specificity **[19]**. However, **Li and his colleagues [20]** tried to improve the detection rate using the ultrasonography. Several biomarkers such as DCP, AFP-L3, human hepatocyte growth factor, and insulin like growth factor-1 as well as AFP are promising, but none of these markers has been validated enough for clinical use. Thus, there is an urgent need for new biomarker for the detection of early HCC. DCP is a well recognized tumor marker for its high sensitivity and specificity in the screening and diagnosis of HCC **[21]**. Although some studies have identified serum GP73 as a potential biomarker for HCC **[21,22]**, the GP73 functions and the mechanisms of regulation in normal and neoplastic tissues are still unclear.

We determined the serum level of GP73 in 31 patients of HCC and 35 patients have liver cirrhosis without HCC to find its sensitivity and specificity in early detection of HCC comparing with conventional markers as DCP and AFP serum levels.

In the current study, the serum levels of AFP, DCP are significantly elevated in LC and more elevated in HCC cases. As well as the serum level of GP73 was significantly higher in HCC cases. These findings are in agreement with **Tian et al**. **[23]** who reported that, serum GP73 in LC was higher than in HCC and In all two groups were higher than those in healthy individuals. In addition, the most profound elevation of serum levels of GP73 was detected in patients who had developed an HCC on the background of HCV infection [**24**]. **Gu et al. [14]** reported that serum level of GP73 in patients with liver disease was significantly higher than in healthy individuals and in patients with other diseases. In a subsequent study by **Marrero et al. [21**] sGP73 levels were significantly increased in patients with HCV–related HCC in comparison with cirrhotic controls

Similar results were reported in a Chinese study on patients with predominantly hepatitis B virus–related liver cancer. In response to these encouraging reports, GP73 was added to a group of emerging candidate HCC serum markers **[24].** **Mao et al. [18**] have found that the elevation of serum GP73 is mildest in virus carriers, moderate in patients with cirrhosis and dramatic in patients with HCC. Therefore, serum GP73 can be used to monitor disease progression from HBV infection to cirrhosis to HCC. Moreover, they found that both liver benign tumours and non-HCC liver malignant lesions had elevated serum GP73, although the magnitude is much smaller than that in HCC. Serum GP73 can therefore be a useful tool in determining the nature (benign vs. HCC) of hepatic tumours. Furthermore, in patients with non-liver cancers also had moderate elevation of serum GP73, none of which, however, reached the level identified for HCC cases. Serum levels of GP73 diagnostic for HCC thus seemed not to be a pan-cancer marker.

Additionally, **Mao et al**. **[18]** study demonstrated that surgical resection of the tumour results in diminished serum GP73 levels and that tumour recurrence correlates with the recurrence of elevated GP73 in the blood. Reappearance of serum

GP73 indicates the existence of tumour lesions and thus may serve as an indicator for the recurrence of HCC.

The mechanism by which sGP73 reaches the circulation was worked out by **Bachert et al. [13]** in cell culture studies. Despite its steady-state localization within the cis-Golgi complex, GP73 cycles through the distal secretary apparatus and transiently reaches the apical cell membrane, from which it returns to the Golgi complex via an endosomal retrieval pathway **[25].** This secreted form is generated by N-terminal cleavage of the molecule by the proprotein convertase furin after amino acid 55, resulting in the release of the large C-terminal ectodomain into the extracellular space **[13].** Using N-terminal sequencing, **Gu et al. [14]** confirmed that the serum form of sGP73 is identical to the furin cleavage product identified in cell culture supernatants. This finding provides a mechanistic explanation for the appearance of sGP73 in serum.

The need for closer monitoring of patients with chronic hepatitis who have a high risk of developing HCC during the course of the disease has long been stated. In these patients, AFP has been a particularly unsatisfactory screening tool for early detection of HCC [**27**]. **Riener et al. [24]** concluded that GP73 is not a general HCC serum tumor marker but could rather be a valuable complementary tool in the surveillance of at-risk patients. The data presented in **Riener et al. [24**] study provides further evidence that GP73 protein is strongly expressed in HCC and bile duct carcinoma tissues and is secreted into the blood. Possibly, it is either involved in posttranslational protein modification, transport of secretory proteins, cell signalling regulation, or simply maintenance of Golgi apparatus function. GP73 has several potential glycosylation sites and up to 75% of GP73 secreted from hepatocytes is fucosylated **[28].** Endosomal trafficking of the normally membrane-bound GP73 leads to secretion into the blood, making it a potential serum biomarker for HCC **[13].**

The expression levels in benign liver lesions—focal nodular hypertrophy and hepatic adenoma were not significantly different from those of the surrounding areas.

These findings provide evidence that the increased sGP73 in HCC patients originates from cancerous hepatocytes, an important requirement for the validation of tumor biomarkers **[25].** Another novel finding is the marked up-regulation of GP73 expression in cancers of biliary origin **[29].**

In HCC diagnosis, previous studies have shown a better sensitivity of GP73 than AFP in diagnosis of HCC **[21**]. In this study, AFP had a sensitivity of 77.4% and a specificity of 60% at a cut-off 28.51 ng/ml. Parallel to these results of AFP in prediction of HCC, **Hakamada et al., [30]** reported a sensitivity of 69.3%, specificity 60%. Another two studies by **Trevisani et al. [31]** and **Gambarin-Gelwan et al**.**[32],** AFP specificity varies from about 76% to 96% and increases with elevated cut-off value.

In our study, DCP give a sensitivity of 80.6% and specificity of 85% in early HCC diagnosis at a cut-off 32.64 ng/ml. This agreed with the finding of **Durazo et al. [33],** who reported a sensitivity of 87.2 %, and specificity of 85.0% at similar cut-off. Also, in an older study by **Nakagawa et al**. **[34],** the sensitivity of DCP is 48%-62% and the specificity is 81%-98%, because they used a higher cut-off for DCP. However, AFP and DCP

are not correlated, so the combination of these two markers significantly improves HCC detection **[35, 36].**

Furthermore, whether GP73 is a better serum biomarker than AFP is controversial. The sensitivity and specificity of GP73 for HCC were superior to those of AFP, especially in early HCC, in our study; GP73 had a sensitivity of 87% and a specificity of 95% at the optimal cut-off value of 7.62 ng/ml. The area under receiver-operating characteristic curve (AUROC) was 0.87. However, when used in combination with AFP, they lead to an enhanced the sensitivity of detection of HCC up to 90.3% and AUROC was 0.83.

Previous studies by **Marrero & Lok [17]** and **Gomaa et al. [37]** postulated that, GP73 is up-regulated in HCC and measurement of serum GP73 revealed a sensitivity and specificity of 69% and 75%, respectively. In a more recent study by **Tian et al. [24],** AFP/GP73 had a sensitivity of 75.8% and specificity of 79.7% with an AUROC of 0.844. vs. 0.812 for AFP with a sensitivity of 95.2% and specificity of 47.1%; in detecting early HCC, AUROC of AFP/GP73 was 0. 804 vs. 0.766 for AFP alone. Also, **Wang et al**. **[38**] and **Mao et al. [18],** the combined measurement of GP73 and AFP can further increase the sensitivity for the detection of HCC.

These results were disappointing with two previous studies, in the first study, sGP73 was found to be elevated in patients with liver disease but did not distinguish between HCC, cirrhosis, and chronic hepatitis **[14]**. In the second study, which was reported in an abstract form, sGP73 was surprisingly found to be decreased in HCC patients **[39]**. The results of **Riener et al. [24]** study cast doubt on the diagnostic utility of sG73 as a serum marker of HCC. However, a few methodological questions will need to be addressed before the authors' interpretation is endorsed. First, the sGP73 serum levels reported by **Riener et al. [24]** were approximately 80-fold higher than those reported by **Gu et al. [14]** with a median serum concentration in normal subjects of 4 ug/mL, which is well within the range of many classical plasma proteins **[40].**

The correlation study, revealed that, a significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and child score) .This in agreement with the finding of **Tian et al. [23]**, who reported that, serum GP73 in LC patients with Child-Pugh class A was lower than in class B and C and GP73 correlated with AST, AST/ALT, albumin, A/G and alkaline phosphatase in liver cirrhosis.

Other, interesting findings in our study is the level of GP73 correlated with more aggressive tumor characters (tumor size and vascular invasion). These are similar to **Sun et al. [41**], who reported that, a significant overexpression of GP73 at both protein and mRNA levels along with overexpression of GP73 protein is associated with aggressive behavior of HCC. **Fimmel & Wright** [**29]** recorded that, the degree of GP73 expression correlated with the tumor grade. In contrast, serum levels of GP73 in patients with HCC were not consistently affected by the tumour sizes and the status of tumour differentiation [**18]**.

Conclusion:

The serum levels of GP73 concentration in patients with LC and HCC was significantly higher than in healthy individuals and it had a high sensitivity and specificity for early prediction of HCC cases. Level correlated with disease progression to HCC. In combination, measurement of AFP and GP73 has the promise to further improve the detection and treatment of HCC. Further research is needed to determine the potential of GP73 as a therapeutic target.

Conflicts of Interest:

There were no Conflicts of Interest in this study.

Authors' contributions:

All authors' contributed equally to this study.

Correspondence author

Amal M Fawzy

Clinical Pathology, National Cancer Institute- Cairo University, Egypt

amalfawzy69@hotmail.com

References:

1-El-Serag HB, Rudolph KL (2007): Hepatocellular carcinoma: epidemiology andmolecular carcinogenesis**.** *Gastroenterology*, 132:2557-2576.

2- Altekruse SF, McGlynn KA, Reichman ME (2009): Hepatocellular carcinoma ,incidence, mortality, and survival trends in the United States from 1975 to 2005**.** *J Clin Oncol*, 27:1485-1491.

3-Nguyen MH, Whittemore AS, Garcia RT, et al (2004): Role of ethnicity in risk for hepatocellular carcinoma in patients with chronic hepatitis C and cirrhosis. Clin Gastroenterol Hepatol, 2:820-824.

4-Farazi PA, DePinho RA (2006): Hepatocellular carcinoma pathogenesis: from genes to environment. Na Rev Cancer, 6:674-687.

5-El-Serag HB (2007): Epidemiology of hepatocellular carcinoma in USA. Hepatol Res, 37(Suppl-2):S88-94.

6- El-Serag HB (2004): Hepatocellular carcinoma: recent trends in the United States**.** *Gastroenterology*, 127:S27-34.

7- Di Bisceglie AM and Hoofnagle JH. (1989): Elevations in serum α-fetoprotein levels inpatients with chronic hepatitis B. Cancer; 64:2117–20.

8- Sherman M. Hepatocellular carcinoma (2005): epidemiology, risk factors, and screening.Semin Liver Dis;25:143–54.

9- Liebman HA, Furie BC, Tong MJ, et al. (1984): Des-gamma-carboxy (abnormal)

prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med;* 310:1427-1431.

10-Weitz IC, Liebman HA. (1993): Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. *Hepatology*; 18: 990-997.

11- Kladney RD, Bulla GA, Guo L, Mason AL, Tollefson AE, Simon DJ, et al. (2000): GP73, a novel Golgi-localized protein upregulated by viral infection. Gene;249:53-65.

12- Iftikhar R, Kladney RD, Havlioglu N, Solomon H, et al. (2004): Disease- and cell-specific expression of GP73 in human liver disease. Am J Gastroenterol; 99:1087-1095.

13- Bachert C, Fimmel C, Linstedt AD. (2007): Endosomal trafficking and proprotein

convertase cleavage of cis Golgi protein GP73 produces marker for hepatocellular carcinoma. Traffic; 8:1415-1423.

14- Gu Y, Chen W, Zhao Y, Chen L, Peng T. (2009): Quantitative analysis of elevated serum Golgi protein-73 expression in patients with liver diseases. Ann Clin Biochem;46:38-43.

15- Block TM, Comunale MA, Lowman M, et al. (2005): Use of targeted glycopro-teomics to identify serum glycoproteins that correlate with liver cancer in woodchucks and humans. Proc Natl Acad Sci USA; 102:779-784.

16- Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. (2005): GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol; 43:1007-1012.

17- Marrero JA and Lok AS. (2004): Newermarkers for hepatocellular carcinoma.Gastroenterology ; 127(5 Suppl 1):S113-119.

18- Mao Y,Yang H, Xu H, Lu X, et al. (2010): Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. Gut, doi10.1136/gut. 2010.214916.

19- Soresi M, Magliarisi C, Campagna P, et al. (2003): Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. *Anticancer Res*; **23**: 1747-1753

20- Li H, Wetten S, Li L, St Jean PL, Upmanyu R, Surh L, et al. (2008): Candidate single nucleotide polymorphisms from a genome wide association study of Alzheimer disease. Arch Neurol; 65:45-53.

21- Marrero JA, Romano PR, Nikolaeva O, Fimmel CJ, et al. (2005): GP73, a resident

Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol;43:1007-1012.

22- Mao YL, Yang HY, Xu HF, et al. (2008): Significance of Golgi glycoprotein 73, a

New tumour marker in diagnosis of hepatocellular carcinoma: a primary study. Zhonghua Yi Xue Za Zhi (In Chinese); 88:948-951

23-Tian L, Wang Y, Xu D, Gui J, et al. (2010): Serological AFP/ golgi protein 73 could be a new diagnostic parameter of hepatic diseases. Int J Cancer Dec 7.

24-Riener MO, Stenner F, Liewen H et al. (2009): Golgi Phosphoprotein 2 (GOLPH2) Expression in Liver Tumors and Its Value as a Serum Marker in Hepatocellular Carcinomas. Hepatology;, 49:1602-1609.

25- Block TM, Marrero J, Gish RG, Sherman M, London WT, Srivastava S, et al. (2008): The degree of readiness of selected biomarkers for the early detection of hepatocellular carcinoma: notes from a recent workshop. Cancer Biomark; 4:19-33.

26- Puri S, Bachert C, Fimmel CJ, Linstedt AD. (2002): Cycling of early Golgi proteins via the cell surface and endosomes upon lumenal pH disruption. Traffic; 3:641-653.

27- Chen TM, Huang PT, Tsai MH, Lin LF, Liu CC, Ho KS, et al. (2007): Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. J Gastroenterol Hepatol; 22:669-675.

28- Norton PA, Comunale MA, Krakover J, Rodemich L, Pirog N, D'Amelio A, et al. (2008): N-linked glycosylation of the liver cancer biomarker GP73. J Cell Biochem;104:136-149.

29- Fimmel and Wright .(2009): Golgi Protein 73 as a Biomarker of Hepatocellular Cancer: Development of a Quantitative Serum Assay and Expression Studies in Hepatic and Extrahepatic Malignancies. Hepatology, Vol. 49, No. 5,:1421-1423.

30- Hakamada K, Kimura N, Morohashi H et al. (2008): Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma.World J Gastroenterol , March 7; 14(9): 1370-1377.

31- Trevisani F, D'Intino PE, Morselli-Labate AM, et al. (2001): Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* ; 34: 570-575.

32- Gambarin-Gelwan M, Wolf DC, Shapiro R, et al. (2000): Sensitivity of commonly available screening tests in detecting hepatocellular carcinoma in cirrhotic patients undergoing liver transplantation. *Am J Gastroenterol*; **95**: 1535-1538.

33- Durazo FA, Blatt LM, Corey WG, Lin JH, et al., (2008): Des-γ-carboxyprothrombin, α- fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellularcarcinoma. J Gastroenterol Hepatol. Oct;23(10):1541-8.Epub Apr 19.

34- Nakagawa T, Seki T, Shiro T, Wakabayashi M, et al. (1999): Clinicopathologic significance of protein induced vitamin K absence or antagonist II and

alpha-fetoprotein in hepatocellular carcinoma. *Int J Oncol*; 14: 281-286.

35- Sterling RK, Jeffers L, Gordon F, Sherman M, et al. (2007): Clinical utility of AFP-L3 measurement in North American patients with HCV-related cirrhosis. *Am J Gastroenterol*; 102: 2196-2205.

36- Sterling RK, Jeffers L, Gordon F, et al. (2009): Utility of Lens culinaris agglutinin-reactive fraction of alphafetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. *Clin Gastroentero-Hepatol*; 7: 104-113.

37- Gomaa AI, Khan SA, Leen EL, Waked I, Taylor-Robinson SD. (2009): Diagnosis of hepatocellular carcinoma. World J Gastroenterol; 15(11):1301-1314.

38-Wang M, Long RE, Comunale MA, Junaidi O et al. (2009): Novel Fucosylated Biomarkers for the Early Detection of Hepatocellular Carcinoma. *Cancer Epidemiol Biomarkers Prev*; 18:1914-1921.

39- Sangiovanni A, Romeo R, Prati GM, Manini MA, et al. (2007): Serum expression of lectin reactive alpha-fetoprotein, des-gammacarboxy prothrombin, golgi protein-73 antigen and antibody, for the diagnosis of hepatocellular carcinoma [Abstract]. Hepatology,;46(Suppl):406A.

40- Anderson NL and Anderson NG. (2002): The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics; 1:845-867.

41-Sun Y, Yang H, Mao Y et al. (2011): Increased GP73 expression in hepatocellular carcinoma tissue correlates with tumor aggression but not survival. Journal of Gastroenterol Hepatol; 26(7):1207-12. doi: 10.1111/j.1440-1746.2011.06733.x.

4/25/2012