

# Study of Serum Golgi Protein 73 Level as a Marker for Diagnosis of Hepatocellular Carcinoma

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**Background and study aim:** Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death. Golgi protein 73 is normally expressed in epithelial cells of many human tissues. GP73 expression is upregulated in hepatocytes, and in serum from patients with hepatitis and liver cirrhosis regardless the etiology. This work aimed to study the diagnostic role of serum Golgi protein 73 level as a marker for HCC.

**Patients and methods:** This study was conducted on 48 patients with HCC on top of liver cirrhosis (GI), 20 patients with liver cirrhosis (GII), and 20 healthy controls (GIII). Patient and controls were subjected to careful medical history, full clinical examination and laboratory investigations including CBC, ESR, liver function tests, renal function tests, viral markers, serum AFP and Serum Golgi protein 73 by ELISA.

**Results:** Serum GP73 showed highly significant increase (with P value <0.001) in HCC group X±SD (1765.92±747.99) in comparison with cirrhotic X±SD (772.45±73.84) and control X±SD (458.30±103.03) groups, also significantly increased in cirrhotic group in comparison with control group. There was significant increase in mean values of serum GP73 in patients with HCC associated with portal vein thrombosis or lymph node enlargement also there was significant positive correlation between GP73 and tumor size. In diagnosis of HCC, at cut off point 55 ng/ml, AFP had sensitivity 81.3% and specificity 70.0%, and Gp73 at cut off point 847.5 ng/l, the sensitivity was 93.8% and specificity 90.0%. With combined use of AFP and Gp73: the sensitivity of diagnosis of HCC increased to 95.8%.

**Conclusion:** Significant increase in sensitivity, accuracy and negative predictive value with combined use of AFP and Gp73 than AFP alone in diagnosis of HCC.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death. The burden of this devastating cancer is expected to increase further in coming years [1]. As most patients with HCC are diagnosed at an advanced stage with underlying liver dysfunction, the mortality rate of HCC is similar to the incidence rate. Early detection of HCC is therefore extremely important in improving the survival of patients [2].

Approximately 70%-90% of patients with HCC have an established background of chronic liver disease and cirrhosis, with major risk factors for

developing cirrhosis including chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease, and nonalcoholic steatohepatitis (NASH). Patients with cirrhosis are thus usually included in surveillance plans [3].

AFP is the most widely tested biomarker in HCC. But the clinical value of AFP is challenged due to low sensitivity and specificity. AFP is not elevated in all patients with HCC. Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP, even without the presence of a tumor [4]. Alternative serum biomarkers are being actively sought include prothrombin induced by vitamin K absence (PIVKA), glypican-3,

and squamous cell carcinoma antigen-1; however, none of these have been adequately investigated to be recommended as a screening test [5].

Golgi protein 73 (GP73) also named Golgi phosphorprotein 2 (GOLPH2), is a type II Golgi localized integral membrane protein that is normally expressed in epithelial cells of many human tissues. GP73 was first identified in a genetic screen for proteins with differential expression in adult giant-cell hepatitis (GCH) [6]. It is consistently present in biliary epithelial cells in normal livers, and hepatocytes show little or no signal. However, GP73 expression is upregulated in hepatocytes, and in serum samples from patients with acute & chronic hepatitis and liver cirrhosis regardless the etiology [7]. The aim of the present work was to study the diagnostic role of serum Golgi protein 73 level as a marker for HCC in comparison with serum Alpha-fetoprotein.

## PATIENTS AND METHODS

This study was conducted on 68 patients with liver cirrhosis with or without HCC and 20 healthy subjects of matched age and sex as controls. Patients and controls were selected from inpatients and outpatients clinic of Tropical Medicine Department Menoufia University Hospital in the period between June 2012 and December 2013. Patients were 52 (76.5%) males and 16 (23.5%) females. Their ages were ranging between 31-76 years old. An informed consent was obtained before patients entered the study. Diagnosis of cirrhosis was done by clinical examination, ultra-sonographic finding and laboratory investigations; while diagnosis of HCC was done by their characteristic features in 2 imaging methods (abdominal ultrasonography and triphasic CT).

**Patients and controls were classified into the following groups:**

**Group I:** Comprised 48 patients with HCC on top of liver cirrhosis. All patients did not receive prior treatment for HCC.

**Group II:** Comprised 20 patients with liver cirrhosis.

**Group III:** Comprised 20 healthy subjects as a control group.

**Exclusion criteria:**

1. Focal hepatic lesions other than HCC (cholangiocarcinoma, hemangioma, hepatoblastoma, metastatic focal lesions...etc).
2. Malignancy elsewhere

**Patients and controls were subjected to the following:**

- 1- Careful medical history
- 2- Full clinical examination
- 3- Laboratory investigations including complete blood picture, ESR, liver function tests, renal function tests and viral markers
- 4- Serum AFP level by ELISA
- 5- Serum Golgi protein 73 was quantitatively determined by ELISA GP73 provided by Glory science catalog number 11598. The range of the kit was: 50ng/L→1500ng/L. Principle of test; the kit used a double-antibody sandwich ELISA to assay the level of Human Golgi protein-73(GP-73) in samples. Add (GP-73) to monoclonal antibody Enzyme well which is pre-coated with Human (GP-73) monoclonal antibody, incubation; then, add (GP-73) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color of the Human Substance (GP-73) of sample was positively correlated.
- 6- Imaging studies (abdominal ultrasonography, triphasic CT).

**Statistical Analysis:**

The data were collected, tabulated, and analyzed by SPSS (statistical package for social science) version 17.0 on IBM compatible computer. Significance of results: Non-significant difference if  $P > 0.05$ , Significant difference if  $P < 0.05$  and highly significant difference if  $P < 0.01$ .

## RESULTS

**Demographics of the studied groups**

There was no significant difference between the three studied groups as regards age and sex. Patients were 52 (76.5%) males and 16 (23.5%) females. Their ages were ranging between 31-76 years old as well as 20 healthy subjects of matched age and sex as controls. The mean of age in HCC group was  $57.85 \pm 7.23$ , in cirrhotic group was  $48.25 \pm 7.99$  and in control group was  $54.70 \pm 8.12$ . In GI, there were 38 (79.2%) males and 10 (20.8%) females, in GII there were 14 (70%) males, and 6 (30%) females and in GIII there were 14 (70%) males, and 6 (30%) females. Manifestations of cirrhosis were

present in various proportions in all GI and GII patients. Some manifestations consistent with HCC (anorexia and loss of weight) were present in various proportions in HCC patients.

#### **Biochemistry of the studied groups**

There was highly significant decrease in mean values of hemoglobin concentration and platelet count in HCC and cirrhotic groups in comparison with control group. While there was no significant difference between GI & GII as regards platelets count and Hb concentration. There was no significant difference between studied groups as regards total WBCs.

Statistical analysis revealed that there was highly significant increase in mean values of AST, ALT and serum bilirubin as well as highly significant decrease in mean values of serum albumin and prothrombin concentration in HCC and cirrhotic groups in comparison with control group.

Chronic HCV was the commonest etiology of cirrhosis in GI (85.4%) and GII patients (80.0%) while chronic HBV was far less common etiology. There was non-significant difference between HCC group and cirrhotic group as regards the Child classification.

Triphasic CT revealed evidence of cirrhosis in all GI & GII patients as well as imaging evidence consistent with HCC in all GI patients (Table 1).

There was highly significant increase in mean values of serum AFP & GP73 in HCC group in comparison with other groups and in cirrhotic group in comparison with control group (Table 2).

There was no significant difference in mean values of AFP as regard tumor size in the HCC group, While there was statistical significant increase in

the mean values of Gp73 with increasing the tumor size (more than 5 cm) (Table 3 and Figure 1).

Statistical analysis revealed significant increase in mean values of serum GP73 in patients with HCC complicated by portal vein thrombosis in comparison with patients without portal vein thrombosis, while there was no significant difference between patients with HCC with or without portal vein thrombosis as regards mean values of AFP. Also there was significant increase in mean values of serum GP73 in patients with HCC associated with lymph node enlargement in comparison with patients without, while there was no significant difference between patients with HCC with or without lymph node enlargement as regards mean values of AFP (Table 4).

Statistical analysis revealed no significant difference in mean values of AFP as regard Child-Pugh grades (A, B, C) in the HCC group, while there was significant increase in the mean values of Gp73 with increasing Child grades in HCC. There was no significant difference in mean values of both AFP and GP73 as regard Child-Pugh grades (A,B,C) in the cirrhotic group (Table 5).

Receiver operating characteristic curve (ROC curve) of AFP and GP73 for diagnosis of HCC versus cirrhotic cases showed that the cutoff point was 55ng/ml for AFP and 847.5ng/l for GP73 (Figure 2).

At cut off point 55 ng/ml, AFP had sensitivity 81.3%, specificity 70.0%, and accuracy 77.9%, while for Gp73 at cut off point 847.5 ng/l, the sensitivity was 93.8%, specificity 90.0% and accuracy 92.6% in diagnosis of HCC. With combined use of AFP and Gp73: the sensitivity increased to 95.8% (Table 6).

**Table (1):** Triphasic CT findings among the studied patients

Triphasic CT findings	GI (N = 48)		GII (N = 20)	
	No	%	No	%
Liver size				
Average	3	6.25	1	5.0
Hepatomegaly	18	37.5	1	5.0
Shrunken	27	56.25	18	90.0
Wavy or lobulated Outline	48	100	20	100
Size of the focal lesion(s)				
X ± SD	2.30 ± 2.36			
Range	1.7 – 12		-	-
<b>Number of the focal lesion(s)</b>				
One	32	66.7		
Two	4	8.3	-	-
Multicentric	12	25.0		
<b>Ascitis</b>	38	79.2	17	85.0
<b>Spleen</b>				
- Splenomegaly	47	97.9	19	95.0
- Splenectomy	1	2.1	1	5.0
- Dilated collaterals	10	20.8	5	25.0
<b>Portal vein (PV)</b>				
Normal	6	12.5	2	10.0
Dilated	42	87.5	18	90.0
PV Thrombosis	10	20.8	1	5.0
<b>Lymph nodes enlargement</b>	6	12.5	0	0.0

**Table (2):** Comparison between studied groups as regards AFP and Gp73

	The studied groups			Mann Whitney U test	P value
	Group I N = 48	Group II N = 20	Group III N = 20		
<b>AFP(ng/ml)</b>					
X ± SD	771.89±1002.99	30.0±27.17	1.15±0.94	4.86 6.44 5.31	<0.001 <sup>1</sup> <0.001 <sup>2</sup> <0.001 <sup>3</sup>
Range	19 – 2850	1 – 90	0.8 – 5		
<b>Gp73(ng/l)</b>					
X ± SD	1765.92±747.99	772.45±73.84	458.30±103.03	6.23 6.41 4.92	<0.001 <sup>1</sup> <0.001 <sup>2</sup> <0.001 <sup>3</sup>
Range	810 – 3515	635-890	350 - 665		

1 = Comparison between HCC cases and cirrhotic cases

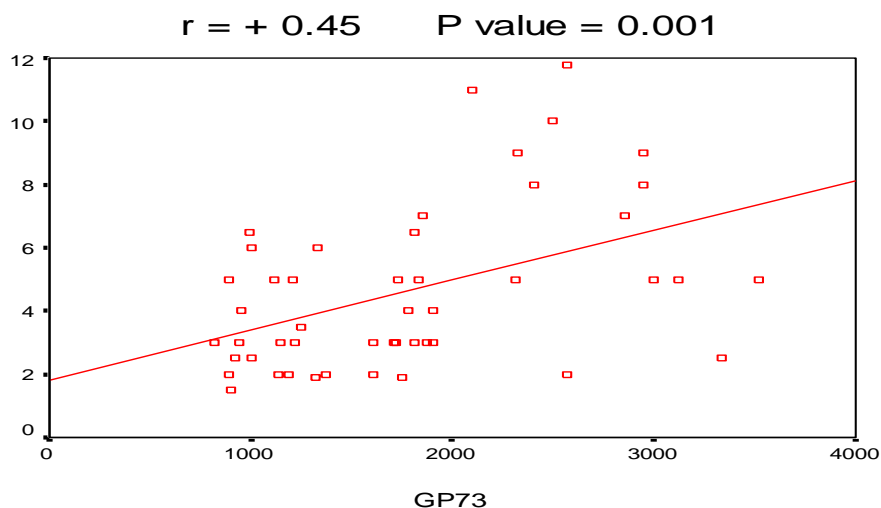
2 = Comparison between HCC cases and control

3 = Comparison between cirrhotic cases and control

**Table (3):** The relation between tumor size and AFP & GP73 levels in GI

Variable	Tumor size		Kruskal Wallis Test	P value
	< 5 cm (n= 26)	≥ 5 cm (n= 22)		
<b>AFP (ng/ml)</b> X±SD Range	705.38±967.58 7 – 2850	850.5±1060.69 19 – 2800	0.17	0.87
<b>Gp73 (ng/l)</b> X±SD Range	1485.85±575.02 815 – 2900	2106.0±787.99 895 – 3515	2.76	0.006

**Correlation between GP73 and tumor size**



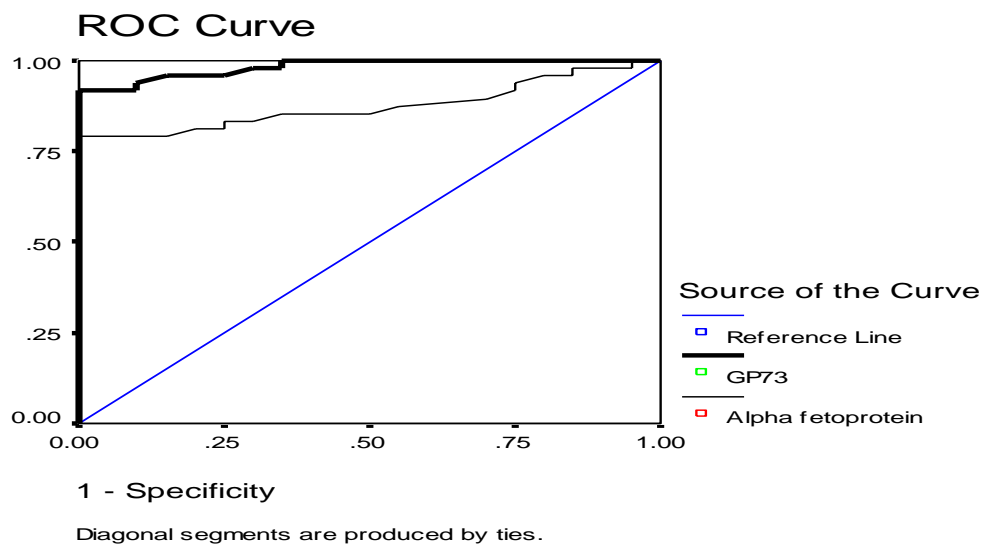
**Figure 1** Showed significant positive correlation between GP73 and tumor size

**Table (4):** The relation between AFP & GP73 levels and PV thrombosis or LN enlargement in GI

Variable	Portal vein		Mann Whitney U	P value
	Patent(n=38)	Thrombosis(n= 10)		
<b>AFP (ng/ml)</b> X±SD Range	743.84±1014.92 19 – 2800	878.5±1001.46 10 – 2850	0.56	0.58
<b>Gp73 (ng/l)</b> X±SD Range	1660.63±726.35 815 – 3515	2186.0±681.76 895 – 2915	2.07	0.04
	Lymph nodes enlargement			
	LN enlargement (n=6)	No LN enlargement (n= 42)		
<b>AFP (ng/ml)</b> X±SD Range	1455.0±1438.95 49 – 2850	674.31±907.01 19 – 2800	1.28	0.20
<b>Gp73 (ng/l)</b> X±SD Range	2487.67±455.41 1781 - 3515	1662.81±727.29 810 – 2995	2.56	0.01

**Table (5):** The relation between Child classification and AFP & GP73 in GI & GII

Variable	Child-Pugh classification in GI			Kruskal Wallis Test	P value
	A (n= 5)	B (n=11 )	C (n= 32)		
<b>AFP(ng/ml)</b> X±SD Range	1510.80±1355.54 13 – 2750	536.45±790.44 7 – 2750	737.37±988.75 19 – 2850	0.64	0.72
<b>Gp73 (ng/l)</b> X±SD Range	986.0±132.64 895 – 1220	1661.45±785.76 895 – 3515	1929.94±709.9 815 – 2915		
	Child-Pugh classification in GII				
	A (n= 3)	B (n= 6)	C (n= 11)		
<b>AFP (ng/ml)</b> X±SD Range	26.33±29.16 9 – 60	53.17±33.82 13 – 90	18.36±13.65 1 – 44	3.93	0.14
<b>Gp73 (ng/l)</b> X±SD Range	703.0±96.16 645 – 814	776.5±44.13 699 – 815	789.18±75.96 655 – 890		

**ROC curve of AFP and GP73 for diagnosis of HCC versus cirrhotic cases****Figure (2) :** ROC curve of AFP and GP73 for diagnosis of HCC versus cirrhotic cases.



**Table (6):** Diagnostic performance of AFP and gp73 in diagnosis of HCC

Variable	AFP	GP73	Both
AUC	0.88	0.98	---
P Value	<0.001	<0.001	---
Cutoff	55	847.5	----
Sensitivity (%)	81.3	93.8	95.8
Specificity (%)	70.0	90.0	70.0
Positive predictive value (%)	86.7	95.7	88.5
Negative predictive value (%)	60.9	85.7	87.5
Accuracy (%)	77.9	92.6	88.2

## DISCUSSION

Regarding age distribution in HCC group, the present study showed that age was ranging from 43 to 76 years with mean age of the patients  $57.85 \pm 7.23$ . This was in agreement with Amer et al. [8] who reported that mean age of the patients with HCC was  $54 \pm 8.6$  years, while Yang and Roberts [9] found HCC to be more frequent in individuals of an average age of about 64 years and El-Serag [10] reported that HCC is rare before 40 and peaks around age 70. The endemicity of HCV infection in Egypt, in addition, other contributing environmental factors such as the presence of aflatoxin in many food stuff, and contamination with insecticides may explain the occurrence of HCC at younger age groups [11].

Regarding the gender distribution, the present study showed that HCC is more prevalent in men than in women with 3.8 times higher in men, these results were in agreement with El-Serag [10] who reported that there was a striking male HCC predominance, with the highest male: female ratios (averaging between 2:1 and 4:1) in high HCC incidence areas. Male sex predominance of HCC may be attributed to sex hormones and sex-specific differences in exposure to risk factors. Men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes, and have increased iron stores. However, experiments show a 2 - 8 fold increase in HCC development in male mice. These data support the hypothesis that androgens influence HCC progression rather than sex-specific exposure to risk factors [10]. Several studies conducted in Taiwan reported a positive association between increased circulating testosterone levels and HCC in HBV-infected men [12].

In the present study, Chronic HCV was the commonest etiology of cirrhosis in HCC group

(85.4%), HBV was less frequent (8.3%) and coinfection with both viruses in (6.3%), this was in agreement with Amer et al. [8] who found that the major risk factor for cirrhosis and the subsequent development of HCC was chronic hepatitis C (67.7%) and to a lesser extent chronic hepatitis B (8.8%) and coinfection with both viruses (4.8%). The major factors increasing the risk of HCC are chronic hepatitis B and C as well as cirrhosis, irrespective of its etiology. In North America, Europe, and other areas of low prevalence of HCC, most patients have underlying cirrhosis unrelated to HBV or HCV infection [13]. The etiological contribution of viral hepatitis toward the risk of HCC differs in different countries according to the prevalence of viral infection and other causes of liver cirrhosis. Kumar et al. [14] found that chronic HBV infection is the major factor for the development of HCC in China.

As regards the relation between HCC and the severity of cirrhosis, the present study revealed that about two thirds of HCC patients (66.7%) were Child C cirrhosis, 22.9% of the patients were Child B and 10.4% were Child A liver cirrhosis). These results were in agreement with Amer et al. [8] who found that 54.65% of HCC patients were Child C cirrhosis and HCC occurred least frequently in patients with Child A liver cirrhosis (15.4%).

The present study revealed a higher level of AFP in both cirrhotic and HCC groups than the control group and it is significantly higher in HCC group when compared to cirrhotic and control groups. AFP levels in cirrhotic group ranged from 1 to 90ng/ml and in HCC group it was 1.9–2850ng/ml. The value of AFP in diagnosis of HCC was variable in different studies. Chan et al. [15] found elevated serum AFP levels not more than 200ng/ml in patients with benign liver conditions such as hepatitis and

cirrhosis. Also Yoshida et al. [16] reported normal AFP levels in approximately one-third of patients with HCC a large number of HCC patients have AFP values <400 ng/mL, making them very difficult to undergo detection and prognosis of HCC. Similarly, Tsai et al. [17] observed that an AFP level less than 400ng/ml was noted in 51% of HCC patients, furthermore, at least one third of small HCC and up to 30% of advanced HCC will be missed unless other diagnostic tools are used. In addition, AFP may be elevated in non-malignant liver diseases, so, it is obvious that AFP alone is not a reliable indicator for detection and prognosis of HCC.

In our study, statistical analysis revealed that there was highly significant increase in mean values of serum GP73 in HCC group in comparison with other groups and in cirrhotic group in comparison with control group. GP73 in HCC group ranged 810 – 3515 ng/l and in cirrhotic group ranged 635-890 ng/l while in control group it was 350 - 665. These results were in agreement with Mao et al. [18] have found that the elevation of serum GP73 is mildest in chronic viral hepatitis, moderate in patients with cirrhosis and dramatic increased in patients with HCC. Similarly Tian et al. [19], El Shafie et al. [20] and Wang et al. [21] reported that, serum GP73 in HCC was higher than in liver cirrhosis and chronic hepatitis and in all patients were higher than those in healthy individuals. On the other hand Ozkan et al. [22] and Shi et al. [23] failed to find significant elevation of serum GP73 in HCC groups compared with that in liver cirrhosis groups and that the potential clinical value of GP73 as a better serum biomarker than AFP remains controversial.

The relation between elevation of serum GP73 in patients with HCC and the etiology of liver cirrhosis couldn't be assessed in the present study because of the small number of patients with etiology other than HCV (only 4 patients with HCC have chronic HBV infection), although it was found that serum GP73 was increased in HCC that developed on top of cirrhosis caused by either HCV or HBV. Previous study reported that 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]. Similarly Riener et al. [25] found significant (3- to 5-fold) increases the serum GP73 levels were seen in HCC patients with underlying HCV infection (especially HCV genotype 1b) when compared with patients with

HCC unrelated to HCV. However Mao et al. [26] showed that serum GP73 levels in HCC patients with underlying HBV infection were significantly higher than those of the HBV carriers, patients with liver cirrhosis, and healthy controls. They reported that serum GP73 had a higher sensitivity and specificity in diagnosis of hepatitis B-related HCC than AFP, and that it could be as an effective HCC tumor marker in Chinese Patients.

In the current study there was no significant difference in mean values of AFP as regard tumor size however Ba et al. [27] reported that serum AFP levels have been shown to correlate with tumor size. There was significant increase in mean values of serum GP73 in patients with HCC with increased tumor size (more than 5cm), this was in agreement with Sun et al. [28]. In contrast, Ozkan et al. [22] and Mao et al. [18] reported that serum levels of GP73 were not correlated with tumor sizes.

In the current study there was significant increase in mean value of serum GP73 in patients with HCC complicated by portal vein thrombosis while there was no significant difference as regards mean value of AFP. These results were similar to those obtained by El Shafie et al. [20] who reported that the level of GP73 correlated with more aggressive tumor characters including vascular invasion with no significant difference as regards mean value of AFP, On the other hand Ozkan et al. [22] reported that there was no correlation between GP73 levels portal vein thrombosis. In the current study there was significant increase in mean value of serum GP73 in patients with HCC associated with lymph nodes, this was in agreement with Fimmel and Wright [29] who recorded that, the degree of GP73 expression correlated with lymph nodes invasion while Ozkan et al. [22] did not find significant correlation.

The present study revealed significant increase in the mean values of GP73 in HCC group in relation to the severity of liver cirrhosis (the highest value was in patients with Child-Pugh grade C. This was in agreement with El Shafie et al. [20] who found significant correlation between serum GP73 level and child score in HCC patients on the other hand Mao et al. [18] reported that serum levels of GP73 in patients with HCC were not correlated with Child-Pugh grades (A, B,C). our study revealed no significant difference in mean values of GP73 as regard Child-Pugh grades (A, B, C) in the cirrhotic group, on the



other hand Tian et al. [19] reported that, serum GP73 in patients with Child-Pugh class A was lower than in class B and C in liver cirrhosis.

In the current study the area under AUROC for AFP was 0.88 and at cut off point 55 ng/ml, the sensitivity of AFP was 81.3% and specificity was 70.0%. Trevisani et al. [30] reported that specificity of AFP varied from about 76% to 96% and increased with elevated cutoff value, also El Shafie et al. [20] found that AFP had a sensitivity of 77.4% and a specificity of 60% at a cut-off 28.51 ng/ml.

In our study the area under AUROC for GP73 was 0.98 with sensitivity 93.8% and specificity 90.0% at cut off point 847.5ng/l. Marrero and Lok[31] postulated that, GP73 is up-regulated in HCC and measurement of serum GP73 revealed a sensitivity and specificity of 69% and 75%, respectively. Also El Shafie et al. [20] postulated that GP73 had a sensitivity of 87% and a specificity of 95% at the optimal cut-off value of 7.62 ng/ml. These results were disappointing with Gu et al. [32] who found that GP73 elevated in patients with liver disease but did not distinguish between HCC, cirrhosis, and chronic hepatitis. Riener et al. [25] reported GP73 was surprisingly found to be decreased in HCC patients and doubt on the diagnostic utility of GP73 as a serum marker of HCC.

In our study we found that with combined use of AFP and GP73 there was significant increase in sensitivity of detection of HCC up to 95.8% than using either of them alone, these results were in agreement with Wang et al. [33] and Mao et al. [18] who reported that the combined measurement of GP73 and AFP can further increase the sensitivity for the detection of HCC, also Omran et al. [34] reported that by combining serum GP73 and AFP for the diagnosis of HCC, it was found that sensitivity rises to 93% and El Shafie et al. [20] reported that when GP73 used in combination with AFP, they lead to an enhanced sensitivity of detection of HCC up to 90.3%.

## CONCLUSION

The sensitivity, accuracy and negative predictive value for diagnosis of HCC in cirrhotic patients increased to 95.8%, 88.2% and 87.5% respectively with combined assay of serum GP73 and serum AFP. Serum GP73 levels correlated positively with tumor size, portal vein thrombosis and lymph node involvement in cirrhotic patients with HCC.

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**Ethical approval:** Was granted by the Institutional Review Board and informed consent was obtained from each patient prior to inclusion in the study.

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