Assessment the level of Golgi protein 73 and Clusterin among Egyptian patients for detection of Hepatocelluar Carcinoma

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Abstract: Objective: The aim of this study was to assess the level of Golgi protein 73 and clusterin among Egyptian patients for detection of HCC. In addition, the levels of Golgi protein 73 and clusterin are evaluated after surgical resection of hepatocellular carcinoma. **Background:** Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is the third leading cause of cancer-related deaths worldwide. Golgi protein 73, is a resident Golgi-specific membrane protein expressed by biliary epithelial cells in normal liver, and its expression is increased markedly in chronic liver diseases, especially in HCC cells serum clusterin is promising biomarker for detection of HCC. **Materials and methods**: The study was conducted on 76 patients selected from the Hepatology Department of National Liver Institute, Menoufyia University. The patients were divided into 3 groups: Group I (hepatocellular carcinoma (HCC)) included 38 patients (10 of them were followed after hepatectomy to detect level of GP73 and clusterin after treatment); Group II (Chronic liver diseases (CLD)) included 25 patients with either liver cirrhosis or hepatitis; Group III served as the control group and consisted of 13 apparently healthy subjects. Clinical examination, abdominal ultrasonogrphy, and triphasic CT to patients with focal lesion were performed. Liver function tests, serum AFP, serum GP73 was measured using ELISA method. Hepatitis markers and clusterin were determined by ELISA Kit. **Results**: There was highly statistically significant difference in GP73 and clusterin between the HCC group and the CLD group ($P < 0.001$). There was highly statistically significant difference in GP73 and clusterin between the HCC group and the control group ($P < 0.001$). There was no statistically significant difference in GP73 and clusterin between the CLD group and the control group ($P > 0.05$). Moreover, GP73 and clusterin were significantly lower after hepatectomy. For discrimination of the HCC group from the healthy control, ROC curve showed that the serum GP73 levels had the AUROC of 0.89 (95% CI: 0.81–0.98) and a sensitivity of 76.3%, specificity of 92.3%, the diagnostic accuracy was 80.4%, positive predictive value was 96.7%, negative predictive value was 57.1% at cut off point 192ng/L. On the other hand, clusterin level had AUROC of 0.99 (95% CI: 0.99–1.01) and a sensitivity of 97.4%, specificity of 100%, the diagnostic accuracy was 98%, positive predictive value was 100%, negative predictive value was 92.9% at cut off point 105.45 ng/L. For discrimination of the HCC group from the chronic liver disease cases, ROC curve showed that the serum GP73 levels had the AUROC of 0.88 (95% CI: 0.79–0.96) and a sensitivity of 76.3%, specificity of 84%, the diagnostic accuracy was 83.3%, positive predictive value was 87.8%, negative predictive value was 70% at cut off point 195ng/L. On the other hand, clusterin level had AUROC of 0.97 (95% CI: 0.93-1.0) and a sensitivity of 84.2%, specificity of 92%, the diagnostic accuracy was 87.3%, positive predictive value was 94.1%, negative predictive value was 79.3% at cut off point 127.5ng/L. **Conclusion**: clusterin expression is highly increased in HCC patients. Its diagnostic performance is superior to that of GP73. Moreover GP73 and clusterin are useful markers for follow up of HCC patients after surgical hepatectomy. [Waleed Mohamed Fathy, Dalia Abo-Elela and Osama Hegazy. **Assessment the level of Golgi protein 73 and Clusterin among Egyptian patients for detection of Hepatocelluar Carcinoma.** *J Am Sci* 2015;11 (11): 189-197]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 20. doi: 10.7537/marsjas111115.20.

Key words; Serum Golgi protein 73, clusterin, α-fetoprotein, HCC.

1. Introduction:

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is the third leading cause of cancer-related deaths worldwide[1]. In Egypt, the overall frequency of HCC is 2.3% among other types of cancer. Over a decade, there was nearly a twofold increase in the proportion of HCC among chronic liver disease patients in Egypt, where 48% of HCC cases were attributed to hepatitis C virus (HCV) related liver cirrhosis. In fact, it has now become widely accepted that HCC nearly exclusively arises in chronic HCV after cirrhosis is established[2].

The global distribution varies by region due to factors at the origin of the disease. HCC is an end result of some chronic infections with the hepatitis B (HBV) or the hepatitis C (HCV)[2, 3]. Treatment options for HCC are very limited, as it is often being diagnosed at a late stage[4]. Laboratory diagnosis of HCC is established either by measurement of circulating biomarkers or by fine-needle cytology which is invasive[5].

The American Association for the Study of Liver Diseases (AASLD) guidelines recommended that serum levels of AFP ≥ 200 ng/ml may be used instead of fine-needle cytology for diagnosis, especially in patients with liver cirrhosis[3]. Nevertheless, the diagnostic performance of AFP is moderate with a sensitivity of 39–65% and specificity of 76–94%, leaving about one-third of cases with early-stage HCC and small tumors (<3cm) undiagnosed. Meanwhile, increased serum AFP concentration in several other types of cancer, chronic hepatitis, and liver cirrhosis should be taken into consideration. Newer markers are needed to overcome these problems and allow the diagnosis of HCC at an earlier stage[1, 5].

Golgi protein 73, is a resident Golgi-specific membrane protein expressed by biliary epithelial cells in normal liver, and its expression is increased markedly in chronic liver diseases, especially in HCC cells[6].

clusterin (CLU) is a 449-amino acid, heterodimeric glycoprotein that is expressed and present in most body fluids. Functionally, CLU exerts a chaperone-like activity with action like small heat shock proteins, by binding to misfolded stressed proteins. In contrast to other heat shock proteins, it is present in the extracellular space, where its expression is altered in various diseases[6, 7, 8]. So far, CLU is thought to play diverse functions both cytoprotective and cytotoxic, thus resulting in conflicting results[8]. For example, its involvement in numerous physiological processes important for carcinogenesis has been reported, including apoptotic cell death, cell adhesion, tissue remodeling, cell cycle regulation, DNA repair, lipid transportation, membrane recycling and immune system regulation[9].

Cytoplasmic CLU immunostaining was noted to correlate with poor prognosis in patients with renal cell carcinoma[10], hepatocellular carcinoma[11], urothelial bladder carcinoma[12], and prostate adenocarcinoma[13]. Also increased expression of secreted CLU was associated with radioresistance, chemoresistance, and hormone resistance, making CLU a promising target for antitumor therapeutics[14]. Both preclinical and clinical phase studies demonstrated that inhibition of CLU expression using antisense oligonucleotides enhances the apoptosis induced by several chemotherapeutic treatments[9]. On the other hand, cytoplasmic CLU staining correlated with good prognosis in pancreatic adenocarcinoma and did not correlate with prognosis in breast carcinoma[15, 16].

Aim:

The aim of this study was to assess the level of Golgi protein 73 and clusterin among Egyptian patients for detection of HCC. In addition, the levels of Golgi protein 73 and clusterin are evaluated after surgical resection of hepatocellular carcinoma.

Patient and Methods:

The study was conducted on 76 patients selected from the Hepatology Department of National Liver Institute, Menoufyia University, and they were divided into three groups:

Group I hepatocellular carcinoma (HCC): This group included 38 patients. This group had 35 males and 3 females with a mean age ± SD of 52.68 ± 8.80. Ten of them were followed after hepatectomy to detect the level of GP73 and clusterin after treatment.

Group II Chronic liver diseases (CLD): They were classified into two subgroups:

A) Liver cirrhosis (n=15): This subgroup included 15 patients with liver cirrhosis. This group had 10 males and 5 females with a mean age ± SD of 51.68 ± 6.85.

B) Chronic hepatitis (n=10): This subgroup included 10 patients with chronic hepatitis. This group had 8 males and 2 females with a mean age ± SD of 51.68 ± 6.85.

Group III Control group: This group included 13 apparently healthy subjects served as a control group. This group had 10 males and 3 females with a mean age ± SD of 51.38 ± 9.91.

Patients were selected as regards the following exclusion criteria: none of the patients had bacterial or other viral infection, chronic renal damage, insulin-dependent diabetes mellitus (IDDM) and other malignant diseases. The patients undergoing interferon administration or immuno-suppressive therapy were also excluded from this study.

All patients and control groups were subjected to the following:
- Collection of full medical history thorough clinical examination.
- Abdominal ultrasonography, and ultrasound guided liver biopsy 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 laboratory investigations were done: liver function tests including: ALT, AST, serum albumin, total and direct bilirubin were done by using Synchron Cx 9 ALX Clinical Autoanalyzer from Beckman Coulter, USA. Prothrombin concentration was done on the Fibrintimer II (Behring, Germany) and INR.

Serum AFP was measured using the commercially available enzyme-linked immunosorbent assay (ELISA) Kit. ELISA method Kit supplied by Glory Science (Immunospec

Corporation, 7018 Owensmouth Ave. Suite 103 Canoga Park, CA, 91303).

Hepatitis markers (HBsAg, anti-HBc, and HCV antibody) were determination by direct sandwich assay using the ELISA Kit supplied by Adltis, Germany.

Determination of Golgi Protein 73 (GP73) was determined by using the commercially available enzyme-linked immunosorbent assay (ELISA) Kit supplied by Glory Science (Glory Science Co., 2400 Veterans Blvd. Suite 16 - 101, Del Rio, TX 78840, USA). The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Golgi Protein 73 (GP73) in samples. Add GP73 to monoclonal antibody enzyme well which is pre-coated with GP73 monoclonal antibody, incubation; then, add GP73 labeled antibodies and enzyme to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogenic solution, the color of the liquid changes into the blue, and by the effect of acid, the color finally becomes yellow. The chroma of the color and the concentration of the GP73 of sample were positively correlated. The concentration of GP73 in the samples was then determined by comparing the O. D. of the samples to the standard curve.

Estimation of serum clusterin using sandwich ELISA method was performed according to manufacturer's instructions (Human clusterin ELISA, Bouendor laboratories Ltd Modrice Czech Republic). All diluted samples, quality controls, were incubated in microtitration wells pre-coated with monoclonal antihuman clusterin antibody. After 10 minutes and washing, biotin-labeled second monocloncal antihuman clusterin antibody was added and incubated with captured clusterin for 60 minutes. After another washing, streptavidin-horseradish peroxidase (HRP) conjugate was added. After 30 minutes incubation and the last washing step, the remaining conjugated was allowed to read with substrate solution hydrogen peroxide and tetramethylbenzidine (TMB). The reaction was stopped by the dilution of acidic solution $(0.2MH_2SO_4)$ and absorbance of the resulting yellow product was measured spectrophotometrically at 450nm. The absorbance was proportional to the concentration of clusterin. A standard curve was constructed by plotting absorbance value versus clusterin concentration of standards, and concentrations of unknown samples are determined using this standard curve.

Statistical analysis:
Data are expressed as mean ± SD. The SPSS computer program version 12.0 was used for

statistical analysis. Chi-square test ($\chi2$) was used to study association between two qualitative variables. Mann-Whitney test (nonparametric test) was used to compare significance between two groups not normally distributed having quantitative variables. Kruskal-Wallis test (nonparametric test) was used to compare significance between three or more groups not normally distributed (if one or both variables are skewed) having quantitative variables. Wilcoxon signed-rank test was used for non-parametric statistical hypothesis test when comparing two related samples, matched samples, or repeated measurements on a single sample to assess whether their population mean ranks differ. 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.

Results:
Comparison between the three studied groups with regards to alpha fetoproteins, GP73, and clusterin were made. Table 1 revealed that there was highly statistically significant difference in AFP, GP73, and clusterin between the HCC group and the CLD group ($P < 0.001$). There was highly statistically significant difference in AFP, GP73, and clusterin between the HCC group and the control group ($P < 0.001$). There was no statistically significant difference in alpha fetoproteins, GP73, and clusterin between the CLD group and the control group ($P > 0.05$).

Correlations study between GP73 and the other studied parameters in Table 2 revealed highly significant positive correlations between GP73 and AST, ALT, AFP, and clusterin ($P <0. 001$), and significant positive correlations between GP73, ALP, and total and direct bilirubin. There is no significant correlation between GP73 and age, albumin, and INR

Correlations study between clusterin and the other studied parameters in Table 2 revealed highly significant positive correlations between clusterin and AST, ALT, AFP, and GP73 ($P < 0.001$) and significant positive correlations between clusterin and ALP. There is no significant correlation between clusterin and age, albumin, total and direct bilirubin, and INR.

Table 3 showed that the mean ± SD of AFP in the HCC cases before treatment was 44.76 ± 90.08. The mean ± SD of GP73 in the HCC cases before treatment was 270.0 ± 134.68. The mean ± SD of clusterin in the HCC cases before treatment was 194.09 ± 49.37.

The mean ± SD of AFP in the HCC cases after treatment was 12.80 ± 8.34. The mean ± SD of GP73 in the HCC cases after treatment was 143.0 ± 63.43.

The mean ± SD of clusterin in the HCC cases after treatment was 116.16 ± 35.20.

There were statistically significant differences in the level of AFP, GP73, and clusterin before and after treatment ($P < 0.001$).

For discrimination of the HCC group from the healthy control, ROC curve showed that the serum GP73 levels had the AUROC of 0.89 (95% CI: 0.81–0.98) and a sensitivity of 76.3%, specificity of 92.3%, the diagnostic accuracy was 80.4%, positive predictive value was 96.7%, negative predictive value was 57.1% at cut off point 192ng/L. AFP had an AUROC of 0.83 (95% CI: 0.71–0.95) and a sensitivity of 73.7%, specificity of 61.5%, the diagnostic accuracy was 70.6%, positive predictive value was 84.8%, negative predictive value was 44.4%, at cut off point 10.5ng/ml. Clusterin level had AUROC of 0.99 (95% CI: 0.99–1.01) and a sensitivity of 97.4%, specificity of 100%, the diagnostic accuracy was 98%, positive predictive value was 100%, negative predictive value was 92.9% at cut off point 105.45 ng/L.

For discrimination of the HCC group from the chronic liver disease cases, ROC curve showed that the serum GP73 levels had the AUROC of 0.88 (95% CI: 0.79–0.96) and a sensitivity of 76.3%, specificity of 84%, the diagnostic accuracy was 83.3%, positive predictive value was 87.8%, negative predictive value was 70% at cut off point 195ng/L. AFP had an AUROC 0.83 (95% CI: 0.72–0.93) and a sensitivity of 73.7%, specificity of 68%, the diagnostic accuracy was 71.4%, positive predictive value was 77.8%, negative predictive value was 63% at cut off point 10.5ng/ml. Clusterin level had AUROC of 0.97 (95% CI: 0.93–1.0) and a sensitivity of 84.2%, specificity of 92%, the diagnostic accuracy was 87.3%, positive predictive value was 94.1%, negative predictive value was 79.3% at cut off point 127.5ng/L.

Table (1): Comparison between the three studied groups as regards alpha fetoproteins, GP73 and clusterin:

	The studied groups			**Mann Whitney**	*P* value
	HCC Case **N = 38**	**CLD** **& Cirrhotic cases** **N = 25**	**Control** **N = 13**		
AFP (ng/ml) X ± SD	212.11 ± 243.39	25.57 ± 72.30	9.28 ± 5.54	4.36 3.49 0.05	<0.001[1] <0.001[2] 0.96[3]
GP73 (ng/L) X ± SD	382.34 ± 292.22	154.0 ± 67.87	152.23 ± 36.96	5.01 4.20 0.43	<0.001[1] <0.001[2] 0.67[3]
clusterin (ng/L) X ± SD	168.24 ± 42.51	92.93 ± 18.77	89.29 ± 11.06	6.25 5.32 0.17	<0. 001[1] <0. 001[2] 0. 87

1 = comparison between HCC group and CLD group 2 = comparison between HCC group and control group.
3 = comparison between CLD group and control group *P* < 0. 05 = significant   *P* >0. 05 = non significant   n=number.
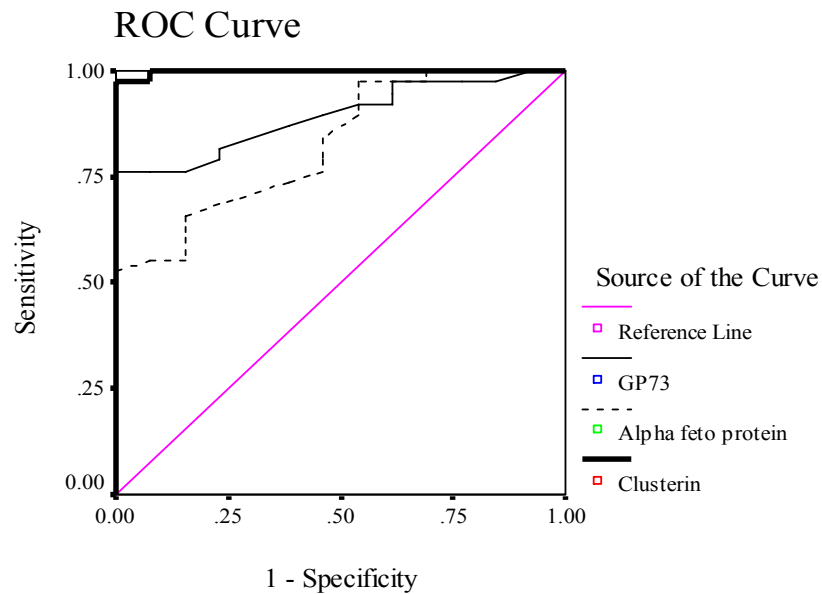
Table (2): Spearman's Rank Correlation between both GP73 and clusterin and other parameters

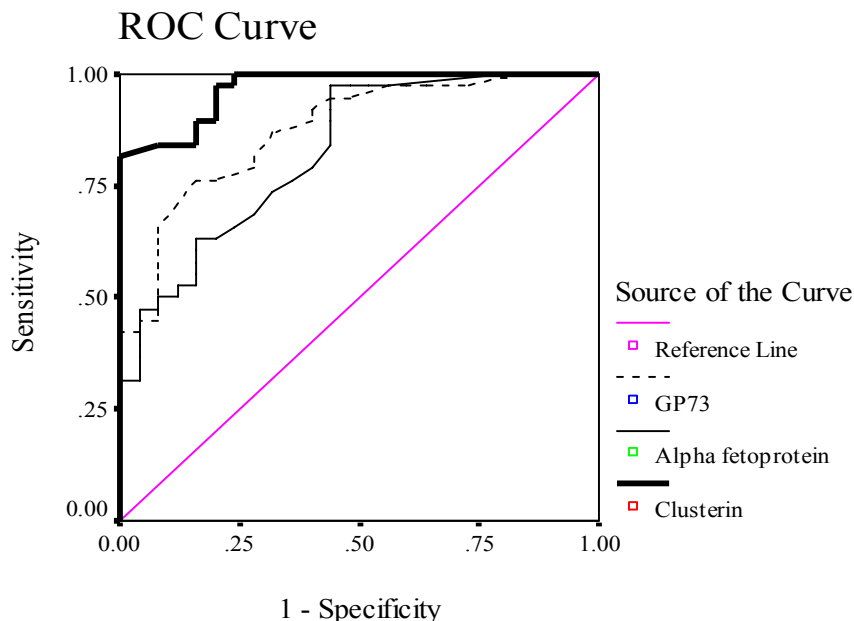|	**GP73**		**clusterin**	
	Correlation coefficient	P value	Correlation coefficient	*P* value
Age	+ 0.04	0.74	+ 0.02	0.84
AST	+ 0.40	<0.001	+ 0.39	<0.001
ALT	+ 0.36	0.001	+ 0.39	<0.001
ALP	+ 0.29	0.01	+ 0.31	0.006
Total bilirubin	+ 0.30	0.008	+ 0.18	0.13
Direct bilirubin	+ 0.31	0.007	+ 0.20	0.09
Albumin	- 0.15	0.18	- 0.07	0.53
INR	+ 0.18	0.11	+ 0.09	0.45
Alpha fetoprotein	+ 0.40	<0.001	+ 0.46	<0.001
GP73	1.0	-----	+ 0.65	<0.001

P < 0. 05 = significant   *P* >0. 05 = non significant   n=number

Table (3): Comparison of alpha fetoprotein, GP73, and clusterin in a group of HCC cases before and after treatment

	HCC cases		Wilcoxon signed rank	P value
	Before treatment N = 10	After treatment N = 10		
AFP (ng/ml) X ± SD	44. 76±90. 08	12. 80±8. 34	2. 81	0. 005
GP73 (ng/L) X ± SD	270. 0±134. 68	143. 0±63. 43	2. 80	0. 005
clusterin (ng/L) X ± SD	194. 09±49. 37	116. 16±35. 20	2. 80	0. 005

$P < 0. 05$ = significant $P > 0. 05$ = non -significant n=number

ROC Curve

Diagonal segments are produced by ties.

Fig. (1): ROC curve of alpha feto protein and GP73 for discrimination of HCC from healthy control cases

Table (4): Diagnostic performance of AFP, GP73, and clusterin for discrimination of the HCC group from the healthy control group

	Alpha feto protein	GP73	clusterin
AUC	0.83	0.89	0.99
P value	<0.001	<0.001	<0.001
95% CI	0.71–0.95	0.81–0.98	0.99–1.01
Cut off point	10.5	192	105.45
Sensitivity	73.7%	76.3%	97.4%
Specificity	61.5%	92.3%	100%
+ve predictive value	84.8%	96.7%	100%
-ve predictive value	44.4%	57.1%	92.9%
Accuracy of the test	70.6%	80.4%	98%

ROC Curve

Diagonal segments are produced by ties.

FIG (2): ROC curve of alpha feto protein, GP73 and clusterin for discrimination of HCC from chronic liver disease cases

Table (5): Diagnostic performance of AFP, GP73, and clusterin for discrimination of HCC from chronic liver disease cases

	Alpha feto protein	GP73	clusterin
AUC	0. 83	0. 88	0. 97
P value	<0. 001	<0. 001	<0. 001
95%CI	0. 72 – 0. 93	0. 79 – 0. 96	0. 93 – 1. 0
Cut off point	10. 5	195	127. 5
Sensitivity	73. 7%	76. 3%	84. 2%
Specificity	68%	84%	92%
+ve predictive value	77. 8%	87. 8%	94. 1%
-ve predictive value	63%	70%	79. 3%
Accuracy of the test	71. 4%	83. 3%	87. 3%

4. Discussion:

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is the third leading cause of cancer-related deaths worldwide. Treatment options for HCC are very limited, as it is often diagnosed at a late stage[1].

Therefore, the use of AFP as a primary screening test for HCC has been questioned, so more sensitive and specific serum biomarkers for HCC are desired[4].

Studies have identified Golgi protein 73 (GP73; also named Golgi phosphoprotein 2 (GOLPH2)), as a HCC serum marker. GP73 is an 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[7].

Although upregulated GP73 was initially identified in hepatic viral infections with unknown function[11]. Subsequent studies showed that the GP73 serum level is elevated in viral and non-viral liver diseases, including hepatitis, cirrhosis and HCC, and also in non-liver malignancies[11].

clusterin (CLU) is a chaperone that inhibits protein aggregation and precipitation, otherwise induced by physical or chemical stresses. CLU is a protective molecule by helping cells to cope with stress condition in cancer cells. Many studies demonstrated that CLU conferred resistance to anti-cancer agents in many kinds of cancer[22]. Although number of reports has purported to explain clusterin functions in various cell types and tissue, including senescent and cancer cells, an understanding of

clusterin function has remained elusive, especially in term of apoptosis and tumorigenesis[8].

The present study revealed that was high statistically significant difference between HCC group and control group regarding GP73 and clusterin (P<0.001) and also there was high statistically significant difference between HCC group and CLD group regarding GP73 and clusterin (P<0.001).

This was supported by Marrero et al. who reported that sGP73 levels were significantly increased in patients with HCV– related HCC in comparison with cirrhotic controls[6].

Mao and colleagues showed that the serum GP73 levels in HCC patients that were HBV positive were significantly higher than those of the HBV carriers, patients with non liver diseases, and healthy control[11].

Tian et al. reported that serum GP73 in HCC was higher than in chronic liver diseases and in all two groups were higher than those in healthy individuals[12].

The study of Hou et al. demonstrated that patients with HCC exhibit markedly higher levels of GP73 in the serum compared with patients with chronic hepatitis, liver cirrhosis and healthy controls[21].

This result agreed with Ramadan et al., who found higher serum clusterin in the HCC group than control &HCV related liver cirrhosis denoting its role in carcinogensis[18].

Nafee et al. reported a significant rise in serum clusterin in viral related HCC patients[19].

Also Kang et al., with the use of a tissue microarray method, examined clusterin overexpression immunohistochemically in surgically resected HCCs[20], and found that HCCs exhibited clusterin overexpression. These findings lead us to hypothesize that clusterin secretion occurs from tumor cells in HCC and is reflected in its serum level.

Wang et al. reported that serum clusterin level in HCC patients were significantly lower than those with chronic hepatitis and healthy subjects, but it was higher than in those with cirrhosis this result disagreed to our results[22].

Additionally, there was significant decrease of the level of GP73 and clusterin in group HCC cases before and five days post hepatectomy (p<0.05)[22].

Mao et al. showed that in a few HCC patients, the GP73 levels were not markedly lower a week after surgical resection, but became lower 1.5 to 2 years after surgery. However, AFP levels usually decrease substantially within a week post-resection. This result demonstrates that serum GP73 levels change slower than serum AFP levels[11].

Results of this study is in agreement with Mao et al., which demonstrated that Serum GP73 values were monitored after surgical resection of tumors in patients with HCC[3]. GP73 in patients with HCC dropped dramatically following hepatectomy. Serum GP73 dropped after hepatectomy and returned to high levels after HCC relapse between 3 and 18 months. So surgical resection of the tumor results in diminished serum GP73 levels and that tumor recurrence correlates with the recurrence of elevated GP73 in the blood. Reappearance of serum GP73 indicates the existence of tumor lesions and thus may serve as an indicator for the recurrence of HCC.

In the present study there were high positive correlations between GP73 and clusterin and both AST and AFP (P<0.001), and significant positive correlations between GP73 when compared to ALT, ALP, and total and direct bilirubin. Additionally, there is no significant correlations between GP73 and age, albumin, and INR.

Analysis of clusterin showered that there were significantly positive correlations between clusterin when compared to ALT, AST, AFP and GP73. There were positive correlations between clusterin and ALP. However, there were no significant correlations between clusterin and age, direct, total bilirubin, albumin and INR.

This in agreement with the finding of Tian et al., 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[12].

Furthermore, El–Shafie et al. revealed that a significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and child score)[4].

Results of this study disagreed with Nafee et al., who reported that there was no correlation between serum clusterin and the degree of deterioration of functional liver status with advancement of Child-Pugh score, which indicated that increased serum clusterin levels in HCC patients could be related to the process of carcinogenesis rather than cirrhosis or fibrosis[19].

This study showed that serum GP73 levels were significantly higher in patients with HCC compared to those with chronic liver disease patients. Serum GP73 levels had the AUC of 0.88 (95% CI: 0.79–0.96) and a sensitivity of 76.3%, specificity of 84%, while, AFP with an AUC 0.83 (95% CI: 0.72–0.93) and a sensitivity of 73.7%, specificity of 68% at cut off point 195ng/L and 10.5ng/ml for GP73 and AFP respectively. This demonstrating the utility of GP73 in the diagnosis of HCC in patients with normal or mildly elevated AFP, and the performance of GP73

as determined by the AUC was found to be better than AFP.

These findings are in agreement with Marrero *et al.*, which showed that serum GP73 levels were significantly higher in patients with HCC compared to those with cirrhosis[6]. Serum GP73 levels had the AUC of 0.79 (95% CI: 0.72–0.82) and a sensitivity of 69%, specificity of 75%, while, AFP with an AUC 0.61 (95% CI: 0.59–0.71) and a sensitivity of 30%, specificity of 96%.

Mao *et al.* reported that serum GP73 had a higher sensitivity and specificity in diagnosis of hepatitis B-related HCC than AFP, and that it could be a new effective HCC tumor marker in Chinese Patients[11].

Tian *et al.* reported that GP73 had a sensitivity of 75.8% and specificity of 79.7% with the AUC of 0.844 versus 0.812 for AFP with a sensitivity of 95.2% and specificity of 47.1%; in detecting early HCC, the AUC of AFP/GP73 was 0.804 versus 0.766 for AFP alone[12].

Furthermore, El Shafie demonstrated that 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 (AUC) was 0.87[4].

This study showed that serum clusterin levels were significantly higher in patients with HCC compared to those with chronic liver disease patients. Serum clusterin levels had the AUC of 0.97 (95% CI: 0.93–1.0), a sensitivity of 76.3%, and specificity of 84%, respectively.

Nafee *et al.* showed that the sensitivity and specificity of serum clusterin in differentiation of HCC patients from cirrhosis were 90% and 87%, respectively, using a cutoff value of 128 ug/ml, while cutoff value of 100 ng/ml AFP had 75% sensitivity and 80% specificity[19].

Our results also agreed with Wang *et al.*, who found that at a cutoff value 50 ug/ml serum clusterin had 91% sensitivity and 83% specificity[22]. The area under receiver operating characteristic curve (AUC) was (0.937) for serum clusterin and (0.85) for AFP.

Conclusion:

Serum level of clusterin and Gp73 are highly increased in the HCC patients in comparison to either the benign liver diseases or the healthy controls. . Gp73 and clusterin are valuable serum markers for follow up of HCC patient after surgical treatment. Clusterin has a better diagnostic performance than AFP and Gp73 for detection of HCC. Finally, in addition to AFP, measurement of serum GP73 and clusterin can further improve the diagnosis and

follow up of HCC which is one of the most serious malignancies all over the world.

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