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# Serum Level of Golgi Protein 73 as a Tumor Marker in Patients with Hepatocellular Carcinoma and Hepatitis C Virus-Related Liver Cirrhosis

**Roqaya Salman[1], Huda Mohammed El Sayed[2], Omar Heikal[1], Shahinaz El Attar[3] and Fatma M Kotb[2]**

**[1]Department of Internal Medicine, Faculty of Medicine, Misr University for Science and Technology, Cairo, Egypt.**
**[2]Department of Internal Medicine, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.**
**[3]Department of Medical Biochemistry, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.**

***E-mail: roqayasalman91@gmail.com**

## Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors with a low survival rate. Screening of early HCC diagnosis is the greatest challenge & thus decrease HCC related morbidity & mortality. Golgi protein 73 (GP73), a resident Golgi type-II membrane protein, is often upregulated in hepatocytes in different liver diseases. The aim of the paper is to study the usefulness of serum GP73 as a biomarker of HCC and hepatitis C virus (HCV) related liver cirrhosis. This study was conducted on 90 individuals who attended the Gastroenterology and Hepatology unit, (Department of Internal Medicine, Al-Zharaa University Hospital, Al-Azhar university) & Theodor Bilharz research Institute. The patients are divided into three groups: Group I: included 30 patients with HCC on top of liver cirrhosis. Group II: included 30 HCV positive patients with cirrhosis. Group III (Control group): included 30 HCV positive patients without cirrhosis. *Results:* highly statistically significant increase in serum GP73 in group I and II in comparison to control group and statistically significant increase in group I in comparison to group II. Serum GP73 can be used to discriminate between group I and II at a cutoff level of > 14, with 86.7% sensitivity 93.3% specificity and area under curve = 0.954. Also, it used to discriminate between group I and III at a cutoff level of 6, with 100% sensitivity & 100% specificity with area under curve = 1.0. GP73 is superior to Alpha-Fetoprotein (AFP) in early detection & diagnosing HCC.

**Keywords:** Golgi Protein 73, Hepatocellular Carcinoma, Hepatitis C Virus, Liver Cirrhosis.

## 1. Introduction

HCV is recognized as a major cause of HCC globally. HCC represents the sixth most common cancer worldwide. In Egypt, it represents the fourth common cancer and the most common cause of mortality- and morbidity-related cancer [1]. Globally, HCC surveillance includes both ultrasound and AFP level measurement [2] [3]. High level of AFP levels may be seen in patients with cirrhosis or exacerbation of chronic hepatitis. Some studies have indicated that AFP has substantially limited diagnostic

accuracy in detecting small HCC [4]. Therefore, a novel serum biomarker that exhibits superior diagnostic accuracy is required for diagnosing HCC [5]. Golgi protein 73, also called GP73, is a Golgi type II transmembrane protein, GP73 expression in biliary epithelial cells of normal livers, but found it was rarely expressed in hepatocytes. It has also been reported that GP73 expression is upregulated in HBV or HCV infection, alcoholic liver disease, or autoimmune hepatitis, and is found at strikingly high serum concentrations in HCC patients. Moreover, serum concentrations of GP73 in HCC patients are significantly higher than those in patients with liver cirrhosis [6]. The aim of this work is to study the usefulness of serum Golgi protein 73 (GP73) as a tumor marker with hepatocellular carcinoma (HCC) in relation to conventional marker α-fetoprotein (AFP).

## 2. Patients and Methods

This case control study was conducted during the period from December 2019 till October 2020 & included 90 participants divided into three groups **GI**: HCC on top of liver cirrhosis (n=30), **GII:** HCV with cirrhosis (n=30). **GIII (Control group**): HCV without cirrhosis (n=30). Samples were collected from AL-Zahra University Hospital (Internal Medicine Department) & Theodor Bilharz research Institute, and investigations were carried out at Medical Biochemistry Department, Faculty of Medicine for Girls, Al-Azhar University. This study was approved by the medical ethics committee in the faculty and informed consent was obtained from all participants. The IRB no was 201909139.

### 2.1 Inclusion criteria

adult >+18 year with positive HCV antibody.

### 2.2 Exclusion criteria

Patients with viral infection rather than HCV, Patients with another solid tumor, Diabetic with complications or known to have collagen disease.

### 2.3 All the studied groups were subjected to:

Full history taking**,** clinical examination, (CBC), Liver function tests (Serum transaminases, albumin, bilirubin), Kidney function tests which were done on Cobas C 3 11 auto analyzer using Roch Reagent Kits (Hitachi, Roch Diagnostics, Gmbh, Germany), Prothrombin concentration which (were done on Stago)**,** Viral Hepatitis markers {including HCV Ab and HBs Ag were done by ELISA using kits purchased from Human (Cat. no. 51275) (Cat. no. 51048) respectively}**,** HCV polymerase chain reaction (PCR), Alpha-fetoprotein (done by ELISA purchased from USCN Life Sience Inc. E91117Hu) & serum Golgi protein 73 also done by ELISA. Radiological investigations including abdominal ultrasound & Abdominal triphasic CT &/or MRI abdomen for detection of hepatosplenomegaly, liver cirrhosis, ascites, and hepatic focal lesions.

### 2.4 Measurement of GP-73 serum level:

Measured by a commercially available enzyme linked immunosorbent assay ELISA Kit for Human Golgi protein 73, supplied by SHANGHAI KORAIN BIOTECH CO., LTD. Cat.No.E1432Hu. Room 1713, (Block A), Junjiang Global Building, 218 Ningguo Rd, 200090 Yangpu District, Shanghai, China.

### 2.5 Assay Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human GP-73 in samples. GP-73 was added to monoclonal antibody enzyme well which was pre-coated with Human GP-73 monoclonal antibodies, incubation; then, GP-73 antibodies labeled with biotin was

added and combined with Streptavidin-HRP to form immune complex; then incubation and washing again were carried out to remove the uncombined enzyme. Then Chromogen Solution A, B were added, and the color of the liquid changed into the blue, at the effect of acid, the color finally become yellow. The color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of the Human GP-73 in the samples was then determined by comparing the Optical Density (OD) of samples to the standard curve.

### 2.6 Statistical analysis of results:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum, and maximum in quantitative data and using frequency and percentage for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests [7]. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [7]. Correlations between quantitative variables were done using Spearman correlation coefficient [7]. ROC curve was constructed with area under curve analysis performed to detect best cutoff value of AFP and Golgi 73 for detection of HCC. P-values <0.05 were considered statistically significant.

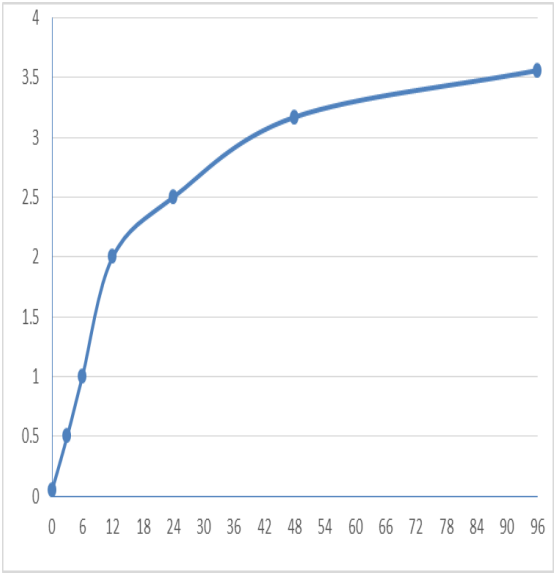


**Figure (1):** Standard curve for measuring Golgi 73.

## 3. Results

The demographic and clinical characteristics of the patients in this study are listed in Table 1. Table 2 illustrates the hematological and biochemical data of the patients.

**Table (1):** Demographic and clinical characteristics of the patients in this study.

| | Group 1 | | | Group 2 | | | Group 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Range** | **Mean** | **SD** | **Range** | **Mean** | **SD** | **Range** | **P value** |
| **Age (years)** | 63.30 | 7.68 | 50.00-77.00 | 61.80 | 8.23 | 46.00-78.00 | 59.13 | 7.19 | 41.00-72.00 | 0.247 |
| | | | **Conut** | **%** | **Count** | | **%** | **Count** | **%** | **P value** |
| **Sex** | **Male** | | 18 | 60.0% | 16 | | 53.3% | 15 | 50.0% | 0.731 |
| | **Female** | | 12 | 40.0% | 14 | | 46.7% | 15 | 50.0% | |

**P-value<0.05 Statistically significant & P-value >0.05 statistically non-significant.**

**Table (2):** Comparison between the studied groups as regards laboratory investigation.

| | Group 1 | | | Group 2 | | | Group 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Range** | **Mean** | **SD** | **Range** | **Mean** | **SD** | **Range** | **P value** |
| **HB (g/dl)** | 10.73 | 2.48 | 6.10-15.80 | 9.65 | 2.33 | 6.20-16.20 | 12.77 | 1.51 | 9.70-15.30 | < 0.001 |
| **RBC count (mill/cmm)** | 4.03 | 0.87 | 2.50-5.80 | 3.69 | 0.89 | 2.50-5.90 | 4.75 | 0.64 | 3.90-5.90 | < 0.001 |
| **TLC (*103/iu)** | 8.35 | 4.50 | 2.90-22.20 | 6.92 | 3.94 | 2.30-17.80 | 7.90 | 3.36 | 2.20-18.00 | 0.287 |
| **PLT *1000 (count x 103 iu)** | 114.54 | 100.69 | 18.00-227.10 | 110.26 | 54.39 | 38.00-265.00 | 210.57 | 124.02 | 33.00-410.00 | 0.008 |
| **AST (IU/L)** | 222.03 | 416.13 | 23.00-2285.00 | 64.50 | 46.06 | 19.00-246.00 | 25.20 | 13.12 | 13.00-84.00 | < 0.001 |
| **ALT (IU/L)** | 97.63 | 121.61 | 10.00-623.00 | 29.70 | 20.48 | 9.00-107.00 | 24.30 | 16.35 | 8.00-91.00 | < 0.001 |
| **ALP (IU/L)** | 369.63 | 145.77 | 178.00-690.00 | 211.33 | 93.15 | 70.00-420.00 | 124.30 | 60.51 | 49.00-275.00 | < 0.001 |
| **BIL T (mg/dl)** | 4.97 | 7.49 | 0.40-36.40 | 3.11 | 5.03 | 0.50-27.00 | 1.42 | 1.47 | 0.40-7.20 | < 0.001 |
| **BIL D (mg/dl)** | 2.81 | 4.58 | 0.20-20.80 | 1.55 | 3.10 | 0.10-17.03 | 0.68 | 0.82 | 0.20-4.10 | < 0.001 |
| **ALB (g/dl)** | 2.56 | 0.68 | 1.70-4.10 | 2.30 | 0.48 | 1.40-3.50 | 3.58 | 0.90 | 2.00-5.00 | < 0.001 |
| **PC %** | 53.57 | 18.79 | 22.00-100.00 | 55.91 | 17.62 | 24.00-89.00 | 90.87 | 8.18 | 59.00-100.00 | < 0.001 |
| **INR** | 1.73 | 0.64 | 1.00-3.40 | 1.61 | 0.49 | 1.00-2.94 | 1.04 | 0.08 | 1.00-1.40 | < 0.001 |
| **Urea (mg\dl)** | 100.00 | 84.95 | 13.00-333.00 | 90.82 | 73.86 | 17.00-309.10 | 26.60 | 11.00 | 16.00-76.00 | < 0.001 |
| **CREAT (mg\dl)** | 1.90 | 1.47 | 0.34-6.80 | 1.58 | 1.03 | 0.50-4.95 | 0.80 | 0.23 | 0.50-1.80 | < 0.001 |
| **AFP (ng/dl)** | 7156.50 | 13906.87 | 2.00-60800.00 | 11.69 | 14.01 | 0.80-68.00 | 4.94 | 2.23 | 1.34-8.80 | < 0.001 |
| **GOLGI 73 (ng/dl)** | 38.87 | 23.40 | 9.00-96.00 | 9.13 | 4.99 | 4.00-27.00 | 2.37 | 0.49 | 1.50-3.00 | < 0.001 |
| **PCR for HCV** | 155527.33 | 202592.07 | 90.00-800000.00 | 30182.39 | 16379.33 | 20.00-100000.00 | 88381.35 | 228615.90 | 70.00-800000.00 | 0.005 |

***P*-value<0.05 Statistically significant & *P*-value >0.05 statistically non-significant.**

Haemoglobin (Hb), red blood cells (RBC), platelet (PLT), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin total (BIL T), bilirubin direct (BIL D), albumin (ALB), prothrombin concentration (PC), international normalization ratio (INR), alpha fetoprotein (AFP)

**Table (3):** Multiple Comparisons between the studied groups as regards the CBC components, liver function test, AFP & GP73.

| | Group 1 vs 2 | Group 1 vs 3 | Group 2 vs 3 |
|---|---|---|---|
| **HB (g/dl)** | 0.192 | 0.003 | < 0001 |
| **RBC count (mill/cmm)** | 0.337 | 0.008 | < 0001 |
| **PLT *1000 (count x 103 iu)** | 0.315 | 0.422 | 0.006 |
| **AST (IU/L)** | 0.047 | < 0001 | < 0001 |
| **ALT (IU/L)** | 0.002 | < 0001 | 0.767 |
| **ALP (IU/L)** | 0.001 | < 0001 | 0.003 |
| **BIL T (mg/dl)** | 0.358 | < 0.001 | 0.040 |
| **BIL D (mg/dl)** | 0.610 | 0.002 | 0.097 |
| **ALB (g/dl)** | 0.878 | < 0.001 | < 0.001 |
| **PC %** | 1 | < 0.001 | < 0.001 |
| **INR** | 1 | < 0.001 | < 0.001 |
| **AFP (ng/dl)** | < 0.001 | < 0.001 | 0.295 |
| **GOLGI 73 (ng/dl)** | < 0.001 | < 0.001 | < 0.001 |

***P*-value<0.05 Statistically significant & *P*-value >0.05 statistically non-significant.**
Hemoglobin (Hb), red blood cells (RBC), platelet (PLT), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin total (BIL T), bilirubin direct (BIL D), albumin (ALB), prothrombin concentration (PC), international normalization ratio (INR), alpha-fetoprotein (AFP).

**Table (4):** Serum levels of Golgi 73 and AFP.

| | Area Under ROC Curve | P value | 95% Confidence Interval | | Cut off | Sensitivity% | Specificity% |
|---|---|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | | | |
| **AFP (ng/dl)** | 0.877 | < 0.001 | 0.784 | 0.971 | 23.5 | 80 | 86.7 |
| **Golgi 73(ng/dl)** | 0.954 | < 0.001 | 0.907 | 1.001 | 14 | 90 | 90 |

***P*-value<0.05 Statistically significant & *P*-value >0.05 statistically non-significant.**

**Table (5):** Diagnostic performance of Golgi 73 and AFP between GI and GII.

| | Area Under ROC Curve | P value | 95% Confidence Interval | | Cut off | Sensitivity% | Specificity% |
|---|---|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | | | |
| **AFP (ng/dl)** | 0.877 | < 0.001 | 0.784 | 0.971 | 43.5 | 76.7 | 96.7 |
| **Golgi 73(ng/dl)** | 0.954 | < 0.001 | 0.907 | 1.001 | 16 | 86.7 | 93.3 |

***P*-value<0.05 Statistically significant & *P*-value >0.05 statistically non-significant.**

**Table (6):** Diagnostic performance of Golgi 73 and AFP between GI and GIII.

| | Area Under ROC Curve | P value | 95% Confidence Interval | | Cut off | Sensitivity% | Specificity% |
|---|---|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | | | |
| **AFP (ng/dl)** | 0.926 | < 0.001 | 0.850 | 1.001 | 8.9 | 83.3 | 100 |
| **Golgi 73(ng/dl)** | 1.000 | < 0.001 | 1.000 | 1.000 | 6 | 100 | 100 |

**Table (7):** Diagnostic performance of Golgi 73 and AFP between GII and GIII.

| | Area Under ROC Curve | P value | 95% Confidence Interval | | Cut off | Sensitivity% | Specificity% |
|---|---|---|---|---|---|---|---|
| | | | Lower Bound | Upper Bound | | | |
| **AFP (ng/dl)** | 0.662 | 0.026 | 0.519 | 0.804 | 6.35 | 56.7 | 70 |
| **Golgi 73 (ng/dl)** | 1.000 | < 0.001 | 1.000 | 1.000 | 3.5 | 100 | 100 |

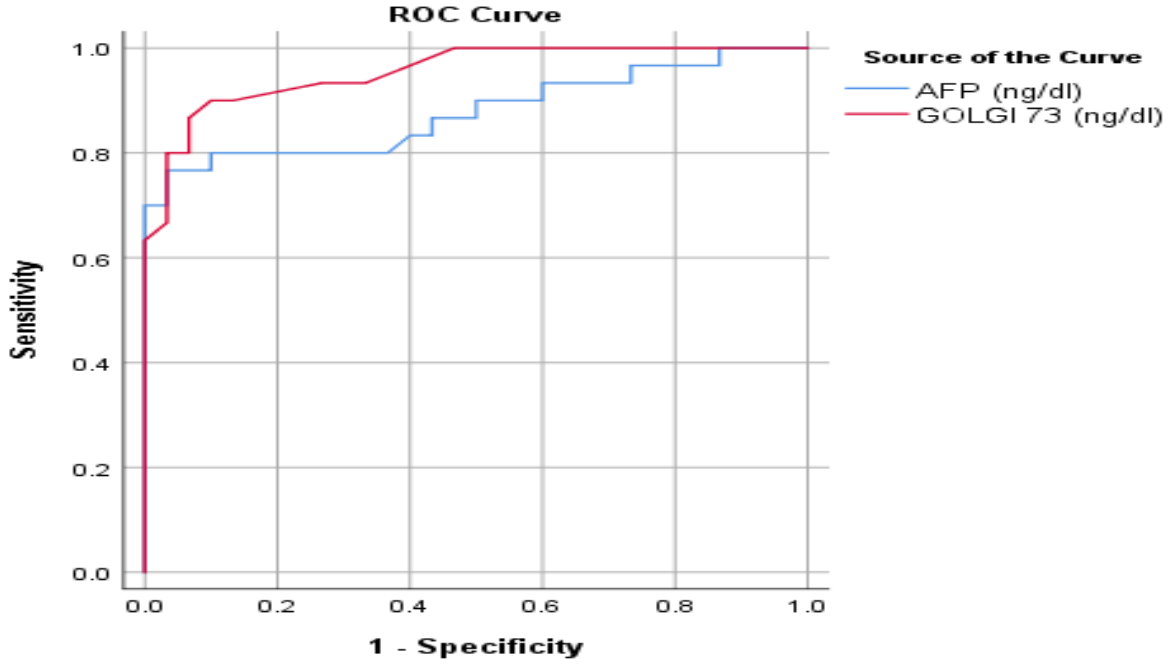


**Figure (2):** ROC curve detecting HCC using AFP (ng/dl) and Golgi 73 (ng/dl)..

## 4. Discussion

Hepatitis C virus infection leads to cirrhotic liver that may progress to HCC (Chen, 2003) [7]. HCC is the leading cause of cancer-related deaths worldwide with limited treatment options (Zhang et al. 2015.) [8]. This study was conducted to evaluate serum level of GP73 as a diagnostic biomarker for HCC & to clarify its sensitivity and specificity in HCC in relation to conventional marker AFP. In this study, regarding the PLT there was statistically significant difference among the studied groups, which came in agreement with Zekri et al [9], who reported statistically significant difference between HCC, cirrhotic, and non-cirrhotic patients as regard Platelets.

Comparing liver functions in the three groups; there was no significant difference as regard Bili (total, direct), Alb, INR in

HCC vs cirrhotic group while regarding AST, ALT & ALP there was statistically significant difference between GI and GII which came in agreement with El Shafie et al. [10]. Who reported that serum levels of AFP, DCP and GP73 were significantly elevated in LC and HCC patients as compared to control group and more elevated in HCC than in LC cases and control group, ($p<0.001$) for each. There was statistically significant difference when compare HCC &HCV without cirrhosis as regard AST, ALT ALP, Bil (total, direct), Alb& INR, which came in agreement with Liu et al. [11]. Also there was statistically significant difference on comparing Cirrhotic vs non cirrhotic HCV group as regard AST, ALP, Bil(total, direct), Alb, INR while regarding ALT no significance was found & this results came in agreement with Abdel-Azeez et al. [12], who reported that; there is significant difference in between chronic HCV without fibrosis and chronic HCV with fibrosis group as regard INR, BIL( total, direct), ALB & ALT and GP73. AFP was higher in group I than group II and in group II than group III, we found that, the difference between the three groups was with significant p value which came in disagreement with Sai et al. [13] who reported that no significant differences between GP73 expression and patients' sex or age, tumor size, or AFP level group $P < 0.05$. and in agreement with Sanai et al. [14]. Who reported that Serum AFP was significantly higher in HCC than cirrhotic $p<0.0001$ and non-cirrhotic chronic hepatitis controls $p<0.0001$.

**Post hoc pair wise comparison (P value between each 2 groups) in significant variables:**

1) GI vs GII regarding Age, HB, RBC count, PLT, Bili (total, direct), Alb, INR, Urea and Creatinine there was no significance difference while as regard AST, ALT, ALP, PCR for HCV, AFP and Golgi 73 protein there was statistically significant p value which came in agreement with El Shafie et al. [15].
2) GI vs GIII regarding Age, HB, RBC count, AST, ALT ALP, Bil (total, direct), Alb, INR, Urea and Creatinine, AFP and Golgi 73 protein there was statistically significant p value while as regard PLT and PCR for HCV no significance of p value which came in agreement with Liu et al. [16].
3) GII vs GIII regarding Age, HB, RBC count, AST, ALP, Bil( total,direct), Alb, INR, Urea and Creatinine, AFP and Golgi 73 protein there was statistically significant p value while as regard ALT, bili direct, PCR for HCV and AFP showed no significance of p value our results as regard AST, BIL direct, PC, GP73, Albumin and PT came in agreement with Abdel-Azeez et al. [17].

**ROC curve for detection of HCC using AFP (ng/dl) and Golgi 73 (ng/dl)**

We concluded that the sensitivity and specificity of AFP were (80 and 86.7%) respectively, while the sensitivity and specificity of Golgi 73 were (90 and 90%) respectively which is in accordance with Ziada et al. [18] who reported that accordingly, AFP has sensitivity, specificity positive predictive value and negative predictive values of 76.7%, 88.6%, 62.7%, 93.8% and 75.7%, 97.3%, 87.6%, 94.1% at cut off value 100 and 200 ng/ml, respectively. This also agrees with Eissa et al. [19] who reported that the cut-off values for GP73 and AFP were 534.5 ng/L and 32 ng/mL, respectively. The specificity and the sensitivity of GP73 were (87%, 88%), while the specificity and sensitivity of AFP levels were (80% and 72%) respectively. We concluded that there was significant p value between GI and GII as regard AFP and Golgi 73 which came in agreement with Omran et al. [20] reporting that Mean serum GP73 and AFP in the HCC group shows significant statistical

difference (P < 0.01), (P < 0.0001) respectively. Moreover, the median level of GP73 was 24.5 ng/ml in HCC patients, whereas it was 11.3 ng/ml in the LC groups. AFP median level was 23 in HCC patients and 16.7 ng/ml for LC patients. There was significant p value between GI and GIII as regard AFP and Golgi 73 which agrees with Ibrahim et al. [21] who reported that Semi-quantitation of the three studied genes revealed a significantly higher means of circulating AFP and GP73 mRNAs in HCC patients than CHC and control group (P<0.05). There was significant p value between GII and GIII as regard AFP and Golgi 73 which came in agreement with Yao et al. [22]. who reported that serum levels of Golgi 73 were significantly higher in cirrhotic patients when compared with those of the healthy controls and the pre-cirrhotic CHC ($p < 0.001$).

## 5. Conclusion

- Both AFP & GP73 had important diagnostic value & increased sensitivity for early detection of HCC.
- Serum GP73 could be an important biomarker and a valuable diagnostic tool for the early diagnosis of HCC in cases of hepatitis C virus-related cirrhosis.

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**Conflicts of interest:** No competing interests.

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