

Serum Golgi Protein 73 as a Biomarker for Liver Fibrosis in Chronic Hepatitis C Patients

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ABSTRACT

Background: Serum Golgi protein 73 (GP73) is a promising biomarker of liver fibrosis, but most data are from hepatitis B virus-related liver diseases rather than hepatitis C virus (HCV).

Objective: To evaluate the use of GP73 as a marker for liver fibrosis in chronic HCV patients and to correlate it with fibrosis staging of liver biopsy and other laboratory findings.

Subjects and Methods: The study included 37 chronic HCV patients who were subdivided according to liver biopsy results into chronic HCV patients without fibrosis (10) and chronic HCV patients with fibrosis (27). 37 healthy individuals were taken as control. All participants were subjected to determination of liver function tests with calculation of AST-to-Platelet Ratio Index (APRI) and serum GP73 by ELISA.

Results: GP73 was significantly increased in chronic HCV patients with fibrosis when compared to patients without fibrosis and healthy control. A significant positive correlation was observed between GP73 and advanced liver function tests, increased fibrosis stage and APRI in chronic HCV patients with fibrosis. Based on receiver operating characteristic (ROC) curve analysis, GP73 had an area under curve (AUC) of 0.909 with 81.8% sensitivity and 93.3% specificity for prediction of significant fibrosis. On combination with APRI, the sensitivity was increased to 90.9%.

Conclusion: GP73 can be used as serum marker for prediction of significant liver fibrosis in chronic HCV patients either alone or in combination with APRI. It can be also useful in monitoring fibrosis progression.

Keywords: Hepatitis C, Golgi protein 73, APRI, Liver fibrosis.

INTRODUCTION

Hepatitis C virus (HCV) represents a significant global public health burden, and long-term infection can lead to many serious liver-related outcomes including liver cirrhosis, hepatocellular carcinoma and liver-related mortality⁽¹⁾. The event with the greatest impact on the natural history of chronic HCV is the progressive formation and accumulation of liver fibrosis. This accumulation of fibrillar extracellular matrix components is the hallmark of the natural history of the disease and significantly influences prognosis⁽²⁾. Liver fibrosis may progress to cirrhosis and eventually death. However, increasing evidence suggests that even advanced fibrosis is reversible, although end-stage cirrhosis is irreversible and affected patients can only survive with a liver transplant. Estimating the current degree of fibrosis is crucial for determining whether the fibrosis could be reversed with treatment⁽³⁾.

Liver biopsy is an invasive method that has long been regarded as the 'gold standard' for staging liver fibrosis. However, several issues prevent the routine use of liver biopsy as a clinical tool, including risk of injury to the patient, variable accessibility of the damaged section of the liver, high cost, sampling errors and inaccuracy due to inter- and intra-observer variability of pathologic interpretations⁽⁴⁾. Instead, several noninvasive methods for fibrosis assessment have been

proposed as the alternatives to liver biopsy, such as fibrosis index based on four factors (FIB-4), the AST-to-platelet ratio index (APRI), and transient elastography, which are based on blood indices and imaging modalities, respectively⁽⁵⁾. Non-invasive assessment of liver fibrosis is a focus of continuing interest to find a marker that is cost-effective, easy to perform with standard laboratory tests, accurately reflective of the stage of hepatic fibrosis and valid over a wide patient population⁽⁶⁾.

In normal livers, Golgi protein 73 (GP73) is a 73 kDa transmembrane glycoprotein constitutively expressed by biliary epithelial cells, but only minimally by hepatocytes. Hepatocellular expression of GP73 mRNA and protein is dramatically upregulated in acute or chronic hepatitis, regardless of the etiology, and accompanies the advanced fibrogenesis stage. With the recovery of acute hepatitis, GP73 expression reverses, whereas biliary epithelial cell expression remains unchanged. GP73 overexpression also occurs in activated hepatic stellate cells, which are the major feature of liver fibrosis. GP73 has been considered a useful indicator in evaluating liver impairment or fibrosis⁽⁷⁾.



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Previous researches about GP73 were mainly focused on hepatitis B virus (HBV) related liver diseases and others reported it as a potential diagnostic marker for hepatocellular carcinoma, the diagnostic potential of serum GP73 in chronic HCV-related liver diseases remains to be investigated. Our study aimed to evaluate the use of GP73 as a marker for liver fibrosis in chronic hepatitis C patients and to correlate it with fibrosis staging of liver biopsy and other laboratory findings.

SUBJECTS AND METHODS

This is a case-control study, which was carried out on 37 patients suffering from chronic hepatitis C virus, and 37 age and sex matched healthy individuals, taken as control. Patients were recruited from the Inpatient Sections and Outpatient Clinics of Tropical Medicine Department, Zagazig University Hospitals during the period from March 2019 to August 2019.

Chronic HCV patients were diagnosed by RT-PCR and elevated AST, ALT more than 6 months⁽⁸⁾. Patients were subdivided according to liver biopsy results into chronic HCV patient without fibrosis (10) and chronic HCV patient with fibrosis (27). Staging of fibrosis was done according to **French Metavir Cooperative Study Group**⁽⁹⁾. Significant liver fibrosis was defined as \geq F2. Patients under therapy for HCV, with hepatocellular carcinoma, liver cirrhosis F4 and other systemic diseases were excluded from the study.

Ethical approval:

Written informed consent was obtained from all individual participants included in the study.

The study was approved by the Local Ethics Committee of Faculty of Medicine, Zagazig University.

Laboratory investigations:

All participants were subjected to determination of platelets count (Sysmex XS 5001), prothrombin concentration (CA 1500 Sysmex), liver function tests (Cobas 8000, Roch Diagnostics) and calculation of AST-to-Platelet Ratio Index (APRI)⁽¹⁰⁾.

A cut-off value > 0.5 was previously established for APRI for diagnosis of significant fibrosis⁽¹¹⁾. Détermination of serum GP73 was performed by Enzyme-Linked Immunosorbent Assay using Uscn life ELISA kit (Wuhan USCN Business Co).

Statistical analysis

Statistical analyses were performed by SPSS 22.0 software (International Business Machines Corporation, New York, USA). Continuous variables were expressed as mean \pm standard deviation (SD) or median and interquartile range (IQR). Categorical variables were expressed as percentages. The differences of groups were analyzed by One-Way ANOVA, or Kruskal-Wallis (KW) rank sum test according to the data's distribution.

Post-hoc Fisher's Least Significant Difference test (LSD) or Tamhane's T tests were used according to homogeneity of variances. In addition, the chi-square test (χ^2) was applied to compare the rates of the classification data. Pearson and Spearman's rank correlation coefficient tests were used to describe the association between two variables.

The diagnostic effectiveness of variables was assessed by the area under the receiver operating characteristic (AUROC) curve with 95% confidence interval (CI), was used to identify optimal cut-off values with maximum sensitivity and specificity. All tests of significance were two-tailed, and $P \leq 0.05$ was considered statistically significant.

RESULTS

Demographic data and laboratory findings of the studied groups are presented in table (1).

Table (1): Demographic data and laboratory findings of the studied groups

Demographic data & Laboratory finding	Healthy control (n=37)	Chronic HCV without fibrosis (n=10)	Chronic HCV with fibrosis (n=27)	p-value
Age (years)				
Median	43	47	44	0.245
Range	22 – 55	39 – 55	20 – 53	
Sex##				0.165
Male	18 (48.6%)	7 (70%)	19 (70.4%)	
Female	19 (51.4%)	3 (30%)	8 (29.6%)	
Fibrosis stage (F0/F1/F2/F3)	----	10/0/0/0	0/5/17/5	---
Platelets count (x10³/mm³)	250 (180 – 300)	252.5 (150 – 280)	180 ¹ (107 – 280)	<0.001
Prothrombin conc. (%)	99 (92 – 105)	98 (92 – 102)	93 ² (65 – 105)	0.037
Total bilirubin (mg/dl)	0.6 (0.3 – 0.9)	0.8 ¹ (0.7 – 2)	1.4 ¹ (0.5 – 4)	<0.001
Direct bilirubin (mg/dl)	0.2 (0.1-0.2)	0.2 ¹ (0.1-0.5)	0.6 ¹ (0.1-1.8)	<0.001
Albumin (g/dl)#	4.4 ± 0.4	4.4 ± 0.2	3.4 ± 0.3 ¹	<0.001
Alkaline phosphatase (U/L)	160 (100 – 200)	119 (100 – 190)	152 (86 – 240)	0.096
ALT (U/L)	22 (18 – 26)	37 ¹ (28 – 42)	48 ¹ (12 – 117)	<0.001
AST (U/L)	22 (18 – 30)	37 ¹ (32 – 55)	45 ¹ (38 – 105)	<0.001
APRI	0.2 (0.1 – 0.3)	0.3 ¹ (0.2 – 0.7)	0.6 ¹ (0.4 – 2)	<0.001
GP73 (ng/ml)	4 (1 – 5)	3.9 (2.5 – 4)	5 ¹ (3.5 – 9.5)	<0.001

Data are presented as median (range), mean ± SD #, or number (percentage) ##

P ≤ 0.05 is significant. ¹ significant with other groups ² significant with control group

GP73 was significantly increased in chronic HCV with fibrosis compared to HCV without fibrosis and control groups while no significant difference in GP73 between chronic HCV without fibrosis and control group (figure 1).

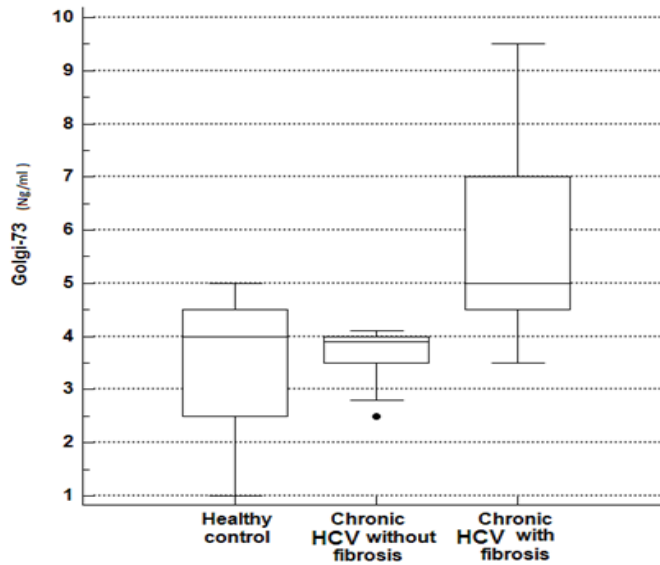


Figure (1): Box-plot of GP73 in the studied groups. Horizontal lines represent median values, boxes represent 25, 75 percentile and error bars represent ranges.

GP73 was significantly positively correlated with total and direct bilirubin in chronic HCV patients without fibrosis. There was a significant negative correlation between GP73 & platelet count, prothrombin concentration and albumin. While, there was a significant positive correlation between GP73 & total bilirubin, direct bilirubin, alkaline phosphatase, ALT, AST, fibrosis staging and APRI in chronic HCV patients with fibrosis (table 2, figures 2 & 3).

Table (2): Correlation between GP73 and selected study parameters

Laboratory findings	GP73			
	Chronic HCV without fibrosis (n=10)		Chronic HCV with fibrosis (n=27)	
	r	p	r	p
Platelets count (X10³/mm³)	-0.324	0.361	-0.418	0.030
Prothrombin conc. (%)	-0.282	0.430	-0.477	0.012
Total bilirubin (mg/dl)	+0.638	0.047	+0.775	<0.001
Direct bilirubin (mg/dl)	+0.646	0.043	+0.569	0.002
Albumin (g/dl)	-0.466	0.174	-0.540	0.004
Alkaline phosphatase (U/L)	-0.558	0.094	+0.390	0.044
ALT (U/L)	-0.205	0.569	+0.795	<0.001
AST (U/L)	+0.105	0.772	+0.715	<0.001
Fibrosis staging	---	---	+0.653	<0.001
APRI	+0.284	0.427	+0.680	<0.001

P ≤ 0.05 is significant

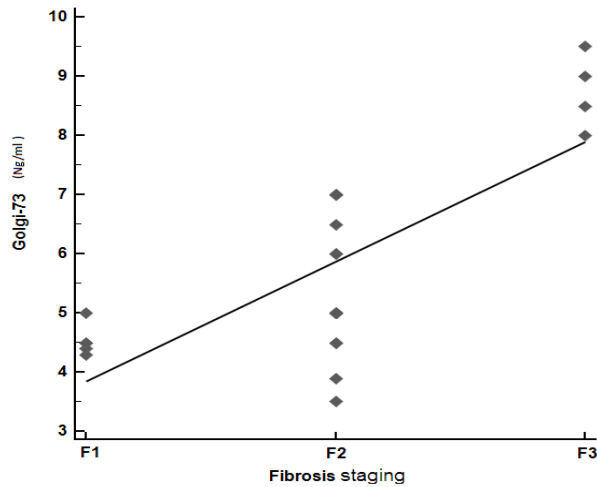


Figure (2): Correlation between fibrosis staging and GP73 in chronic HCV patients with fibrosis

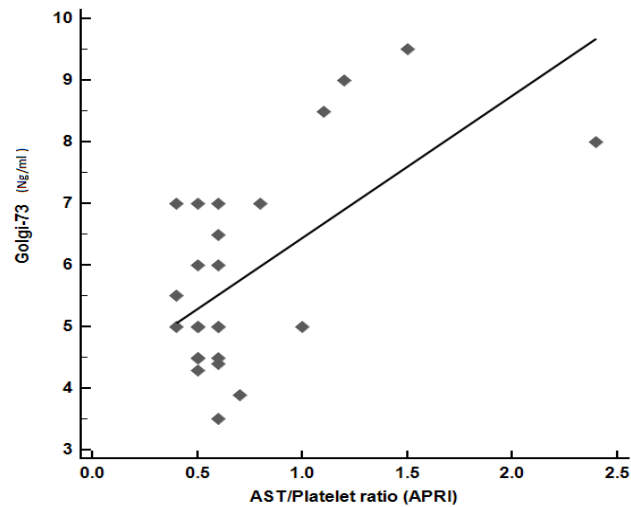


Figure (3): Correlation between APRI and GP73 in chronic HCV patients with fibrosis

ROC curve analysis of GP73 as a predictor of significant fibrosis (\geq F2) in chronic HCV patients revealed an AUC of 0.909. At cut-off value $>$ 4.5 ng/ml for GP73, 18 out of 22 chronic HCV patients with significant fibroses were correctly diagnosed and 1 out of 15 chronic HCV patients without fibrosis was misclassified as having fibrosis. When GP73 and APRI were used in combination for diagnosis of liver fibrosis, 20 out of 22 were correctly diagnosed (figures 4 & 5, table 3).

Table (3): GP73 and combined APRI & GP73 as a predictor for significant fibrosis in chronic hepatitis patients; ROC curve analysis

Parameter	Sensitivity %	Specificity %	Positive Predictive Value % (PPV)	Negative Predictive Value % (NPV)	Accuracy	AUC (95% CI)
GP73	81.8 %	93.3 %	94.7%	77.8 %	86.5%	0.909 (0.809-1.009)
Combined APRI and GP73	90.9%	73.3%	83.3%	84.6%	83.3%	0.935 (0.854-1.015)

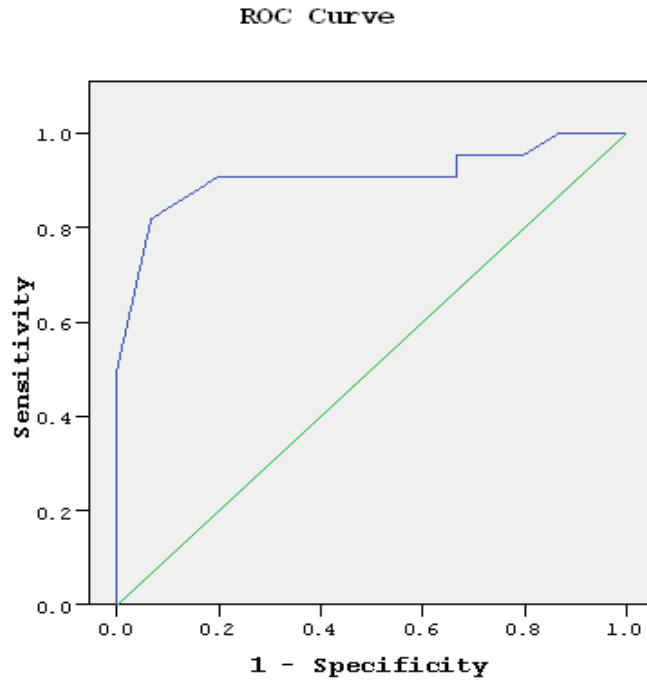


Figure (4): Receiver operating characteristic (ROC) curve of GP73 as a predictor for significant fibrosis in chronic hepatitis patients.

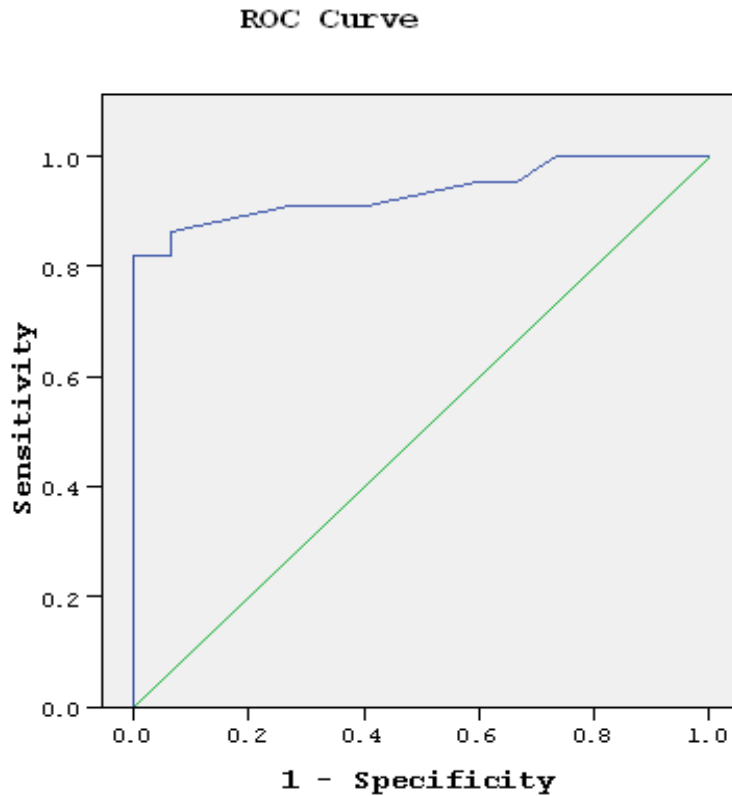


Figure (5): Receiver operating characteristic (ROC) curve of APRI & GP73 as a predictor for significant fibrosis in chronic HCV patients

DISCUSSION

Chronic HCV patients with no or minimal fibrosis at presentation appear to progress slowly and treatment possibly could delay or withheld progression. On the other hand, patients with significant fibrosis (i.e. septal or bridging fibrosis) progress almost invariably are going to cirrhosis over 10 to 20 years period⁽¹⁰⁾. Fortunately, antiviral treatment can reverse even early cirrhosis^(12,13). So, to better manage the chronic hepatitis HCV patients, it is critical to evaluate and monitor the grade of inflammation and the stage of liver fibrosis and cirrhosis. Interest in identifying and describing liver fibrosis using serum markers has been on the rise. Serum markers offer a cost effective alternative to liver biopsy for both patients and clinicians. In addition, being less invasive, there is a low risk of sampling error and no observer-related variability. Moreover, measurements may be performed repeatedly over time, allowing for ongoing monitoring of fibrosis⁽¹⁴⁾.

Our study showed that GP73 was significantly increased in chronic HCV patients with fibrosis when compared to patients without fibrosis and healthy control. A significant positive correlation was also observed between GP73 and increased fibrosis stage in chronic HCV patients. The relationship between GP73 levels and lesions of HCV-related liver disease was investigated by **Qian et al.**⁽¹⁵⁾ who found that serum GP73 rose in parallel with the severity of hepatitis, fibrosis and even cirrhosis. Moreover, several studies have demonstrated that GP73 was increased in HBV-related liver diseases. **Xu and his colleagues**⁽¹⁶⁾ reported an increase in serum GP73 with increasing fibrosis in HBV patients. Also, **Liu et al.**⁽¹⁷⁾ demonstrated that GP73 was significantly increased in HBV-related liver fibrosis patients compared to healthy controls. **Gu et al.**⁽¹⁸⁾ reported that GP73 concentrations in patients with liver disease were three-fold higher than in healthy individuals.

In chronic liver diseases, GP73 and mRNA expression increases gradually, not only in the hepatocytes, but also in activated stellate cells⁽¹⁹⁾, which is commonly considered the most important cell type involved in hepatic fibrogenesis⁽²⁰⁾. Therefore maximal GP73 expressions are seen in fully established cirrhosis⁽¹⁹⁾. GP73 over-expression is induced initially by inflammation, specifically by inflammatory cytokines such as IL-6, possibly in response to chronic active viral infection⁽²¹⁾.

We observed that serum GP73 was significantly positively correlated with advanced liver function tests in HCV patients with fibrosis. Similar results were reported by **Qian et al.**⁽¹⁵⁾ in chronic HCV patients and **Xu et al.**⁽²²⁾ in chronic HBV patients. A significant positive

correlation was observed between GP73 and APRI in chronic HCV patients with fibrosis in this study. **Liu et al.**⁽¹⁷⁾ and **Xu et al.**⁽²²⁾ reported significant positive correlation between serum GP73 and other serum markers of liver fibrosis; serum hyaluronic acid, laminin, type III procollagen protein and type IV collagen.

In our study, ROC curve analysis revealed an AUC of 0.909, at cutoff > 4.5 ng/ml, GP73 had 81.8% sensitivity, 93.3% specificity, PPV 94.7% and NPV 77.8 % with 86.5% accuracy in prediction of significant fibrosis in chronic HCV patients. A comparable results were reported previously in prediction of significant fibrosis in HBV patients by **Wei et al.**⁽²³⁾. Serum GP73 is considered as a biomarker for liver diseases with clinically proven sensitivity and specificity^(24,25). **Gu and his colleagues**⁽¹⁸⁾ used GP73 to detect liver cirrhosis with a sensitivity of 88.4%.

When we combined APRI and serum GP73 as a diagnostic marker for significant liver fibrosis, the ROC-AUC increased to 0.935. Twenty out of 22 chronic HCV patients with significant fibrosis were correctly diagnosed with increased sensitivity (90.9%) and negative predictive value (84.6%).

CONCLUSION

Serum GP73 is significantly increased in chronic HCV patients with fibrosis and is correlated with fibrosis staging determined by liver biopsy. It can be used as serum marker for prediction of significant liver fibrosis in chronic HCV patients either alone or in combination with APRI. It can be also a useful marker in monitoring fibrosis progression. Further studies on large sample size and in different liver diseases are recommended to confirm these results.

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