Original Article

Significance of growth differentiation factor 15 in chronic HCV patients

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Mohab H. Halim [a], Nour A. Abdulla [b], Abdelkarim Kamel [c], Nabila Abd El Maksoud [a], Halla M. Ragab [a],*

[a] Biochemistry Department, National Research Center, Dokki, Giza, Egypt
[b] Hepatology Department, National Hepatology & Tropical Medicine Research Institute, Cairo, Egypt
[c] Medical Biochemistry, Faculty of Medicine, Cairo University, Egypt

A R T I C L E   I N F O

A B S T R A C T

Background and objective: Hepatitis C virus is the most common cause of chronic liver disease in Egypt. This work aims to assess the use of the simple and noninvasive biomarker Serum Growth differentiation Factor 15 (GDF-15), along with Alpha Fetoprotein (AFP) and Ferritin for the diagnosis of advanced liver disease in chronic hepatitis C patients. Subjects and methods: This study was conducted on 60 patients, who were recruited from the National Liver and Tropical Diseases Institute, Cairo, Egypt, who were suffering from early & advanced liver cirrhosis and chronic active hepatitis. Twenty cases of healthy subjects served as controls. Serum (GDF-15), (AFP), Ferritin and Hepatitis markers were measured by ELISA method. Measurement of different liver enzyme activity was done by the kinetic methods. Results: Data analysis revealed significant increase in serum levels of GDF15 in patients with Hepatocellular carcinoma (HCC) and Liver Cirrhosis (LC) compared to the healthy subjects. These results were parallel to those of serum levels of AFP, which also demonstrated significant increase in all patients groups as compared to normal control. A moderate increase in the GDF15 level was detected in the patients with chronic hepatitis C (CHC) compared to normal healthy subjects. Conclusion: This study demonstrated that GDF15 and AFP detection can help in the diagnosis and prediction of complications associated with CHC including liver cirrhosis and HCC. Also GDF15 can be used as a satisfactory serum marker of HCC and LC.
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1. Introduction

Hepatitis C virus has been completely outlined by molecular biology techniques. Although the infection often remains asymptomatic for many years, it can still lead to considerable damage to the liver, as liver cirrhosis (LC) and primary liver cancer [1]. In fact, it is now considered to be the leading indication for liver transplantation [2]. Early diagnosis and treatment have been found to significantly improve outcome in these patients [3]. Liver biopsy is the gold standard test for accurate diagnosis, but this procedure has potentially life-threatening complications [4]. Finding non-invasive biomarkers for liver cirrhosis and carcinoma is urgently needed.

Many markers to detect fibrosis have been introduced [5]. Hepatocellular carcinoma has the worst prognosis worldwide [6]. Similarly, liver cirrhosis is accompanied with a very high mortality

rate. Alpha-fetoprotein is recognised as a serum marker for the detection of liver cancers, but its sensitivity and specificity are unsatisfactory for cirrhosis; it has unclear results and is not significant.

GDF15 is a member of the transforming growth factor-β (TGF-β) cytokine superfamily involved in fibrosis, infection, and apoptosis pathways in the presence of tissue damage or disease [7]. It was first isolated in macrophages after cytokine stimulation [8].

GDF15 mRNA is known to be distributed particularly in the liver but also in the heart, lung, and kidneys [7]. It is expressed and secreted in response to short-wave length exposure, inflammation, oxidative stress, hypoxia, telomere erosion, and tissue injuries and oncogene activation [9].

Iron in the body found in hemoglobin, hemosiderin, myoglobulin and the cytochromes [10]. Ferritin is a major iron-storage protein, and acute phase protein which may be increased in conditions of inflammation, malignancy and chronic liver disease. In this study, the ferritin levels were measured in serum to show if its levels have a significant difference with the severity of disease, and whether its levels in serum can be used to detect liver cirrhosis and hepatocellular carcinoma.

The aim of this study was to measure Serum Growth Differentiation Factor 15 (GDF15) together with Alpha Fetoprotein to determine their efficiency as serum biomarker for diagnosis of liver cirrhosis and HCC in Egyptian patients with chronic viral hepatitis C.

2. Subjects and methods

This study was carried out on patients who were recruited from National Liver and Tropical Diseases Institute, Cairo, Egypt between November, 2014 to April, 2015. They are suffering from chronic liver diseases including hepatic portal hypertension, liver cirrhosis with chronic active hepatitis.

Patients were classified into three groups: Group I – Include 20 cases of pure chronic viral hepatitis C (HCV) with early liver cirrhosis without peri-portal fibrosis, no history of antibilharzial treatment and negative rectal snips for bilharzial ova. Group II – Include 23 cases of mixed viral hepatitis C (HCV) and with cirrhotic changes of the liver with peri-portal fibrosis, history of antibilharzial treatment and positive rectal snips for bilharzial ova. Group III – Include 17 cases of HCC with peri-portal fibrosis and viral hepatitis C (HCV), with history of antibilharzial treatment and positive rectal snips for bilharzial ova. Control group Include 20 cases of normal healthy subjects with no known history of cancer, HBV, HCV infection, antibilharzial treatment or positive rectal snips for bilharzial ova.

2.1. Sample collection

Blood samples were obtained from the patients, and serum was separated at 3000 rpm and stored at −80 °C. Patients were subjected to complete medical history, full clinical examination including examination of chest and abdomen with stress on manifestation of liver cell failure.

All Patients and controls were subjected to thorough history taking, clinical examination., and laboratory investigations including complete blood count, liver and kidney functions, History of antibilharzial treatment, Rectal snips for bilharzial ova examined by transparency technique, test for hepatitis B surface antigen (HBsAg) by ELISA and test for HCV antibodies by ELISA and by quantitative polymerase chain reaction (PCR) assay.

Abdominal Ultrasonography and Duplex Doppler examination was done using a Toshiba SSA, 340A machine to measure liver and splenic size, portal vein diameter, presence of peri-portal fibrosis and liver cirrhosis. Ultrasonography was used for assessment of liver injury and its staging in all patients V) Liver biopsy was taken if feasible and the histopathological changes were assessed according to Knodell's score [11].

We excluded patients with any cause of liver disease other than chronic HCV based on the patient history, laboratory or liver biopsy findings as: HBV/HIV coinfection, serological evidence of autoimmune hepatitis, inheritable disorders as hemochromatosis, Wilson's disease or Alpha 1-antitrypsin deficiency, alcoholic liver disease and drug induced liver disease. Also, patients on anti-inflammatory drugs or those with autoimmune diseases arthritis are excluded from the study.

2.2. Methods

Biochemical blood tests were carried out to evaluate liver functions in the form of serum transaminases (SGOT and SGPT), alkaline phosphatase (ALP), gamma glutamate (GGT), total bilirubin, as well as albumin. Serum SGPT, SGOT, GGT and ALP levels were measured by the kinetic methods according to the recommendations of the Committee on the Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974 & 1976). Serum albumin was measured by colorimetric methods.

Patients were diagnosed as having hepatitis C by detecting HCV antibodies using a third generation enzyme immunoassay, ELISA.

In CHC patients, the baseline HCV RNA load were measured using a quantitative polymerase chain reaction (PCR) assay according to the available technique (Applied Biosystem, USA), with detection limit of 30 IU/ml.

GDF15 was measured in serum using a specific ELISA kit (Cat# KT-16893, R&D Systems, Kamiya Biomedical Co., Seattle, WA), according to the manufacturer's recommended protocol.

Determination of serum Alfa fetoprotein: Serum samples were analyzed for Alfa fetoprotein using a commercially available sandwich ELISA kit (Cat# DAFP00, R&D Systems, USA & Canada).

Determination of serum Ferritin: serum samples were analyzed for Ferritin using a commercially available sandwich ELISA obtained from Sigma-Aldrich).

2.3. Statistical analysis

Data management and statistical analysis were performed using Statistical Package for Social Sciences [12] (SPSS) version 16. Student's *T*-test for unpaired data was used for identifying differences between the groups. A level of probability $P < 0.05$ was accepted as statistically significant, $P > 0.05$ as non-significant, $P < 0.01$ as highly significant and $P < 0.001$ as very highly significant.

3. Results

The patients included in this study were those who suffering from chronic liver diseases such as hepatic portal hypertension, liver cirrhosis with chronic active hepatitis and hepatocellular carcinoma. Clinical data of the patients are shown in Table 1.

Levels of AST, ALT, ALP, GGT, total bilirubin and albumin levels in the different studied groups were showed in Table 2, their levels were significantly higher in Group (I) ($P < 0.01$) and very significantly higher in Groups (II) and (III) ($P < 0.001$) when compared with normal controls. Serum albumin levels were significantly decreased in group I (chronic hepatitis) ($P < 0.05$) and showed a very high significant decrease in groups II and III ($P < 0.001$) when compared with controls and with each other.serum levels of GFD 15 were significantly higher ($P < 0.001$) in Group II and III patients when compared to the normal controls while a moderate increase was shown in patients with Chronic hepatitis C (CHC) with a P value <0.01 when compared with normal healthy subjects, data was showed in Table 3.

Levels of AFP showed very highly increased levels in all studied groups when each was compared to normal controls ($P < 0.001$). Similarly, serum levels of Ferritin showed very high significant increases ($P < 0.001$) in all studied groups when compared with normal controls.

4. Discussion

Hepatitis C virus is the most common cause of chronic liver disease in Egypt. The prevalence of antibodies to HCV is approximately 10-fold greater than that in the United States and Europe. Anti-HCV-positive patients are more likely to have liver enzyme elevations, liver cirrhosis, portal hypertension and spleen enlargement [13]. Schistosomal liver disease in Egypt is significantly accompanied with HCV infection, with the predominance of genotype 4 [14–16].

Both liver cirrhosis and hepatocellular carcinoma have a high mortality rate. HCC is a consequence of HCV infection, rates as

Table 1
Demographic and clinical data of patients (no = 60).

Parameter	Group I Number of cases (%)	Group II Number of cases (%)	Group III Number of cases (%)
Age			
Mean	57 years	55 years	63 years
Range	(45–62)	(43–70)	(56–79)
Sex			
Male	15 (75%)	17 (73.9%)	13 (76.5%)
Female	5 (25%)	6 (26.1%)	4 (23.5%)
Live size			
Normal	6 (30%)	11 (47.8%)	6 (35.3%)
Enlarged	14 (70)	11 (47.8%)	8 (47.1%)
Shrunken		1 (4.4%)	3 (17.6%)
Bilharziasis treatment			
Positive	0 (0%)	12 (52.2%)	9 (52.9%)
Negative	60 (100%)	11 (47.8%)	8 (47.1%)
Spleen			
Splenomegaly	13 (65%)	16 (69.6%)	17 (100%)
Normal splenic size	7 (35%)	7 (30.4%)	0 (0%)
Hypertension			
Portal hypertensive	0 (0%)	14 (70%)	7 (35%)
Portal vein thrombosis	0 (0%)	0 (0%)	
Gall stones			
Positive	9 (45%)	3 (13%)	2 (11.8%)
Negative	11 (55%)	20 (87%)	15 (88.2%)
Focal lesions			
Hepatic focal lesion	0 (0%)	0 (0%)	8 (40%)
Multiple focal lesions	0 (0%)	0 (0%)	9 (45%)
Oesophageal varices	0 (0%)	14 (70%)	14 (70%)
Periportal fibrosis	0 (0%)	5 (25%)	3 (15%)
Ascites			
Positive	0 (0%)	7 (30.4%)	11 (64.7%)
Negative	0 (0%)	16 (69.6%)	6 (35.3%)

the fifth most common cancer in the world [17]. There is a need to update tools for early and non-invasive diagnosis of these conditions since the earlier the intervention, the better the outcome. Patients suffering from liver disease need liver biopsy to diagnose fibrosis histologically which is risky and expensive. Imaging tools are informative mainly for cirrhosis and not for lesser stages of fibrosis. The necessity to find a biochemical marker for the diagnosis of the disease is clear and increased [18].

This study demonstrated no significant difference among the various groups regarding age and sex differences. Levels of AST, ALT, ALP, GGT, and total bilirubin were significantly increased in all patient groups compared to controls while levels of serum albumin were significantly decreased. These differences were more pronounced in Groups II and III.

These findings denote that as the liver condition deteriorates from fibrosis to early cirrhosis passing to advanced cirrhosis, serum levels of liver enzymes increase progressively and serum albumin decreases. In the present study, it is noted that all parameters were more severely increased in those who had mixed infection bilharzial and HCV (Group II and III patients) than those without bilharzial infection (Group I patients).

These finding agrees with that of Giannini and his colleagues, 2002 who reported that in patients with virus-related liver cirrhosis, the AST and ALT have an important prognostic value in following up the severity of liver affection and their combined assessment increases the prognostic accuracy [19]. In addition, Kao study showed that AST as well as ALT levels are related to hepatocyte injury. Thus, higher serum ALT and AST levels indicated ongoing viral replication and inflammation due to HCV infection at the time of surgery [20].

Levels of serum ferritin revealed very highly significant increases in the three study groups with a P value <0.001 compared to normal healthy subjects and were particularly high in cirrhotic patients. Increased serum ferritin levels indicate iron deposition and may indicates advanced hepatic fibrosis in patients with hepatitis C infection [21].

It is important to find reliable serological screening tests for both LC and HCC as this may reduce further morbidity and mortality from cancer. Some serum biomarkers are available for the diagnosis of liver cancer, including alpha-fetoprotein. Filmus et al., (2004) reported that the only serological marker widely used for the diagnosis of HCC was alpha-fetoprotein [22].

In this work, GDF15 was studied to assess its value as a screening test for LC and HCC and results were very satisfactory. Studies have shown a normal reference range for serum GDF15 in healthy subjects of approximately 0.2–1.2 ng/mL [23], which is consistent with our results where levels ranged between 0.3 and 1.05 ng/ml in controls. Levels were highly increased in serum of patients both with hepatocellular carcinoma or/and cirrhosis compared with controls. In Group I, levels of GDF15 were not as high as in patients in Groups II and III, but were still significantly higher than controls.

Table 2
Serum values of AST, ALT, ALP, GGT, total bilirubin and albumin in normal healthy controls and studied groups.

Parameter	Statistics	Normal control (NC) (N = 20)	Chronic hepatitis C G (I) (N = 20)	Liver cirrhosis G (II) (N = 23)	Hepatocellular carcinoma G (III) (N = 17)
AST (U/L)	Range	8–42	15–82	34–157	53–275
	Mean ± S.E.	24 ± 2.07	35 ± 3.46	83 ± 6.97	140 ± 13.72
	P-value <		<0.01	<0.001	<0.001
ALT (U/L)	Range	12–65	20–95	43–187	34–133
	Mean ± S.E.	28 ± 3.63	43 ± 3.78	90 ± 10.3	64 ± 6.09
	P-value <		<0.01	<0.001	<0.001
ALP (U/L)	Range	35–124	52–195	86–220	103–317
	Mean ± S.E.	74 ± 5.57	106 ± 10.01	159 ± 9.73	223 ± 12.74
	P-value <		0.01	<0.001	<0.001
GGT (U/L)	Range	10–37	17–75	22–133	59–231
	Mean ± S.E.	20 ± 2.23	34 ± 3.53	77 ± 7.31	131 ± 10.35
	P-value <		<0.01	<0.001	<0.001
T. BIL. (mg/dl)	Range	0.3–1.12	0.64–1.8	0.82–2.21	1.02–2.82
	Mean ± S.E.	0.75 ± 0.064	1.04 ± 0.074	1.52 ± 0.082	2.1 ± 0.112
	P-value <		<0.01	<0.001	<0.001
ALB (g/dl)	Range	3.4–5.5	2.7–5	2.1–3.9	2.02–3.23
	Mean ± S.E.	4.46 ± 0.14	3.96 ± 0.16	3.07 ± 0.11	2.56 ± 0.09
	P-value <		<0.05	<0.001	<0.001

P < 0.05 statistically significant.

Table 3
Serum values of growth differentiation factor-15 (GDF-15) AFP, and ferritin in controls as and studied groups.

Parameter	Statistics	Normal control (NC) (N = 20)	Chronic hepatitis C G (I) (N = 20)	Liver cirrhosis G (II) (N = 23)	Hepatocellular carcinoma G (III) (N = 17)
GFD15 (ng/ml)	Range	0.30–1.05	0.45 – 3.50	3.4–6.70	4.5–8.79
	Mean ± S.E.	0.70 ± 0.052	1.41 ± 0.21	5.20 ± 0.21	6.49 ± 0.27
	P-value <		<0.01	<0.001	<0.001
AFP (ng/ml)	Range	2.1–5.7	3.5–9.3	13.8–36.4	218–351
	Mean ± S.E.	3.88 ± 0.25	6.63 ± 0.29	28.04 ± 1.85	255 ± 7.53
	P-value <		<0.001	<0.001	<0.001
Ferritin (ng/ml)	Range	30–203	75–273	124–382	243–764
	Mean ± S.E.	95 ± 11.28	162.5 ± 14.13	252 ± 16.06	504 ± 11.96
	P-value <		<0.001	<0.001	<0.001

$P < 0.05$ as statistically significant.

Increased levels of serum GDF15 have been associated with several human diseases, including cardiovascular diseases, prostate, breast and colon cancer [24–28]. The exact role of serum GDF15 in liver disease has not been elucidated. Zimmers and colleagues, 2008 found no effect on HCC carcinogenesis after genetic ablation of GDF-15 on hepatocellular carcinogenesis and growth rate in an HCC mouse model [29]. Moreover, Hurst et al., revealed that Patients with LC show significantly elevated concentrations of GDF-15 as compared to patients with liver fibrosis [30]. Also, Vocka et al., demonstrated that serum GDF-15 levels were significantly higher in patients with colorectal cancer compared to healthy controls, and serum levels of GDF-15 correlated with extent of liver involvement and presence of primary tumor or local relapse [31]. This result also in agreement with the Lee results which concluded that GDF-15 comprised a useful biomarker for the prediction of liver fibrosis and severity in chronic liver disease and Liu results which indicated that Serum GDF15 levels were significantly increased in patients with HCC and cirrhosis compared with healthy controls, also GDF15 protein expression in HCC was significantly higher than that in the corresponding adjacent paracarcinomatous tissue and normal liver [8,32].

The understanding of the exact role of this biological marker has the potential to revolutionise the utilisation of GDF15 in the diagnosis of HCC and targeted therapy.

Levels of AFP which is a tumor marker used in the diagnosis and prognosis of hepatocellular carcinoma were greatly elevated in our patients with cancer group compared to the two other groups ($P < 0.001$). Furthermore, mean levels of AFP were significantly higher in the CHC and LC groups compared to the control group. AFP was shown by filmus and capurro, to be limited to 41–65% [25]. Given the high heterogeneity of HCC, it is believed that the diagnosis of HCC will be based on the simultaneous measurement of two or three highly specific serological markers.

In this study, it was found that the combination of AFP and GDF15 is very effective in reflecting liver status especially in those with HCC. This finding has great potential value in screening HCC risk early in the disease, in large populations as well as in those with liver cirrhosis. This finding supports the previously reported results that indicated using a combination of GDF15 and AFP will improve the sensitivity and specificity of HCC diagnosis [8,33].

5. Conclusion

Serum levels of GDF15 can be used as a biomarker for diagnosis and prognosis of hepatocellular carcinoma and liver cirrhosis.

Conflict of interest

There is no conflict of interest with regard to this manuscript.

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