Association of serum growth differentiation factor 15 and hepatocellular carcinoma in Egyptian patients

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Received 31 May 2018
Accepted 1 September 2018

The Egyptian Journal of Internal Medicine
2019, 31:57–63

Background
Liver cirrhosis and hepatocellular carcinoma (HCC) are consequences of chronic hepatitis C virus (HCV) infection. HCC is one of the fastest rising causes of cancer-related mortality. This dismal prognosis is related to late diagnosis with currently available screening methods. The aim of our study was to evaluate the diagnostic power of serum growth differentiation factor (GDF) 15 in HCC detection and its ability to distinct HCC from cirrhosis in chronic hepatitis C Egyptian patients.
Patients and methods
Ninety participants were included in the study; 30 patients with HCV-cirrhosis, 30 patients with HCV-Cirrhosis and HCC, and 30 gender and age-matched healthy subjects as the control group. The patients were subjected to history taking, clinical examinations, routine laboratory analysis, and α-fetoprotein (AFP) determination. Serum GDF15 was measured using an enzyme-linked immunosorbent assay kit.
Results
The mean level of GDF15 in HCV-cirrhosis patients was 140.28±128.66 pg/ml, in HCV-HCC patients 154.45±123.74 pg/ml, and in the control group it was 81.19 ±42.53 pg/ml. Statistically significant difference in GDF15 level was found between HCV-HCC patients and controls, P=0.012, while no statistically significant difference was found on comparing HCV-cirrhosis patients to controls or to HCV-HCC patients, P=0.064 and 0.473, respectively. The cut-off value of GDF15 to discriminate HCV-HCC patients from controls was 122.3 pg/ml with 53.3% sensitivity and 86.7% specificity and an area under the receiver operating characteristic (AUROC) of 0.692. AFP at a cut-off value of 20.85 ng/l was able to discriminate HCV-HCC patients from HCV-cirrhosis patients with 73.3% sensitivity and 73.3% specificity and an AUROC of 0.744. AUROC for combined AFP and GDF15 showed lower performance than AFP alone in discrimination of HCV-HCC from HCV-cirrhosis patients (AUROC=0.642).
Conclusion
GDF15 is not a potential diagnostic marker for the distinction of HCC from cirrhosis in chronic hepatitis C Egyptian patients.

Keywords:
α-fetoprotein, chronic hepatitis C, Egyptian, growth differentiation factor 15, hepatocellular carcinoma, liver cirrhosis

Egypt J Intern Med 31:57–63

1110-7782

Introduction

The incidence of hepatocellular carcinoma (HCC) has been increasing during the last decades [1] to become the fifth most common cancer worldwide [2] and the second leading cause of cancer-related mortality [3]. HCC etiology is mostly related to hepatitis B virus (HBV) and hepatitis C virus (HCV) infection [4–6], HCV antibodies are positive in the sera of up to 70% of HCC patients [7]. Chronic persistent nature of HCV leads to serious complications with the development of liver cirrhosis in 5–20% of patients [8]. HCC is more likely to develop in cirrhotic liver [7], the 5-year cumulative risk of developing HCC in cirrhotic patients ranges between 5 and 30% and the prevalence of cirrhosis among patients with HCC is 85–95% [9,10].

The rise of HCC incidence is related to the high prevalence of HCV in the patients [11].

HCC has a poor prognosis with a 5-year survival less than 5%, probably due to late diagnosis; however, early discovery improves 5-year survival by up to 70% [12,13]. HCC screening in cirrhotic patients is recommended every 6 months using ultrasound (US) with or without α-fetoprotein (AFP) measurement [14]. US has a sensitivity of ∼60% and specificity of 85–90% [15], which is highly

dependent on operator experience, in addition US may not be applicable for all patients as obese patients present a challenge leading to limited sensitivity of early HCC detection, ranging from 32 to 65% [15,16]. AFP is relatively inexpensive, simple to perform, and is widely available, but it has limitation in sensitivity and specificity, its sensitivity ranges from 18 to 60% and its specificity ranges from 85 to 90% [17]. The low sensitivity of the current measures necessitates the study of other alternatives that may help in early detection of HCC.

Growth differentiation factor 15 (GDF15) is a transforming growth factor β protein expressed in placental tissue under physiological conditions, but it can be induced in activated macrophages by proinflammatory cytokines in the presence of tissue damage or disease [18]. GDF15 plays a dual role in cancer development; it inhibits early cancer genesis but promotes tumor progression at later stages [19]. Its tumor-promoting effects were demonstrated in malignant glioma, glioblastoma, melanoma, myeloma, gastric, prostate, and breast cancer [20]. GDF15 is also upregulated in HCV infection [21,22]. The possible involvement of GDF15 in HCV-related liver carcinogenesis was suggested which needs further verification [22].

The aim of our study was to evaluate the diagnostic power of serum GDF15 in HCC detection and its ability to distinct it from cirrhosis in chronic hepatitis C Egyptian patients.

Patients and methods

This cross-sectional study was conducted at the Internal Medicine Department, Kasr Alainy Hospital, Cairo University. Ninety participants were included in the study; 30 patients with HCV-cirrhosis with no radiological evidence of hepatic focal lesions and normal AFP level, 30 patients with HCV-cirrhosis and HCC, and 30 gender and age-matched healthy subjects as the control group. Patients with liver cirrhosis secondary to HBV infection, history of hepatotoxic drugs, anti-inflammatory drugs, bilharzia infection, autoimmune disease, and metabolic liver diseases were excluded because these factors could affect GDF15 concentrations.

Informed consent was obtained from all participants before enrollment in the study. The study was approved by Faculty of Medicine, Cairo University Ethics Committee and was conducted according to the ethics guidelines of the Declaration of Helsinki.

The patients were subjected to full history taking, clinical examination, laboratory investigations including liver function tests (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, albumin, prothrombin time, prothrombin concentration, international normalized ratio, bilirubin total and direct), complete blood count, creatinine, HCV antibodies, HBsAg, and serum AFP level. The Child–Pugh score was estimated. Abdominal US and computed tomography were done. Serum GDF15 was measured using a specific ELISA kit (kit no. ABIN365728; CUSABIO, China) with assay range 7.8–500 pg/ml, according to the manufacturer's recommended protocol. HCC was diagnosed according to american association for the study of liver diseases (AASLD) practice guidelines [23].

The diagnostic performance of GDF15 was evaluated by calculating the area under the receiver operating characteristic (AUROC) curve.

Statistical analysis

Data were coded and entered using the statistical package SPSS (statistical package for the social sciences; SPSS Inc., Chicago, Illinois, USA) version 23. Data were summarized using mean, SD, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the nonparametric Kruskal–Wallis and Mann–Whitney tests. For comparing categorical data, χ^2-test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman's correlation coefficient. Logistic regression was done to detect an odds ratio of GDF15 and AFP independent predictors of HCC. The ROC curve was constructed with the area under curve analysis performed to detect the best cut-off value of GDF15 and AFP for the detection of HCC. *P* values of less than 0.05 were considered as statistically significant.

Results

Ninety participants were included in the study. They were 52 (57%) men and 38 (42%) women with age ranging from 30 to 80 years. Baseline demographic and laboratory data of the participants are shown in Table 1; baseline radiological findings are shown in Table 2.

Comparison between HCV-cirrhosis and HCV-HCC patients regarding baseline laboratory data showed no statistically significant difference except for AFP,

Table 1 Demographic and laboratory data of the participants

	Cirrhosis (*n*=30)		HCC (*n*=30)		Control (*n*=30)	
Age (years)	40–80	55.53±8.01	42–73	57.6±6.95	30–70	44.93±14.68
Gender						
Male	17	56.7%	22	73.3%	13	43.3%
Female	13	43.3%	8	26.7%	17	56.7%
Child–Pugh score						
B	13	43.3%	11	36.7%	—	—
C	17	56.7%	19	63.3%	—	—
Hb (g/dl)	6.60–14.30	9.57±2.04	4.80–14.20	9.41±1.93	10.00–15.30	12.48±1.50
TLC ($\times 10^3/\mu l$)	1.20–28.80	8.60±6.25	3.20–23.00	9.97±5.83	4.30–11.50	7.40±2.08
Platelets ($\times 10^3/\mu l$)	17.00–230.00	118.11±57.75	13.00–425.00	131.83±84.66	155.00–342.00	222.97±60.41
ALT (U/l)	20.00–455.00	77.02±77.70	18.00–1009.00	109.62±187.32	20.00–44.00	34.67±9.84
AST (U/l)	22.00–216.00	89.46±40.13	37.00–2609.00	257.94±468.11	18.00–42.00	30.93±11.83
ALP (IU/l)	42.00–600.00	113.93±97.05	58.00–240.00	114.53±44.15	18.00–81.00	45.40±15.27
GGT (U/l)	19.00–590.00	76.30±103.28	14.00–176.00	62.93±32.89	7.00–61.00	23.90±11.79
Albumin (g/dl)	1.30–3.50	2.26±048	1.10–3.30	2.14±0.58	3.50–4.10	3.66±.31
Total bilirubin (mg/dl)	0.80–24.60	3.56±5.24	0.70–11.50	3.85±3.79	0.40–1.20	0.81±0.20
Direct bilirubin (mg/dl)	0.20–10.20	1.74±2.60	0.20–8.80	2.33±2.74	0.00–0.70	0.41±0.19
Creatinine (mg/dl)	0.40–5.22	1.30±0.97	0.62–5.00	1.54±0.96	0.30–1.30	0.80±0.30
PT (seconds)	13.90–30.10	18.79±3.90	13.20–32.70	18.26±3.76	11.90–12.90	12.26±0.29
PC (%)	25.00–81.00	52.13±13.41	18.00–78.00	50.42±14.37	96.00–100.10	99.24±1.03
INR	1.15–3.10	1.67±0.44	1.20–4.20	1.80±0.71	1.00–1.38	1.09±0.11
AFP (ng/ml)	1.20–311.00	65.16±113.73	1.50–953.00	185.42±194.28	0.00–13.90	3.55±3.26
GDF15 (pg/ml)	9.5–566	140.28±128.66	10.90–471.70	154.45±123.74	11.50–202.40	81.19±42.53

Data are presented as number (%) and mean±SD. AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GDF, growth differentiation factor 15; GGT, γ-glutamyl transferase; Hb, hemoglobin; INR, international normalized ratio; PC, prothrombin concentration; PT, prothrombin time; TLC, total leukocytic count.

Table 2 Baseline radiological findings of the participants

	Cirrhosis (*n*=30) [*n* (%)]	HCC (*n*=30) [*n* (%)]
US: liver cirrhosis		
Yes	30 (100.0)	30 (100.0)
No	0 (0.0)	0 (0.0)
US: focal lesions		
Yes	0 (0.0)	30 (100.0)
No	30 (100.0)	0 (0.0)
US: ascites		
Yes	22 (73.3)	24 (80.0)
No	8 (26.7)	6 (20.0)
US: splenomegaly		
Yes	23 (76.7)	28 (93.3)
No	7 (23.3)	2 (6.7)
CT: focal lesions		
Yes	0 (0.0)	30 (100.0)
No	30 (100.0)	0 (0.0)
CT: number of lesions		
0	30 (100.0)	0 (0.0)
1	0 (0.0)	9 (30.0)
2	0 (0.0)	11 (36.7)
3	0 (0.0)	4 (13.3)
4	0 (0.0)	6 (20.0)
CT: intra-abdominal lymph nodes		
Yes	0 (0.0)	5 (16.7)
No	30 (100.0)	25 (83.3)

CT, computed tomography; HCC, hepatocellular carcinoma; US, ultrasound.

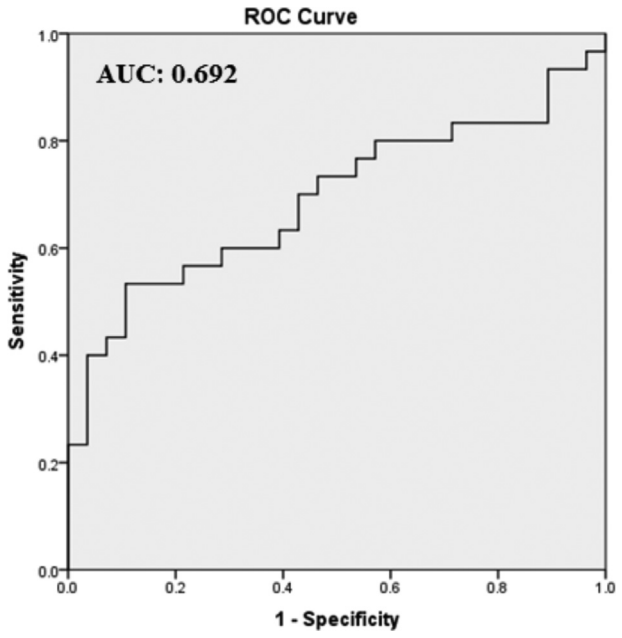
which was significantly higher in HCC patients with mean±SD of 185.42±194.28 ng/l compared with HCV-cirrhosis patients with mean±SD of 65.16 ±113.73 ng/l (P=0.002).

Regarding GDF15, a statistically significant difference was found between HCV-HCC patients and controls, with mean±SD of 154.45±123.74 and 81.19±42.53 pg/ml, respectively (P=0.012). However, no statistically significant difference was found between HCV-cirrhosis patients and controls, with mean±SD of 140.28±128.66 and 81.19±42.53 pg/ml, respectively (P=0.064), or between HCV-cirrhosis and HCV-HCC patients with mean±SD of 140.28±128.66 and 154.45±123.74 pg/ml, respectively (P=0.473).

The areas under the ROC curves showed good performance (AUROC: 0.692) of GDF15 in the detection of HCC. A cut-off value of GDF15 to discriminate HCV-HCC patients from control is 122.3 pg/ml with 53.3% sensitivity and 86.7% specificity [95% confidence interval (CI): 0.552–0.831] (Fig. 1). But GDF15 was not able to discriminate HCV-cirrhosis from HCV-HCC patients with (AUROC: 0.554).

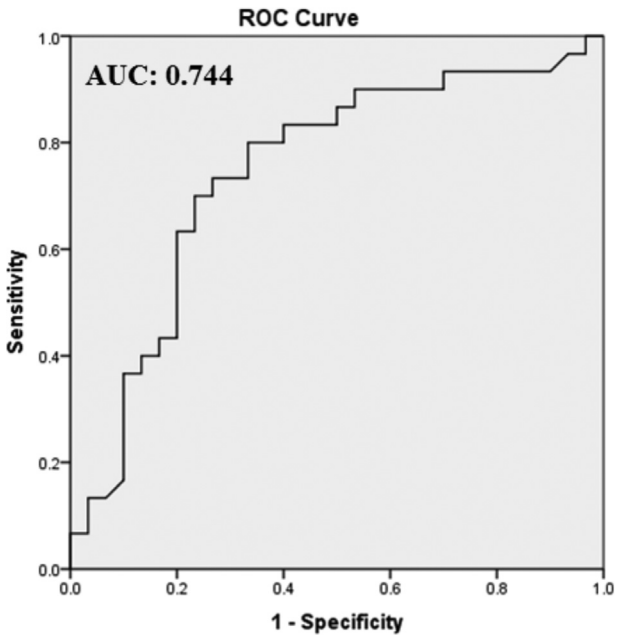
The areas under the ROC curves showed good performance (AUROC: 0.744) of AFP for the

Figure 1



The ROC curve for the discrimination of HCC cases from controls using GDF15. HCC, hepatocellular carcinoma; GDF15, growth differentiation factor 15; ROC, receiver operating characteristic.

Figure 2



The ROC curve for the discrimination of HCC cases from cirrhosis cases Using AFP. AFP, α-fetoprotein; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic.

detection of HCV-HCC patients and their discrimination from HCV-cirrhosis patients. A cut-off value of AFP to discriminate HCV-cirrhosis from HCV-HCC patients is 20.85 ng/l with 73.3% sensitivity and 73.3% specificity (95% CI: 0.615–0.874) and *P* value of 0.001(Fig. 2). With an AUROC of 0.942 for the discrimination of HCV-HCC patients from controls at a cut-off value of 8.75 ng/l, 86.7% sensitivity, 96.4% specificity, 95% CI: 0.880–1.000, and *P* value less than 0.001.

The areas under the ROC curves for combined AFP and GDF15 showed lower performance than AFP alone in the discrimination of HCV-HCC from HCV-cirrhosis patients (AUROC: 0.642) (Fig. 3).

There was positive correlation between GDF15 and AFP in HCV-cirrhosis and HCV-HCC patients with a *P* value of 0.007 and less than 0.001, respectively.

No correlation was found between either AFP or GDF15; and Child–Pugh score, or number of lesions in the liver.

Discussion

Accurate diagnosis of early stages of HCC can significantly improve patient survival. Currently, HCC surveillance is performed by diagnostic imaging techniques and AFP assay. However, these methods have limitations regarding sensitivity and specificity, especially in early-stage HCC [17]. Unfortunately, most HCC cases are diagnosed at a late stage with the resulting median survival of less than 1 year [24,25]. As curative therapies are restricted to patients in early-stage HCC [26] improving diagnostic accuracy for the detection of early-stage HCC is of utmost importance, especially in high-risk patients with chronic viral hepatitis B and post-HCV-cirrhosis [26].

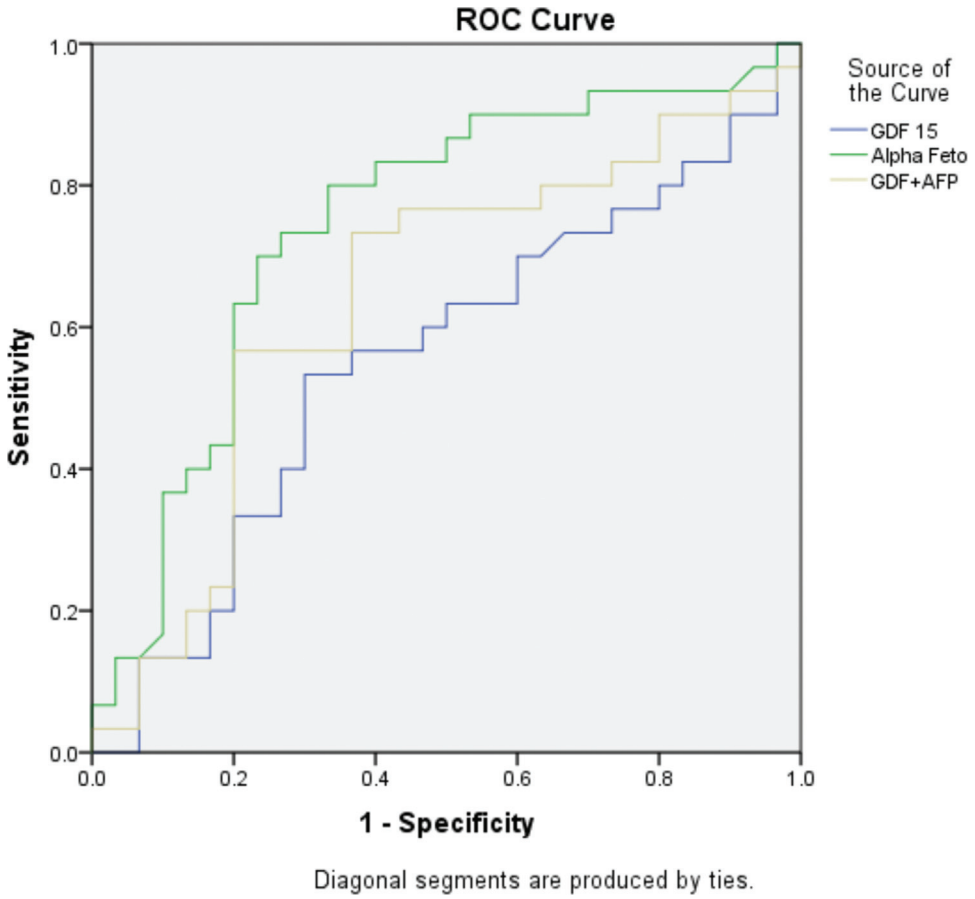
Egypt has the highest prevalence of HCV infection worldwide and incidence of HCC in Egypt. It is reported to be 21% in cirrhotic patients [27]. In the past 10 years, the incidence rate of HCC doubled [28].

In the present study, we evaluated the diagnostic power of serum GDF15 in HCC detection and distinction from cirrhosis in chronic hepatitis C Egyptian patients.

GDF15 is a serum marker associated with carcinogenesis [29–31]. GDF15 has a diagnostic role in gastrointestinal cancers [32,33], and was as well reported to have a diagnostic role in HCC accompanied with cirrhosis [21].

In our study, GDF15 level was significantly elevated in HCV-HCC patients compared with normal controls with *P* value of 0.012, but no statistically significant difference was found between GDF15 levels in HCV-cirrhosis and HCV-HCC patients with *P* value of

Figure 3



The ROC curve for the discrimination of HCC cases from cirrhosis cases using both GDF15 and AFP. AFP, α-fetoprotein; HCC, hepatocellular carcinoma; GDF15, growth differentiation factor 15; ROC, receiver operating characteristic.

0.473. Therefore, GDF15 level was not able to discriminate between HCV-cirrhosis and HCV-HCC patients. Our results are in agreement with the Liu *et al.* [21] study, where 233 HCC patients were studied and only 30/233 (12.8%) were HCV positive. They reported that the highest GDF15 level was found in HCV-HCC patients. They did not study the discriminative power of GDF15 to differentiate HCV-cirrhosis from HCV-HCC because they omitted HCV-cirrhosis cases from the study due to the small number of samples.

Although cirrhosis is the common pathway by which HCV promotes carcinogenesis, HCV may have a direct role in inducing liver cell proliferation through the involvement of viral gene products [34].

On the other hand, Zimmers and colleagues investigated the effect of GDF15 loss *in vivo* on hepatocellular carcinogenesis in a diethylnitrosamine-induced HCC mouse model, and no apparent effect was found on the HCC tumor formation rate, growth rate, or invasiveness after genetic ablation of GDF15 and it was concluded that the biological significance of GDF15 induction in HCC pathogenesis is unknown [35].

The areas under the ROC curves showed good performance (AUROC: 0.692) of GDF15 in the detection of HCV-HCC. The cut-off value of GDF15 to discriminate HCV-HCC from control is 122.3 pg/ml with 53.3% sensitivity and 86.7% specificity (95% CI: 0.552–0.831). But GDF15 was not able to discriminate HCV-cirrhosis from HCV-HCC patients with an AUROC of 0.554.GDF15 was inferior to AFP in its ability to detect HCV-HCC and the AUROC was 0.744 for the detection of HCV-HCC patients and their discrimination from HCV-cirrhosis patients at a cut-off value of AFP 20.85 ng/l with 73.3% sensitivity and 73.3% specificity.

A combination of GDF15 and AFP even compromised the diagnostic accuracy of AFP in discrimination between HCV-cirrhosis and HCV-HCC. On the other hand, Liu *et al.* [21] reported that GDF15 combined with AFP increased the diagnostic accuracy of HCC accompanied with cirrhosis.

At the same cut-off level of 20 ng/ml, our study had higher sensitivity for detecting HCC than the previously published studies where AFP had a sensitivity ranging from 25 to 65% for detecting HCC [36,37].

In conclusion, the HCC burden is increasing worldwide and with Egypt, having the highest HCV prevalence. Improving diagnostic accuracy for detecting HCC is fundamental to reduce the rates of cancer-related mortality, as HCC is one of the fastest rising causes of cancer-related mortality. Several serum biomarkers have been studied, but none was found superior to the AFP [17]. We have to admit limitations of AFP; only one-third of HCC patients have high AFP levels [38,39] as 80% of small HCC nodules do not display increased AFP levels and that AFP sensitivity is restricted to 25% for tumors smaller than 3 cm [40,41]. Furthermore, high AFP levels can be expressed in the absence of malignancy in patients with chronic liver disease, especially HCV patients [42,43]. Continuous research is needed to find the ideal markers for HCC, preferably serum marker with high diagnostic power in both HCC detection and distinction from cirrhosis, while tumor stage and patient etiology are taken into consideration.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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