Vascular endothelial growth factor (VEGF) as a biochemical marker for the diagnosis of hepatocellular carcinoma (HCC)

Abstract

Hepatocellular Carcinoma (HCC), a malignant tumor is common in Egypt. Early diagnosis of HCC is important; therefore, this study evaluated the diagnostic potential of VEGF for diagnosis of HCC and its capability to differentiate HCC from benign tumor and CLD in early stages. Three groups of patients: Group I with HCC (n=116), Group II with Chronic Liver Diseases (CLD) (n=30) and Group III control (n=30) were subjected to full history, clinical examination, liver function tests and radiological investigations. Peripheral blood samples were assayed for VEGF by sandwich ELISA technique, quantitation was achieved by the construction of a standard curve. Serum VEGF and AFP were significantly higher in HCC. A significant positive correlation between VEGF and AFP, Child score and class, Okuda class and total bilirubin and a significant negative correlation with albumin was observed. The cut-off to discriminate HCC and CLD from the healthy group was ≥ 280 pg/mL. VEGF is a highly useful marker for the diagnosis of HCC in patients with normal serum AFP concentrations. Its serum levels beyond threshold show its discriminatory potential for HCC and CLD suggesting that suspected patients with high ALT, AST and albumin levels may be screened for AFP and VEGF.

Keywords: alpha fetoprotein, chronic liver disease, hepatocellular carcinoma, vascular endothelial growth factor, Hepatitis C-virus

Abbreviations: AFP: Alpha Feto Protein; ALT: Alanine Transaminase; AST: Aspartate Transaminase; CLD: Chronic Liver Diseases; CT: Computed Tomography; ELISA: Enzyme Linked Immune Sorbent Assay; GGT: Gamma-Glutamyl Transferase; HBV: Hepatitis B-Virus; HCC: Hepatocellular Carcinoma; HCV: Hepatitis C-Virus; PT: Prothrombin Time; VEGF: Vascular Endothelial Growth Factor; VEGFR: Vascular Endothelial Growth Factor Receptor

Introduction

Hepatocellular Carcinoma (HCC) is the second common cause of cancer-related death mortalities [1]. Its incidences are increasing worldwide ranging between 3% and 9% annually [2]. The rates of HCV in Egypt are among the highest in the world [3,4]. In Egypt, the annual proportion of HCC showed a significant rising trend from 4.7% in 1993 to 7.2% in 2002 [5]. Studies show that there are 6 million cases of HCC per year with annual 600,000 deaths [6,7]. Hypoxia is the major trigger for HCC; thus, it is hypervascular in nature, with an important role of angiogenesis in its pathophysiology [8,9]. Deregulation of several angiogenic pathways are been reported in HCC [10]. There are diverse causes of HCC as chronic Hepatitis B and Hepatitis C infections, alcohol use or non-alcoholic fatty liver disease in association with cirrhosis, hereditary hemochromatosis, obesity or primary biliary cirrhosis, with cirrhosis as the most important risk factor [11-13].

If HCC is left untreated, liver cancer has a poor prognosis [14]. Most of HCC patients visit clinics at advance disease with the survival of approximately 5 years [15,16]. Complete surgical resection and liver transplant are at present the only curative treatment options [17]. Unfortunately, the majority of patients with liver cancer present advanced unresectable disease, therefore, they are not surgical candidates [17,18]. Hence, screening programs of patients at risks, such as chronic infections of hepatitis B and C represent attractive strategies for potential improvement of the outcome of HCC patients. Therefore, it is very important to detect this disease and the recurrence during its early phase.

Serum Alpha Fetoprotein (AFP) is the most widely used tumor marker for diagnosis as well

MN Hamdy[1], KY Shaheen[2], Mona AM Awad[3], Eman MF Barakat[4], SI Shalaby[5], Neelima Gupta[6*] and Varsha Gupta[7]

[1]Department of Clinical and Chemical Pathology, Medical Division, National Research Center, Egypt
[2]Department of Clinical and Chemical Pathology, Ain Shams University, Egypt
[3]Department of Chemical and Clinical pathology, Medical Division, National Research Center, Egypt
[4]Departmentof Tropical Medicine, Ain Shams University, Egypt
[5]Department of Complementary Medicine, Medical Divisions, National Research Center, Egypt
[6]Centre of Excellence Laboratory, Department of Animal Science, MJP Rohilkhand University, India
[7]Chhatrapati Shahu Ji Maharaj University, India

**Author for correspondence: guptagrawal@rediffmail.com*

as surveillance of HCC [19-21]. However, AFP levels may be normal in up to 40% of patients with HCC, particularly during the early stages (low sensitivity) resulting in 40% cases of missed diagnosis [22]. Furthermore, elevated AFP levels may be seen in patients with cirrhosis or exacerbations of chronic hepatitis (low specificity), normal levels of serum AFP are shown in 15%-20% while advanced stages may have AFP concentration as low as <20 ng/ml which account for 30%-40% of HCC [22,23]. Thus, the identification of novel biochemical markers for HCC remains an important goal for many laboratories around the world [24]. It has been suggested that elevated ALT levels are strongly associated with the incidence of HCC regardless of hepatitis virus positivity. Therefore, the ALT level may be considered as a good independent determinant for intervention [25]. Serum ALP level is increased up to 4 times in HCC patients. Total GGT activity increases in both hepatic and extra-hepatic tumors. This mostly decreases both the sensitivity and specificity of this enzyme in detecting HCC [20].

Hepatocellular carcinoma is a highly vascular tumor characterized by neovascularization and a high propensity for venous invasion [26]. Vascular Endothelial Growth Factor (VEGF) is the most potent directly acting angiogenic factor known so far. It is a soluble, homo-dimer of 34-42 kDa heparin-binding glycoproteins that specifically stimulates endothelial cell proliferation and enhances vascular permeability [26]. VEGF promotes extravasation of plasma fibrinogen, leading to the formation of fibrin scaffolding that facilitates cell migration during invasion [19,27]. As an endothelial growth factor, VEGF stimulates endothelial cell proliferation, thus inducing the budding of new blood vessels around the growing tumor masses [19]. Serum VEGF levels were significantly elevated in HCC patients as compared to patients with benign liver lesions and normal controls, however, VEGF levels of serum in the previous reports were not significantly different between the patients with benign liver lesions and normal controls [19,20]. However, many recent studies have reported significantly higher levels of VEGF in HCC as compared to others [8,9,28]. These show that it has the potential to be used as a diagnostic and prognostic marker as well as VEGF/VEGFR pathway as a potential therapeutic target for HCC [29,30].

The aim of the present study was to investigate the clinical utility of VEGF in HCC patients and to compare the results with those having CLD.

Patients and Methods

Patients

The present study included 116 patients from the Department of Tropical Medicine, Ain Shams University Hospital, Cairo, Egypt with HCC, 30 with CLD as pathological control and 30 apparently healthy individuals as the healthy control group. The HCC group was included prior to starting any kind of treatment. The diagnosis was confirmed by a triphasic spiral abdominal CT. They were 95 males and 21 females ranging in age from 40 to 77 years (mean 56.1 ± 7.7 years). The CLD group was matched in age and sex with HCC patients. The diagnosis was based on clinical, biochemical, and sonographic criteria of chronic liver disease. They were 24 males and 6 females ranging in age from 40 to 72 years (mean 55.0 ± 8.4 years). The healthy individuals were matched in age and sex with HCC patients. They were 24 males and 6 females whose age ranged from 43 to 72 years (mean 56.8 ± 7.9 years).

Clinical examinations

All subjects of the study were subjected to full history, detailed clinical examination, routine liver function tests including AST, ALT, albumin, total bilirubin, Prothrombin Time (P.T.), and serum AFP as follows.

AST and ALT

The analysis was done on Synchron CX-5 autoanalyzer by a kinetic method using reagents provided by humans [31].

Albumin

Albumin was analysed on the Synchron CX-5 autoanalyzer by albumin-bromocresol green (BCG) binding method using reagents provided by Spectrum [32].

Total bilirubin

Total bilirubin was estimated on Synchron CX-5 autoanalyzer by a timed endpoint Diazo method using reagents supplied by Synchron System Beckman Coulter [33].

Prothrombin time (PT)

The analysis was done on STA- Stago Compact

C.T. autoanalyzer using reagents supplied by Dade- Behring [34].

■ Alfa feto protein

Assay was carried out by a sandwich ELISA technique using reagents provided by BioCheck, Inc. [35].

■ Radiological investigations

Radiological investigations included CT scan and abdominal ultrasound of the HCC patients using abdominal Ultrasound apparatus and abdominal Triphasic Spiral Computed Tomography (CT).

■ Histopathology

Liver biopsy and histopathological examinations were done whenever needed following routine techniques.

■ VEGF quantitation

Peripheral blood samples were assayed for VEGF by sandwich ELISA technique employing two VEGF specific antibodies. VEGF quantitation was performed by Sandwich Enzyme-Linked Immunosorbent Assay using reagents provided by Bender MedSystems. Quantitation was achieved by the construction of the standard curve using known concentrations of VEGF.

Results

The study was initiated with age and sex-matched control, CLD and HCC subjects. **TABLE 1** shows the patients of HCC and CLD groups classified according to Child-Pugh criteria. A maximum number of patients in both groups HCC and CLD were in B class followed by A and C.

The HCC and CLD groups have been compared according to Okuda **TABLE 2** classification. Group II Okuda showed the highest values for both HCC and CLD groups, followed by I and III.

TABLE 3 shows that there is no significant difference between HCC and CLD groups regarding a number of cases and percentage of HBV and HCV, however, HCV is more common in HCC and CLD patients as compared to HBV.

TABLE 1. Comparison between HCC and CLD groups with respect to child classification.

Group	HCC	CLD
Child classification		
A	44 (37.9%)	11 (36.7%)
B	48 (41.4%)	13 (43.3%)
C	24 (20.7%)	6 (20.0%)
Child score	7.5 ± 2.0	7.5 ± 1.9

TABLE 2. Classification of HCC group according to Okuda classification.

	N	%
Okuda I	49	42.2
Okuda II	54	46.6
Okuda III	13	11.2
Total	116	100

TABLE 3. Comparison between HCC and CLD with respect to the number of cases and percentage of HBV and HCV infection.

	HCC	CLD	χ^2	p
HBV	11 (9.5%)	2 (6.7%)	0.233	0.629
HCV	107 (92.2%)	27 (90.0%)	0.159	0.69

χ^2: Chi square test; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

TABLE 4 with respect to the clinical picture, the only difference between the CLD and HCC group was that tremors were significantly more frequent in the former group as compared to the latter.

Different radiological aspects showed non-significant differences between the two groups in spleen size, ascites, portal vein, its diameter, common bile duct, IHBRD, and collaterals. However, hepatomegaly was significantly more frequent in CLD group than HCC group **TABLE 5**.

ALT, albumin, hemoglobin was significantly higher in HCC group than CLD group, though AST and INR were also higher in HCC than CLD group, but the difference was statistically non-significant, while there was no significant difference between HCC and CLD groups with regards to platelets **TABLE 6**. On the other hand, total bilirubin was significantly higher in CLD group as compared to HCC group.

AFP was significantly different between HCC, CLD and healthy groups being highest in HCC group, followed by CLD group and lowest in healthy group **TABLE 7**.

VEGF was significantly different amongst HCC, CLD and healthy groups were highest in HCC group followed by CLD group and lowest in healthy group **TABLE 8**.

There was a significant positive correlation between AFP and VEGF, ALT and AST as well as AFP and VEGF **TABLE 9**.

TABLE 4. Clinical comparison between HCC and CLD groups.

	HCC	CLD	χ^2	p
Bleeding Tendency	68 (58.6%)	12 (40.0%)	3.336	0.068
Hematemsis	19 (16.4%)	4 (13.3%)	0.167	0.683
Jaundice	34 (29.3%)	11 (36.7%)	0.605	0.437
Ascites	51 (44.0%)	11 (36.7%)	0.52	0.471
Edema	62 (53.4%)	17 (56.7%)	0.099	0.753
Fever	11 (9.5%)	5 (16.7%)	1.261	0.262
Encephalopathy	20 (17.2%)	5 (16.7%)	0.006	0.941
Tremors	1 (0.9%)	2 (6.7%)	3.99	**0.046***
DM	33 (28.4%)	8 (26.7%)	0.037	0.847

χ^2: Chi square test; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01: Highly significant difference

TABLE 5. Radiological comparison between HCC and CLD groups.

	HCC	CLD	χ^2	p
Liver size				
Average	71 (61.2%)	5 (16.7%)	31.55	**<0.001***
Shrunken	19 (16.4%)	2 (6.7%)		
Enlarged	26 (22.4%)	23 (76.7%)		
Spleen size				
Average	78 (67.2%)	21 (70.0%)	2.249	0.325
Shrunken	30 (25.9%)	9 (30.0%)		
Enlarged	8 (6.9%)	0 (0.0%)		
Ascites				
Present	46 (39.7%)	11 (36.7%)	0.089	0.765
Absent	70 (60.3%)	19 (63.3%)		
Portal Vein				
Thrombosed	10 (8.6%)	1 (3.3%)	0.956	0.328
Not	106 (91.4%)	29 (96.7%)		
Portal Vein Diameter				
Dilated	19 (16.4%)	3 (10.0%)	0.758	0.384
Not	97 (83.6%)	27 (90.0%)		
Common Bile Duct				
Dilated	0 (0.0%)	0 (0.0%)	0	1
Not	116 (100.0%)	30 (100.0%)		
IHBRD				
Present	2 (1.7%)	1 (3.3%)	0.307	0.58
Absent	114 (98.3%)	29 (96.7%)		
Collaterals				
Present	11 (9.5%)	0 (0.0%)	3.077	0.079
Absent	105 (90.5%)	30 (100.0%)		

χ^2: Chi square test; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

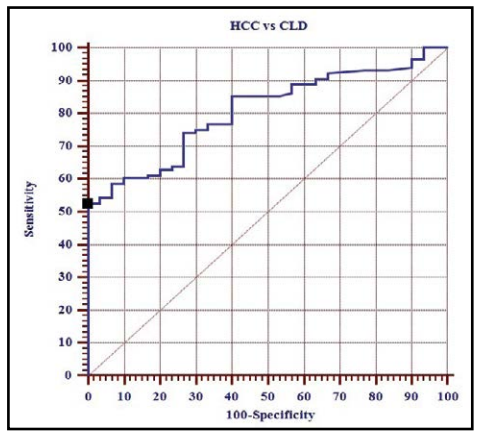

FIGURE 1. Receiver Operating Characteristic (ROC) curve to evaluate the value of VEGF to differentiate between HCC patients versus CLD control group with AUC: 0.807.

TABLE 6. Comparison between HCC and CLD groups based on laboratory results.

	HCC (N=116)		CLD (N=30)		Z	p
	Median (IQR)	Range	Median (IQR)	Range		
ALT	47.5 (37.3-67.0)	8.0-200.0	35 (19.5-53.8)	120.0-135.0	-2.715	**0.007***
AST	65 (46.5-84.5)	10.0-215.0	52 (33.5-84.3)	15.0-285.0	-1.444	0.149
Albumin	3.2 (2.7-3.5)	1.8-4.9	2.9 (2.3-3.2)	1.7-4.6	2.268	**0.023***
Bilirubin	1.5 (1.0–2.5)	0.3–5.7	1.8 (1.5-3.6)	0.7–19.4	-1.953	**0.050***
Hb	12 (10.8-13.5)	8.1-20.7	10.6 (8.8-12.0)	7.1-16.0	3.294	**0.001***
Platelets	110.5 (81.3-151.5)	15.0-661.0	112 (56.3-192.8)	1.0–376.0	-0.242	0.809
INR	1.4 (1.2-1.7)	0.9-2.7	1.3 (1.1-1.5)	0.9-2.6	1.49	0.136

*Z: Mann Whitney test; **IQR: Inter-quartile range; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

TABLE 7. Comparison between HCC, CLD and healthy control groups with respect to AFP.

Group	N	Median (IQR)	Range	HCC	HCC	CLD
I (HCC)	116	25.9 (10-281.3)	0.9-3308	CLD	Healthy	Healthy
II (CLD)	30	6.66 (4.1-15)	2-107.5	Z**=4.318	Z=5.789	Z=1.079
III (Healthy)	30	6.1 (5.3-7.4)	2.9-10.2	p<0.0001*	p<0.0001*	p=0.0280*

IQR*: Inter-Quartile Range; Z**: Mann Whitney test; p>0.05: Non-significant difference, p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

TABLE 8. Comparison between HCC, CLD and healthy groups with respect to VEGF.

Group	N	Median (IQR)	Range	HCC	HCC	CLD
I (HCC)	116	530 (248.5-915)	40.0-3028.0	CLD	Healthy	Healthy
II (CLD)	30	166 (113-340)	13.0-482.0	Z=5.168	Z=6.606	Z= 2.285
III (Healthy)	30	129 (37-212)	12.0-280.0	p<0.000*	p<0.0001*	p=0.0223*

IQR*: Inter-Quartile Range; Z**: Mann Whitney test; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

TABLE 9. Correlation between AFP and other parameters.

	r_s	p
VEGF	0.347	<0.001*
Age	-0.01	0.91
Child Score	0.021	0.807
Child Class	0.016	0.851
ALT	0.268	<0.001*
AST	0.212	0.011*
Albumin	0.135	0.109
Total Bilirubin	0.002	0.983
Hemoglobin	0.157	0.062
Platelets	-0.056	0.56
INR	-0.106	0.209

r_s: Spearman correlation test; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

TABLE 10. Correlation between VEGF and other parameters.

	r_s	p
AFP	0.347	<0.001*
Age	-0.025	0.768
Child Score	0.388	<0.001*
Child Class	0.372	<0.001*
Okuda Class	0.309	<0.001*
ALT	0.114	0.172
AST	0.13	0.118
Albumin	-0.186	0.024*
Total Bilirubin	0.204	0.013*
Hemoglobin	-0.06	0.472
Platelets	-0.059	0.531
INR	0.102	0.221

r_s: Spearman correlation test; p> 0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

There was a significant positive correlation between VEGF and AFP, Child Score, Child class and total bilirubin and a significant negative correlation with albumin **TABLE 10**.

The Receiver Operating Characteristic (ROC) curve analysis was done to assess the diagnostic performance of serum VEGF (pg/mL) in HCC

patients versus CLD control group **TABLE 11**. The curve is represented in **FIGURE 1** whereas the ROC curve to evaluate the value of VEGF to differentiate between HCC and CLD groups versus healthy group is shown in **TABLE 12 and FIGURE 2**.

TABLE 13 shows that VEGF was significantly elevated (>482 pg/ml) in both groups of AFP especially in a group with normal levels of AFP (<200 ng/ml), representing its useful role as a serological marker for diagnosis of HCC patients with a normal level of AFP.

TABLE 14 The levels of AFP in different classes of OKUDA showed significant differences in class wise. Our study shows high levels of AFP in OKUDA I with reduced levels in II but >200 in OKUDA II and very high levels in OKUDA III, while VEGF was very high in all three classes of OKUDA.

TABLE 11. Receiver operating characteristic (ROC) curve analysis to assess the diagnostic performance of serum VEGF (pg/mL) in HCC patients versus CLD control group.

	Cut-off	SN%	SP%	PPV%	NPV%	EFF%
HCC *vs* CLD	>482	52.59	100	100	35.3	61.6

EFF: Efficacy (Diagnostic Accuracy); PPV: Positive Predictive Value; SN: Sensitivity; SP: Specificity; NPV: Negative Predictive Value

TABLE 12. Receiver operating characteristic (ROC) curve analysis to assess the diagnostic performance of serum VEGF (pg/mL) in HCC and CLD groups versus Healthy group.

	Cut-off	SN%	SP%	PPV%	NPV%	EFF%
HCC and CLD vs Healthy	> 280	60.27	100	100	34.1	67

EFF: Efficacy (Diagnostic Accuracy); PPV: Positive Predictive Value; SN: Sensitivity; SP: Specificity; NPV: Negative Predictive Value

TABLE 13. Classification of HCC patients according to their AFP and VEGF levels.

	AFP>200	AFP<200	χ^2	p
VEGF>482	29 (25%)	32 (27.6%)	11.387	0.0007
VEGF<482	9 (7.8%)	46 (39.6%)		

χ^2: Chi square test; p>0.05: Non-significant difference; p<0.05: Significant difference; p<0.01, <0.001: Highly significant difference

TABLE 14. Comparative analysis of AFP and VEGF in different classes of Okuda using one-way analysis of variance. a and b represent significant differences in the means of I versus II and II versus III.

	AFP			VEGF		
Okuda classes	**I**	**II**	**III**	**I**	**II**	**III**
N	49	54	13	49	54	13
Mean	452.67	272.2	2931.9	525.55	626.44	1324.54
Std. Error	156.03	81.05	1907.62	63.78	71.63	181.87
F-value	6.99**			12.85***		

p<0.001 is represented as**; p<0.0001 is represented as***

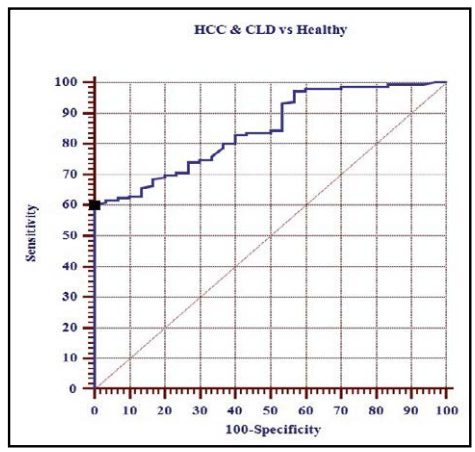

FIGURE 2. Receiver Operating Characteristic (ROC) curve to evaluate the value of VEGF to differentiate between HCC and CLD groups versus healthy group with AUC: 0.847.

Discussion

Hepatocellular carcinoma is the fifth most common malignancy in the world and the third most common cause of cancer-related mortality [19,36,37]. In Egypt, it has been reported that the incidence rate of HCC increases annually and the burden of HCC has been increasing with doubling of the incidence rate in the past 10 years [38].

Hepatocellular carcinoma is often asymptomatic at the early and most curable stages. Unfortunately, patients are usually diagnosed at very advanced stages when effective treatments are not possible. Therefore, early detection of HCC is a critical goal to improve patient health [39].

Our study revealed that 44, 48 and 24 patients with HCC were included in Child's classification, while the majority of HCC patients were in OKUDA II. The results suggest that initial changes based on Child's classification should be taken up seriously for the diagnosis of HCC.

With respect to the number of cases and percentage of HBV and HCV, the present study revealed that there is no significant difference between HCC and CLD regarding HBV, HCV, also in patients with HCC, HCV was positive in 92.2% and HBV in 9.5% cases, therefore HCV is more common in HCC patients than HBV. Hepatitis C virus (HCV) infection was commonly observed in both HCC and CLD groups. HCV infection was also found to be associated with the onset of CLD. In patients with HCC, HCV was positive in 73.3% and HBV in 16.3% cases and our results are in agreement [5].

However, the majority of our HCC patients showed average liver size suggesting ultrasonography may not be a useful modality for diagnosis of HCC (22.4% patients) while CLD was significantly associated with hepatomegaly (76.7% patients). Thus, radiological findings indicated that there is no significant difference between HCC and CLD except for hepatomegaly which is significantly more frequent in CLD group than HCC group; this was similar to earlier results [40,41].

The laboratory results revealed that ALT, AST, Albumin, and hemoglobin are significantly higher in HCC group than CLD group. The results suggest their upregulation in HCC. Results also showed that AST and INR are higher in HCC group than CLD group, but the difference was statistically non-significant. This was similar to the results of Zekri et al. who found that aminotransferases were significantly elevated in HCC when compared to CLD patients [42]. On the other hand, no significant difference between HCC patients and CLD patients regarding aminotransferases were reported, none of the liver function tests were specific enough to be diagnostic of HCC and were not distinct from those found in cirrhosis [43]. The present study indicated that there is no significant difference between HCC and CLD with regard to platelets, while total bilirubin was significantly higher in CLD group than HCC group.

Taking the clinical picture into consideration, there is no significant difference between HCC and CLD except for tremors which were more frequent in CLD group than HCC group, this finding was in agreement where many patients of HCC could be asymptomatic and diagnosed during screening of high-risk patients [44].

AFP was significantly high in HCC group as compared to CLD group. High AFP is suggestive of its diagnostic potential in HCC. Currently, AFP represents the most commonly used serological marker for the diagnosis of HCC, despite its low sensitivity which reaches only 45%. Moreover, alterations of AFP serum levels are commonly observed in cirrhotic patients [45]. The development of false-negative or false-positive rates with AFP has been reported to be as high as 30%-40% respectively for patients with small hepatocellular carcinomas [23]. This prompted the search for more reliable, noninvasive biochemical markers with better sensitivity and specificity for the early diagnosis of HCC. VEGF is one of the platelet-derived growth factor family members who have been found to be overexpressed in HCC [46]. The increase in the VEGF level in CLD patients and the marked increase in HCC patients may be attributed to the increased hepatocyte and hepatoma cell production of VEGF in response to hypoxia resulting from regeneration or proliferation [47].

Our results showed significantly higher VEGF levels in HCC as compared to CLD and healthy controls. Therefore, we evaluated the clinical utility of serum levels of VEGF in HCC patients and compared the results with those having CLD. Yvamoto et al. demonstrated VEGF to be a useful biomarker for the detection

of HCC [48]. However, AFP was important to discriminate patients with HCC and cirrhosis or HCV.

Our study was in accordance with other studies who reported that AFP levels were higher in HCC as compared to CLD patients with statistically significant differences between the two [45,49,50]. Di Bisceglie et al. observed that AFP production is enhanced in the presence of inflammation, necrosis and hepatocellular injury, possibly resulting from increased hepatocyte turnover [51]. However, Abdelgawad et al. opined that elevated serum AFP levels may be the result of altered hepatocyte-hepatocyte interaction associated with a loss of normal architectural arrangements rather than necrosis or active regeneration [52].

In the present study, serum VEGF level in HCC patients was significantly higher than the healthy controls and CLD groups. This finding coincides with other reports and they demonstrated that VEGF levels in HCC patients has shown promise for HCC screening, whether used alone or combined with serum AFP [19,49,53].

Angiogenesis is an important requirement of almost every solid tumor. HCC being highly invasive and vascular tumor has angiogenesis for tumor progression and metastasis, which is also a leading cause of mortality of advance HCC after curative resection. High expression of VEGF and its receptors (VEGFR-1, -2 and -3) are observed to be high in HCC cell lines and serum of HCC patients [8,9,54,55].

VEGF overexpression was reported in pre-cancerous stages as dysplastic and cirrhotic liver tissues, suggesting its important role in angiogenesis in liver cancer [56]. High expression of VEGF was associated with the development of HCC, but was also correlated with the tumor grading of HCC [57]. VEGF and its receptor VEGFR-2 are important angiogenic pathways, and its blockade is the first strategy for cancer therapy [58].

In a case control immune histochemical study performed on 36 patients with HCC and 6 healthy controls, VEGF was detected in 32 out of 36 patients (89%) tumor specimens, but none in the 6 healthy controls [59]. In the present study, there was a significant difference between CLD patients and healthy control groups in the level of VEGF which agreed with El-Houseini et al. [49].

In contrast to our finding, Zhao et al. had detected that serum levels of VEGF were insignificantly higher in CLD patients than in healthy controls [60].

The results of the current study revealed a positive correlation between AFP levels and VEGF and serum aminotransferases. There is a significant positive correlation between VEGF and AFP, Child score class and Okuda class and total bilirubin and a significant negative correlation with albumin. In addition, the present study showed no correlation between VEGF and aminotransferases, hemoglobin, platelets, and INR which is in agreement with Assy et al. who stated that no correlation was found between VEGF serum levels and aminotransferases [61]. On the contrary, Makhlouf et al. found that there was a significant positive correlation between circulating VEGF and aminotransferases which indicates a degree of hepatic dysfunction and this may be attributed to the release of VEGF from the damaged hepatocytes [47]. However, studies reported that VEGF showed no significant correlation to any of the clinicopathological variables in HCC patients [19,53,60].

Also, this study shows no significant correlation between serum VEGF and platelet count, thereby resembling the data given by Assy et al. who stated that no correlation was found between VEGF serum levels and platelets count [61]. In addition, Li et al. reported that there was a weak correlation of platelet number and VEGF level [62]. On the other hand, few studies found that there was a significant positive correlation between the circulating VEGF and the platelet count [47,63,64].

Obtaining plasma VEGF levels is an easy and simple procedure, which makes long-term monitoring of VEGF levels feasible, even after local intervention and therapies for HCC. Moreover, the absence of correlation between plasma VEGF levels with aminotransferases, hemoglobin, platelets and INR suggests that VEGF is not affected by minor changes in blood metabolism or inflammation.

Our findings are in accordance with El-mezayen and Darwish [65]. These also support the work of Mukozu et al., who reported significantly high serum VEGF levels in HCC patients than in non-HCC patients [66].

Likewise, Guo et al. also reported that the median serum VEGF level in the HCC patients (285 pg/mL) was significantly higher than that of healthy controls (p=0.021) [29,67-69]. El-mezayen generated a score incorporating both plasma VEGF and serum AFP beside other parameters for early detection of HCC [65].

Assessment of the diagnostic performance of serum VEGF in our study revealed that the best cut-off for serum VEGF to discriminate HCC from CLD patients was ≥ 482 pg/mL. This cut-off provides sensitivity, specificity, PPV, NPV and efficacy of 52.59%, 100.0%, 100.0%, 35.3% and 61.6% respectively. These results were consistent with the results of El-Houseini et al. who stated that in order to discriminate between HCC patients and CLD patients, the cut-off point for VEGF was established at 355.2 pg/mL with a sensitivity, specificity, PPV, NPV and efficacy of 86.4%, 60.0%, 78.1%, 82.6%, and 33.3% respectively [49]. The results on the diagnostic performance of VEGF in HCC and CLD patients versus the healthy control group revealed that the best cut-off was ≥ 280 pg/mL that yielded sensitivity, specificity, PPV, NPV and efficacy of 60.27%, 100%, 100%, 34.1% and 67% respectively.

According to the European Association for the study of the liver, the AFP level is diagnostic for HCC above 200 ng/mL [50,70]. In the present study, there were 78 HCC patients with AFP <200 ng/mL and 38 patients with AFP level >200 ng/mL. From the 78 HCC patients with AFP <200 ng/mL, there were 32 (27.6%) patients with VEGF >482 pg/mL and 46 (39.7%) patients with VEGF <482 pg/mL and from the 38 patients with AFP level >200 ng/mL there were 29 (25%) patients with VEGF >482 pg/mL and 9 (7.8%) patients with VEGF <482 pg/mL. Therefore, VEGF was significantly elevated (>482 pg/ml) in both groups of AFP especially in the group with a normal level of AFP (<200 ng/ml), representing its useful role as a serological marker for diagnosis of HCC patients with the normal level of AFP.

In a study by Atta et al. for diagnosis of HCC the cutoff value of VEGF in plasma was 271.85 pg/mL that had a sensitivity of 90%, the specificity of 90% with 87.3% accuracy and 92.3% positive predictive value [28]. Using AFP and VEGF both for diagnosis of HCC, 100% and 98.7% increase was observed in the sensitivity and specificity respectively and accuracy of 98.9% [28]. Thus using both the markers simultaneously has increased the HCC detection sensitivity [28]. In our study, AFP and VEGF both were significantly different in different classes of Okuda. The study shows AFP levels were reduced in Okuda II while very high values were observed for Okuda I and III. However, the increase in VEGF levels was found with increasing classes, suggesting its reliability for use as a diagnostic marker and therapeutic target. It suggests that VEGF can serve as a diagnostic marker for detection of HCC in Okuda I, II and III with or without AFP but also that these patients are suitable for anti-VEGF/VEGFR therapy as well. A study by Shigeta et al., suggest that anti VEGFR-2 therapy combined with other therapies as PD1 can overcome resistance to treatment and increase survival in HCC patients [71]. It can thus be used with other therapies for increasing the survival of HCC patients.

A study by Zhuang et al. showed that HCC tissues have a high expression of VEGFR-1, and VEGFR-3 and VEGF-C [72]. It may have an important role in progression of HCC.

Expression of these three factors in the peritumoral tissues may contribute to the assessment of the risk of tumor recurrence in HCC patients and to optimize postoperative treatment to prevent tumor recurrence [72].

For targeting the cancerous cells usage of Sorafenib (multiple kinase inhibitors) prolongs the survival time of advanced HCC patients [73]. Sorafenib specifically acts on VEGFR1, VEGFR2, VEGFR3, PDGFRβ, c-KI, and Raf kinases (Raf1, B-Raf, MEK, ERK). Major targets of sorafenib are VEGF and its receptor VEGFR. Peng et al, have shown that autocrine VEGF is capable of promoting the proliferation of HCC cells. Sorafenib treatment is believed to target the autocrine VEGF signaling pathway in HCC patients [74].

Thus, our study is able to establish that patients with HCV infection with a liver abnormality, high ALT, AST and Albumin should be evaluated for the levels of AFP and VEGF in their serum. If the values of these markers, particularly VEGF are above a definite level, they should be seriously monitored for HCC diagnosis and treatment.

Conclusion

It can be concluded that the results of the current study are encouraging, as serum VEGF could be a promising tumor marker that could be added to the current standard tests for diagnosis of HCC in order to detect the disease at an early stage and hence improve the prognosis and survival rate of the patient. Thus, measurement of VEGF especially in patients with normal AFP is highly recommended, especially in known cirrhotic patients who deteriorate rapidly without any apparent etiology.

Inclusion of serum VEGF evaluation to the current standard tests for HCC would provide diagnostic, prognostic as well as a therapeutic target for better management. This, in turn, could greatly improve the ability to identify such patients and thus could allow them to benefit from earlier treatment. The validation and cost-effectiveness of VEGF as a diagnostic and prognostic tool along with therapeutic target requires further confirmation for large-scale studies in clinical practice.

Declaration of Interests

None

Acknowledgment

National Research Centre, Cairo, Egypt for providing funds for the execution of the work and Ain Shams Faculty of Medicine Hospital, for providing necessary facilities.

Authorship

Guarantor of the article: Neelima Gupta

Author Contributions

1. Concept and design of the study: KYS, EMB
2. Acquisition of data: MNH, MAMA, EMB
3. Analysis and interpretation of data: MNH, KYS, MAMA
4. Drafting and revision of the article: MNH, KYS, SIS, NG, VG
5. Final approval of the version to be published: SIS, NG

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