Study of the Diagostic Role of Vascular Endothelial Growth Factor in Hepatocellular Carcinoma

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Abstract: OBJECTIVE: To evaluate the diagnostic role of vascular endothelial growth factor (VEGF) in detection of hepatocellular carcinoma. **METHODS**: Forty patients with HCC, 20 patients with liver cirrhosis, and 10 healthy control subjects were included in this study. Serum alphafetoprotein (sAFP) and VEGF levels were measured by enzyme-linked immunosorbent assay.**RESULTS:** The serum VEGF levels in the HCC group (4189. ±2831 pg/ml) was significantly elevated as compared with those in patients with cirrhotic liver (470.05±283 pg/ml) and those in normal controls (172.8± 82.71 pg/ml). The VEGF levels were not significantly different between the patients with liver cirrhosis and the normal controls. In the 40 HCC patients, the serum VEGF levels in patients with portal vein (PV) thrombosis (n = 25, 5851.9±2629.4 pg/ml) or with large HCC lesions (>/= 5 cm in diameter) (n = 22, 5686.95 ± 1594.2 pg/ml) were significantly higher than those without PV thrombosis (n = 15, 3294.5 ± 2544.9 pg/ml) or with small HCC lesions (n = 18, 2359.5± 2966.45 pg/ml), while the serum AFP levels in patients with portal vein (PV) thrombosis (711.67 ± 585.97 ng/ml) or with large HCC lesions (>/= 5 cm in diameter) (740.67 ± 645.31 ng/ml) were not significantly higher than those without PV thrombosis (538.34 ± 619.05 ng/ml) or with small HCC lesions (425.86± 519.7 ng/ml) **CONCLUSION:** combined measurement of serum AFP and VEGF significantly increases the sensitivity, accuracy and negative predictive value in detection of HCC in cirrhotic patients rather than using of AFP or VEGF separately.

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1. Introduction

Hepatocellular carcinoma (HCC) accounts for 80% to 90% of primary liver cancer. HCC is a major health problem worldwide, with an estimated incidence ranging between 500 000 and 1 000 000 new cases annually. It is the fifth most common cancer in the world, and the third most common cause of cancer-related death **(Lau and Lai, 2008; Hussain and El-Serag, 2009)**.

The prognosis of hepatocellular carcinoma (HCC) still remains dismal, although many advances in its clinical study have been made. It is important for tumor control to identify the factors that predispose patients to death. With new discoveries in cancer biology, the pathological and biological prognostic factors of HCC have been studied quite extensively **(Qin and Tang, 2002)**.

Tumor progression is angiogenesis dependent, and hepatocellular carcinoma (HCC) is a typical hypervascular tumor characterized by neovascularization, which plays an important role in the growth and progression of HCC **(Pang and Poon, 2006)**.

Vascular endothelial growth factor plays an important role in neovascularization both in normal tissues and most tumors. It has been extensively investigated recently in various hepatic diseases such as primary and secondary hepatocellular carcinoma, liver cirrhosis, hepatitis and even benign tumors in liver. Vascular endothelial growth factor has been verified to be closely involved in the development and metastases of hepatocellular carcinoma and correlated to the high risk of hepatic metastases and a poor prognosis in gastrointestinal cancer. Using antibodies to vascular endothelial growth factor or other drugs to suppress its expression has also been successfully tried to restrain hepatocellular carcinoma cells and metastases *in vitr*o and in animal models **(Shi *et al.*, 2001)**.

Aim of the work:

The aim of the present study was to evaluate the diagnostic role of vascular endothelial growth factor (VEGF) in detection of hepatocellular carcinoma (HCC).

2. Subjects and Methods:

A total number of 60 patients were selected from 103 patients selected from patients attending the Outpatient and /or Inpatient Department of Tropical Medicine, Minoufiya University Hospital and the National Liver Institute in the period between September 2005 to November 2006. They were 47 males (78.3%) and 13 females (21.7%) and their ages were ranging between 38-80 years old, with mean (57.5±10.84years) as well as 10 healthy persons of matched age and sex as a control group. An informed consent was obtained before patients enter the study.

Patients and control were classified into the following groups: -

GI: Comprised (40) patients with HCC on top of cirrhosis. They were subdivided into (3) subgroups according to the results of abdominal ultrasonography, triphasic CT, bone scan and according to TNM classification into:

GIa: Comprised (16) patients with stage I (T1, N0, M0) and II (T2, N0, M0) HCC.

GIb: Comprised (11) patients with stage IIIA (T3, N0, M0) and IIIB (T4, N0, M0) HCC.

GIc: Comprised (13) patients with stage IIIC (Any T, N1, M0) HCC.

GII: - Comprised (20) patients with liver cirrhosis.

GIII: - Comprised (10) healthy persons as a control.

All patients and control were subjected to: full and detailed history taking, complete clinical examination, routine laboratory investigation, viral markers, abdominal ultrasonography and doppler, triphasic CT, CT guided Liver biopsy(this was done for GI only), quantitative measurement of serum alpha-fetoprotein and quantitative measurement of VEGF by ELISA kit. Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 11

3. Results

A total number of 60 patients were selected from 103 patients selected from patients attending the Outpatient and /or Inpatient Department of Tropical Medicine, Minoufiya University Hospital and the National Liver Institute in the period between September 2005 to November 2006. They were 47 males (78.3%) and 13 females (21.7%) and their ages were ranging between 38-80 years old, with mean (57.5±10.84 years) as well as 10 healthy persons of matched age and sex as a control group. An informed consent was obtained before patients enter the study. The results of the present study are presented in the following tables and figures:

Table (1): Statistical comparison between different studied groups as regard mean AFP and VEGF levels

Variable	Group I (n=40)	Group II (n=20)	Group III (n=10)	Kruskal-Wallis test	*P*- value
• **AFP** (ng/ml)					
X±SD	599.01±605.9	141.2±196.4	5.11±3		
Median	490	22.5	4.5	44.02	<0.001
Range	10-2464	2-650	1.5-10		
• **VEGF** (pg/ml)					
X±SD	4189.6±2831	470.05±283	172.8±82.71		
Median	4760	500	147.5	25.98	<0.001
Range	200-9010	102-1000	79-290		

There was significantly higher level of AFP and VEGF in group I than group II and controls.

Table (2): Statistical comparison between different subgroups of HCC, cirrhotics and controls as regard AFP level

Variable	GI (n=40)		
	GIa (n=16)	GIb (n=11)	GIc (n=13)
• **AFP**(ng/ml)			
X±SD	446.53±537.8	587.79±722.44	796.15±568.6
Median	225	400	816
Range	10-1820	17-2464	105-2049

The mean value of serum AFP showed no significant difference between HCC subgroups and no significant difference between HCC subgroups (Ia&Ib) and cirrhotic group and no significant difference between cirrhotic and control groups. The mean value of AFP in HCC subgroup Ia was significantly higher than that of GII. There was a highly significant difference in AFP level between HCC subgroups (Ia, Ib & Ic) and GIII.

The mean value of serum VEGF showed no significant difference between HCC subgroups Ia and (Ib, GII, GIII), nor GIb and GIc, nor between cirrhotic and control groups. The mean value of VEGF in GIb was significantly higher than GII. And there was a highly significant difference between GIc and (GIa, GIb, GII, GIII).

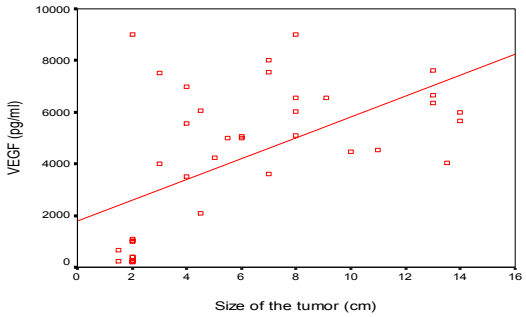
Table (3): Statistical comparison between different subgroups of HCC, cirrhotics and controls as regard serum VEGF level

Variable	GI (n=40)			GII (n=20)	GIII (n=10)	Kruskal-Wallis test	*P*-value
	GIa (n=16)	GIb (n=11)	GIc (n=13)				
• **VEGF** (pg/ml) X±SD Median Range	1346.3±1722.7 535 200-6045	4996.27±960.4 5000 3600-6545	7006.3±1264.7 7000 5000-9010	470.05±283.04 500 102-1000	172.8±82.7 147 79-290	51.07	<0.001

Table (4): The relationship between portal vein patency and AFP and VEGF levels in group I (HCC group).

Variable	**Portal Vein**		**Mann-Whitney test**	***P*-value**
	Patent (n=15)	**Thromposed** (n=25)		
• **AFP** (ng/ml) X±SD Median Range	538.34±619.05 400 10-2464	711.67±585.97 700 17-2049	141.5	>0.05
• **VEGF** (pg/ml) X±SD Median Range	3294.5±2554.9 3800 200-7610	5851.9±2629.4 6275 223-9010	82.00	<0.001

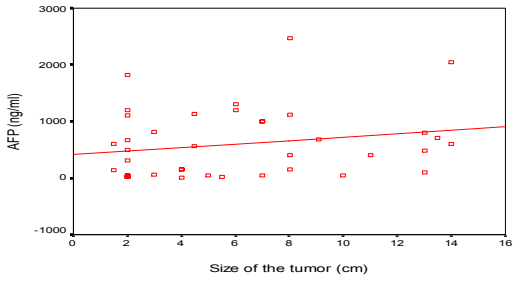
There was a significantly higher VEGF levels in HCC cases with portal vein thrombosis than those with patent portal vein



r=0.5 *P*=0.0001

Figure (1): Correlation between VEGF and tumor size in group I.

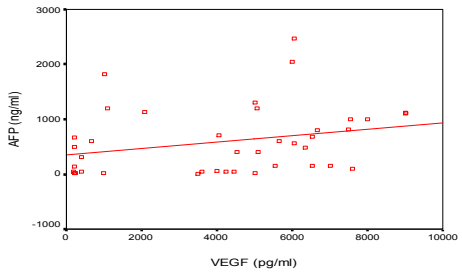
There was a significantly higher level of VEGF in large sized tumor than small sized ones in group I.



r=0.2 P=0.2

Figure (2): Correlation between AFP and tumor size in group I.

There was no significant difference between large and small sizes HCC as regard mean value of AFP.



r=0.27 *P*=0.087

Figure (3): Correlation between AFP and VEGF group I (HCC group).

There was no correlation between AFP and VEGF in group I.

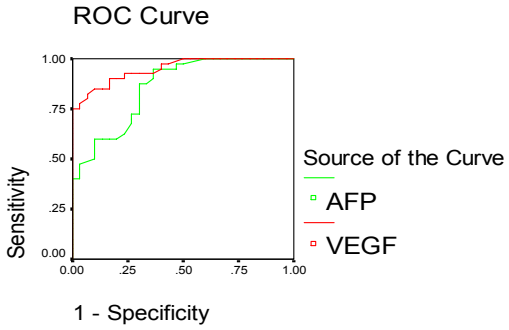


Figure (4): Receiver Operating Characteristic (ROC) curve for AFP and VEGF in HCC versus cirrhotic and control.

The cut off point was chosen from ROC curve as the point of highest accuracy (highest sensitivity and specificity). It is **70.5** ng/ml for AFP and **228** pg/ml for VEGF.

Table (5): Diagnostic performance of AFP and VEGF in diagnosis of HCC

Variable	Cutt off (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)		Negative predictive value (%)
AFP	70.5	72	80	74	94	42
VEGF	228	88	60	82	90	55
Both(AFP +VEGF)	*	95	60	88	90	75

There was a significant increase in sensitivity, accuracy and negative predictive value in diagnosis of HCC when we use VEGF with AFP than AFP alone

4. Discussion:

The primary marker for HCC is alpha-fetoprotein (AFP), generally it shows acceptable sensitivity. However, AFP is not secreted in all cases of HCC and may be normal in about 40% of patients with early HCC **(El-Houseini *et al.*, 2005)**.

Angiogenesis plays an important role in the early stage of multistep hepatocarcinogenesis **(Pang and Poon, 2006)**. HCC develops from dysplastic nodules in a cirrhotic liver; this development is also associated with a change in blood supply from the portal vein to a predominantly arterial blood supply **(Terada and Nakamura, 1995)**. The unpaired arteries stained by angiogenic factors were reported to increase significantly in HCC, and this may have important implications in the diagnosis of HCC **(Roncali *et al.*, 1999).**

Vascular endothelial growth factor (VEGF) is one of the first isolated angiogenic peptides, and is the most well studied angiogenic factor so far. The role of VEGF in HCC angiogenesis has been widely studied, and VEGF appears to be the most important angiogenic factor in HCC **(Amaoka *et al.*, 2006)**.

This work aimed to study methods which improve the early detection of HCC by measuring serum VEGF as well as AFP to improve the diagnostic ability. The present study revealed a higher level of AFP in both cirrhotic and HCC groups than the control group and it is significantly higher in HCC group when compared to cirrhotic and control groups (p<0.001). These results agree with those reported by **Johnson, 2001** who found that serum AFP is increased in cases of cirrhosis as well as non-malignant conditions such as viral hepatitis and pregnancy particularly if pregnancy is complicated by a spinal cord defect or other abnormality.

Perkins *et al.*, 2003 found that AFP may be elevated up to 500 ng/ml in non-malignant conditions such as cirrhosis. They reported also AFP levels are abnormal in 80% of patients with HCC and exceed 1000 ng/ml in 40% of these patients.

Chan *et al.*, 2006 found also that 965 of patients with benign liver conditions such as hepatitis and cirrhosis have AFP levels lower than 200 ng/ml.

Zhou *et al.*, 2006 concluded that serum AFP is the most widely studied screening test for detection of HCC. They found that over 70% of HCC patients have high serum concentrations of AFP. The authors suggest that, a level more than 400 ng/ml is usually regarded as diagnostic. However, in 35- 45% of HCC patients, the AFP level may be normal particularly in patients with small HCC **(Cedrone *et al.*, 2000 and Nguyen *et al.*, 2002)**. On the other hand, elevated AFP level may be observed in patients with cirrhosis or chronic hepatitis. This is the reason why some cases of HCC couldn't be correctly diagnosed by AFP alone **(Wang *et al.*, 2005)**. Similarly, **Tsai *et al.*, 2003** observed that an AFP level less than 400ng/ml was noted in 51% of HCC patients, furthermore, at least one third of small HCC and up to 30% of advanced HCC will be missed unless other diagnostic tools are used. In addition, AFP may be elevated in non-malignant liver diseases, so, it is obvious that AFP alone is not a reliable indicator for detection of HCC.

The present study revealed no statistical difference of AFP in HCC group as regard child Pugh's classification, tumor size and portal vein thrombosis. This is in agreement with **Kew, 1974; Bolondi *et al.*, 1990 and Arrieta *et al.*, 2007** who found that there was no difference between AFP elevation and the Child-Pugh score, age, gender, tumor differentiation and tumor size. In contrast, **Nomura *et al.*, 1989 and Chen *et al.*, 2006** found that, serum level of AFP correlates with the size of the tumor. Also, **Tangkijvanich *et al.*, 2000** found that HCC patients with high AFP concentrations (more than 400ng/ml) tend to have greater tumor size, bilobar involvement, portal vein thrombosis and a lower median survival rate.

Looking at the relation between AFP and the stage of HCC, the present study revealed that AFP is significantly increased in late stage (stage IIIC). This is in accordance with **Peng *et al.*, 2004** who elucidated the pathologic significance of AFP elevation through correlating the AFP levels with tumor grade and stage; they found that high AFP levels were found more in large, high grade, and late

stage tumors which had vascular invasion (stages IIIA to IV).

Nomura *et al.*, 1989 concluded that some HCCs are AFP-negative tumors at an early stage and may turn into AFP-producers later on in an advanced stages, where others may not produce AFP throughout the clinical course. **Chen *et al.*, 1984** concluded that in the early stages of HCC, serum AFP level is frequently normal and thus determination of serum AFP only is not a reliable indicator in the early detection of HCC. Also, **Peng *et al.*, 2004** found that high serum AFP level is more frequent in advanced stages of HCC especially complicated with vascular invasion.

The present study revealed a higher level of VEGF in both cirrhotic and HCC groups than the control group and it is significantly higher in HCC group when compared to cirrhotic and control groups (p<0.001). This is in agreement with **Makhlouf *et al.*, 2002** who measured the level of VEGF in various chronic liver diseases (Chronic hepatitis, Liver cirrhosis) and HCC; they found that a statistically significant higher level of circulating VEGF in HCC patients than in patients with chronic hepatitis and liver cirrhosis.

Also, ***Poon et al., 2004*** studied the prognostic significance of serum VEGF and endostatin in patients with HCC; serum VEGF and endostatin levels were measured by enzyme immunoassay in 108 patients with HCC before surgical resection and in 20 healthy controls. Serum VEGF levels in patients with HCC were significantly higher than those in controls, but serum levels of endostatin were similar in the two groups. High serum levels of VEGF, but not endostatin, were significantly associated with venous invasion and advanced tumour stage.

El Gendy *et al.*, 2005, who compared the circulating levels of some molecular markers (bcl-2, transforming growth factor beta 1 (TGF-ß1), VEGF and beta2-microglobulin) between HCV-infected and HCV-free HCC patients; found that, although the level of serum VEGF was significantly higher in all HCC patients than in healthy control, no significant difference, however was observed between HCV infected and HCV-free groups.

As regard the relation between serum level of VEGF and tumor size, the present study revealed a highly significant difference between small size HCC (<5cm) and large ones (>5cm). This is in accordance with **Zhao *et al.*, 2003** who determine the pre-therapeutic serum level of vascular endothelial growth factor (VEGF) in 150 patients with hepatocellular carcinoma (HCC) to elucidate the relation between the serum level and clinical characteristics and metastasis of HCC, they found that; serum VEGF levels in patients with large HCC lesions (>5cm) were significantly higher than those small HCC lesions (<5cm).

In contrast, **Li *et al.*, 1998; Poon *et al.*, 2001; El Gendy *et al.*, 2005** and **El-Hoseini *et al.*, 2005** didn't find any correlation between serum VEGF levels and tumor size.

The present study revealed that, a significantly higher VEGF levels were found in HCC patients with portal vein thrombosis compared to HCC patients who didn't have portal vein thrombosis. This is in agreement with **Yao *et al.*, 2005**.

Yao *et al.*, 2005 studied the expression of VEGF and microvessel density (MVD) in the serum and liver tissues and their clinicopathological features in HCC; they found that, the serum VEGF levels in patients with PV emboli, with metastasis or large HCC lesion (>5cm) were significantly higher than those without PV emboli, without metastasis or small HCC lesions.

Li *et al.*, 2004 investigated the expression level of plasma vascular endothelial growth factor (P-VEGF) in patients with HCC and its relationship with the clinicopathologic characteristics, and examined the changes of P-VEGF in the course of transcatheter arterial chemoembolization (TACE) in 45 HCC patients, they found a significant difference when plasma VEGF level categorized by tumor size, portal vein thrombosis, arterio-portal vein shunting and advanced TNM stage and the patients who achieved a partial or complete response to TACE therapy showed significantly less pre-treatment P-VEGF than those nonresponders. And a high pre-therapeutic P-VEGF level was associated with poor response to treatment so, P-VEGF may be useful in predicting treatment response, monitoring disease course after TACE and judging the effect of different TACE regimens.

Looking at the relation between serum level of VEGF and the stage of HCC, the present study revealed that, serum VEGF level increases along with the progress of the disease from stage I to stage IIIC, and its level is significantly higher in stage IIIC more than other stages. This is in agreement with **Zhao *et al.*, 2003** who concluded that, the later the stage, the higher the possibility of metastasis, and the higher the level of VEGF expression.

The present study revealed that, there is no correlation between serum VEGF and AFP levels in HCC patients. This is in agreement with **Jinno *et al.*,1998; Poon *et al.*, 2001; Chao *et al.*, 2003 and Poon *et al.*,2007** indicating that VEGF is an independent tumor biomarker for HCC.

In this study, the validity and cut off points of VEGF and AFP in diagnosis of HCC from cirrhotics and controls were calculated using the ROC curve.

As regard AFP, the cut off point (70.5 ng/ml) was the optimal cut off point with a sensitivity of (72%), specificity (80%), accuracy (74%), positive predictive value (94%) and a negative predictive value (42%). While the optimal cut off VEGF for diagnosing HCC from cirrhotics and controls was (228 pg/ml) and with a sensitivity of (88%), specificity (60%), accuracy (82%), positive predictive value (90%) and a negative predictive value (55%).

The present study found that both cut off points of AFP and VEGF have approximately the same positive predictive value and a negative predictive value and VEGF is more sensitive (sensitivity 88%) but less specific (specificity 60%) than AFP. And with combined use of AFP and VEGF for diagnosing HCC from cirrhosis and controls, there is increased sensitivity (95%) and accuracy (88%) but decreased specificity (60%), positive predictive value (90%) and a negative predictive value (75%), than the use of AFP alone. Thus the simultaneous determination of both markers improves the overall sensitivity for diagnosing HCC from cirrhotics and controls.

This is in agreement with **El-Houseini *et al.*, 2005** who studied methods to improve the detection of HCC by measuring AFP in addition to other biochemical factors in the same sample like alpha-L-fucosidase (AFU), VEGF and insulin like growth factor II (IGF-II). They found that, the combination of AFP and VEGF produced enhancement of sensitivity (95.5%) and specificity (85%) compared to their individual sensitivities (68.2% and 86.4, respectively) and specificities (75% and 60%, respectively). The same combination improved the diagnostic accuracy and positive predictive values, and reduced the negative predictive values compared to the individual values.

Conclusion:

From the present study, it is concluded that combined measurement of serum AFP and VEGF significantly increases the sensitivity, accuracy and negative predictive value in detection of HCC in cirrhotic patients rather than using of AFP or VEGF separately.

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