RESEARCH ARTICLE

STUDY OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR VERSUS ALPHA FETOPROTEIN IN HCV CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC) BEFORE AND AFTER THERAPEUTIC INTERVENTION

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ABSTRACT

Introduction: Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers in the world. More than one million cases of HCC occur in the world each year (Jain *et al.,* 2010). Clearly, the available screening methods are inadequate for early detection and follow up of HCC, so there is need for other markers have to high sensitivity in early diagnosis of HCC as well as the specificity in differentiation between HCC and benign lesions (Padma *et al.,* 2009). Human HCC is a highly angiogenic tumor with several studies showing a strong correlation between tissue VEGF levels and HCC (Semela and Dufour, 2004).

The aim of the work:
•To study the clinical significance of serum VEGF in hepatitis C cirrhotic patients with HCC as a diagnostic marker before intervention and a prognostic marker after intervention to improve the outcome of HCC diagnosis and treatment
• To study the VEGF correlation with hepatocellular carcinoma in hepatitis C cirrhotic patients as a simple non invasive tool

Patients and Methods: This study was carried on 80 subjects at the Internal Medicine Department of Ain Shams University Hospitals. classified into 3 groups: Group I was 40 patients with hepatocellular carcinoma and liver cirrhosis, subclassified into Group 1A: Included 20 patients with HCC had been subjected to Radiofrequency ablation Group 1B: Included 20 patients with HCC had been subjected to Transarterial chemo-embolization, Group II was 20 patients with liver cirrhosis only without HCC, Group III was 20 healthy subjects served as control group. HCV related liver cirrhosis and HCC diagnosis were confirmed based on clinical, laboratory and radiological data in addition to serum alpha-fetoprotein (AFP) and Measurment of serum vascular endothelial growth factor (VEGF)

Results: The sensitivity and specificity of VEGF has been shown to vary with the different cutoff values used. According to these results the sensitivity and specificity of VEGF for selective detection of the HCC group over the cirrhotic group were 97.5% and 95% respectively, at a cut-off value of 118ng/mL In addition, the accuracy of VEGF was 96.7%, similar to that of AFP 96.7%.

Conclusion: Plasma VEGF is a sensitive and specific serum marker for the diagnosis of HCC, also VEGF may play a prognostic marker in HCC management.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers in the world. More than one million cases of HCC occur in the world each year (Jain *et al*., 2010). Clearly, the available screening methods are inadequate for early detection and follow up of HCC, so there is need for other markers have to high sensitivity in early diagnosis of HCC as well as the specificity in differentiation between HCC and benign lesions (Padma *et al*., 2009). Human HCC is a highly angiogenic tumor with several studies showing a strong correlation between tissue VEGF levels and HCC (Semela and Dufour, 2004).

The aim of the work

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intervention to improve the outcome of HCC diagnosis and treatment

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MATERIALS AND METHODS

This study was carried on 80 subjects at the Internal Medicine Department of Ain Shams University Hospitals. Classified into 3 groups: Group I was 40 patients with hepatocellular carcinoma and liver cirrhosis, sub classified into Group 1A: Included 20 patients with HCC had been subjected to Radiofrequency ablation. Group 1B: Included 20 patients with HCC had been subjected to Transarterial chemo-embolization, Group II was 20 patients with liver cirrhosis only without HCC, Group III was 20 healthy subjects served as control group. HCV related liver cirrhosis and HCC diagnosis were confirmed based on clinical, laboratory and radiological data in addition to serum alpha-fetoprotein (AFP) and Measurment of serum vascular endothelial growth factor (VEGF) was assayed for all patients and controls Also it was assayed for those patients with HCC subjected to intervention either by RF or TACE after 1 month of the intervention

RESULTS

This table shows statistically significant positive correlation between VEGF versus bilirubin, liver enzymes, PLTs and AFP by spearman correlation test.

Table 1. Correlation between VEGF versus different variables among HCC group before intervention

Variables	VEGF	
	R	P
Age (Y)	0.11	>0.05
HB (mg/dL)	0.21	>0.05
Platelets (10^3/mm^3)	0.335	<0.05
Bilirubin (mg/dL)	0.287	<0.05
INR	0.18	>0.05
Albumin (mg/dL)	-0.14	>0.05
AST (IU/L)	-0.013	<0.05
ALT (IU/L)	0.021	<0.05
AFP (mg/dL)	0.23	<0.05
Urea (mg/dL)	0.03	>0.05
Creatinin (mg/dL)	0.31	>0.05
Na (mmol/L)	-0.10	>0.05
K (mmol/L)	0.33	>0.05

Table 2. Correlation between VEGF versus different variables among HCC group after intervention

Variables	VEGF	
	R	P
Age (Y)	0.19	>0.05
HB (mg/dL)	0.30	>0.05
Platelets (10^3/mm^3)	0.335	>0.05
Bilirubin (mg/dL)	0.09	>0.05
INR	0.06	>0.05
Albumin (mg/dL)	-0.14	>0.05
AST (IU/L)	-0.04	<0.05
ALT (IU/L)	0.001	<0.05
AFP (mg/dL)	0.23	<0.05
Urea (mg/dL)	0.05	>0.05
Creatinin (mg/dL)	0.20	>0.05
Na (mmol/L)	-0.29	>0.05
K (mmol/L)	0.04	>0.05

This table shows statistically significant positive correlation between VEGF versus liver enzymes and AFP by spearman correlation test.

Table 3. Correlation between AFP versus different variables among HCC group before intervention

Variables	AFP	
	R	P
Age (Y)	0.122	>0.05
HB (mg/dL)	0.37	>0.05
Platelets (10^3/mm^3)	0.02	>0.05
Bilirubin (mg/dL)	0.16	>0.05
INR	0.05	>0.05
Albumin (mg/dL)	-0.33	>0.05
AST (IU/L)	-0.47	<0.05
ALT (IU/L)	0.40	>0.05
Urea (mg/dL)	0.31	>0.05
Creatinin (mg/dL)	0.97	>0.05
Na (mmol/L)	-0.17	>0.05
K (mmol/L)	0.21	>0.05

This table shows statistically significant positive correlation between AFP versus AST before therapeutic intervention by spearman correlation test.

Table 4. Correlation between AFP versus different variables among HCC group after intervention

Variables	AFP	
	R	P
Age (Y)	0.017	>0.05
HB (mg/dL)	0.04	<0.05
Platelets (10^3/mm^3)	0.21	>0.05
Bilirubin (mg/dL)	0.26	>0.05
INR	0.1	>0.05
Albumin (mg/dL)	-0.25	>0.05
AST (IU/L)	-0.05	<0.05
ALT (IU/L)	0.28	>0.05
Urea (mg/dL)	0.34	>0.05
Creatinin (mg/dL)	0.019	<0.05
Na (mmol/L)	-0.11	>0.05
K (mmol/L)	0.02	>0.05

This table shows statistically significant positive correlation between AFP versus AST, serum creat, Hb after therapeutic intervention by spearman correlation test.
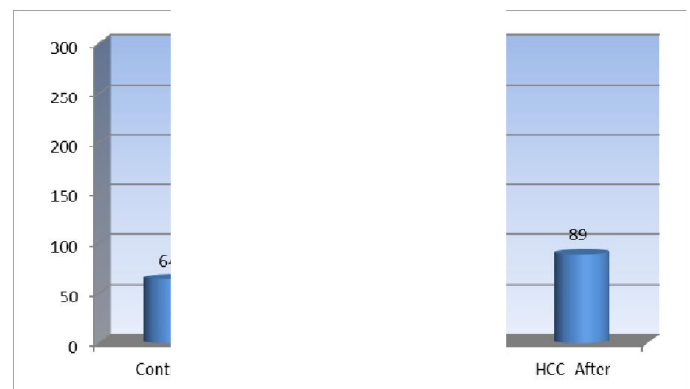

Fig. 1. Shows VEGF in different studied groups

As shown in the figure VEGF level was statistically high in the HCC group versus cirrhotic group and control group. Its level is significantly decreased in the HCC group after intervention

Table 5. VEGF findings in the three studied groups

Laboratory parameters	HCC before (n = 40)		HCC after (n=40)		Cirrhotic (n = 20)		Control (n=20)		Used test	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
VEGF	255	73.46	89	65	165	69	64	35	F=21.1	<0.0001*

Table 6. Alpha fetoprotein findings in the three studied groups

Laboratory parameters	Before RF (n =20)		After RF (n=20)		Before TACE (n = 20)		After TACE (n=20)		Used test	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
VEGF	366	37.	83	44	230	35	100	55	F=21.1	<0.0001*

Table 7. VEGF findings in both subgroups of HCC patients before and after intervention

Laboratory parameters	Before RF (n =20)		After RF (n=20)		Before TACE (n = 20)		After TACE (n=20)		Used test	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
AFP	104	55.	5.5	50	226.5	55	25	40	F=21.1	<0.0001*

Table 8. AFP findings in both subgroups of HCC patients before and after intervention

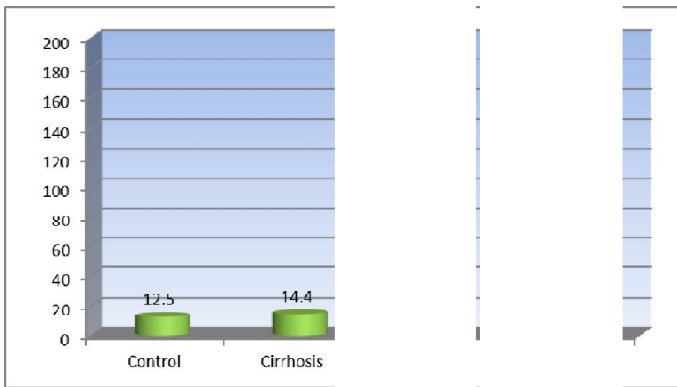| Laboratory parameters	Before RF (n =20)		After RF (n=20)		Before TACE (n = 20)		After TACE (n=20)		Used test	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
AFP	104	55.	5.5	50	226.5	55	25	40	F=21.1	<0.0001*

Fig. 2. shows AFP in different groups

As shown in the figure AFP level was statistically high in the HCC group versus cirrhotic group and control group. Its level is significantly decreased in the HCC group after intervention
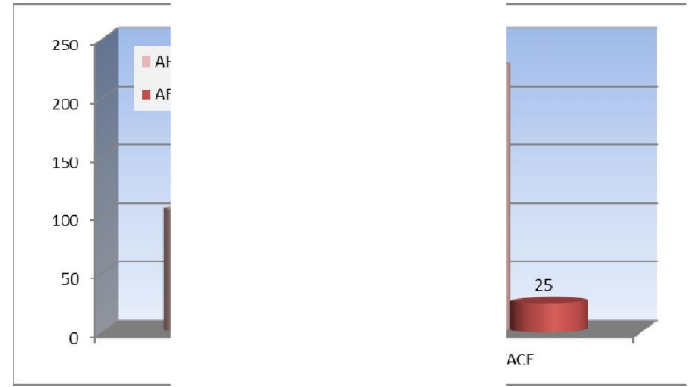

Fig. 3. VEGF findings in both subgroups of HCC patients before and after intervention

As shown in the figure VEGF levels were statistically decreased in both subgroups of patients with HCC after intervention either by RFA or TACE

Fig. 4. AFP findings in both subgroups of HCC patients before and after intervention

As shown in the figure AFP levels were statistically decreased in both subgroups of patients with HCC after intervention either by RFA or TACE
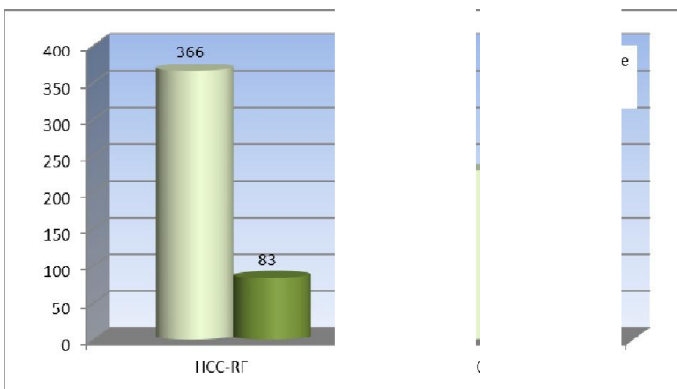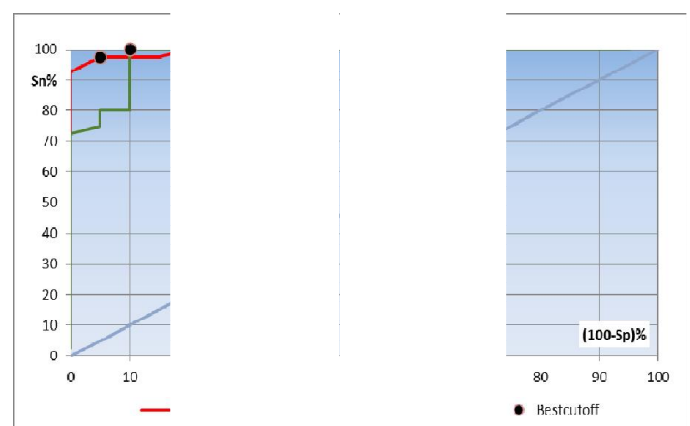

Fig. 5. ROC curve analysis showing the diagnostic performance of AFP and VEGF for discriminating patients with HCC from chronic liver disease

As shown in the figure the best cutoff of VEGF and AFP were 118 and 8.14 respectively with a sensitivity and specificity of VEGF OF 97.5% and 95% respectively versus 100% and 95.2% for AFP. The efficacy of both markers were similar (96.7%)

Table 9. Validity of VEGF in prediction of HCC versus AFP in HCC

Variables	VEGF	AFP
Best cut off	118	8.14
AUC	0.982	0.916
Sensitivity	97.5%	100%
Specificity	95%	90%
PPV	97.5%	95.2%
NPV	95%	100%
Efficacy	96.7%	96.7%

DISCUSSION

In this study six (30%) of cirrhotic patients presented in Child Pugh A while 4(20%) presented in Child-Pugh B and 10 patients presented in Child- Pugh C. On the other hand, thirteen (32.5%) of the HCC patients presented in Child- Pugh C while twenty seven (67.5%) presented in Child-Pugh B. A retrospective study found that 73.6% of the patients presented in Child-Pugh C while 26.4% presented in Child-Pugh B (Makhlouf *et al*. 2002). The study found that there is a significant difference in VEGF level between the cirrhotic group and the control group. These results were not in agreement with those Genesca *et al*. (1999) who found that there was no significant difference of VEGF between the control group (35.78± 19.0 pg/ml) and the liver cirrhosis group (49.48±34.4 pg/ml), but the results were in agreement with those made by Uematsu *et al*. (2005) who stated that the VEGF expression scores in cirrhosis (P<0.001), and HCC (P<0.0011) were significantly higher than in control.

The study showed that there is a positive correlation between the VEGF serum level and tumor size or the number of hepatic focal lesions with P-value=0.027 and 0.012 respectively. This proves that the larger the tumor size the more ischemic effect causing more stimulation of VEGF secretion this agrees Stroescu *et al*., 2008 who reported that overexpression of VEGF was more frequent in large HCCs than small HCCs and that VEGF expression was far stronger in patients with poorly differentiated HCC Suzuki *et al*., 1996., reported that large HCC nodules (>3 cm) tended to have internal hypoxia and necrosis, with up-regulation of the expression of VEGF mRNA. In contrary these results are against those obtained by (Poon and colleagues, 2004) who reported that there were no significant differences among the serum level of VEGF in single or multiple hepatic focal lesions and that whether the size of the focal lesion is giant or small this doesn't affect the serum level of VEGF. Also these results didn't coincide with (Assay *et al*., 1999) who did not find any correlation between VEGF and tumor size.

This study showed significant elevation of plasma VEGF levels in HCC patients with a mean level (255 ng/ml) than cirrhotic patients levels which showed a mean level(165 ng/ml) and lower levels in normal control group with a mean level (64 ng/ml) (P<0.001). So the plasma VEGF level in the present

study increased in HCC patients, these results are in agreement with those of (Makhlouf *et al*., 2002 and Salcedo *et al*., 2005). Who found that The serum VEGF level of the HCC group (206.65±109.23pg/ml) was significantly higher than that of the control group (35.78±19.0pg/ml).This proves that HCC is really a highly vascular tumor.

On the other hand Genesca *et al*. (1999) demonstrated that there is no significant difference in VEGF levels between HCC group and cirrhotic group (P>0.05) and this doesn't coincide with our study or with Kamel *et al*. (2005) who found that VEGF levels in HCC patients were significantly higher than in cirrhotic patients. It indicates that VEGF could play an important role in transforming liver cirrhosis into HCC this is due to less number of patients were included in their study. In the present study the serum level of VEGF in HCC patients was significantly decreased after radiofrequency ablation being 366Pg/ml   before intervention and 83 Pg/ml after, this agree with Ronnie *et al*. (2007) who found a significant decrease in VEGF levels after one month of RF they also found that high pre-treatment serum VEGF levels predict poor prognosis after RFA of HCC. This study highlights the importance of tumor biomarker as a prognostic predictor in ablative therapy for HCC, which has an intrinsic problem of unavailability of histopathological prognostic features.

Also the serum level of VEGF in HCC patients was significantly decreased after TACE, being 230 Pg/ml   before intervention and 100Pg/ml after. These results agree with You *et al*. (2014) who reported that Serum VEGF concentrations decreased in 44 (26.0 %) patients at week 4. Patients who had a VEGF response at week 4 had a longer median survival than those who did not have a VEGF decrease (19.0 vs. 9.8 months, p\0.001). VEGF decrease after TACE (p = 0.012) and presence of extrahepatic metastases (p= 0.02) were independently associated with overall survival by multivariate analysis. They cocluded that a serum VEGF concentration decrease at 4 weeks after TACE may predict favorable overall survival in patients with advanced HCC. The sensitivity and specificity of AFP for selective detection of HCC has been shown to vary with the different cutoff values used. According to the results, at a cutoff >8.14ng/ml the sensitivity was 100% and the specificity was 90%, accuracy 96.7%, positive predictive value 95% and negative predictive value 100%. The present results were not comparable to those of Poon and colleagues (2004) who reported sensitivity 68.2%, and specificity75%, when the cutoff value 19.8ng/ml, accuracy 70.3%, positive predictive value 85.7% and negative predictive value 48.3%. This proves that AFP can be used as a tumor marker for HCC.

Also these results were not comparable to those obtained by Genesca *et al*. (1999) who found that the sensitivity of AFP was 76% and the specify was 62% at a cut of value of15 ng/ml. Also in this study the AFP in HCC patients shows a significant reduction of its level in a subgroup of patients subjected to RF ablation being 104ng/ml before and 15.5 ng/ml after. Again in this study the AFP in HCC patients shows a significant reduction of its level in a subgroup of patients subjected to TACE being 226.5   ng/ml before and 25 ng/ml after. The sensitivity and specificity of VEGF has been shown to vary with the different cutoff values used. According to these results

the sensitivity and specificity of VEGF for selective detection of the HCC group over the cirrhotic group were 97.5% and 95% respectively, at a cut-off value of 118ng/mL these results were comparable to those of Poon and colleagues (2004) who reported the diagnostic sensitivity and specificity of VEGF for selective detection of the HCC group over the non-HCC group (CLD group + healthy control group) were 98% and 46% respectively, at a cut-off level of 108 ng/mL. In addition, the accuracy of VEGF was 96.7%, similar to that of AFP 96.7%. Those results were in consistency with those of Poon and colleagues (2004) who stated that the accuracy of VEGF was 89.4% and that of AFP was 71.4%. Also these results agree with Uematsu *et al*. (2005) who found that the sensitivity of VEGF is 86.4% and the specificity is 60%, accuracy is 78.1 %, positive predictive value 82.6% and the negative predictive value is 33.3% with a cut off value of 355. These results indicate that the serum VEGF level was useful for the diagnosis of HCC as AFP in patients with liver cirrhosis (the same efficacy 96.7%). The study showed that there is a correlation between VEGF and AFP level, these were against Genesca *et al*. (1999) who demonstrated that there is no significant correlation between serum levels of VEGF and serum AFP concentrations in patients with HCC (P=0.178).

Conclusion

Plasma VEGF is a sensitive and specific serum marker for the diagnosis of HCC and we recommend combination of AFP and VEGF in screening and diagnosis of HCC. Plasma VEGF could be considered as a good prognostic tumor marker in HCC management. Plasma VEGF levels directly correlated with the tumor number and also with the overall size of the tumor. Also the marked significant reduction of serum VEGF levels in HCC patients subjected to either RF ablation and TACE proved that VEGF may play a prognostic marker in HCC management.

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