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RESEARCH ARTICLE

Evaluation of hepatocellular carcinoma-vascular endothelial growth factor score for early detection of hepatocellular carcinoma among hepatitis C virus patients

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common tumor worldwide. The burden of HCC has been increasing in Egypt. Most HCC are diagnosed at intermediate or advanced stages beyond any curative therapies.Serum AFP has a low sensitivity, and about one third of early stage HCC patients have low level of AFP. HCC is a hypervascular tumor and vascular endothelial growth factor (VEGF) is pathogenically involved in HCC. We aimed to evaluate the clinical usefulness of VEGF and HCC-VEGF score in the diagnosis of HCC. The study was conducted on 89 subjects including 34 patients with HCC, 35 patients with HCV related liver disease and 20 healthy controls. Full history taking, clinical examination, routine laboratory and radiological investigations were done. Serum VEGF and AFP were measured.HCC was presented more in males. AFP and VEGF levels were significantly higher in HCC group than in cirrhotic group. HCC-VEGF score was significantly higher in patients with HCC group with a mean value of 11.96±10.40 compared to 1.24±1.32 in cirrhotic group and -2.73±0.53 in healthy controls ($p< 0.001$). HCC-VEGF score was positively correlated with size of hepatic focal lesion whereas it showed no statistically significant correlation with Child-Pugh score or MELD score. HCC-VEGF score at a cutoff point of 2.59 showed a sensitivity and specificity of 100 and 82.86 % respectively with area under the curve 0.97. So **,** VEGF and HCC-VEGF score may represent a non invasive marker for prediction of HCC in patients with chronic HCV related liver disease.

Introduction

Hepatocellular carcinoma is the fifth most common cancer in men andthe second most common cause of cancer related death worldwide **(*World Health Organization,2012*).** More than 90% of HCC cases developed in chronically inflamed liver as a result of viral hepatitis including virus C and B **(*Welzel et al.,2011*).** Most hepatocellular carcinomas are diagnosed at intermediate or advanced stages and only 30% of patients benefit from curative therapies ***(Cheng et al .,2008).*** Alpha-fetoprotein (AFP) is the most established tumor marker in HCC and the gold standard by which other markers for the disease are judged ***(Lopez, 2005).*** However, it has a low sensitivity, and about one third of early stage HCC patients with small tumors have low level of AFP which makes the AFP test insufficient for the early detection of HCC in at-risk populations **(*Chen et al ., 2010*).** HCC is a hyper-vascular tumor ***(Brodsky et al., 2007).*** Growing tumors secrete a number of growth factors that can induce angiogenesis. Vascular endothelial growth factor (VEGF) is a primary driving force for both physiological and pathological angiogenesis and overexpression of VEGF is observed in HCC **(*Korpanty et al., 2010*).** It was reported that serum levels of VEGF might be useful predictor of the presence of HCC in patients with chronic liver cirrhosis ***(Mukozu et***

al.,2013). El-mezayen and Darwis (2014) developed a novelsimple diagnostic score namely HCC-VEGF score. That score was based on combination of VEGF, AFP, and routine liver function tests including, albumin, platelet count, and international normalized ratio (INR) for early detection HCC. The aim of our study is to evaluate the clinical usefulness of both VEGF and HCC-VEGF score in the diagnosis of HCC.

Material and Methods

This study was conducted on 89 subjects attending or admitted to the Department of Hepatology, Gastroenterology and Infectious Diseases, Benha University Hospital during the period from December 2014 to August 2015 after approval of BenhaUniversity ethical committee. The study population included 34 patients with HCC (proved by triphasic CT abdomen and serum alpha fetoprotein level) as well as 35 patients with HCV related chronic liver disease . Another 20 persons of apparently healthy individuals with normal routine laboratory investigations and negative for both HCV Ab and HBsAg served as a control group. Patients who have had prior VEGF targeted therapy were excluded. All the patients and controls were subjected to full history taking, complete clinical examination, complete liver function tests , serum alpha fetoprotein (AFP). Abdominal ultrasonography and triphasic Computed tomography were done to diagnose and evaluate hepatic focal lesion(s). Serum VEGF concentration were quantitatively measured using an Enzyme–Linked Immuno-Sorbant Assay (ELISA) kit (Quantikinehuman VEGF Immunoassay; R&D Systems, Minneapolis, MN, USA) according to manufacturers' instructions**.**

Statistical analysis:

The statistical analysis was conducted using STATA/SE version 11.2 for Windows (STATA corporation, College Station, Texas).The collected data were summarized in terms of mean± Standard Deviation (SD) and range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the Chi-square test (χ^2) and Fisher Exact test (FET) to compare proportions as appropriate. The Student t-test (t) was used to detect difference in the mean between two parametric data, while the Mann-Whitney test (z) was used to compare two non-parametric data. Receiver Operating Curve (ROC) analysis was carried out to evaluate the diagnostic performance of studied parameters for HCC screening among cirrhotic patients. The best cutoff point and the corresponding sensitivity and specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Area Under the Curve (AUC) were estimated.Pearson correlation coefficient (r) and Spearman Correlation coefficient (rho; ρ) were used to test for the correlation between the plasma VEGP levels and HCC-VEGF scores and some variables.After the calculation of each of the test statistics, the corresponding distribution tables were consulted to get the "P" (probability value). Statistical significance was accepted at P value <0.05 (S). A P value <0.001 was considered highly significant (HS) while a P value >0.05 was considered non-significant.

Results

The age ranged between 39 and 74 years in HCC cases with the mean age being 57.56±8.02 years. For patients with cirrhosis, the age ranged between34 and 79 with a mean age of 57.77±9.07 years (P=0.92). Male predominance in HCC patients was noted as males represented 79.41% (27/34) compared to 20.59% (7/34) for females whereas there was a female predominance in cirrhotic patients with a percentage of 62.86% (22 /35) compared to 37.14% (13/35) for males (p<0.001). History of Smoking was significantly more frequent in HCC group; 12 patients (35.29 %) compared to 4 patients (11.43 %) in cirrhotic group (p= 0.02). Clinical history and laboratory findings were summarized in table (1) and (2) respectively.

Serum level of alpha fetoprotein was significantly higher in patients with HCC with a mean value of 156.08±190.70 ng/ml compared to 22.03±17.22 ng/ml in cirrhotic patients (p<0.001). Serum VEGF level was significantly higher in patients with HCC than the cirrhotic (164.30±40.52 pg/ml Vs 49.33±14.12 pg/ml) and healthy control group (29.51±10.71) (p < 0.001). When we calculated the HCC-VEGF score, it was significantly higher in patients with HCC group with a mean value of 11.96±10.40 compared to 1.24±1.32 in cirrhotic patients and was -2.73±0.53 in healthy control group (p < 0.001) as showed in table (3). HCC-VEGF score was calculated as =1.26 + (0.05× AFP (ng/ml)) + (0.038× VEGF (pg/ ml)) + (0.004×INR) – (1.02× albumin (g/dl)) – (0.002 × platelet count (10^3/dl)). Table (4) showed that, serum VEGF level was positively correlated with tumor size (p <0.001) and also with MELD

score (p=0.03) but not with Child score (p=0.25). HCC-VEGF score was positively correlated with the tumor size (p<0.001) whereas it showed no statistically significant correlation with Child score or MELD score (p=0.69 and 0.47 respectively).The ROC curves for AFP, VEGF and HCC-VEGF score were studied to compare diagnostic power for each of them in HCC prediction. AFP at a cutoff point of 18.66 ng/ml showed a sensitivity and specificity of 79.41 and 60 % respectively with area under the curve 0.766 while VEGF at a cutoff point of 111.2 pg/ml showed a sensitivity and specificity of 100% of both with area under the curve 1. HCC-VEGF score at a cutoff point of 2.59 showed a sensitivity and specificity of 100 and 82.86 % respectively with area under the curve 0.97 (figure 1 and 2).

Table (1): Clinical history of the patients:

Variable		Group I (HCC group) (No.=34)		Group II (cirrhotic group) (No.=35)		Test	P
		No.	%	No.	%		
Abdominal pain	No	11	32.35	16	45.71	χ^2= 1.29	0.26
	Yes	23	67.65	19	54.29		
Weight loss	No	24	70.59	31	88.57	χ^2= 3.45	0.06
	Yes	10	29.41	4	11.43		
Jaundice	No	25	73.53	22	62.86	χ^2= 0.90	0.34
	Yes	9	26.47	13	37.14		
GI bleeding	No	23	67.65	25	71.43	χ^2= 0.12	0.73
	Yes	11	32.35	10	28.57		
Ascites	No	20	58.82	6	17.14	χ^2= 12.76	<0.001
	Yes	14	41.18	29	82.86		
Encephalopathy	No	31	91.18	26	74.29	χ^2= 3.42	0.06
	Yes	3	8.82	9	25.71		
Fever	No	30	88.24	29	82.86	FET	0.73
	Yes	4	11.76	6	17.14		
	Yes	18	52.94	22	62.86		
History of bilharziasis	No	6	18.18	15	42.86	χ^2= 4.84	0.03
	Yes	27	81.82	20	57.14		
DM	No	24	70.59	25	71.43	χ^2= 0.006	0.94
	Yes	10	29.41	10	28.57		
Hypertension	No	28	82.35	33	94.29	FET	0.15
	Yes	6	17.65	2	5.71		

Table (2): Laboratory findings in all patients:

Variable	Group I (No.=34)		Group II (No.=35)		Test	P
	Mean ±SD	Range	Mean ±SD	Range		
FBS (mg/dl)	122.23±58.74	79-376	142.08±68.63	75-312	z= 1.31	0.19
HB (gm/dl)	11.82±2.25	7.4-16.9	10.41±2.06	7.3-16.1	t= 2.71	0.009
WBCs (10^3/cmm)	6.32±3.49	1.37-17.4	5.06±3.0	1.37-15.9	t= 1.61	0.11
Platelets (10^3/cmm)	124.03±70.86	38-289	75.26±40.12	27-180	t= 3.53	<0.001
S. creatinie (mg/dl)	1.12±0.41	0.6-2.3	1.02±0.34	0.6-2.6	z= 1.11	0.26
ALT (IU)	54.62±27.54	15-159	52.37±41.15	9-253	z= 0.98	0.33
AST (IU)	65.41±38.96	20-220	54.34±32.68	12-185	z= 1.87	0.06
T. bilirubin (mg/dl)	2.34±3.05	0.4-16.6	2.68±2.01	0.6-10	z= 1.74	0.08
D. bilirubin (mg/dl)	1.42±2.01	0.1-10.8	1.43±1.24	0.1-6.2	z= 1.03	0.30
S. albumin (gm/dl)	3.05±0.67	1.8-4.2	2.80±0.58	1.9-4.0	t= 1.65	0.10
INR	1.46±0.42	1-2.51	1.46±0.36	1-2.3	t= 0.06	0.95

Table (3): AFP, VEGF and HCC- VEGF score in the study groups:

Variable	Group I (HCC group) (No.=34)		Group II (Cirrhotic group) (No.=35)		Group III (Healthy group) (No.=20)		Test	P
	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range		
Serum AFP	156.08±190.70†‡	4.47-853	22.03±17.22†	1.8-68.0	1.59±1.53	0.03-4.86	χ^2= 53.49	<0.001
serum VEGF	164.30±40.52†‡	111.2-284	49.33±14.12	30.8-82.46	29.51±10.71	10.1-46.39	F= 217.02	<0.001
HCC-VEGF score	11.96±10.40 †‡	(2.59-46.42)	1.24±1.32; †	(-1.06-4.26)	-2.73±0.53;	(-3.47- -1.73)		

† Significant differences compared to Group III.
‡ Significant differences compared to Group II.

Table (4): Correlation between VEGF and HCC-VEGF scores with some variables in patients with HCC:

Variable (No.=34)	**serum VEGF level**		**HCC-VEGF score**	
	r	P	ρ	**P**
Tumor size	0.81	<0.001	0.76	<0.001
Child score	0.20	0.25	0.07	0.69
MELD score	0.37	0.03	0.13	0.47

r : Pearson correlation coefficient
ρ : Spearman correlation coefficient.

Figure (1): ROC curve for of VEGF.

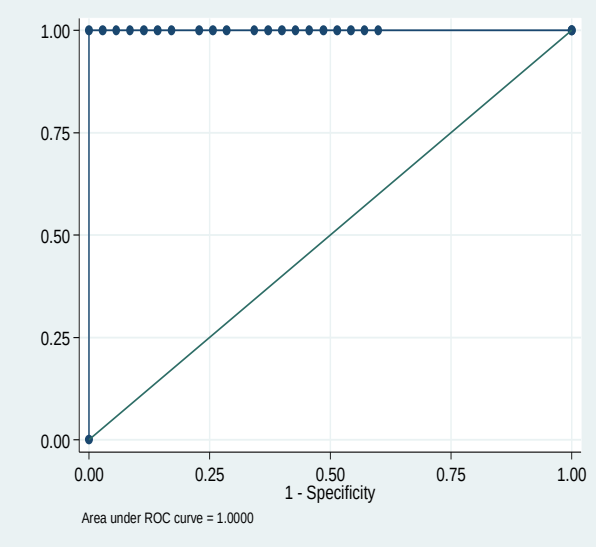
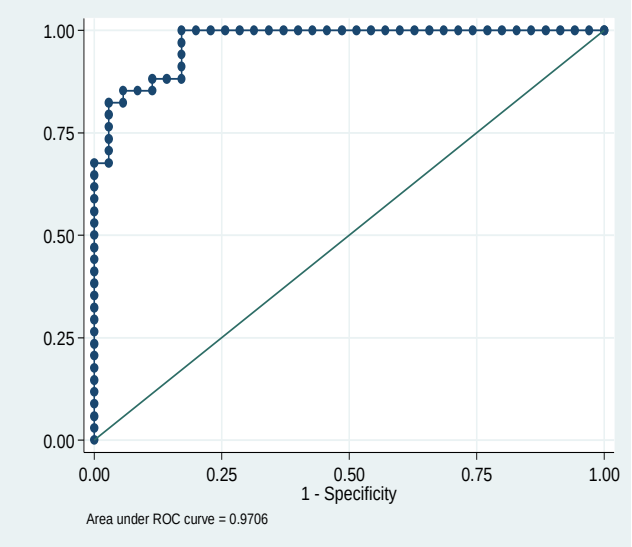


Figure (2): ROC curve for HCC-VEGF score.



Discussion

HCC is a complex disease associated with many risk factors and cofactors ***(Gomaa et al., 2009***). Most hepatocellular carcinomas are diagnosed at intermediate or advanced stages and only 30% of patients benefit from curative therapies such as resection, liver transplantation or percutaneous ablation ***(Cheng et al., 2008).*** Because the majority of HCC develop in cirrhotic livers, HCC surveillance with AFP and ultrasonography have been recommended for persons with cirrhosis ***(Chen et al ., 2010).***US has an acceptable diagnostic accuracy, widespread popularity, good acceptance by patients and relatively moderate cost. Nevertheless, US detection of early HCC on a

cirrhotic background is a challenging issue due to the coarse pattern and the presence of regenerative nodules which may impair identification of small tumors. Also, US is highly dependent on the expertise of the operator and the quality of the equipment ***(Lencioni et al., 2008).*** Serum AFP test has a low sensitivity, and about one third of early stage HCC patients with small tumors have low level of AFP which makes the AFP test insufficient for the early detection of HCC in at-risk populations ***(Chen et al., 2010).*** Activation of VEGF is expected during liver carcinogenesis as the transformation of a cirrhotic nodule into a distinct tumor node is characterized by accumulation of unpaired arteries ***(Lavarone et al., 2007).*** VEGF was found to be upregulated in HCC, and it was also shown to be associated with the carcinogenesis, metastasis, recurrence and prognosis of HCC ***(Shen et al ., 2010)*** . It has been reported that VEGF expression is correlated with tumor vascularity and serum levels of VEGF might be useful predictor of the presence of HCC in patients with liver cirrhosis ***(Mukozu et al .,2013).*** In our study we aimed to assess the potential role of VEGF in the diagnosis of HCC either alone or incorporated in HCC-VEGF score.

In the current study, serum level of VEGF was significantly higher in HCC patients (164.30 pg/ml) than cirrhotic patients (49.33pg/ml) and both were significantly higher than the healthy controls (29.51pg/ml). This was in agreement with ***Mukozu et al., (2013)*** who reported in their study included 124 patients, 49 of them were with HCC that, VEGF level was significantly higher in HCC patients than non HCC patients however in their study, there was no significant difference between the control group and the cirrhotic group.Similar results were found by ***El-mezayen and Darwish (2014)*** who reported that, there was a significant elevation of VEGF serum levels in HCC patients compared to both of the control and cirrhotic group. The variation between our results and the latter two studies in the cirrhotic patients without HCC may be explained by the variation in the study population as most of our patients were Child class B and C i.e at more advanced stages of hepatic decompensation. ***El-mezayen and Darwish (2014)*** explained the insignificant variation of serumVEGF levels between control group and cirrhotic group the possibility that the two groups had benign liver tissue without hypoxia and there is no need for expression of angiogenic markers. On the contrary ***Li et al., (2003)*** found that the mean serum VEGF level was 1.5-fold increase in cirrhotic patients than in healthy controls. Also ***Giatromanolaki et al., (2007)*** reported that VEGF was significantly higher in cirrhotic patients than controls and concluded that growth factors such as VEGF produced by hepatocytes in patients with liver cirrhosis may have an important role in the development of hepatic fibrosis through progressive stimulation of fibroblasts. In the same aspect ***Kemik et al., (2010)*** noted a highly significant association between HCC and VEGF and ***Kaseb et al., (2009)*** reported a potential role for VEGF in screening and surveillance of HCC.

In our study, serum VEGF level was positively correlated with tumor size and also correlated with MELD score but not correlated with Child score. ***Shim et al., (2008)*** also noted that serum VEGF levels had a significant correlation with tumor size. Similar findings were reported by ***Sergio et al., (2008)*** and ***Guan et al., (2015)***. On the other hand , ***Elgendy et al., (2005)*** reported in a study including 77 HCC cases that, VEGF did not have any correlation with tumor size and ***Yamaguchi et al., (1998***) reported that VEGF positivity by immunohistochemical study may gradually decrease with increasing tumor size. The insignificant correlation between VEGF and Child score noted in our study coincide with ***Assy et al., (1999)*** who reported no significant difference in serum VEGF levels among the different Child-Pugh's classes and concluded that circulating VEGF level in patients with liver cirrhosis could not serve as an indicator of the progression of chronic liver disease**.** ***Mukozu et al., (2013)*** also concluded that, there was no significant difference on serum VEGF level between different HCC stages. Serum VEGF level in the present study showing a positive correlation with MELD score. This agrees with ***Stroescu et al., (2008)*** who concluded a high VEGF association with poor survival in patients with HCC.

In the current study, VEGF was analyzed by ROC curve showed a sensitivity and specificity of 100% of both at a cutoff point of 111.2 pg/ml with an area under the ROC curve (AUC) of 1. This result agrees with ***Mukuzo et al., (2013)*** who reported that the AUC for serum VEGF was 0.980. These results indicated that, VEGF might be a useful diagnostic biomarker of HCC patients with HCV-related liver disease.

El-mezayen and Darwish, 2014 developed a novel simple noninvasive diagnostic score namely hepatocellular carcinoma – vascular endothelial growth factor score (HCC-VEGF score) for early detection HCC. Their study included 123 patints with HCC on top of HCV related liver disease, 210 patients with compensated HCV related liver cirrhosis and 53 healthy controls. That score was based on combination of VEGF, AFP, and liver function tests including, albumin, platelet count, and international normalized ratio (INR) . HCC-VEGF score was calculated as

=1.26 + (0.05× AFP (ng/ml)) + (0.038× VEGF (pg/ ml)) + (0.004×INR) – (1.02× albumin (g/dl)) – (0.002 × platelet count (103/dl)). According to our knowledge, no other studies had evaluated such a score in prediction in HCC. In our study, we evaluated this score as a possible diagnostic tool for prediction of HCC. In our study, the five parameters used in HCC-VEGF score were analyzed by ROC curve, showing area under the ROC curve (AUC) were in order of INR (0.483), albumin (0.604), platelet (0.735), AFP (0.766) and VEGF (1). The diagnostic value of HCC-VEGF score was analyzed by the ROC curve showing an AUC of 0.9706. In the current study, the best sensitivity (100 %) and specificity (82.86 %) for HCC-VEGF score were at cutoff value of 2.59 for the best for discrimination of patients with HCC from those with liver cirrhosis (i.e., less than 2.59 indicated patients with liver cirrhosis and greater than 2.59 indicated patient with HCC). Similar findings were obtained by ***El-mezayen and Darwish (2014)*** who reported that the AUC for VEGF score for discriminating HCC patients from liver cirrhosis was 0.98 with sensitivity of 91 % and specificity of 82 % at cutoff 4.4. In our study, HCC-VEGF score was positively correlated with tumor size ($p<0.001$). This also came in agreement with ***El-mezayen and Darwish (2014)*** who reported that HCC-VEGF score was positively correlated with tumor size, tumor number and vascular invasion**.** Our results together with those of ***El-mezayen and Darwish (2014)*** may suggest that the development of HCC in patients with liver cirrhosis might be predicted by an increasing HCC-VEGF score. And so, the addition of this score might improve the performance of HCC screening.

In conclusion, serum level of VEGF either alone or incorporated in HCC-VEGF score may represent a useful marker that complement the role of AFP in the screening for HCC in patients with chronic HCV.

References

Assy N , Paizi M , Gaitini D , Baruch Y and Spira G (1999): Clinical implication of VEGF serum levels in cirrhotic patients with or without portal hypertension. World J Gastroenterol; 5(4): 296-300.

Brodsky S , Mendelev N , Melamed M and Ramaswamy G (2007): Vascular Density and VEGF Expression in Hepatic Lesions. J Gastrointestin. Liver Dis; Vol.16 No 4, 373-377.

Chen L , Ho DW , Lee NP , Sun S and Lam B ,Wong KF, Yi X , Lau GK , Ng EW, Poon TC , Lai PB , Cai Z , Peng J , Leng X , Poon RT and Luk JM (2010): Enhanced detection of early hepatocellular carcinoma by serum SELDI-TOF proteomic signature combined with alpha-fetoprotein marker. Ann SurgOncol ;17:2518–25.

Cheng A , Kang Y , Chen C , Tsao CJ , Qin S , Kim J , Burock K , Zou J , Voliotis D and Guan ZZ (2008): Randomized phase III trial of sorafenib versus placebo in Asian patients with advanced hepatocellular carcinoma. J ClinOncol; 26: 215.

Elgendy SM , Hessien M , Elsherbiny MM , Abd El-salam IM , El-attar IA and El-asser AA (2005): Panel of Molecular Markers in Hepatitis C Virus-Related . Hepatocellular Carcinoma Journal of the Egyptian Nat. Cancer Inst.; 17 (4): 270-78.

El-Mezayen HA and Darwish H (2014): Development of a novel score for early detection of hepatocellular carcinoma among high-risk hepatitis C virus patients.TumorBiol;(35):6501-9.

Giatromanolaki A , Kotsiou S , Koukourakis M and Sivridis E (2007): Angiogenic Factor Expression in Hepatic Cirrhosis. Hindawi Publishing Corporation. Mediators of Inflammation; Article ID 67187.

Gomaa AI, Khan SA, Leen E, Waked I and Taylor-Robinson SD (2009): Diagnosis of hepatocellular carcinoma. Online Submissions: wjg.wjgnet.com World J Gastroenterol; 21; 15 (11): 1301-14.

Guan Q , Gu J , Zhang H , Ren W, Ji W and Yuxiang Fan (2015) : Correlation between vascular endothelial growth factor levels and prognosis of hepatocellular carcinoma patients receiving radiofrequency ablation. Biotechnology & Biotechnological Equipment;29(1): 119-123.

Kaseb AO , Hanbali A , Cotant M , Hassan MM , Wollner I and Philip PA (2009): Vascular endothelial growth factor in the management of hepatocellular carcinoma: a review of literature. Cancer; 1; 115 (21): 4895-906.

Kemik O , Sumer A , Kemik SA , Purisa S and Tuzun S (2010): Circulating levels of VEGF family and their receptors in hepatocellular carcinoma. BratislLekListy; 111 (9): 485-8.

Korpanty G , Sullivan LA , Smyth E , Carney DN and Brekken RA (2010): Molecular and clinical aspects of targeting the VEGF pathway in tumors. J Oncol; 652320.

Lavarone M ,Lamertico P, Lannuzzi F , Manenti E , Donato MF, Arosio E , Bertolini F , Primignani , Sangiovanni A and Colombo M (2007): Increased expression of Vascular Endothelial Growth Factor in Small Heepatocellular carcinoma. Journal of Viral Hepatitis; 14 (2): 133-39.

Lencioni R , Piscaglia F and Bolondi L (2008): Contrast-enhanced ultrasound in the diagnosis of hepatocellular carcinoma. J Hepatol ; 48: 848–857.

Li C , Lee F, Hwang S , Lu R , Lee W, Chao Y , Wang S , Chang F , Whang-Peng J and Lee S (2003): Spider angiomas in patients with liver cirrhosis: role of vascular endothelial growth factor and basic fibroblast growth factor. World Journal of Gastroenterology; 9 (12): 2832–35.

Lopez JB (2005): Recent development in the first detection of hepatocellular carcinoma. Clin.Biochem. Rev; 26: 65-79.

Mukozu T , Nagai H , Matsui D , Kanekawa T and Sumino Y (2013): Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. Anticancer Res ; 33:1013–1021.

Sergio A , Cristofori C , Cardin R , Pivetta G , Ragazzi R, Baldan A , Girardi L , Cillo U , Burra P , Giacomin A and Farinati F (2008): Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. Am J Gastroenterol; 103 (4): 914-21.

Shen YC, Hsu C and Cheng AL (2010) : Molecular targeted therapy for advanced hepatocellular carcinoma: current status and future perspectives. J Gastroenterol ;45: 794-807.

Shim JH , Park J , Kim JH , An M , Kong S , Nam B , Choi J , Kim HB , Lee WJ and Kim C (2008) : Association between increment of serum VEGF level and prognosis after transcatheter arterial chemoembolization in hepatocellular carcinoma patients . Cancer Science ; 99(10): 2037–2044.

Stroescu C, Dragnea A, Ivanov B, Pechianu C, Herlea V, Sgarbura O, Popescu A and Popescu I (2008): Expression of p53, Bcl-2, VEGF, Ki67 and PCNA and prognostic significance in hepatocellular carcinoma. J Gastrointestin Liver Dis; 17 (4): 411-7.

Welzel TM , Graubard BI , Zeuzem S , El–Serag HB , Davila JA and McGlynn KA (2011):Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the Surveillance, Epidemiology, and End Results (SEER)-Medicare database. Hepatology ;(54):463–71.

World Health Organization (2012) : GLOBOCAN 2012: Estimated Incidence, Mortality and Prevalence Worldwide in 2012. Available online: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

Yamaguchi R , Yano H , Iemura A , Ogasawara S , Haramaki M and Kojiro M(1998): Expression of vascular endothelial growth factor in human hepatocellular carcinoma. Hepatology; 28: 68-77.