Serum Interleukin-6 (IL-6), Vascular Endothelial Growth Factor (VEGF), and VEGF/Platelets Ratio as Markers for Hepatocelluar Carcinoma

Ehab F. Moustafa[1], Ghada M. Galal[2], Sahar M. Hassany[1], Mohamed Z. Abd Elrahman[3], and Madleen Adel A. Abdou[3]

[1]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Assiut University
[2]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Sohag University
[3]Clinical Pathology Department, Faculty of Medicine, Assiut University
ehabmostafa_99@yahoo.com

Abstract: Background: Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality. **Aim of the work**: To evaluate the usefulness of serum IL-6, serum VEGF, and VEGF/Platelets ratio in hepatocellular carcinoma (HCC) diagnosis. **Patients and methods:** Fifty-eight cirrhotic patients with hepatocellular carcinoma were included in the study (51 males and 7 females) and 18 liver cirrhosis patients without HCC (15 males and 3 females) were recruited as a control group. All patients were subjected to full medical history, clinical examination, laboratory investigations complete blood count, liver function tests, AFP, serum IL-6, serum VEGF and calculation of VEGF/platelets ratio. **Results**: Patients had significantly higher values of AFP (*P*=0.0001), IL-6 (*P*=0.004), VEGF (*P* =0.001) and VEGF/Platelets ratio (*P* =0.005) than cirrhotic patients without HCC (control group). Sensitivity and specificity of serum IL-6, VEGF and VEGF/Platelets in detecting HCC, was found to be 34.5 % & 94.4%, 43.1 % & 88.9% and 41.4 % & 88.9% respectively. Sensitivity and specificity of serum IL-6, serum VEGF and VEGF/platelets ratio for detection of portal vein thrombosis were 65.5% & 83.3%, 63,8% & 77,8%, and 58,6% & 72,2% respectively. There was significant positive correlation between VEGF and AFP (r=0.794, *P* =0.0001), VEGF/Plat and AFP (r=0.760, *P* =0.0001) and IL-6 and AFP (r=0.804, *P* = 0.0001). **Conclusion:** Serum IL-6, serum VEGF, and VEGF/platelets ratio are significantly higher in HCC patients than liver cirrhosis patients without HCC. The clinical utility of these biomarkers in HCC diagnosis is still doubtful because their sensitivity is not more than that of AFP. They may have a good role in detection of portal vein thrombosis (tumor invasion).

[Ehab F. Moustafa, Ghada M. Galal, Sahar M. Hassany, Mohamed Z. Abd Elrahman, and Madleen Adel A. Abdou. **Serum Interleukin-6 (IL-6), Vascular Endothelial Growth Factor (VEGF), and VEGF/Platelets Ratio as Markers for Hepatocelluar Carcinoma**. *Life Science Journal*. 2012;9(2):930-938]. (ISSN:1097-8135). http://www.lifesciencesite.com. 138

Key words: Hepatocellular carcinoma, diagnosis, vascular endothelial growth factor, IL-6, alpha feto protein.

Abbreviations: hepatocellular carcinoma (HCC), vascular endothelial growth factor (VEGF), Plt (platelets), AFP (alpha feto protein).

1. Introduction

Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality [(1)]. Hepatocellular carcinoma (HCC) accounts for between 85% and 90% of primary liver cancers [(2)]. Its incidence is increasing worldwide ranging between 3% and 9% annually [(3)]. In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients [(4)]. More than 80% of cases of HCC occur in a background of cirrhosis and most frequently involve the right lobe [(2, 5)]. Major causes of cirrhosis are HBV, HCV, and alcohol. Investigations in Egypt during the last decade have shown the increasing importance of HCV infection in the etiology of liver cancer, estimated to account for 40–50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15%, respectively) [(6,7)].

Recently, the survival of patients with HCC after diagnosis has improved (8), which is attributed to advances in diagnostic techniques and to the application of various curative treatment options (surgical resection, liver transplantation, and percutaneous ablation). The major diagnostic modalities for HCC include serum markers, various imaging modalities and histological analysis. The overall sensitivity and accuracy of US-guided biopsy generally exceeds 85% [(9)]. There are virtually no false-positive findings. The negative predictive value of biopsy remains low. Therefore, in patients with negative biopsy findings, HCC cannot be definitely ruled out.

Pathologically, patients with chronic liver disease, particularly those associated with a high degree of hepatocyte regeneration, can express AFP in the absence of cancer. Also, AFP is elevated in

hepatocarcinogenesis, embryonic carcinomas [10] and in gastric [11] and lung cancer [12]. Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP, even without the presence of a tumor. The test had a sensitivity of 39% - 65%, a specificity of 76% - 94%, and a positive predictive value of 9% - 50% for the presence of HCC in previously published studies [13].

Tumor angiogenesis is essential for tumor growth, invasion and metastasis [14]. Tumor angiogenesis is mediated by a number of angiogenic factors and vascular endothelial growth factor (VEGF) is one of these factors. It was demonstrated that there is strong VEGF expression in various solid tumor types. In HCC, VEGF expression in tumor tissue has been found to be correlated with aggressive behavior, and poor prognosis [15]. By measuring circulating VEGF concentrations with the enzyme-linked immunosorbent assay (ELISA), the expression of VEGF in patients with various malignancies has been made possible [16].

VEGF content of platelets is higher in cancer patients than in healthy subjects [17]. It is known that platelets concentrate plasma proteins such as VEGF and later transport them into their granules [15]. So, authors have suggested that VEGF secreted from tumor cells could be stored and transported by platelets in the blood stream, and that this reservoir of VEGF might have a role in tumor angiogenesis and invasion [17,18]. Accordingly, VEGF load in platelets may predict tumor angiogenic activity better than serum VEGF. **George *et al.*** [19] used serum VEGF per platelet count to correct variation of serum VEGF levels in patients with different platelet counts. Serum VEGF per platelet count correlated with advancing stage of colorectal cancer, suggesting its role as a standard measure of circulating VEGF. There is still little data regarding the prognostic significance of serum VEGF per platelet count in patients with HCC. **Kim *et al.*** [20] reported that serum VEGF per platelet count was higher in patients with HCC than those with liver cirrhosis and it was an independent prognostic factor with the presence of portal vein thrombosis in their study. They concluded that serum VEGF per platelet count could be a feasible prognostic indicator during the follow-up of patients with HCC.

Interleukin-6 (IL-6) is a multifunctional cytokine largely responsible for the hepatic response to infections or systemic inflammation. Serum IL-6 levels are elevated in patients with chronic liver inflammation including alcoholic hepatitis [21], hepatitis B [22], HCV infections [23] and steatohepatitis [24]. Furthermore, serum IL-6 levels are reportedly higher in patients with HCC than in those without [25]. In chronic hepatitis, IL-6, produced mainly by activated Kupffer cells, intensifies local inflammatory responses and induces compensatory hepatocyte proliferation, facilitating malignant transformation of hepatocytes [26]. Also, IL-6 induces the hepatic acute phase response by modulating the transcription of several liver specific genes during inflammation [27]. **Malaguarnera *et al.*,** found that there was a significant positive correlation between IL-6 and the size of the tumor [27]. Moreover, Giannitrapani et al., indicated that IL-6 could be more sensitive marker in identifying HCCs than AFP [28].

Aim of the work:

To evaluate the usefulness of VEGF, VEGF/Platelets, interleukin-6 (IL-6) in HCC diagnosis

2. Patients and Methods:

Patients:

Fifty-eight patients with hepatocellular carcinoma (HCC on top of L.C) were included in the study (51 males and 7 females, mean age 59.6± 8.3 years, range 27 – 85 years). They were admitted to Tropical Medicine and Gastroenterology Department, Assiut University Hospital, Egypt from January 2008 to January 2010. The diagnosis of HCC was based mainly on the typical findings of triphasic abdominal computed tomography. Eighteen patients with liver cirrhosis without HCC (15 males and 3 females, mean age 60.2±9.7 years, range 40– 75 years) were also admitted to the department and served as a control group (Table 1). Tumor characteristics such as size, number, and portal vein patency were assessed by real time abdominal ultrasonography (Table 2).

Specimen collection and analysis:

For patients and control subjects, blood was obtained by venipuncture without a tourniquet. Complete blood count was done on Coulter Hmx, USA. Prothrombin time and concentration were estimated according to standard procedure using Sysmex CA500 coaglometer, Germany. Liver function was measured by Hitachi 911, Roche, Germany autoanalyser. Serological tests for hepatitis B surface antigen (HBsAg) and anti-HCV were carried out by Micro particle Enzyme Immunoassay (MEIA) technology using Abbot AXSYM System, USA autoanalyser.

Serum alpha foetoprotein was measured by ELISA DS-EIA-AFP from Diagnostic system Ltd, Germany. Serum vascular endothelial growth factor was measured by ELISA (according to the manufacturer instruction from Biotechnology, USA). Serum IL-6 was measured by ELISA from Orgenium kit Finland, catalogue no.ILO6001).

Statistical analysis:

Data entry and analyses were performed using a statistical software package (SPSS, Version 10.0, Inc., Chicago, IL). Student's t- test, ANOVA test, Chi-square test, Fisher exact probability test, correlation coefficient (r) and spearman's rank correlation (r) were used when appropriate. P<0.05 was considered statistically significant.

Ethical approval:

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. An ethical approval was obtained from our local Ethics Committee (Medical Ethics Committee, Faculty of Medicine, Assiut University). Patients were enrolled after written informed consent was obtained.

3. Results:

The base line clinical and laboratory data of patients (HCC on top of liver cirrhosis) and controls (liver cirrhosis) are demonstrated in Table 1. According to Child-Pugh classification, 21 (36.2%) patients were Child A cirrhosis, 20 (34.5%) were Child B and 17 (29.3%) were Child C. Cirrhotic patients of the control group were 6 (33.3%) Child B and 12 (66.7%) Child C.

In HCC group 33 patients were HCV Ab positive, 19 patients were HBsAg positive and 6 patients were both HCV Ab and HBsAg positive, while in control group (cirrhosis without HCC) 10 patients were HCV Ab positive and 8 patients were HBsAg positive.

Abdominal ultrasound data of patients and controls revealed that thirty- seven patients (63.8%) had single hepatic focal lesion and 21 (36.2%) had multiple hepatic focal lesions varying in size between 1 to 11 cm. Portal vein was patent in 51 (87.9%) patients with focal lesions and was thrombosed in 7 (12.1%). All cirrhotic patients without focal lesions (controls) had patent portal vein (Table 2).

Table 3 shows that HCC patients had significantly higher values of AFP (401.5±665.4 vs 31.2±104.6; *P*=0.0001), IL-6 (138.1 ±257.6 vs 31.2±104.6; *P* =0.004), VEGF (398.9±67.2.5 vs 52.3±150.6; *P*=0.001) and VEGF/Platelets ratio (3.4±6.1 vs 0.7±2.2; *P* =0.005) than cirrhotic patients without hepatic focal lesions (control group).

Studying serum markers of HCC in patients with single and multiple focal lesions revealed that no significant difference in all the studied markers between both groups (Table 4).

Diagnostic value of IL-6, VEGF and VEGF/Platelets

By using the mean levels of IL-6, VEGF and VEGF/Platelets in patients with liver cirrhosis without HCC (23.1 pg/ml, 52.3 pg/ml and 0.7) as cut-off levels, the sensitivity and specificity of these 3 markers in detecting HCC, were found to be 34.5 % with 94.4% specificity, 43.1 % with 88.9% specificity and 41.4 % with. 88.9% specificity respectively (Table 5).

To evaluate the diagnostic value of these markers for detection of portal vein thrombosis, mean levels of IL-6, VEGF and VEGF/Platelets in HCC patients with absence of portal vein thrombosis was used as cut-off level, serum VEGF was found to have a sensitivity of 63.8% and a specificity of 77.8% in detecting portal vein thrombosis, serum IL-6 was found to have a sensitivity of 65.5% and a specificity of 83.3% in detecting portal vein thrombosis and VEGF/Platelets ratio was found to have a sensitivity of 58.6% and a specificity of 72.2% in detecting portal vein thrombosis (Tables 6-8).

Results of correlation studies:

Correlation studies of the tested variables revealed that there was significant positive correlation between VEGF and AFP (r=0.794, *P* =0.0001; Fig 1), VEGF/Plat and AFP (r=0.760, *P* =0.0001; Fig 2) and IL-6 and AFP (r=0.804, *P* = 0.0001; Fig 3)

Table 1: Base line clinical and laboratory data of patients (HCC on top off liver cirrhosis) and controls (liver cirrhosis)

	Cases (n=58)	Control (n=18)
Age(ys) Mean Range	 59.6 (27 – 85)	 60.2 (40 – 75)
Sex Male/Female	 51/7	 15/3
Prothrombin Time(sec)	16.3	19.0
Prothrombin concentration(٪)	63.3	48.4
platelets(x10^9/l)	163.0	126.4
ALP(IU/l)	169.3	121.6
Positive HCV Ab	33 (56.9%)	10 (55.6%)
Positive HBsAg	19 (32.8%)	8 (44.4%)
Positive HCV Ab & HBsAg	6 (10.3%)	0
Child classification: Child A Child B Child C	 21 (36.2%) 20 (34.5%) 17 (29.3%)	 0 6 (3.3%) 12 (66.7%)

Table 2. Sonographic data of patients (LC+HCC) and controls (LC)

Variable	Patients (LC+HCC) n=58	Controls (LC) n=18
Size of focal lesion (cm)		
Range	1-11	----
Mean±SD	4.2±2.1	----
N of focal lesions		
Single	37 (63.8%)	---
Multiple	21 (36.2%)	---
Portal vein		
Patent	51 (87.9%)	18 (100%)
Thrombosed	7 (12.1%)	0 (0%)
Ascites		
Yes	31 (53.4%)	18 (100%)
No	27 (46.6%)	0 (0%)

Data are expressed as mean±SD or as number, (%).

Table 3. Platelets count, Alkaline phsphatase (ALP), α feto protein (AFP), serum IL-6, serum vascular endothelial growth factor (VEGF) nd VEGF/platelets ratio in patients (HCC) versus control (liver cirrhosis without HCC).

Variable	Patients (LC+HCC) n= 58		Controls (LC) n= 18		P value
	Range	Mean±SD	Range	Mean±SD	
AFP (ng/dl)	1-2502	401.5±665.4	2-450	31.2±104.6	0.0001
IL-6 (pg/ml)	1-1100	138.1±257.6	1-355	23.1±83	0.004
VEGF(pg/ml)	4-2300	398.9±672.5	5-650	52.3±150.6	0.001
VEGF/ Platelets	0.03-21.4	3.4±6.1	0.01-9.6	0.7-2.2	0.005

Table 4. Serum markers of HCC in patients with single vs multiple focal lesions.

	n. of focal l (single)		n. of focal l (multiple)		P value
	Mean	SD	Mean	SD	
AFP (ng/dl)	340.89	611.943	508.25	754.453	0.362
IL6 (pg/ml)	138.54	269.82	137.4	240.98	0.987
VEGF(pg/ml)	419.15	734.47	363.13	562.03	0.763
VEGF/plat	3.93	6.77	2.54	4.66	0.361

Table 5. Sensitivity and specificity of serum IL-6, VEGF and VEGF/Platelets in detecting HCC.

	Cut off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IL6(pg/ml)	23.1	34.5	94.4	95.2	30.9
VEGF(pg/ml)	52.3	43.1	88.9	92.6	32.7
VEGF/plat	0.7	41.4	88.9	92.3	32.0

Table 6. Performance of VEGF at cut off point for detection PV thrombosis

	Cut off VEGF value (pg/ml)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PV thrombosis	19.5	63.8	7.8	90.2	740.0

Table 7. Performance of IL-6 at cut off point for detection PV thrombosis

	Cut off IL6 value (pg/m)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Portal V TH	5.6	65.5	83.3	92.7	42.9

Table 8. Performance of VEGF/Plat at cut off point for detection PV thrombosis

	Cut off VEGF/plat value (pg/ml)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PV thrombosis	0.162	58.6	72.2	87.2	35.1

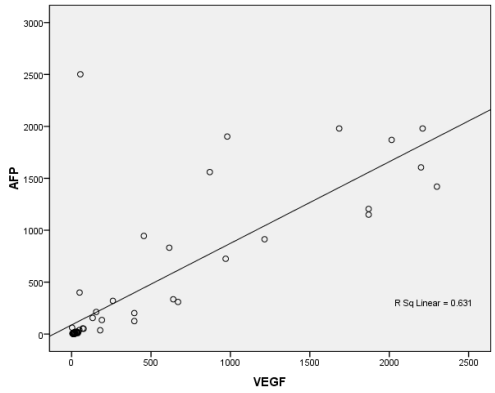

Fig 1. Correlation between AFP and VEGF in HCC patients

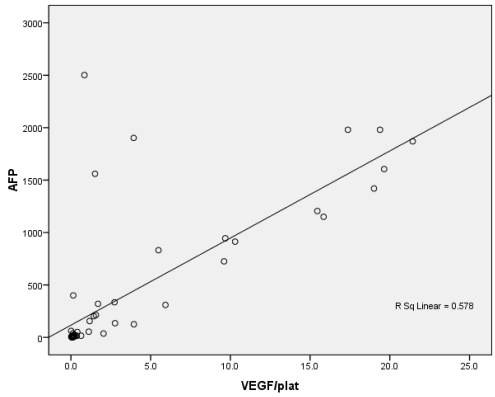

Fig 2. Correlation between AFP and VEGF/Plat in HCC patients

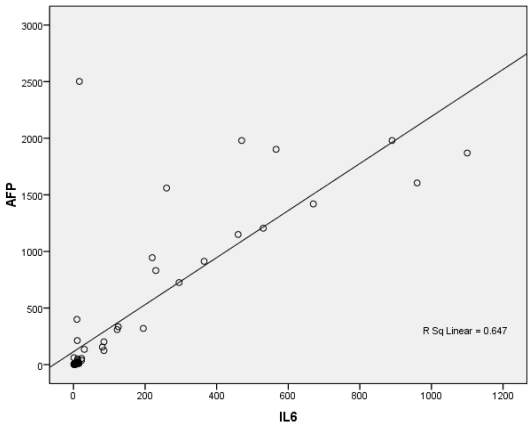

Fig 3. Correlation between AFP and IL-6 in HCC patients

4. Discussion

Surveillance of cirrhotic patients for early diagnosis of HCC has been recommended by all practice guidelines for optimal HCC management [29-31]. The AASLD practice guidelines on the management of HCC were published in 2005 in order to provide an evidence-based approach to screening of HCC, in addition to diagnosis, staging and management of this tumor [30].

The most important tumor marker for HCC is alpha fetoprotein (AFP). The common method for screening high risk patient using AFP marker can detect more early tumors and prolong the survival of patients [32]. The sensitivity of AFP assays has increased. In HCC, the reported range of AFP is 10 to more than 100,000 µg/L. Approximately 40% of patients with HCC have AFP levels greater than 1,000 µg/L [33].

Serum AFP is the most commonly used marker for this neoplasm, but its real clinical usefulness is unclear, a systematic review and critical analysis [34] of the use of this marker in HCC detection yielded sensitivity and specificity rates ranging from 41% to 65% and 80 to 94%, respectively. AFP levels greater than 400 µg/L, however, are generally considered diagnostic of HCC, especially when a liver mass is visualized at ultrasound (US) or computed tomography (CT) [35].

Our results showed that HCC patients have significantly higher values of AFP, serum IL-6, serum VEGF, and VEGF/Platelets ratio than cirrhotic patients without HCC (control group).

Our findings showed significant positive correlations between VEGF and AFP, VEGF/Plat and AFP and IL-6 and AFP.

Many studies showed that serum VEGF has a prognostic value in various cancers [36-38]. But the issue of whether serum VEGF level can reflect tumor VEGF expression has become a major obstacle in its clinical application. Indirect evidence has suggested that the tumor is a major source of serum VEGF in cancer patients. A significant decrease in serum VEGF after tumor resection has been documented [37,38]. Previous study [39] reported that serum VEGF levels in mesenteric venous blood draining from the tumors were several-fold higher compared with peripheral blood in colorectal cancer patients, this remark suggested the secretion of VEGF from the tumors into the circulation. On the other hand, **Salgado *et al.*** [40] found no significant increase in

serum or plasma VEGF levels in the vein draining the tumors in patients with various carcinomas.

Hepatocellular carcinoma (HCC) is a highly vascular tumor characterized by Neo vascularization and a high propensity for venous invasion. A strong VEGF expression was observed in the tissue of HCC and associated with tumor progression and metastasis [13]. **Poon *et al.*** [41], reported that serum VEGF level showed significant elevation in patients with HCC, and high serum VEGF levels were associated with the absence of tumor capsule, the presence of venous invasion and microsatellite nodules, and advanced TNM stage. **Li *et al.*** [42] also reported that serum VEGF was a predictor of invasion and metastasis of HCC. Other previous studies suggested that platelets aggregate at metastatic sites, due to factors released from metastatic cells and vascular invasion, resulting in microthrombosis, tumor adhesion, and may release VEGF to the circulation [43, 44].

Jinno *et al.* [45] concluded that P-VEGF (plasma VEGF) was a promising tumor marker for remote metastasis of HCC and its specificity, sensitivity and overall accuracy were high enough to be clinically useful.

Moreover, **Li *et al.*** [46] found that pre-TACE (transarterial chemoembolization) plasma VEGF was found to be markedly elevated in the majority of patients with HCC and the increase was closely related to a more advanced stage of diseases. This finding suggested that HCC cells were an important source of P-VEGF. But the lower VEGF levels in HCC patients overlapped considerably with those in normal controls, thus limiting the application of VEGF as a tumor marker in early detection of HCC. Also, they found that high plasma VEGF levels were associated with the presence of extrahepatic metastasis and portal vein involvement. Because vascular invasion by tumor cells is indispensable for remote metastasis, portal vein involvement and A-V shunting can reflect the ability of tumor cells to invade blood vessels. They suggested that plasma VEGF could be used as a tumor marker in detecting vascular invasive phenotypes of HCC. They concluded that, Plasma VEGF level is an independent predictive factor of tumor progression, especially vascular invasion.

On the other hand they found no correlation between plasma VEGF and serum AFP, suggesting that they had different mechanisms of production, and P-VEGF might be an independent predictive factor.

Kim *et al.* [19] suggested that serum VEGF per platelet count predicts tumor aggressiveness and treatment outcome. In the current study, serum VEGF per platelet count was higher in patients with HCC than in those with liver cirrhosis. The presence of HCC could contribute to this difference, because both groups had liver cirrhosis and similar characteristics such as liver function and viral markers. This may suggest the possible role of serum VEGF per platelet count as an indicator of the development of HCC in patients with liver cirrhosis during follow-up. In the multivariate analysis for factors predicting survival, high serum VEGF per platelet count (>1.4 pg/106) and portal vein thrombosis were independent prognostic indicators for overall survival. This suggests that serum VEGF per platelet count could be a better prognostic factor than serum VEGF itself.

It was noticed that serum IL-6 level was elevated in patients with primary liver cancer [47] and its levels were significantly higher in HCC patients than in healthy controls [48, 49]. High serum IL-6 may promote HCC development in hepatitis B patients [50]. Therefore, IL-6 could be considered a HCC biomarker and a high risk factor for HCC. Several studies revealed that increased IL-6 is positively correlated with the occurrence and progression of primary liver cancer. Karin group showed that obesity can promote liver inflammation and augment cancer risk via the increased expression of IL-6/STAT3 and TNF/STAT3, where STAT3 mean Signal Transducer and Activator of Transcription 3, an acute phase response factor [51]. Also, they found that diethylnitrosamine (DEN) can cause HCC in 100% of male mice but in lower than 30% of female mice. Other studies concluded that DEN increases serum IL-6 much more remarkably in male mice. Blockade of IL-6 result in absence of this difference in both sexes, so this indicates that IL-6 is an important risk factor of HCC [52]. All the above, suggest that inflammation and IL-6 may be correlated with the development of HCC.

There is strong evidence about the role of the cytokine IL-6 in the process of liver damage and carcinogenesis. Liver cirrhosis is associated with increased hepatic expression of several cytokines, including IL-6 [53]. IL-6 was also shown to induce the expression of the mitogenic, motogenic, morphogenic and pro-neoangiogenic hepatocyte growth factor which are expressed at high levels in HCC [54]. Also, the expression of IL-6 into non-metastatic HCC cells makes them highly metastatic (55). Beside this IL-6 may also decrease HCC cell apoptosis. In one study in a mouse model, IL-6 proved able to reduce Fas-induced apoptosis [56]. It has been postulated that IL-6 may directly stimulate hepatic DNA synthesis, in mice showing a lack in DNA synthesis following hepatectomy [57]. Also, it was reported that IL-6 is considered a cause of natural killer cell dysfunctions, resulting in tumor escape from immune surveillance [58].

Several reports indicate a potential role for IL-6 as a tumor marker for HCC [27, 28, 49].

The sensitivity and specificity of serum IL-6, VEGF and VEGF/Platelets in detecting HCC, was found to be 34.5 % with 94.4% specificity, 43.1 % with 88.9% specificity and 41.4 % with. 88.9% specificity. **Li *et al.*** [46], Found that sensitivity and specificity of P-VEGF in detecting HCC were 73.3% and 62.6%.

Our results showed that serum VEGF had a sensitivity of 63.8% and a specificity of 77.8% in detecting portal vein thrombosis. Serum IL-6 had a sensitivity of 65.5% and a specificity of 83.3% in detecting portal vein thrombosis and VEGF/Platelets ratio was found to have a sensitivity of 58.6% and a specificity of 72.2% in detecting portal vein thrombosis. These also agree with **Li *et al.*** [46], where they found that sensitivity and specificity of P-VEGF in detecting portal vein thrombosis of positive tumors were 62.5 % and 64.9%.

5. Conclusions:

In conclusion, serum IL-6, serum VEGF, and VEGF/platelets ratio are significantly higher in HCC patient than liver cirrhosis patients without HCC. The clinical benefit of using these biomarkers in HCC diagnosis is still doubtful because their sensitivity is not more than that of AFP. They may have a good role in detection of portal vein thrombosis (tumor invasion), with sensitivity and specificity of 65.5% and 83.3%, 63, 8% and 77,8%, and 58,6% and 72,2% respectively.

Correspondence author
Ehab F. Moustafa
Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Assiut University
ehabmostafa_99@yahoo.com

References

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol. 2001; 2:533–543.
2. Yamamoto M, Ariizumi S, Yoshitoshi K, Saito A, Nakano M, Takasaki K. Hepatocellular carcinoma with a central scar and a scalloped tumor margin resembling focal nodular hyperplasia in macroscopic appearance. J Surg Oncol. 2006;94: 587–591.
3. Velazquez RE, Rodriguez M, Navascues CA, Linares A, Perez R, Sotorrios NG, Martinez I, Rodrigo L. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. *Hepatology* 2003; 37: 520-527
4. Rahman El-Zayadi A, Abaza H, Shawky S, Mohamed MK, Selim OE, Badran HM. Prevalence and epidemiological features of hepatocellular carcinoma in Egypt-a single center experience. *Hepatol Res.* 2001; 19: 170-179
5. Fattovich G, Stroffolini T, Zagni I, *et al.* Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004; 127(5 Suppl 1): S35–S50.
6. El-Zayadi AR, Badran HM, Barakat EM *et al.* Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J Gastroenterol.* 2005; 11: 5193–8.
7. Hassan MM, Zaghloul AS, El-Serag HB *et al.* The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. *J Clin Gastroenterol.* 2001; 33: 123–6.
8. Durand F, Belghiti J, Paradis V. Liver transplantation for hepatocellular carcinoma. Liver Transpl. 2007; 13:S17–S23
9. Grizzi F, Franceschini B, Hamrick C, Frezza EE, Cobos E, Chiriva-Internati M. Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. J Transl Med. 2007; 5: 3
10. Chen ZM, Liu BQ, Boreham J, Wu YP, Chen JS, Peto R. Smoking and liver cancer in China: case-control comparison of 36,000 liver cancer deaths vs. 17,000 cirrhosis deaths. Int J Cancer, 2003;107:106–112.
11. Hiroshima K, Iyoda A, Toyozaki T, Haga Y, Baba M, Fujisawa T, Ishikura H, Ohwada H. Alpha-fetoprotein-producing lung carcinoma: report of three cases. Pathol Int. 2002; 52: 46-53
12. Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha fetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterology 2004; 127: S108-S112
13. Gupta MK Qin RY. Mechanism and its regulation of tumor induced angiogenesis. World J Gastroenterol. 2003; 9: 1144-1155.
14. Poon RT, Lau CP, Ho JW, Yu WC, Fan ST, Wong J. Tissue factor expression correlates with tumor angiogenesis and invasiveness in human hepatocellular carcinoma. Clin Cancer Res. 2003; 9: 5339-5345.
15. Kondo S, Asano M, Matsuo K, Ohmori I, Suzuki H. Vascular endothelial growth factor/vascular permeability factor is detectable in the sera of tumor-bearing mice and cancer patients. Biochem Biophys Acta. 1994; 1221: 211-214
16. Vermeulen PB, Salven P, Benoy I, Gasparini G, Dirix LY. Blood platelets and serum VEGF in cancer patients. Br J Cancer 1999; 79:370–6.
17. Benoy I, Salgado R, Colpaert C, Weytjens R, Vermeulen PB, Dirix LY. Serum interleukin 6, plasma VEGF, serum VEGF, and VEGF platelet

load in breast cancer patients. Clin Breast Cancer 2002; 2: 311–5.
18. George ML, Eccles SA, Tutton MG, Abulafi AM, Swift RI. Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: Clinical evidence of platelet scavenging? Clin Cancer Res. 2000; 6: 3147–52.
19. Kim SJ, Choi IK, Park KH, Yoon SY, Oh SC, *et al.* Serum Vascular Endothelial Growth Factor per Platelet Count in hepatocellular Carcinoma: Correlations with Clinical Parameters and Survival. Jpn J Clin Oncol. 2004; 34(4)184–190.
20. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol. 1993; 54: 1–78.
21. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. J Hepatol. 2008;48(Suppl 1):S20–37
22. Deviere J, Content J, Denys C, Vandenbussche P, Schandene L, Wybran J, Dupont E. High interleukin-6 serum levels and increased production by leucocytes in alcoholic liver cirrhosis. Correlation with. IgA serum levels and lymphokines production. Clin Exp Immunol. 1989;77:221–5.
23. Lee Y, Park US, Choi I, Yoon SK, Park YM, Lee YI. Human interleukin 6 gene is activated by hepatitis B virus-X protein in human hepatoma cells. Clin. Cancer Res. 1998; 4:1711–7.
24. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. Am J Gastroenterol. 2008;103:1372–9.
25. Oyanagi Y, Takahashi T, Matsui S, Takahashi S, Boku S, Takahashi K, Furukawa K, Arai F, Asakura H. Enhanced expression of interleukin-6 in chronic hepatitis C. Liver 1999;19:464–72.
26. Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med. 2008; 14:109–19.
27. Malaguarnera M, Di Fazio I, Laurino A et al. Role of interleukin 6 in hepatocellular carcinoma. Bull Cancer 1996; 83: 379–384.
28. Giannitrapani L, Cervello M, Soresi M *et al.* Circulating IL-6 and sIL-6R in patients with hepatocellular carcinoma. Ann N Y Acad Sci. 2002; 963: 46–52.
29. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. EASL Panel of Experts on HCC. Clinical Management of hepatocellular carcinoma. Conclusion of Barcelona-2000 conference. European Association for the study of the Liver. J Hepatol. 2001; 35: 4210-4230.
30. Bruix J, Sherman M. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular Carcinoma. Hepatology 2005, 42: 1208-1236.
31. Poon D, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, *et al.* Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Submmit 2009. Lancet Oncol. 2009; 10: 1111-1118.
32. Lopez J. Recent development in the first detection of Hepatocellular Carcinoma . Clin Biochem Rev. 2005; 26: 70-71.
33. Colombo M. Screening for cancer in viral hepatitis. Clin liver Dis. 2001; 5 (1): 109-22.
34. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. Ann Intern Med. 2003; 139: 46–50.
35. Trevisani F, D' Intio PE, Morselli-Labate AM, *et al.* Serum alpha-fetoprotein for diagnosis of hepatocellular cacinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol. 2001; 34(4): 570-5.
36. Poon R T, Fan S T, and Wong J. Clinical implications of circulating angiogenic factors in cancer patients. J. Clin. Oncol. 19: 1207–1225, 2001.
37. Karayiannakis A J, Syrigos K N, Polychronidis A, Zbar A, Kouraklis G, Simopoulos C, and Karatzas G. Circulating VEGF levels in the serum of gastric cancer patients: correlation with pathological variables, patient survival, and tumor surgery. Ann. Surg., 236: 37–42, 2002.
38. Yamamoto Y, Toi M, Kondo S, Matsumoto T, Suzuki H, Kitamura M, Tsuruta K, Taniguchi T, Okamoto A, Mori T, Yoshida M, Ikeda T, and Tominaga T. Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. Clin Cancer Res. 2: 821–826, 1996.
39. Landriscina M, Cassano A, Ratto C, Longo R, Ippoliti M, Palazzotti B, Crucitti F, and Barone C. Quantitative analysis of basic fibroblast growth factor and vascular endothelial growth factor in human colorectal cancer. Br. J. Cancer 78: 765–770, 1998
40. Salgado R, Benoy I, Weytjens R, Van Bockstaele D, Van Marck E, Huget P, Hoylaerts M, Vermeulen P, and Dirix L Y Arteriovenous gradients of IL-6, plasma and serum VEGF and D-dimers in human cancer. Br. J. Cancer 87: 1437– 1444, 2002.

41. Poon RT, Ng IO, Lau C, Zhu LX, Yu WC, Lo CM, Fan ST, Wong J. Serum vascular endothelial growth factor predicts venous invasion in hepatocellular carcinoma: a prospective study. Ann Surg. 2001; 233: 227-235
42. Li XM, Tang ZY, Qin LX, Zhou J, Sun HC. Serum vascular endothelial growth factor is a predictor of invasion and metastasis in hepatocellular carcinoma. J Exp Clin Cancer Res. 1999; 18: 511-517
43. Pinedo HM, Verheul HM, D'Amato RJ, Folkman J. Involvement of platelets in tumor angiogenesis. Lancet. 1998; 352: 1775-1777
44. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. Proc Natl Acad Sci U S A 1997; 94: 663-668
45. Jinno K, Tanimizu M, Hyodo I, Nishikawa Y, Hosokawa Y, Doi T, Endo H, Yamashita M, Okada Y. Circulating vascular endothelial growth factor (VEGF) is a possible tumor marker for metastasis in human hepatocellular carcinoma. J Gastroenterol 1998; 33: 376-382
46. Li Xin, Feng Gan-Sheng, Zheng Chuan-Sheng, Zhuo Chen-Kai, Liu Xi. Expression of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma and effect of transcatheter arterial chemoembolization therapy on plasma vascular endothelial growth factor level. World J Gastroenterol 2004;10 (19):2878-2882
47. Goydos JS, Brumfield AM, Frezza E, Booth A, Lotze MT, Carty SE. Marked elevation of serum interleukin-6 in patients with cholangiocarcinoma: Validation of utility as a clinical marker. Ann Surg 1998; 227:398-404.
48. Giannitrapani L, Cervello M, Soresi M, Notarbartolo M, La Rosa M, Virruso L, et al. Circulating IL-6 and sIL-6R in patients with hepatocellular carcinoma. Ann NY Acad Sci 2002; 963:46-52.
49. Porta C, De Amici M, Quaglini S, Paglino C, Tagliani F, Boncimino A, et al. Circulating interleukin- 6 as a tumor marker for hepatocellular carcinoma. Ann Oncol 2008; 19:353-8.
50. Wong VW, Yu J, Cheng AS, Wong GL, Chan HY, Chu ES, et al. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. Int J Cancer 2009; 124:2766-70.
51. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 2010; 140:197-208.
52. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007; 317:121-4.
53. Napoli J, Bishop GA, McCaughan GW. Increased intrahepatic messenger RNA expression of interleukins 2, 6, and 8 in human cirrhosis. Gastroenterology 1994; 107: 789–798.
54. Zhu M, Paddock GV. Expression of the hepatocyte growth factor-like protein gene in human hepatocellular carcinoma and interleukin-6-induced increased expression in hepatoma cells. Biochem Biophys Acta 1999; 1449: 63–72.
55. Reichner JS, Mulligan JA, Spisni R et al. Effect of IL-6 overexpression on themetastatic potential of rat hepatocellular carcinoma cells. Ann Surg Oncol 1998; 5: 279–286.
56. Kovalovich K, Li W, DeAngelis R et al. Interleukin-6 protects against Fas-mediated death by establishing a critical level of anti-apoptotic hepatic proteins FLIP, Bcl-2, and Bcl-xL. J Biol Chem 2001; 276: 26605–26613.
57. Wallenius V, Wallenius K, Hisaoka M et al. Retarded liver growth in interleukin-6- deficient and tumor necrosis factor receptor-1-deficient mice. Endocrinology 2001; 142: 2953–2960.
58. Tanner J, Tosato G. Impairment of natural killer functions by interleukin 6 increases lymphoblastoid cell tumorigenicity in athymic mice. J Clin Invest 1991 88: 239–247.