Assessment of the Role of Interleukin-6 in Diagnosis of Hepatocellular Carcinoma

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Interleukin-6 (IL-6) is a promising tumor marker for hepatocellular carcinoma; HCC. IL-6 may help to identify a subset of HCC patients with low alpha-fetoprotein (AFP) level, and may serve as complementary tumor marker, however, this has to be clarified. This study assesses the value of measuring serum level of interleukin-6 in patients with chronic liver disease and HCC, and evaluates its sensitivity and specificity in comparison to AFP in early diagnosis of HCC. Seventy five patients with chronic liver disease (CLD) with or without HCC and 25 healthy controls were included. Patients were divided into Group I: 25 patients with CLD but no evidence of HCC. Group II: 25 patients with HCC on top of post-viral hepatitic with elevation in AFP (>200ng); and Group III: 25 patients with HCC on top of post-viral hepatitic but without elevation in AFP (<200ng). Analysis of the mean serum IL-6 levels revealed a statistically significant difference between all groups (*P*<0.01). A significant positive correlation was found between mean levels of IL- 6 & AFP in HCC (*P*< 0.05), the mean IL-6 levels in patients with Child classification C was higher than in those with Child A and B.After adjustment using multiple logistic regressions, only loss of weight and AFP were found to be significantly associated with HCC (*P*< 0.05). It is concluded that the diagnostic value of IL-6 increased when it is associated with AFP measurement. Combining the two markers can provide a new perspective in the diagnosis of HCC.

Hepatocellular carcinoma (HCC) accounts for 90% of primary liver neoplasms, represents the fifth most common cancer in the world (Fung & Marsh, 2002). In Egypt, there is a rising trend of HCC as there was nearly a two fold increase of the proportion of HCC among chronic liver disease patients over the last decade (El-Zayadi *et al.,* 2005). This malignancy is becoming recognized as an early complication and the most frequent cause of death in persons with viral-associated cirrhosis (Benvegnù *et al.,* 2004). It is very important to detect this disease and the recurrence at its earlier period. Alpha-Fetoprotein (AFP) has been the serum marker that is most widely used for diagnosis as well as surveillance of HCC. However, AFP levels may be normal in up to 40% of patients with HCC, particularly during the early stages (low sensitivity) (El-Folly *et al.,* 2007).

Interleukin-6 (IL-6) is an intriguing cytokine to be studied in hepatocellular carcinoma (HCC), a major health problem worldwide due to both its increasing frequency and its poor prognosis (Kaplan *et al.,* 2003). *It* is considered to be a hepatoprotective cytokine. It induces the hepatic acute phase response by modulating the transcription of several liver-specific genes during inflammation, and has a key role in the process of liver damage and carcinogenesis. IL-6 titers have been found to be four-fold higher in cancer than in cirrhotic patients and 25-fold higher than in healthy controls (Porta *et al.,* 2008) and therefore considered as a promising tumor marker for HCC (Porta *et al.,* 2008).

It has been found to be more sensitive in identifying HCCs than AFP, which still remains the most commonly used marker for this cancer Giannitrapani *et al.* (2002). It may help to identify a subset of HCC patients with low AFP level, and may serve as complementary tumor markers in these patients (Hsia *et al.,* 2007).

The Aim of this work is to assess the diagnostic value of measuring serum interlukin-6 levels in chronic liver disease and HCC, in comparison to AFP.

Patients and Methods

Study Design

This study is a prospective controlled study.

Study Setting and time

This study was done in co-operation between the Tropical Medicine Department in Faculty of Medicine (Ain Shams University) and Professor Doctor Yassin Abdel Gaffer Charity Center for Liver Disease and Research, in the period from August 2008 to June 2009.

Patients Selection

Patients enrolled in our study were taken from outpatient clinic and inpatient department in Professor Doctor Yassin Abdel Gaffer Charity Center for Liver Disease and Research. We recruited 75 cases in whom (clinical, biochemical, and sonographical criteria of chronic liver disease and HCV-Ab+ve or HBsAg+ve). They were categorized into four groups as follows: Group I; Diseased control formed of 25 patients diagnosed as post-viral hepatitic chronic liver disease with no evidence of HCC. Group II; formed of 25 patients diagnosed as HCC on top of post-viral hepatitic chronic liver disease with elevation in AFP. HCC diagnosed by (characteristic features of HCC by spiral abdominal CT and High AFP more than cut-off limit 200ng According to American Association for the study of liver diseases (Bruix & Sherman, 2005). Group III; formed of 25 patients diagnosed as HCC on top of post-viral hepatitic chronic liver disease without elevation in AFP. HCC diagnosed by (subjected focal lesion by ultrasound diagnosis and characteristic features of HCC by spiral abdominal CT) (Esmat *et al.,* 2009). Control Group; Healthy Control formed of 25 age- and sex-matched healthy volunteer controls, with no evidence of liver disease and/or of neoplasm.

Inclusion Criteria

For the three studied groups, they are diagnosed clinically by the presence of one or more stigmata of chronic liver disease and liver cell failure: (jaundice, palmar erythema, ecchymosis, pallor, flapping tremors, hepatic encephalopathy, and ascites), biochemical criteria (elevation of liver enzymes more than two folds, decreased serum albumin<3gm/dl, increased serum bilirubin>3mg/dl, INR>1.2) and sonographical criteria suggestive of chronic liver disease (uneven hepatic margins, increased parenchymal reflectivity, splenomegaly, presence of ascites). All patients were positive for either HCV-Ab or HBsAg.

Exclusion Criteria

Patients with other causes of liver disease (Auto-immune hepatitis, Hemochromatosis, Wilson's disease and Budd Chiarri). Patients with advanced systemic disease as heart failure, renal failure or any depleting disease. Metastatic liver disease.

Sample Size Determination

Sample size was calculated to include 75 patients who fulfilled the predesigned inclusions criteria at 95% confidence interval and a power of 0.80 and an expected effect size of 50%.

Ethical Consideration

The objectives of the study and the possible complications were explained to all patients who met the eligibility criteria and they were asked to sign a consent form.

Tools of the Study

All the studied cases were subjected to the following:

- Complete clinical evaluation.
- Laboratory investigations: [To detect the etiology of liver disease and to evaluate the liver function]. Detection of serum alpha-fetoprotein (AFP) levels: detection of AFP was done by ELIZA immune-assay, using (FUJIREBIO Diagnostics AB).
- Determination of serum Interlukin-6(IL-6) level: using IL-6 ELISA kit supplied by (R and D systems, MN, Minneapolis, USA).
- Classification of Patients according to Child-Turcotte Pugh scoring system (Pugh, 1973).

Items	Score (points)		
	1	2	3
Ascites	Absent	Slight-moderate	Tense
Encephalopathy	None	1-2	3-4
S. albumin (gm/dl)	>3.5	2.8-3.5	<2.8
S. bilirubin (mg/dl)	1-2	2-3	>3
PT in sec. above control	1-3	4-6	>6
Grading	A: 5-6, B: 7-9, C: 10-15		

- Abdominal ultrasonography. with special stress on stigmata of chronic liver disease: Uneven hepatic margins, increased parenchymatous reflectivity, coarseness, increased echographic contrast between right lobe of liver and right kidney, hypertrophied caudate lobe and attenuated hepatic veins. Any focal lesion and its size. Portal vein (patency and its diameter). Splenic size and presence of ascites.
- Abdominal Triphasic Spiral Computed Tomography (CT): was used to confirm the findings of abdominal ultrasonography and for studying of suggestive criteria for HCC (early enhancement in arterial phase, rapid washout in subsequent phases – portovenous and delayed phases).

The Technique of Interleukin-6 Measurement

The IL-6 ELISA kit supplied by R and D systems was used as described by the company. It is designed for quantitative determination of IL-6 in human plasma, serum, or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay that measures IL-6. In principle, a murine monoclonal antibody specific for human IL-6 has been pre-coated onto a microtitre plate. IL-6 in samples, standards is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human IL-6, which is recognized by a streptavidin- peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The colour development is stopped and the intensity of the colour is measured at 450 nm. The mean value of the duplicate readings of the standards and the samples are calculated and a standard curve is generated (using a log-log graph), plotting the standards concentrations on the x-axis and the corresponding mean 450nm absorbance on the y-axis. The unknown sample concentrations are determined from the standard curve in pg/ml, sensitivity is less than 0.70 pg/ml, specificity; this assay recognizes both natural and recombinant human IL-6 with no significant cross reactivity or interference with other cytokines and growth factors tested by the manufacturer, positive control was set as low level (range: 21-45 pg/ml), medium(range:75-111 pg/ml) and high(range:145-228 pg/ml).

Statistical Analysis

The data were processed and analyzed using the statistical package for social sciences (SPSS) program. Expression of data in the form of mean, S.D. (standard deviation) and range for quantitative variables, description of qualitative variables by frequency and percent, comparison between 2 groups' quantitative variables was carried out by student t-test; comparison of more than two groups' quantitative variables was carried out by one way ANOVA test. And chi-square test (Pearson chi-square) was used to compare between qualitative variables. Variables that were significantly associated with HCC were included in a logistic regression analysis model using the stepwise method, to examine the factors affecting serum IL-6 levels in HCC. Areas under the curve (AUC) calculations of nonparametric receiver operating characteristic (ROC) curves were used to assess the best cut-off points for IL-6, to obtain the optimum sensitivity and specificity for the diagnosis of HCC. The area under the curve represents the accuracy of a diagnostic test, where an AUC of 1 represents a perfect test (with a sensitivity of 100% and a specificity of 100%), and an AUC of 0.5 represents a worthless test (Zweig & Campbell, 1993). A p-value of less than 0.05 was considered statistically significant.

Sensitivity (ability of the test to detect positive cases) = true positive/(true positive + false negative).

Specificity (ability of the test to exclude negative cases) = true negative/(true negative + false positive).

Results

The study included 100 cases arranged into four groups according to the following inclusions criteria: Group I; Diseased control formed of 25 patients diagnosed as post-viral hepatitic chronic liver disease with no evidence of HCC. Group II; Formed of 25 patients diagnosed as HCC on top of post-viral hepatitic chronic liver disease with elevation in AFP. Group III; Formed of 25 patients diagnosed as HCC on top of post-viral hepatitic chronic liver disease without elevation in AFP, and Control Group; Healthy Control formed of 25 age- and sex-matched healthy volunteer controls, with no evidence of liver disease and/or of neoplasm.

Age and sex distribution as well as laboratory findings of the studied groups are illustrated in Table 1. Figure 1 illustrates the prominent symptoms in the studied patients. Pattern of hepatitis viruses is shown in figure 2, while the risk factors for their acquisition

are shown in figure 3. Findings of the abdominal ultrasound of the studied groups are illustrated in Table 2. Table 3 shows that there was highly statistical significant difference in the mean serum interleukin-6 levels between the four studied groups (P<0.01).

Table 1. Demographic Data of Patients and Controls

Variable		Group I (n=25)	Group II (n=25)	Group III (n=25)	Control Group. (n=25)	*P*-value
Age (mean ±SD)		49.9±10.2	55.9±6.5	53.9±10.3	34±9.58	NS
Sex	*Male*	21(84%)	15(60%)	19(76%)	14(56%)	NS
	Female	4(16%)	10(40%)	6(24%)	11(44%)	
Liver Function Tests	ALT (up to 40 IU/L)	51.9±46.36	75.7±59	85.6±73.8		< 0.01
	AST (up to 37 IU/L)	73.4±55.7	141±189.2	123±58.9		< 0.01
	Total Bilirubin (up to 1.2 mg/dl)	2.58±5.22	5±5.4	4.1±3.44		< 0.01
	Direct Bilirubin (up to 0.3 mg/dl)	1.5±3.1	3.88±4.5	3±2.8		NS
	Albumin (3-5 mg/dl)	2.72±0.78	2.5±0.59	2.66±0.56		< 0.01
Complete Blood Picture	WBCs (4-10x10^3cells/mm3)	6.46±3.7	9±4.2	8.17±4		< 0.05
	Haemoglobin (12-16g/dl)	11.6±2.31	10.26±2.61	11.34±1.99		< 0.01
	Platelets (148,000 cells/mm3)	127.24±54.55	124.3±53.2	116.4±51.3		< 0.01

P-value of less than 0.05 was considered statistically significant NS= not significant

ALT= alanine aminotransferase AST= aspartate aminotransferase WBCs=white blood cells

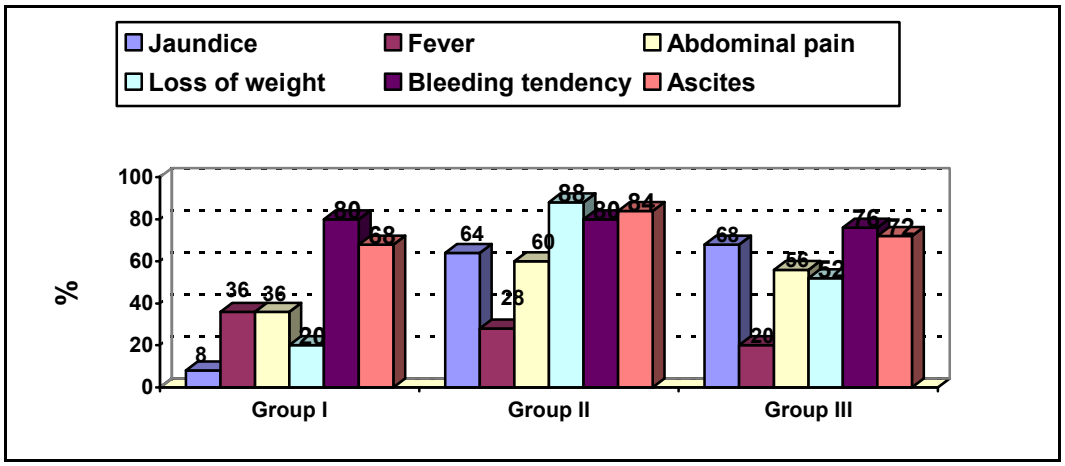

Figure 1. Prominent Symptoms of Patients in the Studied Groups.

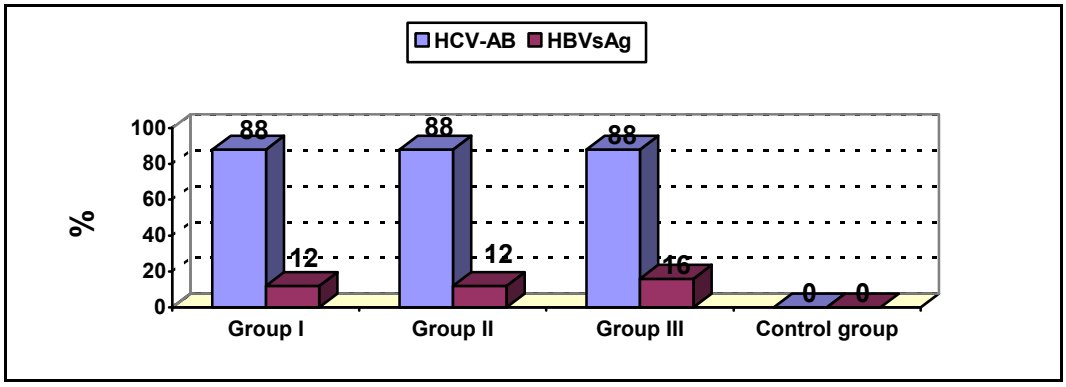

Figure 2. Hepatitis Markers of Patients in the Studied Groups.

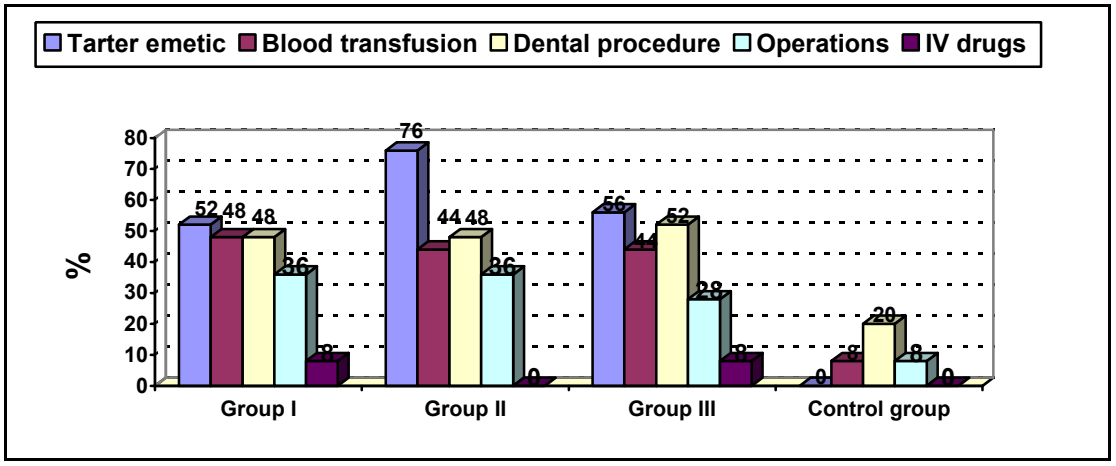

Figure 3. The Risk Factors of Hepatitis Patients in the Studied Groups.

Table 2. Abdominal Ultrasound Findings of the Three Studied Groups (I, II and III):

Variables	Group (n=25)		Group II (n=25)		Group III n=25)		*P*-value
	No	%	No	%	No	%	
Liver echogenicity							
Cirrhosis	21	84	25	100	25	100	<0.01
Collaterals	7	28	7	28	5	20	<0.05
Splenomegaly							
Mild	6	24	16	64	14	56	
Moderate	13	52	8	32	8	32	<0.01
Marked	3	12	0	0	0	0	
Ascites							
Mild	3	12	6	24	6	24	
Moderate	13	52	14	56	10	40	<0.01
Marked	2	8	3	12	6	24	

P-value of less than 0.05 was considered statistically significant.

Table 3. Comparison between the Three Studied Groups (I, II and III) as Regards the Mean Serum Levels of Interleukin-6 (pg/ml):

	IL-6				
	Mean (pg/ml)	SD	F-Value	*P*-value	LSD*
Group I (CLD) (No=25)	5.19	1.36			
Group II (HCC +High AFP) (No=25)	16.95	4.03	90.32	<0.01	Group I, II (+) Group II, III (++)
Group III (HCC +Low AFP) (No=25)	9.05	5.63			Group I, III
Control Group (No= 25)	1.01	1.03			

*LSD=least standard deviation. *P*-value of less than 0.05 was considered statistically significant.

Table 4 revealed a statistically significant positive correlation between mean IL-6 & AFP levels in HCC patients (no= 50) ($P<$ 0.05). Figure 4 shows the Child classes of the three studied groups, a statistical significant difference was obtained in comparing the groups together. Table 5 revealed that there was no statistical significant difference regarding the correlation between the Child-Pugh Staging System in group I (CLD), II (HCC with high AFP), III (HCC with normal AFP) and the mean levels of IL-6.

Table 4. Correlation coefficient between the mean serum levels of IL-6 and AFP in the three groups.

	r -value	*P*-value
Group I (CLD) (No=25)	0.1	NS
Group II (HCC +High AFP) (No=25)	0.32	NS
Group III (HCC +Low AFP) (No=25)	0.12	NS
Group II & III (HCC) (No=50)	0.36	<0.05

P-value of less than 0.05 was considered statistically significant. NS= not significant

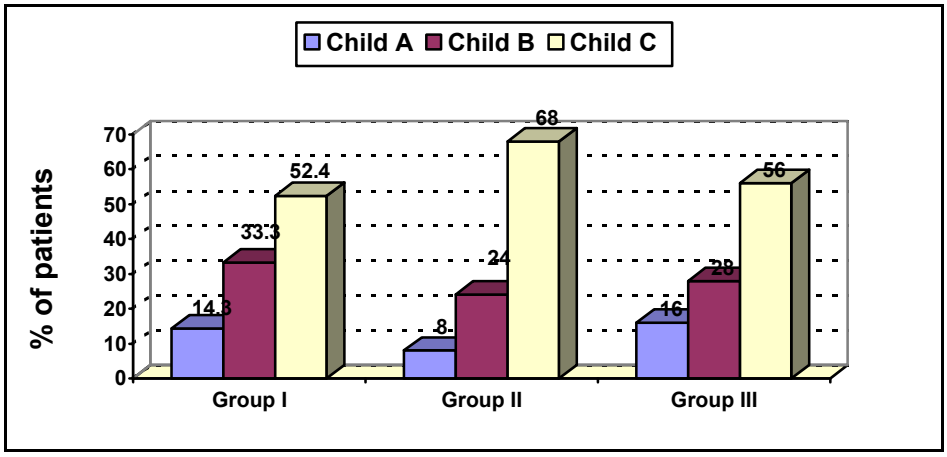

Figure 4. Child's Classification of patients in the studied groups.

Table 5. Mean serum IL-6 levels among patients in each Child-Pugh Staging System in group I (CLD), II (HCC with high AFP) and III (HCC with normal AFP):

Child-Pugh Classes	IL-6				
	Mean	SD	*F-value*	*P*-value	LSD
Class A (No.=9)	9.31	6.75	0.70	NS	Child A, B Child A, C Child B, C
Class B (No.=20)	9.7	5.8			
Class C (No.=42)	11.4	6.7			

P-value of less than 0.05 was considered statistically significant. NS= not significant

The mean IL-6 levels was higher in cases with multiple focal lesions (13.97±6.21) and those with portal vein thrombosis (PVT) (14.35±5.85) than those with single focal lesion (12.03±6.32) and without PVT (11.93±6.50), but without significant difference (*P*>0.05) as illustrated in table 6.

Table 6. Association between mean serum IL-6 levels and number of hepatic focal lesion (single & multiple) and Portal vein thrombosis in groups II & III:

IL-6		Group II & III (No=50)				*P-value*
		No.	%	Mean	SD	
Single		25	44	12.03	6.32	NS
Multiple		25	56	13.97	6.21	
Portal vein thrombosis	+ve	22	44	14.35	5.85	NS
	-ve	28	56	11.93	6.50	

P-value of less than 0.05 was considered statistically significant. NS= not significant

We used the significant variables in univariate analysis and included them into multivariate analysis by the logistic regression stepwise method to examine the factors affecting serum IL-6 levels in HCC. The presented one (Loss of weight and serum AFP levels) gave the highest likelihood ratio in Table 7.

Table 7. Stepwise Regression analysis for factors affecting serum IL-6 levels:

	R^2	B (SE)	F value	*P* value
Loss of weight	0.39	0.32	7.26	< 0.05
AFP	0.49	0.31	6.29	< 0.05

P-value of less than 0.05 was considered statistically significant.

Table 8 shows the results of ROC curve analysis, which present the sensitivity and specificity IL-6 at different cut-off values for the diagnosis of HCC [Figure 5]. The area under the curve is 0.83 (95% confidence interval: 0.73-0.92), which means that IL-6 is a good test to detect HCC. At the same time, the area under the curve for AFP is 0.88 (95% confidence interval: 0.80 – 0.96), which means it is a somewhat better diagnostic test for HCC.

Table 8. ROC curve of serum IL-6 levels to determine optimum cut-off points for diagnosis of HCC cases:

IL-6 (Cut off value)	Sensitivity	Specificity
6.05	76%	80%
10.70	61%	100%
15.45	55%	100%

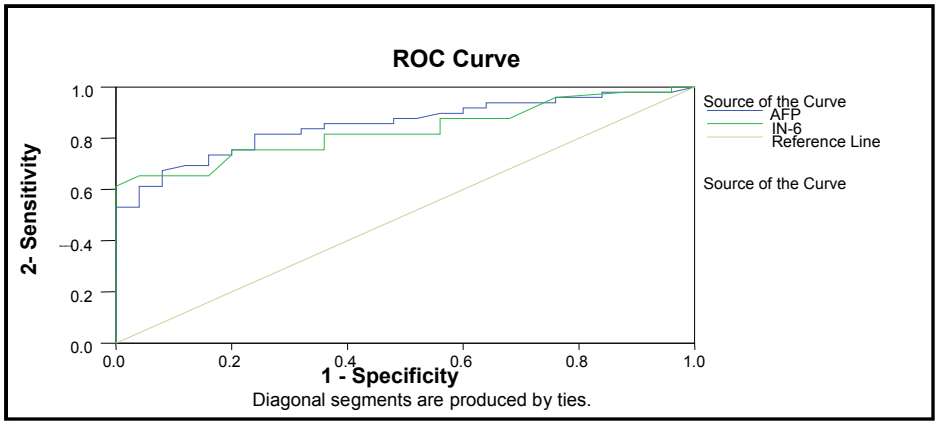

Figure 5. ROC curve for serum IL-6 and AFP levels in the three studied groups

To determine the optimum cut-off levels for diagnosis of HCC: Area under the curve for IL-6= 0.83 CI (0.73-0.92); Area under the curve for AFP = 0.88 CI(0.80-0.96)

Discussion

Hepatocellular carcinoma (HCC) is a major type of primary liver cancer and one of the most frequent human malignant neoplasms (El-Serag *et al.,* 2001; Momosaki *et al.,* 2005) and is estimated to cause more than a quarter of a million deaths each year throughout the world (Katyal *et al.,* 2000*;* Steel *et al.,* 2004). Early diagnosis of HCC is the only hope for cure, as most patients have inoperable disease at time of diagnosis (Kemp *et al.,* 2005). Thus, new serologic markers with sufficient sensitivity and specificity are required to detect HCC at early stages. So far, several candidates for serum proteins, such as lectin-reactive AFP or des-gamma-carboxy prothrombin, have been studied to find a better more convenient surveillance tool. However, most of those markers have been shown to be unsatisfactory in diagnosing small HCC because of low sensitivity, which ranged from 20-48% (Song *et al.,* 2002)

Interlukin-6 (IL-6) could be considered a promising tumor marker for HCC (Porta *et al.,* 2007). Concentrations of IL-6 in serum are increased in situations of chronic liver inflammation including alcoholic hepatitis, HBV, and HCV infections, and steatohepatitis, conditions that may lead to development of HCC (Abiru *et al.,* 2006).

The aim of the current study was to assess the value of serum interlukin-6 levels in

patients with chronic liver disease and its level in patients with HCC, as well as to evaluate its sensitivity and specificity in comparison to AFP in early diagnosis of HCC.

In the present study, there was higher mean age in the group II (55.9±6.5) compared to group I and III (49.9±10.2 and 53.9±10.3 respectively) but without statistically significant difference between the three groups.

This is in consistence with Hopf (2005) who reported that the incidence of HCC increases progressively with age, although this varies by country. Thus, in high-incidence countries, the mean age at time of diagnosis is in the third decade of life, and in low-incidence countries, it occurs 2 to 3 decades later. Also, Velazquez *et al.,* (2003), found that cirrhotic patients older than 54 years are at 4 times greater risk to develop HCC whereas patients of age group older than 60 years were at 11 times more risk to develop HCC. But, Montalto *et al.,* (2002) demonstrated that there is shift to younger age group over the last two decades mostly in the developing countries which may be attributed to emergence of both hepatitis B and or C infections at younger age. We think that this not available in Egypt due to defective surveillance system of HCV infection and HCC on top.

As regards gender distribution in the three groups, group I included 21 male patients (84%) and 4female patients (16%), group II included 15 male patients (60%) and 10 female patients (40%), while group III included 19 male patients (76%) and 6 female patients (24%). There is a higher percentage of males over females in the three studied groups but without significant difference (P >0.05). This is in agreement with the statistical data of National Cancer Institute (NCI) in Egypt that was presented in the 3rd Annual Meeting of Tanta Cancer Center, Alexandria in May (2004). It showed that HCC now ranks as the 2nd malignancy in males and 8th malignancy in females. It represents 10.5% of all male malignancies and 3.6% of all female cancers. The male to female ratio is 3:1. Also, this variability may be explained by the differences in exposure to risk factors as hepatitis B and C which are more prevalent in male patients (Yu et al., 2003).

In the current study, the underlying viral infection of liver disease did not differ significantly between the three studied groups. Hepatitis C virus infection was on the top of the list in all groups being 22 patients (88%) in group I, 22 patients (88%) in group II and 22 patients (88%) in group III. While, Hepatitis B virus infection was documented in a limited number of patients either in group I 3 patients (12%), 3 patients (12%) in group II and 4 patients (16%) in group III.

Our findings were in consistent with El-Zayadi *et al.* (2005), Hussein *et al.* (2008) and El-Folly *et al.,* (2007) who revealed the rising trend of HCC with increasing risk among HCV-infected men of older age groups; such patients should be carefully followed-up and screened for early detection of HCC.

The serum level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was elevated in HCC especially the advanced cases; the AST level was usually higher than that of ALT and the difference becomes greater as the disease progresses (Bruix, 2005). This is in accordance with our findings as serum ALT and AST levels were more elevated in group II (mean 75.7 ± SD 59 and mean 141 ± SD 189.2, respectively) and in group III (mean 85.6 ± SD 73.8 and mean 123 ± SD 58.9, respectively), than that in group I (mean 51.9 ± SD 46.36 and mean 73.4 ± SD 55.7, respectively), with highly significant difference between the three groups.

Our study revealed that serum IL-6 levels correlated with AST levels in group II, this is in accordance to Giannitrapani *et al.* (2002) who found a positive correlation between AST and IL-6 values in cases with HCC.

Alpha-fetoprotein (AFP) has been the serum marker that is most widely used for diagnosis as well as surveillance of HCC. However, AFP levels may be normal in up to 40% of patients with HCC, particularly during the early stages (low sensitivity). Furthermore, elevated AFP levels may be seen in patients with cirrhosis or exacerbations of chronic hepatitis (low specificity) (Kawakita *et al.,* 2003). The level of AFP ≥ 200 ng/mL was considered diagnostic for HCC according to Kreczko *et al.* (2000), Caturelli *et al.* (2002), Taketa *et al.* (2002) and Wu *et al.* (2004) who stated that raised serum levels of AFP ≥ 200 ng/mL in a cirrhotic patient at presentation predict development of HCC in those patients. This diagnostic level of AFP above 200 ng/ml, its sensitivity was 40.8%, while the specificity was 100% (Hussein *et al.,* 2008). Surveillance for HCC should be performed using both AFP (>200ng) and ultrasonography. Patients should be screened at 4 month intervals (Esmat *et al.,* 2009).

In our study, the serum alpha-fetoprotein levels showed a significant elevation in hepatocellular carcinoma patients group II (mean 13882 ± SD 26654.9 IU/ml) and group III (mean 40.2 ± SD 38.49 IU/L) than in group I (mean 18.09 ± SD 22.1 IU/L) and that agreed with Nagaoka *et al.* (2003) and Ibrahim, (2005) who found that the mean plasma concentration of alpha-fetoprotein was significantly higher in untreated patients with hepatocellular carcinoma as compared to patients with chronic liver disease.

In our series, 25 out of 75 patients had HCC by abdominal ultrasound and triphasic spiral CT in group III showed low serum alpha-fetoprotein levels. This in agreement with Esmat *et al., (2009)* who reported that a single imaging modality showing a lesion with the characteristic arterial hypervascularity and washes out in the early or delayed venous phase is required for diagnosis of HCC with low AFP.

Concerning the interlukin-6 (IL-6), a *high statistically significant difference* was elicited between the three studied groups. However, the serum IL-6 levels in group II (mean 16.95 ± 4pg/ml) was higher than group III and group I with (mean 9 ± 5.63 pg/ml and mean 5.19± 1.36 pg/ml respectively). This finding was in contrast to that found by Giannitrapani *et al.,* (2002) who reported that IL-6 could be more efficacious than AFP in detecting patients with HCC. Also, there was definitely a poor performance of AFP relative to IL-6 which had a high diagnostic accuracy in discriminating among healthy control (Porta *et al.,* 2007).

Moreover, the present study revealed that there was a statistically significant positive correlation between the elevation of mean serum IL-6 and AFP levels in group II. Our findings were in agreement with Porta *et al.,* (2007) who reported that the diagnostic value of IL-6 is significantly increased when it is associated with the level of AFP, combination between the two markers provides a new perspective in the diagnosis of HCC.

Regarding the correlation between the Child-Pugh Staging System and the mean serum IL-6 levels in group I, II and III. The mean IL-6 was higher in Child-Pugh class C (11.4 ± 6.7 pg/ml) than Class A and B (9.3 ± 6.75 pg/ml and 9.7 ± 5.8 pg/ml, respectively) but without statistical significant difference between them.

Concerning the correlation between mean serum IL-6 levels and number of hepatic focal lesion (single or multiple) in group II and III, the mean serum IL-6 levels was slightly higher in cases with multiple hepatic focal lesions but without statistical significant

difference between both. As well as no statistical significant difference between the mean serum IL-6 levels and the presence of portal vein thrombosis in HCC patients in group II and III. This is in contrast to Giannitrapani *et al.,* (2002) who demonstrated that in patients with HCC higher levels of IL-6 have been correlated with tumor mass and cancer invasiveness.

The Receiver Operator Characteristic (ROC) curve elicited the sensitivity and specificity of serum IL-6 levels in diagnosis of HCC at different cut-off value. At cut-off value of serum IL-6 levels ≥ 6.05, 10.70 and 15.45 pg/mL, the sensitivity was (76%, 61% and 55% respectively) and specificity was (80%, 100% and 100% respectively). While Porta *et al.,* (2008) reported that at cut-off value of IL-6 (12 pg/ml), the sensitivity was 73% and specificity was 87%.

It is concluded that the diagnostic value of IL-6 is significantly increased when it is associated with AFP measurement. Combining the two markers provides a new perspective in the diagnosis of HCC.

Further studies are recommended to evaluate the diagnostic value and prognostic role (i.e before and after) of Interleukin-6 in patients with HCC (with serum alpha-fetoprotein levels below 200ng/ml).

References

1. Abiru S, Migita K, Maeda Y, Daikoku M, Ito M, Ohata K, Nagaoka S, Matsumoto T, Takii Y, Kusumoto K, Nakamura M, Komori A, Yano K, Yatsuhashi H, Eguchi K, Ishibashi H. (2006). Serum cytokine and soluble levels in patients with non-alcoholic steatohepatitis. Liver Int. 26, 39.
2. Benvegnù L, Gios M, Boccato S, Alberti A. (2004). Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complication. Gut. 53:744-749.
3. Bruix J, Sherman M. (2005). Management of Hepatocellular carcinoma; AASLD Practice Guideline. Hepatology, 42(5); 1209-1236.
4. Bruix J. (2005). Hepatocellular carcinoma. Semin Liver Dis., 25(2):123.
5. Caturelli E, Bartolucci F, Biasini E, Vigliotti ML, Andriulli A, Siena DA, Attino V, Bisceglia M. (2002). Diagnosis of liver nodules observed in chronic liver disease patients during ultrasound screening for early detection of hepatocellular carcinoma. Am. J. Gastro-enterol.,; 97 (2): 397-405.
6. El-Folly FN, Hussein MM, Ibrahim AA, khattab NF, EL-Folly RF. (2007). Serum Transforming Growth Factor Beta 1 in Hepatitis C Virus Related Chronic Liver Disease and Hepatocellular Carcinoma Patients. Thesis submitted for partial fulfillment of Master Degree in Tropical Medicine. Faculty of Medicine. Ain Shams University.
7. El-Zayadi AR, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, Selim O, Saeid A. (2005). Hepatocellular carcinoma in Egypt. A single study over decade. World J Gastroenterol. 11(33): 513 -9.
8. Esmat G, Shaker MK, El-Folly RF, Omer A, El-Metanawy W, El-Dorry A, Tawfik M.M, Waked I, Salama M, Helmy A, El-Meteny M, Kamel RR, Hamada E, El-Zawahry H and AbdelGhaffar TY. (2009). Towards an Egyptian Guideline from screening to treatment of hepatocellular carcinoma (part I). The Afro-Arab Liver Journal, 8(2):77-81.
9. Fung J, Marsh W. (2002). The quandary over liver transplantation for hepatocellular carcinoma: The greater sin?
10. Giannitrapani L, Cervello M, Soresi M, Notarbartolo M, La Rosa M, Virruso L, D'Alessandro N, Montalto G. (2002). Circulating IL-6 and sIL-6R in Patients with Hepatocellular carcinoma. Ann. N.Y. Acad. Sci. 963:46-52.
11. Hassan MM, Zaghloul AS, El-Serag HB, Soliman O, Patt YZ, Chappell CL, Beasley RP, Hwang LY (2001). The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. J. Clin. Gastroenterol. 33(2):123-126.
12. Hopf U. (2005). The elder patient with advanced liver disease. Schweiz Rundsch Med. Prax., 94 (18): 743-750.
13. Hsia CY, Huo TI, Chiang SY. (2007). Evaluation of interleukin-6, interleukin-10 and human hepatocyte growth factor as tumor markers for hepatocellular carcinoma. Eur J Surg Oncol., 33(2):208-12.

14. Hussein MM, Ibrahim AA, Abdella HM, Montasser IF, Hassan MI. (2008). Evaluation of serum squamous cell carcinoma antigen as a novel biomarker for diagnosis of hepatocellular carcinoma in Egyptian patients. Indian J. Cancer, 45(4);

15. Kaplan DE, Reddy KR. (2003). Rising incidence of hepatocellular carcinoma: the role of hepatitis B and C; the impact on transplantation and outcomes. Clin Liver Dis., 7:683–714s

16. Katyal S, Oliver JH, Peterson MS, Ferris JV, Carr BS, Baron RL. (2000). Extrahepatic metastases of HCC. Radiology, 216: 698-703.

17. Kawakita T, Shiraki K, Yamanaka Y, Yamaguchi Y, Saitou Y, Enokimura N, Yamamoto N, Okano H, Sugimoto K, Murata K, Yamakado K, Takeda K, Nakano T. (2003). A new prognostic scoring system involving des-gamma-carboxy prothrombin as a useful marker for predicting prognosis in patients with hepatocellular carcinoma. Int. J. Onco. l23 (4): 1115-1120.

18. Kemp W, Pianko S, Nguyen S, Bailey MJ, Roberts SK. (2005). Survival in hepatocellular carcinoma: Impact of screening and etiology of liver disease. J. Gastroenterol. Hepatol. 20(6):873-881.

19. Kreczko S, Lipska A, Wysocka J. (2000). Alpha-fetoprotein: diagnostic value in hepatic disorders. Merkuriusz Lek., 8 (48): 420-423.

20. Momosaki S, Umemura T, Scudamore CH, Kojiro M, Alter HJ, Tabor E. (2005). SEN virus infection in patients with hepatocellular carcinoma. J. Viral Hepat. 12(4):435-438.

21. Montalto G, Cervello M,Giannitrapani L, Dantona F, Terranova A, Castagnetta LA. (2002). Epidemiology, risk factors and natural history of HCC. Ann N Y Acad Sci, 963: 13-20.

22. Nagaoka S, Yatsuhashi H, Hamada H, Yano K, Matsumoto T, Daikoku M, Arisawa K, Ishibashi H, Koga M, Sata M, Yano M. (2003). The des-gamma-carboxy prothrombin index is a new prognostic indicator for hepatocellular carcinoma. Cancer. 15; 98 (12): 2671-7.

23. National Cancer Institute. (2004). Abstract from Annual Meeting of Tanta Cancer Center, Alxandria, (2004).

24. Porta C, De amici M, Quaglini S, Paglino C, Tagliani F, Boncimino A, Moratti R, Corazza GR. (2008). Circulating Interlukin-6 as a tumor marker for hepatocellular carcinoma. Annals of Oncology, 19(2):353-358

25. Pugh R.N.H. Murray-Lyon I.M. Dawson J.L. Pietroni MC, Williams R. (1973). “Transection of the oesophagus for bleeding oesophageal varices”. Br.J.Surg. 60 (8): 646-649.

26. Song BC, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, Shin JW, Lee HC, Lee YS, Suh DJ. (2002). Transforming growth factor-beta 1 as a useful serologic marker of small hepatocellular carcinoma. Cancer, 94 (1): 175-180.

27. Steel J, Carney M, Carr BI, Baum A. (2004). The role of psychosocial factors in the progression of hepatocellular carcinoma. Med. Hypotheses. 62(1): 86-94.

28. Taketa K, Okada S, Win N, Hlaing NK, Wind KM. (2002). Evaluation of tumor markers for the detection of hepatocellular carcinoma in Yangon General Hospital, Myanmar. Acta. Med. Okayama, 56(6): 317-320.

29. Velazquez RE, Rodriguez M, Navascues CA. (2003). Analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology, 37:13-7.

30. Wu F, Wang ZB, Chen WZ, Zhu H, Bai J, Zou JZ, Li KQ, Jin CB, Xie FL, Su HB. (2004). Extracorporeal high intensity focused ultrasound ablation in the treatment of patients with large hepatocellular carcinoma. Annals of Surgical Oncology, 11 (12): 1061-1069.

31. Yu MW, Chang HC, Chang SC. (2003). Role of reproductive factors in HCC: Impact on hepatitis B- and C-related risk. Hepatology, 38: 1393-1400.

32. Zweig MH, Campbell G. (1993). Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 39:561-77.