Assessment of the Role of Interleukin-18 in diagnosis of Hepatocellular Carcinoma related to Hepatitis C Virus infection

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Abstract: Background: Hepatocellular carcinoma (HCC) accounts for 90% of primary liver neoplasms. Representing one of the most common cancers and is responsible for up to 1 million deaths annually worldwide. Egypt has the highest prevalence of Hepatitis C Virus (HCV) worldwide and has rising rates of Hepatocellular carcinoma (HCC). The prognosis of most patients is unsatisfactory due to rapid clinical deterioration after the initial diagnosis. Therefore, it is very important to detect HCC and the recurrence at its earlier period. Alpha Feto protein (AFP) has been the most widely used plasma marker for diagnosis, surveillance and as a prognostic indicator of HCC patients' survival. Several studies indicated that high plasma levels of AFP are related to poor prognosis, as well as histological grades of malignancy. However, it has been recognized that AFP has a low sensitivity in detection of HCC, and that AFP level often increases in the absence of HCC. Thus the identification of novel biochemical markers for HCC remains an important goal for many laboratories around the world. Interleukin 18 (IL-18) plays a critical role in the host defense against intracellular microbe's infection and also it induces autoimmune diseases and propagating inflammatory process also it was found that, IL-18 could play a key role in the pathogenesis of HCC. **Methods:** This study was conducted on a total number of 120 patients admitted to Hepatology and Gastroenterology Department in Faculty of medicines, Ain Shams University. The patients of this study were subdivided as follows. Group I: included 20 normal healthy subjects (as controls). Group II: included 100 patients with Hepatocellular carcinoma confirmed by pathology, cytology, imaging (computer tomography and ultrasound) and serum α -fetoprotein. **Results:** The mean level of IL-18 was significantly higher in HCC patients (238.69±145.5 pg/ml) compared to the controls (52.8±13.32 pg/ml), P <0.001). There was significant positive correlation between IL18 and Tumor size. **Conclusion:** IL-18 could be used as an additional non invasive marker for monitoring the degree of disease severity in Hepatocellular carcinoma.
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Key words: HCC, HCV, AFP, IL-18.

1. Introduction

Hepatocellular carcinoma (HCC, also called malignant hepatoma) is a primary malignancy of the hepatocyte, the major cell type in the liver **(Motola-kuba *et al.*, 2006)** generally leading to death within 6-20 months. The disease is often clinically silent until it is well advanced or tumor diameter exceeds to 10 cm. Hepatocellular carcinoma frequently arises in the setting of cirrhosis, appearing 20-30 years following the initial insult to the liver. However, 25% of patients have no history or risk factor for the development of cirrhosis. HCC with more than 250000 new cases annually and a 5year survival rate of less than 5% is the five leading causes of cancer death in the word (**Dong *et al.*, 2009**). Egypt has the highest prevalence of HCV worldwide and has rising rates of hepatocellular carcinoma (HCC). Egypt's unique nature of liver disease presents questions regarding the distribution of HBV and HCV in the etiology of HCC. **Lehman and Wilson (2009**) reported prevalence for HBV and HCV to be 6.7% and 13.9% among healthy populations, and 25.9% and 78.5% among HCC cases. Detection and characterization of all hepatic focal lesions are critical especially in patients with liver cirrhosis, as those patients are at high risk to develop hepatocellular carcinoma (**Zhou *et al.*, 2006).** Serum α-fetoprotein (AFP) is the only marker that has been widely used for screening and diagnosis of HCCs. However, development of false-negative or false-positive rates with (AFP) was as high as 30%-40% for patients with small hepatocellular carcinomas (**Wei *et al.*, 2006**). Liver biopsy is the traditional gold standard method to establish the diagnosis and to determine the extent of inflammatory changes and the extent of fibrosis and cirrhosis. However this procedure has many disadvantages, it is invasive, coasty and difficult to standardize (**Bonny *et al.*, 2003).** Patients with chronic HCV are often anxious regarding undergoing a liver biopsy. Biopsy results show significant variability up to 40% for fibrosis diagnosis which can lead to a

wrong diagnosis, indeed the result depends on the representatively of the punctured sample (**Andriulli *et al.*, 2001).** That is why there has been increasing interest in noninvasive assessment of liver fibrosis by the use of surrogate serum markers (**Saadeh *et al.*, 2001).** Besides these features, a number of biological markers including cytokines and growth factors have been demonstrated to be increased in the sera of patients with HCC and may be associated with a poor prognosis. Interleukin-18 (IL-18), originally known as interferon-γ (IFN-γ)-inducing factor (IGIF), is a cytokine that shares structural and functional properties with interleukin-1(IL-1). This cytokine is mainly produced by activated macrophages, but may also be expressed by Kupffer cells, T cells, B cells, keratinocytes, astrocytes, and osteoblast **(Tangkijvanich *et al.*, 2007).** IL-18 has multiple biological activities via its capacity to stimulate innate immunity and both Th1 and Th2 mediated response. It also exerts anti-tumor effects that are mediated by enhancement of NK cell activity, reduction of tumorigenesis, induction of apoptosis and inhibition of angiogenesis in tumor cells **(Tangkijvanich *et al.*, 2007). Chiac *et al.*, 2002** approved that IL-18 play a key role in the pathogenesis of HCC and its levels can be utilized as a possible marker in the diagnosis of HCCs and so, this study was aiming to evaluate IL-18 as non invasive marker of the severity of liver damage in HCC patients and comparing these results by the results of AFP.

2. Study Population

This study was conducted on a total number of 120 patients admitted to Hepatology and Gastroenterology Department in Faculty of Medicines, Ain Shams University. The patients of this study were subdivided as follows. Group I: included 20 normal healthy subjects (as controls), age and sex matched, Group II: included 100 patients with Hepatocellular carcinoma (HCC). All HCC patients were newly diagnosed and none had received any form of anti-cancer therapy before collection of blood samples for biochemical analysis. Diagnosis of HCC was confirmed by pathology, cytology, imaging (computer tomography and ultrasound), and serum a-fetoprotein (AFP).

Blood samples

Ten ml of venous blood were withdrawn from each patient in dry sterile vacutainers. After centrifugation, the serum was tested for: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), G-glutamyltranspeptidase (GGT), total bilirubin, direct bilirubin, albumin and glucose concentrations were assayed using Beckman CX4 chemistry analyzer (USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). AFP and viral markers were measured using Abbott, Axyam (USA, Supplied by al kamal company Cairo, Egypt). II-18, test kit was purchased from Medical & Biological Laboratories CO., LTD Nogoya, Japan. Level of II-18 (pg/ml) was calculated by interpolation from a reference curve generated in the same assay with reference standards of known concentrations. This assay was performed in duplicate according to the manufacturer's instructions.

Statistical analyses

Statistical analysis was performed using the statistical package for social sciences (SPSS, USA). Data are expressed as means ± standard error. The chi-square test was used for the comparisons of proportions. A $p < 0.05$ was considered significant.

3. Results

This study was conducted on 100 patients and 20 healthy volunteers as controls. The Group (1), which included 20 subjects with male to female ratio 9/11, and Group (2), which included 100 patients with Hepatocellular carcinoma with male to female ratio 60/40, was illustrated in table (1). The mean age of control groups was 33.1±8.16 years versus 58.8±9.77 years in HCC group. There was a significant increase in serum levels of Albumin, Total Bilirubin, Direct Bilirubin, ALT, AST, GGT, INR, Creatinine and Glucose were detected in HCC patients when compared to normal healthy controls, while Albumin was lower in HCC group when compared with group I ($P < 0.001$; Table 3). IL18 and AFP are significantly higher in patients with HCC (G2) than in healthy normal subjects (G1), ($P <0.001$).

Table (2) was revealed, a significant positive correlation between IL18 and Tumor size), while there is no significant correlation between AFP and tumor size. By comparing receiver-operating characteristic (ROC) curves for both IL18 and AFP, we found that the areas under the curves were 0.704 and 0.296, respectively and IL18 curve is closer to the upper left corner than that for AFP as shown in Figures (1&2) which means the higher the overall accuracy for IL18 over AFP. Also by comparing the validity and reliability of Il -18 compared to α-Fetoprotein regarding the exclude of control from our calculations, serum AFP performs moderately well as a biomarker of HCC but it showed lower sensitivity 71% compared to serum IL18 (100%).

Table (1): Comparison between all groups according to gender

		Groups		
		Group I	Group II	Total
sex	Female	11	37	48
		55.0%	37.0%	40%
	Male	9	63	72
		45.0%	63.0%	60%
Total count		20	100	120

Table (2): Correlation between IL- 18, AFP & Tumor size

		Tumor size
IL-18	Pearson Correlation	0.583**
	Sig.(2-tailed)	0.000
	N	100
AFP	Pearson Correlation	0.101
	Sig.(2-tailed)	0.318
	N	100

**Correlation is significant at the 0.01 level (2-tailed).

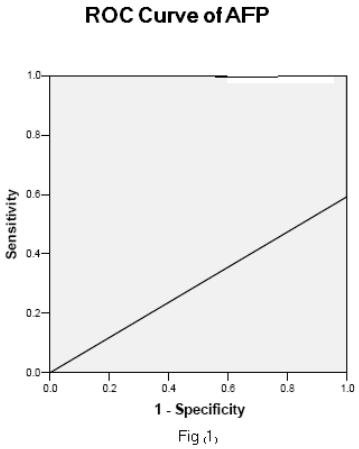

Fig (1)

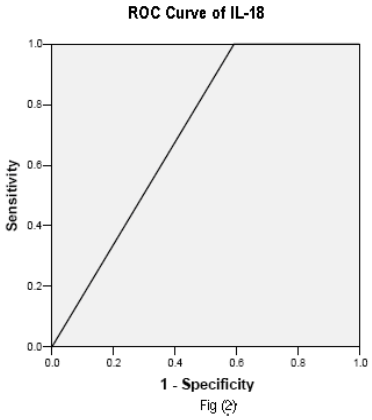

Fig (2)

Table (3): Clinical and biochemical characteristics among studied groups.

Variables	Normal control N=20	HCC N=100	*P*-value (Control vs HCC)
	Mean±SD	Mean±SD	
Age	33.1±8.16	58.8±9.77	0.000 (Significant)
Glucose(mg/dl)	101.8±13.9	211.0±150.2	0.002(Significant)
ALT (IU/L)	30±6	65.7±18.5	0.000 (significant)
AST (IU/L)	32±9	154.8±67.2	0.000 (significant)
T. Bil (mg/dl)	.74±.20	2.71±1.0	0.000 (significant)
D. Bil (mg/dl)	.5±.067	.77±.409	0.000 (significant)
Albumin (g/dl)	3.85±.21	2.74±.53	0.000 (significant)
INR (%)	1.0±.07	1.27±.17	0.000 (significant)
GGT (IU/L)	34.2±9.5	218.7±122.7	0.000 (significant)
TG (mg/dl)	156±30.6	195.1±30.9	0.000 (significant)
Cholesterol(mg/dl)	161.85±20.63	201.69±40.76	.000 (significant)
AFP(ng /ml)	5.86±2.02	316.65±291.60	0.000 (significant)
IL-18(ng/ml)	2.8±13.32	238.69±145.5	0000 (significant)

ALT, Alanine aminotransferase, AST, Aspartate aminotransferase, INR, International normalization ratio of prothrombin time, GGT, Gamma Glutamyltransferase, TG, Triglycerides, Glu, Glucose, Chol, Cholesterol, AFP, Alpha fetoprotein, IL- 18, Interleukin 18, P-value: (comparison between patients with HCC & control group).

4. Discussion

HCC is the fifth leading cause of cancer death in the world with a 5 year survival rate of less than 5% (**Dong *et al.*, 2009)**. Egypt has the highest prevalence of chronic hepatitis c virus infection world wide ranging from 6% to more than 40% among regions and demographic groups, and has rising rates of hepatocellular carcinoma development (**Leman and Wilson, 2009**). Many patients are diagnosed in the late stage and cannot tolerate hepatectomy because of advanced cancer or poor liver function reserve (**Huang *et al.*, 2010)** because this disease is often clinically silent until it is well advanced or tumor diameter exceeds 10 cm. Given the poor prognosis and lack of effective therapies for hepatocellular carcinoma, prevention programs are desperately needed. Surgical resection is the treatment of choice for patients with HCC when the tumor is small and limited to one lobe of the liver. There is increasing interest in non invasive markers to assess inflammatory activity and degree of fibrosis in chronic hepatitis C infection **(Finotto,2004).** Some tumor markers, such as glypican-3, gamma-glutamyltransferase II, alpha-l-fucosidase, transforming growth factor-beta1,tumor-specific growth factor, have been indicated to be available supplementaries to AFP in the detection (**Zhou *et al.*, 2006)**. Some other markers, such as vascular endothelial growth factor and interleukin-8 could also be used as available prognostic indicators and the simultaneous determination of AFP and these markers may detect the recurrence of HCC at its earlier period (**Zhou *et al.*, 2006**). IL-18, previously known as interferon-gamma-inducing factor, is a pleiotropic pro-inflammatory cytokine that is expressed mainly by peripheral blood mononuclear cells and macrophages. In the liver, besides its expression in Kupffer cells, IL-18 can also be synthesized by injured hepatocytes (**Fantuzzi and Dinarello, 1999)**. IL-18 plays a critical role in the host defense against intracellular microbe's infection and also it induces autoimmune diseases and propagating inflammatory process **(Gracie, et al., 2003).** IL-18 increases the susceptibility of liver endothelial cells to undergo apoptosis **(Mohran, 2011).** Elevated levels of interleukin-18 (IL-18) were described previously for chronically HCV-infected patients with different disease severities (chronic hepatitis C, liver cirrhosis and HCC) with an association between IL-18 plasma concentration with the outcome of chronic HCV infection (**Bouzgarrou *et al.*, 2008).** While other investigators describe increased levels of inflammation associated interleukins IL-15, IL-17, IL-18 and IL-18, and their binding proteins tumor tissue. Furthermore, RT-PCR and Western blot analysis revealed that IL-18 was up regulated in tumor tissues and contributed to tumor progression through their proangiogenic effect. In this study, regarding the results of serum alpha fetoprotein (AFP) levels, it was found that, the mean values of AFP levels were significantly increased in HCC group (GII) patients as compared to control group (GI) with the highest values towards HCC patients. This is due to AFP is a well-recognized tumor marker for HCC and elevated serum AFP concentration is found in approximately 60% of HCC patients in agreement with **Goldman *et al.*(2009**).

To validate the up regulation of IL-18 levels in HCC patients, these levels in serum were measured and compared to those of controls. In this study, it was found that serum IL-18 levels were significantly higher in patients with HCC than in controls (the mean level of IL-18 cases was 238.69± 145.5 pg/ml versus the mean IL-18 level in controls 52.8±13.32 pg/ml, p <0.001). These data are in accordance with earlier findings observed by **Mc Guineness *et al.* (2000)** who noted that IL-18 mRNA was up regulated in chronic and cirrhotic HCV patients. The same findings were observed by **Ludwiczek *et al.* (2002**) who found that disease progression from non cirrhotic to cirrhotic disease was accompanied by an increase in plasma IL-18 level, he also found that the deterioration of cirrhosis from Child-pugh stage A to B and C further increased IL-18 levels. Also, **Bouzgarrou *et al.* (2008)** found that patients with cirrhosis and HCC presented a higher increase in plasma IL-18 concentration than chronically infected patients. Our data revealed that, there was a significant positive correlation between IL18 and Tumor size), while there was no significant correlation between AFP and tumor size so, we recommended that simultaneous determination of IL-18 and AFP might significantly increase the Sensitivity and specificity in the diagnosis of HCC.

Conclusion:

It can be concluded that IL-18 levels are elevated in hepatocellular carcinoma patients than in healthy subjects. IL-18 level is significantly increased with the increase the tumor size and its concentration may predict the degree of hepatocellular damage. Thus IL-18 could be used and nominated as an additional non invasive marker for monitoring the degree of liver damage.

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