Evaluation of the Role of IL 18 in Diagnosis of HCC in critical Egyptian Patients with Chronic Hepatitis C

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Abstract: Background: Hepatocellular carcinoma is a major type of primary liver cancer and it is one of the most frequent human malignant neoplasms. It is estimated to cause more than a quarter of a million deaths each year throughout the world. So, early diagnosis of HCC is the only hope for cure, as most patients have inoperable disease at time of diagnosis. The objective of this study is to assess the role of IL-18 in diagnosis of HCC in patients with chronic Hepatitis C. **Method:** 80 patients with chronic viral hepatitis and HCC divided into 2 equal groups; group I diagnosed as chronic hepatitis C and Group II diagnosed as HCC on top of post-viral chronic hepatitis C. All cases are subjected to full labs, Abdominal ultrasound, triphasic C.T. of the abdomen and determination of IL 18 level and AFP level. **Result:** Obtained results revealed that serum IL-18 level was significantly higher in patients with HCC than those with chronic liver disease. It was not correlated to either AFP level nor to any laboratory parameter. Also, it is not correlated to vascular invasion, number or size of the focal lesion. The efficacy of serum IL-18 level at cut-off value 7.25pg/mL was 84.6% with sensitivity 97% and specificity 100%. **Conclusion:** These results indicate that IL-18 was a good test for HCC diagnosis in patients with chronic hepatitis C and may be of value in diagnosis of patients with hepatic focal lesion with AFP less than diagnostic values.

1. Introduction

Hepatitis C virus has been estimated by the World Health Organization (WHO) to infect 170 million patients worldwide, with the highest prevalence rate among Egyptians (14%-18%; approximately 10-fold greater than in the United States and Europe) (1). Because of the very high prevalence rate of HCV in the general Egyptian population, it accounts for most chronic liver disease and HCC cases in Egypt (2 and 3).

Hepatocellular carcinoma is a major type of primary liver cancer and one of the most frequent human malignant neoplasms (4) and is estimated to cause more than a quarter of a million deaths each year throughout the world (5 and 6). Early diagnosis of HCC is the only hope for cure, as most patients have inoperable disease at time of diagnosis (7).

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-carboxyprothrombin, 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% (8).

Interleukin-18, originally known as interferon-γ inducing factor, is a cytokine that shares structural and functional properties with 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 (9).

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 tumor genesis, induction of apoptosis and inhibition of angiogenesis in tumor cells (9).

Aim of the work

Assess the role of IL-18 in diagnosis of HCC in patients with chronic Hepatitis C

2. Patients and Methods

This study is a cross sectional study carried in Internal Medicine Department, Faculty of Medicine, Ain Shams University and included80 patients with chronic viral hepatitis and HCC divided into 2 equal groups: Group I: Diseased control formed of 40 patients (25 male, 15 female) diagnosed as chronic hepatitis C. Group II: Formed of 40 patients (33 male, 7 female) diagnosed as HCC on top of post-viral

chronic hepatitis C (Table 1).

Exclusion Criteria: Metastatic liver disease, Patients with associated severe systemic illness (cardiac, renal, hepatic and neoplastic) and Hepatocellular carcinoma with previous intervention (Radiofrequency ablation, alcohol injection, etc.).

Tools of the study:

All the studied cases are subjected to the following:

Full history taking, Clinical examination, Laboratory investigations included: Complete blood count (CBC). Liver profile (ALT, AST, serum albumin, serum bilirubin (total and direct), prothrombin time and international normalization ratio (INR) and Hepatitis markers (HCV Ab, HBs Ag and HBc IgG). Other markers when indicated like (ANA, ASMA, LKM, Transferrin saturation, ceruloplasmin level, etc.), Renal function tests (blood urea nitrogen and serum creatinine). Patients were classified according to Child-Turcotte Pugh scoring system. Triphasic CT scan of the abdomen for diagnosing HCC and tumor size and site and local spread.

Determination of Interlukin-18 serum level.

Calculation of result:

Average the duplicate reading for each standard. Control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit as an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw the best fit curve through the point on the graph. The data may be linearized by plotting the log of the IL-18 concentration versus the log of O.D and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation. This procedure will produce an adequate but less precise fit of the data. If sample have be diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Statistical Methodology

The data were processed and analyzed using the statistical package for social sciences (SPSS) program.

3. Results

The patients' mean ages were 57 in cirrhotic group and 58in HCC group. Regarding gender distribution in cirrhotic 62.5% were male and 37.5% were female while in HCC group 82.5% were male and 17.5% were female. 27.5% of cirrhotic group were smoker while 45% of HCC were smoker (Table 1).

This study showed significance increasing in total and direct bilirubin level in both groups, there was no significance found regarding ALT, AST and platelets (Table 2). It shows also that IL 18 level was significantly higher in HCC group than that of Cirrhotic group (Table 3).

There were no significance differences found between degree of cirrhosis classified by Child Pugh score and IL18 level (Table 4). There were no significant differences between number of liver foci in HCC group and level of IL 18 and alpha-FP (Table 5). There were also no significance in correlation between ALT, AST, total bilirubin, direct bilirubin, platelets, IL18 & alpha-FP in two groups (Table 6).

In this study, AUC was 0.5 for both IL-18 and Alpha-FP which means that they can be used as predictors of HCC however, IL18 is more reliable with AUC, sensitivity and specificity were 0.98, 97% and 100% respectively (Table 7). Assignment of cutoff point for IL18 & alpha-FP as discriminators between patients with HCC and those without using ROC curve are shown in the figure below.

Table 1	Cirrhotic Group	HCC Group
Mean Age	57 ± 12	58 ± 12
Male %	62.5 %	82.5 %
Female %	37.5 %	17.5 %
Smoking %	27.5 %	45 %

Table 2	Cirrhotic Group	HCC Group
Total Bilirubin	2.2 ± 1.6	3.4 ± 2.6
Direct Bilirubin	1.3 ± 1.0	2.3 ± 1.9

Table 3	Group		Test value	p-value
	Cirrhosis group	HCC group		
	Mean ± SD	Mean ± SD		
IL 18	0.87 ± 2.48	12 ± 7.99	.117	<0.001**

Table 4	Child Pugh classification			Kruskal – wallis	p-value
	A	B	C		
	Mean ± SD	Mean ± SD	Mean ± SD		
IL 18	17.50 ± .42	10.70 ± 6.63	12.17 ± 8.77	5.742	> 0.05

Table 5	HCC groups		Test value	p-value	
	Single focus (n=17)	Multiple foci (n=9)			
	Mean ± SD	Mean ± SD			
IL 18	10.74 ± 3.7	13.86 ± 15.7	1.952	>0.05	
alpha-FP	473 ± 426	332 ± 372	1.209	>0.05	

Table 6	IL 18		alpha-FP	
	Correlation Coefficient (r)	p-value	Correlation Coefficient (r)	p-value
alpha-FP	.151	>0.05		>0.05
IL 18		>0.05	.151	>0.05
age	-.121	>0.05	-.144	>0.05
Spiral CT (Foci size cm)	-.091	>0.05	.200	>0.05
ALT	-.015	>0.05	-.155	>0.05
AST	.153	>0.05	-.091	>0.05
T. Bilir	.104	>0.05	.148	>0.05
D. Bilir	.149	>0.05	.114	>0.05
platelets	.049	>0.05	-.019	>0.05

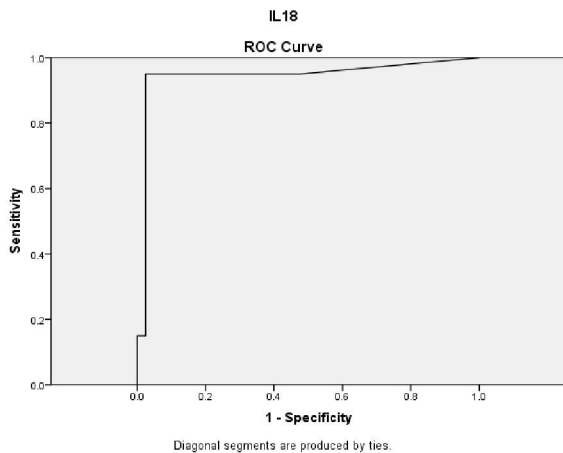| Table 7				
Variable(s)	AUC	HCC if ≥	Sensitivity	Specificity
alpha-FP	0.63	400	37%	100%
IL 18	0.98	7.25	97%	100%

Figure 1. assignment of cutoff point for IL18 & alpha-FP as discriminators between patients with HCC and those without using ROC curve.

The Figure 1 shows assignment of cutoff point for IL18 & alpha-FP as discriminators between patients with HCC and those without using ROC curve.

4. Discussion

Inappropriate production of IL-18 contributes to the pathogenesis of cancers and may influence the clinical outcome of patients. Specifically, it has been demonstrated that serum IL-18 level may have prognostic significance in some types of cancer including colonic carcinoma, gastric carcinoma, esophageal carcinoma, breast cancer, and hematologic malignancies (9).

Elevated levels of interleukin-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 concentrations with the outcome of chronic HCV infection (10).

The present work was designed to study the usefulness of IL-18 as a serological marker for diagnosis of HCC. two groups of patients were studied; Group I consisted of 40 patients with HCV related chronic liver disease, Group II formed of 40patients with HCC on top of viral hepatitis and with characteristic features of HCC by spiral abdominal CT.

In the present study, the percentage of males (82.5%) was higher than that of females (17.5%) in HCC patients. The rates of HCC were consistently higher for males than females, but the male to female ratio differs with the country as well as with the year of survey, because of the changing time trends. Male to female ratio was greater in the high-incidence regions (i.e., Africa, China, Taiwan and Japan) (11 and 12).

In our study, the age of HCC patients ranged (58 ± 12) years. 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 was in the third decade of life, and in low-incidence countries, it occurs 2 to 3 decades later (13). This finding was close to several previous studies reported that the mean age among HCC cases was 53.7±10.1 years, 57.4±8.7 years and 55.2±8 years, respectively (14, 15 and 16).

In our study, we found that IL-18 was not correlated to any biochemical or hematological parameter in all the studied groups. These findings were in accordance with study conducted by Asakawa (17).

In our study concerning IL-18, a high significant difference was elicited between the two groups. The serum level of IL-18 in CLD group I (8.7 ± 2.48) was lower than HCC group II (12 ± 7.99). This finding was in agreement with that found by Bouzgarrou who reported higher levels of plasma IL-18 in the patients with HCC than those with chronic hepatitis and cirrhosis (10).

In our study, we found that IL-18 was not correlated to size and number of hepatic focal lesion. Mohran agree with us as he reported that IL-18 level was not correlated with the size or number of hepatic focal lesions neither with the presence of lymphovascular invasion or abdominal lymphadenopathy (18).

Regarding correlation between Child-Pugh staging system and the mean IL-18 in HCC patients, no significance between the two groups. This finding was in agreement with previous study done by Tangkijvanich (9).

Alpha-fetoprotein 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) (19).

Regarding correlation between the IL-18 and AFP the specificity and sensitivity of AFP were 37% and 100% respectively. Serum IL-18 level is a suitable marker for the diagnosis of HCV-related HCC complementary to AFP, especially in cases with AFP level less than the diagnostic value (2).

Constructing the ROC curve, the best cut-off value of IL-18 was 7.25 pg/mL. At that value the sensitivity of serum IL-18 in diagnosis of HCC was 97%, specificity was 100% and our results was in accordance with previous studies that revealed that IL-18 may be a potential tumor marker in combination with AFP in the diagnosis of HCC (9, 17).

In our study, the high sensitivity and specificity of IL18 in HCC group in agree with Tangkijvanich (9) but differ in cut of point as Tangkijvanich (9) revealed that the best cut-off value of IL-18 for the diagnosis of HCC is 500pg/ml with 84% sensitivity and 86.7% specificity. The difference in cut of point to this study may be attributed to; selection of patient according to etiology of liver disease , AFP level and interventions done prior to the study to patient as Tangkijvanich (9) has Twenty-seven patients (38.6%) had serum AFP higher than 400 ng/mL, 9 patients (12.8%) were associated with alcohol-dependent. Thirty-nine patients (55.7%) were associated with HBsAg-positive, and 7 patients (10%) were associated with anti-HCV-positive, Two patients (2.9%) had both HBsAg-positive and anti-HCV-positive, Seven patients (10%) had undergone surgical resection, 19 patients (27.1%) had been treated with trans-arterial chemoembolization (TACE), and the remaining 44 patients (62.9%) had received no specific treatment because of their advanced tumor stage or refusal to therapy Tangkijvanich (9), in our study selection of patient all of them had HCV infection, no selection to level of AFP, no intervention to patient.

Conclusion

Our study aimed to evaluate the role of IL-18 serum level as a tumor marker in diagnosis of HCC; we found that serum IL-18 level was higher in patients with HCC than those with chronic liver disease. There was non-significant correlation between alpha-feto protein and IL-18 in the studied groups.

The efficacy of serum IL-18 level at cut-off value 7.25pg/mL was 84.6% with sensitivity 97% and specificity 100%. These results indicate that IL-18 was a good test for HCC diagnosis in patients with chronic hepatitis C & may be of value in diagnosis of

patients with hepatic focal lesion with AFP less than diagnostic values.

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