Orginal Article

Study of interleukin 8 (IL8) serum level in patients with chronic liver disease due to hepatitis C virus (HCV) with and without hepatocellular carcinoma (HCC)

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Abstract

Background: HCC is a common complication of chronic hepatitis caused mainly by HCV. Interleukin-8 (IL8) is a cytokine involved in the cellular response to inflammation, being a powerful chemoattractant for neutrophils. Our objective was to estimate serum level of interleukin 8 in patients with chronic liver disease due to hepatitis C with and without hepatocellular carcinoma. **Methods:** This study included forty patients with chronic liver disease due to hepatitis C (twenty with and twenty without hepatocellular carcinoma) and ten control individuals. They were subjected to history taking, physical examination. liver function test and abdominal ultrasound. Serum level of interleukin 8 will be assayed by ELISA test for all patients. Serum alpha fetoprotien and CT abdomen were done according to each case. **Results:** From our study we found that serum IL-8 levels were higher in patients with HCV compared with control subjects, and in patients with HCC associated with HCV infection compared with control subjects. We found also that serum level of IL-8 is significantly higher in patients of chronic hepatitis C with HCC than patients of chronic hepatitis C without HCC. This study revealed also a positive significant correlation between size of tumor and IL-8 in HCC patients, while serum AFP level was not correlated to size of tumor. Serum level of IL-8 was found to be significantly correlated to platelet count and AST but not correlated to ALT in patients with HCC associated with HCV. **Conclusions:** From this study we conclude that serum IL-8 levels were higher in patients with HCV and patients with HCC associated with HCV infection compared with control subjects. Patients with HCC had higher serum IL-8 than patients with HCV without HCC. Serum interleukin 8 can be considered as a marker of HCC in patients with HCV related chronic liver disease.

Key Words

Chronic Hepatitis C virus, Hepatocellular carcinoma, Interleuken 8

Introduction

Hepatitis C virus is one of the major etiological agents of chronic viral hepatitis. It often progresses to chronic hepatitis, cirrhosis and hepatocellular carcinoma [1]. HCC is the malignant transformation of hepatocytes and is a common complication of chronic hepatitis caused mainly by HCV [2]. The factors involved in the progression of liver disease in HCV-infected patients and occurrence of HCC are not well characterized. Cellular immunity to HCV appears to participate in the pathogenesis of the disease. A failure of efficient immune response to HCV, either because of selective defects in the host immune system or because of viral interference of the functions of immune cells, could account for the inability to eradicate the viral infection [3]. It is thought that cytotoxic T lymphocyte responses early in infection may be important for viral clearance. Several cytokines and chemokines induced by viral infection play direct or indirect roles in antiviral defense [4]. Most of acute and chronic liver diseases are characterized by inflammatory processes with enhanced expression of various pro- and anti-inflammatory cytokines in the liver [5]. It was reported that the serum concentrations of (TNF-alpha, IL-6, IL-8 and IL-10) are correlated to the histopathological spoilages of the liver [6]. Interleukin-8 (IL8) is a pro-inflammatory cytokine involved in the cellular response to inflammation, being a powerful chemoattractant for neutrophils. IL-8 is produced by a wide variety of cell types, including monocytes, neutrophils, fibroblasts, and endothelial cells. It serves as a chemical signal that attracts neutrophils at the site of inflammation, and therefore is also known as neutrophil chemotactic factor [7]. Accumulating data indicate that

distinct chemokines and chemokine receptors may be associated with different stages of the chronic hepatitis C virus infection-associated liver disease. Furthermore, the clinical implications of these findings which mark chemokines as prognostic markers and therapeutic targets for immune-modulation during chronic liver viral infection are still under study [8]. Hepatocellular carcinoma is one of the known complications of chronic hepatitis C and the correlations of serum IL-8 levels with tumor size and tumor stage suggest that IL-8 may be directly or indirectly involved in the progression of HCC. These findings suggest that serum IL-8 may be a useful biological marker of tumor invasiveness and an independent prognostic factor for patients with HCC [9]. The aim of this study is to estimate serum level of interleukin 8 in Egyptian patients with chronic hepatitis C with and without hepatocellular carcinoma as this may have diagnostic and therapeutic significance and it may also through a spot light on the pathogenesis of hepatocellular carcinoma in such patients.

Material and Methods

This study has been carried out on 40 patients with their age ranged from 30-70 years. Those patients were admitted or followed up in Cairo University Hospitals. The patients were compared with 10 healthy subjects. All 50 subjects consented to the study and were divided into 3 groups: (Table-1)

Group I

Included 10 healthy subjects as the control group.

Group II

Included 20 patients with chronic liver disease due to hepatitis C virus infection. They were classified according to Child - Pugh classification into:
Child A: 6 patients
Child B: 9 patients
Child C: 5 patients.

Group III (Table-3)

Included 20 patients with HCC associated with HCV.
They were classified according to Child - Pugh classification into:

Child A: 2 patients,
Child B: 14 patients
Child C: 4 patients.

Exclusion Criteria for the Selected Patients

1. Patients associated with infection other than HCV.
2. Patients with HCV under antiviral therapy.
3. Patients with tumors other than HCC.

All patients and controls were subjected to:

- Complete medical history and clinical examination.
- Liver enzymes: ALT, AST.
- Liver function tests as: total bilirubin, direct bilirubin and S. albumin.
- Complete blood picture.
- Bleeding profile (pt, ptt, INR).
- Serum creatinine.
- Blood glucose.
- Abdominal ultrasound.
- CT scan abdomen and alpha feto protein in patients with hepatic focal lesion.
- Serum level of interleukin 8 was assayed by enzyme linked immunosorbent assay (ELISA).The ELISA test is done in the biochemistry department of Cairo University.

Sample Collection

Following an overnight fast, venous blood samples were collected from both patients and control subjects. The blood sample was left to clot at room temperature then centrifuged at 3000 rpm for 20 minutes. The separated serum was kept frozen at -80º C until further analysis of IL-8 by the ELISA kit provided by (EMELCA Bioscience, Minervum, The Netherlands). The IL-8 assay was performed following the manufacturer's instructions using an antibody that has 100% specificity for human IL-8 and no cross-reactivity with any other cytokines. The analytical sensitivity is <1 pg/ml, and normal range in human sera between 7.8-500 pg/ml.

Data Interpretation

Data were statistically described in terms of range and mean ± standard deviation (± SD). Comparison of quantitative variables between the study groups was done using Mann Whitney *U* test for independent samples. Correlation between various variables was done using Spearman rank correlation equation for non normal variables. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for

the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Comparison of patients (Group II – III) and controls (Group I) as regard clinical data is shown in table 1 and comparison between patients with chronic liver disease due to hepatitis C virus infection (Group II) with patients with HCC associated with HCV (Group III) in table 2. Comparison between patients (Group II – III) and controls (Group I) as regard laboratory parameters is shown in table 3 and comparison between patients with chronic liver disease due to hepatitis C virus infection (Group II) with control (Group I) as regarding some laboratory parameters show a significant correlations as regards hemoglobin level, platelet, serum albumen, ALT, AST, bilirubin, random blood glucose level and IL8 levels (table 4) and comparison between group I and group III regarding some laboratory parameters show a significant correlations as regards hemoglobin level, platelet, serum albumen, ALT, AST, bilirubin, random blood glucose level and IL8 levels (table 5). Comparison between group II and group III regarding some Laboratory parameters show no significant correlations as regards hemoglobin level, platelet, serum albumen, ALT, AST, bilirubin and random blood glucose level but there is a significant correlations as regard IL8 levels (table 6). So serum interleukin-8 was found to be significantly higher in patients of HCV without HCC (group II) than control group (group I) ($p<.001$) (table 4).And also we found that serum interleukin-8 was significantly higher in patients of HCV with HCC (group III) than control group (group I) ($p<0.001$) (table 5). When taking all patients as one group (group II,III), Serum interleukin-8 was found to be significantly higher in patients than control group (group I) ($p<0.001$). Serum interleukin-8 was found to be significantly higher in patients of HCV with HCC (group III) than patients of HCV without HCC (group II) ($p=0.013$) (table 6). When we compare between serum interleukin-8 levels in different child groups we found that serum interleukin-8 was found to be significantly higher in patients of child B than patients of child A ($p=0.042$) and there is no significant difference between patients of other child groups as regard IL-8 (pg/ml) (table 7). On studying the correlation between laboratory parameters in group II with IL8 levels we found no linear correlation between IL-8 and platelets count, AST or ALT (table 8). Studying the correlation between laboratory parameters in group III in comparison to AFP and IL8 levels we conclude that AFP was not found to be linearly correlated to platelets count, AST, ALT and tumor size. While IL-8 was found to be linearly correlated to platelets count, AST and tumor size, and no linear correlation was found between IL-8, ALT and AFP. (table 9)

Discussion

Patients with chronic liver disease demonstrate diminished humoral and cellular immune functions. Initiation of the antigen specific immune response requires proper interaction between effectors cells (eg. T cell) and an antigen- presenting cell (APC) [10]. Interleukin-8 (IL8) is a pro-inflammatory cytokine involved in the cellular response to inflammation, being a powerful chemo-attractant for neutrophils. IL-8 is produced by a wide variety of cell types, including monocytes, neutrophils, fibroblasts, and endothelial cells [11]. There are 2 similar cell surface receptors for IL8: type 1 (IL-8RA) is a high affinity receptor for IL8 alone; while type 2 (IL-8RB) is a high affinity receptor for IL8, growth related gene (GRO) and neutrophil-activating protein-2 (NAP-2). The affinity of type 1 receptors for IL8 is higher than that of type 2 receptors [12]. Primary function of IL-8 is the induction of chemotaxis in its target cells (e.g. neutrophil granulocytes) and therefore is also known as neutrophil chemotactic factor [13]. In the current study, we aimed to assess serum IL-8 in Egyptian patients with chronic liver disease due to hepatitis C with and without HCC and healthy subjects as control. We found that serum IL-8 was significantly higher in patients of chronic hepatitis C with and without HCC than controls .The higher serum IL-8 in patients with chronic hepatitis C related liver diseases goes with what has been reported previously that serum IL-8 were significantly higher in HCV patients than in control subjects [14], it also goes with Polyak et al who showed that high serum IL-8 in chronic HCV patients and that was associated with the lack of a biochemical response to IFN therapy [15]. Polyak et al reported also that the core and NS5A proteins of HCV induce the expression of the IL-8 gene, and that serum IL-8 levels in chronic hepatitis C patients are associated with resistance to interferon treatment, suggesting that IL-8 plays an important role in the maintenance of persistent infection with HCV [15]. Serum IL-8 levels increased as the disease progressed from chronic hepatitis to liver cirrhosis and further to HCC, suggesting that the increase may be due not only to immune response against persistent HCV infection but also to the development of HCC [15]. Intrahepatic IL-8 mRNA levels were positively correlated with severity of hepatic inflammation and injury in patients with chronic hepatitis C [16]. In HCV patients with liver inflammation and fibrosis, elevated serum levels of IL-8 have been reported [17]. Moreover, it has been reported that

there is a correlation between IL-8 levels and the severity of liver disorders, including HCV infection [18]. The marked elevation of serum IL-8 level in patients of HCC observed in our study goes with what has been reported by Yin et al, who showed that serum IL-8 were significantly higher in HCC patients than in control subjects [19]. Also, we found that the serum IL-8 is significantly higher in patients of chronic hepatitis C with HCC than the patients of chronic hepatitis C without HCC**.** The expression of IL-8 has been found in various human cancers [20]. Akiba *et al,* found that IL-8 was over expressed in HCC tumors cells [21]. IL-8 was identified to be an angiogenesis regulating molecule that induced angiogenesis by excessive production in tumor cells and release in circulation [21]. Tachibana et al, reported that IL-8 production increases progressively with escalating severity of hepatitis and the development of HCC and the level of IL-8 was significantly increased in patients with advanced HCC with distant metastasis, and then this leads to poor prognosis and IL-8 level was found to be a significant prognostic factor in terms of disease-free survival and overall survival [22]. In our study, we found that the serum IL-8 is significantly higher in patients of child B classification than patients of child A, and this is in disagreement with Homann et al., and we also found that there is no statistically significant difference between other child groups as regard serum IL-8 and this in accordance with Homann et al, who showed that high serum IL-8 didn't significantly correlated with different child classifications in alcohol induced cirrhosis [23]. Current study disclosed positive linear correlation between size of tumor in HCC patients and serum IL-8 and this is in accordance with Yi Ren et al, who showed that high serum IL-8 was significantly correlated with a more aggressive tumor behavior in patients with HCC [24], but this is in disagreement with Kubo et al., who showed no correlation between size of tumor in HCC patients and serum IL-8 and this finding in our study supports the role of IL-8 in progression of HCC this study also shows the usefulness of IL-8 as a more reliable indicator of the size of tumor as AFP which was not correlated to size of tumor [25]. We identified also positive correlation between platelet count in HCC patients and serum IL-8 and this is in accordance with Yi Ren et al., who showed that high serum IL-8 was significantly correlated with platelet count in HCC Platelets may play a role in the transporting circulating IL-8 in cancer patients [24]. IL-8 and its receptors have been stored in nucleus of platelets. There is linear correlation between AST and IL-8 in HCC patients. This may be due to the fact that the majority of our patients are cirrhotics. There is no linear correlation between ALT and IL-8 in HCC patients. The ratio of the ALT and AST may also provide useful information regarding the extent and cause of liver disease. Most liver diseases are characterized by greater ALT elevations than AST elevations. Two exceptions to this rule exist. Both cirrhosis and/or alcohol abuse are associated with higher AST levels than ALT levels, often in a ratio of approximately 2:1 [26]. We didn't identify positive correlation between AST, ALT, PLT count and serum IL-8 in HCV patients but this is in disagreement with Chung-Pin et al., who showed positive correlation between plasma IL-8 and AST, ALT levels in post hepatitis cirrhosis. This may be due to the heterogenicity of cases of HCV without HCC some of cases are with CAH and others are with cirrhosis [27].

Conclusion

From our study we conclude that serum IL-8 levels were higher in patients with HCV and patients with HCC associated with HCV infection compared with control subjects. Patients with HCC had higher serum IL-8 than patients with HCV without HCC. There is positive significant correlation between size of tumor and IL-8 in HCC patients suggesting that IL-8 may be directly and indirectly involved in progression of HCC. IL-8 may be a more reliable indicator of tumor size than AFP. There is positive significant correlation between AST and IL-8 in HCC patients. There is positive significant correlation between PLT and and IL-8 in HCC patients. These finding indicate that serum IL-8 may be a useful biological marker of tumor invasiveness and may be a prognostic marker in patients of HCV. Additional studies on a large number of patients may be required to observe the clinical changes after resection and a radiofrequency ablation therapy as well studies to clarify the relation ship between circulating IL-8 and platelet count in HCC and its biological significance are also required. Targeting IL-8 can be a potential approach to control tumor size invasion

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Table 1. Clinical data of studied groups

Group I1I No=20	Group I1 No=20	Group 1 No=10	
57.05±7.81	50.85±9.19	49.9±7.34	**Age (years) mean±SD**
11/9	10/10	6/4	**Sex (M/F)**
6 (30%)	5 (25%)	0 (0%)	**No of patients with hepatomegaly (%)**
15 (75%)	17(85%)	0 (0%)	**No of patients with splenomegaly (%)**
17 (85%)	12 (60%)	0 (0%)	**No of patients with jaundice (%)**
13 (65%)	10(50%)	0 (0%)	**No of patients with ascities (%)**
2 (10%) 14 (70%) 4 (20%)	6 (30%) 9 (45%) 5 (25%)	0 (0%) 0 (0%) 0 (0%)	**Child classification** **No of child A (%)** **No of child B (%)** **No of child C (%)**

Table 2. Comparison between group II and group III regarding some clinical parameters.

S	P	Group I1I No=20	Group I1 No=20	
Significant	<0.05	57.05±7.81	50.85±9.19	**Age(years) mean±SD**
Not significant	>0.05	11 9	10 10	**Sex** **M** **F**
Significant	>0.001	6 (30%)	5 (25%)	**No of patients with hepatomegaly %**
Significant	0.045	15 (75%)	17(85%)	**No of patients with splenomegaly %**
Not significant	0.258	17 (85%)	12 (60%)	**No of patients with jaundice %**
Significant	<.005	13 (65%)	10(50%)	**No of patients with ascities %**
Significant Not Significant Significant	<0.001 0.343 0.001	2 (10%) 14 (70%) 4 (20%)	6 (30%) 9 (45%) 5 (25%)	**Child classification** **No of child A %** **No of child B %** **No of child C %**

Table 3. Laboratory parameters in the three studied groups (expressed as mean±SD)

Laberatory parameters	group I no=10 Mean±SD	group II no=20 Mean±SD	group III no=20 Mean±SD
Hb g/dl	14±1.1	12.2±13.7060	9.81±2.5941
Plat. (10^3/m)	291±72	108.9±96.014	120.77±105.763
Alb g/dl	4.3±0.4	2.825±0.5149	2.75±0.5898
PT sec	13±0.8	15.26±2.603	16.38±2.133
ALT U/L	14±2.6	66.7±112.380	65.15±50.278
AST U/L	14±3.6	81.95±77.034	122.05±102.674
T.Bil Mg/dl	0.7±0.2	4.21±5.4027	4.7±4.6338
D.Bil Mg/dl	0.3±0.1	2.585±3.6826	2.667±2.0322
S.Cr.Mg/dl	0.79±0.12	0.98±0.4980	1.578±2.0097
RBS Mg/dl	89.6±5.59	130.1±56.356	147.75±60.089
AFPIu/ml	-	-	2290.9±6,882.312
IL-8 (Pg/ml)	37.07±14.3633	184.555±51.8586	273.485±119.1620
HCC Size cm	-	-	5.085±2.2056

Table 4. Comparison between group I and group II regarding some laboratory parameters

P	S	group II no=20 Mean±SD	group I no=10 Mean±SD	Laberatory parameters
Significant	>0.001	12.2±13.7060	14±1.1	Hb(g/dl)
Significant	>0.001	108.9±96.014	291±72	plt (10^3/m)
Significant	>0.001	2.825±0.5149	4.3±0.4	Alb(g/dl)
Significant	.003	15.26±2.603	13±0.8	PT (sec)
Significant	>0.001	66.7±112.380	14±2.6	ALT(U/L)
Significant	>0.001	81.95±77.034	14±3.6	AST(U/L)
Significant	.003	4.21±5.4027	0.7±0.2	T.Bil(Mg/dl)
Significant	.004	2.585±3.6826	0.3±0.1	D.Bil (Mg/dl)
Non significant	0.182	0.98±0.4980	0.79±0.12	S.Cr.(Mg/dl)
Significant	.015	130.1±56.356	89.6±5.59	RBS (Mg/dl)
Significant	>0.001	184.555±51.8586	37.07±14.3633	IL-8 (Pg/ml)

Table 5. Comparison between group I and group III regarding some laboratory parameters

P	S	group III no=20 Mean±SD	group I no=10 Mean±SD	Laberatory parameters
Significant	>0.001	9.81±2.5941	14±1.1	Hb(g/dl)
Significant	.002	120.77±105.763	291±72	plt (10^3/m)
Significant	>0.001	2.75±0.5898	4.3±0.4	Alb(g/dl)
Significant	>0.001	16.38±2.133	13±0.8	PT (sec)
Significant	>0.001	65.15±50.278	14±2.6	ALT(U/L)
Significant	>0.001	122.05±102.674	14±3.6	AST(U/L)
Significant	>0.001	4.7±4.6338	0.7±0.2	T.Bil(Mg/dl)
Significant	>0.001	2.667±2.0322	0.3±0.1	D.Bil (Mg/dl)
Non significant	0.340	1.578±2.0097	0.79±0.12	S.Cr.(Mg/dl)
Significant	0. 010	147.75±60.089	89.6±5.59	RBS (Mg/dl)
Significant	>0.001	273.485±119.1620	37.07±14.3633	IL-8 (Pg/ml)

Table 6. Comparison between group II and group III regarding some Laboratory parameters

P	S	group III no=20 Mean±SD	group II no=20 Mean±SD	Laberatory parameters
Non significant	0.755	9.81±2.5941	12.2±13.7060	Hb(g/dl)
Non significant	0.946	120.77±105.763	108.9±96.014	plt (10^3/m)
Non significant	0.946	2.75±0.5898	2.825±0.5149	Alb(g/dl)
Non significant	0.070	16.38±2.133	15.26±2.603	PT (sec)
Non significant	0.253	65.15±50.278	66.7±112.380	ALT(U/L)
Non significant	0.107	122.05±102.674	81.95±77.034	AST(U/L)
Non significant	0.399	4.7±4.6338	4.21±5.4027	T.Bil(Mg/dl)
Non significant	0.068	2.667±2.0322	2.585±3.6826	D.Bil (Mg/dl)
Non significant	0.849	1.578±2.0097	0.98±0.4980	S.Cr.(Mg/dl)
Non significant	0.185	147.75±60.089	130.1±56.356	RBS (Mg/dl)
Significant	>0.001	273.485±119.1620	184.555±51.8586	IL-8 (Pg/ml)

Table 7. Serum interleukin-8 in different child groups

Child classification	Serum IL-8 (Pg/ml)
Child A no = 8	169.15±42.78
Child B no = 23	258.17±115.567*
Child C no = 9	205.2±61.418

*= significant p = 0.042

Table 8. Correlation between laboratory parameters in group II

	IL-8 Pg/ml	
ALT U/L	r=0.078	p=0.743
AST U/L	r=0.099	p=0.677
PLT 1 0^3/ML	r=0.247	p=0.295

Table 9. Correlation between laboratory parameters in group III.

	AFP Iu/ml	IL-8 Pg/ml
plt 10^3/ml	r=0.246 P=0.295	r=0.473 P=0.035
ALT U/L	r=0.232 p=0.326	r=0.302 p=0.196
AST U/L	r=0.260 P=0.268	r=0.646 p=0.002
Size cm	r=0.176 p=0.457	r=0.512 p=0.021
AFP Iu/ml	-	r=0.390 p=0.089
IL-8 Pg/ml	r=0.390 p=0.089	-