RESEARCH PAPER | Biochemistry | Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

A Study of Serum Interlukin-8 Level in Chronic Hepatitis C Infected Patients in Suez Canal Area

KEYWORDS: Chronic Hepatitis C virus, Interleukin 8

Fatma M. Hefny
Biochemistry Demonstrator, Faculty of Science, Port Said University, Port Said, Egypt

Mohamed Mohy Eldin M.
MD Int. Medicine, Faculty of Medicine, Suez Canal University (SCU), Ismailia, Egypt

Mohamed Ezel Dean R.
MD Organic Chemistry, Faculty of Science, Port Said University, Port Said, Egypt

Manal S. Fawzy
MD Biochemistry and Molecular Biology, Faculty of Medicine, Suez Canal University (SCU), Ismailia, Egypt

ABSTRACT *Background: Hepatitis C virus (HCV) is one of the major etiological agents of chronic viral hepatitis. It often progresses to chronic hepatitis, cirrhosis and hepatocellular carcinoma. Interleukin-8 (IL-8) is a cytokine involved in the cellular response to inflammation, being a powerful chemoattractant for neutrophils. This work aimed to estimate serum level of IL-8 in chronic liver disease (CLD) patients due to HCV.*

Methods: This study included fifty CLD patients due to HCV and fifty controls. They were subjected to history taking, liver biopsy, liver function tests and other laboratory tests. Serum IL-8 had been assayed by IMMULITE® 1000 IL-8.

Results: Serum IL-8 levels were significantly higher in chronic HCV patients compared to controls. Non-responders had higher baseline interleukin-8 levels than responders.

Conclusions: Serum IL-8 is a significant predictor for interferon/ribavirin therapy response in studied chronic HCV patients. Responders are found to have lower pretreatment serum IL-8 than non-responders.

Introduction

Hepatitis C virus (HCV) infects approximately 170 million people worldwide, and a proportion of them develop cirrhosis and hepatocellular carcinoma. [1] Chronic hepatitis C (CHC) is characterized by the relentless deposition of liver fibrosis, a process that may take decades before the cirrhotic stage is reached. [1] Several cytokines and chemokines induced by viral infection play direct or indirect roles in antiviral defense. [2] 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. [3] 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. [4] 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. [5] IL-8 has been shown to be involved in the pathology of a wide range of disorders such as arthritis, psoriasis, acute inflammation, infection, chronic asthma, cancer, systemic lupus erythromatosis [6], trachomatis prostatic infection and nephritis. [7] There was found that the HCV NS5A protein induces expression of IL-8 to partially inhibit the antiviral actions of IFN in vitro. [8] There is a dearth of data on the association of IL-8 serum levels in HCV patients with HCV infection and resistance to interferon. This needs to be investigated so that therapeutic strategies can be developed to provide efficient defense against the virus by manipulating the immune response. The present study was therefore undertaken to determine IL-8 levels in HCV patients and correlate it to response to combined therapy.

Subjects and Methods

Subjects:1-Chronic hepatitis C patients group: A total of 50 patients (31 males and 19 females, aged 21-60 years) with CHC undergoing a percutaneous liver biopsy in the Endoscopy Unit of SCU hospital for fibrosis scoring as a line of their management were recruited from November 2011 to January 2012. The patients were divided into two groups based on early virologic response (EVR) to combined PEG-IFN α-2a (Reiferon Retard®/Ribavirin) therapy after 24 weeks of starting treatment. EVR is considered if the HCV RNA level is undetectable or if a greater than 2-log-fold reduction in HCV RNA level is present**.** [9] **2-Control group**: Fifty healthy blood donors (27 males and 23 females, aged 19-56 years) with normal liver function tests (LFTs) were included as control group. Informed consent was obtained from all the participants prior to inclusion in the study**.**

Method

All patients were subjected to history taking, laboratory investigations including; LFTs, alpha fetoprotein (AFP), prothrombin time (PT), complete blood count (CBC), serum creatinine, thyroid stimulating hormone (TSH) and anti-Schistosomal antibody test that were done at clinical pathology department of SCU hospital. Quantitative HCV RNA analysis was done using Real Time PCR technique in an API PRISM® 7000 thermocycler (applied biosystems, Foster city, CA) at the Oncology Diagnostic Unit of Suez Canal University hospital. For all patients, conventional liver histology was performed on formalin-fixed liver biopsy by pathologists at pathology department of SCU hospital. The liver fibrosis was staged and evaluated by the modified Knodell system of Ishak *et al*., [10] without knowledge of the patient's biochemical or clinical data. Histological activity index (HAI) reflecting the severity was determined. Quantification of IL-8 levels in the sera of all studied subjects was performed as described by the manufacture with a commercially available IMMULITE® 1000 IL-8 kit (Siemens, UK).[11]

Statistical analysis: The data were presented as mean ± standard deviation (SD). Student t-test was applied to compare between two independent groups. Chi-square test or Fisher's exact test where appropriate used for comparison between two or more independent percentages. IL-18 concentrations were compared between groups using the Mann–Whitney U-test. The Pearson's correlation coefficient

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

test was used to find associations between parameters. The odds ratio (OR) was calculated by means of logistic regression and the confidence interval (CI) was calculated at the 95% level. Receiver operating characteristic curve (ROC) used for detection of area under curve (AUC) and cutoff value (COV) for best sensitivity and specificity of serum IL-8 levels. Data was analyzed by Texasoft WINKS, 4.651 software (Texasoft,Texas, USA). Statistical significance was assumed for P values less than 0.05.

Results

Demographic and Laboratory Parameters of the Studied Populations

Patients and controls were matched as regards age and sex. Tables (1 and 2) summarize some laboratory data of HCV patients group. As compared with normal subjects, patients had significant higher levels of ALT, AST and interleukin-8 levels (table 3).

Table (1): Laboratory Data of the HCV Patients Participating in the Study

Laboratory data	Mean±SD	Range
PCRx10^3 (IU/ml)	1134.7±2288.5	1.11-9980
WBCs x10^3	6.55±1.82	3.3-12.3
HB (g/dl)	13.8±1.96	8.1-18.0
AFP (ng/ml)	4.65±4.08	0.3-20.0
TSH (mU/L)	1.66±1.07	0.2-4.75
Total Bilirubin (mg/dl)	0.76±0.26	0.3-1.3
Albumin (g/dl)	4.1±0.49	2.9-5.3
Creatinine (mg/dl)	0.81±0.19	0.5-1.26

PCR: polymerase chain reaction, WBCs: white blood cells, HB; hemoglobin, AFT: alpha fetoprotein, TSH: thyroid stimulating hormone.

Table (2): Anti-schistosomal antibodies and degree of fibrosis of the HCV patients participating in the study

	Freq. (n=50)	%
Anti-schistosomal antibodies (+ve)	19	38
Degree of fibrosis:		
0	1	2
1	8	16
2	19	38
3	15	30
4	3	6
5	3	6
6	1	2

Table (3): Comparison between Controls and HCV Patient Groups Regarding Their Liver Enzymes and Interleukin-8 levels

	Controls (n = 50) (Mean±SD)	HCV-patients (n =50) (Mean±SD)	P-value
ALT (U/l)	22.2±7.1	51.9±27.6	<0.001[a*]
AST (U/l)	22.7±6.4	46.5±20.2	<0.001[a*]
Interleukin-8 (pg/ml)	2.0±3.4	78.5±216.3	<0.001[a*]

[a] Mann-Whitney test
* Statistically significant at 95% confidence level
ALT: alanine transaminase, AST: aspartate transaminase,

Comparison between responders and non-responders of HCV infected subjects regarding their demographic data and some laboratory parameters shows that ALT, AST and Interleukin-8 levels were higher among non-responders than responders, although this difference not reaches statistically significance (table 4). There was, in addition, no significant difference between responders and non responders regarding their anti-schistosomal antibodies and degree of fibrosis (table5).

Table (4): Comparison between Responders and Non-Responders HCV Infected Subjects Regarding Their Laboratory Data

Laboratory parameter	Responders (n = 44) (Mean±SD)	Non responders (n =6) (Mean±SD)	P value
PCRx10^3 (IU/ml)	100.6±2.2	205.6±306.0	0.10[b]
WBCs x10^3	6.5±1.8	7.1±1.8	0.47[b]
HB (g/dl)	13.6±1.7	14.7±2.9	0.19[a]
AFP (ng/ml)	4.6±4.3	4.5±3.1	0.83 [b]
TSH (mU/L)	1.7±1.1	1.3±0.7	0.38 [a]
Total Bilirubin (mg/dl)	0.7±0.2	1.1±0.2	0.001[a*]
Albumin (g/dl)	4.1±0.5	3.9±0.7	0.34 [a]
Creatinine (mg/dl)	0.8±0.2	0.8±0.1	0.56 [a]
ALT (U/l)	50.7±27.9	61.0±25.8	0.44[a]
AST (U/l)	45.8±20.4	51.8±19.9	0.54[a]
Interleukin-8 (pg/ml)	41.8±145.5	347±421.3	0.3[b]

[a] Student-t test

[b] Mann-Whitney test

* Statistically significant at 95%confidence level

PCR: polymerase chain reaction, WBCs: white blood cells, HB; hemoglobin, AFT: alpha fetoprotein, TSH: thyroid stimulating hormone, ALT: alanine transaminase, AST: aspartate transaminase.

Table (5): Comparison between Responders and Non-Responders HCV Infected Subjects Regarding Their Anti-Schistosomal Antibodies andDegree of Fibrosis

	Responders (n = 44)	Non responders (n =6)	P value
Anti-schistosomal antibodies [freq.(% of column)]	15(34.1)	4(66.7)	0.14[c]
Degree of fibrosis [freq. (%of column)]			
0	1(16.7)	0(0)	0.4[d]
1	8(18.2)	0(0)	
2	18(40.9)	1(16.7)	
3	12(27.3)	3(50.0)	
4	2(4.5)	1(16.7)	
5	2(4.5)	1(16.7)	
6	1(16.7)	0(0)	

[c] Fisher exact test
[d] Likelihood ratio

Correlation between HCV Patients' Response to Treatment and Some Demographic and Laboratory Data:

There was significant moderate negative correlation between response to treatment and each of Interleukin-8 (r= -0.5, p=0.001) and total Bilirubin (r= -0.5, p=0.001). In addition, there is a significant weak negative correlation between response to treatment and degree of fibrosis (r= -0.3, p= 0.03) (table 6). Interleukin-8, anti-schistosomal antibodies and degree of fibrosis were found to be predictors for response to

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

treatment as detected by logistic regression analysis (table 7).

Table (6): Correlation between HCV Patients Response to Treatment and Their Demographic, Laboratory and Clinical Data

	Correlation	P value
Age	-0.1	0.48
ALT	-0.1	0.44
AST	-0.1	0.54
Interleukin-8	-0.5	0.001*
PCR	-0.2	0.30
WBCs	-0.1	0.47
Hb	-0.2	0.19
AFP	0.0	0.97
TSH	0.2	0.38
Total Bilirubin	-0.5	0.001*
Albumin	0.1	0.39
Creatinine	0.1	0.56
Degree of fibrosis	-0.3	0.03*
* Statistically significant at 95%confidence level		

Table (7): Logistic Regression Analysis of Predictors for Response to Treatment

Predictors	B	p value	OR	95.0% C.I. for OR	
				Lower	Upper
Interleukin8	-0.01	0.02*	1.0	0.99	0.999
Anti-schistosomal antibodies	2.18	0.09	8.9	0.69	114.30
Degree of fibrosis	-0.90	0.06	0.41	0.16	1.06
Constant	4.21	0.008	67.56		

Interleukin-8 was found to be highly accurate test in diagnosis for response to treatment (AUC=62%). Best cut-off value of IL-8 level that gives best sensitivity and specificity for response to treatment was ≤ 272.5 pg/ml (Figure 1).

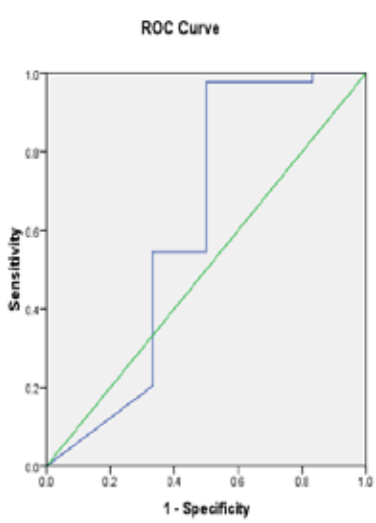

Figure (1): ROC curve of interleukin-8 for response to treatment prediction

Discussion

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. [12] In the current study we found that serum IL-8 was significantly higher in patients of CHC than controls (p<0.001). This is in agreement with Mihm *et al.*,[8] and Polyak *et al* who showed that high serum IL-8 in chronic HCV patients was associated with the lack of a biochemical response to IFN therapy.[13] Polyak *et al* reported that the core and NS5A proteins of HCV induce the expression of the IL-8 gene, and that contributing to elevated serum IL-8 in chronic HCV patients who are associated with resistance to interferon treatment, suggesting that IL-8 plays an important role in the maintenance of persistent infection with HCV.[13] Elewa *et al*, in addition, found that serum IL-8 levels were higher in patients with HCV compared with controls, and in patients with hepatocellular carcinoma associated with HCV infection compared with controls.[12] It has been reported that there is a correlation between IL-8 levels and the severity of liver disorders, including HCV infection. [14] In HCV patients with liver inflammation and fibrosis, elevated serum levels of IL-8 have been reported [15] and intrahepatic IL-8 mRNA levels were positively correlated with severity of hepatic inflammation and injury in those patients. [16] Moreover, at the end of 24 weeks in our study of interferon/ribavirin therapy, PCR showed that 88% of the studied patients have virological response with negative PCR while 12% were non- responders. Non-responders were found to have higher baseline interleukin-8 levels (347±421.3pg/ml) than responders (41.8±145.5pg/ml). Responders, in addition, were found to have better liver enzymes (ALT, 50.7 U/l versus 61 U/l in non-responders; AST, 45.8 U/l versus 51.8 U/l in non-responders) and lower quantitative viral load ($100.6*10^3$ versus $205.6*10^3$ in non- responders). These findings are consistent with those reported by Akbar *et al*, who aimed to prospectively utilize the baseline IL-8 levels in the HCV infected serum and predict its role in sustained virological response (SVR) to IFN-α plus ribavirin therapy, in chronic HCV patients in Pakistan. [17] They have found that non-responders have higher baseline pretreatment levels of IL-8 than responders and they have concluded that increased levels of IL-8 in HCV infection might be involved in pathogenesis, persistence and resistance to IFN-α plus ribavirin combination therapy. [17] In the present study, we found that there was a moderate negative correlation between response to treatment and each of Interleukin-8 and total bilirubin and there was a significant difference between responders and non responders regarding their total bilirubin. This finding is in agreement with Hosogaya *et al*, who reported that low total bilirubin level is significantly associated with SVR. [18] We noted in this study that there is a significant weak negative correlation between response to treatment and degree of fibrosis (r=-0.3, p= 0.03). This finding is in agreement with previous studies of Poynard *et al*, who found that patients with established cirrhosis are resistant to IFN-α therapy than those who have fibrosis, whereas patients with fibrosis are less responsive to IFN-α therapy than those without fibrosis. [19] This also agree with findings of Romero-Gomez *et al*, who found that the mean fibrosis was lower among responders (1.41±0.88 vs2.16±1.39; P=0.0001). [20] In this study we found that there are a high percentage of non-responders who have positive anti-Schistosomal antibody test, although this difference didn't reach statistically significance. This could be explained by limited number of non-responders in this study. This finding is in agreement with Kamal *et al*, who reported that CHC patients with Schistosoma co-infection responded poorly to interferon therapy and had a higher relapse rate than patients not having concomitant schistosomiasis. [21]

Conclusion

These data, although carried out in a small group of patients, provide evidence that serum levels of the proinflammatory chemokine IL-8 in patients with CHC are significantly higher in comparison with healthy controls and these could be one of the predictors for response to interferon/ribavirin therapy

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

in patients with chronic HCV infection. Responders are found to have lower pretreatment serum levels of IL-8 than non-responders. Low fibrosis stage and low viral load are associated with response to therapy.

Acknowledgements:
We thank all the patients and control subjects for agreeing to join this work.

REFERENCE

1- Clement S., Pascarella S., Conzelmann S. et al., (2010), "The hepatitis C virus core protein indirectly induces alpha-smooth muscle actin expression in hepatic stellate cells via interleukin-8." J Hepatol 52, 635–643. | 2- Najafizadeh M., Farhadi N., Sarkari B. et al., (2007), "Th1 cytokine profiles in hepatitis C virus infected patients and their contribution to inflammatory responses." Shiras E-medical journal January 8 (1), 1-49. | 3- Tilg H., Kaser A., Moschen AR. et al., (2006), "How to modulate inflammatory cytokines in liver diseases." Liver International, 26 (9), 1029-1039. | 4- Avramescu CS., Comanescu V., Popescu SN. et al., (2008), "Correlations among the serum levels of some interleukins and histopathological aspects in chronic viral hepatitis C". Room journal Morphol Embryol, 49(1):57-62. | 5- Holmes W.E, Lee J., Kuang W.J., et al., (1991), "Structure and function expression of human IL-8 receptor". Science 13; 253(5025):1278-80. | 6- Anthony R., Das R. G. and Thorpe R. (1997), "Implication for the assay and biological activity of interleukin-8: results of a WHO international collaborative study." Journal of Immunological Methods, 200, 1- 16. | 7- Mazzoli S., Cai T., Rupealta V. et al., (2007), "Interleukin 8 and Anti-Chlamydia trachomatis Mucosal IgA as Urogenital Immunologic Markers in Patients with C. trachomatis Prostatic Infection." European urology, 51, 1385–1393. | 8- Mihm U., Herrmann E., Sarrazin U. et al., (2004), "Association of serum interleukin-8 with virologic response to antiviral therapy in patients with chronic hepatitis." Journal of Hepatology, 40,845–852. | 9- Zeuzem S., Berg T., Moeller B., et al., (2009), "Expert opinion on the treatment of patients with chronic hepatitis C." J Viral Hepat, 16(2): 75–90. | 10- Ishak K., Baptista A., Bianchi L., et al., (1995), " Histological grading and staging of chronic hepatitis. "J Hepatol, 22(6):696-9. | 11- Siemens Healthcare Diagnostics Products Ltd., Llanberis, Gwynedd LL55 4EL, United Kingdom. | 12- Elewa H., Abd-Elmeneem M., Hashem A. M. and Aml Alshehaby. (2010), "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)." International Journal of Hepatology, 1, 3. | 13- Polyak S. J., Khabar K. S., Rezeiq M. et al., (2001), "Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy." J Virol, 75, 6209–6211. | 14- Neuman, J.P. Benhamou, I.M. Malkiewicz et al. (2001), "Cytokines as predictors for sustained response and asin situ messenger RNA hybridization technique." Am. J. Pathol, 150, 1571–1582. | 15- Baggiolini M. and Clark-Lewis I. (1992), "Interleukin-8, a chemotactic and inflammatory cytokine". FEBS Lett., 307(1), 97-101. | 16- Shimoda K., Begum N.A., Shibuta K. et al. (1998), Interleukin-8 and h1RH (SDF1-αPBSF) mRNA expression and histological activity index in patients with chronic hepatitis C.Hepatology, 28:108-115. | 17- Akbar H, Idrees M, Butt S. et al. (2011), High baseline interleukine-8 level is an independent risk factor for the achievement of sustained virological response in chronic HCV patients. Infect Genet Evol, 11(6): 1301 – 1305. | 18- Hosogaya S, Ozaki Y, Enomoto N, Akahane Y. 2006; Analysis of prognostic factors in therapeutic responses to interferon in patients with chronic hepatitis C. transl Res, 148(2): 79-86. | 19- Poynard T, McHutchison J, Manns M. et al., (2002), "Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C." Gastroenterology, 122:1303-1313. | 20- Romero-Gómez M., Salemerón J., Diago M. et al., (2005), "Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in Chronic hepatitis C patients." J Gastroenterology, 128,636-641. | 21- Kamal S. M., Madwar M. A., Peters T. et al., (2000), "Interferon therapy in patients with chronic hepatitis C and Schistosomiasis." J Hepatol, 32, 172-174.