RESEARCH PAPER | Biochemistry | Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

A Study of Serum Interlukin-18 Level in Chronic Hepatitis C Infected Patients in Suez Canal Area

KEYWORDS: Chronic Hepatitis C virus, Interleukin 18

Nadia A. Ahmed
Biochemistry Demonstrator, Faculty of Science, Port Said University, Port Said, Egypt

Faten Z. Mohamed
Professor of Biochemistry, Faculty of Science, Zagazig university, Al-Sharqia, Egypt

Mohamed S. EL-ghareb
Professor of 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) infection is a global health problem. Interleukin-18 (IL-18) plays a critical role in T helper type 1 (Th1) response required for host defense against viruses and antibodies to IL-18 have been found to prevent liver damage in a murine model. This work aimed to estimate serum level of IL-18 in chronic liver disease (CLD) patients due to HCV and correlate it to response to combined therapy.*

Methods: This study included 45 CLD patients due to HCV and 45 controls. They were subjected to history taking, liver biopsy, liver function tests and other laboratory tests. Serum IL-18 levels were assayed by an enzyme-linked immunosorbent assay.

Results: Serum IL-18 levels were significantly higher in chronic HCV patients compared to controls. Non-responders had higher baseline interleukin-18 levels than responders.

Conclusions: Serum IL-18 could be a significant predictor for interferon/ribavirin therapy response in chronic HCV patients with other tests. The best cut-off value of IL-18 for the prediction of the response is ≤ 482.5 pg/ml with 58.3% sensitivity and 66.7% specificity and the area under curve (AUC) is 0.62.

Introduction

Hepatitis C virus (HCV) infection is a global health problem, being the second most common chronic viral infection in the world with a global prevalence of about 3% (about 180 million people). [1] Egypt has the highest prevalence of HCV worldwide (15%) and the highest prevalence of HCV-4, which are responsible for almost 90% of HCV infections. [2] Patients infected with hepatitis C virus (HCV) have different clinical outcomes, ranging from acute resolving hepatitis to chronic liver disease including liver cirrhosis or hepatocellular carcinoma. [3] The currently recommended therapy of chronic HCV infection is the combination of a pegylated interferon (IFN) alpha and ribavirin for a period 24-72 weeks. [4]

Improvements in existing therapies as well as development of new antiviral agents or vaccines for the treatment or prevention of chronic infection are thus highly desirable. The cytokines prompting development of the T helper type 1 (Th1) immune response are of particular interest for potential immunotherapy against HCV. [5] Interleukin-18 (IL-18) described as a member of the IL-1 cytokine superfamily [6] and it has the capacity to activate both Th1 and Th2 responses. [7] Many reports suggest that IL-18 might play a role in viral infections. [8] An increase in its expression has been shown to correlate with IFN- γ production in chronic hepatitis C and cirrhosis, [5] and it could be related to disease persistence. [5] Ludwiczek *et al* found that, IL-18 and IL-18 binding protein (IL-18 BP) are elevated in chronic liver disease as compared with healthy controls and its levels correlate with severity of the disease. [9] Sharma et al.,[5] suggested that IL-18 antagonists are promising candidates for therapeutic interventions to combat the pathological consequences of HCV infection. As few studies (if any) correlate IL-18 levels in HCV patients to response to treatment in our area, we aimed in the current work to estimate serum IL-18 concentration in HCV patients in Suez Canal area and correlate it to response to combined therapy (peg-INF-α plus ribavirin).

Subjects and methods

Subjects: 1-Chronic hepatitis C (CHC) patients group: A total of 45 patients (30 males and 15 females, aged 19-66 years) with CHC attending Endoscopy Unit of SCU hospital from November 2011 to January 2012 to perform percutaneous liver biopsy for fibrosis scoring as a line of their management, included in this study. The patients were divided into two groups based on early virologic response (EVR) to combined PEG-IFN α-2a (Reiferon Retard®/Ribavirin) therapy after 12 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. [10] **2-Control group**: Forty five healthy blood donors (26 males and 19 females, aged 21-60 years) with normal liver function tests (LFTs) and negative results for both anti-HCV antibodies and HBV surface antigen (HBsAg) were included as control group. Informed consent was obtained from all the participants prior to inclusion in the study.

Methods

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.*, [11] without knowledge of the patient's biochemical or clinical data. Histological activity index (HAI) reflecting the severity was determined.

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

Quantitative determination of IL-18 by ELISA technique:
Quantification of IL-18 levels in the sera of all studied subjects was performed as described by the manufacture with a commercially available Enzyme linked Immunosorbent Assay Kit for IL-18 [Uscn; Life Science Inc., available at (www.uscnk.us; www.uscnk.cn; www.uscnk.com)]. Briefly, the assay uses two monoclonal antibodies against two different epitopes of human IL-18. In the wells coated with anti-human IL-18 monoclonal antibody, samples to be measured or standards were incubated. After washing, a peroxidase conjugated anti-human IL-18 monoclonal antibody was added into the microwell and incubated. After another washing, the peroxidase substrate was mixed with the chromogen and allowed to incubate for an additional period of time. An acid solution was then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density (OD) of each well was then measured at 450 nm using a microplate reader (Ceres UV900 HDI, Bio-Tek Instrument Inc., USA). The concentration of serum IL-18 was calibrated from a dose response curve based on reference standards.

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 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-18 levels (table 3).

"Table 1, 2 and 3 about here'

Comparison between responders and non-responders of HCV infected subjects regarding their demographic data and some laboratory parameters shows that ALT, AST and Interleukin-18 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 and 5 about here'.

Correlation between serum IL-18 levels and some demographic and laboratory data:
There was only a significant week correlation between Interleukin-18 levels and AST levels (r=0.3, p=0.04) (data not shown).

Receiver operating characteristic (ROC) curve:
ROC curve was done to test accuracy of Interleukin-18 level as a predictor for response to treatment. ROC showed the best cut-off value which gives 58.3% sensitivity and 66.7% specificity for response to treatment was ≤ 482.5 pg/ml and area under ROC curve was 62% (Figure 1).

"Figure 1 about here'.

Discussion
Hepatitis C virus (HCV) has chronically infected more than 3% world population especially in developing countries. [12] IL-18 originally known as interferon-γ (IFN-γ)-inducing factor (IGIF) has multiple biological activities via its capacity to stimulate innate immunity and both Th1 and Th2 mediated responses. [13] Our data confirm previous reports of elevated plasma levels of IL-18 in HCV patients compared to healthy subjects ($p<0.001$). Mc Guineness *et al.,* [14] for instance, documented that IL-18 mRNA expression is significantly up-regulated in HCV-associated chronic hepatitis. [14] Ludwiczek *et al.,*[9] and Vecchiet et al., [15] suggested that elevated plasma levels of IL-18 may have an important role as a marker of both inflammation and hepatic-biliary injury progression. Stanislawska *et al.,* [16], Bouzgarrou *et al.,* [17] and selim *et al.,*[18] have also observed the same finding.

When correlations between serum IL-18 levels and various laboratory and clinical parameters were analyzed, we found a significant moderate correlation between IL-18 of HCV patients and their AST levels (r=0.3, p=0.04). This finding is in agreement with Ludwiczek *et al.,* [9] who found that IL-18 level was correlated with serum AST as a marker of hepatocyte damage, and Sharma et al., [5] who reported that concentrations of IL-18 were not associated, neither with age nor with ALT levels, however, did it correlate with disease progression.

Moreover, at the end of 24 weeks in our study of interferon/ribavirin therapy, PCR showed that 80% of the studied patients have virological response with negative PCR while 20% were non- responders. Non-responders were found to have higher baseline interleukin-18 levels (513.9±119.4 pg/ml) than responders (471.7±192.1 pg/ml). Although these figures with ALT and AST levels were higher among non-responders than responders, these findings didn't reach statistically significance.

This could be explained by small number of non-responders in the current study. It has been shown that the administration of IFN-γ exerts an anti-inflammatory action in vivo by induction of IL-18 BP and late suppression of IL-18 [19, 20] and this could explain the low level of IL-18 in responders compared to non-responders.

We found, in addition, that there are a high percentage of non-responders who have positive anti-Schistosomal antibody test, although this difference didn't reach statistically significance as explained above. This finding is in agreement with Kamal *et al,* [21] who found that CHC patients with Schistosoma co-infection responded poorly to interferon therapy and with our colleague Hefny *et al.,* [22] who reported the same results in their CHC patients group in the researcher area.

Conclusion
We concluded that IL-18 levels were elevated in HCV patients than controls. IL-18 baseline levels were higher among non-responders than responders and it could be used with other tests as a monitoring tool to assess response to therapy.

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

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

REFERENCE

1- Craxi A. , Laffi G. and Zignego A. L. (2008), "Hepatitis C virus (HCV) infection: A systemic disease." Molecular Aspects of Medicine 29, 85–95. | 2- Al Swaff R. (2012), "Correlation between alanine aminotransferase level, HCV-RNA titer and fibrosis stage in chronic HCV genotype 4 infection." The Egyptian Journal of Medical Human Genetics, 13, 207–12. | 3- Zaltron S., Spinetti A. and L Biasi et al., (2012), "Chronic HCV infection: epidemiological and clinical relevance." BMC Infect Dis, 12 (2). | 4- Ghany M., Strader D. and Thomas D. et al., (2009), " Diagnosis, Management, and Treatment of Hepatitis C: An Update." J. Hepatology, 1335-1374. | 5- Sharma A., Chakraborti A., Das A., et al., (2009), "Elevation of interleukin-18 in chronic hepatitis C: implications for hepatitis C virus pathogenesis." J. Immunology, 128 , 514–22. | 6- Mu¨hl H. and Pfeilschifter J. (2004), "Interleukin-18 bioactivity: a novel target for immunopharmacological anti-inflammatory intervention." European Journal of Pharmacology 63 – 71. | 7- Tsutsui H., Yoshimoto T., and Okamura H. (2003), "Interleukin-18, Encyclopedia of Hormones" (book). (Elsevier Science). 493-500. | 8- Choi HJ, Dinarello CA, and Shapiro L. (2001)," Interleukin-18 inhibits human immunodeficiency virus type 1 production in peripheral blood mononuclear cells." J Infect Dis 184,560–568. | 9- Ludwiczek O, Kaser A, and Novick D et al (2002), "Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease." J Clin Immunol 22,331–337. | 10- 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. | 11-Ishak K., Baptista A., Bianchi L., et al., (1995), " Histological grading and staging of chronic hepatitis. "J Hepatol, 22 (6), 696-699. | 12- Khaliq S., Jahan S. and Pervaiz A. (2011), "Sequence variability of HCV Core region: Important predictors of HCV induced pathogenesis and viral production, Infection, Genetics and Evolution." 11, 543–556. | 13- Tangkijvanich P., Thong-ngam D. and Mahachai V. et al.,(2007), "Role of serum interleukin-18 as a prognostic factor in patients with hepatocellular carcinoma." World J Gastroenterol 13(32), 4345-4343. | 14- McGuinness P. H., Painter D. and Davies S., et al., (2000), "Increases in intrahepatic CD68 positive cells, MAC387 positive cells, and proinflammatory cytokines (particularly interleukin 18) in chronic hepatitis C infection." Gut, 46, 260–269. | 15- Vecchiet J., Falasca K. and Cacciatore P., et al., (2005), "Association between Plasma Interleukin-18 Levels and Liver Injury in Chronic Hepatitis C Virus Infection and Non-Alcoholic Fatty Liver Disease." Annals of Clinical and Laboratory Science, 35 (4), 415-422. | 16- Stanisławska M. E., Pietrzak A. and Brzezi□ska et al., (2008), "Serum IL-18 concentration does not depend on the presence of HCV-RNA in serum or in PBMC." Hepatogastroenterology, 55 (81), 212-215. | 17- Bouzgarrou N., Hassen E. and Schvoerer E., et al., (2008), "Association of interleukin-18 polymorphisms and plasma level with the outcome of chronic HCV infection." J Med Virol, 80, 607–614. | 18- Selim H. S., El-Barrawy M. A., Taha H. A., et al., (2009), "Evaluation of Interleukin-18 as a Non Invasive Marker of Liver Fibrosis among Chronic Hepatitis C Virus Patients." J Egypt Public Health Assoc, 84 ,392-403 | 19- Kaser A., Novick D. and Rubinstein T., et al., (2002), "Interferon-alpha induces interleukin-18 binding protein in chronic hepatitis C patients." Clin Exp Immunol,129,332-336. | 20- Jia H., Du J. and Zhu S., et al., (2003), "Clinical observation of serum IL-18, IL-10 and sIL-2R levels in patients with chronic hepatitis C pre-and post antiviral treatment." Clin Med J,116, 605-608. | 21- Kamal S. M., Madwar M. A. and Peters T. et al., (2000), "Interferon therapy in patients with chronic hepatitis C and Schistosomiasis." J Hepatol, 32, 172-174. | 22- Hefny F. M., Mohy Eldin M. and Eze Eldin M. et al., (2013), "A Study of Serum Interlukin-8 Level in Chronic Hepatitis C Infected Patients in Suez Canal Area." Indian Journal of Applied Research, 3 (7), 56-59. |