The Impact of Serum Interleukin-17 on Chronic Hepatitis C and Its Sequelae

Elham Ahmed Hassan*, Abeer Sharaf EL-Din Abd El-Rehim†, Asmaa Omar Ahmed‡, Nahla Mohamed Elsherbiny§ and Noha Abd El-Rehim Abo Elhagag¶

1Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assiut University, Assiut, Egypt
2Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt
3Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt
4Department of Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

Abstract

**Background:** Recently, interleukin-17 (IL-17) cytokine family plays important roles in the host immunity in chronic inflammatory conditions, autoimmune and viral liver diseases. However, studies on the IL-17 role in the immunopathogenesis of hepatitis C virus (HCV) infection are limited.

**Objectives:** to assess serum IL-17 levels in chronically HCV infected patients and their relations to the severity of liver disease.

**Patients and methods:** The study included 200 chronically HCV infected patients; 100 chronic hepatitis C, 100 liver cirrhosis including 35 with hepatocellular carcinoma (HCC) and 30 controls. Serum IL-17 levels were quantified by ELISA.

**Results:** Serum IL-17 levels were significantly higher in chronically HCV infected patients than controls and cirrhotics had the highest levels (P<0.001). These levels were positively related with inflammation grade and fibrosis stage. Serum IL-17 was significantly higher in HCC than controls. IL-17 was significantly correlated with prothrombin time, ALT, serum albumin, viral load, and alpha fetoprotein-L3.

**Conclusion:** IL-17 levels were increased with increasing liver disease progression and chronicity. Thus, IL-17 may be an important biological marker for the immunopathogenesis of chronic hepatitis, liver fibrosis and HCC.

**Keywords:** Chronic viral hepatitis immunopathogenesis; Chronic viral liver disease; Serum IL-17

Introduction

Hepatitis C Virus (HCV) infection affects an estimated 180 million people globally, and is a leading cause of chronic hepatitis, cirrhosis, liver cancer, and primary indication for liver transplantation [1,2]. This RNA virus primarily infects hepatocytes and induces an immune-related necroinflammatory hepatic reaction, frequently leading to significant fibrosis or cirrhosis [3]. However, many aspects of immune dysregulations in hepatitis C are still unclear, especially the inflammatory pathways.

IL-17A (IL-17) is one of T cell–derived cytokines produced by Th17 CD4+ T cells specifically memory CD4+ T cells [4]. IL-17 is also produced by a wide variety of cell types, including neutrophils, CD8+ T cells, γδ T cells, NKT cells and Tregs [5].

IL-17 is a powerful chemoattractant for neutrophils and has been reported to be involved in many immune processes, most notably in inducing and mediating proinflammatory responses e.g. several autoimmune diseases, allergic diseases, asthma and pulmonary infection [6-8].

Several groups of investigators recognized the role of IL-17 in hepatitis B and its sequelae [9-11]. However, there has been limited data of the role of IL-17 in HCV infected patients, especially in relation to hepatic inflammation and fibrosis and hepatic cell carcinoma. In addition, Egyptian studies on the role of IL-17 in the immunopathogenesis of HCV are limited. Therefore, the aims of this study were to assess serum IL-17 levels in HCV-positive patients with chronic liver disease and their relation to the severity of liver disease.

Patients and Methods

**Study design**

This was a prospective study carried out at Assiut University Hospital (AUH), Egypt, from February 2013 to February 2014. The study was approved by the Ethics Committee of AUH and an informed, written consent was obtained from all the participants before enrollment.

**Study population**

The study group comprised 200 chronically HCV infected patients and they were divided into:

- 100 patients with chronic hepatitis C “CHC”
- 100 patients with liver cirrhosis including 35 with HCC.

Diagnosis of chronic hepatitis “C” was based on laboratory findings of fluctuations of serum transaminases levels for more than 6 months and positive HCV antibodies and serum HCV-RNA for HCV

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*Corresponding author: Elham Ahmed Hassan, MD, Assiut University Hospital, Tropical Medicine and Gastroenterology, Assiut, Egypt, Tel: +201002963415; Fax: +20882333327; E-mail: mam_elham75@yahoo.com

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infection and absence of sonographic findings of liver cirrhosis and histopathologic evidence of chronic hepatitis by liver biopsy.

Cirrhotic patients had diagnostic criteria of Liver Cirrhosis (LC) by clinical, biochemical and ultrasonographic findings. Severity of liver cirrhosis was assessed according to Child-Pugh classification [12]. Further, cirrhotic patients were divided according to hepatic focal lesion(s) by imaging and elevated alpha fetoprotein-L3 (AFP-L3).

The controls were 30 healthy volunteers and they had negative markers for HBV and HCV infections had no hepatic diseases and they were sex and age matched with patients. These patients were selected from outpatient clinics and inpatient wards of the department of Tropical Medicine and Gastroenterology, Assiut University Hospital (AUH), while controls were selected randomly from outpatient clinic and relatives of the patients. Patients with evidence of infection, co-infection of HCV and HBV, HIV, autoimmune hepatitis, alcoholic hepatitis, and hepatic metastasis or under antiviral therapy were excluded. At the study entry, a thorough medical history and clinical examination were taken and measurement of serum IL-17 level, other biochemical parameters and liver biopsy were undertaken.

Methods
Specimen collection and processing
Five milliliters of blood were withdrawn from each patient under complete aseptic conditions. Sera were separated and stored frozen at -70°C until analysis.

Laboratory diagnosis
- Antibodies to HCV using a third-generation enzyme immunoassay, and HCV RNA was quantified by Taq Man Assay Reagents (Applied Biosystems ) using the 7500 fast Real Time PCR system.
- Biochemical tests: liver function tests (Aspartate aminotransferase (AST), alanine transaminase (ALT), albumin, bilirubin and prothrombin time) and Alpha fetoprotein-L3 (AFP-L3 for cirrhotic patients with hepatic focal lesions).
- Serum IL-17 levels were measured by enzyme-linked immunosorbent assay (ELISA) (IL-17: R&D Systems; Quantikine Biosystems ) using the 7500 fast Real Time PCR system.

Liver histology
Liver biopsies were taken percutaneously with a 1.4 mm diameter Menghini needle and consisted of 3-5 mm long liver tissue cylinders. Biopsies were formalin fixed and then embedded in paraffin cut and stored until use. Biopsies were histologically examined using hematoxylin–eosin stain. The Scheuer score was used to classify hepatic necroinflammatory activity and fibrosis [13].

Statistical analysis
All statistical analyses were conducted using SPSS for windows version 17 (SPSS Inc., Chicago, IL, USA). The Kruskal Wallis and Mann-Whitney tests were used to compare the continuous variables. Categorical variables were expressed as percentage and compared using chi-square (χ2) or Fisher's exact test (two-tailed) test. Spearman's correlation coefficient (r) was used to find correlations. All data were summarized as mean and standard deviations. For all analyses, P value<0.05 was considered statistically significant.

Results
Characteristics of the study population
The baseline sociodemographic and biochemical characteristics of the study population were summarized in Table 1. The study included 200 chronically HCV infected patients; 128 males and 72 females with mean age of 47.7 ± 5.3 years. Control group was formed of 11 females and 19 males with a mean age of 45.5 ± 7.6 years.

Serum IL-17 levels
Compared with controls, serum IL-17 levels were significantly higher in chronically HCV infected patients and those with LC exhibited the highest IL-17 levels (P<0.001) as shown in Table 2. Patients with CHC had significantly higher levels than controls (P=0.03). Furthermore, serum IL-17 levels were significantly higher in patients with LC compared to CHC group (P<0.001). Additionally, cirrhotic patients with Child-Pugh class C had higher serum IL-17 levels (71.9 ± 41.9 pg/ml) than Child-Pugh class A and B (59.8 ± 29.5 and 65.5 ± 33.4 pg/ml respectively) but without statistical significant differences (P=0.07).

Among the cirrhotic group, patients with HCC (n=35) had significantly higher serum IL-17 levels than controls (67.3 ± 40.4 vs. 19.4 ± 7.3 pg/ml, P<0.001) and insignificantly higher values than patients without HCC (n=56) and patients with hemangioma(s) (n=...
patients with chronic liver disease goes with what has been reported previously that serum IL-17 level was increased in liver injuries following chronic hepatitis and cirrhosis supporting a role for IL-17 as a chronic disease inducer in the pathogenesis and/or progression of liver fibrosis [15]. However, IL-17A levels were not significantly different between Child A and B and C. This lack of difference may be attributed to the small sample size within the cirrhosis subgroups.

In this study, serum IL-17 levels of CHC patients were positively related with the grades of liver inflammation and fibrosis; the higher the grade, the higher the serum IL-17 levels indicating that IL-17 takes part in chronic inflammation and in the development of hepatic fibrosis. These results were compatible with previous studies that reported a close correlation between virus-induced liver inflammation, infiltration in CHC infections and activation of Th17 cells and the amount of liver damage caused by the antiviral immune response [15,16]. IL-17 exerts its proinflammatory effects through recruitment of neutrophils and monocytes, T cell infiltration facilitation and production of variety of inflammatory factors as IL 1,6, TNF-α,...etc. It also induces the expression of several inflammation-associated genes in primary hepatocytes [6,17-19]. The relation of IL-17 to liver fibrosis remains elusive. Liver fibrosis is a severe, life-threatening clinical condition resulting from non-resolving hepatitis of different origins. IL-17 may have a pro-fibrogenic effect through two independent mechanisms [9,20]: first, IL-17 stimulates Kupffer cells to express inflammatory cytokines IL-6, IL-1β, and TNF-α, as well as the major fibrogenic cytokine TGF-β1 which in turn, induce activation of Hepatic Stellate Cells (HSC) into myofibroblasts. Second, IL-17 directly stimulates HSCs to express collagen Type I and promotes their activation into fibrogenic myofibroblasts via Stat3 signaling pathway. Moreover, it has been reported elsewhere that intrahepatic IL-17 expression was positively correlated with the serum indices of hepatic fibrosis that is an important pathological process in the development of liver cirrhosis [6].

Table 3 showed that CHC patients with the highest serum IL-17 levels had the highest necroinflammatory activity (P = 0.02) and highest fibrosis stage (P=0.04). Additionally, there were significant correlation between serum IL-17 and grades of inflammation (r=0.98, P<0.001) and stages of fibrosis (r=0.57, P=0.02). Serum IL-17 levels were significantly correlated with ALT, viral load in CHC patients and prothrombin time and negatively correlated with serum albumin in cirrhotic patients (Table 4). Regarding HCC, serum IL-17 level positively correlated with AFP-L3 (r=0.55 and P=0.02), however, no correlation was found between serum IL-17 levels and tumor size or number (r=-0.13, P=0.3 and r=- 0.21, P=0.2 respectively).

**Discussion**

Hepatitis C Virus (HCV) infection primarily infects hepatocytes and induces an immune-related necroinflammatory hepatic reaction, frequently leading to significant fibrosis or cirrhosis [3]. The present study was conducted to investigate the involvement of the Th17 immune response which is not fully understood by determining serum levels of the IL-17 cytokine, in patients with chronic hepatitis C infection as well as cirrhosis with or without HCC aiming to reach an immunotherapeutic target at the future for the prevention of liver tissue damage. As a major function of most cytokines is to act in intercellular communication, it has been demonstrated that cytokine levels in serum and plasma provide at best an indirect measure of this key cytokine function [14].

We found that serum IL-17 levels were significantly higher in chronically HCV infected patients than controls and the highest serum IL-17 levels were observed in LC patients. This higher serum IL-17 in patients with chronic liver disease goes with what has been reported previously that serum IL-17 level was increased in liver injuries following chronic hepatitis and cirrhosis supporting a role for IL-17 as a chronic disease inducer in the pathogenesis and/or progression of liver fibrosis [15]. However, IL-17A levels were not significantly different between Child A and B and C. This lack of difference may be attributed to the small sample size within the cirrhosis subgroups.

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We reported significant correlations between the serum concentration of IL-17 and that of some indicators of liver function e.g. ALT, prothrombin time and serum albumin suggesting that IL-17 is, to a certain extent, associated with the extent of liver damage. Additionally, this significant correlation between serum IL-17 and ALT was in accordance with several studies which showed that IL-17 level correlated directly with severity of liver inflammation [10,15]. This is probably because IL-17 activates a variety of immune cells to release inflammatory mediators as mentioned in details before, leading to repeated inflammation of the liver and deterioration of liver function [20]. However, Du et al’s earlier report showed no correlation between serum IL-17 and ALT where the latter can be easily affected by drugs which decrease its level [6].

Our study revealed a positive linear correlation between serum IL-17 and viral load that was similar with previous studies [21]. This finding which is in agreement with that of Hou et al. suggested that IL-17 may contribute to the immunopathogenesis of chronic viral hepatitis by persistent viral infection due to IL-17 up-regulated anti-apoptotic molecules and thus enhancing the survival of virus infected cells and blocking target cell destruction by cytotoxic T cells [22,23]. Also, Oyoshi et al. [24], reported that IL-17 contributes to viral replication in a model of disseminated vaccinia infection rather than providing host defense against viral infection. However, Balanescu et al. [5], reported the demonstrated lack of correlation between serum IL-17 levels and viral markers owing to multiple IL-17 sources that might account for their serum levels and fluctuation of viral loads.

In the present study, we found that serum IL-17 levels were significantly higher in patients with HCC compared to controls and were insignificantly higher than in those without HCC and hepatic haemangioma(s). This result was agreed with Liao et al. [25] who reported that serum IL-17 levels to be higher in cirrhotic patients with HCC than those with hepatic haemangioma which suggested that IL-17 takes part in the pathogenesis of HCC reflecting the systemic immune status of individuals with tumor. The contribution of IL-17 and Th17-related immunity during carcinogenesis has been demonstrated recently [25,26]. The potential mechanisms involve angiogenesis and promotion of tumor growth by cytokine induction in the tumor microenvironment and activating the oncogenic signal Stat3 [27-29]. As we reported that serum IL-17 levels were positively correlated with serum AFP, it may act as a noninvasive marker for HCC screening and recurrence monitoring [29]. Our study was in accordance with Wu et al. [11] where the increased serum IL-17 level was not significantly influenced by the tumor intrinsic characteristics (tumor size and number). Liao et al. [30] found that IL-17 was over expressed in HCC tumors cells and associated with HCC recurrences and poor survival [31].

In conclusion, IL-17 levels were increased with increasing liver disease progression and chronicity. Thus, IL-17 may be an important biological marker for the immunopathogenesis of chronic hepatitis, liver fibrosis and HCC. Thus, Blocking of IL-17 expression may be a potential target for controlling the inflammatory response in chronic hepatitis and liver fibrosis, hence, the development of cirrhosis and HCC. Further studies are recommended to establish the role of infiltrating IL-17+ cells in the pathogenesis of liver diseases in conjunction with the measurements of serum IL-17 cytokine.

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