Role of Th1/Th2 Balance in the Pathogenesis of Diabetes Mellitus Complicating Chronic Hepatitis C

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Abstract

Background and Aim: Hepatitis C virus (HCV) infection is a major cause of morbidity and mortality worldwide. HCV mainly affects the liver, but also several other tissues outside the liver have been reported to be involved, resulting in a wide spectrum of extra hepatic manifestations. The association between HCV and type 2 diabetes mellitus has been described. Although the specific mechanisms involved in the pathogenesis of diabetes associated with HCV remain to be elucidated, High levels of proinflammatory cytokines have been found in HCV-infected patients and, thereby, they could be involved in the pathogenesis of insulin resistance associated with HCV.

The aim of present work was to study the role of Th1/Th2 balance (by assessing the levels of IL-2 IL-10) in the pathogenesis of DM in patients with chronic HCV infection.

Methods: Twenty diabetic patients with HCV and 20 non diabetic patients with HCV were studied in addition to 15 healthy control subjects after full history taking and clinical examination, IL-2 and IL-10 were assayed for the all studied groups.

Results: Both HCV groups had higher IL-10 and lower IL-2 in comparison to control group. Diabetic patients showed significantly lower IL-2 levels compared to non diabetic patients (2.81 ±0.67 versus 3.69±0.92 pg/ml, p<0.05). On the other hand, IL-10 levels were found to be significantly higher in diabetics compared to non diabetic patients (10.11 ±2.90 versus 7.85±1.94pg/ml, p<0.05). The correlation between IL-2 and different variables in the different studied groups showed that IL-2 correlated positively to serum albumin and negatively to ALT, AST, serum bilirubin (total and direct), FBS and IL-10. On the other hand, The correlation between IL-10 and different variables in the different studied groups showed that IL-10 correlated positively with ALT, AST, S.bilirubin (total and direct), FBS and negatively to serum albumin and IL-2.

Conclusion: It was concluded that Th1/Th2 (i.e. IL2/IL10) imbalance in favor of Th2 is present in chronic HCV infected patients and these changes are much more apparent in those who developed DM. This may open a gate for trials to treat chronic HCV infection and prevent the occurrence of DM in patients with HCV infection by restoring the disturbed Th1/Th2 balance. Therapy with IL-2 cytokines that elevate Th1 response may be tried to prevent the development of DM in HCV infected patients.

Key Words: Hepatitis C virus – Type 2 diabetes mellitus – Th1/Th2 balance – Interleukin 2, 10.

Introduction

HEPATITIS C virus (HCV) infection is a major cause of morbidity and mortality worldwide [1]. There are about 170 million persons having hepatitis C infection, all over the world [2]. Its prevalence varies from region to region with the highest prevalence (15-20%) has been found in Egypt [3]. According to an estimate, at least 50% of infected persons will develop chronic hepatitis and 20% of them will pass to cirrhosis [4]. Also it is estimate that 27% of cirrhotic patients and 25% of patients with hepatocellular carcinoma are infected by HCV [5,6].

The overall majority of experimental studies and clinical observations among patients with HCV infection favour immune mediated hepatocellular damage over direct viral cytopathic effect. An imbalance between T helper-1 (Th1) and T helper-2 (Th2)-like cytokines has been described in several chronic infectious diseases [7]. In many cases, HCV infection is fought with both arms of the immune system (i.e., humoral mediated immunity by Th2 lymphocytes and cellular mediated immunity Th1 lymphocytes. The interplay between Th1 and Th2 cytokines may be important in regulating hepatocellular damage and disease progression. The Th1 cytokines, mainly IL-2 and interferon (INF- γ) are required for host antiviral immune response [8], on the other hand, the Th2 cytokines, mainly IL-10 and IL-4 can inhibit these effector mechanisms [9]. So activation of Th2 lymphocytes in acute hepatitis C is correlated with chronicity, whereas predominant Th1 activity is predictor of recovery [7].

HCV mainly affects the liver, but also several other tissues outside the liver have been reported to be involved, resulting in a wide spectrum of
extra hepatic manifestations [10]. Mixed cryoglobulinemia, Sjögren syndrome, and chronic polyarthritis are the most documented rheumatologic extrahepatic manifestations of HCV infection. The most frequent and clinically important extrahepatic endocrine manifestations of chronic HCV infection are thyroid disorders and type 2 diabetes mellitus [11].

The association between hepatic cirrhosis and type 2 diabetes mellitus has been described since the last decade [12]. The prevalence and incidence of type 2 diabetes are higher in individuals infected by the HCV with or without cirrhosis, when compared to patients with other chronic hepatic diseases. These findings suggest that HCV would be a factor leading to glucose intolerance and diabetes [13]. Although the specific mechanisms involved in the pathogenesis of diabetes associated with HCV remain to be elucidated, it seems that insulin resistance plays an essential role [14,15]. High levels of proinflammatory cytokines have been found in HCV-infected patients and, thereby, they could be involved in the pathogenesis of insulin resistance associated with HCV [16].

The aim of present work was to study the role of Th1/Th2 balance (by assessing the levels of IL-2 IL-10) in the pathogenesis of DM in patients with chronic HCV infection.

Subjects and Methods

This study was conducted on 55 subjects who were selected from patients in internal medicine department at Ain Shams University Hospitals. All participants were subjected to the following after written consent: full history taking and detailed clinical examination Fasting (FBS) and postprandial blood sugar, liver function tests, viral marker including (HCV Abs and HBs Ag using ELISA technique), abdominal ultrasonography, liver biopsy in HCV infected patients and assay of serum IL-2 and IL-10. Subjects were divided into three groups matched in age and sex.

Group I: Included 20 HCV seropositive patients with liver cirrhosis and Diabetes mellitus. They were diagnosed to be diabetic after discovery of HCV infection; this group consisted of 13 males and 7 females with mean age of 49 ±3.6.

Group II: Included 20 HCV seropositive patients with liver cirrhosis, they were non diabetics 13 males and 7 females, with their mean age 48 ±4.9 years.

Group III: Include 15 control subjects with no history of previous liver affection, normal liver function test, normal abdominal ultrasonography, all of them were non-diabetics and seronegative for HCV antibodies and HBs Ag. They were 10 males and 5 females with mean age of 50 ±6.4.

Methods:

Specimen collection:

Three milliliters of venous blood were collected using aseptic venipuncture technique. Serum was separated by centrifugation and kept at -20ºC till being tested.

IL-10 assay:

The serum level of IL-10 were determined by human IL-10 which is a quantitative microtitrative solid phase competitive enzyme immunoassay kit. IL-10 antibodies, biotinylated IL-10 conjugate and non-biotinylated IL-10 (either in the standard or unknown sample) are mixed. The IL-10 antibodies bind specifically to the wall of the wells, the biotinylated IL-10 and the non-biotinylated IL-10 compete for the antibody binding sites. By the end of the assay, after addition of a streptavidin alkaline phosphatase conjugate and a chromogenic substrate, a color was developed which is inversely proportional to the concentration of IL-10 in the sample. This concentration was determined by the reading of the OD at 490nm using a spectrophotometer, then plotting the reading on a standard curve. This curve was plotted on a semi-log graph paper having a sigmoidal shape that showed an inverse relationship between OD and IL-10 concentration.

IL-2 assay:

Human IL-2 kit was used to detect the levels of this cytokine. It is also a competitive enzyme immune assay. It has the same principle, test procedure and interpretation as Human IL-10 assay kit.

Statistical analysis:

Data were analyzed statistically using t test, Chi square tests, ANOVA test (analysis of variance) and pearson correlation coefficient test.

Results

Tables (1, 2 and 3) showed comparison between IL-10 levels in the three studied groups. IL-10 (Th2 activity) is significantly increased in both patients groups (group I, II) as compared to control group (group III) (10.11 ±2.90 and 7.85 ±1.94 versus 3.80±0.65 pg/ml, p<0.01) (Tables 1,2) and on comparing IL-10 levels between both patients groups, HCV diabetic patients (group 1) showed significant higher levels compared to non diabetic patients (group 2) (10.11 ±2.90 versus 7.85 ±1.94 pg/ml, p<0.05) (Table 3).
Tables (4, 5 and 6) showed comparison between IL-2 levels in the three studied groups. IL-2 (Th1 activity marker) is significantly reduced in patients groups (group I and group II) as compared to control group (group III) (2.81 ± 0.67 and 3.69 ± 0.92 pg/ml versus 6.59 ± 1.53, \( p<0.01 \)) (Tables 4, 5). While on comparing IL-2 in both patients groups (Table 6), diabetic patients infected with HCV (group I) showed lower IL-2 compared to non-diabetic infected patients (group II) (2.81 ± 0.67 versus 3.69±0.92 pg/ml, \( p<0.05 \)).

Fig. (2) show comparison between IL-2 levels in the three studied groups. There was a highly significant difference between the three groups (\( p<0.01 \)).

The correlation between IL-10 and different variables in the different studied groups showed that IL-10 correlated positively with ALT, AST, S.bilirubin (total and direct), FBS (\( p<0.01 \)) and negatively to serum albumin and IL-2 (\( p<0.01 \)). On the other hand, the correlation between IL-2 and different variables in the different studied
groups showed that IL-2 correlated positively to serum albumin \((p<0.01)\) in all the studied groups and negatively to ALT, AST, serum bilirubin (total and direct), FBS and IL-10 \((p<0.01)\).

In the present study, diabetic patients with HCV infection (group I) have shown a significant increase in IL-10 (marker of Th2) and significant decrease in IL-2 (marker of Th1) in comparison to non-diabetic patients with HCV (group II) and control (group III) i.e., Th2 predominance.

Several possible mechanisms have been postulated to link HCV infection to diabetes mellitus. It may be possible that HCV could infect the pancreatic B cell or HCV may trigger autoimmune destruction of endocrine pancreatic tissue. Autoimmunity directed to B-cells was suggested but disproved as no correlation was found between HCV infection and islet cell antibodies [18].

In the present study, development of type 2 diabetes mellitus in HCV infected patients was associated with increased Th2 predominance. The role of raised Th2 type cytokines in developing diabetes in HCV patients is not so far understood. Is it islet cell function inhibition? Is it due to acute phase response-induced by the cytokines acting on pancreatic \(\beta\)-cell? Or on adipose tissues? Is it due to brain stimulating diabetogenic hormone secretion? or through inhibition of tyrosin kinase activity of the insulin receptors? All these questions need to be verified [23].

In a designed study to assess plasma IL-10 dynamics in chronic hepatitis C patients during combined antiviral treatment with pegylated interferon + Ribavirin, there was a statistically significant decrease in IL-10 plasma values after 12 weeks of Pegylated interferon and Ribavirin in patients with chronic HCV [24]. That is to say that treatment with interferon decreased the level of Th2 cytokines (IL-10) which paralleled a decrease in HCV RNA. Thus, modulation of the T cell function and cytokine production may be one mechanism whereby INF therapy results in reduced viral burden [28]. Also, immunization with HCV structural and non-structural antigens could induce IL-2 (T helper-1 response) which is likely a pivotal factor influencing the control and clearance of HCV infection [26].

Also a recent study to assess the association of DM in HCV patients to the severity of hepatic fibrosis and to the response to antiviral treatment and to assess the safety of pegylated interferon and ribavirin combination therapy in diabetic HCV patients showed that diabetic patients with HCV

**Fig. (2):** Comparison between IL-2 levels (pg/ml) in the three studied groups.

**Discussion**

In addition to liver involvement, HCV infections can cause extrahaepatic diseases such as essential mixed cryoglobulinemia, sporadic porphyria cutanea tarda, DM and thyroid disorders, probably due to an interaction between HCV and the host immune system [17]. A previous work reported that patients infected with HCV are 3-5 times more likely to have type-2 DM than those without HCV [18]. Type-2 DM and insulin resistance syndrome have been hypothesized to constitute manifestations of an ongoing acute-phase response. Thus HCV might stimulate an immunological reaction that leads to a high prevalence of type-2 DM [19].

In the present study, non-diabetic patients with HCV infection (group II) have shown a significant increase in IL-10 (marker of Th2) and a significant decrease in IL-2 (marker of Th1) when compared to controls (group III) i.e., Th2 predominance.

Experimental data from animal models and clinical data from patients suggest that inflammation-associated cytokines including pro-inflammatory cytokines such as IL-2, (INF\(\gamma\)), TNF-\(\alpha\) and TGF-\(\beta\), and anti-inflammatory cytokines such as IL-10, are involved in the development of liver injury [9,20]. The effects of IL-10 have been observed in viral or autoimmune hepatitis, alcoholic liver disease, and animal models. Patients with a strong Th1 response (high levels of IL-2) during acute HCV infection can clear the virus, while patients presenting with a Th2 response (high levels of IL-10) evolve into chronicity [21,22].

In the present study, diabetic patients with HCV infection (group I) have shown a significant increase in IL-10 (marker of Th2) and significant decrease in IL-2 (marker of Th1) in comparison to non-diabetic patients with HCV (group II) and control (group III) i.e., Th2 predominance.

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Also a recent study to assess the association of DM in HCV patients to the severity of hepatic fibrosis and to the response to antiviral treatment and to assess the safety of pegylated interferon and ribavirin combination therapy in diabetic HCV patients showed that diabetic patients with HCV
was associated with more hepatic fibrosis, poor response to antiviral therapy and more adverse events during antiviral therapy compared to non-diabetic HCV patients [27].

Our study showed that IL-2 (marker of Th1 response) was correlated positively with serum albumin and negatively with liver enzymes and blood glucose i.e., the high Th1 response, the better the liver function and less development of DM. On the contrary increased IL-10 (marker of Th2) was positively correlated to liver enzymes and blood sugar, and negatively correlated to serum albumin i.e., Th2 response was associated with more deterioration of liver functions and increase in blood glucose level. It was reported that individuals who have vigorous Th1 response to HCV antigen seem to be able to clear the virus, in particular as the production of Th1 cytokines is associated with protective antiviral immune responses, whereas the predominance of Th2 cytokines may lead to disease exacerbation [26].

In conclusion, Th1/Th2 imbalance in favor of Th2 (indicated by high IL-10 and low IL-2) is present in chronic HCV infected patients and this may explain the association of HCV with DM as these changes were much more apparent in patients with HCV who developed DM. This may open a gate for trials to treat chronic HCV infection and prevent the occurrence of DM in patients with HCV infection by restoring the disturbed Th1/Th2 balance. Therapy with IL-2 cytokines that elevate Th1 response may be tried to prevent the development of DM in HCV infected patients.

References


