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ALTERATION OF IMMUNE MEDIATORS IN PATIENTS WITH HEPATITIS B AND C VIRUS INFECTION

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ABSTRACT

HBV and HCV are the major causes of chronic liver diseases throughout the world, and constitute a major global health risk. There is accumulated evidence that the imbalance of pro-inflammatory and anti-inflammatory cytokine production may play an important role in the pathogenesis of viral hepatic infections and may influence the clinical outcome and disease progression.

This study was undertaken to analyze the circulating levels of Tumor Necrotic Factor (TNF-α) and Th2 cytokine IL-10 in patients infected with Hepatitis B and C virus. The study population consisted of 30 patients with chronic HBV, in addition to other 30 patients with chronic HCV infection were recruited on their first examination at the Al-Kindy General Hospital in Baghdad city. Another 12 healthy individuals with negative hepatitis serology as normal controls were observed.

TNF-α level was significantly increased in chronic HBV infected patients compared with normal controls (6.81± 1.25 vs. 5.62± 1.71 pg/ml, p= 0.001). Similarly, the levels of the TNF-α was significantly elevated in HCV patients (8.62± 0.79 pg/ml) after comparison with its level in HBV patients (p= 0.023). Serum levels of Th2 cytokines IL-10 were also elevated in chronic HBV infected patients (25.05± 3.90 pg/ml) and in HCV infected patients (28.07± 3.35 pg/ml)

but these values failed to reach the significant level when compared with normal control (17.54± 1.39 pg/ml). Furthermore, the serum levels of TNF-α and IL-10 were lower in HBV infected individuals compared with that of HCV infected individuals, but these levels were not significant. There were no significant differences in the serum levels of ALT and AST, although the levels were higher in patients with HBV than HCV infected patients (P >0.05). Serum level of bilirubin was significantly higher in patients with HCV than its corresponding level in HBV infected patients (p= 0.001). In conclusion, our data indicate higher levels of TNF-α particularly in chronic HBV+ patients than controls**,** but less than its level in HCV+ patients with significant correlation between serum TNF-α and IL-10 levels and indices of hepatic injury and both cytokines participate in the immune impairment of HBV and HCV infections..

Keywords: HBV, HCV, TNF-α, IL-10, ALT, AST, Bilirubin

INTRODUCTION

Hepatitis is a serious global problem due to its high morbidity and mortality worldwide. HBV and HCV are the major causes of chronic liver diseases throughout the world, and constitute a major global health risk. WHO estimated that about 350 million people are chronically infected with hepatitis B and 170 million people or 3% of the world's population are infected with HCV **[1]**. Infection with hepatitis B virus (HBV) leads to a wide spectrum of clinical presentations ranging from a symptomatic carrier state to self- limited acute or fulminate hepatitis to chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma **[2]**.

HBV is a member of hepadnavirus group, is small DNA (double stranded circular DNA molecule), which replicated usually by reverse transcription **[3]**. HBV was originally the cause of serum hepatitis, and is the most common form of parentally transmitted viral hepatitis, about 90% of infants infected parentally **[4]**.

Hepatitis C virus (HCV) is a member of the Hepacivirus, has single strand RNA genome. It is a virus predominantly of humans although primates can be infected. This virus, like HBV is transmitted by blood transfusion and body fluids. In 80% of cases the virus infection fails to be cleared and a persistent infection is established **[4]**. Hepatitis C virus mainly affects the liver, but also several tissues outside the liver have been reported to be involved, resulting in a wide spectrum of extrahepatic manifestations **[5]**. The most frequent and clinically important endocrine HCV are thyroid disorders and type 2 diabetes mellitus **[6]**. Gulcan *et al.*, estimated that

seropositivity rates of HbsAg and anti- HCV were 5.1% and 3.2% in diabetic patients, and were 3.8% and 1.3% in control group respectively **[7]**.

The host response to hepatitis involves different components of the immune system including T- cell cytokines which have various roles in the clinical manifestation of the disease. The T- helper (Th1) cytokines interferon- gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and interleukin (IL) - 2 are related with cell- mediated immunity and suggested to be associated with recovery **[8]**, while the Th2 cytokines IL-4 and IL-10 mediates humoral immune responses and may take place in the progression or persistence of chronic infection **[9,10]**. There is accumulated evidence that the imbalance of pro-inflammatory Th1 and anti-inflammatory Th2 cytokine production may play an important role in the pathogenesis of viral hepatic infections and may influence the clinical outcome and disease progression **[10, 11]**. Many researchers believe that cytokines play important roles in both immunoregulation and immune impairment **[12]**.

This study was undertaken to analyze some circulating cytokine levels in individuals infected with Hepatitis B and C virus. We measured serum levels of proinflammatory cytokine TNF-α, and anti-inflammatory Th2 cytokine IL-10 in such patients.

MATERIALS AND METHODS

The study was approved by the Medical Ethics Committee of Al-Kindy College of Medicine, University of Baghdad (Iraq). The study population consisted of 30 patients with chronic HBV, in addition to another 30 patients with chronic HCV infection were recruited on their first examination at the Al-Kindy General Hospital in Baghdad city. Those patients showed the characteristic clinical symptoms of hepatitis infection: jaundice, anorexia, and serum alanine aminotransferase (ALT) concentrations above the normal value of 40 U/ ml. Another 12 uninfected healthy individuals of both genders matched for sex and age without any clinical history of hepatitis, with negative hepatitis serology, and considered healthy through routine laboratory analysis were recruited as a control group. All subjects gave written informed consent. All patients underwent a complete medical and laboratory evaluation including a liver ultrasound scan. The results of which were used to divide the patients into two groups on the basis of the presence of HBV infection or HCV infection and the demographic distribution of the study groups were represented in **Table 1**.

Sample Collection and Methods

A 10 ml sample of peripheral blood was withdrawn from each individual. The blood sample was centrifuged at 3000 rpm for 10 minutes to separate out the serum, divided into aliquots and stored at – 20^0C until it was analyzed. Hepatocellular injury, as revealed by alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum bilirubin levels was detected by Abbot Diagnostic System (Abbott Diagnostic- USA). According to manufacturer's instruction, normal values of (ALT= 6-55 U/L, AST= 5-34 U/L, S. bilirubin= 0.2-1.2 mg/dl).

Hepatitis viruses were determined in serum samples of patients by serological methods of the immunoglobulins IgG class specific for HBV, and HCV. The diagnosis of chronic HBV infection was based on the detection of HBsAg using EIA Test Kit (Foresight, ACON, USA) in the patient's serum, in association of the detection of high levels of ALT above the normal value. The samples initially reactive for HBsAg were analyzed in duplicate to confirm the infection. According to manufacturer's instruction, the specimens with absorbance greater than or equal to the Cut-off value are considered positive. RecombiLISA HCV Ab test is a solid phase ELISA for the qualititative detection of IgG antibodies to HCV infection in human serum (CTK- BIOTECH, USA). According to manufacturer's instruction if the specimen OD ratio is $\geq$ 1.00, it considered positive result. The serum samples of the patient and control groups were analyzed for TNF-α and IL-10 by sandwich ELISA technique using commercial kits according to the manufacturer's instructions (US Biological, USA).

Patients show serological evidence of co-infection with other hepatotropic viruses, and those with autoimmune diseases and who had been vaccinated against HBV were excluded from the study. Other possible cause of hepato-cellular injury, such as alcohol and drug-related injuries were also excluded.

Statistical Analysis

SPSS.15 under window XP for statistical analysis is used for descriptive (mean± SE) and inertial use t-test for significant. P values less than 0.05 were considered as statistically significant while P-value more than 0.05 was considered as statistically not significant. Sperman- Person correlation (r) test was used in the evaluations of the relation among cytokines, and between cytokines and other parameters (biochemical liver function test).

RESULTS

Results represented in **Table 2** demonstrated there were no significant differences in the serum levels ALT and AST, although the

levels were higher in patients with HBV than HCV infected patients (P >0.05). The serum level of bilirubin was significantly higher in patients with HCV than its corresponding level in HBV infected patients (p= 0.001).

The mean levels of serum cytokines TNF-α and IL-10 was represented in **Table 3** and **Figure 1**. TNF-α level was significantly increased in chronic HBV infected patients compared with normal controls (6.81± 1.25 vs. 5.62± 1.71 pg/ml, p= 0.001). Similarly, the levels of the TNF-α was significantly elevated in HCV patients (8.62± 0.79 pg/ml) after comparison with its level in HBV patients (p= 0.023).

Serum levels of IL-10 were also elevated in chronic HBV infected patients (25.05± 3.90 pg/ml) and in HCV infected patients (28.07± 3.35 pg/ml) but these values failed to reach the significant level when compared with normal control (17.54± 1.39 pg/ml). Furthermore, the serum levels of TNF-α and IL-10 were lower in HBV infected individuals compared with that of HCV infected individuals, but these levels were not significant.

Sperman- Person correlation test is used to analyze the relation of TNF-α and IL-10 cytokines with the serum activity of ALT, AST and bilirubin in HBV and HCV infected patients. There was a significant negative linear correlation between TNF-α and serum AST level in HBV infected patients (r = -0.583, p= 0.022) as represented in **Figure 2**. Anon significant linear correlated were demonstrated between the TNF-α and serum ALT and bilirubin in HBV infected patients (r = - 0.386, 0.381 respectively, p> 0.05).

On the other hand, there was a significant negative linear correlation between IL-10 and serum ALT level in HBV infected individuals as represented in **Figure 3**, (r = 0.410, p= 0.048). Anon significant linear correlation between the IL-10, serum AST and bilirubin were found in HBV infected patients (r = 0.198, 0.251 respectively, p> 0.05). Concerning the HCV infected patients, there were negative linear correlation between TNF-α and serum ALT, AST levels failed to reach significant levels (r= -0.361, -0.023 respectively, p> 0.05). On the other hand a significant negative linear correlation was demonstrated between the TNF-α and serum bilirubin (r = -0.441, P= 0.048) as shown in **Figure 4**. **Figure 5**, represented a significant negative linear correlation between the IL-10 and serum AST (r= -0.663, P= 0.006) and also IL-10 with serum bilirubin (r = -0.491, P= 0.049) in HCV infected persons. But there was no significant linear correlation between the IL-10 and serum ALT in those patients (r=0.099, p>0.05).

Table 1: Demographic Characteristics of the Patients and Control Group

Control group	HBV	HCV	Patients groups
Number	30	30	12
Sex: Males (%)	22 (73.3)	18 (60)	7 (58.3)
Females (%)	8 (26.7)	12 (40)	5 (41.7)
Age interval (year)	14-65	11-75	13-60
Mean± SE	42.3±3.9	47.6±4.4	38.3±4

Table 2: Biochemical Characteristics of the Patients Groups (Mean ± SE)

Groups / Characteristics	HBV	HCV
Serum ALT level (IU/L)	218.96 ± 25.58	199.57 ± 45.94
Serum AST level (IU/L)	171.34 ± 13.98	156.56 ± 13.56
Serum total bilirubin level (mg/dL)	22.53 ± 0.72	*32.39 ± 2.04

NOTE: SE: Standard Error, *p= 0.001

Table 3: Serum Cytokine Levels of the Study and Control Groups (mean ± SE)

	Patients		Control
	HBV	HCV	
TNF-α (pg/ml)	*6.81± 1.25	†P 8.62± 0.79	5.62± 1.71
IL-10 (pg/ml)	25.05± 3.90	28.07± 3.35	17.54± 1.39

NOTE: Mean± Standard Error, *p= 0.001 HBV Infected Patients vs. Controls, †P= 0.023 HCV Infected Patients vs. HBV Infected Patients

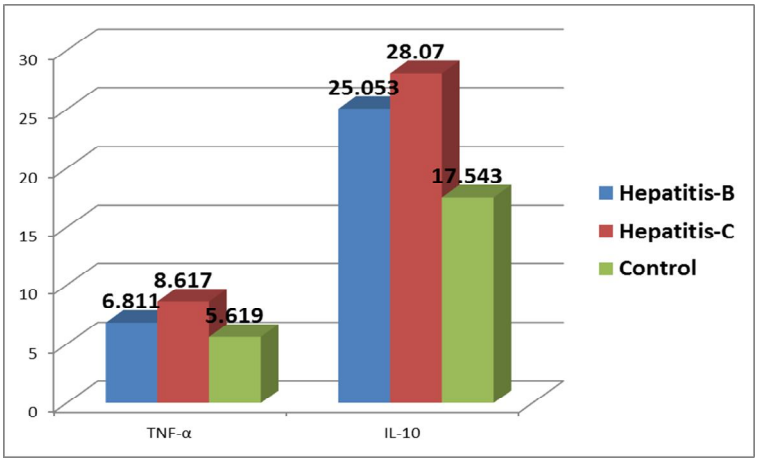

Figure 1: TNF-α levels and IL-10 levels in HBV, HVC Infected Patients and Healthy Controls

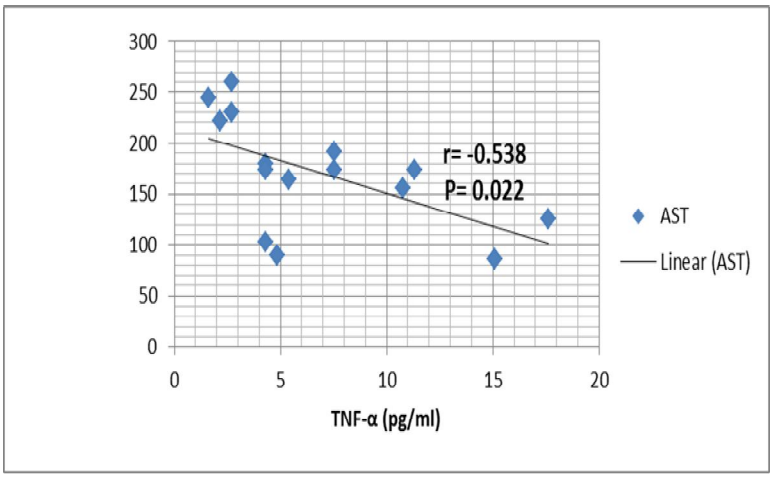

Figure 2: Person Correlation of TNF-α and Serum AST in HBV Infected Patients

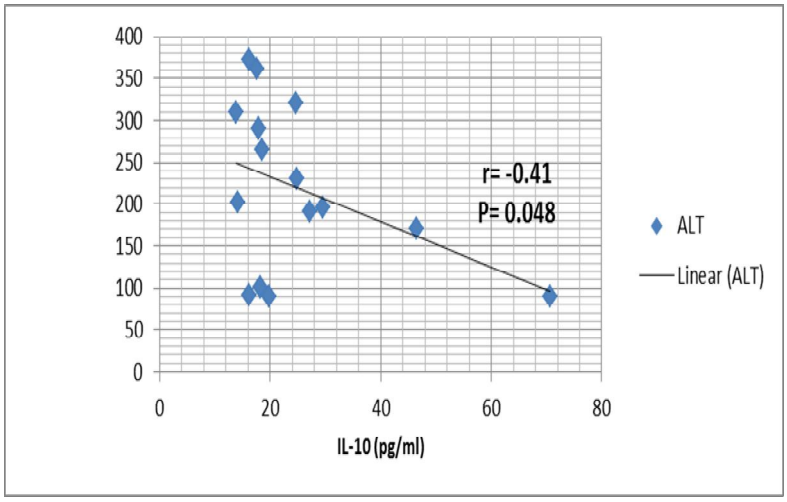

Figure 3: Person Correlation of IL-10 and Serum ALT in HBV Infected Patients

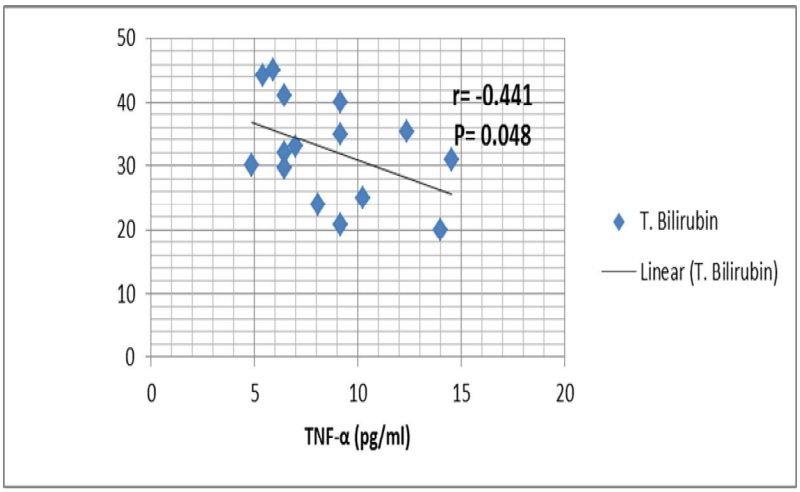

Figure 4: Person Correlation of TNF-α and Total Serum Bilirubin in HCV Infected Patients

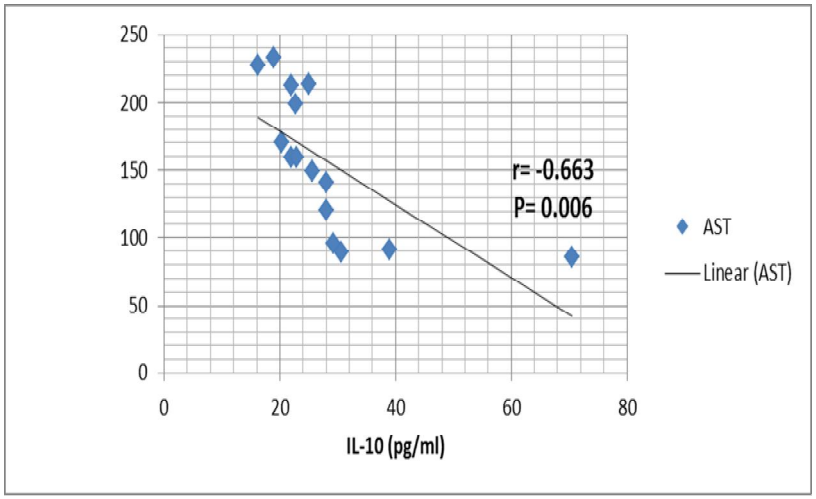

Figure 5: Person Correlation of IL-10 and Serum AST in HCV Infected Patients

DISCUSSION

Cytokines are mediators of various biological processes including inflammation, apoptosis, necrosis, and fibrosis. Viral dissemination is mainly limited by cellular immunity of the host particularly cytotoxic T lymphocytes (Tc). T-cell receptors are activated by the complex of HLA class I and II and viral proteins, during which CD+4 and CD+8 T lymphocytes play a role. The CD+4 cells contribute the central role in cytokine secretion and enhance the CD+8 cells to produce cytokines **[13]**. The Tc lymphocytes response to HBV is relatively weak in patients with chronic HBV infection, except during interferon induced recovery. Studies in the HBV transgenic mouse model revealed that, virus- specific T cells as well as NK cells can abolish HBV expression and replication without killing the hepatocytes and this antiviral activity is mediated by interferon gamma and TNF-α **[14]**.

In this study, high levels of TNF-α was demonstrated in patients infected with HBV and was significantly higher than those of the controls (p= 0.001), and also significantly elevated in HCV after comparison with HBV infected individuals (p= 0.023). This result is in agreement with the finding of Akpolat *et al*., and Falasca *et al*., **[15, 16]** and other *in vitro* studies **[17, 18]**, suggesting that TNF-α may play a key role in inhibition of viral replication and ongoing process of HBV and HCV elimination. Moreover, TNF-α resembles a multifunctional cytokine and has been shown to be essential for normal liver regeneration and proliferative response **[19]**. Since TNF-α may not has a mitogenic effect on hepatocytes, it has been suggested that it may primes hepatocytes to responds to growth factors such as hepatocyte growth

factor **[20]**, and has been shown to act as an anti-fibrotic agent in experimental liver fibrosis **[21]**. Although HCV is a hepatotropic virus, there is some evidence that it may also be lymphotropic. Although HCV is not known to be cytopathic, HCV infection of Dendritic Cells (DC) appears to impair function **[22]**. Flower, *et al.*, found that TNF-α is considered to be a weak inducer of DC maturation in HCV- positive patients and allow persistent viral infection **[23]**.

IL-10 is an important anti-inflammatory cytokine secreted from Th2 cells. The levels of IL-10 production determine immunoregulation, and the balance between the inflammatory and humoral responses. The current study showed that the increased production of Th2 (IL-10) cytokine is present in both HBV and HCV infected patients, but not significantly different from controls. This result is in agreement with another observation reported by **[10]**. It is also found that persistent infection with HBV and HCV-RNA positive expression was associated with IL-10-1082 AA genotype which showed a positive correlation with clinical progression of disease **[24]**. The value of IL-10 showed a significant correlation with AST in HCV infected patients which agree with **[25]**. In contrast, no significant association had shown with plasma ALT level, which agrees with the results of **[26]**. Fan *et al*., documented a Th2 type immune response during chronic active hepatitis C infection **[10]**. A strong positive correlation of IL-10 cytokines and glutathione reductase with increasing viral load in HBV and HCV was observed, suggested of its involvement in the impairment of the immune system, and such impairment can increase the risk of developing hepatic carcinoma **[27]**. These cytokines recruit inflammatory cells, promote fibrogenesis and further activate oxidative burst **[21]**.

Previous studies demonstrate that Th2 cells may be associated with the persistence of HBV infection **[28]**. The value of IL-10 showed a significant correlation with ALT in HBV infected patients. Moreover, Hepatocellular injury was probably caused by the decreased production of the anti-inflammatory cytokine IL-10 **[24]**. In contrast, there is evidence that HCV+ patients present a Th1 type immune response with over-production of IFN-γ **[29, 11]**. In view of negative regulation of Th2 cytokines for immune functions, we consider that enhanced Th2 reaction is at least partly responsible for immunopathogenesis of chronic HCV infection. The enhanced Th2 responses may allow the human host to suppress the inflammatory immune responses, resulting in reducing the hepatic tissue injury through

down-regulation of the inflammatory/immune reaction and leading to inability to eliminate the virus **[30]**. This is one possible explanation why HBV and HCV infection tends to be a chronic condition.

In conclusion, our data indicate higher levels of TNF-α particularly in chronic HBV+ patients and HCV+ patients than controls, and significant correlation between serums TNF-α and IL-10 levels and indices of hepatic injury. Both TNF-α and IL-10 participated in the immunopathogenesis and immune impairment of HBV and HCV infections.

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