

Assessment of IFN-Gamma and IL-10 in Acute HBV Patients

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The host immune response to hepatitis B virus is a critical factor in determining the outcome of HBV infection. An effective cytokine response is thought to be one of the reasons for the failure to suppress hepatitis B virus (HBV) replication and to eliminate the virus. We investigated the serum levels of Interferon (IFN)- γ and Interleukins (IL)-10 in acute HBV infected patients to assess whether they were related to the outcome of HBV infection. Ninety consecutive patients of acute hepatitis B were included in this study. They were first screened by all serological markers for HBV followed by polymerase chain reaction (PCR). IFN- γ and IL-10 levels were estimated in the serum of all acute HBV patients and 20 healthy blood donors by ELISA (Orgenium, Finland). Among 90 acute HBV patients, there were 54 males and 36 females and their age group ranged from 18 to 70 years. Elevated SGOT, SGPT and serum bilirubin level were found in majority of patients. Necrotizing inflammation was observed in all the patients. Mean value of IFN- γ and IL-10 in healthy blood donor were 11.329 pg/ml and 12.7902, respectively. While mean value of IFN- γ and IL-10 in acute HBV infected patients were 17.37pg/ml and 83.497pg/ml, respectively ($p < 0.001$). Measurable amount of these two cytokines were significantly higher in acute HBV infected patients compared to healthy blood donors. 60 and 63 patients showed increased IFN- γ and IL-10 serum level respectively. However, IL-10 serum levels were more significantly elevated than IFN- γ . IL-10 is a potent immunosuppressive cytokine that can inhibit both innate and adaptive immunity. Based on our results, it can be concluded that higher IL-10 level can suppress immune response to HBV infection in acute HBV patients. IL-10 may play an anti-inflammatory role. IL-10 therapy could prove useful in controlling necrotizing inflammation in acute HBV patients.

Key words: Hepatitis B , IL-10,IFN Gamma, ELISA.

Hepatitis B virus (HBV) is a hepatotropic, non-cytopathic DNA virus (3.2 kb partially double-stranded DNA) that causes acute and chronic hepatitis. Hepatitis B virus (HBV) infection results in a broad spectrum of liver diseases, ranging from asymptomatic carriers to self-limiting acute hepatitis, chronic infection and fulminant hepatitis leading to liver failure (Acharya *et al.*, 2000). The

interactions between HBV replication and immune responses against HBV infection play an important role in determining the outcome of virus infection (Rehermann B *et al.*, 2003). The mechanism of hepatocellular injury in patients with chronic HBV infection is immune-mediated (Dudley *et al.*, 1972; Millich, 1991). The host immune response to hepatitis B virus is a critical factor in determining the outcome of HBV infection. An ineffective cytokine response is thought to be one of the reasons for the failure to suppress hepatitis B virus (HBV) replication and to eliminate the virus (Le song *et al.*, 2002).

IL-10 is a multifunctional cytokine with closely related homologues in several virus

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genomes, testifying to its crucial role in regulating immune and inflammatory responses (Moore and de Waal Malefyt, 2001). IL-10 inhibits a broad spectrum of activated macrophages monocyte functions including cytokine synthesis, production of reactive oxygen intermediates. Also it downregulates class-II MHC expression and consequent impairment of the accessory cell functions of macrophages and dendritic cells.

In this study, we investigated the serum levels of IFN- γ and IL-10 in acute HBV infected patients to assess whether they were related to the outcome of HBV infection.

MATERIALS AND METHODS

Study subjects

90 consecutive cases with symptoms of acute hepatitis and 30 healthy controls were enrolled in the study and detailed clinical history was elicited from them. The patients (54 men and 36 women) ranged in age from 18 to 70 years.

Exclusion criteria

Patients with autoimmune hepatitis, alcoholic hepatitis, drug induced hepatitis, patients giving history of recent infection, surgery, trauma within the preceding two months, renal insufficiency or with other acute or chronic inflammatory diseases were excluded from this study. None of the participants had received any antiviral or immunosuppressive therapy before or during the course of study.

Serological investigations

They were first screened by various serological markers like HBsAg, HBeAg, anti-HBe, anti-HBc IgM (DRG International, Inc., USA), HBe total antibody (Bio-Rad, France), anti-HBs (Organics) followed by PCR amplification in all cases.

PCR amplification

The amplification for surface gene and core gene of HBV was carried out in a 25 μ L reaction mixture containing 12.5 μ L of 1x Master mix (Fermentas, India), 8.5 μ L of nuclease free water, 1 mL of forward primer and 1 μ L of reverse primer. To this reaction mixture 2 μ L of the extracted DNA was added. PCR amplification was carried out using a thermocycler (Labnics, USA). The cycling condition for PCR amplification includes 40 cycles with initial denaturation at 95 for 5 min followed

by 95 for 50 sec denaturation, 58 for 60 sec annealing and extension at 72 for 1 min with different sets of primers.

Primer sequences for core gene of HBV:

Primer 1; 5'-TA(C/T)TAG GAG GCT GTAGGC AT-3'

Primer 2; 5'-AGAATA GCT TGC CTGAGT GC-3'

Other investigations

Liver function tests (LFT) like serum amino alanine transaminase (ALT), serum aspartate amino transferase (AST) and alkaline phosphatase (ALP), bilirubin (direct and indirect), total bilirubin, albumin, globulin, creatinine and international normalized ratio for prothrombin time were performed.

Healthy blood donors

20 Healthy blood donors were included in the study. They had no serological markers of HAV-HEV, CMV, EBV infection and liver functions were normal.

Detection of cytokines

The serum levels of a Th1 cytokine, IFN-gamma and Th2 cytokine, IL-10 were measured by ELISA (Diaclone, France and Orgenium, Finland) in 20 healthy controls and in 90 acute HBV infected patients. The ELISA kit protocols were followed as per the manufacturer's instructions. Absorbance was read at 450nm in an automated ELISA plate reader (thermo scientific).

RESULTS

Among 90 acute HBV patients, there were 54 males and 36 females and their age group range was from 18 to 70 years (mean age: 42.5 years). Elevated SGOT, SGPT and serum bilirubin levels were found in majority of patients. All patients had necrotizing inflammation.

Serological Profile

Serological profile is given in Table 1. HBs Ag was positive in all cases, 30 (30.33) were positive for HBeAg. All cases of AHB were positive for anti HBc IgM

HBV DNA Analysis

HBV DNA was detected in 84 (93.3%) of these patients.

Association of cytokines with HBeAg status

IFN gamma levels were higher in HBeAg seronegative individuals, mean levels being 20.77

Table 1. Distribution of HBV serological markers in hepatitis B virus positive patients

Cases	HBsAg	HBeAg	anti-HBe	HBc IgM	HBc (total Antibody)	Anti-HBs	HBV DNA
AcuteHBV patients(n=90)	90(100%)	30(33.3%)	80(88.8%)	90(100%)	90(100%)	20(22.2%)	84(93.3%)

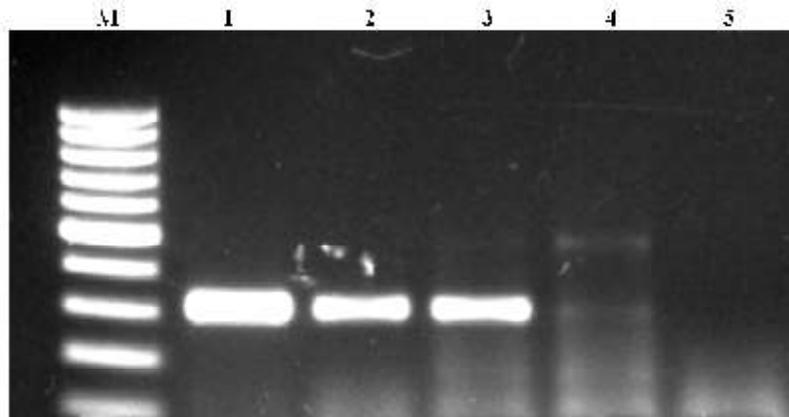
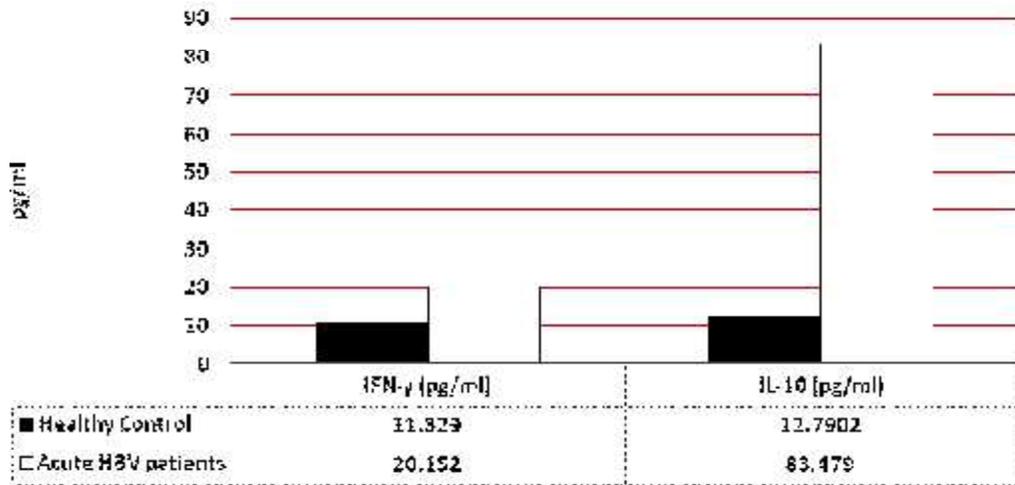


Fig. 1. Showing M, 100 bp ladder; lane 1, 2, 3, & 4 show the 307 bp product obtained with P1 and P2 primers of core gene of HBV lane 5 is an negative control

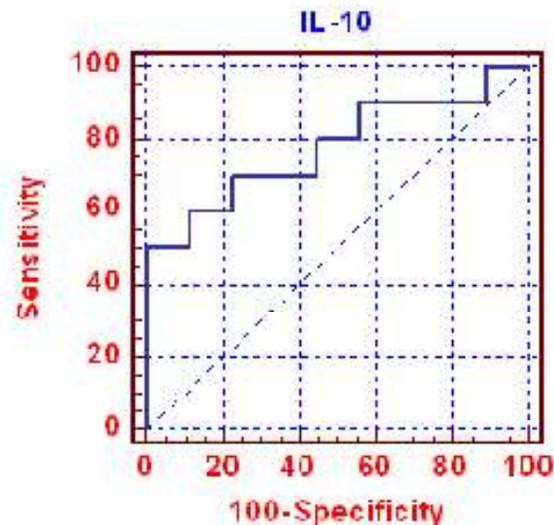


On comparison of cytokine levels between patients and controls, IL-10 and IFN- γ levels were significantly higher in the patients than in the controls. Mean serum levels of IFN- γ & IL-10 in healthy controls were 11.329 pg/ml & 12.79pg/ml respectively. Mean serum level of IL-10 (83.479pg/ml) was found more elevated in patients than healthy controls ($p < 0.001$), while mean serum level of IFN- γ were 20.152 pg/ml, $p < 0.05$

Fig. 2. Serum Cytokine levels

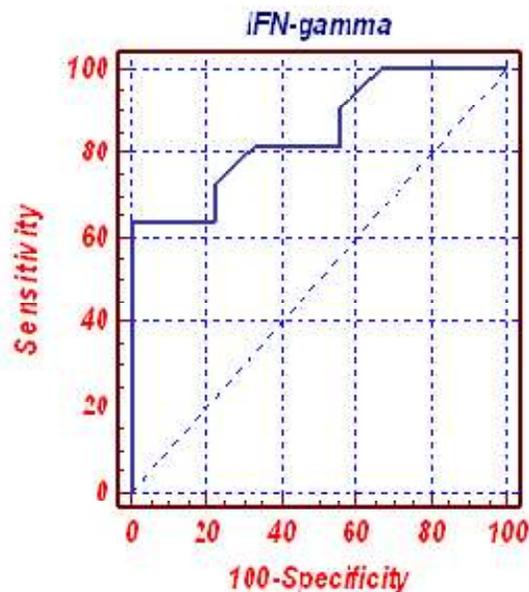
pg/ml while IL-10 levels were lower in HBeAg seronegative individuals mean levels being 73.56pg/ml in comparison to HBeAg positive

patients showing the inversely proportional relationship between IFN gamma and IL-10.



On examining the ROC curve of IL-10 (Fig. 2) in relation to acute HBV infection, significant cut off level for IL-10 was 14.99 pg/ml. The sensitivity was 60%; specificity was 100%; positive predictive value was 100%; negative predictive value was 64.3%; area under curve (AUC) was 0.778 with 95% CI= 0.531 to 0.932, $p < 0.0105$

Fig. 3. Showing Receiver Operating Characteristic (ROC) curve for IL-10



On examining the ROC curve of IFN-gamma (Fig. 6) in relation to acute HBV infection, significant cut off level for IFN-gamma was 12.466 pg/ml. The sensitivity was 63.64%; specificity was 100%; positive predictive value was 100%; negative predictive value was 69.2%; area under curve (AUC) was 0.848 with 95% CI= 0.619 to 0.965, $p < 0.001$

Fig. 4. Showing Receiver Operating Characteristic (ROC) for IFN-Gamma

DISCUSSION

In this study, we investigated the serum levels of interferon (IFN)- γ and IL-10 in acute HBV infected patients to assess whether they were related to the outcome of HBV infection. For this purpose, we measured cytokine concentrations in serum samples from patients with acute Hepatitis B infection as well as from a group of healthy uninfected control individuals. The interactions between HBV replication and immune responses against HBV infection play an important role in determining the outcome of virus infection (Rehermann *et al.*, 2003). Cytokines are likely to be involved in both the regulation of the immune responses and the direct inhibition of HBV replication. Recent studies have reported either very low or very high levels in different clinical groups. Our observations are in agreement with other studies showing that serum IFN- γ levels are elevated in HBV patients with different clinical presentations.

While T-helper type 1 cytokines (IFN- γ) are required for host antiviral immune response and are involved principally in cell-mediated immunity, T-helper type 2 cytokines (IL-10) mostly regulate humoral immune response (Bertoletti *et al.*, 1997). The immune response which is associated with a T-helper (Th)1 cytokine profile, suggests that cell-mediated immunity is associated with recovery (Hultgren *et al.*, 1998), while Th2 cytokine response takes place in development of persistent infection (Fan X G *et al.*, 1998).

The serum levels of IFN- γ and IL-10 in patients with HBV infection were higher than those in healthy blood donors. These results may suggest that IFN- γ and IL-10 are involved in the pathogenesis of acute hepatitis B. IFN Gamma levels were higher in HBeAg negative cases suggesting its greater role in controlling HBV infection in non cytolitic conditions. IFN Gamma was associated with absence of HBeAg in the serum, pointing to their possible role in decreasing viral load.

These results demonstrate that non-cytopathic antiviral mechanisms may contribute to viral clearance in acute HBV infection. On the other hand elevated levels of IL-10 were associated with HBeAg positivity which may be associated with hepatocellular damage.

Based on our results, it can be concluded that higher IL-10 level can suppress immune response to HBV infection in acute patients. IL-10 is a potent immunosuppressive cytokine that can inhibit both innate and adaptive immunity. IL-10 may play an anti-inflammatory role and monoclonal antibodies against IL-10 could prove useful in controlling necrotizing inflammation in acute HBV patients.

CONCLUSION

Based on our results, it can be concluded that higher IL-10 level can suppress immune response to HBV infection in acute HBV patients. IL-10 may play an anti-inflammatory role. IL-10 therapy could prove useful in controlling necrotizing inflammation in acute HBV patients.

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