Evaluation of Serum levels of INF-γ and IL-12 in patients with chronic hepatitis B

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The specific cellular immune response play a main role in the hepatic necrosis that occur with hepatitis B virus (HBV) infection and in the persistence or lack of viral infection.

The aim of this study was to evaluate the cellular immune response in patients with chronic hepatitis B virus (CHBV) infection, via estimation of serum levels of interferon-gamma (IFN-γ) and interleukin-12 (IL-12).

Venus blood samples were collected from 45 patients with CHBV infection. Other 20 blood samples were collected from healthy individuals as control group. Enzyme linked immune sorbent assay (ELISA) was used to estimate the serum levels of (IFN-γ) and (IL-12).

The results showed insignificant drooping in serum levels of IFN-γ and IL-12 respectively in CHBV patients group as compared with healthy control group.

ABSTRACT

The specific cellular immune response play a main role in the hepatic necrosis that occur with hepatitis B virus (HBV) infection and in the persistence or lack of viral infection.

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INTRODUCTION

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family, is a small DNA virus with unusual features similar to Retrovirus through an RNA intermediate and can integrate in host genome (1,2). Hepatitis B virus is a major cause of acute and chronic hepatitis in human. As HBV itself is currently viewed as non-cytopathic virus, the liver pathology associated with hepatitis B is mainly thought to be due to immune response directed against HBV antigens (3,4,15). In approximately 95% of adults exposure to HBV lead to acute infection that is rapidly resolved without long-term consequences, whereas the remaining 5% fail to control viral infection, leading to chronicity(5,6). The clearance of virus was clearly associated with an efficient adaptive immune response (7).

Substantial evidence exist to indicate that host innate and adaptive immune responses play a crucial role in controlling HBV replication in vivo (8). Infection with HBV in adults usually results in self-limiting acute hepatitis, which confers protective immunity and causes no farther disease. Patients with a chronic HBV infection lack a vigorous polyclonal and multispecific T-cell response and instead exhibit a weak, infective or undetectable virus-specific T-cell response.

The host protective immune response against HBV infection is mainly mediated by CD4+ and CD8+ T-cells, which secrete IFN-γ and activate cytotoxic T lymphocytes, which directly eliminate infected cells(9,10). In addition, type 2 cytokines, such as IL-4 and IL-5, may also be involved in the clearance of circulating virus by promoting the production neutralizing antibodies against the HBV surface and core antigens (11). In chronic hepatitis B (CHB), the T-cell response and circulating cytokines profile are associated with viral replication and liver function (9). Low dose of virus may trigger Th1 and cytotoxic T-lymphocytes responses, whereas high dose of virus induce a Th2 mediated, non-protective humoral response (12). The T-cell response is relatively mild infective in chronically infected patients compared to acute incidence (13), both Th1 and Th2 immunity are functionally impaired in chronic HBV patients (14). Because HBV is non-cytopathic, liver damage is thought to be immune mediated. Immune liver damage in HBV patients has conventionally been attributed to cytotoxic killing of infected hepatocytes by virus specific CD8 T-cells(15,16).

The distinguishing feature between patients with or without HBV-related chronic liver disease was the presence of large, non-antigen-specific lymphocytic infiltrate in the livers of the former group (17). Liver inflammation initiated by virus-specific CD8 is amplified by other lymphocytes (18). One of the largest constituent of lymphocytic infiltrate in HBV infection is NK cells (19-23). The CD56 subset of NK cells is known a potent source of cytokines such as...
IFN-γ (20). NK expressed tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) may play a role in non antigen-specific mediation of liver damage in CHBV infection. NK cells can directly activated to promoting cytotoxicity(24), and TRAIL expression(25), and IL-12 favoring IFN-γ production(24,26). CD4+CD25+ regulatory T-cells (Tregs) are immunosuppressive T-cells that play an essential role in controlling immune response and autoimmunity(27). Tregs may also play a role in regulating immune responses to HBV infection(28). High levels of Tregs have been detected in CHB and are thought to be responsible for chronicity of hepatitis(29).

MATERIAL AND METHODS
Forty five (15-65 years old, mean 39.5 years, 25 males and 20 females) patients with CHBV infection in Kadhamiya teaching hospital, during period from October 2008 to march 2010 were used for this study. the cause of chronic liver disease was determined using standard diagnostic criteria, chronic hepatitis B was diagnosed by positive serological tests for serum hepatitis B surface antigen (HBsAg) for at least 6 months. Twenty (18-55 years old, mean 33.4 years, 10 males and 10 females) healthy individuals were used as control group.

Five ml of venous blood were collected from each individual in plain tube. Serum were separated, put in eppendorf tubes and stored at (deep freezing -20 C°).

Immunological assays:
Enzyme-linked immune-sorbent assay (ELISA), (e Bioscience, England) and Biosource, Belgium) was used to estimate serum levels of IFN-γ and IL-12.

STATISTICAL ANALYSIS
Statistical analysis has been performed using (SPSS, version 11.0) for windows. Continuous variables were expressed as mean ±standard error (SE). data were analysed using independent sample student’s t test. Significance was assigned for p values (<0.05) with 95% confident interval.

RESULTS AND DISCUSTION
Interferon-gamma: serum levels of IFN-γ showed insignificant dropping in CHB patients group (532.77 ± 79.73 pg/ml) as compared with healthy control group (665.00 ± 100.97 pg/mi) Fig.1
Evaluation of serum levels of INF-γ and IL-12 in patients with chronic hepatitis B.

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**Interleukin-12**: serum levels of IL-12 showed insignificant dropping in CHB patients group (23.43 ± 4.60 pg/ml) as compared with healthy control group (32.67 ± 13.08 pg/ml). Fg.2

HBV-specific T-cells responses in the chronic stage of HBV infection are functionally impaired and much weaker than those detectable in acute self-limited infection (30-33). The response associated with particular cytokines IFN-γ, IL-12, IL-2 and TNF-β. These cytokines profile promotes delayed-Type hypersensitivity response, cytotoxic cell response and macrophages activation or cellular inflammatory reaction (34). Primarily Th1 response characterized by IL-2, IL-12 and IFN-γ (34-36). The Th1 cytokines are effective to eliminate cancer cells whereas the Th2 cytokines are inhibitory on Th1 mediated anticancer effects (37,38). Th1/Th2 cytokines producing T-cells were significant lower in CHBV patients compared to normal individual (14).

The current study results shows dropping of serum levels of IFN-γ in CHB patients these data agree with Francisca et al (39), which revealed that concentration of IFN-γ in hepatitis B patients were significantly decreased. The decrease or not affect of serum levels of IFN-γ in CHB patients (3,13,14), refer to impairment of Th1 immunity which revealed by a weak infective or un detectable virus-specific T-cell response (8). The decrease in serum levels of IFN-γ coincides with the increase in the levels of IL-10 which inhibits IFN-γ synthesis (40) which may lead to suppression of cytotoxic killing of infected hepatocytes by virus-specific CD8+ T-cells. Gorelik et al (37) showed that the normal level of IFN-γ in the serum of acute HBV infection this results explained by fact ,DNA viruses are poor inducer of IFN-γ. Hence, the immunotherapeutic strategies have proved to be useful in hepatitis B infection (4,41).
Matured dendritic cells (DCs) activate naive helper cells efficiently through stimulation with HLA class II, co-stimulatory molecules (CD80 and CD86), and cytokines such as IL-12. The stimulated cells in turn activate DCs by expression CD40 ligand and secreting TNF.

IL-12 produced by myeloid DCs stimulate The cells to differentiate towards Th1 cells (42). Furthermore IL-12 which produced by DCs and Th1 cells promoting the activation of NK cells and CTL cells (42-46). Macrophages stimulated by Th1 cell produce TNF which accelerates local inflammation (42). Rossol et al (47) showed that HBV patients undergoing therapy demonstrating increases in serum IL-12. IL-12 and IL-18 released by macrophages was shown to contribute to non-cytopathic antiviral effect by inducing IFN-γ in other immune cells (48,49).

Significant dropping of serum level of GM-CSF which produced by macrophages, macrophages suppression will lead to decrease its ability to produce IL-12 and inhibits of IFN-γ production by other cells consisted of Th1 (50,51)

REFERENCES


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