Advances in Bioresearch
Adv. Biores., Vol 9 (1) January 2018: 145-151
©2018 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.1.145151

Advances in Bioresearch

ORIGINAL ARTICLE

Evaluation levels of Immunoactive, Immunoinflammation and Immunoregulation cytokines in serum of the patients with acute and chronic Hepatitis B Virus infection

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ABSTRACT
HBV infection, one of the most prevalent viral infections in humans, is a serious public health problem in many countries. It is of two types: (1) acute and (2) chronic. Productions of various cytokines, both in vitro and in vivo, play a role in susceptibility to a number of clinical conditions, such as HBV infectious, and it also plays a role in the antiviral T cell response to HBV. In this study, the plasma samples were selected and ELISA technique were performed to examine the serum level of IL-2, 4, 10, 12, 27 and IFN-γ in three groups of control, acute and chronic. The results showed that there was significant difference in serum level of IL-2, 4, 10, 12, 27 and IFN-γ between itself in acute and chronic patients and controls. There is no significant meaningful difference between all age groups and genders, but changing the level of cytokines is influenced from host immune response and HBV infection period. Balances between cytokines, especially cytokines from Th1, 2 and Treg are very important and for manipulation of each cytokine, attention to host conditions is necessary.
Key Words: *Th1/Th2/Treg Cytokine, Acute/Chronic, HBV*

Received 10.07.2017 Revised 11.08.2017 Accepted 09.12.2017

How to cite this article:
A Khodadadi, M Taghavi, S S Athari·Evaluation levels of Immunoactive, Immunoinflammation and Immunoregulation cytokines in serum of the patients with acute and chronic Hepatitis B Virus infection. Adv. Biores., Vol 9 [1] January 2018.145-151.

INTRODUCTION

Globally, an estimated 350 million people are infected with hepatitis B. According to the recent WHO estimates, approximately one-third of the world's population has serologic evidence of Hepatitis B virus (HBV) infection, and 350 million live with chronic infection.[1]

Epidemiology of chronic HBV infection is one of the most prevalent viral infections in humans, and is a serious public health problem in many countries. HBV was among the first viruses to be implicated as a cause of a human cancer, and it is now believed to be second in importance only to tobacco as an environmental carcinogen to which man is exposed.[2, 3]

The hepatitis B virus (HBV) genome is one of the smallest viral genomes (approximately 3200 base pairs) and encodes only one viral enzyme, namely the HBV reverse transcriptase. The use of antiviral agents licensed for the treatment of chronic HBV infection, namely nucleoside analogs (NUCs), can lead to the development of resistance.[4, 5]

According to the natural history, chronic HBV carriers include three main states: immune-tolerance state, chronic hepatitis B state, and inactive hepatitis B surface antigen (HBsAg) carrier state. T-cells response during acute self-limited hepatitis B patients is characterized by a vigorous, polyclonal, and multi-specific cytotoxic and T helper cells response. By contrast, the immune response in chronic HBV carriers, not able to eliminate the virus, is weak or undetectable.[6, 7]

The specific cellular immune response plays a main role in the hepatic necrosis that occurs with HBV infection and in the persistence or lack of persistence of viral infection. Certain cytokines can contribute

to this process by efficiently inhibiting viral replication when the subtype Th1 cytokine secretion pattern is predominant or by facilitating the propagation of the pathogens in the patient if the subtype Th2 cytokine secretion pattern is predominant Some of these cytokines lead to activities against pathogens, activate effector cells involved in the cellular interactions that occur during inflammation, and are part of the acute and chronic stages of viral hepatitis. Production of various cytokines, both in vitro and in vivo, plays a role in susceptibility to a number of clinical conditions, such as HBV infectious. Cytokines play an important role in the antiviral T cell response to HBV.[8, 9]

IL-12 plays a critical role in cell-mediated immunity and regulates the balance between Th1/Th2 lymphocyte subsets, promotes naive T cells differentiation into Th1 cells, and inhibits naive T cells differentiation into Th2 cells.[10, 11]

IFNs are responsible for mediating both immunopathogenesis and HBV inactivation in the target cells by noncytolytic mechanisms, are among the most important cytokines involved in the immune response to HBV infection.[12]

Cytokines have been used as vaccine adjuvants in antitumor vaccines. IL-2 and IL-12 have been promising in conferring improved Th1 type immune responses to various tumor antigens. But if IL-2 is increased that can active Regulatory T cells (Treg) and suppress Immune system.[13]

IL-27 is a member of the IL-6/IL-12 family that consists of an IL-12 p40-related protein (EBI3). The receptor complex for IL-27 is composed of WSX-1 and gp130. IL-27 stimulates naive CD4+ T cells to produce IFN-γ in synergy with IL-12 and plays an important role in bridging innate and adaptive immunities. IL-27 inhibits HIV-1 replication and hepatitis C virus (HCV) infection.[14] It has been reported that serum IL-10 levels are higher in HBV infected patients. It could be suppress activated T cells and torn off the activated Immune system.[15]

IL-4 is an important cytokine from Th2 and whet this is released, the immune system response shifting to inflammation and allergen pathway. Increasing of this cytokine, inactive Th1 response and inhibit of adoptive cellular response again pathogens.[16]

So in this study there are special attentions to several important cytokines that have main role in Immune responses. Present study aimed at evaluation of cytokine production (IFN-γ, IL-2, IL-4, IL-12, IL-27 and IL-10) by Th lymphocytes in peripheral blood of Patients with acute and chronic hepatitis B infection. In the studies, cytokines linked to both Th1 and Th2 lymphocytes are analyzed.

PATIENTS AND METHODS

Study population: The study population consisted of three groups: group 1 consisted of 80 healthy individuals of both genders between 15 to 60 years old with no clinical laboratory evidence of hepatic illness, group 2 consisted of 80 patients (age range, 20 to 60 years; mean age standard deviation, 29.2±8.3 years) with chronic hepatitis, which had HBsAg and immunoglobulin M (IgM) antibodies against the HBV core (IgM anti-HBc). They were HBsAg- and HBV DNA-positive in serum (viremia levels from 10 to 500 pg/ml); They persisted with abnormal ALT values (within five times the upper limit of the normal range documented at least three times within the 6 months screening period); and had chronic hepatitis B without cirrhosis documented in a liver biopsy obtained within 6–18 months prior to inclusion. These were continuing more than 180 days. Group 3 consisted of 80 patients (age range, 20 to 60 years; mean age standard deviation, 29.1 ± 8.4 years) with acute hepatitis which had HBsAg and immunoglobulin M (IgM) antibodies against the HBV core (IgM anti-HBc). The diagnosis of this infection was based on the detection of ALT and AST at levels 10-fold above the normal value in association with the detection of HBsAg, the HBV e antigen (HBeAg), and IgM anti-HBc antibodies. These were continuing less than 180 days.

Criteria for exclusion: Pregnant women, patients with leukemia, hemophilia, or autoimmune illnesses, patients who had been vaccinated against HBV, patients with hepatitis A, C, and D virus infections, patients with human immunodeficiency virus infection, or patients under dialysis were excluded from the study.

Sample collection: Peripheral blood was withdrawn from each individual. Serum was obtained by centrifugation at 1600g (Damond Mod. PR-J) for 15 min and divided into aliquots of 500 ml each and stored at -70°C until it was analyzed.

ELISA Test: The samples used for detection of HBV infection markers were analyzed by the enzyme-linked immunosorbent assay (ELISA) technique. The samples initially reactive for HBsAg and IgM anti-HBc were analyzed in duplicate to confirm. The concentration of each cytokine, IL-2 (Human IL-2 Kit II, BD Biosciences, Cat. No. 550611), IL-4 (Human IL-4 Kit II, BD Biosciences, Cat. No. 550614), IL-10 (Human IL-10 Kit II, BD Biosciences, Cat. No. 550613), IL-12 (Human IL-12 p40 Kit II, BD Biosciences, Cat.

No. 551116), IL-27 (Human IL-27 ELISA Kit, GenWay Biotech Inc™, Cat. No. GWB-SKR145) and IFN-γ (Human IFN-γ Kit II, BD Biosciences, Cat. No. 550612) in serum was determined by the ELISA.

Statistical Analysis: All data were subjected to one-way ANOVA using SPSS 15.0 for Windows (SPSS, Chicago, IL). If appropriate post-hoc analyses were carried out using the Bonferroni's test for multiple comparisons. Statements of statistical significance are based on $P < 0.05$.

Ethics approval: studies were approved by the ethics committees, and all patients gave written informed consent before enrollment.

RESULTS

Serum concentrations of IL-2, IL-4, IL-10, IL-12, IL-27 and IFN-γ for patients with acute hepatitis, chronic hepatitis and controls were presented in Table 1. In this study according to analysis, Serum levels of IL-2 between the groups have meaningful significance (P<0.001). Between acute group with other groups, there is significant differences and was higher than two groups but between the two groups of control and chronic there were no differences.

Serum levels of IL-4 in control were significantly (P<0.001) higher than the other groups, but between the two groups of acute and chronic there were no significant differences.

The values of IL-10 in acute group were significantly (P<0.001) higher than the other groups but there were no differences between the two groups of control and chronic.

Results obtained from IL-12 represented a significant difference (P<0.001) between control and chronic groups with acute, but there was not any statistical difference between control and chronic groups.

Control and chronic groups had lower IL-27 level than the acute group (P<0.001).

The concentrations of INF-γ between the groups have significant difference (P<0.001). INF-γ level in control group was upper than acute group and acute group was upper than chronic group. The result was showed in Figure 1.

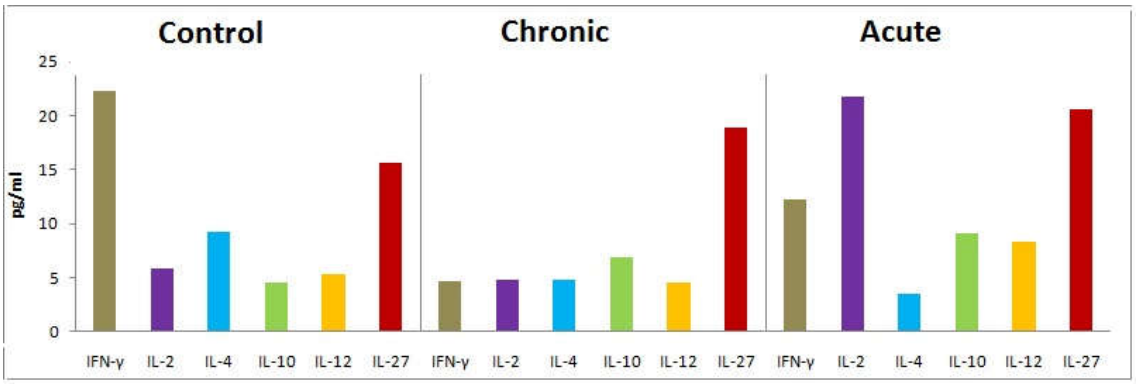

Figure 1. Cytokine levels in three groups.

Table 1. Individual data and mean serum concentrations of IL-2, IL-4, IL-10, IL-12, IL-27 and IFN-γ for patients with acute hepatitis, chronic hepatitis and controls. The mean values were compared by analysis of variance (ANOVA) with Bonferroni's correction for multiple comparisons.

	Treatment			SEM	P value
	control	chronic	acute		
IL-12	5.34[a]	4.33[a]	9.60[b]	0.148	P<0.001
IL-2	5.88[a]	4.60[a]	25.30[b]	0.633	P<0.001
IFN-γ	22.31[c]	4.47[a]	14.21[b]	0.490	P<0.001
IL-10	4.48[a]	6.69[a]	10.62[b]	0.205	P<0.001
IL-4	9.21[a]	4.59 [b]	4.06[b]	0.164	P<0.001
IL-27	15.68[a]	18.31[a]	23.98[b]	0.262	P<0.001

Means within the same row with different superscripts differ significantly.
SEM: standard error mean.

DISCUSSION

Many studies have suggested that hepatitis viral proteins play direct roles in interfering with cytokine production. HBV causes an inflammatory cellular infiltrate that produce some important cytokines which

mediate the inflammatory process and which contribute to the successful clearance of the virus, avoiding the mechanisms of the immune response or the progression of infection and persistence of the virus. Investigations carried out with IL-2 report decrease the levels of its production in patients with chronic HBV infections. The increased levels of IL-2 during the acute phase and until the total resolution of the HBV infection could allow for higher levels of T-lymphocyte activation during this period. Increasing level of IL-2 leads to activation of T cells and acute Immune response against of HBV that Th and Tc are activated and continued with switch in of Treg CD25+(IL-2R), Immune response is suppressed and IL-2 level is decreased. Therefore, IL-2 level is more than range of normal and chronic. IL-2 could be a stimulus for the activation of NK cells and CD8+ lymphocytes participating in the development of immunity.[17, 18]

In this study, serum levels of IL-4 for control group was significantly higher ($P<0.001$) than patient groups. In HBV infection, IL-4 level is decreased that shows Th1 and Tc are activated. In this study IL-10 level for acute patients is more than from chronic and control groups that shows when type1 immune response acutely active, in parallel IL-10 level is increased to modulate immune response. Therefore it could be a negative feedback again type 1 cytokines to regulation of responses.

The lymphocyte Th2-typical and HBcAg-specific defect in release of IL-4 and IL-5 remained unchanged following co-stimulation with IL-12 and IL-18, similarly to the unchanged high IL-10 production. The latter effect may decrease efficiency of the antiviral response. Nevertheless, the anti-HBV activity of Th1 lymphocytes, strongly induced by IL-12 and IL-18 and reflected by a high secretion of IFN-γ, markedly surpassing IL-10 production, might be sufficient for viral clearance in children with chronic hepatitis B infection. In acute self-limited B hepatitis, HBcAg specific significant increase was noted in IFN-γ and IL-2 secretion as compared to secretion of IL-4, IL-5 and IL-10. Secretion of IFN-γ and IL-2 was significantly higher in the course of recovery as compared to the acute stage of the disease. Thus, the data may confirm importance of IFN-γ for HBV Elimination.[19-21]

In this study, IL-12 levels for acute group was upper than two other groups. Serum levels of INF-γ between the groups have significant difference. INF-γ level in control group was more than patient groups. The ability of IL-12 to enhance cell-mediated immunity through its effects in promoting Th1 responses, inducing IFN-γ production by both T and NK cells, and augmenting specific CTL responses suggested a utility in the treatment of chronic hepatitis. Histological examination of livers from IL-12-treated animals revealed randomly distributed, multifocal parenchymal infiltrates composed of cells of lymphocytic. The mononuclear infiltrates were very often associated with liver necrosis.[22, 23] Current therapies are aimed at reducing the virus load and at the same time improving the patient's immune system. IL-12 may contribute by upregulating Thl cell responses and exerting antiviral effects through HBV antigen-specific CTL responses. Moreover, the importance of IL-12 in HBV clearance has been reported recently in patients with chronic hepatitis B undergoing treatment with IFN-α.[22] According to our observation, with silencing of HBV, production of IFN-γ is decreased and levels of this remain in low range.

The type and degree of cellular immunity response correlate with the outcome of HBV infection. Individuals with a strong and broad immune response develop an acute self-limited hepatitis. Individuals who do not mount a vigorous immune response fail to clear the virus but develop persistent infection and become chronic carriers of the HBsAg. IL-12 play an important role in the defense against viral infections, both indirectly, through promoting naive T cells differentiation into Th1 cells, and directly, through inhibition of viral replication. Elevation in IL-12 levels is associated with accelerating HBeAg seroconversion and HBV DNA clearance. A study among patients with chronic hepatitis B undergoing interferon-alpha treatment demonstrated that only those who clear HBV show a substantial increase in the production of biologically active IL-12. This may suggest that the high levels of IL-12 be an essential factor for HBeAg seroconversion.[24-26]

Some data were demonstrated that serum levels of IL-12 presented two types with significant difference. In majority of this state, lower IL-12 levels may be one explanation for the poor response to antivirus treatment (interferon-alpha or nucleoside analogues). In the contrary, significant elevation in IL-12 levels was observed in minority of this state. Promoting naive T cells differentiation into Th1 cells, elevation in IL-12 levels may be a sign of immune activation and a key factor related with spontaneous HBeAg seroconversion.[27, 28]

Chronic HBV infection is an important factor to induce liver cirrhosis and hepatocellular carcinoma. To accelerate HBeAg seroconversion, the significant elevation serum levels of IL-12 may be an ideal phase point to be given antivirus treatment for immune-tolerance state. IL-12 is an important element for establishing the host's immune ability on HBV replication and its detection is convenient. Serum levels of IL-12 may be a marker to evaluate immune state of the patients with chronic HBV infection.[20, 25, 26,

29] IL-12 is a very important cytokine in immune response. Several study showed that insufficient level of IL-12 causes HBV couldn't be clearance from body that leads to latent infection in patients. There are direct correlation between IL-12 level and clinical symptoms. when IL-12 level is low, intensity of clinical symptoms are very low ore not and with high level of IL-12, clinical symptoms of infection appear and are high intensity but clearance of virus and DNA of virus from body would be very high and virus could be eliminated perfectly from body. Because IL-12 powerfully actives immune response that is cause clearance of virus with killing and elimination of infected hepatocytes and injury of liver tissue. So here there is contradictory relation that is influenced with perfect clearance of virus and appearing injury of hepatocytes. Because liver could repair itself, maybe increasing IL-12 with some injury could be benefit for clearance of virus. Here liver would have some injury that could be repaired but HBV is clearance from liver which could prevent of liver cirrhosis and hepatocellular carcinoma in long time.

Serum IL-12 level plays a vital role in viral clearance during acute infection. Being a member of IL-12 family, IL-27 promotes the development of naive CD4+ T cells into Th1 cells and synergizes with IL-12 to trigger the production of Th1 cytokine in naive CD4+ T cells and natural killer cells. IL-27 plays an important role in promoting Th1 responses during the early phase of HBV infection as clearance of HBV needs a co-ordinated innate and adaptive cell-mediated immune response. IL-27 acts on naive CD4+ T cells and regulates only the initiation phase of Th1 responses, but not the induction and maintenance phases of effecter Th1 responses.[21, 25, 29-31]

In later stage of hepatitis B, IL-27 seems to help the immune system to generate a mild protective inflammatory response. We investigated that Serum levels of IL-27 in acute group have significant difference with the two groups of control and chronic and was upper than two groups that was according other researches that were demonstrated HBV infected patients have higher serum IL- 27 levels than healthy individuals. IL-27 level was influenced by the presence of HBeAg which has been proposed to modulate the host immune response.[30, 31]

In this study, there is no significant difference between all groups of age and genders, therefore changing in cytokines is influenced from host immune response and HBV infection period.

According all recommends, Compared to previous years, the infection rates have decreased among blood donors and that transfusions have been carried out more safely because with screening and vaccination than could be controlled and to overcome this problem improvement in predonation screening tests and educational policies is being proposed. So vaccination against hepatitis B should take its place in the routine vaccination. With vaccination, in the next generation of people, it will be observed that all people will be health and safe and this is a big economic burden for blood centers.[32-34]

Changing in level of cytokines is influenced from host immune response and HBV infection period. Balances between cytokines, especially cytokines from Th1, 2 and Treg is very important and for manipulation of each cytokine, attention to host conditions is necessary. If host conditions are acceptable (for example, host is young and could repair his/her liver and etc.), it could be recommend to cytokines therapy, especially IL-12, but host conditions and immune response aren't acceptable, cytokines therapy could have high risk. HBeAg purportedly acts via interference with Th1/Th2 cross-regulation, and prevention of severe liver injury during adult infections. Not only in HBV infection, but also in other problems, attention to level of one cytokine solely isn't correct. In this condition, it should be intentioned that balance of cytokines is important and when one type 1 cytokine increases, in parallel one type 2 and regulatory cytokine increase, this is in balance and manipulation of this balance isn't good. That is similar to each cytokines are in normal range and didn't increase. For example, in this study there is correlation between INF-γ and IL-10 and when IL-10 increased, INF-γ increased too. Therefore these are in balance and have no meaningful change in balance. So balance of these is important, not absolutely increase level of these. Response of T cell immunity, an increase of serum Th1, Th2 and Treg cytokines in chronic HBV patients aren't important, balance between these are important.

CONFLICT OF INTEREST STATEMENT

We declare that there is no conflict of interest in the publication of this paper.

FINANCIAL SUPPORT

The authors received support from Islamic Azad University of Malekan Branch.

ACKNOWLEDGEMENT

This study was supported by the Islamic Azad University of Malekan Branch in East Azerbaijan. We are grateful to Dr. R.Omidi and M.Athayi for their helpful suggestions, discussions and excellent technical

assistance. Thanks are due to all staff members of the Department of Immunology and Mycology, colleagues and our parents who sponsored this study.

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