Int J Clin Exp Med 2016;9(6):9437-9442
www.ijcem.com /ISSN:1940-5901/IJCEM0015838

Original Article

Analysis of IL-6, IL-10, IL-17 levels in the blood of patients with chronic hepatitis B

De-Song Ming*, Zhen-Zhong Lin*, Xiao-Dong Qiu

*Department of Clinical Laboratory, The First Affiliated Hospital of Fujian Medical University, Quanzhou 362000, China. *Equal contributors.*

Received September 9, 2015; Accepted March 15, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Objective: To investigate the role and significance of serum IL-6, IL-10, IL-17 in the occurrence and development of chronic hepatitis B. Methods: Enzyme-linked immunosorbent assay (ELISA) was used to detect serum IL-6, IL-10, IL-17 levels and liver function in patients with hepatitis B and relationship between serum viral load in 70 cases of CHB patients and 20 healthy subjects (control group). Results: Compared with healthy controls, IL-6, IL-10, IL-17 in CHB patients were significantly increased ($P<0.05$); compared with HBeAg negative patients, IL-6 levels were decreased in HBeAg positive group ($P<0.05$), while IL-17 levels were significantly increased ($P<0.05$) and there was no significant difference in the level of IL-10; three kinds of cytokine levels did not show difference between HBV-DNA ≥105 copies/ml and <105 copies/ml^2 group; IL-6 was positively correlated with AST, TBIL, HBV-DNA. Conclusion: IL-6, IL-10, IL-17 are involved in the pathogenesis of CHB, the change of its content is closely related to the degree of liver inflammation and viral replication.

Keywords: HBV, IL-6, IL-10, IL-17

Introduction

Hepatitis B is one of common chronic diseases, which was serious harm to people's health. After the body is infected with hepatitis B virus (hepatitis B virus, HBV), clinical outcome showed significant difference, the role of cytokines attracted more and more attention. When people were infected with HBV, immune cells secreted large amounts of cytokines, which may cause the virus to clear or persistent infection or even liver damage in different outcomes. In these factors, IL-6, IL-10 and IL-17 represented different subsets of features and functions respectively, which have important significance in immune regulation reaction. In this study, by comparing chronic hepatitis (chronic hepatitis B, CHB) serum expression of these three factors, we explored its role and significance in the pathogenesis of CHB.

Subjects and methods

Objective

Between July 2010 and August 2011, 70 cases of CHB patients hospitalized in Ningde hospital attached to Fujian Medical University and Quanzhou First Hospital attached to Fujian Medical University Hospital while 54 cases were male and 16 females, aged between 20 and 58 years old, with an average of (30.65± 14.26) years. There were 46 cases of mild to moderate, and 24 cases of severe patients; 41 cases were HBeAg-positive and 29 cases were negative. All patients were consistent with the diagnosis of viral hepatitis prevention and treatment programs and chronic hepatitis B prevention and treatment guidelines [1, 2]. All patients accepted at least three months without immunomodulatory agents (such as thymosin, glucocorticoids), protective liver and anti-viral treatment, elimination of overlaps A, C, D, hepatitis E virus, human immunodeficiency virus and other viral infections, and other known genetic metabolic liver disease, cholestatic liver disease, obesity or autoimmune liver disease, or primary liver cancer patients. Alternatively at the same period, 20 cases of normal healthy control group from Quanzhou first hospital outpatient were enrolled, including 13 males and 7 females, aged between 21 and 62 years with mean age of (36.50±13.26) years.

Table 1. Comparison of general clinical data in each group

Group	Cases		Age (Years)	Liver function		
	Male	Female		ALT/(IU·L^{-1})	AST/(IU·L^{-1})	TBiL/(μmol·L^{-1})
Mild and moderate CHB group	35	11	34.61±10.89	332.11±309.46[a]	171.03±165.07[a]	21.24±11.6
Severe CHB group	19	5	30.29±8.64	816.06±839.61[a,c]	457.48±446.73[a,c]	158.53±116.96[b,c]
Control group	14	6	36.5±13.26	16.4±1.91	13.35±1.12	19.00±4.75

Note: a: P<0.05, vs. control group; b: P<0.05, vs. mild and moderate CHB group; c: P<0.05, vs. control group.

Table 2. Serum levels of the three cytokines in each group

Group	Cases	IL-6	IL-10	IL-17
Mild and moderate CHB	46	4.83±6.23	95.73±107.97	125.95±43.19
Severe CHB	24	11.32±20.37▽	69.52±66.49	137.38±54.56
Normal control	20	2.78±2.00*	26.74±19.21*	91.42±35.48*

Note: *P<0.05, vs. each CHB group; ▽P<0.05, vs. mild and moderate CHB group.

Table 3. Comparison of the expression of 3 cytokines between HBeAg positive and negative groups

Group	Cases	IL-6	IL-10	IL-17
HBeAg positive	41	4.74±2.97	92.13±110.89	142.20±43.67*
HBeAg negative	29	10.47±20.28	79.12±71.32	112.44±47.47

Note: *P<0.05, vs. HBeAg negative group.

Methods

Samples: After collecting 5 ml fasting peripheral venous blood from 70 cases of CHB patients and 20 healthy, they were treated with 3000 r/min centrifugation for 3 min. They were separated and stored at -80°C for ELISA test.

Detection of serum cytokines: The above frozen serum samples were thawed at room temperature; serum IL-6, IL-10, IL-17 expression levels were detected by the ELISA kit (MAPKET company, USA), strictly in accordance with the kit instructions; data were read in a microplate reader (Alisei Quality System, Italy SEAC company) at 450 nm.

Other index detection: The USA Abbott reagents were used to detect HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc). Serum HBV-DNA quantification was performed using real-time quantitative fluorescence detector PE9700 (US PE Company) and serum HBV-DNA kit (Zhongshan Medical University, Da An Gene Co., Ltd.). Automatic ELISA biochemical analyzer (LX-20, Beckman, USA) was used to detect alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin Su (DBIL) and other biochemical indicators.

Statistical analysis

Statistical analysis was performed using SPSS17.0 software; results were expressed as mean ± standard deviation ($\bar{x}$±s); The results between the groups were compared using the Mann-Whitney U test; Spearman's test was used for correlation analysis; P<0.05 represents that the difference was statistically significant.

Results

Comparison of general clinical data

There were no statistically significant differences in age, gender ratio, etc. between CHB group and the healthy control group; the groups were comparable (P>0.05). ALT and AST in mild, moderate and severe CHB patients were significantly increased compared with the control group (P<0.05); TBiL in severe CHB group was significantly higher than that in the control group (P<0.05); ALT, AST and TBiL in severe CHB group were significantly higher than those in mild and moderate group (P<0.05); the results were shown in **Table 1**.

Comparison of cytokine levels in peripheral blood

Compared with the healthy control group, IL-6, IL-10, IL-17 levels in CHB patients were significantly increased (P<0.05); the results are shown in **Table 2**. According to HBeAg-positive,

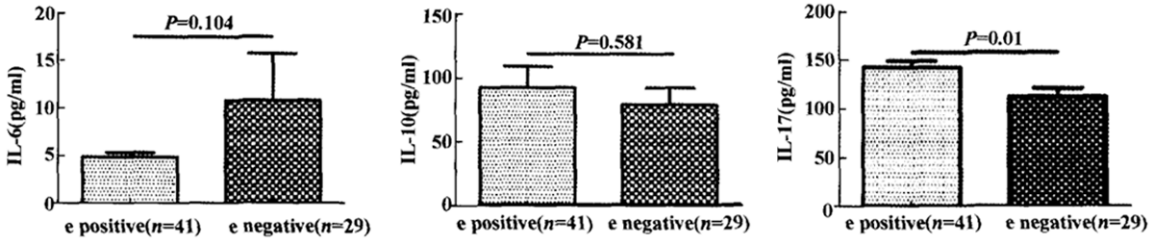

Figure 1. Comparison of the serum levels of 3 cytokines between HBeAg positive and negative groups.

Table 4. Comparison of the expression of 3 cytokines between the groups of HBV-DNA ≥10^5 copies/ml and <10^5 copies/ml

HBV-DNA	Cases	IL-6	IL-10	IL-17
≥10^5 copies/ml	55	7.18±14.43	90.75±90.64	128.38±47.32
<10^5 copies/ml	15	5.68±3.53	68.83±113.08	135.34±48.56

70 cases of CHB patients were divided into e-antigen positive and e-antigen negative groups; the levels of three kinds of cytokines were compared between the two groups; IL-6 levels in HBeAg-positive group were lower than those in negative group, but the difference was not statistically significance (P = 0.104); IL-10 level in HBeAg positive group was higher than that in negative group, but the difference was not statistically significant (P = 0.104); and IL-17 level was higher than that in e antigen-negative group (P<0.05); the results were shown in **Table 3** and **Figure 1**. According to the level of HBV-DNA titer, the 70 CHB patients were divided into CHB HBV-DNA ≥10^5 copies/ml and <10^5 copies/ml groups; the difference in three kinds of cytokines between the two groups were compared; the results showed no significant differences in three kinds of cytokines (P>0.05), shown in **Table 4** and **Figure 2**.

Correlation analysis of three kinds of cytokine with ALT, AST, TBiL and HBV-DNA in CHB patients

The results showed that, IL-6 was positively correlated with AST, TBIL, and HBV-DNA (γ were respectively 0.416, 0.374 and 0.317; all *P* values were less than 0.05); there was no significant correlation between IL-10 and AST, TBIL, HBV-DNA (γ were 0.113, -0.025, -0.12, *P* values were 0.353, 0.837, 0.33). IL-17 had no significant correlation with AST, TBIL, HBV-DNA (γ were respectively -0.084, 0.012, 0.135, *P* values were 0.489, 0.921, 0.272).

Discussion

Hepatitis B is an immune-related disease; HBV infection has no obvious damage to human hepatocytes, which damages liver cells indirectly by inducing the body's immune response; meanwhile the body's immune response plays a role in clearing the virus [3]. The outcome of HBV infection depends on virus and the immune response; immune disorders, especially disorders in T lymphocyte subsets and their cytokines, are closely related to the occurrence and development of chronic liver diseases [4, 5]. The outer circumferential mature T lymphocytes are heterogeneous groups neither in terms of phenotype or function, which can be divided into $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes according to phenotypes and can be divided into helper T lymphocyte (Th), suppressor T lymphocyte (Ts) and cytotoxic T lymphocyte (CTL) according to their different functions. Now we believe that naive $CD4^+$ T lymphocytes can differentiate into4 major mature-functional subsets (Th1, Th2, Th17 and regulatory T lymphocytes (Treg)) in the role of different cytokines, respectively; they through different transcriptional pathways and characteristic cytokines perform different biological functions [6], forming a huge cytokine network. A cytokine often has a variety of biological activities, and a variety of cytokines can share a common biological activity; different cytokines form a network and interact with each other to regulate the production and effect; and the dysregulation of these cytokines is closely related to the occurrence and development of chronic liver disease. Wherein, Th1 mainly produces IL-2, IL-12, TNF-β, IL-18 and interferon-γ (IFN-γ) to mediate cell immune response; Th2 mainly produces IL-4, IL-5, IL-10 and IL14 to mediate humoral immune response;

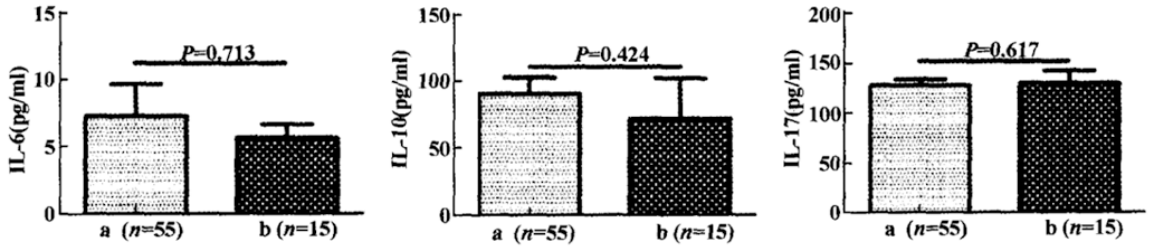

Figure 2. Comparison of the serum levels of 3 cytokines between HBV-DNA $\geq 10^5$ copies/ml (a) and $<10^5$ copies/ml[2] groups (b).

Th17 is characterized by expressing IL-17A, which also secretes IL-17F, IL-22, IL-21, IL-6 and other factors; it is a major proinflammatory subset of HBV infection; Treg cell maintains immune tolerance [7-10] by direct cell-cell contact and the release of IL-10 and TGF-β and other inhibitors. In these factors, IL-6, IL-10 and IL-17 respectively represent the features and functions of different subsets, which are important in regulating immune responses.

IL-6 is an important proinflammatory cytokine in vivo, which can regulate the synthesis of many acute phase proteins; it is also a factor associated with the immune regulation of chronic liver disease [11]; it is highly expressed in the early stage of acute and chronic liver injury. The concentration of IL-6 is very low of normal in normal human peripheral blood, often less than 5 kIU/L [12]; it is not expressed in most normal resting cells, but under the stimulation of infection or other factors, T lymphocytes and single macrophages are activated, resulting in increased synthesis and release of IL-6, which in turn promotes the proliferation of activated T cells and stimulates immature thymocytes differentiate into mature CTL, thereby clearing the virus within the cell; but it also enhances the inflammation of the liver, thereby increasing the damage to liver cells [13]. Studies found that serum IL-6 level in the acute phase of hepatitis and cirrhotic cirrhosis was significantly higher than the normal level, and it increased more significantly in serious or dead population; it was positively correlated with the number of white blood cells and neutrophils, C-reactive protein (CRP) and TBIL levels, and negatively correlated with serum total protein level; and elevated IL-6 was correlated with the severity of CHB [14]. This study found that for CHB patients, IL-6 level in severe group significantly increased compared with mild group, moderate group and the healthy control group; IL-6 had a positive correlation with AST and TBIL; serum AST and TBiL levels are common indicators of liver function, so the level of serum IL-6 was closely related to the degree of necrosis of liver cells. The correlation study between IL-6 and HBV-DNA showed that IL-6 was positively correlated with HBV-DNA, which may be due to that the increase in HBV DNA load may further lead to the increased cellular immune dysfunction in hepatitis B patients, thereby producing and releasing IL-6 to promote inflammatory responses. It has been reported that, reducing viral load and thereby reducing the IL-6 by anti-HBV treatment have a certain role in promoting functional recovery of liver cells [15], further indicating that serum IL-6 levels are closely related to the extent of necrosis of the liver cells.

IL-10 is an important anti-inflammatory cytokine in vivo; by inhibiting the proliferation of Th1 cells and the production of IL-2, INF, leukotrienes and other cytokines, it can inhibit Th1-type immune response [16], reduce the patient's immune cell function, and promote Treg proliferation and activation, which is conducive to induce tolerance to foreign pathogens, leading to persistent hepatitis B virus [17]. This study shows that, serum IL 10-level in CHB groups significantly increased compared with the control group; the difference was statistically significant; IL-10 level in mild and moderate groups is higher than that in the severe group, suggesting that the advantaged Th2 cytokine IL-10 can inhibit Th1 cell response, so that the body cannot effectively remove virus-infected liver cells, causing persistent HBV infection, and with the aggravation of inflammation, IL-10 shows a downward trend. The study also found that there was no statistically significant difference in IL 10 level between two

different e-antigen groups, which may be associated with that there were no significant differences in HBV-DNA levels between the two groups. The study of Lai Yafang et al [18] found that, serum HBV-DNA load was positively correlated with serum levels of IL-10, suggesting that elevated levels of IL-10 can suppress host anti-HBV cell immune activity, resulting in continued HBV replication and expression in vivo, which is the main reason for chronic hepatitis B virus infection. However, in this study, patients were grouped according to HBV-DNA load level, and the results showed that there was no statistically significant differences in IL-10-level between the two groups, possibly due to that the detection of peripheral blood reflects the total effect and the overall level rather than the local inflammatory cytokine expression [19].

IL-17 is a specific pro-inflammatory factor mainly secreted by activated memory $CD4^+$ T lymphocytes (TH17 cells) [20], which can promote the body to locally produce chemokines such as IL-8 and IL-6, lead to rapid growth of monocytes and neutrophils, enhance local inflammation and induce proinflammatory cytokines, thereby resulting in tissue damage. Studies have found that IL-17 in the serum and liver tissue of most patients with CHB was positively correlated with HBV-DNA and ALT levels [8]. This study found that, IL-17 levels in each CHB group were significantly higher than those in the control group, and the difference was statistically significant, suggesting that IL-17 was closely related with liver inflammation; but correlation analysis did not find the positive correlation with liver inflammation levels. Therefore, IL-17 may be involved in HBV-induced pathological lesions of the liver, but whether it is involved in the T cell immunity of clearing viruses is still not clear and needs further study. Meanwhile, according to the level of HBV-DNA, grouping was performed in this study, which found that there was no statistically significant difference in IL-17 level between the two groups; and further correlation analysis had not found that IL-17 and HBV-DNA levels were positively correlated, which may because that IL-17-mediated liver inflammatory injury did not play a major role in long-term chronic hepatitis B infection.

By comparing serum IL-6, IL-10 and IL-17 expression in CHB patients, this study primarily elucidates the role and significance of these three kinds of cytokines in the chronic HBV infection, providing theoretical basis and new ideas for HBV immune therapy from the cytokines aspect.

Disclosure of conflict of interest

None.

Address correspondence to: De-Song Ming, Department of Clinical Laboratory, The First Affiliated Hospital of Fujian Medical University, Dong Street 250# of Quanzhou, China. Tel: +86-0595-2227407; Fax: +86-0595-22278131; E-mail: mingdesongm@163.com

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