Int J Clin Exp Med 2020;13(8):5838-5842
www.ijcem.com /ISSN:1940-5901/IJCEM0096904

Original Article

The expression and clinical significance of PD-1 in chronic hepatitis B

Qiaomei Dong[1], Xiaochun Liu[2], Junfang Zhu[1], Li Zhao[1], Xiaowei Zhang[1], Xiaoling Xie[1]

[1]The Central Laboratory of The First Hospital of Lanzhou University, Lanzhou, Gansu, P. R. China; [2]The People's Hospital of Qingcheng, Qingyang, Gansu, P. R. China

Received May 12, 2019; Accepted July 16, 2019; Epub August 15, 2020; Published August 30, 2020

Abstract: Objective: To investigate the expression and significance of Programmed cell death receptor 1 (PD-1) in the peripheral blood of chronic hepatitis B (CHB) patients. Methods: Flow cytometry was used to detect PD-1 and $CD4^+CD25^+$ regulatory T cell (Treg) levels in 126 cases of CHB and 46 healthy controls. The correlation between PD-1, $CD4^+CD25^+$ Treg and clinical parameters of CHB were analyzed. Results: Both PD-1 and $CD4^+CD25^+$ Treg levels in CHB patients were significantly higher than that in healthy controls, and both were slightly increased with the copy number of HBV-DNA in CHB patients. Receiver operating characteristic (ROC) curve showed that the area under the curve (AUC) values of PD-1 and $CD4^+CD25^+$ Treg to distinguish CHB patients from healthy controls were 0.8 and 0.5, respectively. Conclusion: PD-1 is an independent factor to distinguish CHB from healthy controls. It may be used as a potential molecular biomarker for early diagnosis and screening of CHB.

Keywords: Chronic hepatitis B, PD-1, $CD4^+CD25^+$ Treg, early diagnosis

Introduction

Chronic hepatitis B (CHB) is a global disease caused by hepatitis B virus (HBV) [1]. The immune system plays an important role in the process of HBV infection [2]. Programmed cell death receptor 1 (PD-1), also known as CD279, which belongs to the immunoglobulin superfamily, is a newly discovered immune inhibitory molecule [3]. It can be induced in the activation of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, B cells and mononuclear cells. The interaction between PD-1 and its ligands PD-L1 and PD-L2 may inhibit T lymphocyte responses through different mechanisms [4, 5], including T lymphocyte deletion and apoptosis, anti-toxic killing, inhibition of proliferation, as well as induction and maintenance of T lymphocyte failure [6]. $CD4^+CD25^+$ regulatory T cells (Tregs) are important indicators to reflect the immune status of the body [7], $CD4^+CD25^+$ Tregs increased in patients with chronic HBV infection. In addition, studies have confirmed that $CD4^+CD25^+$ Treg could inhibit the initiation and expansion of antigen-specific $CD8^+$ T cell response during HBV-DNA immunization [8]. Meanwhile the PD-1/PD-L1 pathway is closely related to Tregs [9, 10]. The PD-1/PD-L1 pathway inhibits the immune response of T cells by inducing and maintaining Tregs. In this study, to explore the feasibility of using PD-1 as a molecular biomarker to diagnose CHB, we compared the expression levels of PD-1 in the peripheral blood of CHB patients and healthy controls, and statistically analyzed the correlation between PD-1 and liver function indexes of CHB patients. Meanwhile, the difference of $CD4^+CD25^+$ Treg level between patients with CHB and healthy controls was also detected and analyzed.

Materials and methods

Specimens

A total of 126 CHB patients (72 males and 54 females, aged 13-80 years, with an average age of 38 years) who were admitted to our hospital from 2013 to 2014 were selected for PD-1 and $CD4^+CD25^+$ Treg detection. The diagnosis was made according to the "guidelines for the prevention and treatment of CHB" (2005) by the liver disease branch, and infection epidemiology branch of the Chinese medical associa-

Table 1. The percentage of PD-1 and $CD4^+CD25^+$ Treg in peripheral blood of CHB patients and healthy controls (Student's t-test was used to compare differences between the two groups, $*P < 0.05$, $**P < 0.01$)

Group	n	PD-1 (%)	P	$CD4^+CD25^+$ Treg (%)	P
CHB	126	25.01±11.03**,↑	0.005	19.26±9.25*,↑	0.037
Control	42	17.2±4.37		18.15±5.23	

*The difference is significant. **The difference is very significant. ↑Express elevated.

tion [4]. All patients were screened and excluded from having liver damage caused by overlapping viral infections such as hepatitis A, C, D and E, as well as other causes (alcohol, drugs, etc.). In the experiment, 46 healthy control subjects were selected. The healthy subjects do not have hepatitis history, and were negative for hepatitis B markers.

Reagents

Anti-CD25-APC, anti-CD4-FITC and anti-PD-1 (CD279)-PE kits were purchased from Becton-Dickinson. HBV-DNA quantitative PCR kit was provided by Sun yat-sen medical University Da'an gene co., LTD.

Flow cytometry

Two-3 mL of peripheral venous blood was sampled and anticoagulant EDTA was used. After blending, 100 μL of the whole blood was placed in to two determination tubes. Twenty μL of isotype control and CD4-FITC/CD279-PE/CD25-APC were added to the two tubes, respectively. After mixing, cells were incubated for 20 mins at room temperature in the dark and washed twice with PBS. Samples were immediately measured by Canto II flow cytometry. The percentage of PD-1 and $CD4^+CD25^+$ Treg were obtained by counting 10,000 cells in the determination process.

Quantitative detection of HBV-DNA

Fluorescence quantitative PCR was used. The operation was carried out in strict accordance with the instructions of the HBV-DNA quantitative PCR kit. The diagnostic kit used a nucleic acid lysis buffer to allow rapid lysis and release of HBV-DNA from a serum or plasma specimen. By applying real-time fluorescence quantitative PCR technology, this test utilized a pair of specific primers which are designed to target at a conserved sequence of HBV-DNA a specific fluorescence probe, accompanied with PCR mix, to achieve quantitative detection of HBV-DNA through fluorescent signal changes.

Statistical analysis

Student's t-test was used to compare differences between the two groups or association. $P < 0.05$ was considered as a statistically significant different. Pearson's Correlation test was used to show the correlation of PD-1 and $CD4^+CD25^+$ Treg in CHB specimens. Receiver operating characteristic (ROC) curves were plotted to determine the potential of PD-1 and $CD4^+CD25^+$ Treg to distinguish CHB from control. All statistical comparisons were performed using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA).

Results

PD-1 levels in CHB patients and healthy controls

A total of 172 cases were enrolled in this study. The expression levels of PD-1 in controls ($n = 46$) and patients with CHB ($n = 126$) were assessed by flow cytometry. The results showed that the PD-1 expression level was significantly increased in CHB patients than that of controls (25.01±11.03 vs. 17.2±4.37, $P = 0.005$, **Table 1**).

Association between the value of PD-1 and clinical parameters in CHB patients

We assessed the potential correlations between the value of PD-1 and clinical parameters of CHB patients, including HBV-DNA copy number, the value of $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+CD25^+$ Tregs, and age of patients. The value of PD-1 increased slightly with the increase of HBV-DNA copy number. When HBV-DNA copy number was less than 10^3, the value of PD-1 was 24.81±9.21. When HBV-DNA copy number was greater than or equal to 10^3, the value of PD-1 was 25.27±10.23, but the difference was not significant ($P = 0.668$, **Table 2**). Furthermore, the value of PD-1 was not correlated with the value of $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+CD25^+$ Treg, or the age of CHB patients (**Table 3**).

Table 2. The percentages of PD-1 and $CD4^+CD25^+$ Treg in peripheral blood of patients with different HBV-DNA copy number of CHB (Student's t-test was used to compare differences between the two groups)

Group	n	PD-1 (%)	P	$CD4^+CD25^+$ Treg (%)	P
HBV-DNA ≥ 10^3	64	25.27±10.23↑	0.668	19.66±7.25↑	0.825
HBV-DNA < 10^3	62	24.81±9.21		19.21±8.02	

↑Express elevated.

Table 3. Correlation between PD-1 and clinical parameters of CHB patients (Pearson's Correlation test was used to show the correlation)

Correlation	R	P
PD-1 and $CD4^+$	0.119	0.185
PD-1 and $CD8^+$	0.003	0.971
PD-1 and Treg	0.082	0.361
PD-1 and age	0.336	0.214

$CD4^+CD25^+$ Treg levels in CHB patients and healthy controls

The value of $CD4^+CD25^+$ Treg in the peripheral blood of CHB patients was 19.26±9.40, which was significantly higher than that of the healthy control group (18.15±5.23, $P = 0.037$, **Table 1**). Similar to PD-1, the $CD4^+CD25^+$ Treg level was slightly increased with the increase of HBV-DNA copy number. When HBV-DNA copy number was less than 10^3, the value of $CD4^+CD25^+$ Treg was 19.21±8.02. When HBV-DNA copy number was greater than or equal to 10^3, the value of $CD4^+CD25^+$ Treg was increased to 19.66±7.25, but the difference was not significant ($P = 0.825$, **Table 2**).

PD-1 exhibits sufficient power to distinguish CHB patients from control samples

To further evaluate whether the levels of PD-1 and $CD4^+CD25^+$ Treg could distinguish CHB patients from control samples, ROC curves were plotted. The results demonstrated that AUC values of PD-1 and $CD4^+CD25^+$ Treg were 0.8 and 0.5, respectively; which demonstrated that PD-1 exhibited sufficient power to distinguish CHB patients from control samples (**Figure 1**).

Discussion

CHB is a worldwide disease caused by HBV. Due to its high incidence, long course, easy recurrence and level of harm, it has now become an internationally recognized problem [11, 12]. The immune system plays an important role in the process of HBV infection. The strength and type of immune response directly affect the development and outcome of CHB in patients [13, 14].

PD-1 is a newly discovered immunosuppressive co-stimulator, which is induced in activated $CD4^+$ T cells, $CD8^+$ T cells, B cells, monocytes and NKT cells [15]. Its interaction with PD-L1 and PD-L2 may inhibit T lymphocyte response through different mechanisms [16], including T lymphocyte deletion and apoptosis, anti-toxic killing, inhibition of proliferation, induction and maintenance of T lymphocyte failure. Recent studies have shown that the PD-1/PD-L1 inhibitory pathway is involved in regulating the functional failure of T cells in HBV and HCV patients with chronic infection; which weakens the body's ability to effectively kill viruses and thus leads to chronic viral infection [17]. In this study, we compared the differences of PD-1 between CHB patients and healthy control individuals, and the results showed that the expression of PD-1 in CHB patients was significantly higher than that in healthy controls, and it was slightly increased with the copy number of HBV-DNA in CHB patients, indicating that PD-1 may be used as a molecular biomarker for the early screening and diagnosis of CHB. To further confirm that PD-1 may be used as an independent factor to distinguish CHB from healthy controls, we used ROC curves to statistically analyze the difference between the two. Results of the ROC curve showed that the AUC value of PD-1 to distinguish the two was 0.8. The closer the AUC value is to 1, the stronger the ability of this factor to distinguish between the two [18]. Therefore, the results of this study showed that PD-1 had a strong ability to distinguish CHB from a healthy control. PD-1 may be used as an independent factor to distinguish CHB from healthy controls, and as a potential molecular marker of CHB, it may be used for early diagnosis and screening of CHB.

In addition, studies have pointed out that the PD-1/PD-L1 pathway can induce the differ-

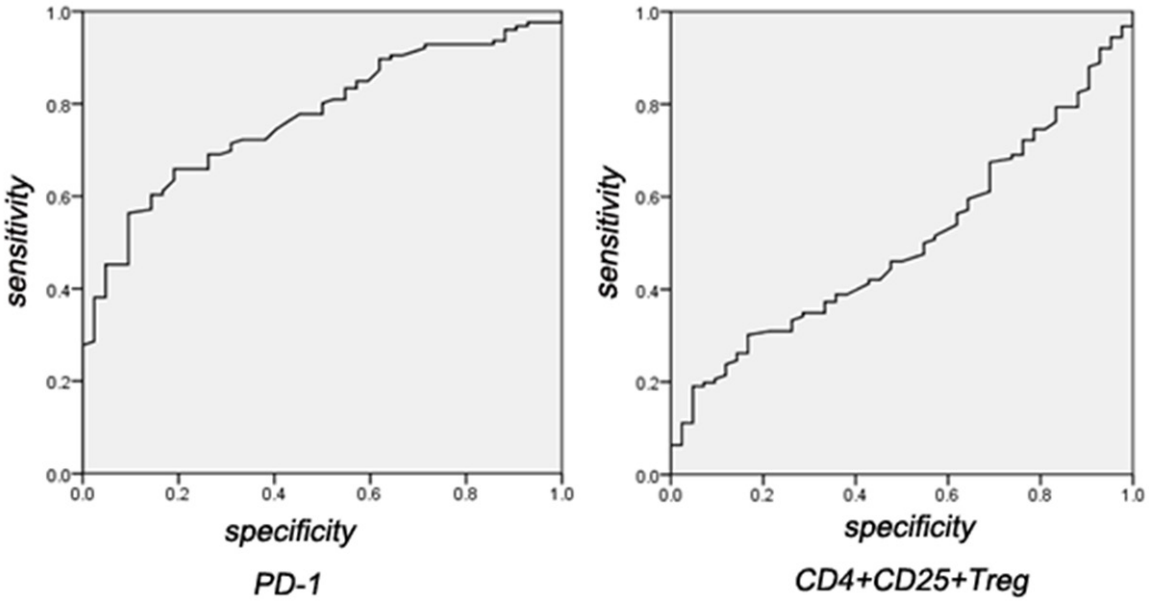

Figure 1. ROC curves showed that, for PD-1, the AUC value is 0.8, and for $CD4^+CD25^+$ Treg, the AUC value is 0.5.

entiation and maintenance of $CD4^+CD25^+$ Treg cells by enhancing the expression of Foxp3, a master transcription factor of Treg cells. Through the induction of Tregs, PD-1 inhibits the T cell immune response. $CD4^+CD25^+$ Tregs are important indicators reflecting the immune status of the body [19, 20]. Peng et al. found that the $CD4^+CD25^+$ Treg levels increased in patients with chronic HBV infection. In addition, studies have confirmed that $CD4^+CD25^+$ Treg could inhibit the initiation and expansion of antigen-specific $CD8^+$ T cell responses during HBV-DNA immunization [8]. $CD8^+$ T cells showed an enhanced response peak after the removal of this cell population [21, 22]. Therefore, in this study, we compared the differences of $CD4^+CD25^+$ Tregs between CHB patients and healthy control individuals. It was also found that $CD4^+CD25^+$ Tregs in CHB patients were higher than that in healthy controls, and $CD4^+CD25^+$ Tregs increased slightly with the increase of HBV-DNA copy number in CHB patients, indicating that the proportion of $CD4^+CD25^+$ Tregs was higher in CHB patients with more active virus replication. In CHB patients, Treg may play a role in the following two aspects: on the one hand, Tregs can inhibit the proliferation and differentiation of self-reactive lymphocytes and secrete cytokines such as IFN-γ; On the other hand, Tregs can also exert a regulatory function by secreting IL-4, IL-10, TGF-β and other inhibitory cytokines [19]. Furthermore, the results of the ROC curve showed that the AUC value of $CD4^+CD25^+$ Treg to distinguish between CHB and healthy controls was 0.5. The AUC value of PD-1 to distinguish the two was 0.8 (which is closer to 1). Detecting the expression of PD-1 may be more effective than detecting $CD4^+CD25^+$ Tregs to distinguish CHB patients from healthy controls.

Disclosure of conflict of interest

None.

Acknowledgements

This work was supported by the Fundamental Research Funds for the Central Universities (lzujbky-2013-150), Science and Technology Plan Projects in Gansu Province (1308RJYA071), and the Research Program of the First Hospital of Lanzhou University (ldyyynlc201101), Health Research Project of Gansu Province (GSWST2011-05).

Address correspondence to: Xiaoling Xie, The Central Laboratory of The First Hospital of Lanzhou University, NO. 1 The West Donggang Road, Lanzhou 730000, Gansu, P. R. China. Tel: +8609318356323; E-mail: dongqm06@126.com

References

[1] Pardee M. Diagnosis and management of hepatitis B and C. Nurs Clin North Am 2019; 54: 277-284.

[2] Gill US and Kennedy PTF. The impact of currently licensed therapies on viral and immune responses in chronic hepatitis B: considerations for future novel therapeutics. J Viral Hepat 2019; 26: 4-15.
[3] Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, Pasdar A and Sahebkar A. PD-1/PD-L1 pathway: basic biology and role in cancer immunotherapy. J Cell Physiol 2019; 234: 16824-16837.
[4] Li M, Sun XH, Zhou ZH, Yu Z, Jin SG, Zhu XJ and Gao YQ. Beneficial therapeutic effect of Chinese herbal Bushen formula on CHB patients with mildly elevated alanine aminotransferase by down-regulating CD4+CD25+T cells. J Ethnopharmacol 2013; 146: 614-622.
[5] Wenjin Z, Chuanhui P, Yunle W, Lateef SA and Shusen Z. Longitudinal fluctuations in PD1 and PD-L1 expression in association with changes in anti-viral immune response in chronic hepatitis B. BMC Gastroenterol 2012; 12: 109.
[6] Song MK, Park BB and Uhm J. Understanding immune evasion and therapeutic targeting associated with PD-1/PD-L1 pathway in diffuse large B-cell lymphoma. Int J Mol Sci 2019; 20: 1326.
[7] Gliwinski M, Piotrowska M, Iwaszkiewicz-Grzes D, Urban-Wojciuk Z and Trzonkowski P. Therapy with CD4(+)CD25(+) T regulatory cells - should we be afraid of cancer? Contemp Oncol (Pozn) 2019; 23: 1-6.
[8] Xibing G, Xiaojuan Y and Juanhua W. PD-1 expression on CTL may be related to more severe liver damage in CHB patients with HBV genotype C than in those with genotype B infection. J Viral Hepat 2013; 20: e1-2.
[9] Wu SP, Liao RQ, Tu HY, Wang WJ, Dong ZY, Huang SM, Guo WB, Gou LY, Sun HW, Zhang Q, Xie Z, Yan LX, Su J, Yang JJ, Zhong WZ, Zhang XC and Wu YL. Stromal PD-L1-positive regulatory T cells and PD-1-Positive CD8-positive T cells define the response of different subsets of non-small cell lung cancer to PD-1/PD-L1 blockade immunotherapy. J Thorac Oncol 2018; 13: 521-532.
[10] Ni L, Ma CJ, Zhang Y, Nandakumar S, Zhang CL, Wu XY, Borthwick T, Hamati A, Chen XY, Kumaraguru U, Moorman JP and Yao ZQ. PD-1 modulates regulatory T cells and suppresses T-cell responses in HCV-associated lymphoma. Immunol Cell Biol 2011; 89: 535-539.
[11] Baqai SF, Proudfoot J, Yi DH, Mangahas M and Gish RG. High rate of core promoter and precore mutations in patients with chronic hepatitis B. Hepatol Int 2015; 9: 209-217.
[12] Zhang Q, Liao Y, Cai B, Li Y, Li L, Zhang J, An Y and Wang L. Incidence of natural resistance mutations in naive chronic hepatitis B patients: a systematic review and meta-analysis. J Gastroenterol Hepatol 2015; 30: 252-261.
[13] Mondal RK, Khatun M, Ghosh S, Banerjee P, Datta S, Sarkar S, Saha B, Santra A, Banerjee S and Chowdhury A. Immune-driven adaptation of hepatitis B virus genotype D involves preferential alteration in B-cell epitopes and replicative attenuation--an insight from human immunodeficiency virus/hepatitis B virus coinfection. Clin Microbiol Infect 2015; 21: 710, e11-20.
[14] Balmasova IP, Yushchuk ND, Mynbaev OA, Alla NR, Malova ES, Shi Z and Gao CL. Immunopathogenesis of chronic hepatitis B. World J Gastroenterol 2014; 20: 14156-14171.
[15] Peng G, Li S, Wu W, Sun Z, Chen Y and Chen Z. Circulating CD4+CD25+ regulatory T cells correlate with chronic hepatitis B infection. Immunology 2008; 123: 57-65.
[16] Zhang HH, Guo F, Fei R, Ma H, Cong X, Wei L and Chen HS. [Inhibition of CD4+ CD25+ regulatory T cells in chronic hepatitis B patients]. Zhonghua Yi Xue Za Zhi 2008; 88: 511-515.
[17] Zhou L, Li X, Huang X, Chen L, Gu L and Huang Y. Soluble programmed death-1 is a useful indicator for inflammatory and fibrosis severity in chronic hepatitis B. J Viral Hepat 2019; 26: 795-802.
[18] Zhang HH, Mei MH, Fei R, Liu F, Wang JH, Liao WJ, Qin LL, Wei L and Chen HS. Regulatory T cells in chronic hepatitis B patients affect the immunopathogenesis of hepatocellular carcinoma by suppressing the anti-tumour immune responses. J Viral Hepat 2010; 17 Suppl 1: 34-43.
[19] Zhang HH, Fei R, Mei MH, Liao WJ, Wang XY, Cong X, Qin LL, Ji Y, Wang SX, Wei L and Chen HS. [The frequency, phenotypes and functions of CD4+ CD25+ regulatory T cells in hepatocellular carcinoma patients]. Zhonghua Gan Zang Bing Za Zhi 2007; 15: 266-272.
[20] Xu P, Chen YJ, Chen H, Zhu XY, Song HF, Cao LJ and Wang XF. The expression of programmed death-1 in circulating CD4+ and CD8+ T cells during hepatitis B virus infection progression and its correlation with clinical baseline characteristics. Gut Liver 2014; 8: 186-195.
[21] Hou FQ, Wu XJ, Wang Y, Chen J, Liu YZ, Ren YY, Song G, Ding YP, Yu M and Wang GQ. Rapid downregulation of programmed death-1 and interferon-gamma-inducible protein-10 expression is associated with favourable outcome during antiviral treatment of chronic hepatitis B. J Viral Hepat 2013; 20 Suppl 1: 18-26.
[22] Arnaout M, Mongardon N, Deye N, Legriel S, Dumas F, Sauneuf B, Malissin I, Charpentier J, Pene F, Baud F, Chiche JD, Mira JP and Cariou A. Out-of-hospital cardiac arrest from brain cause: epidemiology, clinical features, and outcome in a multicenter cohort*. Crit Care Med 2015; 43: 453-460.