PD-1.1 polymorphism associates with liver cirrhosis caused by chronic HBV infection

Pham Thi Minh Huyen, Mai Thanh Binh, Nghiem Xuan Hoan

108 Institute of Clinical Medical and Pharmaceutical Sciences

Summary

Objective: PD-1 is an immunoinhibitory receptor which plays vital role in pathogenesis of hepatitis B virus (HBV) infection. The aim of this study was to understand the relationship between *PD-1.1* polymorphism in HBV infection and liver disease progression. *Subject and method:* 154 chronic hepatitis B virus (CHB), 127 liver cirrhosis (LC), 186 hepatocellular carcinoma (HCC) and 160 healthy controls. The *PD-1.1* polymorphism was genotyped by directed Sanger sequencing. *Result:* The *PD-1.1 TT* genotype was found to be a risk factor for LC predisposition of CHB patients. *Conclusion:* The *PD-1.1* polymorphisms are related to the progession of HBV-related liver disease, especially as a prognostic factor for cirrhosis liver in patients with chronic hepatitis B.

Keywords: Hepatitis B virus, PD-1/PD-L1, PD-1.1, polymorphism.

1. Background

Hepatitis B virus (HBV) is one of the most common pathogens globally. HCC and LC due to chronic HBV infection have been a serious medical problem in Asian countries for their high prevalence of HBV infection [15].

The cellular immune response plays a vital role in HBV infection pathogenesis through the activities of CD4+ and CD8+ T cells, especially HBV-specific CD8+ T cells. Hepatitis B virus is eliminated mainly through the antiviral activity of HBV-specific CD8+ T cells, including both cytotoxic and non-cytotoxic activity [5]. However, in patients with chronic hepatitis B virus, the response of HBV-specific CD8+ T cells is relatively weak [4].

Studies showed that depletion of T-cell immune function is induced by overexpression of immune checkpoint inhibitors such as: PD-1, TIM-3, CTLA-4, 2B4, CD160, LAG-3 in HBV-specific T cells [4]. Among them, the immunosuppressive activity of the PD-1/PD-L1 (programmed cell death 1/programmed cell death ligand 1) signaling pathway plays the role as a central regulator to the dysfunction of T cell in chronic HBV infection by inhibiting the activation of HBV-specific T cells in various ways [13, 17].

To date, there have been a number of several studies evaluating the association between genetic variants *PDCD1,* which encode protein PD-1, in cancer, autoimmune diseases and infections [3, 13]. In which *PD-1.1* is located in the promoter region of the *PDCD1* gene. *PD-1.1* polymorphism is considered to influence the transcription and activation of the *PD-1* gene. Therefore, this polymorphism might affect on PD-1 protein function in inhibiting T-cell activation. Several studies have shown that the *PD-1.1* polymorphism is associated with susceptibility to autoimmune disease and cancers. We have recently conducted a case-control study aiming to investigate the relationship between *PD-1.1* polymorphism with chronic HBV infection and progression of HBV-related liver diseases.

***Received**: 28 September 2023, **Accepted**: 15 November 2023*

***Correspondence to**: Pham Thi Minh Huyen - 108 Institute of Clinical Medical and Pharmaceutical Sciences*

***Email:** minhhuyenv108@gmail.com*

2. Subject and method

2.1. Study subject

The study included 467 adult patients with chronic HBV infection, including: 154 patients with CHB, 127 patients with LC and 186 patients with HCC in accordance with the diagnostic criteria according to "Guidelines for the diagnosis and treatment of hepatitis B virus- Vietnam Ministry of Health-2019" [1] and "Guidelines for the diagnosis and treatment of Hepatocellular carcinoma - Vietnam Ministry of Health" [2]. In addition, 160 healthy individuals were collected as healthy controls.

All study subjects were confirmed negative for anti-HCV and anti-HIV by Elisa assays (Diagnostic automation/Cortez Diagnostics, Inc., Woodland Hills, California, USA).

2.2. Method

Study design: Case-control study.

Time and place: The study was conducted at 108 Military Central Hospital from December 2019 to June 2021.

Techniques

PD-1.1 genotyping: Genomic DNA was isolated from 200µl of whole blood using a DNA isolation kit (Qiagen, Hilden, Germany), following manufacturer's instructions. The process of *PD-1* genotyping was direct Sanger sequencing. The amplicon containing the variants *PD-1.1 (rs36084323)* was amplified by PCR using the specific primer pairs *PD-1.1*_F: 5'-TGG CAC GAG TGG GCT GGA G-3' and *PD-1.1*_R: 5'-GGC TCA GGT TCC TGG GCT G-3'. Primer pair sequence was designed by our team using Primer 3 software. PCR products were purified using Exo-SAP-IT PCR product purification kit (Afymetrix Santa Clara, USA) 5µl of purified PCR products were used as sequencing templates. Sequencing was performed using the BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) on the ABI 3130XL sequencer according to the manufacturer's instructions.

Gene sequencing results:

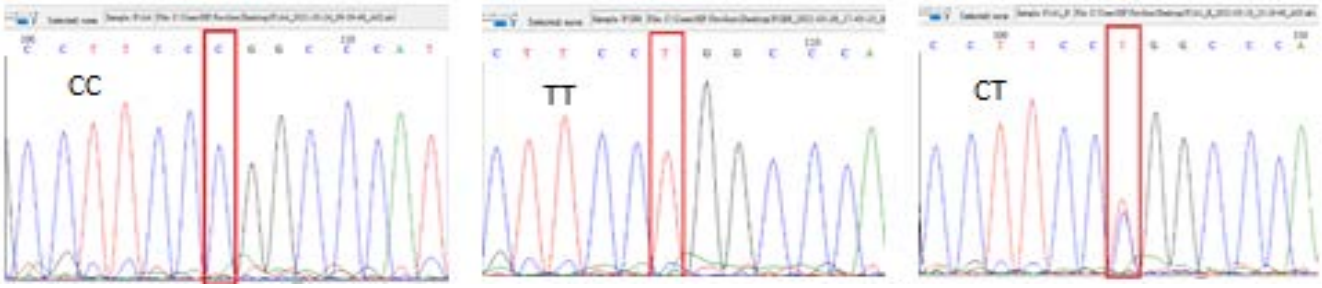

Figure 1. The results of PD-1.1 sequencing.

2.3. Statistical analysis

All statistical analysis was performed using SPSS 25.0 software. Chi-squared tests were used to test for significant differences between categorical variables. Mann-Whitney tests were applied to compare quantitative variables between groups. We applied binary logistic regression model adjusted for age and gender to determine the association between *PD-1.1* genetic variants and progression of HBV-related liver disease. Significance was set at a value of $p<0.05$.

2.4. Ethics statement

This study was approved by Hanoi medical university ethics committee coded 64/ GCN-HĐĐĐNCYSSH-ĐHYHN 25/03/2020. Informed written consent was obtained from all participants after explanation of the study at the time of sampling.

3. Result

3.1. Baseline characteristics of subjects

Table 1. Clinical and subclinical characteristics of patients and healthy control

Character	HBV (n = 467)	Healthy controls (n = 160)	p
Age (years)	54.07 (19-90)	44.33 (19-69)	0.0001[a]
Men/ women	392/75	86/74	0.0001[b]
AFP (IU/mL)	10055.93 (1-524080)	ND	NA
HBV DNA (copies/mL)	22.9×10^7 ($7\text{-}2.25\times10^{10}$)	ND	NA
PLT (x 10^3/L)	183.45 (20-598)	266.15 (118-422)	0.0001 [a]
AST (U/L)	300.31 (3-3679)	22.51 (11-49)	0.0001 [a]
ALT (U/L)	380.87 (8-4926)	20.83 (4-53)	0.0001 [a]
Total Bilirubin (µmol/L)	74.38 (4.5-1504)	11.81 (1.52-15.9)	0.0001 [a]
Direct Bilirubin (µmol/L)	42.11 (0.5-542.7)	6.7 (1.8-8.2)	0.032 [a]
Albumin (g/L)	36.08 (17-49)	43.99 (41-47)	0.0001 [a]
Prothrombin (% of standard)	78.37 (14-136)	ND	

Abbreviation: AST: Aspartate amino transferase, ALT: Alanine amino transferase, AFP: Alpha-feto protein, IU: International unit, ND: Not done, NA: Not applicable. The results are presented in median and range. a: P value calculated by Mann-Whitney test, b: p value calculated by Chi-square test.

Comments: The mean age of the patient group was higher than that of the control group, with male majority in both groups. Platelet count, albumin concentration of the HBV group was significantly higher than that of the control group, while the levels of AST, ALT, total bilirubin, and direct bilirubin were significantly higher than that of the control group.

3.2. Association of PD-1.1 polymorphism with susceptibility to HBV infection

Table 2. Frequencies of PD-1.1 polymorphism in HBV and control groups

PD-1.1	HBV (n = 467) (%)	Controls (n = 160) (%)	OR (95% CI)	p
PD1.1 (rs36084323)				
CC	143 (30.6)	50 (31.3)	1	
CT	238 (51.0)	82 (51.2)	0.94 (0.59-1.51)	0.81
TT	86 (18.4)	28 (17.5)	1.03 (0.56-1.89)	0.93
Allele				
C	524 (56.1)	182 (56.9)	1	
T	410 (43.9)	138 (43.1)	1.0 (0.75-1.35)	0.98
Dominant				
CC	143 (30.6)	50 (31.2)	1	
CT&TT	324 (69.4)	110 (68.8)	0.97 (0.62-1.51)	0.88
Recessive				
CC&CT	381 (81.6)	132 (82.5)	1	
TT	86 (18.4)	28 (17.5)	1.07 (0.62-1.82)	0.82

Abbreviation: OR: Odd ratio, OR and P values were calculated by using binary logistic regression model adjusted for age and gender.

Comments: There was no significant difference in genotype and allele distribution of the PD-1.1 polymorphism between the HBV group and the controls.

3.3. The association of PD-1.1 polymorphism with liver disease progression

Table 3. The association of PD-1.1 polymorphism with liver disease progression

PD1	CHB, n (%)	LC, n (%)	HCC, n (%)	LC vs. CHB		HCC vs. CHB		HCC vs. LC	
				OR (95%CI)	p	OR (95% CI)	p	OR (95% CI)	p
PD1.1 (promoter, *rs36084323)*									
CC	53 (34.4)	38 (29.9)	52 (28.0)	1		1		1	
CT	81 (52.6)	59 (46.5)	98 (52.7)	1.0 (0.6-1.8)	NS	1.1 (0.6-1.9)	NS	1.1 (0.6-1.9)	NS
TT	20 (13.0)	30 (23.6)	36 (19.4)	2.2 (1.04-4.6)	0.04	1.5 (0.7-3.2)	NS	0.9 (0.5-1.8)	NS
Allele									
C	187 (60.7)	135 (53.1)	202 (54.3)	1		1		1	
T	121 (39.3)	119 (46.9)	170 (45.7)	1.38 (0.97-1.98)	NS	1.2 (0.8-1.7)	NS	1.0 (0.7-1.3)	NS
Dominant									
CC	53 (34.4)	38 (29.9)	52 (28.0)	1		1		1	
CT&TT	101 (65.6)	89 (70.1)	134 (72.0)	1.23 (0.72-2.1)	NS	1.2 (0.7-2.0)	NS	1.0 (0.6-1.7)	NS
Recessive									
CC&CT	134 (87.0)	97 (76.4)	150 (80.6)	1		1		1	
TT	20 (13.0)	30 (23.6)	36 (19.4)	2.2 (1.1-4.2)	0.02	1.4 (0.7-2.7)	NS	0.9 (0.5-1.5)	NS

Abbreviation: OR: Odds ratio, CHB: Chronic hepatitis B, LC: Cirrhosis liver, HCC: Hepatocellular cancer, non-HCC: Chronic hepatitis B and liver cirrhosis. OR and 95% CI were calculated by using binary logistic regression model adjusted for age and sex. Bold values present the statistical significance. NS: Non significant.

Comments: The frequency of TT allele of PD-1.1 in liver cirrhosis group was significantly higher than that in chronic hepatitis B group when compared with CC genotype as well as TT versus CC and CT recessive models (Multivariate logistic regression model adjusted for age and sex).

4. Discussion

PD-1 is an immunosuppressive receptor that inhibits the activation of T lymphocytes, enhancing immune tolerance. Increased PD-1 expression on T-lymphocyte is an important mechanism leading to T-lymphocyte depletion in chronic hepatitis B virus infection [15]. Therefore, the level of PD-1

expression is closely related to the pathogenesis of chronic hepatitis B virus infection. The *PD-1.1* polymorphism is located in the promoter region of the *PDCD1* gene (coding for the PD-1 protein) where transcription factors and polymerases cooperate to influence transcription and synthesis of the PD-1 protein. Therefore, it has an impacts on many human diseases such as: Cancer, autoimmune diseases... [10]. In this study, we found that *PD-1.1* polymorphism are related to liver disease progression associated with HBV infection.

PD-1.1 is located in the putative binding site for the UCE-2 transcription regulators. As the consequence, the promoter activity in the construct containing *PD-1.1G* allele was significantly higher than that with the *PD-1.1 A allele* [10]. Hence, it can be assumed that carriers of *PD-1.1 G* allele could have a higher expression of PD-1. Thus, they inhibited activation and proliferation of T cells, which in turn can lead to poor ability to remove cancer cells [11]. However, the results of clinical studies are different. Several studies have shown that the *PD-1.1* polymorphism is associated with susceptibility to autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and certain cancers such as non-small cell lung cancer, breast cancer [9]. Nonetheless, Hashemi's meta-analysis showed there was no association between *PD-1.1* variants with overall cancer risk. Though, in the racial analysis of Da et al. *PD-1.1 TT* increased overall cancer risk in the Asian population [7]. In this study, we found no association between the *PD-1.1* polymorphism and HBV infection. This result once again confirms the conclusion of Guoyu Zhanga et al [6] as well as the study of Feng Lv et al, that there were no relationship between genotype frequencies, alleles of *PD-1.1* and chronic HBV infection in the Chinese population [12].

When analyzing the frequency of *PD-1.1* genotypes among subgroups of chronic hepatitis B virus-infected patients, it was found that the *PD-1.1 TT* genotype increased the risk of cirrhosis liver in chronic hepatitis B patients when compared with *CC* genotype, as well as when analyzed in recessive model *TT* versus *CT* and *CC*. This result is consistent with the conclusion of Zhouhua Hou (2017) that the frequency of *PD1.1 TT* genotype was significantly higher in the chronic HBV infection group in comparision to spontaneously recovered group [9]. However, this result is different from the results of Hong Peng et al. (2015), which is also on the Chinese population, which concluded that *PD-1.1 AG* genotype is a risk factor for cirrhosis liver, HCC in patients with chronic hepatitis B [8]. The difference might due to the distinction in the study sample, the technique of genotyping the SNP of the two studies.

This study demonstrated the role of *PD-1.1* genetic polymorphism in monitoring liver disease progression associated with hepatitis B virus infection. However, the results of this study will be more meaningful if we could have determined the expression of PD-1 protein in PBMC (peripheral blood mononuclear cells) as well as protein expression in liver tissue by immunohistochemical assays. Nevertheless, in this study, neither liver tissue samples nor blood samples were available for isolation of PBMC.

5. Conclusion

This study showed that the *PD-1.1* polymorphisms are associated with the progression of HBV-related liver disease, especially as a prognostic factor for cirrhosis liver in patients with chronic hepatitis B.

Acknowledgments: We would like to thank 108 Military Central Hospital, Hanoi Medical University, and the National Foundation for Science and Technology Development (Nafosted) for their support. We sincerely thank all study subjects for their participation.

References

1. Vietnam Ministry of Health (2019) *Guidelines for the diagnosis and treatment of hepatitis B virus.*
2. Vietnam Ministry of Health (2020*) Guidelines for the diagnosis and treatment of Hepato cellular carcinoma.*

3. Salmaninejad A, Khoramshahi V, Azani A, Soltaninejad E, Aslani S, Zamani MR, Zal M, Nesaei A, Hosseini SM (2017) *PD-1 and cancer: molecular mechanisms and polymorphisms.* Immunogenetics 70(2): 73-86.
4. Chang JJ, Lewin SR (2007) *Immunopathogenesis of hepatitis B virus infection*. ImmunolCell Biol 85(1): 16-23.
5. Guidotti LG, FV C (2006) *Immunobiology and pathogenesis of viral hepatitis.* Annu Rev Pathol 1: 23-61.
6. Zhang G, Liu Z, Duan S, Han Q, Li Z, Lv Y, Chen J, Lou S, Li N (2010) *Association of polymorphisms of programmed cell death–1 gene with chronic hepatitis B virus infection*. Hum immunol 71(12): 1209-1213.
7. Hashemi M, Karami S, Sarabandi S et al (2019) *Association between PD-1 and PD-L1 polymorphisms and the risk of cancer: A meta-analysis of case-control studies*. Cancers (Basel) 11(8): 1150-1172.
8. Hong Peng Q-LL, Si-Hui Hou, Jun Hu, Jia-Hao Fan, Jin-Jun Guo (2015) *Association of genetic polymorphisms in CD8+ T cell inhibitory genes and susceptibility to and progression of chronic HBV infection.* Infect Genet 36: 467-474.
9. Hou Z, Zhou Q, Lu M, Tan D, Xu X (2017) *A Programmed cell death-1 haplotype is associated with clearance of hepatitis b virus.* Ann Clin Lab Sci 47(3): 334-343.
10. Ishizaki Y, Yukaya N, Kusuhara K et al (2010) *PD1 as a common candidate susceptibility gene of subacute sclerosing panencephalitis.* Hum Genet 127(4): 411-419.
11. Li Y, Zhang HL, Kang S, Zhou RM, N W (2017) *The effect of polymorphisms in PD-1 gene on the risk of epithelial ovarian cancer and patients' outcomes*. Gynecol Oncol 144(1): 140-145.
12. Lv Feng, Gao YF, Zhang ZH et al (2011) *Polymorphisms in programmed death-1 gene are not associated with chronic HBV infection in Chinese patients*. World J Hepatol 3(3): 72-78.
13. Rehermann B (2013) *Pathogenesis of chronic viral hepatitis: Differential roles of T cells and NK cells.* Nat Med 19(7): 859-868.
14. Sharpe AH, KE P (2018) *The diverse functions of the PD1 inhibitory pathway.* Nat Rev Immunol 18(3): 153-167.
15. WHO. Presentative Office-Vietnam (2018) http://www.wpro.who.int/vietnam/topics/hepatitis/factsheet/en/
16. WHO. Global hepatitis report (2017) http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/
17. Shuai Z, Leung MW, He X, Zhang W, Yang G, Leung PS, Eric Gershwin M (2016) *Adaptive immunity in the liver*. Cellular & Molecular Immunology 13: 354-368.