Levels and Significance of Serum Adiponectin in Patients with Chronic HBV Infection at Different Clinical Stages

Bei Liu

Department of Medical Technology, Xi'an Medical College, Xi'an 710021, China

Abstract: To analyze the serum APN levels of patients with chronic HBV infection in different clinical stages and their correlation with clinical laboratory examination indicators. A total of 120 HBV-infected patients are included in this study, including chronic HBV carriers, chronic hepatitis B (CHB) and compensated cirrhosis patients, 40 cases in each group, and 40 medical examinees as healthy controls. Compared with the healthy control group, the APN level in the chronic HBV infection group is significantly increased ($p<0.05$), and the APN level in the cirrhosis group is significantly increased compared with the other two groups ($p<0.05$). There is no significant difference between the chronic HBV carrier group and the CHB group. The ratio of people with high APN in the high DNA viral load group is higher ($p = 0.002$, $\chi2 = 9.143$); the APN level of the liver cirrhosis group is significantly different from the non-cirrhosis group ($P = 0.004$, $\chi2 = 8.123$). There is no significant correlation between APN level and ALT, AST and other indicators (P>, 0.05). High serum APN may be used as a marker for the diagnosis of HBV-infected liver cirrhosis.

1 Introduction

China is an area with high incidence of hepatitis B virus (HBV) infection. Chronic HBV infection is prone to develop into chronic hepatitis and liver fibrosis, and some may develop into cirrhosis or liver cancer, which seriously threatens the health of the people. [1]Adiponectin (APN) is a fat factor specifically secreted by adipose tissue and high in plasma, which plays a key role in regulating lipid and sugar metabolism. The liver is the main target organ for APN. APN can promote fatty acid oxidation in the liver, reduce lipid synthesis, inhibit gluconeogenesis, increase insulin sensitivity, and inhibit inflammation [1-3]. The relationship between APN and HBV infection is still unclear, and the relationship between APN level and clinical stage of HBV infection and liver fibrosis has not been elucidated. The purpose of this study is to analyze the serum APN levels of patients with chronic HBV infection in different clinical stages and their correlation with clinical laboratory examination indicators. The reports are as follows:

2 Materials and methods

2.1 Study objects

This study retrospectively analyzes 120 cases of HBV-infected patients in the Department of Infectious Diseases, Tangdu Hospital, Air Force Military Medical University from June 1, 2017 to December 31, 2018, including 40 chronic HBV carriers, 40 chronic hepatitis b (CHB), and 40 patients with compensated cirrhosis. All patients are treated initially (no antiviral treatment in the past six months). The diagnosis and clinical staging of chronic HBV infection are based on the diagnostic criteria of the Guidelines for the Prevention and Treatment for Chronic Hepatitis B (2010 Version). The exclusion criteria are: patients with other hepatitis virus infection, alcoholic hepatitis, autoimmune liver disease and tumor. In this study, 40 healthy people who came to the hospital for medical examination during the same period are used as healthy controls.

2.2 Reagents and methods

Fasting venous blood is collected in the morning and serum is separated by centrifugation. The five Hepatitis B items (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb) are detected by Abbott's automatic chemiluminescence immunoanalyzer, and HBV virus DNA HBV DNA is quantified by real-time fluorescence quantitative PCR (Zhongshan Daan kit). ALT, AST, TBiL and other items are tested by Biochemical analyzer of Olympus Corporation of Japan. PT detection is performed using the automatic coagulation analyzer detection system. ANP is detected by ELISA, a kit from American R&D Systems.

* Corresponding author: liubei83@163.com

2.3 Statistical analysis

SPSS 25.0 software is used for statistical analysis, measurement variables are expressed as x±s, and comparison between groups is by t test. Count variables are described by percentage (%) for descriptive statistical analysis, and comparison between groups is by χ2 test. Correlation is analyzed by Spearman correlation. All statistical results are statistically significant with P<0.05.

3 Result

3.1 General conditions of the subjects and laboratory examination parameters

According to the clinical diagnosis, the patients are divided into three groups: chronic HBV carriers, CHB, and compensated cirrhosis, with 40 cases in each group. The chronic HBV carrier group is younger than the other groups (p<0.05), and there is no statistical difference in age among the other three groups. The gender ratio of each group, ALT, AST, TBIL, PT, viral DNA load, HBeAg positive rate values are shown in Table 1. Among them, the ALT and AST values of the cirrhosis group and the CHB group are significantly higher than those of the HBV carrier group (p<0.05), the PT value of the cirrhosis group is significantly longer than the CHB group (p<0.05), and the DNA load of the HBV carrier group is higher than that of the liver. The sclerosis group increased significantly (p<0.05) (see Table 1).

3.2 Serum APN levels of HBV-infected patients and healthy controls in each group

The detection values of serum APN in each group are 4.1±1.9μg/ml in chronic HBV carrier group, 4.2±2.6μg/ml in CHB group, 5.2±2.2μg/ml in hepatitis B cirrhosis group, and 2.7±1.4μg/ml in healthy control group. Compared with the healthy control group, the HBV chronic infection group is significantly increased (p<0.05). Compared with the HBV infection group, the cirrhosis group is significantly increased compared with the other two groups (p<0.05). There is no significant difference between the chronic HBV carrier group and the CHB group (see Table 1).

Table 1 Basic parameters of laboratory tests for patients with chronic HBV infection and healthy controls

	Chronic HBV carrier （Group A）	CH Group B）	Hepatitis B cirrhosis （Group C）	Healthy controls （Group D）
	n=40	n=40	n=40	n=40
Age	28.1±6.6* a c	44.8±9.2	51.2±7.2	46.0±10.2
Sex F /M	24（60%） /16（40%）	24（60%） /16（40%）	23（58%） /17（52%）	23（58%） /17（52%）
ALT	27.4±7.3 a c	70.5±84.0 *	54.0±21.4*	19.5±13.2
AST	31.6±7.3 a c	51.6±44.0*	53.2±20.1*	21.5±12.8
TBIL	17.1±5.1	20.4±11.3*	26.4±34.6*	10.56±7.1
PT	12.6±1.7	12.44±1.7 b*	13.6±1.6*	10.75±0.6
HBV DNA log10 IU/mL）	6.8±1.5 a	5.36±0.75	5.8±0.74#	-
HBeAg Positive rate	32（80%）	7（17.5%）	24（60%）	-
APN	4.1±1.9* c	4.2±2.6* b	5.2±2.2*	2.7±1.4

*Compared with healthy control group (Group D), P<0.05A
a Chronic HBV carrier group (group A) compared with CHB (group B), P<0.05
b CHB (group B) is compared with hepatitis B cirrhosis (group C), P<0.05C
c Chronic HBV carrier group (Group A) compared with hepatitis B cirrhosis group (group C), P<0.05
#DNA test in 4 patients with cirrhosis <103

3.3 Comparison of clinical and laboratory test parameters grouped with APN detection median 4.2 g/ml

In order to study the relationship between APN and clinical diagnosis and laboratory test parameters, we divide the patient's APN test results into a high APN and a low APN group with a median of 4.2 μg/mL, and divide liver function indicators ALT, AST, HBeAg and whether they occurred Cirrhosis is also grouped according to normal/abnormal (yes/no) results. DNA viral load is divided into two groups of high viral load (≥106IU/mL) and low viral load (<106IU/mL) at 106IU/mL. The difference between the two groups is compared, and the χ2 test is used for statistical analysis. The results show that among patients with high DNA viral load, those with high APN accounted for 57.3%, and among patients with low DNA viral load, those with low APN accounted for 71.1%; there are significant differences in the APN levels between the two groups (p=0.002, X2=9.143); the occurrence of liver cirrhosis is significantly different from the APN level (p=0.004, χ2=8.123). At the same time, among those with normal AST, those with high APN accounted for 42.5%, and those with abnormal AST accounted for 67.5%, with statistical differences (p=0.01, χ2=6.669). The ALT and HBeAg results are not statistically different from the APN level (P>0.05) (see Table 2).

Table 2 Comparison of clinical and laboratory test parameters grouped by APN detection median 4.2 g/ml

	APN（μg/ml）				P value	X^2
	<4.2		≥4.2			
ALT（U/L）	n	%	n	%		
<40	37	48.1	40	51.9	0.369	0.809
≥40	17	41.4	26	58.6		
AST						
<40	46	57.5	34	42.5	0.01	6.669
≥40	13	32.5	27	67.5		
HBV DNA log10 IU/mL）						
<10*6	32	71.1	13	28.9	0.002*	9.143
≥10*6	32	42.6	43	57.3		
HBeAg						
positive	16	33.3	32	66.7	0.641	0.217
negative	27	37.5	45	62.5		
Whether cirrhosis of the liver occurs						
Yes	11	27.5	29	72.5	0.004*	8.123
NO	44	55.0	36	45.0		

* P<0.05

3.4 Correlation between clinical test index and ANP test value

Spearman correlation analysis of ALT, AST, TBIL, PT, DNA virus load values and ANP detection values of all HBV infected patients showed no significant correlation between APN and each indicator (see Table 3).

Table 3 Correlation between clinical indicators and ANP

	ANP	
	r	P
ALT	0.088	0.615
AST	0.478	0.106
TBIL	0.033	0.853
PT	0.246	0.167
DNA（log10 IU/ml）	0.013	0.944

4 Discussion

Chiang et al. [4]and Hsu et al. [5] confirm that serum APN of HBV infected patients is significantly higher than that of healthy controls, which is consistent with the results of this study. We also find that patients with high DNA viral load have a higher proportion of APN. Peroxisomal proliferator-activated receptor (PPAR) is an important molecule for signal transduction after APN binding to the receptor. Cell experiments in vitro shows that viral DNA replication can up-regulate APN expression by promoting PPAR expression [6]. Treatment with PPAR agonist (benzabate), PPAR agonist (Fenofibrate), and PPAR agonist (rosiglitazone) in a mouse model significantly promoted HBV DNA replication [7-9]. The above studies suggest that the regulatory patterns of HBV gene and key molecules of APN signaling pathway in hepatocytes are similar and mutually reinforcing, which may be a reason for the increased APN level in patients with chronic HBV infection. After chronic HBV infection, the immune response of the body to the virus will cause liver cell damage and inflammation necrosis. The pathological manifestations of the liver are as follows: excessive activation of HSC transformed into myofibroblasts and synthesis of excessive extracellular matrix, liver tissue activity increased, and inflammation enhanced. If patients do not receive antiviral treatment, will cause liver fibrosis, and even progress to cirrhosis. In this study, APN levels are significantly increased in the cirrhosis group compared with the other two groups in each clinical stage of chronic HBV infection. The reasons for the significant increase of APN in patients with liver cirrhosis are analyzed: cell and animal experiments suggest that APN might play a protective role in the process of liver cell injury: APN could, on the one hand, inhibit the proliferation and activation of HSC [10,11]; On the other hand, the over-strong immune response can be regulated by regulating the polarization of macrophages toward M2 (anti-inflammatory) and inhibiting the expression of pro-inflammatory factors to play an anti-inflammatory role [12-13]. Different drugs and diets are used to induce mouse liver fibrosis models, and the results all confirm that APN knockout mice has significantly enhanced liver fibrosis degree compared with wild-type mice, while APN overexpression could significantly reduce the degree of liver fibrosis in mice [14]. Therefore, a significant increase in serum APN in patients with cirrhosis may be a protective mechanism against excessive inflammatory response and liver injury.

In addition, the liver is the main site of APN metabolism and clearance, and HBV-induced cirrhosis leads to decreased excretion and clearance of APN in the liver, which is also an important reason for increased SERUM APN in patients with cirrhosis.

Regarding whether the elevated APN level is related to the degree of liver fibrosis and liver function indicators, the current research results are still divided: most studies [15-16] support that high APN levels are positively correlated with the fibrosis grade of patients with chronic viral hepatitis. However, some studies have shown that APN levels have no significant correlation with liver fibrosis stage[17]. Since this study did not include patients with decompensated cirrhosis, the correlation between liver fiber grade and APN stage is not studied; analysis of the relationship between APN and ALT, AST, TBIL, PT and viral DNA load showed that APN level There is no obvious correlation with each index. We speculate that the reasons for these different results may be due to differences in the degree of obesity, insulin resistance, viral genotype, number of cases, and whether or not to receive antiviral treatment in patients included in different studies. Therefore, the relationship between APN and disease progression after HBV infection and liver fibrosis grade needs to be further clarified by large-scale prospective clinical studies.

Conclusion: This study confirms that the serum APN of patients with chronic HBV infection is significantly higher than that of healthy controls, and the serum APN of patients with liver cirrhosis is significantly higher than that of patients with chronic HBV carriers and CHB. Among chronic HBV infected patients, the proportion of patients with high DNA viral load with high APN is higher. The cirrhotic group has higher APN than the non-cirrhotic group. High serum APN may serve as a potential marker for assisting the diagnosis of HBV-infected cirrhosis.

Acknowledgment

This work was supported by Shaanxi Natural Science Basic Research Foundation (2017JM8011), and the Foundation of Xi'an Medical College (2018PT70).

Reference

1. Fang H, Judd RL. Adiponectin Regulation and Function [J]. Compr Physiol, 2018,8(3): 1031-1063.
2. Zhang Delong, Ma Xiaoxia, Li Yufei, et al. Research Progress and Prospect Of Adiponectin and Its Receptor In Mammals [J]. Biotechnology bulletin,2020,36(6):60-68.
3. Gamberi T, Magherini F, Modesti A, et al. Adiponectin Signaling Pathways in Liver Diseases[J]. Biomedicines, 2018,6(2):52-52
4. Chiang CH, Lai JS, Hung SH, et al. Serum adiponectin levels are associated with hepatitis B viral load in overweight to obese hepatitis B virus carriers[J]. Obesity (Silver Spring), 2013,21(2): 291-296.
5. Hsu CS, Liu CH, Wang CC, et al. Impact of hepatitis B virus infection on metabolic profiles and modifying factors[J]. J Viral Hepat, 2012,19(2): e48-57.
6. Yoon S, Jung J,Kim T, et al.Adiponectin, a downstream target gene of peroxisome proliferator-activated receptor gamma, controls hepatitis B virus replication[J]. Virology, 2011,409(2):290-8
7. Du, L., et al.Peroxisome Proliferators Activated Receptor (PPAR) agonists activate hepatitis B virus replication in vivo[J]. Virol J, 2017,14(1): 96-96
8. Adachi, M. and D.A. Brenner. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase[J]. Hepatology, 2008,47(2): 677-85
9. Wulster-Radcliffe M.C, Ajuwon KM, Wang J et al.Adiponectin differentially regulates cytokines in porcine macrophages[J]. Biochem Biophys Res Commun, 2004,316(3): 924-9.
10. Ohashi, K., et al.Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype[J]. J Biol Chem, 2010,285(9): 6153-60.
11. Kamada Y, Tamura S, Kiso S, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin[J]. Gastroenterology, 2003,125(6): 1796-807.
12. Shafiei MS, Shetty S, Scherer PE, et al. Adiponectin regulation of stellate cell activation via PPARgamma-dependent and -independent mechanisms[J]. Am J Pathol, 2011,178(6):2690-2699.
13. Hui CK, Zhang HY, Lee NP, et al. Serum adiponectin is increased in advancing liver fibrosis and declines with reduction in fibrosis in chronic hepatitis B[J]. J Hepatol, 2007,47(2): 191-202.
14. Siagris D, Vafiadis G, Michalaki M, et al. Serum adiponectin in chronic hepatitis C and B[J]. J Viral Hepat, 2007,14(8): 577-83.
15. Tsochatzis E, Papatheodoridis GV, Hadziyannis E, et al. Serum adipokine levels in chronic liver diseases: association of resistin levels with fibrosis severity[J]. Scand J Gastroenterol, 2008,43(9): 1128-1136.
16. Hsu CS, Liu WL, Chao YC, et al. Adipocytokines and liver fibrosis stages in patients with chronic hepatitis B virus infection[J]. Hepatol Int, 2015,9(2): 231-242.
17. Wong VW, Wong GL, Yu J, et al. Interaction of adipokines and hepatitis B virus on histological liver injury in the Chinese[J]. Am J Gastroenterol, 2010,105(1): 132-138.