



Case Report

Analysis of HBV-RNA level in patients with Chronic hepatitis B whose HBV-DNA is negative after NAs antiviral therapy

Wang Chunyan¹, Cao Yu¹, Guo Yuanqiang², Wen Jun¹, Li Jia¹

¹Tianjin Second People's Hospital, Tianjin, China

²Nankai University, Tianjin, China

***Corresponding author:** Wang Chunyan, Tianjin Second People's Hospital, Tianjin, China. Email: water4645@sina.com

Citation: Chunyan W, Yu C, Yuanqiang G, Jun W, Jia L (2020) Analysis of HBV-RNA level in patients with Chronic hepatitis B whose HBV-DNA is negative after NAs antiviral therapy. Ann Case Report 15: 5001. DOI: 10.29011/2574-7754/105001

Received Date: 16 June, 2020; **Accepted Date:** 13 July, 2020; **Published Date:** 17 July, 2020

Abstract

Background: While Nucleotide Analogs (NAs) are widely used in the treatment of chronic Hepatitis B Virus (HBV) infection, it cannot clear covalently closed circular DNA from the liver, making chronic hepatitis B difficult to cure. However, NAs can effectively inhibit retroviral transcription and viral DNA synthesis, and when HBV DNA is reduced to the lower limit of detection, HBV RNA becomes a more valuable diagnostic marker.

Aim: To analyze HBV RNA levels in chronic HBV patients who tested negative for HBV DNA after NAs antiviral therapy.

Methods: We enrolled 60 chronic hepatitis B patients who were admitted to Tianjin Second People's Hospital from September 2018 to March 2019 in this study. Patients were treated with NAs antiviral therapy for at least one year. HBV DNA was detected and measured using Roche reagents and fluorescence quantitative polymerase chain reaction with a detection limit of 20 IU/ml at least two intervals of three months. These patients were also tested for HBV-RNA level, and Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), γ -Glutamyl Transpeptidase (GGT), Hepatitis B Surface Antigen (HBsAg), Hepatitis B E Antigen (HBeAg), Hepatitis B Core Antibody (HBcAb) in the meantime.

Results: Patients were divided into the undetected HBV DNA and < 20 IU/ml HBV DNA groups based on HBV DNA level. The quantitative comparison between these groups showed significant differences in HBV RNA level ($Z = 3.818$, $P < 0.05$). Patients were also divided into the HBeAg-positive and HBeAg-negative groups based on HBeAg level. There were significant differences in HBV RNA level between these groups ($Z = 3.363$, $P < 0.05$). Additionally, patients were divided into the following groups based on HBsAg level: the $\text{HBsAg} \leq 500$ IU/ml, $500 < \text{HBsAg} < 1500$ IU/ml, and $\text{HBsAg} \geq 1500$ IU/ml groups. There were no significant differences in HBV RNA level between these groups (Chi-square value = 5.891, $P > 0.05$). However, there were significant differences in HBV RNA level between the $\text{HBsAg} \leq 500$ IU/ml and $\text{HBsAg} \geq 1500$ IU/ml groups. ($Z = 2.426$, $P < 0.05$). ALT, AST, GGT, HBsAg, HBeAg, and HBcAb were included in the univariate linear regression analysis, which showed that only HBeAg level significantly correlated with HBV RNA level ($P < 0.05$).

Conclusion: HBV RNA may still be detected in chronic HBV patients after long-term NA antiviral therapy, and HBeAg level may be associated with serum HBV RNA level.

Keywords: Chronic Hepatitis B; HBeAg; HBsAg; HBV-DNA; HBV-RNA; NAs

Core Tip

We detected and measured the Hepatitis B Virus (HBV) RNA levels of chronic HBV patients whose HBV DNA levels were lower than the detection limit after undergoing nucleotide analog

antiviral therapy. We also compared HBV RNA to liver function and HBV markers. Based on our results, we learned that HBV RNA can still be detected in the absence of HBV DNA. Ours is also the first study to report the possible relationship between HBeAg and HBV RNA. Further, we believe that HBV DNA and RNA should be used concurrently as indicators of antiviral efficacy.

Introduction

NAs are widely used in the treatment of chronic hepatitis B. Although active antiviral therapy can delay the progression of liver diseases and reduce the occurrence of end-stage liver diseases, few patients have achieved the clinical cure standard, which is defined as HBsAg-negative conversion. About 650,000 people still die every year due to hepatic failure, cirrhosis, and hepatocellular carcinoma caused by HBV infection [1, 2]. Clinical findings have shown that, after NA treatment, while the detection of HBV DNA in chronic hepatitis B patients is lower than the detection limit, the disease may continue to progress, resulting in cirrhosis and liver cancer. However, recent studies have confirmed that serum HBV RNA is associated with the ratio of hepatic RNA to covalently closed circular DNA (cccDNA), which implies the occurrence of viral transcription. Therefore, HBV RNA can be used as a clinical marker for the transcriptional activity of cccDNA [3].

Previously, it was believed that chronic hepatitis B was difficult to cure due to the covalent closure of cyclic DNA in the nucleus of hepatocytes. Although NAs can effectively inhibit retroviral transcription and viral DNA synthesis, it cannot clear the cccDNA from the liver [4, 5]. Theoretically, both serum HBV DNA and RNA can be useful markers for HBV cccDNA activity in treatment-naïve patients. However, in CHB patients under NAs therapy, HBV DNA is suppressed due to halted pgRNA reverse transcription, making RNA a more direct marker for cccDNA [6]. When NAs reduce serum HBV DNA levels to the lower limit of detection, serum HBV RNA level becomes particularly valuable for diagnosis [7]. Therefore, in this study, we analyzed the influence of HBV RNA level on chronic hepatitis B patients whose HBV DNA levels were lower than the detection limit after undergoing NAs treatment in order to guide the formulation and adjustment of clinical antiviral treatment and delay the progress of liver disease.

Materials and Methods

Study Design

We enrolled 60 chronic hepatitis B patients who were admitted to Tianjin Second People's Hospital from September 2018 to March 2019. All patients were diagnosed as having chronic hepatitis B in accordance with the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2015 edition). All patients received NA antiviral therapy for at least one year. The detection of HBV DNA via Roche reagents was less than 20 IU/ml or undetected at least two intervals of three months. Patients were excluded if they had other hepatitis virus and cytomegalovirus infections, a history of taking medication for hepatitis, alcoholic hepatitis, autoimmune liver disease, and hereditary metabolic liver disease, cirrhosis, and hepatocellular carcinoma. This study was approved by the Medical Ethics Committee of Tianjin Second People's Hospital.

Detection Methods

HBV DNA and HBV RNA were detected simultaneously. HBV-DNA was detected by real-time fluorescent quantitative PCR. HBV DNA quantitative detection reagents were purchased from Roche Diagnostic Products (Shanghai) Co., Ltd. HBV DNA detection was carried out via fluorescence quantitative polymerase chain reaction with a detection limit of 20 IU/ml. HBV-RNA was detected by real-time fluorescent quantitative PCR. HBV RNA quantitative reagents were purchased from China Hunan Shengxiang Biological Technology Co., Ltd. HBV RNA was detected with a detection limit of 100 IU/ml. HBV serum markers (quantitative method) were detected via chemiluminescence methods, and reagents were purchased from Abbott Medical (Shanghai) Co., Ltd. Liver function was evaluated using a Hitachi 7180 automatic biochemical analyzer. Whenever HBV RNA was detected, ALT, AST, GGT, and HBV serum markers were detected simultaneously.

Statistical Methods

SPSS 23.0 was used for all statistical analyses. For measurement data, normal distributions were expressed as mean \pm standard deviation and non-normal distributions were expressed as median plus quartile. The Kruskal-Wallis test was used for multigroup comparisons and the rank sum test was used for pairwise comparisons between groups. The influencing factors of HBV RNA were analyzed via single-factor linear regression. A P value of < 0.05 was considered statistically significant.

Results

Patients' Demographic and Clinical Characteristics

A total of 60 patients were enrolled in this study, including 41 males and 19 females who were 27-69 years old (Table 1).

Parameter group (n=60)	
Age	44.3 \pm 9.8
Gender	41/19(Male/Female)
ALT(U/L)	25.16 \pm 5.23
AST(U/L)	26.23 \pm 6.01
GGT(U/L)	33.2 \pm 10.12
ALB(g/L)	44.2 \pm 6.9
TBIL(umol/L)	19.5 \pm 5.2
Antiretroviral drugs	
Entecavir	28(46.7%)
Tenofovir	15(25%)
lamivudine	1(1.7%)
Telbivudine	3(5%)
Adefovir dipivoxil	1(1.7%)
Entecavir plus tenofovir dipivoxil	3(5%)
Entecavir plus adefovir dipivoxil	3(5%)
Lamivudine plus adefovir dipivoxil	1(1.7%)
Telbivudine plus adefovir dipivoxil	5(8.3%)

Table 1: General and clinical characteristics of patients.

Differences in HBV RNA level based on HBV DNA level

Patients were divided into the following two groups based on HBV DNA level: the undetected HBV DNA and < 20 IU/ml HBV DNA groups. There were significant differences in HBV RNA level between the two groups ($Z = 3.818, P < 0.05$) (Table 2).

HBV-DNA undetected group(31case)	HBV-DNA< 20 IU/ml group(29 case)	Z	P
Log10HBV RNA 0.0 (0.0, 2.0)	2.0 (2.0,2.4)	3.818	0.000

Table 2: Quantitative comparison of HBV-RNA with different levels of HBV-DNA.

Differences in HBV RNA level based on HBeAg level

Patients were divided into either the HBeAg-positive or HBeAg-negative groups based on their HBeAg levels. The quantitative comparison between the two groups showed significant differences in HBV RNA level ($Z = 3.363, P < 0.05$) (Table 3).

HBeAg positive group(27 case)	HBeAg negative group(33 case)	Z	P
Log10HBV RNA 2.0 (0.0, 2.7)	0.0 (0.0, 2.0)	3.363	0.001

Table 3: Quantitative comparison of HBV-RNA with the different level of HBeAg.

Note: HBV DNA and HBV RNA levels change exponentially. We converted them into logarithms so that they could become continuous variables with normal distributions.

Differences in HBV RNA based on HBsAg level

Patients were divided into the following three groups based on HBsAg level: the HBsAg ≤ 500 IU/ml, 500 IU/ml < HBsAg < 1500 IU/ml, and HBsAg ≥ 1500 IU/ml groups. The quantitative comparison between these groups showed no significant differences in HBV RNA level. (Chi-square value = 5.891, $P > 0.05$). There were significant differences in HBV RNA level between the HBsAg ≤ 500 IU/ml and HBsAg ≥ 1500 IU/ml groups ($Z = 2.426, P < 0.05$) (Table 4).

HBsAg≤500IU/ m(17case)	500<HBsAg<1500 IU/ml(19case)	HBsAg≥1500 IU/ml(24case)	Chi-square	P
Log10HBV RN 0.0 (0.0, 2.0)	2.0 (0.0, 2.4)	2.0 (0.0, 2.3)	5.891	0.05

Table 4: Quantitative comparison of HBV-RNA with the different levels of HBsAg.

Note: Group A compared to group B, $Z = 1.660, P = 0.097$; Group A compared to group C, $Z = 2.426, P = 0.015$; Group B compared with group C, $Z = 0.572, P = 0.568$.

Analysis of the factors that influence HBV RNA level

In the univariate linear regression analysis, ALT, AST, GGT, HBsAg, HBeAg, and HBcAb levels were independent variables and serum HBV RNA level was a dependent variable. This analysis showed that only HBeAg level significantly correlated with HBV RNA level ($P < 0.05$) (Table 5).

variable	β	SE	β'	t	P
ALT	-0.0181	0.0199	-0.1201	-0.9131	0.365
GGT	-0.0104	0.0083	-0.1622	-1.2412	0.2196
HBsAg	0.0001	0.0000	0.2514	1.961	0.0548
HBeAg	0.0218	0.0074	0.3635	2.946	0.0047
HBcAb	0.0200	0.1448	0.0186	0.1381	0.8907

Table 5: Analysis of influencing factors of HBV-RNA level.

Discussion

In 1996, Kock et al detected HBV RNA in the sera of chronic hepatitis B patients. The clinical value of HBV RNA was gradually discovered over the course of developing antiviral treatment for chronic hepatitis B patients. Furthermore, Wang et al reported that serum HBV RNA mainly exists in the form of pre-genomic RNA and it may be a stable serological marker that can reflect the existence of cccDNA in liver tissue [8]. After NAs treatment, serum HBV RNA level may better reflect the activity of cccDNA in the liver than serum HBV DNA. It has been reported that the only source of HBV RNA in sera is cccDNA located in the nucleus of infected hepatocytes, and its production cannot be inhibited by NAs. So, HBV RNA level can reflect the real level of existence and transcriptional activity of cccDNA in hepatocytes [9, 10]. Therefore, when HBV RNA is not detected in sera, it is a likely indication of the disappearance or silence of cccDNA transcription in patients' hepatocytes.

HBV DNA level can also reflect the replication activity of cccDNA in hepatocytes to some extent. However, the lower detection limit of HBV DNA can only indicate the inhibition of retroviral processes. It does not reflect the transcriptional status of cccDNA. After retroviral processes are inhibited, cccDNA continues to produce progeny viruses in the form of HBV RNA virus-like particles. So, disease rebound and recurrence rates are higher after the withdrawal of medication under the precondition of sustained virological response [11-14]. In our study, patients were divided into the undetected HBV DNA and < 20 IU/ml HBV DNA groups based on HBV DNA level. There was a statistical difference in HBV RNA level between the two groups ($Z = 3.316,$

$P < 0.05$). This suggests that the lower HBV DNA level, the lower the serum HBV RNA level will be after long-term NA antiviral treatment. Even when HBV DNA is below the detection limit, the replication activity of cccDNA continues, which leads to the detection of HBV RNA or at a higher level.

As a result, HBV RNA can more accurately reflect the status of cccDNA than HBV DNA, making HBV RNA level a more powerful indicator of antiviral efficacy [11, 15, 16]. Additionally, a good correlation between the status of HBeAg and HBV DNA quantification has been reported [17]. However, a correlation between serum HBeAg status and serum HBV RNA remains to be seen. Wang et al showed that the status of serum HBeAg potentially has an effect on the correlation between serum HBV RNA and hepatic HBV cccDNA [18]. Moreover, van Bömmel F et al showed that serum HBV RNA is an early predictor of the serological conversion of HBeAg after PEG interferon alpha-2a treatment [19]. And His another study suggests that Serum HBV RNA levels may serve as a novel tool for prediction of serological response during polymerase inhibitor treatment in HBeAg-positive patients [20]. In our study, patients were divided into the HBeAg-positive and HBeAg-negative groups based on HBeAg level.

The quantitative comparison between the two groups showed significant differences in HBV RNA level ($Z = 3.363$, $P < 0.05$), which is similar to the results reported by Jansen et al [21]. Our single-factor linear regression analysis also showed that HBeAg level was the only factor that correlated with HBV RNA level ($P < 0.05$). After our patients underwent long-term antiviral treatment, their HBV DNA levels were lower than the detection limit, and the HBV RNA levels in HBeAg-positive patients were higher than those in HBeAg-negative patients. Therefore, HBeAg level may be associated with HBV RNA level. Further, serum HBsAg is derived from hepatic HBV cccDNA transcription, and its level is closely related to the transcriptional activity of hepatic cccDNA. The removal of serum HBsAg can significantly reduce the risk of liver cirrhosis and hepatocellular carcinoma in chronic hepatitis B patients [22]. HBV RNA can also directly reflect the level and transcriptional status of cccDNA in the liver.

This is why we studied the relationship between HBsAg and HBV RNA. Patients were divided into the three following groups based on HBsAg level: the $\text{HBsAg} \leq 500$, $500 < \text{HBsAg} < 1500$ IU/ml, and $\text{HBsAg} \geq 1500$ IU/ml groups. The quantitative comparison between the three groups showed that differences in HBV RNA level were not statistically significant. (Chi-square value = 5.891, $P > 0.05$). However, there were significant differences in HBV RNA level between the $\text{HBsAg} \leq 500$ IU/ml and $\text{HBsAg} \geq 1500$ IU/ml groups ($Z = 2.426$, $P < 0.05$). There was no significant difference between the remaining two groups ($P > 0.05$). Our univariate linear regression also showed no correlation between HBsAg level and HBV RNA level ($P > 0.05$). It is suggested that the HBV RNA

levels in the $\text{HBsAg} < 500$ IU/ml group are lower than that in $\text{HBsAg} < 1500$ IU/ml group. However, HBsAg level was not a factor that affected HBV RNA level in our study.

Therefore, healthcare practitioners should use the lower detection limit of serum HBV RNA level as a virological response index after reaching the lower detection limit of HBV DNA in chronic hepatitis B patients who have undergone long-term antiviral therapy. It is only when both HBV DNA and RNA are below the detection limit, thereby indicating the disappearance or silence of cccDNA transcription in the liver, can it be possible to completely prevent patients' liver disease and improve the patients' quality of life. Presently, the detection of HBV RNA is not widely used in clinical practice. This study acts as a preliminary exploration of the clinical significance of HBV RNA in chronic hepatitis B patients. Our study had some limitations. A large number of clinical studies are needed to prove the significance of serum HBV RNA as an evaluation standard in antiviral therapy for chronic hepatitis B patients.

Conclusion

HBV RNA can still be detected in the absence of HBV DNA. Ours is also the first study to report the possible relationship between HBeAg and HBV RNA. Further, we believe that HBV DNA and RNA should be used concurrently as indicators of antiviral efficacy. It is only when both HBV DNA and HBV RNA are below the detection limit, thereby indicating the disappearance or silence of cccDNA transcription in the liver, can it be possible to completely prevent patients' liver disease and improve the patients' quality of life.

References

1. Chinese Society of Hepatology, Chinese Medical Association, Chinese Society of Infectious Diseases, Chinese Medical Association, Hou J-L, et al. (2015) The guideline of prevention and treatment for chronic hepatitis B: a 2015 update[J]. *Chin J Hepatol* 23: 888-905.
2. Trépo C, Chan HL, Lok A (2014) Hepatitis B virus infection. *The Lancet* 384: 2053-2063.
3. Wang J, Yu Y, Li G, Shen C, Meng Z, et al. (2017) Relationship between serum HBV-RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. *J Hepatol* 8278: 32261-32264.
4. Wang J, Shen T, Huang X, Kumar GR, Chen X, et al. (2016) Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. *J Hepatol* 65: 700-710.
5. Wang J, Sheng Q, Ding Y, Chen R, Sun X, et al. (2018) HBV RNA virion-like particles produced under nucleos(t)ide analogues treatment are mainly replication-deficient. *J Hepatol* 68: 847-849.
6. Shi Liu, Bin Zhou, Juan D Valdes, Sun J, Guo H (2019) Serum HBV RNA: a New Potential Biomarker for Chronic Hepatitis B Virus Infection. *Hepatology* 69: 1816-1827.

7. Giersch K, Allweiss L, Volz T, et al. (2017) Serum HBV pgRNA as a clinical marker for cccDNA activity. *J Hepatol* 66: 460-462.
8. Ding S and Robek (2014) Cytidine deamination and cccDNA degradation: A new approach for curing HBV? *Hepatology* 60: 2118-2121.
9. Guo JT and Guo H (2015) Metabolism and function of hepatitis B virus cccDNA: Implications for the development of cccDNA targeting antiviral therapeutics. *Antiviral Res* 122: 91-100.
10. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, et al. (2014) Specific and Nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 343: 1221-1228.
11. Lu FM, Wang J, Chen XM, Jiang JN, Zhang WH, et al. (2017) [The potential use of serum HBV RNA to guide the functional cure of chronic hepatitis B] *Zhonghua Gan Zang Bing Za Zhi* 25: 105-110.
12. Wang J, Yu Y, Li G, Chen S, Zhang X, et al. (2018) Natural history of serum HBVRNA in chronic HBV infection. *J Viral Hepat* 25: 1038-1047.
13. Liu RX, Pan XC, Geng J (2017) Clinical value of serum HBV RNA. *Journal of Clinical Hepatology* 33: 2196-2199.
14. Huang Y, Chayama K, Tsuge M, Takahashi S, Hatakeyama T, et al. (2010) Differential effects of interferon and lamivudine on serum HBV RNA inhibition in patients with chronic hepatitis B. *Antivir Ther* 15: 177-184.
15. Butler EK, Gersch J, Mcnamara A, Luk K-C, Holzmayer V, et al. (2018) HBV serum DNA and RNA levels in nucleos(t)-ide analogue-treated or untreated patients during chronic and acute infection. *Hepatology* 68: 2106-2117.
16. Tsuge M and Chayama K (2013) Availability of monitoring serum HBV DNA plus RNA during nucleot(s)ide analogue therapy. *J Gastroenterol* 48: 779-780.
17. Zhou P, Yang F, Wang J, Mao R, Qi X, et al. (2015) Improved Efficacy of a Pegylated Interferon- α -2a Stepwise Optimization Treatment Strategy in the Treatment of Hepatitis B e Antigen-positive Chronic Hepatitis B Patients[J]. *Medicine (Baltimore)* 94: 730-732.
18. Wang J, Du M, Huang H, Chen R, Niu J, et al. (2017) Reply to: "Serum HBV pgRNA as a clinical marker for cccDNA activity": consistent loss of serum HBV RNA might predict the "para-functional cure" of chronic hepatitis B. *J Hepatol* 66: 462-463.
19. van Bömmel F, van Bömmel A, Krauel A, Wat C, Pavlovic V, et al. (2018) Serum HBV RNA as a predictor of peginterferon alfa-2a response in patients with hbeag-positive chronic hepatitis B. *J Infect Dis* 218: 1066-1074.
20. Florian van Bömmel, Anne Bartens, Alena Mysickova, Hofmann J, Krüger DH, et al. (2015) Serum Hepatitis B Virus RNA Levels as an Early Predictor of Hepatitis B Envelope Antigen Seroconversion during Treatment with Polymerase Inhibitors. *Hepatology* 61: 66-76.
21. Jansen L, Kootstra N, van Dort K, Takkenberg RB, Reesink HW, et al. (2016) Hepatitis B Virus Pregenomic RNA Is Present in Virions in Plasma and Is Associated with a Response to Pegylated Interferon Alfa-2a and Nucleos(t)ide Analogues. *J Infect Dis* 213: 224-232.
22. Guner R, Karahocagil M, Buyukberber M, Kandemir O, Ural O, et al. (2011) Correlation between intrahepatic hepatitis B virus cccDNA levels and other activity markers in patients with HBeAg-negative chronic hepatitis B infection. *Eur J Gastroenterol Hepatol* 23: 1185-1191.