American Journal of Medicine and Medical Sciences 2020, 10(5): 290-292
DOI: 10.5923/j.ajmms.20201005.03

The Significance of M2BPGi Marker and CCC DNA HBV in Hepatitis of Unknown Etiology

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Abstract 25 patients with a diagnosis unknown etiology hepatitis were examined. All samples were negative for the surface antigen HBV and antibodies to HCV. Further, testing carried out for the presence of CCC DNA HBV in liver biopsies. The calculation of the number of copies of CCC DNA HBV per one hepatocyte also performed. All patients tested for level of serum marker M2BPGi. Of the 25 patients examined, CCC DNA HBV detected in 13, which indicates an occult form of hepatitis B. The level of serum marker M2BPGi distributed C.O.I. from 0.39 to 14.73.

Keywords M2BPGi, CCC DNA HBV, Hepatitis of unknown etiology

1. Introduction

Laboratory diagnosis of viral hepatitis is an actively developing branch of medical science. Over the 50 years since the opening of the "Australian antigen", many viruses discovered that are responsible for the onset of hepatitis. The variety of serological markers of infection, methods for their detection using highly sensitive and specific methods have opened the possibility of not only the etiological decoding of acute or chronic viral hepatitis, but also monitoring of treatment. Traditionally, methods for laboratory diagnosis of viral hepatitis divided into 2 groups: detection of viral antigens and antibodies to them and molecular biological methods for the detection and analysis of viral nucleic acids. The evolutionary development of the methodological base and diagnostic preparations fully affected the laboratory diagnosis of viral hepatitis. The accumulated knowledge on the etiology and pathogenesis of these human infections is the basis of modern diagnosis. At the same time, we consider it necessary to emphasize that the detection data for specific markers of viral hepatitis infection are important, but only part of the general diagnosis system. Hepatitis of unknown etiology is a great challenge for diagnosis. Hepatitis of unknown etiology can be toxic etiology, alcoholic etiology or infectious etiology. Usually the doctor excludes the toxic and alcoholic components of the liver, and then the assumption remains only about the infectious nature of the disease. In cases where the possibility of alcoholic, toxic liver damage excluded, routine markers of viral hepatitis have a negative test result; it is worth checking the hypothesis of infection of the occult form of viral hepatitis B.

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Published online at http://journal.sapub.org/ajmms

2. Overview

A Viral hepatitis B is a serious problem for modern healthcare. Chronic HBV infection characterized by the presence of surface HBV antigen (HBsAg) in the blood plasma, as well as viremia. It previously accepted that the disappearance of HBsAg from plasma in patients with HBV infection is associated with a cessation of viremia and remission of the disease. However, according to recent data, hepatitis B DNA in low concentrations continues to detected in blood plasma and liver tissues in a certain percentage of patients with undetectable HBsAg levels - in acute infection with independent resolution and in chronic or even after successful treatment with antiviral drugs [1]. The above led to the development of the concept of silent, latent and “occult” HBV infection, which characterized by the presence of the virus in the liver tissue with an undetectable level of HBsAg in blood plasma [2].

There is a wide range of estimates of the prevalence of occult hepatitis B in different countries. According to various sources, the prevalence of occult hepatitis B varies from one to 18% of the total number of patients with chronic hepatitis B [3,4,5,6, and 7]. These data greatly underestimated, because patients with occult hepatitis B have extremely low levels of HBV DNA in serum and liver biopsy specimens. Thus, the level of HBV DNA viremia in

blood plasma in patients with occult hepatitis B indicated below 200 IU / ml [8], but in more than 90% of patients with HBV DNA level will be 20 IU / ml in blood serum [5]. Such low levels of HBV DNA, as well as their fluctuations, make it difficult to detect occult hepatitis B even when using existing standardized and highly sensitive tests for HBV DNA analysis. The occult form of hepatitis B is an urgent problem in modern health care. The diagnosis of occult hepatitis B is difficult to establish, as routine tests for hepatitis B markers may show a negative result. Detection of HBV covalently closed circular DNA HBV (CCC DNA HBV) is the gold standard for the diagnosis of occult hepatitis B. This method is highly accurate and highly specific; however, the complexity of the method is associated with the presence of only in-house methods, which increases the qualification requirements of the staff conducting the test. Nevertheless, now, this is the main method that allows you to determine the CCC DNA HBV integrated into the hepatocyte genome and make the correct diagnosis for the patient with the subsequent administration of etiotropic treatment.

Thus, if the patient has clinical manifestations of viral hepatitis with negative test results for viral hepatitis markers, it is worthwhile to conduct a more in-depth examination. Not only clinical signs, but also studies indicating the presence of progressive liver fibrosis can prompt the doctor to think of the patient having an occult form of viral hepatitis B.

To assess the presence of fibrosis in patients, liver elastography is most often use, and cytology of liver biopsy used as the "gold standard". Both of these methods have several advantages and disadvantages. Liver elastography has become a very affordable study in recent years. The rapidity, cheapness and simplicity of this type of diagnosis has many followers. However, unfortunately, the results of elastography show an approximate level of liver fibrosis due to indirect signs, such as tissue density for example. In the case of liver biopsy cytology, the accuracy of the results is quite high, but the technique is invasive and time-consuming, and also requires highly qualified personnel. However, both methods can only indirectly help the doctor predict the risks of the patient. In this sense, the M2BPGi marker is noteworthy. M2BPGi is Mac-2-binding protein glycosylation isomer, a novel serum marker for liver fibrosis. Determining the level of M2BPGi does not require a surgery operation, as with a liver biopsy, and its accuracy is very high. This non-invasive method has a high correlation with the development in patients of complications in the form of liver fibrosis and hepatocellular carcinoma, and allows the doctor to see the prognosis of the course of the disease [9].

2.1. Purpose of the Study

Determine the presence of CCC DNA HBV and the level of serum fibrosis marker M2BPGi in patients with hepatitis of unknown etiology in order to predict the outcome.

2.2. Materials and Methods

Blood plasma and liver biopsies from 26 patients investigated. All patients admitted to the intensive care unit of the Research Institute of Virology in severe condition. All patients given written agreement for testing.

For the detection of HBV DNA in blood plasma, nucleic acids were isolated using the Ribo-prep commercial kit (AmpliSens, Russia, Moscow). To avoid a false negative result at low viral load, all samples pre-concentrated by ultracentrifugation of blood plasma for 1 hour at 24,000 g, 4 degrees C. The presence of the virus analyzed by real-time PCR with a hybridization-fluorescence detection in real time using a commercial kit HBV-FL (AmpliSens, Russia, Moscow).

For in-depth analysis, nucleic acids were isolated from blood plasma and puncture liver biopsies in accordance with the methodology described in Sambrook J. Et al. [10] with some modifications.

CCC DNA HBV detected according to the Pollicino T. [11] method. Using TaqMan probes for real-time PCR. DNA pretreated with MungBean endonuclease at the rate of one unit enzyme per one μg of DNA to remove single-stranded DNA and RNA and cleavage of genomic DNA of HBV (partially circulated). For each sample, the reaction carried out three times; then, the Ct value for them averaged. Analysis of the results carried out by the method of relative calculation with normalization by the endogenous reference gene GAPDH.

In order to confirm the presence of HBV, untreated endonuclease DNA used as a template for PCR with further sequencing.

The M2BPGi values measured using HISCL M2BPGi kit (Sysmex, Kobe, Japan) on fully automated immunoanalyzer, HISCL-800 (Sysmex). M2BP levels were indexed by the following equation: Threshold index (COI) = ([WFA+M2BP] sample - [WFA+M2BP] NC)/ ([WFA+M2BP] PC - [WFA+M2BP] NC), if [WFA+M2BP] sample - level of WFA+M2BP in sample, [WFA+M2BP] PC - positive control, [WFA+M2BP] NC - negative control. The positive control supplied in the form of a calibration solution standardized to obtain a COI value of 1.0.

3. Results and Discussion

3.1. Results

25 patients with a diagnosis unknown etiology hepatitis were examined. For all samples, ELISA tests for HBsAg and AntiHCV performed repeatedly. All samples were negative for the presence for surface antigen of HBV and antibodies to HCV. Further, testing carried out for the presence of CCC DNA HBV in liver biopsies. The calculation of the number of copies of CCC DNA HBV per one hepatocyte also performed. All patients tested for a level of serum marker M2BPGi.

Of the 25 patients examined, CCC DNA HBV detected in

13, which indicates an occult form of hepatitis B. The average number of copies of CCC DNA HBV per one hepatocyte was 0.2. The level of serum marker M2BPGi distributed C.O.I. from 0.39 to 14.73.

3.2. Discussion

The prevalence of the occult form of hepatitis B in our group of patients was 52%. Such a high percentage explained as follows. Patients examined for the HBV markers after long time infection. Nevertheless, occult form hepatitis B usually have mutant seroconversion of surface antigen and detected usually in resent infection case. Also noteworthy the fact that the quantity of CCC DNA HBV copies for one hepatocyte was small – an average 0.2. This suggests that with the occult form of hepatitis B, the infectious process proceeds with an extremely low amount of the virus. However, the risk of undesirable outcomes in patients with this form of hepatitis B is extremely high. This can be due to the fact that, these patients do not receive etiotropic or symptomatic therapy, which leads to worsening the condition. This also leads to the fact that patients go to the Hospital too late when clinical symptoms appear. The level of M2BPGi in our study did not strongly correlate with the presence of occult hepatitis B. A significant correlation of M2BPGi observed more with the level of liver fibrosis, which confirm with stage of liver fibrosis. However, fibrosis stage in patients with a detected occult hepatitis B was slightly higher than with hepatitis of unknown etiology. The average level of M2BPGi was C.O.I. 5.34 among the group with detected CCC DNA HBV and C.O.I. 4.02 among the group with unknown etiology hepatitis. Thus, we can assume that patients with the occult form of hepatitis B have higher risk of developing liver fibrosis and adverse associated outcomes. Monitoring of level M2BPGi also used for evaluation of treatment success in patients with hepatitis of unknown etiology.

4. Conclusions

There was no correlation between the detection of an occult form of hepatitis B by CCC DNA HBV testing and serum marker M2BPGi level in our study. However, there is good potential for using serum marker M2BPGi to assess liver fibrosis and treatment success in patients with hepatitis of unknown etiology.

ACKNOWLEDGEMENTS

Many thanks to everyone who helped with the research, for the unprecedented support and trust to us.

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