CORRELATION BETWEEN REPLICATION OF HEPATITIS B VIRUS AND DEMOGRAPHIC CHARACTERISTICS AND DIFFICULTIES OF PATIENTS WITH CHRONIC HEPATITIS B

Boban Mugosa¹, Dragica Terzić², Neda Svtitlhi³, Zoran Terzić⁴, Sonja Žerjav⁵, Gordana Rašović⁵

¹Institute of Public Health, Montenegro, ²Clinic for Infectious Diseases, Clinical Center of Montenegro, ³Institute for Infectious and Tropical Diseases, Belgrade, ⁴Surgical Clinic, Clinical Center of Montenegro, ⁵Center for Blood Transfusion, Clinical Center of Montenegro

ABSTRACT

Hepatitis B virus infects more than 2 billion people in the world, and 300 million of them developed chronic infection, that is as long as life lasts, and puts them at risk of developing cancer or cirrhosis of the liver. The aim of the work is to show correlation between Hepatitis B virus replication and demographic characteristics and difficulties in persons with chronic Hepatitis B.

Study sample involved all persons with Hepatitis B in whom diagnosis was made based on epidemiological, clinical, biological and pathohistological parameters in the period from January 2002 to January 2008. In the study sample, out of 30 persons with chronic Hepatitis B, 23 (77%) were men and 7 (23%) were women. Replication of hepatitis B virus was found in higher percentage in men (78,2%). Examinees were from all age groups, and most of them were adults (between 20-59 years of age), 24 patients (80%). Virus replication was found in all groups without statistically significant difference. Difficulties in patients with chronic hepatitis B were fatigue and nausea, and in 54,5 % cases virus replication is mainly found in men of all age groups, with different level of education.

Chronic infection caused by this virus with "mute" clinical picture followed by virus replication accelerates developing of cirrhosis and hepatocellular carcinoma

Key words: Hepatitis B, replication, gender, age group, difficulties

INTRODUCTION

In the last decades of XX century the significant improvement in diagnosis and therapy of liver diseases has been achieved. Many dilemmas related to etiology of viral hepatitis, its pathogenesis, morphological picture and therapy have not yet been resolved detailed. It is especially from the reason that these diseases can be asymptomatic for a long period and they are recognized at the moment of occurrence of irreversible damage of the liver. (1) World Health Organization estimates that Hepatitis B virus infects more than 2 billion people in the world, and 300 million people of them developed chronic infection lasting whole life and which puts them at risk of developing cancer or cirrhosis of the liver. High percentage of Hepatitis B virus is found in many developing countries (2). Transmission results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include (but are not limited to) unprotected sexual contact, blood transfusions, re-use of contaminated needles, syringes, and vertical transmission from mother to child during childbirth. Without interven-

SAŽETAK

Dvije milijarde ljudi širom svijeta inficirano je virusom hepatitisa B od kojih više od 300 miliona ima hroničnu infekciju a koja traje cijeli život i koja ih izlaže riziku da obojevanja od raka ili cirrhose. Cilj rada je prikazati povezanost replikacije virusa hepatitisa B sa demografskim karakteristikama i tegobama oboljelih od hroničnog hepatitisa B. Israživački uzorak čine svi obolijeli od hroničnog hepatitisa B kod kojih je dijagnoza postavljena na osnovu epidemioloških, kliničkih virusololoških i patohistoloških parametara u vremenskom periodu od januara 2002. do januara 2008. god.. U istraživačkom uzorku od 30 obolijelih od hroničnog hepatitisa B bila su 23 (77%) muškaraca i 7 (23%) žena. Replikacija virusa hepatitisa B je bila više prisutna kod obolijelih muških pola (78,2%). Obolijeli ispitanici pripadali su svim starosnim grupama a većina njih činila je grupu odraslih bolesnika (između 20 i 59 godina), tj 24 bolesnika (80%). Replikacija virusa bila je prisutna u svim grupama bez statistički značajne razlike. Tegobe kod obolijelih od hroničnog hepatitisa B su se najčešće manifestovale kroz zatam i mučninu a njih 54.5% imalo je i prisutnu replikaciju virusa. Hronični hepatitisa B sa prisutnom replikacijom virusa je bolest većinom muškaraca, svih uzrasta, različitog obrazovanja. Hronična infekcija ovim virusom uz prisustvo replikacije virusa uz njemu kliničku sliku brže vodi kacirozi i hepatocelularnom karcinomu

Ključne riječi: hepatitis B, replikacija, pol, uzrast, tegobe
tion, a mother who is positive for the hepatitis B surface antigen confers a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for the hepatitis B e antigen. HBV can be transmitted between family members within households, possibly by contact of nonintact skin or mucous membrane with secretions or saliva containing HBV.(3) However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor.(4,5) Chronic infection with Hepatitis B virus may be either asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of liver cancer. Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of Membranous glomerulonephritis (6) The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or HBeAg. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (anti-HBc IgM) may be the only serological evidence of disease.(7) Shortly after the appearance of the HBsAg, another antigen named as the hepatitis B e antigen (HBeAg) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true. During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (anti-HBe) will arise immediately afterwards. (8) This conversion is usually associated with a dramatic decline in viral replication. More recently, PCR tests have been developed to detect and measure the amount of viral nucleic acid in clinical specimens. These tests are called viral loads and are used to assess a person's infection status and to monitor treatment.(9)

The life cycle of Hepatitis B virus is complex. Hepatitis B is one of a few known non-retroviral viruses that use reverse transcription as a part of its replication process. The virus gains entry into the cell by binding to a receptor on the surface of the cell and enters it by endocytosis. Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones. The partially double stranded viral DNA is then made fully double stranded and transformed into closed circular supercoiled DNA (cccDNA) that serves as a template for transcription of four viral mRNAs. The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and re-cycled to produce even more copies.(10,11) The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity.

Presence of active form of chronic hepatitis B supports faster progression from hepatitis B viral infection to cirrhosis and hepatocellular carcinoma. In our conditions through carefully selected group of patients with chronic hepatitis B where other causes of chronic hepatitis were excluded based on virologic and immunologic tests, the demographic characteristics and presence of active disease can be observed. Majority of population has regular vaccination status but also the portion of population with risky behavior without regular vaccination status for hepatitis B viral infection is significantly high. In the last ten years in our country the cases of making diagnosis of hepatitis B viral infection in the stadium of decomposed cirrhosis and hepatocellular carcinoma have not been rarely reported.

AIM

The aim of study was to present correlation between Hepatitis B virus replication and demographic characteristics and difficulties of people with chronic hepatitis B.

MATERIAL AND METHODS

Sample size involving 30 patients was epidemiologically processed at the Institute of Public Health Podgorica and diagnosis and treatment was done at the Clinic for Infectious diseases, Clinical Center of Montenegro and the Institute for Infectious and Tropical Diseases, Clinical Center Serbia, in the period from January 2002 to January 2008.

Epidemiological processing was done by conducting survey in patients using questionnaire specially designed for this purpose, in the Center for Disease Control and Prevention, Institute of Public Health, Podgorica.

Markers of Hepatitis B viral infection: HbgAg, HbeAg, anti HbcAg, anti Hbe, anti HBs were detected by enzymeimmunnoassay (ELISA) at the Institute of Public Health of Montenegro and the Center for Blood Transfusion, Clinical Center of Montenegro.

Presence of HBV DNA in serum was detected by using PCR commercial tests in laboratory for virology, In-
stitute for Infectious and Tropical Diseases in Belgrade and the Institute for nuclear researches in Vinca.

Presence of hepatitis B virus replication was defined through presence of replication markers in serum of patients: HBeAg, HBVDNK, IgM, anti HBc. For this study we used data on Hepatitis B markers before initiating therapy treatment. Pathohistological analyzing of liver tissue was done at the Institute for Pathology, Clinical Center of Montenegro, after sampling by blind aspiration or target biopsy of liver during surgical remove of gall-bladder.

After electronic processing in statistical package SPSS for Windows (version 10) results have been presented in tables.

**RESULTS**

During epidemiological survey conducted in the period from 2002-2008 patients with chronic Hepatitis B indicated different possible modes of infection by hepatitis B virus. In the survey examinees have been selected into two groups depending on mode of transmission and presence of Hepatitis B virus replication (Table 1).

Table 1. Exposure to risk and presence of Hepatitis B virus replication in relation to risk

<table>
<thead>
<tr>
<th>Risk</th>
<th>N</th>
<th>%</th>
<th>Virus replication N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risky sexual contact</td>
<td>10</td>
<td>33,3%</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Transfusion of blood and blood derivates</td>
<td>5</td>
<td>16,6%</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Surgical interventions</td>
<td>5</td>
<td>16,6%</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Narcotics</td>
<td>6</td>
<td>20%</td>
<td>4</td>
<td>66</td>
</tr>
<tr>
<td>Tran placental</td>
<td>2</td>
<td>6,7%</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Accidental- injury with needle</td>
<td>2</td>
<td>6,7%</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100%</td>
<td>22</td>
<td>73,3%</td>
</tr>
</tbody>
</table>

Data presented in the table show that during epidemiological survey the most of the examinees indicated the risky sexual contact as a possible mode of transmission of infection - 33,3% and the same percent of examinees indicated the exposure to infection via blood and surgical interventions.

In the study sample of 30 patients with chronic Hepatitis B there were 23 (77%) men and 7 (23%) women. Hepatitis B virus replication was found in higher percentage in men (78,2%) in relation to total number of diseased with replication (83% 18/22). Table 2

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive replication No</th>
<th>%</th>
<th>Negative replication No</th>
<th>%</th>
<th>Total No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18 (78,2%)</td>
<td>5</td>
<td>23</td>
<td>100%</td>
<td>18/22</td>
<td>78,2%</td>
</tr>
<tr>
<td>Female</td>
<td>4 (57,1%)</td>
<td>3</td>
<td>7</td>
<td>100%</td>
<td>4/7</td>
<td>57,1%</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>8</td>
<td>30</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of obtained results showed that male patients were dominant with 77% in relation to female patients 23% and Hepatitis B virus replication was more frequently found in male patients (78,2%) than in female patients (57,1%).

Hepatitis B is present in all age groups. The least number of case was reported in under 10 age group - 0,6% and over 60 age group - 0,3%. The highest number of cases was reported in the age group between 20-50 years – 19 (63%) cases. There were 5 patients in the age group between 50-60 years (1,7%). Table 3

Table 2. Hepatitis B virus replication in relation to gender

Table 3. Hepatitis B virus replication in relation to age groups

Analysis of results showed that viral replication was found in all age groups of examinees without statistically significant difference p>0,05 in relation to number of diseased in certain age groups. The highest number of cases with replication was registered in age group between 20-50 years – 10 (33%) which was statistically significant difference in relation to number of examinees without replication in the same age group (10/15,66%,p<0,05)

Within demographic characteristics of patients with chronic Hepatitis B the level of education was also considered. The most of examinees had with higher school education 13 (43,4%) but without statistically significant difference in relation to those with elementary school education - 26% and secondary school education – 30%. Table 4
The most of patients were in the group with higher school education - 43.4% and the least number of patients were in the group with elementary school education - 26.6%, while the highest percentage of viral replication was found in the group with secondary school education.

Difficulties of patients with chronic Hepatitis B were mild but they existed and they manifested through fatigue, nausea and pain under right rib edge. Total number of patients who had some of mentioned difficulties was 8 (40%). Hepatitis B virus replication was more frequently present in the group of patients who had difficulties but without statistically significant difference in relation to patients without difficulties (18/12, 60%S.v.s.40%, p>0.05). Table5.

Analysis results showed that most of patients had coincidental viral replication regardless to the presence of difficulties (60%S.v.s 40%).

**DISCUSSION**

Viral hepatitis has significant portion in causing of liver diseases. According to frequency they are placed directly after alcohol diseases depending on geographical distribution of viruses that have ability to persist, causing chronic liver disease.

WHO estimates that Hepatitis B virus infects more than 2 billion people in the world, and 300 million people of them developed chronic infection, that is as long as life lasts, and puts them at risk of developing cancer or cirrhosis of the liver. High percentage of Hepatitis B virus is found in many developing countries (2). Today it is possible to exclude other etiology of chronic liver disease, by using contemporary, commercial, rapid and effective tests for detecting virologic, immunologic and inheritably diseases.

Based on epidemiological survey 33% of our patients reported risky sexual contact as a most common exposure to infection by hepatitis B virus; then exposure to infection via blood transfusion, blood donors and surgical interventions, 16,6 %. Virus replication was found in all groups of patients with highest incidence of 80 % in patients who reported risky sexual contact as a most common mode of hepatitis B virus infection.

Regular follow up of Alfa fetoprotein as a glikoprotein, because its level increase in serum can bi significant for liver disease progression to hepatocellular carcinoma, did not show increase in people with chronic hepatitis B regardless to replication. Injected drug use is common mode of hepatitis B virus transmission (1,2). In our study 20 % of patients reported intravenous drug injection as a possible mode of infection. Hepatitis B virus replication in injected drug users was found in 66 % of cases. Analysis of demographic characteristics in 30 patients with chronic hepatitis B showed that majority of diseased was men-77 %. Higher percent of infected among men can be explained by epidemiological characteristics, mode of transmission and risky behavior which is more common in male population. Hepatitis B virus replication was 78.2 % in men, and 57,1 % in women. More frequent presence of replication in men than in women indicates possible influence of sexual hormones to glucocorticoid receptor in genome of hepatitis B, which accelerate virus replication (12,13).

Analysis of age distribution showed presence of disease in all age groups with highest incidence between 20 and 50 years of age, which can be explained by highest exposure to risks in that period of life (surgical interventions, drug use, blood transfusion and sexual activity) as well as unimplemented vaccination against hepatitis B virus. Viral replication in chronic hepatitis B is found in 63 % of cases in the age group between 20-50 years of age in which the number of diseased was the highest. In regard to total number of patients with viral replication in relation to age distribution selected by age groups there was not significant difference.

**Table 4.** Level of education of patients with hepatitis B and the presence of viral replication

<table>
<thead>
<tr>
<th>Education</th>
<th>No.</th>
<th>%</th>
<th>Viral replication</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elementary school education</td>
<td>8</td>
<td>26,6</td>
<td>6</td>
<td>75%</td>
</tr>
<tr>
<td>Secondary school education</td>
<td>9</td>
<td>30</td>
<td>7</td>
<td>77,7</td>
</tr>
<tr>
<td>Higher school education</td>
<td>13</td>
<td>43,4</td>
<td>9</td>
<td>69%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100%</td>
<td>22</td>
<td>73%</td>
</tr>
</tbody>
</table>

Analysis of age distribution showed presence of disease in all age groups with highest incidence between 20 and 50 years of age, which can be explained by highest exposure to risks in that period of life (surgical interventions, drug use, blood transfusion and sexual activity) as well as unimplemented vaccination against hepatitis B virus. Viral replication in chronic hepatitis B is found in 63 % of cases in the age group between 20-50 years of age in which the number of diseased was the highest. In regard to total number of patients with viral replication in relation to age distribution selected by age groups there was not significant difference.

This finding could indicate that virus replication is not influenced by age group of a host..(14,15)

There was not significant difference in the number of patients with chronic hepatitis B in regard to level of education (elementary, secondary and higher school education).

Number of patients with higher school education was inconisiderably higher -13 (43,4%) but with the group of patients with secondary school education – 9 (30%) it is not statistically significant. Chronic hepatitis B is significantly more often reported in people with certain level of
education and better economic life conditions. Hepatitis B is common in people who travel abroad often, drug users and in people who often change partners of both genders (16,17).

It is generally accepted that people with chronic hepatitis B who have not developed the cirrhosis of the liver and its complications do not have characteristic difficulties.(18).

In our study sample 60% of examinees had difficulties. Analysis of difficulties in our examinees showed that the more common were fatigue and nausea. Viral replication is not associated with presence of difficulties in patients as the patients without manifested difficulties had statistically significant presence of viral replication - 83%, p<0.05.

Vaccination against hepatitis B virus is mandatory but the number of people with hepatitis B is not insignificant especially in men in their most productive period of life and regardless to level of education. Chronic infection with “mute” clinical picture followed by virus replication accelerates developing of cirrhosis and hepatocellular carcinoma.

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