CLINICALLY SIGNIFICANT HEPATITIS B VIRUS MUTATIONS IN A GROUP OF PATIENTS AT THE HEMODIALYSIS CENTER IN THE NORTH-WEST FEDERAL DISTRICT, RUSSIA

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Резюме: *несмотря на снижение распространенности гепатита В среди пациентов гемодиализных центров после введения программ вакцинации, данные пациенты подвержены риску заражения парентеральными гепатитами. Показана широкая распространенность HBsAg-негативной формы ГВ, а также мутаций вакцинного бегства среди пациентов гемодиализных центров.*

Resume: *despite the decrease in the prevalence of hepatitis B among patients in hemodialysis centers after the introduction of vaccination programs, these patients are at risk of contracting parenteral hepatitis. A wide prevalence of HBsAg-negative hepatitis B, as well as vaccine escape mutations among patients of hemodialysis centers was shown.*

Relevance. Due to the invasiveness of the hemodialysis procedure, patients in hemodialysis centers are at risk of contracting blood-borne infections, including hepatitis B, representing the potential spread of the virus both among other patients and among medical personnel [1]. Additional risks for patients with end-stage renal disease may come from the need for blood transfusions, frequent hospitalizations, and surgery. Testing for the presence of HBsAg and the introduction of vaccination programs has reduced the incidence of hepatitis B among patients in hemodialysis centers, but only about 60% of patients with ESRD achieve sufficient titers of anti-HBs Ig G antibodies compared with 95% of healthy individuals [2].

In addition, the control and detection of HBsAg-negative hepatitis B is complicated, which is often also characterized by a low viral load in blood plasma. For patients on hemodialysis therapy, the identification of OBI is vital. The failure to detect OBI with standard HBsAg screening and the absence of clinical symptoms indicative of liver inflammation may result in a long-term exposure risk for healthcare workers and other hemodialysis patients. Patients infected with HBV variants carrying clinically significant mutations in the MHR region are a potential problem for public health in general and for hemodialysis centers in particular, as they can become a source for the spread of HBV genovariants carrying vaccine escape mutations.

Aim: to analyze mutations in the genome of hepatitis B virus (HBV) isolates isolated from blood plasma samples of patients receiving replacement therapy using programmed hemodialysis.

Objectives: 1. To analyze the prevalence of hepatitis B serological markers (HBsAg, anti-HBs IgG, anti-HBcore IgG) in the study group; 2. To analyze the prevalence of molecular markers of hepatitis B (HBV DNA) in the study group; 3. To sequence HBV genomes and identify clinically significant virus mutations, including those associated with the HBsAg-negative form of the disease.

УДК 61:001(06) ББК 5 Ф 94

Materials and methods. The study analyzed 173 blood plasma samples from patients in hemodialysis centers located in St. Petersburg, Russia. To assess the prevalence of serological markers of hepatitis B (HBsAg, anti-HBs IgG, anti-HBcore IgG), the ELISA method was used in accordance with the manufacturer's recommendations.

The samples were also analyzed using molecular biological methods (real-time PCR with hybridization-fluorescence detection using commercial test systems and a previously developed method for detecting HBV DNA at low viral load [3], sequencing of nucleotide sequences).

Results and their discussion. The number of women in the group slightly prevailed compared to men: 54.9% and 45.1%, respectively, the age of patients ranged from 20 to 83 years and averaged 56.8±15.4 years. Antibodies to the hepatitis C virus were detected in 7.5% of patients.

Hepatitis B markers were detected in 92.5% of patients. Most of these cases are associated with anti-HBs Ig G vaccine antibodies. 24.3% of anti-HBCore Ig G cases were detected, 90.5% of them in combination with anti-HBs Ig G. These results indicate that 21 .9% of patients were exposed to the virus and retained detectable levels of antibodies. However, HBsAg was detected only in 1.1% of cases.

HBV DNA using commercial test systems (analytical sensitivity 50 IU/ml) was detected in all HBsAg-positive cases. Additionally, an additional 1.7% of HBsAg-negative DNA-positive cases were detected using a previously developed low viral load HBV DNA detection technique based on nested-PCR. Thus, the frequency of occurrence of HBV DNA was 2.8%. Earlier in the Russian Federation, it was shown that among patients of hemodialysis centers in Moscow, the prevalence of markers of viral hepatitis B in the period 1996-2001. was up to 61%, and the occurrence of HBsAg up to 12%. Around the same time, in different hemodialysis units in St. Petersburg, the prevalence of HBsAg ranged from 0 to 12.9%, while the proportion of anti-HBc IgG varied from 38.6 to 52.7%, and the proportion of anti-HBs IgG from 12.8 to 36.6% [4]. Thus, in the examined group, we found a large representation of anti-HBs IgG antibodies, which, apparently, is associated with the successful implementation of the hepatitis B vaccination program. Investigators in Egypt reported that the prevalence of OBI among hemodialysis patients ranged from 1.5% to 4.1% of cases, in Turkey - 2.7%. However, other studies report a significantly higher prevalence of HBsAg-negative CHB among hemodialysis patients. For example, in Brazil, HBV DNA was detected in 15% of samples obtained from HBsAg-negative patients; in Turkey, when studying the prevalence of OBI in patients of hemodialysis centers, HBV DNA was detected in 16.9% of patients [5]. Such significant variations in the results obtained by different research groups can be associated both with different levels of prevalence of chronic hepatitis B in different countries and regions, and with different sensitivity and specificity of the methods used to detect HBV DNA.

As mentioned above, in patients with chronic renal failure, cellular and humoral immune responses exhibit specific and nonspecific defects, resulting in a low anti-HBs response of hemodialysis patients to vaccination and is estimated at 45-60% [6]. Apparently, the predominance of seronegative HBV among hemodialysis patients in studies of different scientific groups may be associated with the same reasons. At the same time, it is known that serum transaminases tend to decrease in dialysis patients, which makes it diffi-

УДК 61:001(06) ББК 5 Ф 94

cult to determine liver damage using biochemical tests. The analysis of liver enzymes did not show any significant differences in patients with OBI compared with HBV-negative patients [7]. Thus, due to normal biochemical parameters and the absence of detectable HBV antigens and/or corresponding antibodies in most patients, the detection of OBI by molecular methods with high sensitivity remains virtually the only method for laboratory diagnosis of infected patients.

Mutations in the HBsAg region, especially in determinant "a", can affect the antigenicity of this protein due to conformational changes leading to the inability to neutralize the virus with anti-HBs antibodies. To date, more than 30 immune escape mutations have been identified in this region of the virus genome, which may affect detection by standard diagnostic assays for the detection and quantification of HBsAg, and may also interfere with recognition of HBsAg by vaccine-induced antibodies, posing a potential threat to the global program. vaccination. These mutations may pose a public health risk due to their pathogenic potential and the possibility of transmitting the virus to vaccinated individuals.

Phylogenetic analysis of the obtained HBV isolates showed that 80% of cases belonged to the D2 subgenotype and 20% of cases to the D3 subgenotype. In all cases, mutations in the MHR region were detected, but only in HBsAg-negative isolates, mutations in the region of 124-147 amino acids were detected. Mutations P120T, R122K, A128V, Q129R, M133I, G145R are known to affect the recognition of HBsAg by anti-HBs antibodies, while amino acid substitutions P120T, Q129R, M133I, G145R are associated with vaccine resistance.

Conclusions: 1. Due to the high prevalence of HBsAg-negative form of chronic hepatitis B, as well as vaccine escape mutations among dialysis center patients, it is recommended to pay close attention to the occurrence of mutant variants of the virus in hemodialysis centers; 2. It is necessary to use highly sensitive molecular genetic methods to detect the HBsAg-negative form of chronic viral hepatitis B.

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УДК 61:001(06) ББК 5 Ф 94