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АМИНОКИСЛОТНЫЕ ЗАМЕНЫ В РЕГИОНАХ CORE И HBsAg ВИРУСА ГЕПАТИТА В ПРИ МОНОИНФЕКЦИИ И ВГВ/ВИЧ-КОИНФЕКЦИИ В ГВИНЕЙСКОЙ РЕСПУБЛИКЕ

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Целью исследования являлось охарактеризовать генетические варианты ВГВ, циркулирующие в Гвинейской Республике, на основе нуклеотидных последовательностей полного генома вируса, а также проанализировать мутации вируса в регионах Core и HBsAg при моноинфекции и ВГВ/ВИЧ-коинфекции.

Материалы и методы. Материалом для исследования послужили 2616 проб сыворотки крови жителей Гвинейской Республики. Пациенты были обследованы на наличие маркеров ВГВ: HBsAg, HBs IgG и HBCore IgG. Нуклеотидные последовательности полного генома ВГВ были получены для 298 образцов, включая пациентов с коинфекцией ВИЧ/ВГВ. Амплификацию и последующее секвенирование вируса проводили с использованием вложенной ПЦР с парными перекрывающимися праймерами, совместно фланкирующими полный геном ВГВ (гены S, P, C, X).

Результаты. Серологические маркеры ВГВ выявлены в 80,77% проб, HBsAg — у 16,01% обследованных. ДНК ВГВ выявили в 22,36% случаев. Распространенность HBsAg-отрицательного ВГВ у пациентов с РНК ВИЧ составляет 45,16%, что значительно выше, чем в группе без ВИЧ-инфекции (6,07%). В обследованной группе преобладал ВГВ генотипа E — 75,5%, по сравнению с D1 — 9,39%, D2 — 4,02%, D3 — 6,37%, A2 — 4,7%. Вариабельность аминокислот среди образцов ВГВ была выше в регионе PreCore/Core, чем в регионе PreS1/PreS2/S. Мутации SHB были обнаружены в 83,89% случаев, мутации Core у 94,29%, замены аминокислот PreCore у 16,77% пациентов соответственно. Результаты, полученные в этой работе, демонстрируют высокую распространенность ВГВ в регионе и указывают на необходимость дальнейших крупномасштабных исследований мутаций ВГВ с целью улучшения стратегий контроля и профилактики заболеваний в Гвинейской Республике.

Ключевые слова: ВГВ, генотипы, аминокислотные замены, мутации вакцинного бегства, мутации лекарственной устойчивости, молекулярная эпидемиология, Гвинейская Республика

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AMINO ACID SUBSTITUTIONS IN CORE AND HBSAG REGIONS OF HEPATITIS B VIRUS IN PATIENTS WITH MONOINFECTION AND HBV/HIV-COINFECTION IN THE REPUBLIC OF GUINEA

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The aim of this study was to characterize the genetic variants of HBV currently circulating in the Republic of Guinea, based on the nucleotide sequences of the complete virus genome, and to analyze clinically significant mutations in the Core and HBsAg regions during HBV monoinfection and HBV/HIV coinfection.

Materials and methods. The study material was represented by 2616 blood serum samples collected from residents of the Republic of Guinea. The subjects were examined for the presence of HBV markers with a qualitative detection of HBsAg, HBs

IgG, and HBCore IgG. HBV complete genome nucleotide sequences were obtained for 298 samples including HIV/HBV co-infected patients. Amplification and subsequent sequencing of HBV were performed using nested PCR with pair's overlapping primers jointly flanking the complete HBV genome (S, P, C, X genes).

Results. HBV serological markers were detected in 80.77% samples, while HBsAg was detected in 16.01% of the examined group. HBV DNA we detected in 22.36%. The prevalence of HBsAg-negative HBV in patients with HIV RNA is 45.16%, which is significantly higher than 6.07% found in the group without HIV infection. Phylogenetic analysis of HBV in the examined samples showed that HBV genotype E (75.5%) predominates in the group compared to HBV genotype D1 (9.39%), D2 (4.02%), D3 (6.37%), and A2 (4.7%). In the tested group, the variability of amino acids among the HBV samples was higher in the PreCore/Core region than in the PreS1/PreS2/S region. SHB mutations were detected in 83,89%, Core mutations in 94.29%, PreCore amino acid substitutions in 16.77% of the patients, respectively.

The results obtained in this work demonstrate a high prevalence of HBV in the region and indicate the need for further large-scale studies of HBV mutations in order to improve strategies for disease control and prevention in the Republic of Guinea.

Key words: HBV, genotypes, amino acid substitutions, vaccine escape mutations, drug resistance mutations, molecular epidemiology, Republic of Guinea

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Introduction. Hepatitis B virus (HBV) is one of the most common hepatotropic viruses which can cause both acute and chronic liver diseases. More than 240 million people have been diagnosed with chronic hepatitis B infection (CHB) [1]. The main laboratory diagnostic marker is the HBV surface antigen (HBsAg), the occurrence of which in population varies by geographic region.

HBV infection is prevalent in the African continent; the number of people in Africa with CHB is over 75 million, 25% of whom will presumably die from liver disorders. The occurrence of the HBV surface antigen in African countries exceeds 8% and may reach 25% [1]. There is a wide variation in HBsAg prevalence estimates in these countries. The highest prevalence of the virus has been found in sub-Saharan Africa, in such West African countries as Liberia (17.55%), Mali (15.5%), Burkina Faso (14.5%), Ghana (13.8%), Senegal (13.8%), Nigeria (13.6%), and Mauritania (10.9%) [2, 3]. In East and South Africa, the HBsAg prevalence is relatively low and corresponds to the average level [1], whereas in such North African countries as Tunisia, Algeria, Egypt, and Morocco, the level is below 2% [4]. This can partially be explained by differences in risk factors and transmission routes in different countries. In general, the prevalence estimates at the country level indicate a high infection burden in sub-Saharan Africa [1].

Along with the differences in HBsAg prevalence between the countries, the occurrence of HBV surface antigen varies among different groups in the same region or country. However, it should be noted that data on the serological prevalence of HBV in the population are limited, since serological screening is often carried out only in certain population groups — in the high-risk groups (HIV-infected people, prisoners, IDUs, etc.) and in the groups where the prevalence of infection significantly affects the health of the population (blood donors, pregnant women). For instance, in Sierra Leone, the HBsAg+ prevalence among children was 18.18% in 1998, whereas the HBcore IgG antibodies were detected in 71% of children; only seven years later, when assessing the HBV prevalence in pregnant women from the middle and high social and economic classes, the HBsAg+ occurrence was found to comprise 6.2%, which

apparently indicates the importance of social status / living standards for the HBV prevalence [5]. Estimates of the prevalence of the virus in the population may also vary depending on gender and age, which indicates the need for careful analysis and selection of the groups to be examined. For instance, in rural areas of the southwestern Chad, the overall HBsAg+ prevalence was 22.9%, with the youngest age group (6–15 years) and boys/men showing significantly higher HBsAg prevalence than older groups and girls/women, respectively ($p<0.01$) [2].

Such significant differences among different groups may be caused by the development and implementation of new methods for HBV marker identification, although, according to some authors, the heterogeneity of the results obtained by different researchers in the same country is primarily associated with differences in geographic regions, socialization and introduction of universal immunization rather than with the patient's gender, HBV screening instrumentation and methodological characteristics of assays [6].

In fact, differences in the HBsAg prevalence in different groups are also observed in European countries, Russian Federation, USA, etc.; for instance, the HBsAg+ occurrence is higher in the high-risk groups (HIV-infected people, IDUs, prisoners, homosexuals, sex workers, etc.) and is lower among blood donors and pregnant women compared to the general population. In African countries, by contrast, the HBsAg+ prevalence in newborn children (16.3%) and blood donors (23.4%) can be significantly higher than in the general population (13.6%), as described for Nigeria [7].

It is worth noting that in Africa HBV is transmitted mainly at an early age. Horizontal transmission takes place at the age of 2 to 10 years; children with high-level viremia are highly likely to transmit the virus through cuts and scratches to their susceptible siblings and playmates. Although horizontal transmission is the main route of virus transmission, perinatal transmission is believed to cause about 10% of chronic infection, and the low level of HBeAg occurrence in HBsAg-positive pregnant women in most African countries correlates with the low frequency of perinatal transmission. Moreover, 20–30% of patients infected in their early childhood become chronic carriers, while only 10% of them remain HBeAg-positive in adolescence [8].

Regarding the high occurrence of HBV in African countries, it should also be noted that these countries have the high prevalence of hepatocellular carcinoma which is known to account for almost 85% of all primary liver tumours and is the fifth most frequently diagnosed cancer disease in the world. In sub-Saharan Africa, HCC makes a significant contribution to the liver disease mortality due to the occurrence of high risk factors in the continent. West African countries are slightly behind, with HCC mortality in West Africa reaching 200,000 people per year. Moreover, due to late visits to doctors, most patients with HCC die within a few weeks after the diagnosis. In fact, the mortality from HCC in this region is almost the same as the morbidity. Gambia is the most affected country, followed by the Republic of Guinea, Liberia, and Sierra Leone [9]. One of the main reasons for the high prevalence of HCC in African countries is the HBV infection at an early age, late virus detection, and inadequate treatment, which is associated inter alia with insufficient diagnostic means available in the region.

Occult hepatitis B infection (OBI) is a phase of CHB in which HBV DNA is detected in the liver tissue with undetectable HBsAg levels in peripheral blood serum, whether or not HBV DNA is detected in peripheral blood [10]. Despite the absence of HBsAg in peripheral blood, most patients with OBI are seropositive for one or more serological markers, depending on the phase of disease progression, anti-HBs IgG, HBeAg, anti-HBe IgG, anti-HBCore IgG; however, over 20% of the patients are seronegative for all HBV markers [10]. The prevalence of OBI correlates with the prevalence of HBsAg-positive CHB.

A large proportion of HCC cases in African countries is associated with occult HB; OBI is detected in more than 75% of HBsAg-negative HCC patients [11]. Such a high prevalence of HBV is attributed inter alia to the OBI occurrence in the region. It should be noted that the methods for HBV detection and diagnostics of HBV-associated liver disease in low- and middle-income countries differ significantly from those used in countries with access to expensive technologies that require qualified personnel. Most research on this topic in African countries is limited to detection of HBV surface antigen, while molecular genetic methods that make it possible to more accurately assess the HBV prevalence, genotype the virus, quantify HBV DNA, detect the infection at low virus load in the case of occult CHB are available only in central laboratories of large cities.

HBV is characterized by a high degree of genetic heterogeneity; it is currently subdivided into ten geno-

types designated as A-I genotypes which differ from each other in the composition of nucleotide sequences by more than 8%. Additionally, genotypes A, B, C, D, F, and H are subdivided into 45 subgenotypes which show complete divergence of nucleotide sequences in the range from 4 to 7.5% [12]. Studies of the prevalence of HBV variants in different regions of the world have been actively carried out since the discovery of the genotype up to the present. All genotypes and serotypes differ in their geographic distribution which changes very slowly over time, reflecting the virus propagation pathways associated with human migration, as well as the possible different geographical origin of different genotypes, which allows to use them as epidemiological markers [1]. In most regions, with rare exceptions, there are 1–2 prevailing genotypes and several minor ones, including those imported from other regions [13]. Despite significant public health problems associated with HBV in sub-Saharan Africa, many countries in this region do not perform systematic HBV monitoring and there is no information on genetic characteristics of virus variants prevailing in a particular region. Nonetheless, even though molecular genetic methods are insufficiently used in the region, some genetic variants of the virus are known that are characteristic of African countries. A, D and E genotypes are the most common HBV genotypes found in Africa. For instance, although genotype A is widespread throughout the world, on the African continent it is predominant in South, Central, and East African countries. In general, genotype E is prevalent in West and Central Africa. However, the prevalence of these genotypes can vary significantly even within the same country.

Vaccination and anti-viral therapy are key approaches to reducing morbidity and mortality from HBV infection. However, after nearly half a century of the development and implementation of therapeutic drugs and effective HBV vaccine, and significant global vaccination coverage, HBV infection is still a serious health problem worldwide. A high degree of HBV genetic variability allows the virus to respond to endogenous and exogenous selective pressures by further modulating its genome structure. During prolonged infection and under different selective pressures, some variants, particularly in the S gene, might evolve and thereby assist the virus to escape therapeutic, prophylactic, and diagnostic measures.

Mutations in the PreS/S gene. The envelope gene of HBV has three open reading frames (ORFs), PreS1, PreS2, and S, which encode three proteins, the small, middle, and large HBsAg translated from distinct mRNAs. The large S gene of HBV encodes the preS1 protein (108 aa), the preS2 protein (55 aa), and the small S protein — HBsAg (226 aa). Mutations in the S region (the region of the hepatitis B small surface antigen, SHB) mainly occur in the N-terminal region (aa 1–99) and in the major hydrophilic region (MHR) (aa 100–169), rather than in the C-terminal area (aa 170–226), both before and after antiviral therapy. MHR, for which a relatively large number of amino acid substitutions were shown, includes the "α" determinant (aa 124–147), the tertiary structure of which determines antigenic specificity. Thus, the efficacy of vaccination and therapy may be reduced due to the emergence of clinically significant mutations: immune-escape mutations (IEMs), drug resistance mutations (DRMs), and mutations affecting the progression of the disease. Due to the overlap of the S and the reverse transcriptase (RT) genes, DRMs in the RT gene can lead to the emergence of IEMs in the MHR and vice versa.

Mutations occur due to the high error rate of the RT enzyme without the possibility of proofreading and provide a selective advantage in avoiding the effect of drug therapy and spreading in the population. Moreover, some mutations lead to resistance to only one agent, while others are associated with resistance to several agents. Depending on the drug to which mutations cause resistance, resistance to 3TC (primary and compensatory), ETV (combination of amino acid substitutions), and TFV is known. In African countries, drug choices are usually limited to 3TC or tenofovir. Among patients with chronic HBV monoinfection, the incidence of HBV 3TC resistance reaches 20% per year. In patients with HIV + HBV coinfection, this indicator can reach 90% after 5 years of treatment, since the development of resistance is accelerated by HIV coinfection [14]. The presence of amino-acid substitutions in the MHR of HBsAg can alter the immunogenicity of HBsAg and can contribute to immune escape. Immune-escape variants of HBV may be associated with the failure of diagnostic tests. Because an escape mutation alters the protruding loop of the "α" determinant, pre-existing neutralizing antibodies cannot adequately recognize the altered epitope [15]. These mutants can cause HBV reactivation even in anti-HBs-positive patients and can spread despite correct active/passive vaccination strategies. Thus, immune-escape variants of HBV present a clinical challenge, because these mutations can lead to the diagnostic failure of

commercial assays for HBsAg, as well as to the prophylactic failure of immunoglobulins or vaccines and to HBV reactivation. Some Pre-S mutations are associated with the progression of liver disease. For example, Pre-S2 deletions are frequently observed in cases of liver cirrhosis and HCC. Pre-S2 start codon mutations are associated with cases of liver cirrhosis and HCC as well; they can also impair secretion of the virus. In addition, mutations in the vicinity of the Pre-S2/S splice donor site were common in occult HBV infections.

Mutations in the PreCore/Core gene. The HBV core protein encoded by the HBV core gene from the PreCore/Core ORF has 183 aa and is essential for viral replication. It is a 21 kDa structural protein involved in both structural and functional processes. It plays a key role in the HBV life cycle, and alterations in the protein sequence may serve as potential markers of disease progression [16]. Some of the basal core mutations promoters were identified as possible prognostic markers for the development of cirrhosis and HCC.

The Republic of Guinea is a region with a high prevalence of many viral infectious diseases, including those caused by hepatotropic viruses, which emphasizes the importance of the epidemiologic assessment of this territory for determining the HBV prevalence [17]. However, studies in this region are limited in both the quantity and quality of the diagnostic methods used. In Guinea, an assessment of the HBsAg prevalence in various groups showed that the incidence among HIV-infected people, diabetics and prisoners was 8%, 8–9%, and 27.7%, respectively [18]. An earlier phylogenetic analysis of samples obtained in 2006 from asymptomatic HBsAg carriers from Conakry confirmed the prevalence of genotype E which comprised 95.1% and a wide representation of deletions in the core region among circulating strains [19]. While several dozens of studies have been published for some countries, allowing to summarize the available data and obtain reliable HBV information for these territories, very few publications can be found for other countries, and they are frequently not only limited in terms of the methodology, but also contradict each other. We found no publications on the prevalence of OBI or mutations of HBV strains circulating in the Republic of Guinea. In this region, practically no studies on genotypic characterization, analysis of HBV vaccine escape mutants and drug resistance have been carried out.

Aim. The aim of this study was to characterize the genetic variants of HBV currently circulating in the Republic of Guinea, based on the nucleotide sequences of the complete virus genome, and to analyze clinically significant mutations in the Core and HBsAg regions during HBV monoinfection and HBV/HIV coinfection.

Materials and Methods. The study material was represented by 2616 blood serum samples collected from residents of the Republic of Guinea — blood donors, conditionally healthy people. The donors denied the anamnesis of HBV infection.

The subjects were examined for the presence of HBV markers with a qualitative detection of HBsAg, HBs IgG, and HBCore IgG using test-systems manufactured by Vector-Best (Russia) and Diagnostic Systems (Russia) in accordance with the manufacturer's instructions.

For the primary detection of HBV, nucleic acids were extracted from blood serum using the AmpliPrime RiboPrep kit from the Central Research Institute of Epidemiology (CRIE, Russia). The test for the presence of the virus was performed by real-time polymerase chain reaction (RT-PCR) with hybridization fluorescence detection using the AmpliSens® HBV-FL kit (CRIE, Russia) with a sensitivity of 50 IU/ml. Next, we used a PCR-based method developed by the St. Petersburg Pasteur Institute which allows to detect low concentrations of HBV DNA in various clinical samples and uses amplified products for sequence analysis; the sensitivity was 5 IU/ml [20]. Amplification and subsequent sequencing of HBV were performed using nested PCR. At the first stage, asymmetric PCR with extended oligonucleotides was carried out; at the second stage, to increase the sensitivity, PCR was carried out using the amplification products of the first reaction and one of the nested pair's overlapping primers jointly flanking the complete HBV genome (S, P, C, X genes). The products of the sequencing reaction were analyzed using an ABI Prism 3500 genetic analyzer (Applied Biosystems, USA). The primary analysis of the obtained fragments was performed using the BLAST algorithm (http://www.ncbi.nlm.nih.qov/BLAST) on the nucleotide sequences provided in the GenBank sequence database. The resulting sequences were aligned in the MEGAv.7.0 program using the ClustalW algorithm. The phylogenetic tree was constructed using the neighbor-joining method; the significance of the tree was assessed using bootstrap analysis with 1000 replicates. The amino acid sequence of the envelope protein was determined by translating the corresponding nucleotide sequence according to the open reading frame.

The nucleotide sequences of the complete genomes of some HBV isolates examined in this work were deposited to the GenBank database under the accession numbers MN507840-MN507849, MW455161-MW455166.

Statistical data processing was carried out using the MS Excel (Microsoft, USA) and Prizm 5.0 software (GraphPad Software, USA). To assess the statistical significance of the numerical data obtained during pairwise comparison depending on the sample characteristics, the Fisher's exact test or the Chi-square test with Yates' correction were used. The probability value $p<0.05$ was taken as the statistical significance threshold.

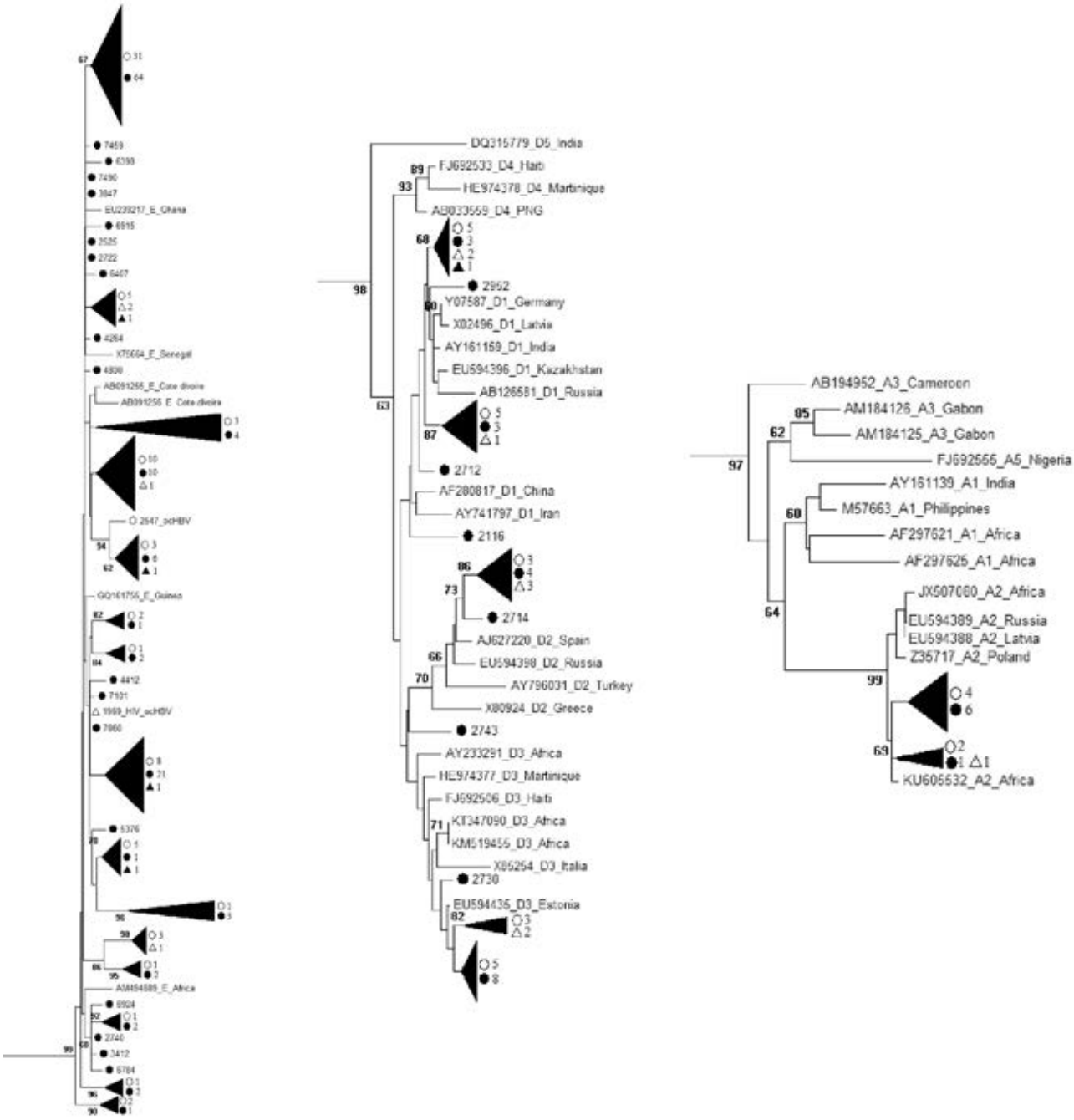
Results and Discussion. We examined 2616 clinical blood serum samples; HBV serological markers were detected in 2113 samples, or 80.77%, while HBsAg was detected in 16.01% of the examined group. When examining 2,616 clinical blood serum samples for the presence of HBV DNA using the AmpliSens® HBV-FL kit, we detected the virus in 426 samples, or 16.28%. However, although HBsAg was detected in combination with other markers in most samples, there were also seven HBsAg-negative samples. In five cases, the viral load was below 100 IU/ml, in two cases it was slightly above 50 IU/ml, however, the viral load of about 50 IU/ml was detected in only one out of seven HBV DNA-positive HBsAg-negative samples. When examining serum samples for the presence of HBV DNA using the PCR-based method developed by the St. Petersburg Pasteur Institute, we detected the virus in 585 samples, or 22.36%.

We previously showed that serological markers of HBV are detected in 29.03% of HIV-positive patients, including 16.12% of HBsAg. HBV DNA was detected in all HBsAg-positive and two anti-HBCore IgG-positive patients, as well as in twelve patients who were negative for all HBV serological markers analyzed in the work. Thus, HBV DNA was found in 61.29% of HIV-positive patients [21].

The probability of detecting HBV serological markers in HIV-infected patients is significantly lower than that in patients from the group without HIV infection: $\chi^2=48.379$, $p<0.0001$, $df=1$, $RR=0.3594$, CI: 0.2072–0.6235. No significant differences were found in the prevalence of HBsAg among patients from the HIV+ and HIV- groups examined in this study ($p>0.05$). Thus, the risk of CHB development in HIV-infected patients is more than twice as high as in patients without HIV: $\chi^2=24.198$, $p<0.0001$, $df=1$. $RR=2.741$, $p<0.0001$, CI: 2,053–3,658. The prevalence of HBsAg-negative HBV in patients with HIV RNA is 45.16%, which is significantly higher than 6.07% found in the group without HIV infection: $\chi^2=115.07$, $p<0.0001$, $df=1$. The risk of developing HBsAg-negative CHB in HIV-infected patients is more than ten times higher than in patients without HIV: $RR=10.969$, $p<0.0001$, CI: 7.823–15.379.

The development of OBI is determined by suppression of the endonuclear transcription of sub-genomic HBV RNA from the covalently closed circular HBV DNA matrix; the RNA is implicated in the synthesis of the viral genome and viral proteins. The suppression may be caused by a variety of factors which are not yet fully understood, including genetic characteristics of the virus itself and/or of its host, or by external interference. HIV coinfection is one of these factors. A reactivation of OBI can be caused by immunodepression/immunosuppression induced by HIV infection. These results are consistent with published reports that the OBI prevalence in HIV-infected patients ranged from 0 to 10% when using PCR-based testing with standard commercial kits and up to 35–89% when using more sensitive versions of the method. OBI has been shown to be associated with high levels of HIV RNA. Since HIV is capable of suppressing HBV replication, this leads to the prevalence of occult CHB among HIV-infected patients, whereas HIV-induced immunosuppression can lead to low antibody response to HBsAg, as well as HBV reactivation [4]. It should be noted that the registered high incidence rates of OBI among HIV-infected patients reflect the assumption of higher rates of HIV/HBV prevalence in West and South African countries that can be as high as 30% [5].

Nucleotide sequences of the complete HBV genome were obtained from 298 samples, including patients with HIV/HBV coinfection. Phylogenetic analysis of HBV in the examined samples showed that HBV genotype E (75.5%) predominates in the examined group compared to HBV genotype D (19.8%) and HBV genotype A (4.7%). It should be noted that genotype A is represented by subgenotype A2, whereas genotype D — by subgenotypes D1, D2, and D3. Thus, HBV genotype E comprising 75.5% predominates in the group compared to HBV genotype D1 (9.39%), D2 (4.02%), D3 (6.37%), and A2 (4.7%). The phylogenetic relationships between the examined HBV isolates from patients living in the Republic of Guinea and the reference sequences from the GenBank database are shown in Figure.



а *б* *в*

Figure. Phylogenetic analysis of HBV entire genome nucleotide sequences obtained from patients living in the Republic of Guinea in comparison with the reference sequences provided in the GenBank international database. The reference sequences are denoted by GenBank accession numbers with the identification of the genotype and origin of the sample. *а* — branch genotype E, *б* — branch genotype D, *в* — branch genotype A. Black triangles indicate HBsAg(+), RNA HIV(+), white triangles indicate HBsAg(-), RNA HIV(+), black circles indicate HBsAg(+), RNA HIV(-), white circles denote HBsAg(-), RNA HIV(-) samples. Genetically related isolates are pooled with an indication of the number/ affiliation of isolates in the pool. Bootstrap values are ⩾60

It should be noted that genotype E and genotype D were detected in both HBsAg-negative and HBsAg-positive samples. We previously showed that among patients with HIV/HBV coinfection, HBV genotype E and HBV genotype D have equal representation in the group (47.36%), while HBV genotype A was detected in only one case (5.26%) [3]. A comparative analysis of the prevalence of HBV subgenotypes among patients with or without HIV infection showed a significant difference: $\chi^2=12.597$, p=0.0134, df=4. Thus, the prevalence of HBV genotype D among patients with HIV/HBV coinfection is higher than among patients with HBV monoinfection: $\chi^2=7.948$, p=0.0048, df=1, RR=2,643, 95% CI=1.546–4.520.

In regions that are geographically close to the Republic of Guinea, the prevalence of HBV genotypes is generally consistent with our results. For example, in Côte d'Ivoire, the distribution of HBV genotypes was 6.3% for genotype A, 6.3% for genotype D, and 87.4% for genotype E. In Burkina Faso, genotype E comprises 72%, while subgenotype A3 — 25%. In Mali and Senegal, HBV genotype E is also predominant — 91.1% and 75%, respectively. In Côte d'Ivoire and Uganda, HBV/A2 was present as a minority HBV/A variant. HBV/A2 strains similar to some isolates characteristic of Europe suggest the spread of HBV from South Africa to other regions [17]. Genotype D is widespread throughout the world, whereas its subgenotypes have distinctive geographic distribution. Subgenotype D1 is found in North African countries, while subgenotype D3 in South African countries. Genotype D which is presumably associated with more severe liver diseases was identified mainly in North Africa and some East African countries [17]. HBV genotype E is highly epidemic in most South and West sub-Saharan countries, where it accounts for more than 90% of total HBV, while regions in which genotype E is endemic have a higher incidence of HCC [9, 17]. Despite its wide geographic distribution, genotype E is characterized by a low genetic diversity, which apparently indicates its short evolutionary history. HBV genotype E is epidemiologically associated with chronic and occult HBV infections, as well as with HCC, drug resistance, and vaccine escape.

In the tested group, the variability of amino acids among the HBV samples was higher in the PreCore/Core region than in the PreS1/PreS2/S region. SHB mutations were detected in 83.89%, Core mutations in 94.29%, PreCore amino acid substitutions in 16.77% of the patients, respectively. Information on the most common clinically significant mutations identified in the examined group is presented in the Table.

Mutations associated with amino acid substitutions in the reverse transcriptase domain are classified as primary or secondary compensatory resistance mutations. None of the patients had known primary drug resistance HBV mutations; however, among samples with HBV genotype E, we identified mutations at specific positions of reverse transcriptase associated with drug resistance, including primary mutations that are potentially associated with reduced sensitivity to antivirals and the secondary compensatory resistance mutations that generally restore the replication capability of the viral polymerase (Table).

Due to the overlap between the genes encoding reverse transcriptase and HBsAg, drug resistance mutations can introduce mutations in the MHR of HBsAg. To date, more than 30 immune-escape mutations in HBsAg have been identified; they allow the virus to evade neutralizing antibodies and facilitate persistent HBV infection and viral fitness. Immune-associated escape mutations can also interfere with HBsAg recognition by antibodies induced by the vaccine, thus posing a potential threat to the global vaccination program [22]. We determined the prevalence of 10 immune-associated escape mutations affecting HBsAg-recognition by antibodies. Most of these substitutions are known to act as vaccine-escape mutations (Table). Mutations in the aa 124–137 region are believed to interact with protective or neutralizing anti-HBs antibodies resulting from natural infection as well as from vaccination with HBsAg. HBV genotype E is known to exhibit clear genotypic divergence from all other genotypes within the «a» determinant which can have escape mutations. The most common polymorphisms in genotype E are known to be T116N, P120L/S, Q129H/R, M133I, D144E, and G145I. In our work, we see the confirmation of this data.

Of particular interest is the detection of HBV DNA in six samples (HBsAg- HBCore IgG+ HBs IgG+). Five samples belonged to genotype E and one to genotype D1. Anti-HBCore IgG+ usually appears in the acute phase of HBV infection and persists for a long time after the virus clearance, while the presence of anti-HBs IgG+ together with anti-HBCore IgG+ usually indicates, resolved infection. In the absence of HBsAg, serum anti-HBs IgG+ indicate protective immunity against HBV acquired through vaccination (HBCore IgG-) or natural infection (HBCore IgG+) [23]. In patients with CHB, the coexistence of HBsAg and HBs IgG+ is associated with an increase in the variability of the "a" determinant, suggesting the selection of HBV immune-escape mutants during chronic carriage. The most frequent changes are localized at positions 123, 126, 129, 130, 133, 144, 145 and 181, as described for immune-escape variants, and some of them, including aa 119–123, have been reported to play a role in the mechanism of occult HBV infection [6, 14, 15, 17]. However, in each of the six cases we described, escape mutations associated with diagnostic failure and vaccine escape were also found. These patients may develop liver cir-

Table

Information on the most common clinically significant mutations identified in the examined group

HBV genome region	Mutation	Prevalence in all group	Genotype; prevalence in genotype	Description
RT	L80F	3.69%	E (4.88%)	The mutations are not described, however, aa change at given positions were can be considered as possible mutations of resistance to lamivudine, telbivudine, and entecavir
RT	S202R	5.03%	E (6.66%)	
RT	S204R	10.4%	E (13.77%)	
MHR	Y100C	4.69%	A (7.14%), E (5.77%)	Associated with HBsAg-negative CHB
MHR	M103I	3.02%	E (4%)	Associated with HBsAg-negative CHB
MHR	T127P	19.12%	D (96.61%)	Escape-mutant (immune escape, vaccine escape, diagnostic escape). Since MHR is the most important antigenic determinant in envelope proteins and is composed of two loops bounded by disulfide bridges between cys124 and cys137, and cys139 and cys147, mutations in this region breaks disulfide bridges. In-silico structure prediction studies suggested that the three dimensional conformation of the extravirion loop of isolates with aa substitutions between residues 133 and 144 are different from that of the wild-type genotype E, and the loop is embedded in the polar head-groups of the extravirion leaflet of the virion membrane. Some of them leading to surface antigen production different from the usual one
MHR	Q129H/R	19.79%	E (20.44%), D (22.03%)	
MHR	M133I	22.14%	E (25.33%), D (11.86%), A (14.28%)	
MHR	Y134H	5.7%	E (7.55%)	
MHR	C137Y	10.4%	E (13.7%)	
MHR	K141E	8.38%	E (11.11%)	
MHR	D144E	5.36%	E (7.11%)	
MHR	G145R	4.69%	E (6.22%)	Escape-mutant (vaccine escape, diagnostic escape). Mutation can impair HBsAg secretion. Reinfection in the liver occurs despite protective anti-HBs titres
MHR	Y147C	3.69%	E (4.88%)	Contribute viral genome replication at the stage viral DNA synthesis, which may be of importance given the significance of Cys-Cys disulphide bond in maintaining the conformation required for HBsAg antigenicity. Breakthrough infection in vaccinated patients
PreCore	H5D	29.19%	E (38.66%)	In HBsAg-negative HBV genotype E. Associated with severe liver disease. This mutation could partially explain the high prevalence of HCC in Africa
PreCore	K21N	6.37%	D3 (100%)	Suspected of being associated with severe disease in HBsAg-negative patients
PreCore	W28* (G1896A)	36.91%	E (47.11%), D + A (5.48%)	To affect HBeAg production negatively. Creates a stop codon and prevents the synthesis of HBeAg. Responsible for more than 90% of defective HBeAg secretion and to affect the HBeAg serostatus
PreCore	G29D	18.79%	E (24.88%)	The mutation is associated with hepatocellular carcinoma development and affects the HBeAg serostatus
Core	L116I	94.63%	E (97.33%), D (88.13%), A (78.57%)	Changes between aa 113 and 143 influence the antigenicity and stability of the particle. May create immune escape mutants leading to chronic viral persistence and severe liver disease. Located within B-cell epitopes, and is associated with disease progression, cirrhosis, and hepatocellular carcinoma development
Core	T146N	75.5%	E (100%)	Possibly characteristic of HBV genotype E

rhosis, HCC, and liver failure. Vaccinated people are at risk of contracting strains with such mutations. Of particular importance are 31 samples with a combination of T127P + S204R mutations in the SHB region: this combination, together with the F170L mutation, has previously been observed in an HBsAg-negative patient with HBV reactivation 3 months after the termination of long-term prophylactic treatment with lamivudine [Ошибка: источник перекрёстной ссылки не найден4]. The authors suggested that these mutations may interfere with the HBsAg recognition in the diagnostic assays.

The incidence of the G1896A mutation was significantly higher among HBV genotype E samples (47.11%) compared to samples of all other genotypes (5.48%): χ^2=39.254, p<0.0001, df=1, RR=8,598, 95% CI=3.282–22.522. The correlation of HBV genotype E with an increased risk of developing HCC can also be explained by the substantial proportion of patients harbouring a viral strain with the G1896A mutation [25].

It is interesting that the same HBV mutations were detected both in the studied group among HIV-infected patients and in patients without HIV infection. Moreover, the incidence of certain mutations in the subgroups did not differ significantly. The absence of significant differences in the prevalence of HBV mutations between mono- and coinfected patients that were either HBsAg-negative or HBsAg-positive may indicate an independent natural development that is not associated with the coinfection or the occult CHB infection. Immune-escape variants do not usually occur alone in HBV strains of infected patients, but in combination with polymerase and/or core mutations. It is therefore important to understand the functional implications of immune-escape mutations for the replication of HBV strains, especially for strains with common treatment-associated mutations, such as drug resistance. Quasispecies with higher complexity should theoretically tend to breach genetic barriers, thus leading to drug resistance. The discovery of the coexistence of complex HBV mutants, for example, immune escape and drug resistance, represents a serious challenge requiring antiviral therapy adjustments.

Screening for HBV DNA is not routinely done in the Republic of Guinea due, at least in part, to the high cost and lack of HBV treatment programs outside of coinfection with HIV. Patients infected with HBV either are left untreated (HBV monoinfection) or undergo ART (HIV + HBV coinfection) without proper monitoring, which greatly increases the risk of developing DRMs.

Conclusion. Although the information on HBV genetic diversity and clinically significant mutations is crucial for patient management, it is scarce for the Republic of Guinea. The results obtained in this work demonstrate a high prevalence of HBV in the region and indicate the need for further large-scale studies of HBV mutations in order to improve strategies for disease control and prevention in the Republic of Guinea. At the same time, methods should be used that allow not only detecting manifest and occult CHB forms, but also sequencing identified isolates, since although vaccination protects against a wide range of strains, it cannot neutralize the so-called vaccine-escape strains, which can lead to seronegative OBI with subsequent reactivation.

In this study, we for the first time showed the presence of HBV isolates with immune-escape mutations in the Republic of Guinea. These findings emphasize the need for careful monitoring of specific mutational patterns, as these data raise the question of possible transmission of HBV genotype E to vaccinated individuals.

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