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Prevalence of viral hepatitis B markers among blood donors in the Republic of Guinea

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Introduction. The problem of transfusion safety in relation to parenteral viral hepatitis still remains relevant. Viral hepatitis B (HB) remains the most common viral infection transmitted through transfusion procedures. One of the natural phases of chronic hepatitis B (CHB) is occult hepatitis B infection (OBI), characterized by an undetectable HBsAg (regardless of the other serological markers content) in the presence of hepatitis B virus (HBV) DNA in the liver tissue and an extremely low, up to undetectable, level of viral load in the blood. In the Republic of Guinea, as in most countries on the continent, the prevention of HBV transmission through transfusion is still based on HBsAg serological testing of donors only. In this connection, OBI remains as a potential threat to blood transfusion safety. Detection of HBV DNA is a reliable preventive measure against transmission of the virus from donors with HBsAg-negative HBV infection, especially in highly endemic regions. In this regard, the study was conducted to substantiate recommendations for improving blood safety against the background of significant HBV prevalence in the Republic of Guinea.

The **aim** of the work was the evaluation of serological and molecular markers of HBV infection in blood donors in the Republic of Guinea.

Material and methods. We examined 250 blood samples obtained from donors living in Conakry, Republic of Guinea. Samples were tested for the presence of serological (surface antigen, HBsAg; antibodies (ABs) to surface (anti-HBs IgG) and core (anti-HBc IgG) antigens) and molecular (DNA) markers of HBV infection.

Results and discussion. The overall detection rate of hepatitis B markers was 83.2%; HBsAg was detected in 16.4% of all individuals. The high incidence of HBsAg in men (19.55%) compared to women (8.45%) was shown, the relative risk of HBV infection with the formation of HBsAg-positive chronic hepatitis B in males was also significantly higher. The prevalence of the HBV DNA in the study group was 30.4%, the OBI cases accounted for 15.6%. The prevalence of this form of the disease was shown in donors aged 30–49 years (24.78%), in the group of people younger than 30 years, the incidence was lower (8.73%), and at the age of over 50 years, OBI was not detected. Based on the phylogenetic analysis of 76 virus isolates, it was shown that genotype E prevails in the examined group (85.53%).

Cases of pathogen DNA detection occurred in HBsAg-negative blood donors in the presence of anti-HBs IgG ($n = 4$), as well as in the simultaneous presence of ABs anti-HBs IgG and anti-HBc IgG ($n = 7$). The viral load exceeded 200 IU/ml in OBI samples. Escape mutations were detected by sequencing in each OBI sample, contributing to the virus escaping from diagnostic based on screening for HBsAg.

Conclusion. Assessment of the prevalence viral hepatitis B markers in blood donors, determination of genotypes and clinically significant mutations of virus variants are necessary to ensure safe medical manipulations, control and prevention of the spread of this infectious agent.

Keywords: *viral hepatitis B (HB); occult hepatitis B infection (OBI); hepatitis B virus (HBV); serological markers; molecular biological markers; laboratory diagnostics; infectious blood safety; blood donors; Republic of Guinea*

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Conflict of interest. The authors declare no conflict of interest.

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Распространенность маркеров вирусного гепатита В среди доноров крови в Гвинейской Республике

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Введение. Проблема трансфузионной безопасности в отношении парентеральных вирусных гепатитов до настоящего времени сохраняет свою актуальность. Вирусный гепатит В (ВГВ) остается наиболее распространенной вирусной инфекцией, передаваемой при трансфузиологических манипуляциях. Одной из естественных фаз течения хронического вирусного гепатита В (ХГВ) является occultный гепатит (ОкГВ), характеризующийся недетектируемым уровнем HBsAg (независимо от содержания иных серологических маркеров) при наличии ДНК ВГВ в ткани печени и крайне низким уровнем вирусной нагрузки в крови вплоть до неопределяемого. В Гвинейской Республике, как и в большинстве государств континента, профилактика трансфузионной передачи ВГВ посредством скрининга доноров до сих пор основывается на изолированном серологическом определении HBsAg, в связи с чем ОкГВ сохраняется в качестве потенциальной угрозы для гемотрансфузионной безопасности. Определение ДНК ВГВ служит надежной профилактической ме-

рой против передачи вируса от доноров с HBsAg-негативным ГВ, особенно в высокоэндемичных регионах. В связи с этим для обоснования рекомендаций по повышению безопасности крови на фоне значительной частоты встречаемости ВГВ на территории Гвинейской Республики проведено настоящее исследование.

Цель работы – оценка распространенности серологических и молекулярно-генетических маркеров вирусного гепатита В (ГВ) у доноров крови в Гвинейской Республике.

Материал и методы. Исследованы 250 образцов крови, полученные от доноров, проживающих на территории г. Конакри (Гвинейская Республика). В пробах определяли наличие серологических (поверхностный антиген – HBsAg; антитела (АТ) к поверхностному (анти-HBs IgG) и ядерному (коровому) (анти-HBc IgG) антигенам) и молекулярно-генетических (ДНК) маркеров этой инфекции.

Результаты и обсуждение. Встречаемость маркеров вирусного ГВ составила 83,2%; HBsAg обнаружен у 16,4% исследуемых. Частота его выявления оказалась более высокой среди мужчин (19,55%) по сравнению с женщинами (8,45%), относительный риск инфицирования вирусом с формированием HBsAg-положительной хронической формы заболевания у лиц мужского пола также достоверно выше. Значение распространенности ДНК возбудителя в исследуемой группе составило 30,4%; при этом 15,6% приходится на ОкГВ. Показано преобладание данного варианта течения инфекционного процесса у доноров 30–49 лет (24,78%). Среди лиц моложе 30 лет встречаемость ОкГВ оказалась ниже (8,73%), а в возрасте от 50 лет и старше occultная форма ГВ не выявлена. На основании филогенетического анализа 76 изолятов вируса установлено преобладание в исследованной группе генотипа Е (85,53%).

Случаи выявления ДНК патогена имели место у HBsAg-негативных доноров крови при наличии АТ анти-HBs IgG ($n = 4$), а также на фоне одновременного присутствия анти-HBs IgG и анти-HBc IgG ($n = 7$). При этом показатель вирусной нагрузки превышал 200 МЕ/мл. В ходе секвенирования в каждом образце обнаружены эскаре-мутации, способствующие ускользанию вируса от диагностики при скринировании на HBsAg.

Заключение. Оценка распространенности маркеров ГВ у доноров крови, определение генотипов и клинически значимых мутаций вируса необходимы для обеспечения биологической и трансфузионной безопасности при медицинских манипуляциях, контроля и предотвращения распространения данного инфекционного агента.

Ключевые слова: вирусный гепатит В (ГВ); occultный вирусный гепатит В (ОкГВ); вирус гепатита В (ВГВ); серологические маркеры; молекулярно-биологические маркеры; лабораторная диагностика; инфекционная безопасность крови; доноры крови; Гвинейская Республика

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Introduction

Parenteral viral hepatitis remains a significant threat to transfusion safety. Viral hepatitis B (HB) is still the most frequent transfusion-transmitted viral infection [1]. Although the amount of transfused plasma or blood components cannot be overlooked, the crucial role in infection belongs to the total concentration of viral particles received by the recipient from the donor, as the minimum infectious dose of the HB virus (HBV, *Hepadnaviridae*; *Orthohepadnavirus*: *Hepatitis B virus*) is ~16 copies (3 IU/ml) of HBV DNA [2]. Therefore, transfusion of blood components remains one of the leading artificial routes of HBV transmission. Over the last decades, the risk of such transmission of HBV has been steadily reduced by the successive implementation of measures aimed at safety of blood component therapy in most of the countries. The measures contributing to a lower risk of infection include recruitment of volunteer donors, medical evaluation, laboratory screening tests based on decreed indicators, and selection of donors based on risk-behavior evaluation. Quality-assured screening of donated blood samples is one of the ways to reduce the risk of HBV transmission during donor blood transfusion. The presence of this infectious agent in serum can be detected using biochemical, histological, and virological characteristics such as the activity level of alanine aminotransferase (ALT), HBV induced antibodies (ABs), and/or viral antigens and DNA. However, most of the laboratories detect the presence of infection in donors only by one serological marker, the HBV surface antigen (HBsAg), which is clearly not sufficient. Firstly, the infection cannot be detected during the “seronegative window” period, which is ~59 days (on average, 45–50 days for most sensitive assays). Secondly, one of the phases in the natural course of chronic viral hepatitis B (CHB) is occult hepatitis B infection (OBI) characterized by an undetectable level of HBsAg (regardless of the presence of other serological markers and their levels), while HBV DNA is present in liver tissue, and by an extremely low or even undetectable viral load in blood [3]. True OBI can be classified as seronegative and seropositive. In the first case, this form of infection can develop immediately (primary infection) and gradually, losing serological markers during the clinical course of the disease. In seropositive OBI, the loss of HBsAg can either result from the resolution of acute HB or represent a successive stage of CHB progression [4]. The third reason for the negative HBsAg test can include mutations associated with conformational and hydrophobic changes within and outside the major hydrophilic region (MHR) of HBsAg, which is the main target for capture of antibodies in commercial assays (the so-called false OBI) [1].

Considering the above, in some countries, donors' screening for detection of HB serological markers has been extended to include detection of anti-HBc IgG ABs [5]. Unlike HBsAg, the latter can be present both in CHB patients and during recovery from acute infection, when HBsAg may not be detected. However, test systems

for detection of HB serological markers were initially intended for examination of patients with suspected diseases rather than for donor screening; they can generate false-positive results, as their sensitivity is frequently higher than their specificity, and false reactivity rates can range from 16 to 75% [1, 5]. In addition, anti-HBc IgG tests cannot detect infection during the “seronegative window” period, while the strategy of donor screening for anti-HBc IgG cannot be used in regions with high HBV prevalence, as it may lead to an unacceptably increased loss of potential blood donors. Nevertheless, tests for anti-HBc IgG can still be significant in screening algorithms due to the reduced residual risk of infection.

To detect infected blood donors, some countries practice mandatory tests for presence of HBV DNA [5]. As mentioned above, OBI is characterized by very low (frequently undetectable) viral loads; therefore, tests are associated with difficulties. The problem was resolved by providing recommendations for using both commercial kits and laboratory-developed oligonucleotide arrays for virus detection by the nested polymerase chain reaction (PCR) [3, 6].

In most cases, OBI is asymptomatic, demonstrating no clinical manifestations or morphological changes for several years [7]. The worldwide OBI prevalence varies across regions, and, generally, its rates tend to increase with the percentage of HBsAg-positive CHB patients. In countries of Africa, HBV is extremely common; therefore, the prevalence of the occult form of CHB infection is also potentially high. For example, the OBI prevalence among urban population in African countries can reach 30%, according to some data [8]. In the Republic of Guinea as well as in most of the countries of the continent, donor-screening for prevention of transfusion-transmitted HBV infection is still limited to isolated serological detection of HBsAg [9, 10]. As a result, OBI remains a potential threat to blood transfusion safety.

Detection of HBV DNA in blood donors can be challenging for low-income countries such as the Republic of Guinea due to expensive diagnostic tests, limited availability of materials and equipment, shortage of qualified personnel. Although through international efforts aimed to end the epidemic of Ebola virus disease, also known as Ebola hemorrhagic fever (EBHF) (*Filoviridae*: *Ebolavirus*: *Zaire ebolavirus*) in 2014–2016, the country received hospitals, equipped diagnostic laboratories, and trained personnel, molecular and genetic methods are still not used in blood donor screening for parenteral hepatitis and human immunodeficiency virus (HIV) (*Retroviridae*: *Orthoretrovirinae*; *Lentivirus*: *Human immunodeficiency virus*). Partially, it can be explained by the fact that most of the aforesaid resources are employed in detection of pathogens causing especially dangerous infections [11]. Nevertheless, HBV DNA detection is a reliable preventive tool against transmission of the virus from donors with HBsAg-negative HB, especially in highly endemic regions. This study was performed to buttress recommendations for blood safety improvement, considering high prevalence rates of HBV in the Republic of Guinea.

The aim of the study was to assess the prevalence of serological and molecular markers of the HBV infection among blood donors in the Republic of Guinea.

Material and methods

The study was performed using plasma samples collected in 2019 from 250 volunteer donors living in Conakry (the Republic of Guinea). The laboratory tests were performed at the Russian-Guinean Scientific and Research Center of Epidemiology and Infection Disease Prevention of the Institute of Applied Biological Research of Guinea (IRBAG) in the Kindia prefecture. This study was approved by the National Ethics Committee of the Ministry of Health of the Republic of Guinea (Approval No. 129/CNERS/16 of August 31, 2015). All the participants provided their written informed consent to participation in the study.

Patients were tested for serological markers of parenteral viral hepatitis using the enzyme immunoassay (EIA) for qualitative detection of HBsAg, anti-HBs IgG, and anti-HBc IgG Abs with commercial DS-EIA-HBsAg, DS-EIA-ANTI-HBsAg, DS-EIA-ANTI-HBc assays (Diagnostic Systems RPC, Russia) and Vektohep B-HBs-antigen, VektoHBsAg-antibodies, HepaBest anti-HBc-IgG kits (Vector-Best JSC, Russia) according to manufacturer instructions.

Genetic markers of the HBV infection were detected using real-time PCR with fluorescent hybridization probes and a commercial AmpliSens HBV-FL kit (Central Research Institute of Epidemiology, Russia) in accordance with manufacturer instructions as well as the technique developed at the Pasteur Institute of Epidemiology and Microbiology (St. Petersburg) for detection of HBV DNA in biological material at low viral loads, including HBsAg-negative HB or OBI cases [6].

To identify genotypes of the virus, the nested PCR with the sequencing was performed. We used overlapping pairs of primers flanking a 1475 bp long fragment, which included an HBs Pre-S1/Pre-S2/S region, which was 1169 bp long (region 2848–3182...1–835 nucleotide positions), according to the Mart-B47 isolate (HE974377.1) in the international GenBank database (HE974377.1) [12].

The statistical analysis of the data was performed using MS Excel professional plus 2013 (Microsoft) software, Prizm v5.0 (GraphPad Software Inc.). The margin of error was represented by the “exact” Clopper–Pearson interval. The results were shown, including the 95% confidence interval (CI). To measure the significance of the differences in quantitative variables received from pairwise comparison, we used (depending on the sample pattern) Fisher’s exact test or Yates’ chi-square test (χ^2_{Yates}). The probability value $p < 0.05$ was set as the significance threshold.

Results and discussion

The age of the examined group ranged from 18 to 72 years. The proportion of male blood donors was 2.5 times as high as that of the female donors: 71.6 and 28.4% (95% CI: 65.58–77.1), respectively. The first stage of the study

included a gender-age analysis of blood donors, whose samples were collected to identify and detect HB markers (Figure).

The total prevalence of serological markers in the examined samples was 83.2% (95% CI: 77.98–87.62), however, HBsAg was detected only in 16.4% (95% CI: 12.03–21.58) of the individuals. The prevalence data for HB markers in the examined group are shown in Table 1.

The high prevalence of HB serological markers in the examined group implies that most of the group participants were exposed to the virus; the prevalence is consistent with the data on the pathogen prevalence in Africa. For example, in Guinea-Bissau, the exposure to HBV was confirmed in 91.9% blood donors [13]. The rate of positive detection of HBsAg was 15% in first-time donors in the Republic of Guinea in the 1999–2000 studies [9]. The results of this study confirm that HB is still a serious concern for the region’s healthcare, and they are basically consistent with the data on marker prevalence among blood donors in West Africa [13, 14], showing the prevalence of 16.4%. The high prevalence of this antigen in the examined group can apparently be explained by the fact that first-time donors accounted for a significant proportion of the examined individuals (64.8%; 95% CI: 58.53–70.71), who frequently donate blood in countries of Africa to receive remuneration, being also interested in free tests for HIV, syphilis, and parenteral viral hepatitis. It has been found that the number of HBsAg-positive donations as compared to HBsAg-negative donations is significantly higher among first-time and/or replacement donors than among non-remunerated volunteer donors, who demonstrate significantly lower prevalence of viral markers [14]. Considering this, transfusion safety can be increased through attracting volunteer non-remunerated donors to blood donation on a routine basis, as regular donors.

The gender-based analysis of HBsAg prevalence in the group showed that this marker was detected more frequently among male participants (19.55%) than among female participants (8.45%), while the relative risk (RR) of infection and developing a HBsAg-positive form of CHB is significantly higher among males than among females (RR = 2.314; 95% CI: 1.018–5.251; $p = 0.0369$).

In the donor group, a total of 53.2% (95% CI: 46.81–59.51) cases of the anti-HBc IgG presence were detected, out of them 45.11% (24% of the total number) were characterized by the concurrent presence of anti-HBs IgG. This fact demonstrates that 24% of the donors were exposed to the virus, recovered, and retained detectable levels of neutralizing ABs after the natural infection. In the Republic of Ghana, which is highly endemic for HB, anti-HBs IgG ABs were detected in 24.5% of anti-HBc-reactive donors [15]. The detection of some donors with anti-HBs IgG, who informed about their absent vaccination against HB, is of great significance.

Special attention should be given to the cases characterized by isolated anti-HBc IgG, as there are several possible explanations of this serological profile: 1) late recovery from acute hepatitis B, when HBsAg is not de-

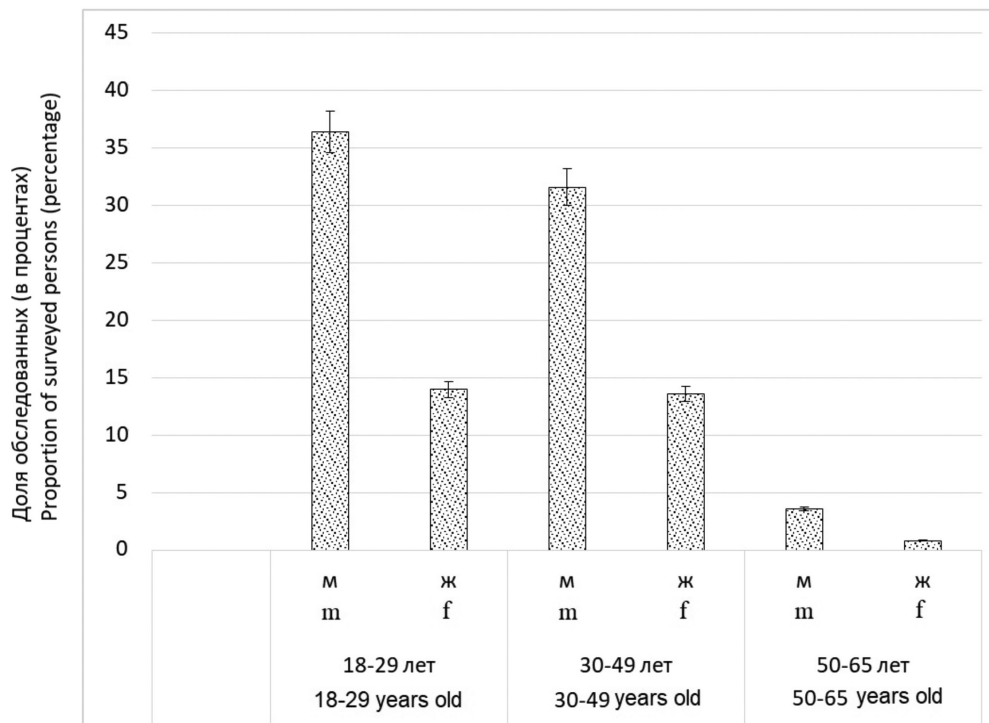


Fig. Gender-age structure of the examined blood donors.

Note. The following designations are given: m, male, f, female.

Рис. Гендерно-возрастная структура обследованных доноров крови.

Примечание. Даны следующие обозначения: м – мужчины, ж – женщины.

Table 1. Distribution of the hepatitis B serological markers (HBsAg, anti-HBc IgG, anti-HBs IgG) in the examined group and among seropositive individuals

Таблица 1. Распределение серологических маркеров гепатита В (HBsAg, анти-HBc IgG, анти-HBs IgG) в обследованной группе и среди серопозитивных лиц

Revealed serological markers in blood serum Выявленные серологические маркеры в сыворотке крови	Number of surveyed individuals (proportion in percentage) <i>n</i> = 250 Число обследованных лиц (доля в процентах) <i>n</i> = 250	Proportion of seropositive donors out of the number of individuals with hepatitis B serological markers (in percentage) <i>n</i> = 208 Доля серопозитивных доноров от числа лиц с серологическими маркерами гепатита В (в процентах) <i>n</i> = 208
HBsAg	41 (16.40)	19.71%
HBs IgG	34 (13.60)	16.35%
HBc IgG	73 (29.20)	35.10%
HBc IgG + HBs IgG	60 (24.00)	28.85%
Seronegative individuals Серонегативные лица	42 (16.80)	–

tected, though the low level of HBV DNA can be preserved for a short while; 2) CHB with present anti-HBc IgG at the HBsAg concentration below the limit of detection of the applicable diagnostic assays; 3) infection with the virus carrying mutations leading to low levels of replication and/or to changed HBsAg epitopes, which cannot be detected by test systems used in healthcare practices. Thus, blood donors with anti-HBc IgG being the only serological marker can have true and false OBI.

As a rule, the literature describing OBI prevalence in different populations provides data on specific ethnic

groups or groups of people at increased risk of infection, which for some reason attracted attention of researchers. In addition, the variability of this parameter depends on the employed screening diagnostic techniques and their sensitivity as well as on analyzed characteristics of the studied cohort. For example, in risk groups (HIV or HCV-infected (the hepatitis C virus, *Flaviviridae: Hepacivirus: Hepacivirus C*), injection drug users, etc.), the OBI prevalence is much higher. Despite the controversial data on its prevalence in populations of healthy individuals, the studies of specific immunity showed that the re-

action intensity of T helper 1 (Th1) cells contributing to development of a cell-mediated immune response mainly by activating macrophages was much higher in OBI patients than in those with HBsAg-positive CHB [16, 17]. The OBI reactivation can be accompanied by increased replication of the virus in patients taking immunosuppressive agents and in HIV-positive patients undergoing long-lasting antiretroviral therapy; this can lead to development of fulminant hepatitis and a fatal outcome. The risk of reactivation ranges from 21 to 67% [18]. The OBI can lead to faster progression of liver fibrosis, development of cirrhosis and hepatocellular carcinoma at low viral loads [3, 7].

In the Republic of Guinea, the OBI data studied for HIV-infected patients show that the prevalence rate reached 45.16% in the above group [19]. The prevalence of HBsAg-negative HB among pregnant women was 9.84% [20].

In this study, the presence of HBV DNA was recorded in 90.24% (14.8% of the total number of the examined) of HBsAg-positive individuals and in 18.66% (15.6%) of HBsAg-negative individuals, respectively. The prevalence of HBV DNA among blood donors was 30.4% (95% CI: 24.76–36.51), the OBI cases accounted for 15.6% (95% CI: 11.33–20.7). The OBI prevalence among blood donors in the Republic of Guinea is basically consistent with the data on countries of West Africa, where the detection rate for OBI was estimated at 4.6–17.0%, reaching 32% in some cohorts of HBsAg-negative donors [21]. The variation of data provided by different studies can be explained by differences in the employed methods and strategies for analyzed samplings [22]. In this study, the tests for viral DNA were performed individually in each sample; diagnostic methods using mini-pools, which may include only 2–3 samples, can affect the sensitivity of the tests due to the dilution effect caused by sample pooling. The low viral loads (<20 IU/ml) observed in most of the

detected cases confirm that donors with true OBI were infected with replication-competent viruses, where the replication activity and genes' expression were inhibited.

Note that the detection rate for infection markers among blood donors in rural areas can be higher than the HBV detection rate in Conakry. It can be explained, firstly, by the limited availability of medical services, including diagnostic resources, in rural communities compared to cities; secondly, by the significantly lower literacy rate among rural residents who may have difficulty understanding information about transmission paths of pathogens [8].

The age distribution of blood donors with OBI was similar to those observed among comparable populations in countries of the South African Region in 1995–2005. OBI was detected primarily among individuals aged 30–49 years (24.78%; 95% CI: 17.14–33.78), while among individuals under 30 years, its detection rate was significantly lower (8.73%; 95% CI: 4.44–15.08; $\chi^2 = 11.236$, $p = 0.0008$, df (degrees of freedom) = 1); among individuals over 50 years old, OBI was not detected in any of the examined. The fact that OBI occurs at a younger age can be explained by the specific natural course and transmission of infection in sub-Saharan Africa rather than by a certain genotype HBV typical of the region. This specific transmission implies the vertical transmission of the virus as the principal route of infection in small children.

The results of distribution of HBV DNA and serological markers of the infection among HBsAg-negative blood donors are presented in **Table 2**.

The obtained results lead to conclusion that the isolated screening of donor blood for HBsAg is not sufficient to eliminate the risk of transfusion-transmitted HBV-infection in the Republic of Guinea. Donation tests aimed at detection of viral DNA reduce this risk by detecting OBI and acute infections during the “serological window” period [22]. When there are no tests for anti-HBc IgG and

Table 2. Hepatitis B virus DNA and serological markers (anti-HBc IgG, anti-HBs IgG) distribution in the HBsAg-negative and in the general groups

Таблица 2. Распределение ДНК ВГВ и серологических маркеров вирусного гепатита В (анти-HBc IgG, анти-HBs IgG) среди HBsAg-негативных доноров крови и в общей группе

Revealed HBV DNA and serological markers in blood serum Выявленные серологические маркеры и ДНК ВГВ в плазме крови	Number of HBsAg-negative donors examined (proportion of individuals with hepatitis B serological markers, in percentage) <i>n</i> = 209 Число обследованных HBsAg-негативных доноров (доля от лиц с серологическими маркерами гепатита В, в процентах) <i>n</i> = 209	Proportion of the total number of surveyed individuals (in percentage) <i>n</i> = 250 Доля от общего числа обследованных лиц (в процентах) <i>n</i> = 250
HBs IgG + HBV DNA HBs IgG + ДНК ВГВ	4 (1.91)	1.60
HBc IgG + HBV DNA HBc IgG + ДНК ВГВ	21 (10.05)	8.40
HBc IgG + HBs IgG + HBV DNA HBc IgG + HBs IgG + ДНК ВГВ	7 (3.35)	2.80
Seronegative individuals with HBV DNA presence Серонегативные лица с наличием ДНК ВГВ	7 (3.35)	2.80

HBV DNA, any blood transfusion practices are associated with the risk that infection from donors with an occult form of the disease can be transmitted to recipients [9].

With the transfusion practices demonstrating a clear trend of successive implementation of safety measures, along with the adopted measures aimed at risk-free transfusion, the respective costs and economic effectiveness are increasingly taking the central place in discussions. In the meantime, in regions highly endemic for HB, any additional donation tests limited to detection of anti-HBc IgG, without concurrent detection of viral DNA, may result in a significant shortage of blood components and products. A solution can be offered by concurrent screening for anti-HBs IgG, as their presence at levels >100 IU/ml in anti-HBc IgG-positive donor media is usually seen as safe in transfusion terms.

In addition, we have identified virus genotypes and sub-genotypes for all samples. The phylogenetic analysis of 76 HBV isolates showed that the examined group was characterized by the prevalence of virus genotype E (85.53%) compared to sub-genotype A3 of genotype A (11.84%) and sub-genotype D2 of genotype D (2.63%). In 2006, in the Republic of Guinea, the distribution of virus genotypes among blood donors also demonstrated the prevalence of genotype E (95.1%) as compared to A3 (1%) and A/E recombinants (4–7%) [23].

Note that in the Republic of Guinea, the distribution of HBV genetic variants among blood donors differs from the distribution among HIV-infected individuals, who were characterized by prevalence of genotype E HBV (47.36%) compared to D1 – 21.05%, D2 – 15.78%, D3 – 10.52%, and A2 – 5.26%, though did not have sub-genotype A3 ($\chi^2 = 29.739$, $p < 0.0001$, $df = 2$) [19].

The detection of viral DNA in HBsAg-negative blood donors having anti-HBs IgG ABs ($n = 4$) as well as anti-HBs IgG and anti-HBc IgG ABs ($n = 7$) is of special interest, being typical of convalescents and indicating the developed protective immunity. In all these cases, the viral load exceeded 200 IU/ml, meaning that all the 11 patients were diagnosed with false OBI. This result can be caused by point mutations in the a-determinant located in the MHR region of the HBV S protein. These mutation-induced changes can cause changes in the immune epitope; they can affect antigenicity, immunogenicity, secretion, and/or expression of HBsAg, and can decrease or completely inhibit replication and/or secretion of the virion, having an adverse effect on HBsAg and thus making the detection of the above marker inefficient. For these isolates, we performed virus whole-genome sequencing, following the procedure described above [24]. The obtained nucleotide sequences were deposited to the international GenBank database under numbers MZ962189–MZ962199. All samples belong to genotype E; in each case, we detected mutations associated with HBsAg-negative CHB (Y100C, M103I) and/or escape mutations located in the MHR region of the S gene, which contributed to virus escape from detection when screened for HBsAg (L115I/E, T127P, Q129H/R, M133I/A/F, C137Y, K141E, D144E, G145A/R, C147T, R149A/D). The above mutations can explain the na-

ture of the OBI cases observed in our study. The same mutation-induced changes were observed when false OBI was identified in HBsAg-negative blood donors in Burkina Faso [21]. The presence of HBV genetic variants with escape mutations is of major clinical significance, as the latter can contribute to HB reactivation even in patients with anti-HBs IgG.

The further plans include the analysis as well as molecular and genetic characterization of all HBV variants detected in the group of blood donors.

Conclusion

Thus, the blood donors in Conakry demonstrated high prevalence of viral HB, including its occult form. The analysis of the virus genome structure, which was performed during the donor blood screening, proves that tests should be aimed not only at HBsAg, but also at anti-HBc IgG and HBV DNA to reduce the risk of virus transmission to recipients from donors with OBI. Countries, which are highly endemic for HB, should assess effectiveness and economic feasibility of high-tech screening of blood donors, using the information about resources of the national healthcare and epidemiological data. All the above leads to conclusion that further studies are required for more accurate assessment of HBV prevalence among first-time and regular blood donors, for identification of genotypes and clinically significant mutations of virus variants circulating in the region.

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