THE PREVALENCE OF HEPATITIS B AND C VIRUS MARKERS AMONG APPARENTLY HEALTHY RESIDENTS OF THE SOCIALIST REPUBLIC OF VIETNAM (SOUTHERN VIETNAM).

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РЕЗЮМЕ

Целью нашего исследования было оценить распространенность серологических и молекулярно-биологических маркеров вирусных гепатитов В и С среди условно здоровых жителей Южного Вьетнама. Материал исследования - 397 образцов плазмы крови условно здоровых жителей Южного Вьетнама. Исследование ИФА на наличие маркеров ВГВ и ВГС включало определение HBsAg, anti-HBs IgG, anti-HBcore IgG и качественного определения анти-HCV. Для обнаружения ДНК ВГВ и РНК ВГС нуклеиновые кислоты выделяли из плазмы крови, и проводили тест на присутствие вирусов с помощью ПЦР в реальном времени с гибридизационно-флуоресцентной детекцией. Амплификацию и последующее секвенирование ВГВ и ВГС проводили с использованием вложенной ПЦР с парными перекрывающимися праймерами, совместно фланирующими целевые регионы. Анализ общей распространенности серологических маркеров показал, что среди условно здоровых лиц HBsAg и антитела к ВГС были обнаружены у 12,3% (95% ДИ: 9,27-15,99%) и 3,27% (95% ДИ: 1,76-5,53) лиц, соответственно. Распространенность HBsAg у мужчин (19,1%) значительно превышала таковую у женщин (5,9%), \( \chi^2 = 14,688 \) с \( p = 0,0001 \), \( df = 1 \), рассчитанное отношение шансов \( OR = 3,751 \) (95% ДИ: 1,892-7,439). Среди условно здоровых пациентов с учетом HBsAg-положительных и отрицательных проб ДНК ВГВ была выявлена у 26,95% (95% ДИ: 22,65-31,6%). Филогенетический анализ ВГВ показал, что распространенность подтипа B4 составляет 64,49%, также были выявлены подтипы C1 - 14,95%, B2 - 9,35%, C2 - 6,54%, C3 - 0,93% и C5 (3,74%). РНК ВГС была обнаружена в 7 образцах, что составило 1,76% (95% ДИ: 0,71-3,6%). Филогенетический анализ показал, что все изоляты ВГС относятся к генотипу 6, подтипу 6а (100%).

Ключевые слова: ВГВ, ВГС, маркеры вирусных гепатитов, молекулярная эпидемиология, генотипы, условно-здоровые жители, Южный Вьетнам.
ABSTRACT

The aim of this study was to assess the prevalence of serological and molecular biological markers of viral hepatitis B and C among apparently healthy residents of the Southern Vietnam. The study material was represented by 397 blood serum samples collected from apparently healthy residents of the Southern Vietnam. The ELISA examination for presence of HBV and HCV markers involved HBsAg, anti-HBs IgG, anti-HBcore IgG, and anti-HCV qualitative determination. For HBV DNA and HCV RNA detection, nucleic acids were extracted from serum blood, and a test for virus detection were carried out by real-time PCR with hybridization fluorescence detection. Amplification and subsequent sequencing of HBV and HCV were performed using nested PCR with paired overlapping primers jointly flanking the target regions. The analysis of the overall prevalence of serological markers showed that among the apparently healthy individuals anti-HBsAg and anti-HCV antibodies were detected in 12.3% (95% CI: 9.27%-15.99%) and 3.27% (95% CI: 1.76%-5.53%) of individuals, respectively. The prevalence of HBsAg in men (19.1%) significantly exceeded that of found in women (5.9%), \( \chi^2 = 14.688 \) with \( p = 0.0001, \) df = 1, calculated odds ratio OR = 3.751 (95% CI: 1.892-7.439).

Among apparently healthy patients, taking into account HBsAg-positive and negative samples, HBV DNA was detected in 26.95% (95% CI: 22.65%-31.6%). HBV phylogenetic analysis showed that subtype B4 prevalence comprised 64.49%, subtypes C1 – 14.95%, B2 - 9.35%, C2 – 6.54%, C3 – 0.93%, and C5 (3.74%) were also identified. HCV RNA was detected in 7 samples, which accounted for 1.76% (95% CI: 0.71%-3.6%). Phylogenetic analysis showed that all HCV isolates belong to genotype 6, subtype 6a (100%).

Keywords: HBV, HCV, viral hepatitis markers, molecular epidemiology, subtype, apparently healthy residents, Southern Vietnam.
Introduction

Hepatotropic viruses, which cause chronic liver diseases, remain one of the most serious public health concerns in the world. Viral hepatitis is the seventh leading cause of death worldwide, and approximately 47% and 48% of these deaths are associated with the hepatitis B virus (HBV) and hepatitis C virus (HCV), respectively. According to preliminary calculations, the cumulative deaths from viral hepatitis in the period from 2015 to 2030 could be approximately 20 million [35].

The epidemiology of viral hepatitis is a dynamic phenomenon, subject to change due to socio-economic development, the development of socio-cultural practices, community activities carried out within the framework of national programs, and the development of hepatitis viruses understanding. Currently, in the understanding of viral hepatitis, great strides have been made, ideas about chronic and acute forms of diseases caused by hepatotropic viruses have changed. The methods have appeared that help identifies viruses in the early stages or with latent forms of the disease course. Antiviral drugs and effective vaccines have been developed against some viruses.

HBV and HCV are found all over the world, and their prevalence differs depending on the geographic region. The worst affected regions with high rates of chronic HBV and HCV infections are in Africa, especially sub-Saharan Africa, Central Asia, and East Asia. Depending on the country, infections may be concentrated in certain population groups (for example, injecting drug user (IDU)).

HBV and HCV belong to parenteral-transmitted infections, which means that virus is transmitted via blood and/or other body fluids upon the condition of skin or mucosal damage. Natural infection routes include sexual transmission (direct sexual contacts), vertical (mother-to-child transmission during or after birth, as well as germinal infection), domestic contacts (direct and indirect including use of common hygiene items with an infected person, etc.). Artificial routes include IDU infection when using infected materials, via medical procedures with the use of
HBV- or HCV-contaminated tools, blood and blood products transfusion, etc. The transmission of HBV can be blocked by vaccination, while it is not available for HCV. The progression of liver diseases associated with parenteral viral hepatitis can be prevented by long-term suppression of viral activity with effective drugs [16].

Both pathogens can lead to both acute and chronic liver disease, and in either case, during the acute stage of infection, most people do not experience any symptoms [0]. According to global statistics, around 15–45% of HCV infected people can spontaneously clear the virus within 6 months after the infection, without any treatment. The other 55–85% of people develop CHC [15]. CHB is very common in infants infected from their mothers or before the age of 5 year when the likelihood of progressing from acute hepatitis B (AHB) to chronic hepatitis is more than 90%. Infection developing at an adult age can lead to chronic hepatitis in less than 5% of cases [37].

The clinical course of infection depends on several factors, including the age at the time of infection, gender, ethnicity, host genetic factors, immune status. Viral genotype, subtype and genomic variability of the virus are also significant factors [31, 36]. HBV and HCV are genetically heterogeneous. HBV is subdivided into 10 genotypes (A-J) and more than 40 sub-types differing in nucleotide sequence composition [25]. HCV is classified into seven genotypes 1–7, genotypes 1–4 and 6 are subdivided into a variable number of closely related subtypes (more than 100 subtypes have now been described) [28].

HBV and HCV genotype and subtype determination are crucial for a better understanding of the disease epidemiological and virological particularities, agent characteristics; besides, this provides additional information for decision taking on antiviral therapy strategies. The genotypes and subtypes of viruses differ significantly concerning the natural course of infection, pathogenesis, modes of transmission, disease progression, treatment regimens, responses to antiviral therapy, and clinical outcome [5, 30].
It should be noted that the methods of HBV and HCV detection, as well as the diagnostics of the associated liver diseases in middle and low-income countries, differ fundamentally from those that are used in countries with access to high-cost technologies requiring special-purpose equipment and skilled personnel. Most of the related tests are limited to the detection of hepatitis B surface antigen (HBsAg) and antibodies to the hepatitis C virus (anti-HCV IgG), while molecular and genetic methods providing a more accurate assessment of virus prevalence are available only in core laboratories in big cities [22]. Data on the prevalence of HBV and HCV markers in a population are bounded, as tests are frequently limited to certain groups of population–risk groups (HIV-infected people, prisoners, IDU, etc.) and groups where the prevalence of infection has a substantial impact on the health of the population (blood donors, pregnant women).

The first global health sector strategy on viral hepatitis addresses with a particular focus on hepatitis B and C, owing to the relative public health burden they represent [35]. One of the tasks required to follow this strategy is to estimate the prevalence of HBV and HCV among conventionally healthy people.

Vietnam is currently one of the countries with the highest mortality rates from liver cancer associated mainly with HBV and HCV infections [9]. Vietnam is amongst the 20 countries with the highest burden of HCV in the world. In Vietnam, the prevalence of HCV infection varies depending on the geographical location and the target population. The prevalence of anti-HCV Ig G antibodies among the population is 1.0–4.7%, but is substantially higher among at-risk groups [18]. The prevalence of chronic HBV infection, as measured by the hepatitis B surface antigen (HBsAg) prevalence, is 8–20% in the general population and 31–54% in the high-risk urban population in Vietnam [11]. Predictive and model studies have predicted approximately 8 million chronic HBV cases and 58,600 HBV-related liver cancers in Vietnam by 2025, and an estimated annual HBV-related mortality rate of 20,000 / year by 2025 [20].
Aim

The aim of this study was to assess the prevalence of serological and molecular biological markers of viral hepatitis B and C among conditionally healthy residents of South Vietnam.

Materials and Methods

The study material was represented by 397 blood serum samples collected from conditionally healthy residents of the Southern Vietnam. The examined persons denied HBV or HCV infection anamnesis. The local ethics committee approved the study. All the patients gave written informed consent to participate in the study.

The ELISA examination for HBV and HCV markers occurrence involved HBsAg, anti-HBs IgG, anti-HBcore IgG, and anti-HCV qualitative determination (test-systems by Vector-Best CJSC, Diagnostic Systems RPC) in compliance with the manufacturer’s manuals.

For primary HBV DNA and HCV RNA detection, nucleic acids were extracted from blood serum using AmpliPrime Ribo-Prep commercial kit (CRIE, Moscow). Virus presence test was executed by real-time polymerase chain reaction (PCR) with hybridization fluorescence detection using AmpliSens® HCV/HBV/HIV-FL commercial kit (CRIE, Moscow).

Further, for HBV DNA detection, PCR-based method was used developed by Saint-Petersburg Pasteur Institute under Federal Service for Epidemiological and Health Surveillance (Rospotrebnadzor), which allows to detect low HBV DNA concentrations in various clinical materials and use amplified products for sequence analysis, sensitivity is 5 ME/ml [4]. Therewith, HBV amplification was applied involving nested-PCR by Taormina Occult HBV Consensus recommendation [26]. At the first stage, asymmetric PCR with extended oligonucleotides was performed, and at the second stage, to increase the sensitivity, PCR was performed using the amplification product of the first reaction.
and one of the nested pairs overlapping primers jointly flanking the complete HBV genome (S, P, C, X genes) [3]. The nucleotide sequences of the 18 complete HBV genomes were deposited in the international GenBank database under the numbers MZ671234-MZ671251.

For HCV RNA analysis the next stage included a reverse transcription reaction with the REVERTA-L reagent kit for cDNA synthesis from an RNA template (CRIE, Moscow), and amplification with specific primers was used to obtain sequences of viral region NS5B.

The amplification products were purified and analyzed for the fragment size and concentration. Sequencing reactions were performed according to the instructions for the ABI PRISM BigDye Terminator v3.1 reagent kit (Applied Biosystems, USA), in triplicate, on forward and reverse primers. The ABI Prism 3500 genetic analyzer (Applied Biosystems, USA) was used to identify nucleotide sequences. The primary analysis of the obtained fragment was performed according to BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST) in comparison with nucleotide sequences given in GenBank international database. The obtained sequences were aligned in MEGAv.7.0 with use of ClustalW algorithm [17].

For phylogenetic trees creation and subsequent phylogenetic analysis, distances between sequences were considered by neighbour-joining allowing to optimize the tree in accordance with the balanced minimum evolution criterion, a bootstrap analysis for 1000 replicas was performed to assess the created trees reliability.

Statistical data processing was carried out using the MS Excel and Prizm 5.0 (GraphPad Software Inc.) software package. The “exact” Clopper-Pearson interval was used to estimate statistical uncertainty. Results are represented as a median (Me) indicating 95% confidence interval (95% CI). Fisher exact test or Yates-corrected Chi-Squared test was used to evaluate statistical significance of numeric
The age of the examined individuals ranged from 18 to 65 years. Among the conditionally healthy individuals who applied not for medical reasons, the ratio of men and women did not differ, amounting to 49.12% and 50.88% (95% CI: 45.85%-55.9%), respectively. The sex and age structure of the surveyed group are shown in Figure 1.

The analysis of the overall prevalence of serological markers showed that among the conditionally healthy individuals HBsAg and anti-HCV antibodies were detected in 12.3% (95% CI: 9.27%-15.99%) and 3.27% (95% CI: 1.76%-5.53%) of individuals, respectively. The results of the distribution analysis for HBV and HCV markers in the examined group are shown in the Table 1.

When analyzing the occurrence of markers in the group of conventionally healthy patients, depending on gender and age, it was shown that among HBsAg-positive individuals, men prevailed (75.5%) compared with women (24.5%). Thus, in the group of conventionally healthy individuals, the prevalence of HBsAg in men (19.1%) significantly exceeded that in women (5.9%), χ² = 14.688 with p = 0.0001, df = 1, calculated odds ratio OR = 3.751 (95% CI: 1.892-7.439). We did not find any dependence of the anti-HCV Ig G distribution on sex and age.

In the examination of 397 clinical blood serum samples for HBV DNA and HCV RNA presence using AmpliSens® HCV/HBV/HIV-FL commercial kit, the HCV was detected in 7 samples, which accounted for 1.76% (95% CI: 0.71%-3.6%), and HBV detected in 42 of HBsAg-positive patients, which accounted for 10.58% (95% CI: 7.73%-14.03%).

With using a method that detects HBV DNA at low viral load, HBV was detected in all HBsAg-positive samples, as well as in 58 HBsAg-negative samples, which amounted to 14.61% (95% CI: 11.28%-18.47%). Thus, among conditionally

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healthy patients, taking into account HBsAg-positive and negative samples, DNA
HBV was detected in 26.95% (95% CI: 22.65%-31.6%).

Researches on HBV and HCV genovariants prevalence in different regions of
the world have been actively carried out from the moment of the genotypes
discovery until the present. All genotypes and serotypes have different geographic
distribution, which is changing very slowly with time reflecting the virus
propagation paths related to people’s migration, as well as possible different
geographical origins of different genotypes, which allows using them as
epidemiological markers [27]. Therewith, in most regions with rare exceptions, 1-2
prevailing genotypes and several minor ones are circulating including those
imported from other areas [28]. The tendency observed over the last years to
prevalence displacement of one or another HBV or HCV genotype in various
geographic areas due to international contact development, labor migration flows,
especially from the regions with high hepatotropic virus prevalence, makes the
clinicians and epidemiologists pay focused attention to hepatitises B and C
epidemiological situation not only in their region but also in neighboring ones [6].

For further HCV analysis, sequencing of the NS5B virus regions of 7 samples
(100%) from a group of apparently healthy patients was carried out. Phylogenetic
analysis showed that all isolates of the conventionally healthy group belong to
genotype 6, subtype 6a (100%). Phylogenetic relationships between the examined
HCV isolates from conventionally healthy patients, and reference sequences from
the GenBank international database are shown in Figure 2.

Phylogenetic analysis of HBV (n=107) showed that the prevalence of HBV
genotype B (73.84%) compared to genotype C (26.16%). With regard to the deep
typing results, subtype B4 prevalence is 64.49%, subtypes C1 – 14.95%, B2 -
9.35%, C2 – 6.54%, C3 – 0.93%, and C5 (3.74%) were also identified.
Phylogenetic relationships between the examined HBV isolates from
conventionally healthy patients, and reference sequences from the GenBank
international database are shown in Figure 3.
Discussion

Out of 397 plasma samples, 13 (3.27%) were with anti-HCV antibodies and 7 (1.76% of all group) of them were confirmed RNA HCV. Our results do not contradict other studies according. The prevalence of anti-HCV in Vietnam is estimated to be 6.1%, however, the prevalence is not uniform throughout the country and in different groups. So, the anti-HCV antibody rate in blood donors is 20.6% in Ho Chi Minh City in Southern Vietnam, 0.8% in Hanoi in Northern Vietnam, 9% in individuals without liver disease in Ho Chi Minh City, and 4% in Hanoi, 22.9–89.0% in HIV-infected individuals, 74.0–87.0% in IDU [18].

We did not reveal any dependence of the anti-HCV Ig G distribution on sex and age, although there is data in the literature on an increase in the prevalence of HCV antibodies in people over 50 in the south of Vietnam [9].

Our findings showed that only subtype HCV 6a is presented in Southern Vietnamese conditionally healthy residents and we did not find any genotype 1. Our results contradict studies according to which genotypes 1 and 6 are widespread in the southern region of Vietnam, but the prevalence of one or the other genotype differs in different sources. For example, some report the predominance of genotype 6 among blood donors and in the general population; others report the predominance of genotype 1, in particular 1b (58%), followed by genotype 6a (17%) among patients with liver disease [19]. The genotype prevalence among conditionally healthy persons has never been clearly determined although Vietnam is among those countries in which genotype 6 variants were first detected, it cannot be argued that only genotype 6a is common among conventionally healthy individuals. We assume that the identification only one of subtype in the surveyed group is associated with limited sample size. An indirect confirmation of this is the work of Le Ngoc C. et al: in their study, genotype 6 prevailed in patients with chronic HCV monoinfection (44.4%) compared with patients with HIV + HCV coinfection [18]. Besides
showed that more than half of the Vietnamese patients who lived in Ho Chi Minh City were infected with genotype 6 variants, and the reason why this HCV variants are circulating in this limited geographical region remains unknown [24]. Talking about other countries, subtype 6a circulate in South-East Asia including Thailand, Myanmar, Laos PDR and in the South-China territories of Hong-Kong and Macau as well as in China’s southernmost provinces of Yunnan and Guangxi [23].

The prevalence of HBsAg, anti-HBs Ig G, and anti-HBcore Ig G in the study groups was 12.34%, 38.53%, and 56.17%, respectively. Thus, over 78% (95% CI: 73.69%-82.06%) have been exposed to the virus, which is confirmed by anti-HBcore IgG and anti-HBs IgG antibody detection. A similar picture of the HBV serological markers occurrence in the population is presented in the works of other researchers. For example, among of 509 adults, comprised of 230 men (45.2%) and 279 women (54.8%), prevalence of HBsAg, anti-HBs Ig G and anti-HBcore Ig G were 15.3%, 60.3% and 71.7%, respectively [9]. In another study, when analyzing for serological markers 837 samples from patients aged 16-82 years prevalence of anti-HBcore and HBsAg was 68.2% and 19.0%, respectively [21]. In our study, the prevalence’s of HBsAg being higher in males than females.

The PCR technique is widely used for molecular diagnostics of HBV. The testing performed with the AmpliSens® HBV-FL commercial kit showed that the HBV DNA prevalence in the group (10.57%) is comparable with the HBsAg detection frequency (12.34%). Nevertheless, both techniques are not sufficiently sensitive, and it is proved by the detection of HBV DNA in another 58 seronegative patients (14.61% of total samples) when we used the technique developed at the Saint-Petersburg Pasteur Institute for detection of HBV DNA at low viral loads (refer to Table 2). Thus, the prevalence of HBV DNA in the examined group (26.95%) exceeds the previously published prevalence rates for the virus in the region. It can be assumed that this is due to the limitation of the methods used, which do not allow detecting the HBsAg-negative form of the course of CHB at a low viral load.
Confidence intervals overlapping in HBV DNA detection in HBV-positive patients indicates two independent phases of CHB natural progression – accompanied and non-accompanied by virus replication with active sub-genome RNA translation with HBV cccDNA persistence in hepatic cell cores, but with entire-genome RNA transcription suppression, which, however, does not evidence virus elimination.

It is known that chronic infection is characterized by persistent HBsAg presence during at least 6 months (with concomitant HBeAg presence or absence), and its level in blood serum constitute the main marker for the disease development risk assessment and CHB forecasting, as well as for HBV diagnostic in general. However, one of CHB natural progression forms is presented by occult hepatitis B infection (OBI), which is characterized by HBV DNA persistence in liver tissue and extremely low HBV DNA concentration with undetectable HBsAg level in peripheral blood [12]. OBI development is conditioned by suppression of sub-genomic HBV RNA endonuclear transcription from the covalently closed circular HBV DNA matrix, based on which viral genome and viral proteins are synthesized [28]. The suppression may be caused by a number of factors, which are not yet fully understood, including genetic traits of the virus itself and/or of its host, or by external interference. Therewith, in most cases, virus replication and gene expression may be suppressed to such extent that viral load in a patient’s peripheral blood is extremely low, down to the impossibility of HBV DNA detection by standard methods, but virus elimination does not happen under the replication suppression [26]. Despite the HBsAg absence in peripheral blood, most patients with OBI are seropositive on one or several serological markers – depending on the disease progression phase, anti-HBs IgG, HBeAg, anti-HBe IgG, anti-HBcore IgG; however, over 20% of patients are seronegative on all HBV markers [26].

Despite the considerable public healthcare problems associated with HBV in Vietnam, it should be noted that HBV detection methods and HBV-related liver
disease diagnostics in low or medium income countries significantly differ from those used in the countries, which have access to more advanced technologies. Most studies on this subject in Vietnam are limited to measurement of HBV surface antigen, while molecular genetic methods allowing more accurate assessing HBV prevalence and genotyping the virus are available only in large cities’ central laboratories [2]. Besides, there are limited data on HBV markers prevalence in population, since screening is performed predominantly in separate population groups – the high-risk groups (HIV-infected persons, IDU, etc.) and in the groups, where infection prevalence significantly influences public health (blood donors, pregnant women). Occult hepatitis B infection occurrence is varying around the world, however, it in general correlates with HBV manifest form occurrence [13]. In our study should be noted high OBI occurrence percentage, which is peculiar to the regions, where HBV is widely occurring. It should be noted, that HBV has the highest prevalence among low-income population groups, including rural population, while our study included mainly persons with relatively favorable social and economic position by this region standards. We suppose that examination in poorer people groups of this geographic region would indicate considerably higher rates of HBV molecular markers prevalence.

Our results of HBV genotyping indicate that HBV genotype B dominates in Southern Vietnam, followed by genotype C. This is consistent with earlier data published in Vietnam. However, while it is known that OBI genotypes and sub-types correlates with HBV genotypes distribution in a particular region [13], literature data on occult HBV epidemiologic situations in Vietnam are very few. With regard to the above, we deemed it necessary to analyze HBV genotypes distribution within the obtained sample collection in two groups – in the HBsAg-positive (n=49) and HBsAg-negative (n=58). When comparing the distribution of genotypes in both groups does not differ from the distribution in the total group, but significant differences from each other (χ2=12.39, p=0.0298, df=5). The
identified HBV genotypes and subtypes are in general peculiar to Vietnam; however, the shown proportion considerably differs from total data. For example, among patients with chronic hepatitis undergoing treatment, genotype B (71.43%) prevailed compared to C (27.55%), one isolate was recombinant (between B and C). Among the isolates of genotype B, 92.86% were subgenotype B4, 7.14% - B2, 92.6% of subgenotype C belongs to C1, 3.7% - subgenotype C2, and the remaining 3.7% - C3 [32]. Our results of HBV genotyping indicate that HBV genotype B occurrence frequency among HBsAg-positive individuals (85.72%) is much closer to the data of other researchers (71.43% among patients with CHB or 75.3% among randomly sampled individuals) [9, 32], than the corresponding frequency among OBI patients (63.79%). Interestingly, a lower representation of genotype B (67.8%) and high genotype C (27.9%), which is similar to our results for OBI patients, was shown among CHB patients in Northern Vietnam [33]. Our study identified that subtype B4 occurrence frequency was significantly lower (51.72%), and that of subtypes C1 (20.69%) and B2 (12.07%) among OBI was higher than the occurrence of those among HBsAg-positive individuals - 79.6%, 8.16%, and 6.12%, respectively. The distribution of HBV genotypes / subtypes in groups is shown in Figure 4.

While it was shown that in Vietnam, genotype C has a higher load than genotype B and is associated with more severe liver diseases [33], while among the HBsAg-negative samples we examined, genotype C isolates are presented with a significantly lower viral load. It can be assumed that the diagnostic methods commonly used in the region do not allow identifying these cases. Due to this, in the cases described in the literature, the prevalence of HBV genotype C is somewhat lower than was found in our study. In addition, in our study, a significant number of C5 subtype samples (3.74%) were identified among HBsAg-negative samples. Previously, cases of HBV C5 detection were reported, but in single quantities, when reported that HBV subtype B4 (82.6%) predominated in in
risk groups, and other genotypes detected included B2 (2.7%), C1 (14.6%) and C5 (0.5%) [10].

Nevertheless, when evaluating the HBV diversity pattern on the entire examined group material, a close genetic relationship of manifest CHB and OBI isolates became apparent, which also evidences OBI prevalence in the region. It should be noted that all HBV subtypes presented in the surveyed group are also common in neighboring countries. However, in Cambodia genotype C (80.49%) was abundantly found throughout the whole of Cambodia whilst genotype B (19.51%) was exclusively found in regions bordering Vietnam [14]. In Thailand, the majority of OBI samples were HBV genotype C (81.3%) with 6.3% of samples being genotype B, although genotype I was also detected [8]. In Laos, multiple genotypes and subtypes cocirculate and many recombinant viruses forms including subtypes B1, B2, B4, C1, C5, I1, and I2 [5]. On the other hand, the demonstrated HBV sub-types distribution and similarity between some of them and isolates from other Asian countries pieces of evidence high irregularity of their prevalence in Vietnam.

Despite their rarity, cases of mixed infection and recombination have been reported in Vietnam. In our study, we could neither detect viral genome recombination or cases of coinfection with different genotypes HBV. Our limited number of samples might account for the apparent absence of both recombination and coinfection.

It should be noted that the results obtained by us for conventionally healthy patients cannot be considered as population data, since we estimated the prevalence of viral hepatitis markers in the population visiting hospitals, while some socioeconomic or occupational groups may have more reasons to visit hospitals than others. Thus, these samples may represent individuals with a higher risk of infection in general, and therefore may not be representative of the population.
Conclusion

Our work has shown a high incidence of parenteral viral hepatitis markers among conditionally healthy residents of southern Vietnam. Particular attention should be paid to the high prevalence of HBsAg-negative HBV in the region, which indicates the insufficiency of the currently used methods both for detecting the virus and for preventing (prophylaxis) infection.
Figure 1. Age-and sex-related sample distribution.

Рисунок 1. Распределение образцов по возрастным группам и полу.

Figure 2. Phylogenetic analysis of NS5B regions HCV nucleotide sequences isolated from apparently healthy residents of the Socialist Republic of Vietnam (Southern Vietnam) in comparison with the reference sequences retrieved from the international GenBank database. Reference sequences are designated with GenBank codes indicating the genotype/subgenotype. Bootstrap values higher than 70% based on 1000 replications are shown at branching points.

Рисунок 2. Филогенетический анализ нуклеотидных последовательностей региона NS5B ВГС, полученных от условно здоровых жителей Социалистической Республики Вьетнам (Южный Вьетнам) в сравнении с представленными в международной базе данных GenBank референсными последовательностями. Референсные последовательности обозначены кодами GenBank с указанием генотипа и региона происхождения образца. Данны значения bootstrap ≥70.
Figure 3. Phylogenetic analysis of HBV genome nucleotide sequences isolated from apparently healthy residents of the Socialist Republic of Vietnam (Southern Vietnam) in comparison with the reference sequences retrieved from the international GenBank database. Reference sequences are designated with GenBank codes indicating the genotype/subgenotype. Woolly monkey HVB (AY226578) was used as an outgroup. Bootstrap values higher than 70% based on 1000 replications are shown at branching points.
Рисунок 3. Филогенетический анализ нуклеотидных последовательностей геномов ВГВ, полученных от условно здоровых жителей Социалистической Республики Вьетнам (Южный Вьетнам) в сравнении с представленными в международной базе данных GenBank референсными последовательностями. Референсные последовательности обозначены кодами GenBank с указанием генотипа и региона происхождения образца. Как внешняя группа использована нуклеотидная последовательность ВГВ шерстистой обезьяны AY226578. Даны значения bootstrap ≥70.
HBV AND HCV MARKERS IN SOUTH VIETNAMESE RESIDENTS

5 ocHBV + 5 HBV
6 ocHBV + 4 HBV
3 ocHBV + 4 HBV
4 ocHBV + 5 HBV
1 ocHBV + 4 HBV
9 ocHBV + 6 HBV
2 ocHBV + 1 HBV

VitocHBV/chr11
VitocHBV/chr296
JQ688405 B2 China
G0924603 B2 Malaysia
VitocHBV/chr252
VitocHBV/chr192
VitocHBV/chr388
VitocHBV/chr153
VitocHBV/chr6

G0924617 B3 Malaysia
G0924640 B5 Malaysia
AB602818 B1 Japan
JN792893 B6 Canada

AB048705 C4 Australia
X75665 C3 Okeania
VitocHBV/chr85
DQ089801 C3 Hong-Kong
HM011493 C4 Malaysia
EU670263 C6 Philippines
EU410080 C5 Philippines
VitocHBV/chr375
JN827414 C5 Thailand
VitocHBV/chr72
1 ocHBV + 2 HBV
VitocHBV/chr228
VitocHBV/chr211
3 ocHBV

VitocHBV/chr8
LC535915 C1 Cambodia
MF674388 C1 Vietnam
VitocHBV/chr37
VitocHBV/chr242
VitocHBV/chr166
1 ocHBV + 1 HBV
1 ocHBV + 2 HBV

KP017269 C2 Japan
2 ocHBV

Genotype A
AB091255 E Cote dIvoire
G0161755 E Guinea
X75664 E Senegal
Genotype D
AF405706 G Germany
AB055615 G USA

X69798 F Mexico
AF223963 F Argentina
AY090460 H USA
AB064315 H USA
AY226578 Woolly-monkey-WMHBV-2
Figure 4. Distribution of HBV subtypes among HBsAg-positive and negative samples.

Рисунок 4. Распределение генотипов ВГВ среди HBsAg-позитивных и негативных образцов.
TABLES

Table 1. Distribution of HBV and HCV serological markers (HBsAg, anti-HBcore IgG, anti-HBs IgG, anti-HCV) in the examined group.

Таблица 1. Распределение серологических маркеров ВГВ и ВГС (HBsAg, anti-HBcore IgG, anti-HBs IgG, anti-HCV) в исследуемой группе.

<table>
<thead>
<tr>
<th>Revealed serological markers in blood serum</th>
<th>The surveyed group (n = 397), proportion of the total surveyed number</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg+</td>
<td>49 (12.3%)</td>
</tr>
<tr>
<td>HBs IgG+</td>
<td>153 (38.53%)</td>
</tr>
<tr>
<td>HBcore IgG+</td>
<td>223 (56.17%)</td>
</tr>
<tr>
<td>Anti-HCV+</td>
<td>13 (3.27%)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>74 (18.63%)</td>
</tr>
</tbody>
</table>

Table 2. Results of HBV DNA low concentration detection in blood serum samples.

Таблица 2. Результаты выявления ДНК ВГВ низкой концентрации в образцах сыворотки крови.

<table>
<thead>
<tr>
<th>HBV DNA</th>
<th>The samples number n = 397 (100%)</th>
<th>HBsAg in blood serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HBsAg (+) n=49 (12.34% от n=397 (CI 95%: 9.27%-15.99%))</td>
</tr>
<tr>
<td>HBV DNA+</td>
<td>107 (26.95%) (CI 95%: 22.65%-31.5%)</td>
<td>49 (45.79%) (CI 95%: 36.12%-55.7%)</td>
</tr>
</tbody>
</table>
МАРКЕРЫ ВГВ И ВГС У ЖИТЕЛЕЙ ЮЖНОГО ВЬЕТНАМА.
HBV AND HCV MARKERS IN SOUTH VIETNAMESE RESIDENTS
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ТАКТУЛЬНЫЙ ЛИСТ

РАСПРОСТРАНЕННОСТЬ МАРКЕРОВ ВИРУСОВ ГЕПАТИТА B И C СРЕДИ УСЛОВНО-ЗДОРОВЫХ ЖИТЕЛЕЙ ЮЖНОГО РЕГИОНА СОЦИАЛИСТИЧЕСКОЙ РЕСПУБЛИКИ ВЬЕТНАМ

THE PREVALENCE OF HEPATITIS B AND C VIRUS’S MARKERS AMONG CONDITIONALLY HEALTHY RESIDENTS OF THE SOCIALIST REPUBLIC OF VIETNAM (SOUTHERN VIETNAM)

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Сокращенное название: Маркеры ВГВ и ВГС у жителей Южного Вьетнама.
HBV and HCV markers in South Vietnamese residents.

Ключевые слова: ВГВ, ВГС, маркеры вирусных гепатитов, молекулярная эпидемиология, генотипы, условно-здоровые жители, Южный Вьетнам.

Keywords: HBV, HCV, markers of viral hepatitis, molecular epidemiology, subtype, conditionally healthy residents, Southern Vietnam.

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