COMPARATIVE ANALYSIS OF THE CHRONIC VIRAL HEPATITIS B MARKERS PREVALENCE IN HIV-INFECTED AND CONDITIONALLY HEALTHY RESIDENTS OF HO CHI MINH CITY

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1. 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% of these deaths are associated with the hepatitis B virus (HBV) [1]. HBV is found all over the world, and its prevalence differs depending on the geographic regions. The worst affected regions with high rates of chronic HBV infections (CHB) are in Africa, especially sub-Saharan Africa, Central Asia, and East Asia [2]. HBV is to parenteral-transmitted infection, which means that the 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 injecting drug users (IDU) infection when using infected materials, via medical procedures with the use of HBV-contaminated tools, blood and blood products transfusion, etc. [3].

It should be noted that the methods of HBV detection 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). It is known that chronic infection is characterised by persistent HBsAg presence during at least 6 months (with concomitant hepatitis B e antigen (HBeAg) presence or absence), and its level in blood is 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 [4].

According to various sources, the incidence of occult HBV in different regions of the world ranges from 1% to 89%, such conflicting results are associated with the surveyed groups, different levels of HBV prevalence in a particular geographical region, and differences in the methods used in studies that have greater or lesser sensitivity [5].

One of the tasks required is to estimate the prevalence of CHB among conventionally healthy people and in risk groups (for example, among HIV-infected individuals).

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 [6]. Vietnam is currently one of the countries with the highest mortality rates from liver cancer associated mainly with HBV infections [7].

The aim of this study was the comparative analysis prevalence of serological and molecular biological markers of viral hepatitis B among HIV-infected and conditionally healthy residents of Ho Chi Minh City.

2. MATERIALS AND METHODS

The material was represented by biosamples without patient identification data (plasma and whole blood remnants) obtained during routine studies. The study was approved by the Local Ethics Committee of the Saint Petersburg Pasteur Institute. The study material was represented by 316 and 397 blood samples collected from HIV-infected and conditionally healthy (outpatients seeking general health checkup) residents of Ho Chi Minh City, respectively.

The ELISA examination for HBV markers occurrence involved HBsAg, anti-HBs IgG, anti-HBcore IgG qualitative determination (test-systems by Vector-Best CJSC, Diagnostic Systems RPC) in compliance with the manufacturer's manuals. For primary HBV DNA detection, nucleic acids were extracted from blood serum using "Ribo-Prep" commercial kit (CRIE, Moscow). Virus presence test was executed by real-time polymerase chain reaction (PCR) with hybridization fluorescence detection using AmpliSens® HBV-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 [8, 9]. Therewith, HBV amplification was applied involving nested-PCR by Taormina Occult HBV Consensus recommendation [4]. 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) [8, 10]. 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.qov/BLAST) in comparison with nucleotide sequences given in GenBank international database. The obtained sequences were aligned in MEGAv.10.0 with use of ClustalW algorithm; 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 [11].

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 data obtained during the paired comparison depending on sample characteristics. The significance threshold was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

The analysis of the overall prevalence of serological markers showed that among the conditionally healthy individuals and HIV-infected persons HBsAg were detected in 12.3% (95% CI: 9.27%-15.99%) and 9.49% (95% CI: 6.5%-13.28%) of individuals, respectively. 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%), $\chi 2 = 14.688$ with $p = 0.0001$, degree of freedom (df) = 1, OR = 3.751 (95% CI: 1.892-7.439). In the HIV-infected group the prevalence of HBsAg among men (12.28%) showed an excess in women (5.52%), OR=2.398, p=0.04 (95%, CI: 1.028-5.592). The prevalence of anti-HBs Ig G, and anti-HBcore Ig G in the study groups were 38.53% and 56.17% for conditionally healthy persons, and 10.44% and 42.08% for HIV-infected individuals, respectively. Thus, over 78% conditionally healthy persons and over 35% HIV-infected individuals had contact with the virus, which is confirmed by anti-HBcore IgG and/or anti-HBs IgG antibody detection. However, the lower prevalence of CHB serological markers in HIV-infected individuals was not associated with actual cases of HBV infection, but with suppression of viral replication, HBsAg synthesis, and reduced immune status of patients due to co-infection with HIV. A similar picture of the HBV serological markers occurrence in the population was present in the works of other researchers. For example, among of 509 adults, prevalence of HBsAg, anti-HBs Ig G and anti-HBcore Ig G were 15.3%, 60.3% and 71.7%, respectively [7].

In the examination of 397 clinical blood serum samples from conditionally healthy persons for HBV DNA presence, taking into account HBsAg-positive and negative samples, CHB was detected in 26.95% (95% CI: 22.65%-31.6%). In the group of HIV-infected individuals (n=316), taking into account HBsAg-positive and negative DNA samples, HBV was detected in 32.59% (95% CI: 27.45%-38.07%) cases. The prevalence of HBV DNA in the examined groups (26.95% and 32.58%) exceeded the previously published prevalence rates for the virus in the population in region. For example, the prevalence of chronic HBV infection in general population is 8.8-12.3% [12]. Pooled HBV prevalence was low in blood donors (1.86%) but high in antenatal populations (10.8%), adults in the general population (10.5%), and in inpatients presenting with non-hepatic illness (16.2%) [13]. 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. Despite the considerable public healthcare problems associated with HBV in Vietnam, it should be noted that 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. Occult hepatitis B infection occurrence is varying around the world, however, it in general correlates with HBV manifest form occurrence [14]. 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 favourable 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.

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. Therewith, in most regions with rare exceptions, 1-2 prevailing genotypes and several minor ones are circulating including those imported from other areas. The tendency observed over the last years to prevalence displacement of one or another HBV 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 hepatitis B epidemiological situation not only in their region but also in neighboring ones [15].

Phylogenetic analysis of HBV from conditionally healthy persons showed the prevalence of HBV genotype B (73.84%) compared to genotype C (26.16%). Phylogenetic analysis of HBV from HIV-infected individuals showed the prevalence of HBV genotype B (69.59%) compared to genotype C (30.41%). With regard to the deep typing HBV results, from conditionally healthy persons subtype B4 prevalence is 64.49%, subtypes C1 - 14.95%, B2 - 9.35%, C2 - 6.54%, C3 - 0.93%, and C5 (3.74%); from HIV-infected individuals subtype B4 (60.89%) prevailed over subtypes C1 (21.73%), B2 (8.7%), C2 (4.34%), and C5 (4.34%). Our results of HBV genotyping indicate that HBV genotype B dominates in residents of Ho Chi Minh City, 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 [8], 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 and HBsAg-negative. 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 ($\chi2=12.39$, $p=0.0298$, $df=5$). The identified HBV genotypes and subtypes are in general peculiar to Vietnam; however, the shown proportion considerably differs from total data. Our results of HBV genotyping indicate that HBV genotype B occurrence frequency among HBsAg-positive individuals is much higher, than the corresponding frequency among OBI patients. Interestingly, a lower representation of genotype B and high genotype C, which is similar to our results for OBI patients, was shown among CHB patients in Northern Vietnam [16]. While it was shown that in Vietnam, genotype C has a higher load than genotype B and is associated with more severe liver diseases [16], 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% conditionally healthy persons, 4.34% HIV-infected individuals) were identified among HBsAg-negative samples. Previously, cases of HBV C5 detection were reported, but in single quantities. 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. 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.

4. CONCLUSION

Our work has shown a high incidence of viral hepatitis B among conditionally healthy and HIV-infected residents of Ho Chi Minh City. 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.

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SUMMARY

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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. Vietnam is currently one of the countries with the highest mortality rates from liver cancer associated mainly with HBV infections. 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. One of the tasks required is to estimate the prevalence of CHB and OBI among conventionally healthy people and in risk groups (for example, among HIV-infected individuals). The analysis of the overall prevalence of serological markers showed that among the conditionally healthy individuals and HIV-infected persons HBsAg were detected in 12.3% and 9.49% of individuals, respectively. The prevalence of HBV DNA in the examined groups (26.95% and 32.58%) exceeds the previously published prevalence rates for the virus in the population in region. Our results of HBV genotyping indicate that HBV genotype B dominates in residents of Ho Chi Minh City, followed by genotype C. However, HBV genotype B occurrence frequency among HBsAg-positive individuals is much higher, than the corresponding frequency among OBI patients. In addition, in our study, a significant number of C5 subtype samples (3.74% conditionally healthy persons, 4.34% HIV-infected individuals) were identified among HBsAg-negative samples. The results obtained in this work demonstrate a high prevalence of CHB/OBI in the region and indicate the need for further large-scale studies of HBV in order to improve strategies for disease control and prevention in the Vietnam.

Keywords: *HBV, markers of viral hepatitis, molecular epidemiology, subtype, conditionally healthy residents, HIV-infected individuals, coinfection, Ho Chi Minh City.*

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