UNDERSTANDING OCCULT HEPATITIS B IN BLOOD DONORS

Albana Gjyzari[1], Irini Kasolli[1,2], Ariana Strakosha[3], Admir Nake[1], Ergys Ramosaçaj[1], Gentian Stroni[1], Floreta Kurti[1], Ariana Stojku[3], Gentjan Asllanaj[1]
[1]Faculty of Medical Technical Sciences, University of Medicine, Tirana, Albania
[2]National Blood Transfusion Center, Tirana, Albania
Nephrologist, Specialties Health Center No. 2, Tirana, Albania
[3]Nephrology Service, University Hospital Center Mother Theresa
Tirana, Albania
Correspondence E-mail: albana.gjyzari@umed.edu.al

Abstract
Understanding and diagnosing occult hepatitis B infection (OBI) still presents an obstacle in managing HBV infection. OBI poses a problem in screening blood donors and managing transfusion procedures. The key identifier of OBI is the presence of HBV-DNA that can only be detected using DNA assays. Nucleic acid testing (NAT) or Real Time Polymerase Chain Reaction (PCR) quantifies HBV-DNA levels in units per ml [IU/ml] or copies, per ml to assess the amount. Even if the donor is HBsAg negative, they can transmit OBI to the recipient by their viremic status (detection of HBV DNA). As a result, it is well worth developing strict screening criteria and reliable tests to identify suspected OBI in blood donors and avoid the chance of HBV transmission through unscreened blood transfusion. In all, we need to consider how prevalent OBI may be among blood donors. Moreover, continuous monitoring is required because the prevalence rates differ across studies conducted in different populations. Furthermore, many infected individuals will eventually manifest liver damage and progress to cirrhosis and hepatocellular carcinoma. Accordingly, OBI is not only important in terms of transmission of infection through blood transfusion, but also has other clinical significance.

Key words: Occult hepatitis B infection, Polymerase Chain Reaction, blood donors, Nucleic acid testing

HEPATITI B OKULT NË DHURUESIT E GJAKUT; ÇFARË DUHET TË DIMË

Abstrakt
Diagnostikimi I infeksionit okult të virusit të hepatit B (VHB) ende paraqet një aspekt të rëndësishëm në menaxhimin e tij. Infeksioni okult I hepatitit B (IOB) merr rëndësi veçanërisht në ekzaminimin e dhuruesve të gjakut dhe menaxhimin e procedurave të transfuzionit. Identifikuesi kryesor I IOB është prania e HBV-AND, që mund të zbulohet vetëm duke përdorur analizat e I-së. Testimi I acidit nukleik (TAN) ose Reaksioni Zinxhiri I Polimerazës në kohë reale (RZP) përcakton nivelet e HBV-I-së në njësi për ml [IU/ml], ose kopje për ml. Edhe nëse dhuruesi është HbsAg negativ, ata mund të transmetojnë IOB te marrësi në varësi të statusit të tyre viremik (zbulimi I I-së së VHB). Zhvillimi I kritereve të rrepta të depistimit dhe testeve të besueshme për të identifikuar IOB të dyshuar te dhuruesit e gjakut mbetet I rëndësishëm dhe I domosdoshëm për të shmangur mundësinë e transmetimit të VHB përmes transfuzionit të gjakut. Në përgjithësi, ne duhet të vlerësojmë prevalencën e IOB midis dhuruesve të gjakut. Për më tepër, kërkohet monitorim I vazhdueshëm, sepse prevalence e IOB ndryshon sipas studimeve të kryera në popullata të ndryshme. Veç kësaj, disa individë të infektuar mund të manifestojnë dëmtime të mëlçisë, të cilat progresojnë në cirrozë dhe karcinomë hepatocelulare. Rrjedhimisht, IOB nuk është

I rëndësishëm vetëm për sa I përket transmetimit të infeksionit përmes transfuzionit të gjakut, por edhe aspektit klinike dhe impaktit në shëndetin publik.

Fjalë kyçe: infeksion okult I hepatitit B, Reaksion zinxhir polimerazë, dhurues gjaku, testimi I acidit nukleik

Introduction

According to the World Health Organization (WHO) 2024 for the European Region 10.6 million people are living with hepatitis B virus (HBV). If absent from proper medical treatment, the infection may lead to severe clinical outcomes such as cirrhosis or Hepatocellular Carcinoma (HCC) (1). It belongs to the Hepadnaviridae family and the main route of transmission is through the infected fluid containing either blood, or semen (2). Through studying the structure and epidemiology of the hepatitis B virus, it becomes clear how its strains and clinical forms alter approaches to prevention and treatment.

- Regarding structure, HBV belongs to the group of double-stranded DNA viruses that have an incomplete internal structure made up of essential surface antigens (HbsAg) for infectivity and immunogenic purposes that are externalized. The core antigen known as HbcAg is found within the viral envelope in the form of a nucleocapsid that encases the viral DNA polymerase and parts of the viral DNA (3).
- In accordance with the transmission section, routes for the potential transfer of HBV include unprotected sexual contact, use of non-sterile syringes or other medical equipment, or transmission from mother to child during birth. More importantly, this virus can survive outside the body for more than seven days without decomposing (4).
- In addition, HBV also possesses additional types of genotypes A through H, bearing in mind their geographical ranges and clinical relevance. Such types of genotypes can affect condition progress, treatment responsiveness, and even the responses to vaccines. For instance, genotype C has a worse liver disease and a higher risk of hepatocellular carcinoma (5).
- In relation to clinical manifestations, infection with HBV can be avirulemic with spontaneous resolution or become chronic. Many of the patients will, at the time of diagnosis, not be symptomatic during the productive phase of the infection, while many others may experience nausea, jaundice, abdominal discomfort, or flu-like illness. Chronic infection with HBV is associated with the development of complications such as liver cirrhosis, liver failure, and HCC (2, 6).
- Furthermore, concerning prevention and treatment, the definite and most effective method for preventing the population from HBV is vaccination. The vaccine is effective against all the well-known genotypes of the virus (1). Antiviral agents can be given to infected patients to control viral replication and prevent the complications (1). There are many strategies that can be employed, but will be influenced greatly by the phase of infection (acute or chronic) and the patient's clinical state (7).

What is occult hepatitis B virus infection and why is it a concern in blood donation?

Occult hepatitis B virus infection (OBI) according to the statements from the Taormina expert meeting, is a form of HBV infection defined by the presence of HBV DNA in the liver, which can be undetectable or detectable in the serum by molecular testing in patients who test negative for the hepatitis B surface antigen (HbsAg), which represents a standard marker for HBV infection (8). In the context of a positive HBV DNA result, a low viral load in the serum (usually <200 or even below 20 IU/ml) in an individual negative for HbsAg is indicative of an OBI (8). HBV DNA

load—amount of HBV genetic material in a blood sample usually is measured as international units per mL (IU/mL) or copies per mL (9). This happens in those who are either positive or negative for HBV antibodies, and according to serologic patterns, OBI is divided into seropositive OBI and seronegative OBI (8). The difference between seropositive and seronegative OBI is clinically important. Seropositivity of OBI could represent a cleared HBV infection, where HBV is no more replicative but HBV DNA remains in the liver. They may be at reduced risk for liver-related complications compared with patients, who may have an active HBV infection, but might benefit from follow-up to monitor for disease progression or the emergence of potential reactivation. By contrast, seronegative OBI demands novel diagnostic strategies in that these individuals are negative for HBV antibodies and therefore not detected by standard serology. Yet they have hepatitis B virus DNA (viral replication) in their blood and can still potentially infect others, particularly via blood transfusion or organ donation (9).

Interest in occult hepatitis B, especially in relation to blood donation, stems from a theoretical risk of transmitting the HBV to recipients by transfusion. Although the HbsAg test for blood donors is a routine introductory screening, it may not be able to identify individuals with occult hepatitis B (10). On the subject of transmissibility, individuals with occult hepatitis B can still transmit the infection through blood transfusions, organ transplants, and other routes of exposure. (11, 12, 13) Others may be at risk for developing chronic HBV infection if they receive blood that contains occult HBV, especially those with compromised or suppressed immune systems (14). Concerning the risk of disease progression, cirrhosis and HCC are two serious liver problems that can result from a persistent HBV infection (15).

Implementation of extra steps by blood banks in the screening of blood donation to lower the risk of occult hepatitis B, included nucleic acid testing (NAT) for HBV DNA in association to the screening for the presence of HbsAg (16). Since NAT can detect HBV DNA in blood samples when no HbsAg is present, it can be used to identify individuals with occult hepatitis B and prevent the transmission of HBV via blood component transfusion.

Epidemiology insights of occult hepatitis B infection

The prevalence of occult hepatitis B infection in blood donors shows significant variability depending on the population and geographical location. Variations in OBI prevalence have been found by epidemiological studies across different locations and populations. OBI occurred more frequently in areas with high HBV endemicity, such as sub-Saharan Africa and parts of Asia (17). Some populations are at an increased risk of OBI due to their prior exposure history to HBV, including health workers, drug abusers, and those who have undergone organ transplantation or blood transfusion. In addition, a higher prevalence of OBI was also evidenced in people whose immune responses are depressed, as seen in HIV-infected patients and organ transplant recipients (1). The rates of occult hepatitis B in blood donors have been variably reported from as low as 0.2 % in some areas to as high as 1 to 10 % or greater in others (18). Assuming the prevalence of hepatitis B among blood donors in Albania, the findings presented at the Transfusiology Conference on New Pathogens and Transfusion Safety in 2023 indicate that the prevalence of infections detected through NAT has raised from 0.24% in the first year of implementation in 2016 to 0.65% in 2022, resulting in an average prevalence of 0.49%. Additionally, at the First International Conference of the Balkan Consortium of Transfusion Medicine, rates for our neighboring country, Kosovo, were reported as dHBV at 0.3%, dHCV at 0.01%, and dHIV at 0.009% for 2023 (19). These findings highlight a concerning trend in transfusion-transmissible infections, particularly with regard to hepatitis B, emphasizing the necessity for ongoing vigilance and improvements in blood testing methodologies to ensure transfusion safety. Among a review of all studies we found that Leontari et al. (2024) reported an occult hepatitis B infection rate of 5.4% among blood donors from northwest Greece, while Manzini et al. (2007) made an estimate

of about 4.86% in Italian blood donors in northwestern Italy (20, 21). So, the prevalence of occult hepatitis B may depend on the HBV prevalence in the general population, sensitivity of screening tests, and demographic features of blood donors (22). As the detection is difficult and diagnostic criteria keep varying, OBI underestimation can occur. More sensitive screening methods, such as nucleic acid testing, identified occult hepatitis B in blood donors and perhaps contributed to a better understanding of its prevalence (23). Understanding the epidemiology of OBI is critical to implementing targeted screening and prevention strategies in high-risk populations and informing public health policy aimed at reducing hepatitis B infections in transmission and burden.

Risk factors for occult hepatitis B infection

Risk factors associated with OBI span a broad spectrum influenced by various demographic, behavioral, and medical factors. Age is an important factor, with people of older ages being more likely to have been exposed to HBV throughout their lives, increasing their risk of OBI, according to Jürgen Ott's study (24) on the global epidemiology of hepatitis B virus infection and new estimates of HbsAg seroprevalence for age. Gender may also play a role, as some studies indicate a higher prevalence of OBI in men compared to women, possibly due to differences in occupational exposure or health care-seeking behavior (25). Geographic location is another crucial factor, with higher prevalence rates observed in regions with endemic HBV transmission, such as parts of sub-Saharan Africa and Asia. Occupational hazards remain important determinants to mention as they include healthcare workers and laboratory personnel at increased risk of OBI due to possible exposure to HBV-contaminated blood or body fluids (26). Behaviors like injecting drug users and high-risk sexual behavior are recognized major behavioral risk factors of OBI since these increase the chances of transmitting HBV. Besides, the risk groups, which have a higher susceptibility to OBI, are the immunocompromised hosts, either through a co-infection, for instance with the hepatitis C virus (HCV) or the human immunodeficiency virus (HIV), or immunosuppression therapy, which can all impair their immune responses (14). Medical case history: including blood transfusions, organs transplantations, or dialysis, further increase the risks in OBI (27, 28). A family history of HBV infection or liver disease may represent familial genetic susceptibility and may mean there is a higher probability for OBI development (29). Furthermore some medical conditions such as thalassemia, hemophilia, cryptogenic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) are also risk factors attached to a significantly increased chance of having OBI (30). Lifestyle factors may aggravate its degree of liver injury in those with OBI and lead to higher morbidity and mortality.

Clinical significance of occult hepatitis B infection

The potential for donors with undeclared infections to have low blood levels of HBV DNA increases the risk of HBV transmission during transfusion. The precise transmission risk from occultly infected donors is still poorly understood, and could lead to a variety of potentially fatal outcomes, including acute HBV infection, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (30, 31). Furthermore, recipients of blood transfusions may themselves be immunocompromised or have underlying liver disease, which increases the risk of serious consequences in the event of HBV infection (32). If there is occult HBV DNA in the liver without detectable HbsAg in the blood, it is probable that there is viral replication and liver damage. This could indicate that individuals with OBI have a higher likelihood of developing liver disease as time goes on (33). The risk of infection by transfusion justifies the need for strict screening and sensitive tests for the detection of latent HBV infection in blood donors. In order to guarantee that, in the unlikely event of the worst occurring HBV transmission the individuals in question will be recognized early and treated right away to protect all transfusion recipients must also be the subject of ongoing monitoring and follow-up.

Occult hepatitis B diagnosis

Challenges in diagnosing occult hepatitis B due to absence of HbsAg

Occult hepatitis B is very challenging to diagnose since HbsAg is absent. Its absence makes conventional diagnosis methods very cumbersome, because HbsAg is one of the principal markers in the identification of active HBV infection (34). In diagnosis, OBI cases need special molecular and serological testing methods since they have undetectable HbsAg, but have HBV DNA circulating within the bloodstream (35). The above-identified challenges are therefore in need of sensitive molecular techniques and expansive serological testing methods so that occult hepatitis B cases are identified correctly for relevant management to be instituted (32).

Laboratory detection tests

Laboratory assessments consist of molecular methods that provide screening such as nucleic acid testing (NAT), real-time and quantitative polymerase chain reaction (qPCR), and transcription mediated amplification (TMA) for OBI detections, and measuring, HBV DNA copies (8). NAT is a direct method of detection of HBV DNA in blood samples through amplification of viral DNA with the use of polymerase chain reaction or other similar methods (36). Real-time PCR is a method that measures DNA during its process of amplification and therefore accurately measures the HBV DNA levels. On the other hand, TMA represents a highly sensitive tool for which many studies have been performed for the detection of HBV DNA, and quantitative PCR quantifies an amount of amplified DNA, thus giving quantitation of HBV DNA load (37, 38). These are supplemented by molecular tests for anti-HBc (hepatitis B core antibodies) and anti-HBs (hepatitis B surface antibodies) serological tests, which have an important role in defining OBI. These tests can make out seropositive and seronegative cases of occult hepatitis B. Seropositive OBI has been defined by the presence of HBV core antibodies (anti-HBc) and/or positive HBV surface antibodies (anti-HBs), which testify to a previous exposure to HBV. In contrast, seronegative OBI is an occult hepatitis B infection characterized by the absence of both anti-HBc and anti-HBs antibodies (8, 39). Given this, a multifaceted approach is needed to diagnose occult hepatitis B infection and prescribe the proper treatment.

Potential role of NAT to improve blood safety

According to the consensus conference Taormina 2018 the gold standard for OBI diagnosis is the detection of HBV DNA in the liver (39). Since, standardised and valid assays for HBV DNA detection in the liver are not yet available; the most commonly used method is detection of HBV DNA in the blood. NAT serves as a critical tool to increase blood safety by significantly reducing the risk of transfusion-transmissible infections, including occult hepatitis B. It was introduced for screening blood donors during the mid to late 1990s (16). This method lets us spot and measure viral nucleic acids. Assays used for NAT in blood products screening have high specificity (99.9%) and a limit of detection of 2–4 IU/ml HBV DNA when applied to individual units (40). NAT can find viral nucleic acids early in the infection process. This makes blood screening more accurate and adds an extra safety step in managing blood supplies. As a result, using NAT has helped reduce HBV infections from transfusions. These methods contribute to understanding the severity of infection and the magnitude of disease transmission risk because they accurately quantify the HBV DNA load in the blood (36). Early diagnosis and timely intervention and appropriate management strategies are thus possible to prevent disease progression and reduce the risk of transmission to others by using sensitive molecular techniques (38).

Management Strategies

The management of occult hepatitis B includes a number of measures that are directed towards the

safety of blood transfusions and reduction of risk for HBV infection. Introduction of NAT reduced the risk of transfusion-transmitted viruses through identification of donors with low HBV DNA levels (16). While, combined screening by highly sensitive serological and molecular assays is warranted in minimizing transfusion transmission risk of HBV (41, 42). Robust screening protocols should be in place to ensure that potentially infectious blood samples are discarded, thereby reducing the risk of HBV transmission through transfusions. Deferral criteria are also helpful in improving the safety of donation by admitting only those who do not have OBI but who are at high risk from other factors such as intravenous drug users or sexual activity that put them at higher risk of contracting OBI entail (43). With these criteria, it is easy to exclude people who are more likely to transmit HBV, thereby keeping infected blood out of the donor pool.

Conclusion

To summarize, OBI is a major blood transfusion risk due to its potential for HBV transmission by infected donors. Global OBI rates are inconsistent, and therefore we need careful review and more research. We have to determine better how the screening can be improved and the blood safer as the OBI rates vary in different groupings or localities. To detect low levels of HBV DNA among blood donors, we should use sensitive molecular tests such as NATs, which minimizes chances of transfusing OBI. Therefore, it is increasingly important to diagnose OBI by reliable molecular and serological methods that are capable of identifying those with a real risk of latent and occult HBV transmission. Moreover, awareness campaigns will guarantee an all-inclusive screening protocol in place, which will maintain the high quality of the blood donated to patients. The prospective working agenda needs researchers, physicians, and policymakers' concerted efforts aimed at understanding occult hepatitis B further and ensuring safety for blood recipients. Ultimately, this will contribute towards faster patient recovery and enhanced public health outcomes.

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