

## Detection of Occult Hepatitis B Virus in Anti-HBc Positive/Anti-HBs Positive Blood Donors in Saudi Arabia

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**Abstract:** Despite the progress made in the prevention of transfusion transmitted infections, transmission of Hepatitis B Virus (HBV) from donors with Occult Hepatitis B virus (OHB) still poses a challenge to the safety of blood donation. The presence of OHB in anti-hepatitis B core antigen (anti-HBc) positive/anti-hepatitis B surface antigen (anti-HBs) negative blood donors has recently been documented worldwide however, its prevalence in anti-HBc positive/anti-HBs positive donors remains obscure. Therefore, this study was aimed to evaluate the presence of OHB among anti-HBc/anti-HBs-positive blood donors. To meet these objectives, a total of 1000 serum samples of consenting blood donors negative for Hepatitis B surface Antigen (HBsAg) were collected at the Hera'a General Hospital, Makkah Al-Mukaramah, Saudi Arabia and screened for anti-HBs and anti-HBc antibodies. According to their serological status, the samples were classified into anti-HBc negative/anti-HBs negative; anti-HBc positive/anti-HBs negative and anti-HBc positive/anti-HBs positive donors. Samples that showed positive reactivity for both anti-HBc/anti-HBs antibodies were then selected for quantitative detection of HBV DNA by using RT-PCR. Results showed that of the 1000 HBsAg-negative donors, 12 (1.2%) were anti-HBc positive/anti-HBs negative and 63 (6.3%) were anti-HBc positive/anti-HBs positive. Additionally, among these 63 anti-HBc positive/anti-HBs positive blood donor population HBV DNA was detected in two of them (3.2%). In conclusion, the results showed the presence of HBV DNA in the sera of anti-HBc positive/anti-HBs positive blood donors in Saudi Arabia. Overall, these results can raise an important public health issue that an anti-HBc positive/anti-HBs positive serum is not a sign of total HBV eradication and in turn, highlight the need for a stringent and better screening system to prevent post-transfusion HBV infection.

**Key words:** Hepatitis B virus, occult hepatitis B, blood donor, HBV DNA, anti-HBc, anti-HBs

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### INTRODUCTION

The safety of blood products is one of the major issues in the area of transfusion medicine. Chronic Hepatitis B Virus (HBV) infection continues to be a global public health problem that affects an estimated 360 million individuals. Two-third of these HBV carriers live in Asia-Pacific region where infection with hepatitis B is the leading cause of chronic hepatitis, cirrhosis and Hepatocellular Carcinoma (HCC) (Liaw *et al.*, 2008; Thedja *et al.*, 2010). The infection is usually defined by the presence of Hepatitis B surface Antigen (HBsAg) in serum or plasma. However, HBV may exist in humans without detectable HBsAg but with presence of HBV DNA in the serum and/or in the liver i.e., the Occult HBV infection (OHB). A 2008 international workshop on OHB, endorsed by the European Association for the Study of the Liver (EASL) defined OHB as the presence of HBV DNA in the liver of individuals testing HBsAg-negative with currently available assays (Raimondo *et al.*, 2007; Gerlich *et al.*, 2010).

Since, OHB has been detected in blood donors and could be transmitted to the recipients (Hoofnagle *et al.*, 1978), the safety of blood transfusion has become an important issue. The occurrence of OHB to a large extent depends on the prevalence of HBV infection in the general population. The occult infection may result from the low viral load in circulation (usually  $<200 \text{ IU mL}^{-1}$ ) or a mutant HBsAg which is not recognized by the commercial monoclonal antibodies against HBsAg (anti-HBs) (Torbenson and Thomas, 2002; Raimondo *et al.*, 2008). There are several conditions for OHB infection including recovery from past infection defined by the presence of anti-HBs; chronic hepatitis with surface gene escape mutants that are not or poorly recognized by current assays; chronic carriage without any marker of HBV infection other than HBV DNA referred to as seronegative and chronic carriage with HbsAg too low to be detected and recognized solely by the presence of anti-HBc (Allain, 2004; Levicnik-Stežinar *et al.*, 2008; Liu *et al.*, 2010).

The prevalence of OHB in anti-HBc-positive blood donors has recently been documented worldwide (Yotsuyanagi *et al.*, 2001; Chemin and Trepo, 2005; Niederhauser *et al.*, 2008; Hollinger and Sood, 2010; Yuen *et al.*, 2011). However, its frequency in anti-HBc positive/anti-HBs positive donors remains obscure. Thus, we performed this study to identify this issue and evaluate the possibility of OHB in anti-HBc/anti-HBs-positive blood donors in Holly Makkah, Saudi Arabia.

**MATERIALS AND METHODS**

**Blood donors and sampling:** A total of 1000 HBsAg negative blood specimens of accepted blood donor volunteers were collected between April 5, 2009 to June 15, 2010 at the Hera'a General Hospital, Holly Makkah, Saudi Arabia. According to the policy set up by the Kingdom of Saudi Arabia Health Ministry for blood donation, an accepted donor is defined as a generally healthy person with normal levels of liver function key enzymes, negative for HBsAg and antibodies against Hepatitis C Virus (HCV) and HIV. The HBsAg in each donor was tested in parallel by two different commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits. In addition, donors with any medical problem or previously received HBV vaccination had been excluded from the populations of the present study. All the experiments were approved in accordance with the Ethics Committee and the guidelines of the Nation Health and Medical Research Council of the Kingdom of Saudi Arabia. Informed written consents had also been obtained from all participants. Strict measures were adopted to avoid any contamination. The collected samples were immediately transferred to the lab and allowed to clot at room temperature for 30 min and centrifuged. Sera samples were separated, aliquoted and stored at -20°C until used.

**Serological and biochemical assays:** The collected samples were screened by ELISA to detect antibodies to Hepatitis B surface antigen (anti-HBs) and core antigen (anti-HBc) using the commercially Diagnostic kits (Dade Behring, Marburg, Germany) according to manufacturer's

instructions. Samples with Optical Density (OD) higher than or equal to the cut-off value had considered to be positive and retested in duplicate before the final interpretation. On the other hand, samples with an optical density less than the cut-off values had considered to be negative. The samples were classified according to their serological status into anti-HBc/anti-HBs-negative; anti-HBc-positive/anti-HBs-negative and anti-HBc positive/anti-HBs positive samples. In addition, the serum levels of liver function key enzymes [Aspartate aminotransferase (AST) and alanine Aminotransferase (ALT)] were performed on all samples.

**Quantitative assay of HBV-DNA by real-time polymerase chain reaction:** Samples that showed anti-HBc/anti-HBs-positivity were selected for detection of HBV DNA by real-time PCR using the Abbott RealTime HBV DNA quantitative assay with m2000<sub>rt</sub> System (Abbott Molecular Inc., Des Plaines, IL 60018 USA) following the manufacturer's instructions. The lower detection limit of the assay was 10 IU mL<sup>-1</sup> for 0.5 mL sample volume.

**RESULTS AND DISCUSSION**

As shown in Table 1 and Fig. 1, the serological screening showed that among the 1000 HBsAg-negative samples, 63 (6.3%) were anti-HBc positive/anti-HBs positive and 12 (1.2%) were anti-HBc positive/anti-HBs negative. HBV DNA was detected by RT-PCR in two (3.2%) samples of the 63 anti-HBc positive/anti-HBs positive samples but it was not detected in the anti-HBc positive/anti-HBs negative samples. The levels of liver function key enzymes (AST and ALT) were within normal values in all samples.

Despite the introduction of reliable serologic screening of blood donations, the identification of blood donors with occult HBV infection (OHB; donors who are negative for HBsAg but have detectable circulating HBV DNA) still has clinical impact with regards to the safety of blood supply (Yuen *et al.*, 2011). Since, the first evidence of OHB was reported in 1979, there has been continuous increase in the number of publications on OHB covering

Table 1: Summary of the serological profile, detection of HBV DNA and the mean of liver function key enzymes among the volunteer blood donors who were negative for hepatitis B surface antigen (HBsAg)

Groups	Number and percentage	Detection of HBV DNA	Liver function key enzymes	
			AST (15-46 IU L <sup>-1</sup> )	ALT (11-66 IU L <sup>-1</sup> )
Anti-HBc negative/anti-HBs negative	925/1000 (92.5%)	0/925 (0.00%)	29.1±2.87	31.6±3.32
Anti-HBc positive/anti-HBs negative	12/1000 (1.2%)	0/12 (0.00%)	32.5±1.42	35.1±3.30
Anti-HBc positive /anti-HBs positive	63/1000 (6.3%)	2/63 (3.17%)	31.5±2.42	33.0±1.50

Anti-HBc, Anti-Hepatitis B core antigen antibodies; Anti-HBs, Anti-Hepatitis B surface antigen antibodies; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

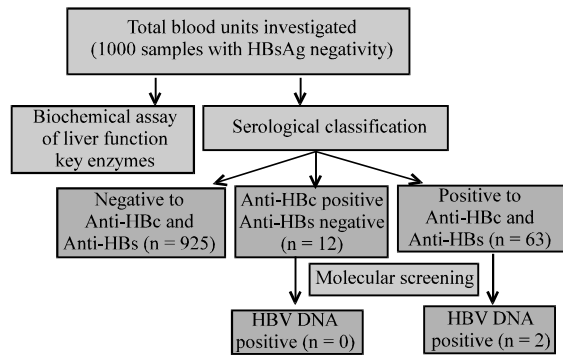


Fig. 1: Schematic presentation of the study design

various areas of bio-medical and public health aspects. In this concept, the prevalence of OHB in anti-HBc-positive alone blood donors has recently been documented worldwide (Yotsuyanagi *et al.*, 2001; Chemin and Trepo, 2005; Hollinger and Sood, 2010; Yuen *et al.*, 2011) however, its frequency in anti-HBc positive/anti-HBs positive donors remains obscure. In the present study, we investigated the possible existence of OHB in anti-HBc positive/anti-HBs positive blood donors in Holly Makkah, Saudi Arabia. The results showed that among a total of 1000 serum samples of regular blood donors negative for HBsAg, 63 samples showed positive reactivity for both anti-HBc and anti-HBs antibodies and HBV DNA was detected in two (3.2%) samples of the 63 anti-HBc positive/anti-HBs positive samples.

The implications of HBV infection involve several clinical aspects varying from chronic asymptomatic carriers to complicated liver diseases including liver cirrhosis and HCC (Allain, 2004). OHB infection has appeared to have similar infectivity and pathogenicity in the development of fulminant hepatitis, liver cirrhosis and HCC (Torbenson and Thomas, 2002) and would possibly affect the safety of blood transfusion (Wang *et al.*, 2002; Ramezani *et al.*, 2010).

The prevalence of OHB in blood donors appears to be varied in different countries or even in different areas in a country and such variations may be associated with the different assays used in the various surveys and the HBV endemicity (Asim *et al.*, 2010). Several factors are involved in the recognition of the OHB infection including viral variants carrying mutant HBsAg that are not recognized by specific antibodies used in assays for HBsAg (Kreutz, 2002; Laulu and Roberts, 2006; Weber, 2006) and/or low level expression of *HBV* genes (Garcia-Montalvo *et al.*, 2005; Niederhauser *et al.*, 2008; Hollinger and Sood, 2010). Although, detection of HBV DNA using liver tissue DNA extracts can increase the positive rate of OHB (Gonzalez *et al.*, 2009), it is not

feasible to get liver tissues from the blood donors. Indeed, the using of Nucleic Acid Amplification Tests (NAT) to screen HBV in blood donors has successfully reduced the risk of post-transfusion HBV infection in certain countries (Bamaga *et al.*, 2009; Gonzalez *et al.*, 2009). However, NAT is sometimes associated with the risk of false-positive results (Liu *et al.*, 2010). Therefore, under the current circumstance the positive rate of OHB that was detected her by using highly sensitive RT-PCR screening is less likely to be underestimated.

In the present study, HBV DNA was detected in 3.2% of anti-HBc positive/anti-HBs positive donors. Previously, the presence of anti-HBc antibodies in HBsAg-negative individuals has been considered as a marker of past HBV exposure and/or of resolved infection. However, the recent application of molecular biology techniques has shown that HBV viremia is detectable in 1.33-38% of HBsAg-negative/anti-HBc-positive donors (El-Sherif *et al.*, 2007). In a consistent line, co-presence of anti-HBs and anti-HBc antibodies is still used as an indication of immunity after HBV infection and in some countries blood units with high levels of anti-HBs antibodies is considered to be safe (Hollinger, 2008). By contrary, the presence of anti-HBs antibodies is not a sign of total HBV eradication has recently been suggested (Thedja *et al.*, 2010). Reactivation of HBV infection despite of high anti-HBs levels has been revealed by Gartner *et al.* (2007) and further confirmed by Levicnik-Stežinar *et al.* (2008). Similarly, Matsumoto *et al.* (2001) has demonstrated the transmission of HBV among Japanese recipients from OHB subjects in the presence of concurrent neutralizing anti-HBs antibodies in the same specimen. A same observation has also been reported in Italy by Manzini *et al.* (2007) that some blood donors with anti-HBs titer over 100 IU L<sup>-1</sup> still had detectable HBV DNA. More importantly in a recent study 4.6% of the HBV DNA positive cases were detected in donors with low titers of anti-HBc positive and anti-HBs positive antibodies (Asim *et al.*, 2010). Based on these collective facts, it is interesting to point out that HBV DNA was detected in 3.2% of the anti-HBc positive/anti-HBs positive donors and this finding re-highlight the importance of HBV DNA detection compared to other serological HBV markers for predicting latent HBV infection and to improve the safety of blood supply. In addition, a further study is also required to determine the precise relationship between the incidence of OHB and the titer values of anti-HBc and anti-HBs antibodies.

## CONCLUSION

In the present study OHB was detected in HBsAg-negative/anti-HBc positive/anti-HBs positive

blood donors. These data will be meaningful in setting up a strategy to prevent posttransfusion OHB. Overall, these results raise an important public health issue that the absence of HBsAg as HBV infection marker does not exclude the possibility of viral transmission and anti-HBc positive/anti-HBs positive sera is not a sign of total HBV eradication.

#### ACKNOWLEDGEMENTS

This research was sponsored by the fund (43109007) of The Institute of Scientific Researches at Umm Al-Qura University, Saudi Arabia. Also, researcher grateful to Dr. Adel G. El-Shemi, Dr. Amr M. Abd Al-Fattah and Dr. Hani Faidah (Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al-Qura University) for their help and excellent technical assistance.

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