Prevalence of HBc-Ab among HBs-Ag negative healthy blood donors in south of Iran

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Abstract:
Background: The risk of infection with transfusion-transmitted viruses, such as Hepatitis B virus (HBV), has been reduced markedly. However, a zero-risk blood supply remains a popular goal. Anti-hepatitis B core antigen (anti-HBc) is considered to be an effective marker for occult HBV infection, and it has served a significant role in improving blood safety. The purpose of this study was to determine the seroprevalence of anti-HBc antibodies among blood donors.
Methods: During a period of six months in 2012, 1000 blood donors in Bandar Abbas were investigated for the presence of HBV infection markers. We used ELISA for the detection of HBsAg and chemiluminescence for the detection of HBsAb and anti-HBc antibodies.
Results: The prevalence of positive anti-HBc among the 1000 donors was 8.3%. Almost 2.3% of the donors were reactive for anti-HBc and negative for HBsAg.
Conclusions: The study showed that more than 2% of healthy blood donors in Bandar Abbas already have been exposed to HBV, although their HBsAg tests were negative. We suggest screening for anti-HBc to improve the safety of the blood supply.

Keywords: Anti-HBc; HBsAg; Bandar Abbas; Blood donor

Additional Information for citing this article:
Title of Journal: Electronic physician; Abbreviated title of journal: Electron. Physician
doi: 10.14661/2013.659-663

Editorial information:
Type of article: Original
Published: August.01.2013

1. Introduction

Hepatitis B virus (HBV) infection is a major global problem, with about two billion people now infected despite the availability of an efficacious vaccine. It has been estimated that there are more than 350 million carriers of HBV in the world and that roughly one million of them die annually from HBV-related liver disease (1-3). The prevalence of hepatitis B infection varies in different parts of the world, ranging from less than 1% to 15%. In the Middle East, the endemicity is intermittent, with a carrier rate of 2 to 8% (4-7). Transmission of HBV can occur through sexual intercourse, parenteral contact, or from an infected mother to a baby at birth (1). HBV transmission has similar characteristics to the transmission of HIV in that the virus has been detected in peripheral mononuclear cells; in tissue from the pancreas, kidneys, skin, and spleen; and in bodily fluids, including semen, breast milk, sweat, tears, urine, and vaginal secretions (1, 8).

Hepatitis B core antibody (HBc Ab) is the first antibody produced after infection with HBV, and it is the only detectable marker in the window period. It is in the serum of recovered individuals and also those with chronic hepatitis B (CHB). Resolved infections usually reveal hepatitis B surface antibodies (HBs Ab) (2, 3). Isolated HBc Ab refers to the presence of HBc Ab in sera without hepatitis B surface antigen (HBs Ag) or HBs Ab. It may be an indicator of resolved HBV infection in which HBs Ab had declined to an undetectable level, or it may be an indicator of chronic infection, such that HBs Ag cannot be detected due to protein mutation, which makes it undetectable by certain diagnostic assays (9). Recent studies have indicated that we cannot judge the HBV infection based only on the presence or absence of HBs Ag and HBs Ab (10). Despite the decrease in HBV infections globally, the use of serological markers for screening blood donors is still important, since HBV remains a great risk for patients who receive blood transfusions (11, 12). The objective of our study was to evaluate the seroprevalence of HBc Ab among healthy blood donors in Bandar Abbas City in the south of Iran.

2. Material and Methods

This cross-sectional study was conducted in 2012 in Bandar Abbas, the capital city of Hormozgan Province in the south of Iran. For the purposes of the study, 1000 people were selected for participation from first-time, voluntary blood donors who came to the Blood Transfusion Organization in Bandar Abbas City. The study was approved by the Ethics Committee at the Hormozgan University of Medical Sciences, and a written, informed-consent form was obtained from the donors.

The participants were asked questions regarding the frequency of blood donation, past surgical procedures, and other risk factors related to hepatitis B and C transmission. After the informed-consent forms were signed, physical examinations were conducted, and blood samples were drawn for testing. All donors who came to the Department of Transfusion Medicine during the study period and met the general criteria for blood donation were included in the study. Donors who did not meet these criteria were not allowed to donate blood, and, hence, they were excluded from the study. Potential donors with medical histories that included symptoms suggestive of acquired immunodeficiency syndrome, a history of jaundice, a blood transfusion in the last year, or close contact with a patient suffering from hepatitis in the last six months also were not allowed to donate blood. Detailed histories were obtained and clinical examinations were performed for all the donors by qualified staff members who had been trained to screen potential blood donors. According to the policy of the Iranian Blood Transfusion Organization, all donors were checked for HBs Ag, HCV Ab, and HIV by the ELISA method. Based on the results of these tests, all of the participants were deemed to be healthy and eligible to donate blood.

Table 1. Classification of HBs Ab and HBc Ab

HBs Ab		HBc Ab	
Titer	Classification	Titer	Classification
<10	Negative	<0.01	Positive
10 – 100	Suspect	0.01 – 0.1	Borderline
>100	Positive	>0.1	Negative

Five milliliters of blood were collected from each donor in a sterile, capped tube. Then, the blood was centrifuged, and two aliquots of the plasma were separated and stored at -70 °C until it was needed for testing. The screening for the hepatitis B core antibody (anti-HBc IgM and IgG) was done by a chemiluminescence kit made by DiaSorin LIAISON®. All of the steps were followed according to the manufacturer's instructions. The presence or absence of anti-HBc antibodies was determined by comparing the recorded absorbance with the calculated cut-off values. The test results were classified according to Table 1. The statistical analyses were done by using the Epi Info® 7 software. A multivariate analysis was done to relate the variables with each other. The chi-squared test was used to detect any significant correlations between the different variables.

3. Results

One thousand samples were collected from participants during one year, and the vast majority of the participants were males (954: 95.4%). The ratio of males to females was 20.7:1. Demographic characteristics of the participants are given in Table 2. Out of the 1000 samples studied, 83 of them were found to be positive for HBc Ab. The prevalence rate of HBc Ab was [(83/1000) x 100% = 8.3%]. Table 3 indicates that there was a greater prevalence of HBc Ab among younger people, but the relationship between age and HBc Ab results was not significant (P value > 0.05).

Among the 83 people who tested positive for HBc Ab positive, 60 of them (72.3%) tested positive for HBs Ab positive as well, and 23 of them (27.7%) tested negative for HBs Ab negative. Isolated HBc Ab was 2.3% [(23/1000) x 100%]. Of the 23 participants who tested positive for anti-HBc and negative for anti-HBs, 21 (91.3%) were males and 2 (8.7 %) were females.

Table 2. Demographic characteristics of blood donors in Bandar Abbas

Characteristics		Frequency	Percent
Gender	Female	46	4.6
	Male	954	95.4
Job	Employee	130	13
	Student	40	4
	Military	220	22
	Housewife	12	1.2
	Jobless	158	15.8
	Others	440	44
Age	Mean age of the donors was 31.2 ± 7.5		

Table 3. Prevalence of HBc Ab by age of the participants

Age group	HBc Ab+ Frequency	HBc Ab + Percent	Total
16 – 25	24	8.2	290
26 – 30	36	8.7	410
31 – 35	12	7	170
36 – 40	8	10	80
41 – 55	4	8	50
Total	83	8.3	1000

4. Discussion

Infection by the hepatitis B virus is a public health problem and a major cause of morbidity and mortality, particularly in developing countries (6). The world can be divided into three areas based on the prevalence of chronic HBV infection, i.e., high (> 8%), intermediate (2-8%), and low (< 2%) (6, 7). Most countries in the world are still considered intermediate to high endemicity for HBV infection (6, 7). In Iran the prevalence of hepatitis B varies from 1.3 to 6.3%, depending on the different areas of the country (13).

Transfusion of blood collected from a donor who is in the window period may lead to post transfusion hepatitis B in the recipient (14, 15). At present, the detection of HBsAg is the only diagnostic screening test for HBV infection in blood transfusion centers in Iran. We examined 1000 serum samples from healthy donors that tested negative for HBsAg and found that 8.3% of them were positive for anti-HBc. Previous studies have demonstrated the presence of HBV DNA in anti-HBc-only donors (16, 17). These findings corroborate the findings of an earlier report that indicated that testing blood donors for HBsAg alone is not sufficient to eliminate HBV from the blood supply (18). Chaudhuri et al. observed the presence of HBV DNA in 20% of anti-HBc-only donors studied and stated that reactivity to anti-HBc can only predict cryptic HBV infection (17). A report from Japan (16) demonstrated the presence of HBV DNA in 38% of donors who were HBsAg-negative and anti-HBc-reactive. It was observed earlier (14, 15, 19) that the screening test for the detection of HBsAg does not rule out the transmission of Hepatitis B, because the donor might be in the "window period," and only the detection of the antibody to the hepatitis B core-type antigen HBcIgM serves as a useful serological marker during this period. A prevalence of 8.3% observed for anti-HBc in this study was significantly greater than the 0.39% observed by Kumar et al. (19). This might indicate that the possibility of transfusing HBV through blood donation is increasing progressively.

Anti-HBc has been found to be an excellent indicator of occult HBV infection during the window period (15, 20, 21). Other markers for detecting occult HBV infection in an HBsAg negative blood donor, such as detection of HBV DNA by polymerase chain reaction (PCR), may not be cost effective (22). Detection of anti-HBc has contributed significantly to the reduction of the incidents of post-transfusion hepatitis B among patients (23, 24). The IgM class of the anti-HBc is a marker that indicates recent infection. The IgG variety of anti-HBc appears later during the infection and points to a past HBV infection. Individuals with the IgG variety of anti-HBc may not be infectious

because they may have sufficiently high titers of antibodies to HBsAg (anti-HBs), which are protective in nature, and the affected individuals may actually be disease free.

5. Conclusion

The results of the study showed that 8.3% of 1000 blood donors tested positive for anti-HBc antibodies. It shows the need to include anti-HBcIgM in routine screening of blood donors in Iran. It also confirmed the fact that testing blood donors for HBsAg alone is not sufficient to eliminate HBV from the blood supply. Although the possibility of achieving zero risk of transfusion-associated HBV infection depends largely on DNA testing of all of the units of blood collected before they are used in transfusions, this cannot done in many developing countries, including Iran, due to cost. Thus, it is recommended that anti-HBcIgM be included in the routine screening of blood donors in countries where DNA testing is not done. This will make a significant contribution to the reduction of transfusion-associated hepatitis B virus infections.

Acknowledgments:

This project was sponsored by the Hormozgan University of Medical Sciences.

Conflict of Interest:

There was no conflict of interests to be declared.

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