

Frequency of Anti-HBc & HBV DNA detection in blood donors of Kerman province, Iran

Maryam Delavari^{1*}, Naser Shahabi-Nejad², Andre M.N. Renzaho¹, Mohamad Javad Zahedi³ and Amin Reza Owahdi³

¹Public Health Research, Evaluation and Policy cluster, Faculty of Health, Medicine, Nursing and Behavioural Sciences, Deakin University

²Research Centre for Tropical and Infectious Diseases, Kerman University of Medical Sciences, P.O. Box 76169-14133, Kerman, Iran

³University of Medical sciences, Kerman, Iran

Abstract

Hepatitis B is a serious global infection disease and a major cause of mortality and morbidity worldwide. However, data on Occult Hepatitis B in Iran are scarce. The current study assessed the frequency of Anti-HBc and HBV DNA in serum sample of healthy blood donors negative for HBsAg stratified by sex and age; and also investigated the relationship between detection of HBV-DNA and anti-HBc positivity. Since anti-HBc screening is not performed in Iranian Blood Bank, we assessed whether anti-HBc could be adopted as a screening assay for the donated blood. The study included a total of 1525 blood samples of blood donors negative for hepatitis B virus surface antigen (87% male with a mean age \pm SD: of 31 \pm 8yr; and 13% female with a mean age \pm SD of 30 \pm 6yr). Eight percent (121 out of 1525) of the blood samples with negative HBs-Ag were positive for Anti-HBc and were all from males. HBV-DNA was detected in 36 out of 121 anti-HBc+ specimens (29.7%). The study found a positive relation between anti-HBc positivity and detection of HBV-DNA in serum samples of HBs-Ag negative blood donors. Findings from this study suggest that, introducing anti HBc screening in Iran maybe very practical in order to limit the transmission risk of Occult Hepatitis B virus through blood transfusion.

Keywords: Occult Hepatitis B; Hepatitis B Virus; Blood donor/s; Anti-HBc; HBV DNA; Kerman; Iran

Introduction

Hepatitis B is a serious global infection disease and a major cause of mortality and morbidity. It is the 10th leading cause of death, with two billion people infected, and an estimated 400 million suffering from chronic hepatitis B virus (HBV) infection worldwide [1-5]. HBV endemicity has varied widely worldwide. It has been estimated that HBV is highly endemic in all of Africa (except Tunisia and Morocco), some parts of South America, Alaska, northern Canada and parts of Greenland, eastern Europe, the eastern Mediterranean area, south-east Asia, China, and the Pacific Islands (except Australia, New Zealand and Japan) [6,7]. In the Middle East, Saudi Arabia, Jordan, Oman, Yemen, and Palestine are areas classified as of high endemicity; Iraq and the United Arab Emirates have intermediate endemicity; whereas Iran, Kuwait, and Bahrain have low endemicity [1,4,8]. In re-examining the epidemiology of HBV infection, Custer and colleagues undertook a structured review of available primary literature for over 30 countries worldwide. They found that the prevalence of chronic HBV infection ranged from over 10% in some Asian and Western Pacific countries to under 0.5% in the United States and northern European countries [9].

Unsafe blood transfusion is one of the routes of transmission for HBV infection. Despite, all blood donations being tested routinely for hepatitis B surface antigen (HBs-Ag) as a marker of transmissible HBV, occasional cases of post-transfusion hepatitis B virus infection are common [8,10-13]. Generally occult HBV infection is defined as the detection of HBV-DNA in the serum or tissue of subjects who have negative test for HBs-Ag [2]. In addition, antibodies to hepatitis B core (HBc) antigen are marker to acute, chronic and resolved HBV infection that remain detectable forever. Consequently anti HBc is detected in anyone who has been infected with HBV, while the level of HBs-Ag in the circulation becomes too low to be distinguished [14]. The frequency of this cryptic infection appears to be varies considerably according to prevalence of the infection. In relatively low prevalence areas of HBV infection such as Europe and North America, than 5% of blood donors

are characterised as having occult hepatitis B. In contrast, occult HBV is the major cause of transfusion transmitted HBV infection in high prevalence areas such as Ghana [1,2, 10,15,16]. In Iran data on Occult hepatitis B are scarce. There are only three studies which demonstrated evidences of HBV-DNA detection in blood donor samples positive for Anti-HBc. Those identified found a range between 11.3% and 28.6 % HBV

DNA positivity among Anti-HBc⁺ blood donor samples (Table 1). A map of Iran provinces mentioned above is shown in Figure 1. These studies conducted in different urban areas of Iran and only one of them [12] considered subjects' sex and age. Given that the multiethnic nature

Reference	Country/City	Number screened	Anti-HBc+ (%)	DNA+ (%)
[25]	Greece	6696	Na*	0
[26]	UK	103869	586(0.56)	0
[27]	USA	4885732	40998(0.84)	3.7
[15]	Ghana	242	110(45.5)	12.7
[1]	India	5511	188(18.3)	21.0
[28]	Lebanon	1027	203(3.7)	5.4
[12]	Iran/Shiraz	2000	6.55	12.2
[21]	Iran/Isfahan	545	8.00	11.26
[13]	Iran/Rafsanjan	270	5.18	28.57

*Not mentioned

Table 1: Reported Prevalence of HBV-DNA* in anti-HBc⁺ blood donors.

***Corresponding author:** Dr. Maryam Delavari Public Health Research, Evaluation and Policy cluster Faculty of Health, Medicine, Nursing and Behavioural Sciences Deakin University 221 Burwood Highway Burwood 3125 Australia Tel: +61 9 244 6036, +61 4 035 42500; E-mail: mdela@deakin.edu.au

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of Iran population can affect the epidemiology of blood-borne occult hepatitis B [4], establishing the prevalence of occult hepatitis B among blood donors in each province, including both urban and rural areas, of Iran can be an appropriate indicator for the risk of transmitting HBV through blood transfusion. Moreover, Iran, like many other countries in the Middle East, has a large number of major beta-thalassemia patients that require regular multi-transfusion for survival [17]. These patients are at high risk for blood transmitted viral infections such as hepatitis B [18]. The purpose of this study was to assess the anti-HBc positivity and presence of HBV-DNA in serum sample of healthy blood donors negative for HBsAg stratified by sex and age. Since anti-HBc detection is not a routine test in Iran Blood Bank, this study assessed whether anti-HBc could be adopted as a screening assay for the donated blood.

Materials and Methods

Procedure

Blood samples of blood donors used in the study were from the whole province of Kerman, Iran. All blood samples were collected over a one year period (2008). Blood donors were interviewed and medically examined as a part of the standard screening process for blood donation in Iran Blood Banks. For example, those with high risk behaviours such as unsafe sexual contact or intravenous drug use and those who recently received HBV vaccination were excluded from donating blood. Consequently they were not included in the current study. As usual process for all blood donated, hepatitis B (surface) antigen (HBs-Ag), antibody to HCV (anti-HCV), Rapid Plasma Reagin (RPR) test, and antibody to human immune deficiency virus (anti-HIV) were tested. To conduct the study, all samples negative for HBs-Ag and other tests mentioned above were tested for Hepatitis B (core)-Antigen (anti-HBc). Samples with repeated anti-HBc positivity were considered to be anti-HBc⁺ and were tested for HBV-DNA. This study was approved by Kerman University Medical Sciences Ethics committee.

Serological assays

HBs-Ag and anti-HBc were detected by enzyme immunoassay available in Iran Blood Bank. HBs-Ag testing was conducted with a commercial EIA (Enzygost HBsAg 6.0) and anti-HBc testing

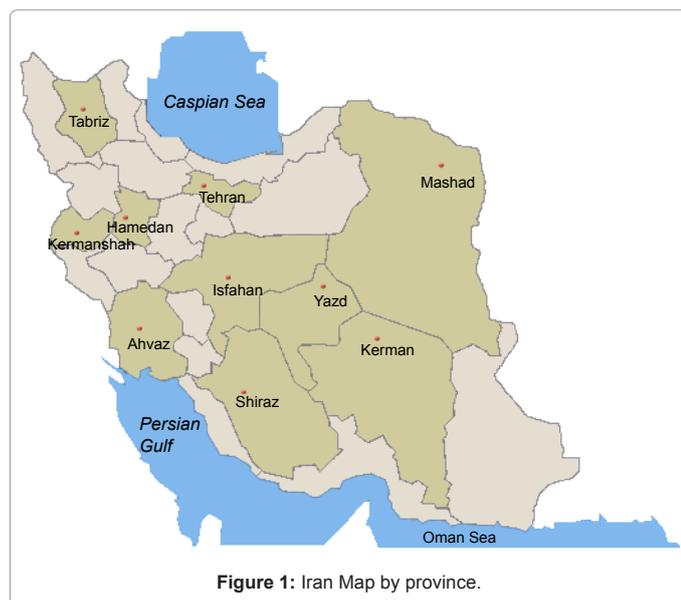


Figure 1: Iran Map by province.

Sex	Age	Number screened (%)	Anti-HBc+ (%)	Anti-HBc+ & HBV-DNA+ (%)
Male	31±8yr	1326(87%)	121(9.13%)	29.75%
Female	30±6yr	199(13%)	0(0%)	0%

Table 2: HBV-DNA⁺ in anti-HBc⁺ blood donors in Kerman province, Iran.

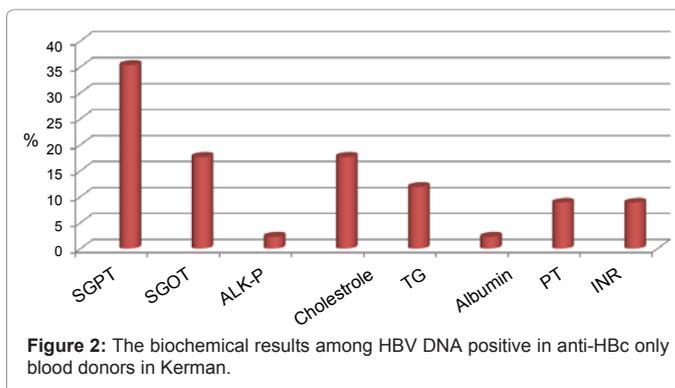


Figure 2: The biochemical results among HBV DNA positive in anti-HBc only blood donors in Kerman.

was conducted with another commercial EIA (Enzygost Anti-HBc monoclonal). All serological tests were performed by a laboratory staff that had been trained before by the manufacturer's representative.

PCR assay

Preparation of DNA samples from the sera-strict measures was adopted to prevent any contamination [19]. Serum HBV DNA was assayed according to the protocol recommended by the manufacturer.

DNA extraction

DNA was extracted from 200ml samples by DNA template extraction kit (Roche, Germany), was eluted in 200 ml elution buffer and then was freezed in - 80 centigrade degree.

PCR

For detection of HBV-load in samples, we used Roboscreen HBV-detection kit (Germany, Roboscreen) with three HBV-DNA, positive and negative standard controls (using Corbett Research Australia, USA). Samples with ≥ 20 copies/ml were considered to be positive. Sensitivity and specificity for this test were 100% and 98% respectively.

Biochemical tests

Performing biochemical tests was not a part of the study aims. However, considering the authors ethical responsibility toward the participants with positive HBV DNA PCR results, we invited them for a free follow-up management. Therefore, biochemical factors such as aspartate aminotransferase (AST=SGOT), alanine amino transferase (ALT=SGPT), prothrombin time (pt) and international normalized ratio (INR) were performed and also essential treatment and/or further follow up, based on medical indications, were also prepared.

Statistical analysis

Using SPSS software version 10.0 was performed to analyse the data. Descriptive tables and summary statistics were constructed to provide basic characteristics of the subjects in the study.

Results

Serological tests

During the time frame for the study, a total of 1525 donation blood

negative for HBs-Ag and all other routine blood donor screening assays were given to Kerman blood bank centres. Of whom 1326 (87.0%) of blood samples were from men (age range 18-50yr, mean \pm SD: 31 \pm 8yr) and 199 (13.05%) were from women (age range 21-48yr, mean \pm SD: 30 \pm 6yr). Eight percent (121/1525) of them were positive for Anti-HBc, and all were from males (Table 2).

HBV-DNA was detected in 36 out of 121 anti-HBc specimens (29.7%).

Positive predictive value for anti-HBc was 83%.

Biochemical markers

Liver markers including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed on HBV-PCR positive samples. The tests' results were in 32.4% of samples higher than the upper range of normal levels for these tests in clinical diagnostic settings. 4.4% of subjects had abnormal PT or INR (Figure 2). Positive predictive value for liver markers was 35.3 %.

Discussion

In the present study, frequency of anti-HBc positivity and presence of HBV-DNA in sera of healthy blood donors negative for HBs-Ag and all other routine blood donor screening assays from Kerman province were investigated. 7.9% of them were positive for anti-HBc and all were from males. None of the women's blood samples were found as having detected DNA for hepatitis B virus. Among studies conducted in Iran only one study stratified in blood donors by gender. 6% of men and 11.6% of women found to be positive for Anti-HBc and negative for HBs-Ag [12]. Our finding is consistent with a previous result by Isfahan in the central of Iran [21], but it is slightly higher than results from blood donors in Shiraz, in the south west of Iran [12], Hamadan in the Northwest of Iran [22], and Rafsanjan which is a small city of Kerman province [13]. The prevalence of anti-HBc in our study was relatively higher than other blood donor population in Iran and some countries that are classified as having low endemicity for hepatitis B infection. These dissimilar findings could be the result of regional differences of the prevalence of HBV infection in Iran [23] (Figure 1).

In this study the frequency of HBV-DNA⁺ among anti-HBc positive blood donors was 29.7% which is similar to that reported by Rafsanjan and Isfahan [13]. However this result is significantly higher than two other studies carried out in Shiraz and Isfahan (Table 1). This dissimilarity in the frequency of occult HBV in different geographical areas in Iran may be attributed to ethnic diversity and PCR detection with different sensitive assays. The frequency of HBV-DNA⁺ also varies considerably according to the prevalence of the hepatitis B virus infection. Current study demonstrated that, the proportion of HBV DNA positive donors is higher than in Europe and USA but relatively similar to other regions in the Middle East.

In blood donors with anti-HBc, most reports did not mention biochemical marker levels such as ALT as donors were asymptomatic. However, studies conducted prior to the availability of sensitive HBV-DNA assays showed that deferring HBs-Ag negative blood donors with elevated ALT decreased HBV transmission [24]. We performed some biochemical tests for PCR positive samples. The tests' results were in 32.4% of samples higher than the upper range of normal levels for these tests in clinical diagnostic settings. In particular, positive predictive value for liver markers was 35.3 %. In a study of anti-HBc and HBV-

DNA detection in blood donors negative for hepatitis B virus surface antigen in Shiraz, liver marker test results were all in normal ranges except in 4 of 16 (25%) of HBV-DNA positive subjects. Therefore an association between occult HBV and elevated ALT in the absence of other causes of liver disease such as HCV infection and alcohol abuse may be proposed.

Conclusion

We found a positive relationship between anti-HBc positivity and detection of HBV-DNA in serum samples of HBs-Ag negative blood donors. Currently a number of countries including United States screen all donations for anti HBc which is not mandatory in some other countries such as Iran [25]. Nevertheless it has been argued that the exclusion of anti-HBc positive donors is impractical in countries where HBV infection is prevalent and more than 20 percent of the population are positive for anti-HBc. At the present Iran is classified as having a low endemicity for hepatitis B infection and 7.93% of blood donors in the study were found to be positive for anti-HBc. Among them 30% was found to have detected HBV-DNA. In addition, while missing anti-HBs test might be a weakness of our study, considering lack of published data in humans, absence of any agreement among professionals regarding the relationship between HBV DNA detection and infectivity of the transfused units in humans with and without anti-HBs, and also taking into account that various blood components contain of plasma, it should be considered that any unit with detectable HBV DNA can potentially infect transfusion recipients with HBV. Therefore, to limit the transfusion transmission risk of occult hepatitis B virus in Iran blood banks, we conclude that introducing anti HBc screening may be practical.

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