

# Hepatitis B Core Antibody Immunoglobulin M in Blood Donors With a History of Hepatitis B Virus Infection

Helia Azimi,<sup>1,2</sup> and Maryam Vaezjalali<sup>1,3,\*</sup>

<sup>1</sup>Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

<sup>2</sup>Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, IR Iran

<sup>3</sup>Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

\*Corresponding author: Maryam Vaezjalali, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. Tel: +98-2123872556, Fax: +98-2122439972, E-mail: maryam.vaezjalali@sbm.ac.ir

Received 2016 April 05; Revised 2016 May 02; Accepted 2016 May 10.

## Abstract

**Background:** Hepatitis B is still an issue after blood transfusion. A reason could be the window period of hepatitis B infection in blood donors. In countries such as Iran hepatitis B surface antigen enzyme-linked immunosorbent assay (ELISA) is the only test to detect the virus in blood donors. This procedure may miss the window period of hepatitis B infected donors.

**Objectives:** The current study aimed to look for hepatitis B core antibody immunoglobulin M in blood samples of Iranian donors with a history of hepatitis B virus infection to detect window period infection.

**Materials and Methods:** Eighty serum samples with hepatitis B core antibody were collected from 1000 healthy blood donors, forty of them had been positive for hepatitis B virus DNA in authors' previous study and were diagnosed as occult hepatitis B infection. All 80 samples were tested for hepatitis B core immunoglobulin M.

**Results:** One thousand blood samples were collected from 64 (6.4%) female and 936 (93.6%) male subjects. None of the blood samples contained hepatitis B core immunoglobulin M. The study found no significant differences between male and female subjects in term of HBcAb positivity.

**Conclusions:** Hepatitis B core antibody immunoglobulin M positivity is different in healthy blood donors of different countries according to the prevalence of chronic hepatitis B and its vaccination. Based on the current study findings, all positive samples of hepatitis B core antibody in Iranian blood banks should be considered as candidates for occult hepatitis B not just the window period infected samples.

**Keywords:** Hepatitis B Virus, Blood Donors, Viral DNA, Immunoglobulin M

## 1. Background

Hepatitis B is still an issue after blood transfusion. Regardless of all efforts to guarantee blood safety, the highest risk is among transfusion-transmitted diseases, accounting for 1 per 63,000 transfused units (1). Thus, the risk of its virus infection through blood transfusion cannot be totally reduced by blood bank donor screening (2).

This occurrence is important because of two reasons. The first one is presence of occult hepatitis B infection in some blood donors. Basically, occult hepatitis B infection refers to the presence of hepatitis B virus DNA in the serum of people without hepatitis B surface antigen (HBsAg) but with serological markers of previous infections such as hepatitis B core antibody (HBcAb) (3). Therefore when using enzyme-linked immunosorbent assay (ELISA), there is still a potential risk of transmission of hepatitis B virus infection in the occult phase of hepatitis B by blood transfusion.

The second reason is the window period of hepatitis B

infection. After hepatitis B virus infection, the first serological marker to appear in the blood is the hepatitis B virus DNA, followed by HBsAg, the DNA polymerase and the hepatitis B envelope antigen (HBeAg). Afterwards, the antibodies to the hepatitis B core antigen, HBeAg and HBsAg can be detected. Screening the donated blood for HBsAg with ELISA test is a common method to detect hepatitis B infection. However, it does not totally rule out the risk of hepatitis B transmission. This is because during the host's serological response to infection, there is a phase during which the HBsAg cannot be detected in the blood, although hepatitis B infection is present. This phase is called the window period (4). It represents a carrier state of the disease. Therefore, there is a definite risk of transmitting hepatitis B to blood recipients. During the window period, detecting the antibody of hepatitis B core antigen is a useful serological marker for hepatitis B infection.

The immunoglobulin M (IgM) of the hepatitis B core antigen is the first to appear and indicates a recent infec-

tion. The immunoglobulin G (IgG) of hepatitis B core antigen appears later during the infection and shows a previous hepatitis B virus infection. People with IgG of hepatitis B core antigen may not be contaminated as they may have sufficiently high titers of antibodies for HBsAg, which are protective in nature. The affected people may actually be disease free (5).

According to some studies, HBsAg tests are not sufficient to detect hepatitis B virus infection in the blood supply (6-9). Thus, finding a marker for hepatitis B infection during the window period is important in blood banks, especially in low income countries in which DNA testing of all collected blood units is not feasible (10).

Based on the population proportion that is seropositive for HBsAg, the global epidemiology of hepatitis B virus infection is described in three categories of endemicity, i.e., high, intermediate, and low (11). With a 3% prevalence of HBsAg, Iran was an intermediate endemic country of hepatitis B virus infection. Nowadays, with the adoption of routine hepatitis B virus neonatal vaccination since 1993, hepatitis B virus endemicity has decreased to 1.7% (12). In a study in Tehran, 2447 HBsAg positive blood donors were compared with 2425 HBsAg negative donors. A major predicting factor of hepatitis B virus infection in that study was a history of receiving blood transfusion (13). Therefore, HBcAb IgM ELISA tests can be used to detect acute hepatitis B virus infection among blood donors.

## 2. Objectives

The current study aimed to determine the presence of HBcAb IgM in Iranian blood donors with HBcAb total who had donated blood to Tehran blood transfusion center in 2011. Also, it sought for a marker which would indicate hepatitis B infection during its window period.

## 3. Materials and Methods

Eighty serum samples were obtained from a thousand apparently healthy blood donors from Tehran blood transfusion center offices, forty serum samples were positive for hepatitis B virus DNA in authors' previous study (14). The inclusion criteria were not having HBsAg, hepatitis C virus and HIV antibodies in ELISA tests. Having HBcAb (total antibody), as an indicator of hepatitis B virus infection was another inclusion criterion.

### 3.1. ELISA Test

Hepatitis B core IgM was tested by ELISA kit (Diapros Diagnostic Bioprobes Milano, Italy). The kit was stored at room temperature. It included diluted samples, washing

buffer, control serum, six calibrators, substrate and sulfuric acid. In the first phase, according to the manufacturer's instruction, 100  $\mu$ L of calibrators, control serum and samples were added to the wells. The plates were incubated at 37°C for 60 minutes. They were washed with buffer five times with 30 seconds soaking between each washing. In the second phase 100  $\mu$ L substrate was added to the wells and they were incubated at 37°C for 60 minutes and then washed again. In the third phase they were put in room temperature for 20 minutes. Finally, 100  $\mu$ L of sulfuric acid was added to the wells. After this phase, positive samples were supposed to be blue while the negative ones were colorless. The results were read in 450 nm optical density.

### 3.2. Statistical Analysis

Data were analyzed by the statistical package for social sciences (SPSS) software version 16. Categorized variables were analyzed by Chi-square or Fisher exact tests as needed. Numerical variables were analyzed by independent sample T-test. P value less than 0.05 was considered significant.

## 4. Results

Based on authors' previous report (14), 1000 blood samples were collected from 64 (6.4%) female and 936 (93.6%) male subjects. The mean age of the subjects was  $37.7 \pm 10.5$  years old. Donation frequency was 1-92 times (mean of 5.67 times). No positive case of HBcAb IgM was detected. This meant that all HBcAb positive cases were HBcAb IgG positive and none of the blood donors were in the window period of hepatitis B virus infection. The study found no significant differences between male and female subjects in terms of HBcAb positivity.

## 5. Discussion

In blood banks of many countries, HBsAg ELISA test is the only way to detect bloods infected with hepatitis B virus. However, it is shown that HBcAb and nucleic acid tests are more effective to detect hepatitis B virus infection in blood donors. Since nucleic acid tests are expensive, it seems that HBcAb can be used for more effective diagnosis. This is because hepatitis B is more frequent after blood transfusion with HBcAb than without it. One possibility is that transfusion of blood collected from a donor in the window period may lead to post-transfusion of hepatitis B in the recipient (15, 16). However, other findings verify that testing blood donors for HBsAg alone is not sufficient to eliminate hepatitis B virus from a blood supply (10).

HBcAb positivity shows a history of hepatitis B virus infection. A national study in Iran estimated that 35% of the

Iranian population is positive for HBcAb (17). Also, a recent study in Iran reported HBcAb records from three crowded provinces in Iran. The study randomly selected 6,583 subjects from Tehran, Golestan and Hormozgan provinces, with the age range of 18 - 65 years old. Serum samples were tested for HBsAg and HBcAb. Various risk factors were recorded and multivariate analysis was conducted. The prevalence of HBsAg and HBcAb were 2.6% and 16.4%, respectively. Predictors of HBsAg or HBcAb in multivariate analysis were being older, not having high-school diploma, living in rural areas, and having a family member with liver disease (18).

Today, HBcAb has decreased among Iranians from 35% to 16.4%. However, in spite of nationwide vaccination of newborns against hepatitis B virus since 1992 in Iran, hepatitis B virus infection remains a very common cause of chronic liver disease which should be dealt with for at least the next 30 - 50 years (18).

All of the study samples were positive for HBcAb total and none had HBcAb IgM as the only serological evidence of hepatitis B virus acute infection. HBcAb positivity among blood donors is reported by other research groups. In a study in Pakistan, HBcAb prevalence in blood donors without HBsAg and hepatitis B virus DNA was 167 out of 966 (17.28%). It shows that more than 17% of healthy, young blood donors are already exposed to hepatitis B virus (19). Therefore, high prevalence of hepatitis B virus infection among blood donors is an important challenge to discard HBcAb positive blood samples in the developing countries. The results are higher than the previous Egyptian studies which reported 10.96% and 7.8% prevalence of HBcAb (20, 21).

Hepatitis B core antigen IgG may remain positive for a lifetime in an affected individual, although the individual has protective levels of HBsAb. Therefore, it does not necessarily mean that blood of such donors is contaminated. HBcAb IgM is a more specific marker for hepatitis B virus infection during the window period (7, 22). In a study in India, 704 blood samples were screened for HBcAb. A total of 11 (0.43%) blood units were reactive for HBcAb IgM (5).

In a study in Nigeria, HBsAg and its antibody were detected in 18 (19.6%) and 14 (15.2%) of 92 blood donors, respectively. Hepatitis B core antigen IgM was found in 12 (13.0%) of 92 blood donors, while HBeAg and its antibody were detected in 4 (8.9%) and 12 (26.7%) of 45 samples, respectively. Five (5.4%) of 92 donors had hepatitis B core antigen IgM as the only serological evidence of hepatitis B virus infection. The researchers suggested using HBcAb IgM in routine screening of blood donors in Nigeria (10).

There is higher prevalence in other countries such as 13.5% in Korea, 15.03% in Greece, 16.4% in Saudi Arabia and 76% in Ghana (23-25). All of these results are comparable to

the previous reports of countries with lower prevalence, including 0.56% in the United Kingdom, 0.84% in the United States, 1.4% in Germany and 4.85% in Italy (26-28). Screening bloods for HBcAb total is practical in the western countries because they have low incidence of HBsAg and HBcAb. Therefore, the HBcAb total positive blood units can be discarded from blood banks. This may not be practical in crowded countries in which the incidence of HBcAb total is high in the population.

In a study in Egypt, 3.8% of 7340 blood units had HBcAb IgM (29). Four of the HBcAb positive samples were also positive for HBsAb. Therefore, researchers suggested that HBcAb and hepatitis B virus DNA should be tested routinely in blood donors. If they were positive regardless of HBsAb titer, the blood should be discarded to reach a completely safe blood transfusion (29). However, factors such as prevalence of hepatitis B virus in different regions, sensitivity and laboratory test costs should be considered (29). According to the authors' previous study, findings of HBsAb and hepatitis B virus DNA with vaccine-escaped mutations among occult hepatitis B infected patients may present a new challenge for this protocol.

### 5.1. Conclusions

HBcAb IgM positivity is different in healthy blood donors of different countries. A reason might be the different prevalence of hepatitis B virus chronic infection in the world. On the other hand, HBcAb is an indicator of past hepatitis B virus infection which differs in different populations based on hepatitis B virus vaccination.

None of the healthy blood donors in the current study had HBcAb IgM. All positive samples of HBcAb in Iranian blood banks should be considered as candidates for occult hepatitis B not just the window period infected samples. However, a larger sample size should be considered to achieve better conclusions.

### Acknowledgments

The authors would like to thank Seyed Muhammed Hussein Mousavinasab for his sincere cooperation in editing the text.

### Footnotes

**Authors' Contribution:** Both authors contributed in isolation of samples, preparing the extract and writing the manuscript.

**Funding/Support:** This study was financially supported in part by Shahid Beheshti University of Medical Sciences, Tehran, Iran.

## References

- Almeida Neto C, Strauss E, Sabino EC, Sucupira MC, Chamone DA. Significance of isolated hepatitis B core antibody in blood donors from Sao Paulo. *Rev Inst Med Trop Sao Paulo*. 2001;**43**(4):203-8. [PubMed: [11557999](#)].
- Jongerijs JM, Wester M, Cuypers HT, van Oostendorp WR, Lelie PN, van der Poel CL, et al. New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion*. 1998;**38**(1):56-9. [PubMed: [9482395](#)].
- Chemin I, Trepo C. Clinical impact of occult HBV infections. *J Clin Virol*. 2005;**34** Suppl 1:S15-21. [PubMed: [16461218](#)].
- Makroo RN, Chowdhry M, Bhatia A, Arora B, Rosamma NL. Hepatitis B core antibody testing in Indian blood donors: A double-edged sword!. *Asian J Transfus Sci*. 2012;**6**(1):10-3. doi: [10.4103/0973-6247.95043](#). [PubMed: [22623835](#)].
- Kumar H, Gupta PK, Jaiprakash M. The role of anti-HBc IgM in screening of blood donors. *Med J Armed Forces India*. 2007;**63**(4):350-2.
- Banerjee K, Sarin SK, Naik SR, Khandekar P. Polymerase chain reaction based diagnostic assay for identification of hepatitis B virus. *Indian J Med Res*. 1992;**95**:173-8. [PubMed: [1398804](#)].
- Niermeijer P, Gips CH. Viral antibodies and the infectivity of serum in hepatitis B. *N Engl J Med*. 1978;**299**(17):958. doi: [10.1056/NEJM197810262991721](#). [PubMed: [692609](#)].
- Vaezjalali M, Norder H, Magnusius L, Jazayeri SM, Alavian SM, Mokhtari-Azad T. A new core promoter mutation and premature stop codon in the S gene in HBV strains from Iranian patients with cirrhosis. *J Viral Hepat*. 2009;**16**(4):259-64. doi: [10.1111/j.1365-2893.2009.01069.x](#). [PubMed: [19222745](#)].
- Vaezjalali M, Rezaee H, Goudarzi H. HBV S Gene Premature Stop Codon in Strains From Middle Eastern Patients. *Arch Clin Infect Dis*. 2012;**8**(1):3-7.
- Japhet MO, Adesina OA, Donbraye E, Adewumi MO. Hepatitis B core IgM antibody (anti-HBcIgM) among hepatitis B surface antigen (HBsAg) negative blood donors in Nigeria. *Viral J*. 2011;**8**:513. doi: [10.1186/1743-422X-8-513](#). [PubMed: [22074048](#)].
- Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev*. 2006;**28**:112-25. doi: [10.1093/epirev/mxj009](#). [PubMed: [16754644](#)].
- Zali MR. Epidemiology of hepatitis B in the Islamic Republic of Iran. *EMRO*. 1996;**2**(2):290-8.
- Alavian S, Mostajabi P, Malekzadeh R, Azimi K, Vosoogh H, Sarrafi M, et al. Evaluation of hepatitis B transmission risk factors in Tehran blood donors. *Govareh*. 2012;**9**(3):169-75.
- Vaezjalali M, Rashidpour S, Rezaee H, Hajibeigi B, Zeidi M, Gachkar L, et al. Hepatitis B viral DNA among HBs antigen negative healthy blood donors. *Hepat Mon*. 2013;**13**(3):e6590. doi: [10.5812/hepatmon.6590](#). [PubMed: [23675384](#)].
- Muhlbacher A, Zdunek D, Melchior W, Michl U. Is infective blood donation missed without screening for antibody to hepatitis B core antigen and/or hepatitis B virus DNA?. *Vox Sang*. 2001;**81**(2):139. [PubMed: [11555477](#)].
- Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. *Blood*. 2002;**100**(7):2637-41. doi: [10.1182/blood-2002-03-0798](#). [PubMed: [12239179](#)].
- Merat S, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. *Arch Iran Med*. 2000;**3**(4):192-201.
- Merat S, Rezvan H, Nouraei M, Jamali A, Assari S, Abolghasemi H, et al. The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: a population-based study. *Arch Iran Med*. 2009;**12**(3):225-31. [PubMed: [19400598](#)].
- Bhatti FA, Ullah Z, Salamat N, Ayub M, Ghani E. Anti-hepatitis B core antigen testing, viral markers, and occult hepatitis B virus infection in Pakistani blood donors: implications for transfusion practice. *Transfusion*. 2007;**47**(1):74-9. doi: [10.1111/j.1537-2995.2007.01066.x](#). [PubMed: [17207233](#)].
- El-Zayadi AR, Ibrahim EH, Badran HM, Saeid A, Moneib NA, Shemis MA, et al. Anti-HBc screening in Egyptian blood donors reduces the risk of hepatitis B virus transmission. *Transfus Med*. 2008;**18**(1):55-61. doi: [10.1111/j.1365-3148.2007.00806.x](#). [PubMed: [18279193](#)].
- Antar W, El-Shokry MH, Abd El Hamid WA, Helmy MF. Significance of detecting anti-HBc among Egyptian male blood donors negative for HBsAg. *Transfus Med*. 2010;**20**(6):409-13. doi: [10.1111/j.1365-3148.2010.01021.x](#). [PubMed: [20573069](#)].
- van Ditzhuijsen TJ, Seltens GC, van Loon AM, Wolters G, Matthyssens L, Yap SH. Detection of hepatitis B virus DNA in serum and relation with the IgM class anti-HBc titers in hepatitis B virus infection. *J Med Virol*. 1985;**15**(1):49-56. [PubMed: [3968544](#)].
- Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giachetti C, et al. The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. *Blood*. 2003;**101**(6):2419-25. doi: [10.1182/blood-2002-04-1084](#). [PubMed: [12393691](#)].
- Zervou EK, Dalekos GN, Boumba DS, Tsianos EV. Value of anti-HBc screening of blood donors for prevention of HBV infection: results of a 3-year prospective study in Northwestern Greece. *Transfusion*. 2001;**41**(5):652-8. [PubMed: [11346702](#)].
- Seo DH, Whang DH, Song EY, Kim HS, Park Q. Prevalence of antibodies to hepatitis B core antigen and occult hepatitis B virus infections in Korean blood donors. *Transfusion*. 2011;**51**(8):1840-6. doi: [10.1111/j.1537-2995.2010.03056.x](#). [PubMed: [21332731](#)].
- Soldan K, Davison K, Dow B. Estimates of the frequency of HBV, HCV, and HIV infectious donations entering the blood supply in the United Kingdom, 1996 to 2003. *Euro Surveill*. 2005;**10**(2):17-9. [PubMed: [15735312](#)].
- Kleinman SH, Kuhns MC, Todd DS, Glynn SA, McNamara A, DiMarco A, et al. Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening. *Transfusion*. 2003;**43**(6):696-704. [PubMed: [12757519](#)].
- Manzini P, Giroto M, Borsotti R, Giachino O, Guaschino R, Lanteri M, et al. Italian blood donors with anti-HBc and occult hepatitis B virus infection. *Haematologica*. 2007;**92**(12):1664-70. doi: [10.3324/haematol.11224](#). [PubMed: [18055990](#)].
- Badrawy H, Bakry R. Anti-HBc and HBV-DNA detection in blood donors negative for hepatitis B virus surface antigen. *Am J Mol Biol*. 2013;**3**:62-6.