

Detection Of Occult Hepatitis B Virus Infections In Surface Antigen Hbs Ag Negative Blood Donors In The Main Blood Bank In Nineveh -Iraq

Ahmed Abd Al-Salam Fawzi Altai*, Dunia Kamal Salim

Department of Biology, College of Science, Tikrit University, Tikrit, Iraq



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

<https://doi.org/10.54153/sjpas.2025.v7i1.877>

Article Information

Received: 08/04/2024

Revised: 10/05/2024

Accepted: 20/06/2024

Published: 30/03/2025

Keywords:

AST , ALT ,Hepatitis B virus, HBs Ag, blood bank

Corresponding Author

E-mail:

ahmedalmosully.aa@gmail.com

07719826740

Abstract

Viral hepatitis B is a virus that causes inflammation of the liver and can lead to both acute and chronic diseases. The purpose of this study was to determine how often healthy blood donors who tested negative for HBs Ag, HCV Ab in serum samples from the Nineveh Central Blood Bank tested positive for anti-hepatitis "B" core and had "HBV DNA" in their bodies I conducted this research and examined 2,000 serum samples taken from donors in who can give blood that was negative for HBs Ag ,HCV Ab. (EIAs) were used to determine the titters of HBV Markers in all samples that tested positive for anti-HBc . In addition to checking for HBs Ag we used (PCR) to check for (HBV-DNA) in any sample that came back positive for (anti-HBc) on its own or in combination with other markers for serology. Out of 2,000 blood samples, 20 (or 1% of the total) tested positive for anti-HBc. Out of 20 specimens that tested positive for anti-HBc, 16 (or 80%) tested positive for HBV DNA. The average viral load was 3500 copies per millilitre. Out of the samples that tested positive for anti-HBc, 50% had anti-HBs Ag, 25% had anti-HBe Ag, and 25% had anti-HBe. Except for four out of sixteen individuals who tested positive for HBV-DNA, Our research has shown, through the results of the ELISA test, that the results of the surface antigen (HBs Ag) screening test are inaccurate. This is due to several reasons, including that the detection of the surface antigen (HBs Ag) may have been conducted during the window period, which is the period when the surface antigen disappears during the examination, and all of the liver function tests came back within normal limits. The average levels of (ALT) in "HBV-PCR" positive participants were 14 IU/l, whereas the levels of (AST) were 23.7 IU/l.

Introduction

In transfusion medicine, one of the main concerns is blood products' safety. Donated blood is a more common vector for the transmission of viral hepatitis B (HBV) than viral hepatitis C (HCV) (1:60000 vs. 1:103000) [1]. Post-transfusion viral hepatitis B infection occurs sporadically despite the availability of a sensitive screening test for HBs Ag [2]. When commercial tests produce false negative results, there could be one of three reasons. The screening assay may miss blood donors infected with HBs Ag mutants or with low levels of

viral protein in their blood, which might endanger the blood supply [3], As indicated by researchers Rana, the year 2022 in Iraq[4]. A second theory is that the antibodies used in the tests do not detect certain viral variations because they produce different sequences [5]. Some variations in various regions of the genome suppress HBs Ag P synthesis [6]. Clearance of hepatitis B may be caused by an extremely high concentration of the hepatitis C virus (HCV). Maybe this is because HCV is so effective at triggering an immune response [7]. Whether an infection is acute, chronic, or resolved, "antibodies" to the hepatitis B core (HBc) "antigen" could be detected forever. These may exist in situations where neither HBs Ag nor anti-HBs antibodies are present, in the time between hepatitis B infection at its acute stage and the emergence of "anti-HBs antibodies", or in individuals who recovered from infection but no longer have detectable anti-HBs antibodies. Due to this, the existence of anti-HBc is linked to HBV infections. [8]. Scientific evidence suggests that some people who test negative for HBs Ag and others who test positive for anti-HBc can nonetheless replicate HBV [9,10]. Based on these results, it seems that the immune system maintains a very low level of the virus rather than eradicating it entirely when a person recovers from an acute viral hepatitis B infection. In serum samples from individuals who do not have "HBs Ag", there is a correlation between the detection of HBV DNA and the "anti-HBc" levels, which is a positive link [11]. According to estimates, viral hepatitis B causes the death of approximately (200,000 to 500,000) individuals per year [12]. Approximately 90% of babies who contract the hepatitis B virus will develop a persistent infection, which can potentially increase the likelihood of liver fibrosis and cancer [13]. Chronic infection is defined as lasting for a duration exceeding six months, and the likelihood of contracting HIV is influenced by its fluctuating association with the individual's age. Due to the replication of viral hepatitis B within hepatic cells and its impact on liver function, around 15–40% of infected persons may encounter many intricate hepatic issues [14]. Viral hepatitis can manifest signs such as fatigue, nausea, loss of appetite, vomiting, abdominal discomfort, and fluctuations in body temperature, along with specific clinical signs of liver and spleen enlargement [15, 16]. Specialized care is typically not utilized for the treatment of acute viral hepatitis B. There is a highly effective vaccine available for immunisation against diseases such as viral hepatitis B. In 2002, the World Health Organisation (WHO) advised that this vaccine be given as a standard measure [17]. In instances of persistent infections, two therapeutic approaches are employed in Europe and certain other nations worldwide (Lamivudine, Conventional Interferon Alfa IFNa). Type B also encompasses hepatitis B immunoglobulin, which prevents vertical transmission of the viral hepatitis B from an infected pregnant woman to her newborn [18].

Amis of Study:

This study was done to determine whether or not donors in excellent health who tested negative for HBsAg and anti-HCV had HBV-DNA in their serum samples. We tested whether anti-HBc could be used to screen donated blood.

Materials and Methods:

Donors tested negative for (HBs Ag) and (HCV Ab) among 2,000 blood samples obtained in Nineveh, Iraq's Central Blood Bank In August and September 2023 .

The Enzyme-Linked Immunosorbent Assay (ELISA):

Tests of RCR in real-time:

Measurement of viral hepatitis B DNA. A real-time PCR detection system developed by the 9 Towers analytical gene company in Germany was used in the HBV DNA quantitation test. The test was conducted with HBV DNA (primer design kit). Added 7.5µl of the mixture into every well using a pipette. Small pipette Each well should have (2.5) µl of "DNA template" and standard, whereas the negative well control should utilize (2.5) µl of (DNase/RNase) free water. Making a series of dilutions according to the standard curve. sorted tubes 2–6 and transfer 900 µl of water that is free of RNase and DNases into each labelled tube. Fill tube 2 with 100 µl of the positive control template using a pipette. Thoroughly detangle. Transferred 100 µl from tube 2 to tube 3 using the Chang pipette tip. Thoroughly detangle. Proceed with steps "4" and "5" once more to finish the dilution sequence (Amplification Protocol).

Table 1: The results were analyzed according to the manual

The number of cycles	Steps	Time	The temperature
50 cycles	Activation of enzymes	10 mints	95 Celsius degree
	Denaturation	5 mints	95 Celsius degree
	Data Gathering	25 mints	60 Celsius degree

Tests for Biochemistry:

Aspartate aminotransferase (AST), alanine(ALT), and other biochemical variables were present in all PCR-positive samples.

Results:

Serological findings:

Acute bilirubinemia (TBIL), aminotransferase (ALT), and alkaline phosphatase are the cutoff values for detecting anti-HBc antibodies in serum samples, which were also conducted using conventional techniques.

OD (450/620) nm (0.62) was discovered in 20 out of 1% of the samples of blood for which negative test results for HBsAg turned out to have HBc antibodies. As shown in the table 2.

Table 2: Rate of (anti-HBc IgM and IgG) among blood donors who tested negative for HBsAg

Blood donors in total	Anti-HBc IgG&IgM	
	Negative%	Positive%
2000	1980(99%)	20 (1%)

It was determined that an OD450/620 (nm) of 0.20 distinguished positive from negative results when testing blood samples for anti-HBs antibodies. Table 3 shows that out of 20

participants tested positive for (anti-HBc Ab), 10 tested positive for (anti-HBs Ab) (>10 mlU/ml), and 5 tested positive for (anti-HBeAg).

Table 3: The percentage of blood donors who had positive hepatitis B serology tests

Total Number of Positive Anti-HBc Tests	markers of HBV		
	Anti-HBeAg	HBeAg	Anti-HBsAg
20	5(25%)	5(25%)	10(50%)

Discoveries at the molecular level:

Table 4 shows that eighty percent, or 16 of the 20 specimens tested positive for HBc Ab, also tested positive for HBV DNA.

Table 4: How much HBV-positive DNA was found in blood donors who tested positive for anti-HBc

Sum of HBc Ab Positive Results	Percentage of positive HBV DNA	
	Negative%	Positive%
20	4(20%)	16 (80%)
Virus load on average	NOT detected	Over 3500 copies per millilitre

Chemical Markers:

Tests of liver function (LFT) were performed on samples that had a positive result for HBV-PCR. These tests included aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, and alkaline phosphatase. The results are displayed in Table 5. Except for four patients whose AST levels were much higher than normal (>33 IU/l), all of the LFT findings were within the normal range. Subjects that tested positive for HBV-PCR had an average ALT level of 14 ± 5.0 IU/l and an average AST level of 23.7 ± 9.9 IU/l. Since the ALT levels of our HBc-positive blood donors were within normal ranges.

Table 5: There were differences in the percentage of blood donors who tested positive for viral hepatitis B DNA based on factors such as height, liver function tests, and altitude.

The sum of all HBV DNA positive cases	Differences in liver function test results			
	Bilirubin (7-15 mmol/l)	ALP (30-85 U/l)	AST N.R (Up to 12 U/l)	ALT N.R (Up to 12 U/l)
16	0%	0%	25%	0%

Discussion:

Healthy blood donors who tested negative for HBsAg and anti-HCV antibodies had their serum tested for the presence of viral hepatitis B DNA and the incidence of anti-HBc positivity. There is currently no other diagnostic examination available for viral hepatitis B infection in Iraqi blood transfusion centers other than HBsAg detection. Compared to the 16.4% reported in Saudi Arabia, our finding of approximately 1% of donated blood that tested positive for anti-HBc Ag was lower. However, they failed to disclose what fraction of the donated blood tested positive for HCV antibodies.

Most research on latent HBV infection has shown that peripheral mononuclear cells or the liver have a greater rate of HBV-DNA detection than serum or plasma [19]. The prevalence of HBV infection (as measured by DNA in serum samples) was 1% in our study cohort of healthy blood donors who tested positive for HBc antibodies.

However, there was no connection between HBc antibodies and viral hepatitis B-DNA positivity. It appears that the undetectable circulating levels of HBsAg for current screening assays are the cause of the high rate of post-transfusion hepatitis (PTH). However, some of these donor units can be ruled out by anti-HBc antibody screening tests. We cannot determine if the removal of (anti-HBc) positive units will result in the eradication of (PTHB) since we have not analyzed (anti-HBc) negative samples for the existence of (HBV DNA).

We separated our pool of potential blood donors into two categories: (HBsAg) positive and (HBsAg) negative, in accordance with the theory put forth by [20]. There were two subgroups of sero-negative subjects: those who tested positive for anti-HBc and those who tested negative. Subdividing the (1%) anti-HBc positive subgroup into 50% with anti-HBsAg and 50% without anti-HBs allows for a more precise analysis of the blood donor population. According to reports, (anti-HBc) positive, (HBsAg) negative, and (HBsAb) positive donors can all be detected with HBV-DNA [21, 22].

Results showed that 50% of people who tested positive for anti-HBc antibodies also tested negative for anti-HBs antibodies, suggesting that these individuals may have had HBV. People with persistently low levels of HBV may have recovered from a prior infection. Individuals who have received a vaccination against hepatitis B and have an HBs antibody titer of more than 10 IU/mL have never shown symptoms of the virus.

Although the advancement of HBc Ab in some vaccine recipients is suggestive of HBV infection, this typically occurs in the absence of disease. In most cases, the protective (HBs Ab) antibody will target the determinant of HBsAg. It is possible for antibodies to target determinants other than "a" and fail to kill the circulating virus in such instances. This means that these instances should be considered chronic infections [23].

People who test positive for (HBc Ab) and (HB Ab) antibodies may be suffering from a chronic, persistent HBV infection if they find HBV DNA.

In nations where viral hepatitis B infection is common and over 20% of the population is (HBc Ab) positive, it is not feasible to exclude donors who test positive for the virus [24]. On

the other hand, our study only discovered that 1 percent of blood donors tested positive for HBc Ab. One way to check for HBV infection in organ transplant patients is with an anti-HBc test. If the results come back positive, it is recommended that sera be tested for viral hepatitis B DNA using polymerase chain reaction (PCR). Donating an organ should only be done in cases of extreme medical need or in the event of a good result.

Conclusion:

Blood donor volunteers should undergo routine anti-HBc antibody testing; the blood should be destroyed if the results come back positive, regardless of the HBsAb titer. Our current study showed, through the results of the enzyme-linked immunosorbent test (ELISA), that the results of the surface antigen (HBs Ag) screening test were inaccurate. This is due to several reasons, including that the detection of the surface antigen (HBs Ag) may have been conducted during the window period of infection of the patient, or the reason may be the presence of a low viral load in the blood and its greater presence in the liver, or the reason may be the use of laboratory equipment of non-reliable origin. These reasons are considered an indicator of the weak sensitivity of this test and giving negative results even though the donor has hepatitis. Therefore, the use of the enzyme-linked immunosorbent test (ELISA) to detect the alloantigen (HBcor) and the polymerase chain reaction technique to detect deoxyribonucleic acid (DNA-PCR) in cases negative for the surface antigen, Real time - PCR is considered one of the tests. Confirmatory confirmation of blood donors at the main blood bank in the city of Mosul. Anti-HBcor (IgM) is the first specific antibody to appear after exposure to acute infection in donors negative for the surface antigen HB Ag, which gives an indication that the infection is recent. Anti-HBcor (IgG) appears at the time of infection. Later, this gives an indication that the infection is old (chronic) [25].

For a more comprehensive assessment of HBV infection in the blood donor, additional HBV-DNA tests could prove beneficial.

The relationship between hepatitis B infection and viral hepatitis B core (hidden) HBcor: This study showed, as this study was compatible to the study conducted in China[26], and is not compatible with the study conducted in Yemen[27].

References

1. Grubyte, S., Urboniene, J., Nedzinskiene, L., Jelinskaite, A., Zagminas, K., Ambrozaitis, A., & Jancoriene, L. (2021). Prevalence, incidence and residual risk of transfusion transmitted viruses (HBV, HCV and HIV infections) in Lithuanian blood donors from 2004 to 2018: The incidence/window-period model study. *Plos one*, 16(2), e0246704.
2. Arababadi, M. K., Hassanshahi, G., Pourfathollah, A. A., Zarandi, E. R., & Kennedy, D. (2011). Post-transfusion occult hepatitis B (OBI): a global challenge for blood recipients and health authorities. *Hepatitis monthly*, 11(9), 714.

3. Jongerius 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* 2008; 38: 56-9.
4. Mohsen, R. T., & Anmar, K. M. (2022). Evaluating of the association of ABO blood groups, age and sex with chronic hepatitis B virus in Iraqi patients. *Samarra Journal of Pure and Applied Science*, 4(2), 16-22.
5. Khedive, A., Sanei-Moghaddam, I., Alavian, S. M., Saberfar, E., Norouzi, M., Judaki, M., ... & Jazayeri, S. M. (2013). Hepatitis B virus surface antigen (HBsAg) mutations are rare but clustered in immune epitopes in chronic carriers from Sistan-Balouchestan Province, Iran. *Archives of Iranian Medicine*, 16(7), 0-0.
6. Carman WF, Mimms LT. Pre-S/S gene variants of hepatitis B virus. In: Rizzetto G, editors. *Viral hepatitis and liver disease*. Torino, Italy: Edizioni Minerva Medica; 2009 p. 108-15.
7. Vaillant, A. (2021). Transaminase Elevations during Treatment of Chronic Hepatitis B Infection: Safety Considerations and Role in Achieving Functional Cure. *Viruses*, 13(5), 745.
8. Lee WM. Hepatitis B virus infection. *N Engl J Med* 2010;337: 1733-45.
9. İnce, N., Tosun, S., Balkan, A., Uğuz, M., Çuvalcı, N. Ö., Yıldız, İ. E., ... & Bekçibaş1, M. (2020). How Aware are We of the Immune Status of Hepatitis B and Hepatitis A in Chronic Hepatitis C Patients? A Multicenter Retrospective Study from Turkey. *VIRAL HEPATITIS DERGISI-VIRAL HEPATITIS JOURNAL*, 26(3).
10. Mortensen, E., Kamali, A., Schirmer, P. L., Lucero-Obusan, C., Winston, C. A., Oda, G., ... & Holodniy, M. (2016). Are current screening protocols for chronic hepatitis B virus infection adequate?. *Diagnostic microbiology and infectious disease*, 85(2), 159-167.
11. Iizuka H, Ohmura K, Ishijima A, Satoh K, Tanaka T, Tsuda F, et al. Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. *Vox Sang* 2009; 63 : 107-11.
12. Karimi, G., Zadsar, M., Vafaei, N., Sharifi, Z., & Falah Tafti, M. (2016). Prevalence of antibody to Hepatitis B core antigen and Hepatitis B virus DNA in HBsAg negative healthy blood donors. *Virology journal*, 13, 1-6.
13. Malekzadeh R, Khatibian M, Rezvan H. Viral hepatitis in the world and Iran. *J Iranian Med Council* 2007; 15 :183- 200.
14. Merat SH, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. *Arch Iranian Med* 2008; 3 : 192-201.
15. Amini S, Mahmoodi MF, Andalibi S, Solati AA. Seroepidemiology of hepatitis B, delta and human immunodeficiency virus infections in Hamadan province, Iran: a population based study. *J Trop Med Hyg* 2009; 96 :277-87.
16. Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 2009; 339 : 237-8.
17. Gish RG, Lau JY, Brooks L, Fang JW, Steady SL, Imperial JC, et al. Ganciclovir treatment of hepatitis B virus infection in liver transplant recipients. *Hepatology* 2006;23: 1-7.

18. Bernvil SS, Andrews V, Kuhns MC, McNamara AL. Hepatitis B core antigen antibody as an indicator of a low grade carrier state for hepatitis B virus in a Saudi Arabian blood donor population. *Transfus Sci* 2007; 18 : 49-53.
19. Torii N, Hasegawa K, Joh R, Hayashi N. Configuration and replication competence of hepatitis B virus DNA in peripheral blood mononuclear cells from chronic hepatitis B patients and patients who have recovered from acute self-limited hepatitis. *Hepatology* 2007; 44: 234-43.
20. Coppola, N., Onorato, L., Pisaturo, M., Macera, M., Sagnelli, C., Martini, S., & Sagnelli, E. (2015). Role of occult hepatitis B virus infection in chronic hepatitis C. *World journal of gastroenterology*, 21(42), 11931.
21. 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* 2009; 100 : 2637-41.
22. Roche B, Feray C, Gigou M, Roque-Afonso AM, Arulnaden JL, Delvart V, et al. HBV DNA persistence 10 years after liver transplantation despite successful anti-HBs passive immunoprophylaxis. *Hepatology* 2008; 38 : 86-95.
23. Seddigh-Tonekaboni S, Waters JA, Jeffers S, Gehrke R, Ofenloch B, Horsch A, et al. Effect of variation in the common "a" determinant on the antigenicity of hepatitis B surface antigen. *J Med Virol* 2008; 60 : 113-21.
24. Nandi J, Benerjee K. Detection of hepatitis B virus DNA in donor blood by the polymerase chain reaction. *Natl Med J India* 2008; 5: 5-7
25. Zangiabadian, M., Zamani, A., Nasiri, M. J., Behzadi, E., & Fooladi, A. A. (2022). Diagnostic accuracy and validity of serological and molecular tests for hepatitis B and C. *Current Pharmaceutical Biotechnology*, 23(6), 803-817.
26. Ye, X., Li, T., Li, Y., Zeng, J., Li, R., Xu, X., ... & Li, L. (2023). Comparative analysis of hepatitis B virus infections in blood donors born before and after the implementation of universal HBV vaccination in southern China. *Transfusion Medicine*, 33(1).
27. Al-khulidi, J. M., Al-Taj, M. A., & Abdullah, A. A. (2023). Detection of Hepatitis B Virus by HBsAg and Total HBc Antibody among Blood Donors at National Blood Transfusion and Research Center in Taiz City, Yemen. *Al-Saeed University Journal of Applied Sci.*

الكشف عن الإصابات بفيروس التهاب الكبد الوبائي الخفي عند المتبرعين بالدم السلبيين للمستضد السطحي في بنك الدم الرئيسي في محافظة نينوى – العراق

أحمد عبد السلام فوزي الطائي*، دنيا كمال سليم
قسم علوم الحياة، كلية العلوم، جامعة تكريت، تكريت، العراق

معلومات البحث:

تاريخ الاستلام: 2024/04/08

تاريخ التعديل: 2024/05/10

تاريخ القبول: 2024/06/20

تاريخ النشر: 2025/03/30

الكلمات المفتاحية:

ناقلة أمين الأسبارتات، ناقلة أمين الألانين، التهاب الكبد الفيروسي نوع ب، المستضد السطحي لفيروس الكبد ب، بنك الدم

معلومات المؤلف

الايمل: ahmedalmosully.aa@gmail.com

الموبايل: 07719826740

الخلاصة:

التهاب الكبد نوع ب هو فيروس يسبب التهاب الكبد ويمكن أن يؤدي لأمراض حادة ومزمنة. وكان الغرض من هذه الدراسة هو تحديد عدد المتبرعين بالدم الأصحاء والذين كانت نتائج اختباراتهم سلبية لـ (HCV) (Ab & HBs Ag) في عينات المصل المأخوذة من بنك الدم الرئيسي في نينوى وإيجابيين للحمض النووي HBV-DNA في أجسادهم ، في هذا البحث تم فحص 2000 عينة مصل من المتبرعين بالدم السلبيين للمستضد السطحي HBs Ag ، تم إجراء اختبار الحمض النووي HBV-DNA PCR للعينات التي جاءت نتائجها إيجابية لفحص لب الفيروس HBcore. من بين 2000 عينة دم، كان اختبار 20 (أو 1% من الإجمالي) إيجابيًا لمستضادات HBc. ومن بين 20 عينة كانت نتيجة اختبارها إيجابية لمستضادات HBc، كانت 16 (أو 80%) إيجابية لـ DNA لفيروس التهاب الكبد B. المتوسط كان الحمل الفيروسي 3500 نسخة لكل مليلتر، ومن بين العينات التي جاءت نتيجة اختبارها إيجابية لمستضادات HBc، كان 50% منها يحتوي على مضادات HBs Ag و25% كانت تحتوي على مضادات HBe Ag و25% كانت تحتوي على مضادات HBe. باستثناء أربعة من أصل ستة عشر شخصًا جاءت نتيجة اختبارهم إيجابية لـ HBV-DNA، لقد أظهر بحثنا وذلك من خلال نتائج اختبار ELISA عدم دقة نتائج اختبار فحص المستضد السطحي (HBs Ag) وهذا يعود لعدة أسباب منها قد يكون الكشف عن المستضد السطحي (HBs Ag) قد اجري في أثناء فترة النافذة وهي فترة اختفاء المستضد السطحي أثناء الفحص، عادت جميع اختبارات وظائف الكبد ضمن الحدود الطبيعية. وكان متوسط مستويات (ALT) في المشاركين الإيجابيين لفيروس 14 وحدة دولية/لتر، في حين كانت مستويات AST (23.7 وحدة دولية/لتر).