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Occult hepatitis B- viral infections among blood donors in Duhok-Iraq

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Abstract

Background & objectives: When a patient has occult hepatitis B virus infection, HBV-DNA is detected in blood or liver tissues with negative HBsAg, with or without anti-HBc antibodies. The study's objective was to ascertain the prevalence of occult HBV infection among Duhok City blood donors.

Methods: A cross-sectional study has been conducted at Duhok blood bank from November 2022 to January 2023. Plasma samples were obtained from 100 willing blood donors (98male, 2 females, 21-67 years old) who tested negative for HBsAg but positive for AntiHBc. HBsAg negative/anti-HBc positive blood donors have been examined by Anti-HBs II (immunoassay for quantitative detection of antibodies to hepatitis B surface antigen). Next, with the use of a Viral Nucleic Acid Extraction Kit, DNA was isolated from plasma samples. An automated real-time PCR approach was used to identify HBV DNA.

Results: Of the 3156 HBsAg negative donors, 100 (3.16%) tested positive for anti HBc antibodies (total), 20 (20%) tested negative, and 80 (80%) tested positive for anti HBs Antibodies. All 100 donors had negative results from the real-time PCR with no HBV-DNA. No significant correlations were found between AntiHBc and AntiHBs results with sex and different age groups of donors.

Conclusion: No occult HBV infection was detected among Anti-HBc positive donors who were deferral from blood bank reserve. Taking in consideration that 80% of them have protective titer of Anti -HBs, reevaluation of the exemption of such blood donors should be done to reduce the deferral of donors and enhance blood bank reserve.

Keywords: Anti HBc, Blood donors, Hepatitis B virus, Occult Hepatitis B

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Introduction

Infection with hepatitis B virus (HBV), which affects about 240 million individuals worldwide, is seen as a serious health issue in Asia, Africa, and the Middle East.[1] The majority of HBV infections are self-limited acute infections, but 5% of them progress to chronic infection and result in consequences including hepatocellular carcinoma (HCC) and liver cirrhosis.[2] The WHO reports that the HBV has infected half of world's population. It has been observed that both chronic and acute HBV infections have the simultaneous presence of HBsAg and HBc antibody (anti-HBc). The persistence of serum HBsAg for longer than six months following acute infection is referred to as chronic HBV infection. Along with HBsAg, chronically infected people most likely have elevated levels of anti-HBc in their serum. Since HBV produces hepatitis B e antigen (HBeAg) throughout the active replication phase, the existence of HBsAg and anti-HBc characterizes the status of chronic HBV carriers.[3] People who have naturally contracted HBV are known to have anti-HBc, and its existence in the absence of HBsAg is typically interpreted as proof of prior HBV exposure. Isolated anti-HBc is the existence of anti-HBc without both HBsAg and anti-HBs antibodies. Anti-HBc positive indicates a risk of continued HBV infection in addition to previous infection history.[4] When HBV DNA is found in the blood or liver tissue of HBsAg negative patients, whether or not they have anti-HBc antibodies, the condition is known as occult HBV infection (OBI). A higher percentage of OBI patients have no symptoms, while viral DNA screening is used to make diagnosis. Regular testing for HBsAg in donated blood does not stop HBV from spreading, which highlights the need of identifying occult HBV infection. Thus, it is believed that the lack of HBsAg in blood lowers the risk of transmission.[5] Almost all transmission routes could be avoided, and blood transfusion is the most. Researches has shown that transmission through HBsAg negative blood elements could still happen throughout the acute phase of infection during seronegative window period or throughout chronic stages of infection with undetectable HBsAg. The first notable success in boosting transfusion safety came with the discovery of HBsAg in early 1970.[6] When immunosuppressed, an individual with OBI has a chance of regaining infection, and since HBsAg is not visible, there is a persistent danger of spreading the infection to other individuals.[7] The frequency of OBI and Anti-HBc depends on the level of HBV endemicity; in low-endemic areas, 10–20% of all HBV-positive individuals have anti-HBc. Within high-endemic areas such as Asia and Sub-Saharan Africa, the incidence of Anti-HBc is about 50% in general populations. Approximately 20% of all OBI have a serological pattern that is seronegative, meaning they are negative for all serological HBV markers. The remaining 80% of OBI have a seropositive pattern, which is defined as positive for anti-HBc and/or anti-HBs.[8] The study was conducted to determine the rate of OBI among blood donors at Duhok Blood Bank, serological techniques were used to identify anti-HBc and anti-HBs antibodies. Real-Time PCR was then used to establish the existence of HBV-DNA in donors' blood.

Patients and methods:

The Study design was a cross-sectional study at the Blood Bank of Duhok City, involving 100 willing blood donors (98 male-2 female) between the ages of 21 and 67 (mean 40.2, SD± 11.35). The three-month period from November 2022 to January 2023 saw it completed. Exclusion criteria involved patients with HBsAg positive result and non-consenting participants. Formal patient permission was applied for, and the Kurdistan Higher Council of Medical Specialties has approved the research ethical

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committee. A total of 100 plasma samples out of 3156 donors with negative HBsAg were randomly collected from the Blood Bank of Duhok city / virology department. After confirmation of negative HBsAg, another test was applied to check for Anti-HBc (total) antibodies by CLIA (LIAISON XL-Anti-HBc). With the use of Anti-HBs II kit (Immunoassay for the quantitative determination regarding antibodies to HBsAg and analyzed by Cobas e411, samples that tested negative for HBsAg but positive for Anti-HBc have been subjected to a second serological test for checking Anti-HBs antibodies. A titer equal to or greater than 10 IU/L has been considered positive. Next, HBV-DNA was found by PCR to validate OBI. With the use of (AddPrep Viral Nucleic Acid Extraction Kit), HBV-DNA was extracted, and HBV-Monitor-L PCR Kit was used for real-time PCR following the manufacturer's instructions. we used SPSS version 26 to analyze all of the data, and the variables have been characterized by their mean, range, and standard deviation. At $p<0.05$, the threshold for statistical significance was established.

Results:

At Blood Bank in Duhok City, Blood samples were collected from a total of one hundred prospective blood donors. They ranged in age from 21 to 27 (mean 40.2, SD± 11.35). Among Anti-HBc antibodies positive donors, 98% were male participants while only 2% were female. 20% of participants were anti-HBs negative, and 80% of participants were anti-HBs positive. No significant correlation was found between sex and anti-HBc or anti-HBs antibodies (p value = 0.563). Regarding the type of donors, Table (1) indicates that 52% of repeat donors and 48% of first-time blood donors had anti-HBc antibodies, while 75% of repeat donors and 85.4 % of first-time donors had anti-HBs antibodies. However, the type of donors was not found to be significantly correlated with positive anti-HBc and anti-HBs antibodies.

Table (1): Serological and molecular results concerning sex and number of donations

	Anti-HBc +ve No. (%)	Anti-HBs +ve No. (%)	Anti- HBs -ve No. (%)	HBV-DNA	p value
Male	98 (98%)	79 (80.6%)	19 (19.4)	0	0.563
Female	2 (%)	1 (50%)	1 (50%)	0	
Donor type					
First time	48 (48%)	41(85.4%)	7 (14.6%)	0	0.848
Repeated donors	52 (52%)	39 (75%)	13 (25%)	0	
Total No. (%)	100	80	20	0	

According to the age groups and the results of anti HBs antibodies, the highest percentage of (92.8%) was detected in the age group 50-59 years old. the lowest percentage of Anti HBs (50%) was detected among the age group 60-69 years old. The rate of anti-HBs antibody positivity in donors in the age range of 30-39 years old was 83.8%, whereas the rate in the age range of 40-49 years old was 74.19%. Therefore, age groups and anti-HBs antibodies did not significantly correlate (p value=0.208), Table (2).

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Table (2): The serological markers and molecular tests among different age groups

Age groups (years)	AntiHBs No. (%)	AntiHBs No. (%)	HBV-DNA	p value
20-29	15 (83.3)	3(16.7)	0	0.208
30-39	26(83.8)	5(16.2)	0	
40-49	23(74.19)	8(25.8)	0	
50-59	13(92.8)	1(7.2)	0	
60-69	3(50%)	3(50)	0	
Total	80	20	0	

Regarding the anti HBc results among different age groups, most of the participants with positive Anti HBc fall in age groups (30-39 and 40-49 years old) with the percentage of 31% in each of them in comparison to other age groups which were less than 31%. Twenty participants had an protective titer of Anti HBs (<10iu/Ml) which is considered a negative result. The other 80 donors had protective titer of Anti HBs (positive) >10iu/Ml which were distributed as follows: 15 of them had a titer of 10-100 iu/Ml and 65 with titer >100 which is considered high positive and they were mostly in age groups 30-39 as well as 40-49 years old as shown in table 3, but no significant correlation was detected between different age groups and titer of AntiHBs antibodies (p value =0.703), Table (3).

Table (3): The titer of Anti HBs antibodies among different age groups

	Anti HBc +ve No. (%)	Anti HBs <10iu/Ml	AntiHBs 10-100 iu/Ml	AntiHBs >100 iu/Ml	p value
Total No. (%)	100	20	15	65	0.703*
male	98 (98%)	19	15	64	
female	2 (2%)	1	0	1	
Age groups					
20-29	18 (18%)	3	2	13	
30-39	31 (31%)	5	4	21	
40-49	31 (31%)	8	3	20	
50-59	14 (14%)	1	4	9	
60-70	6 (6%)	3	2	2	

Discussion:

OBI has been defined as the positive HBV-DNA by PCR with or without serological markers. Most of these cases are associated with serological markers (Anti HBc antibodies ± AntiHBs antibodies) which is about 80% of them, while 20 % were seronegative without any serological markers.[9,10] In this cohort study all suspected blood donors to have OBI were positive for AntiHBc measured by CLIA which clarifies they are considered serological positive suspected cases. The detection of 100 donors with positive Anti HBc among 3156 HBsAg negative donors (3.1%) is an alarming mark, since these cases in Duhok Blood bank are considered potentially infected with HBV without implementation of PCR and are discarded. This rate of total AntiHBc among blood donors in Duhok is consistent with the fact that Iraq is considered as low endemic region of HBV infection. Mohammed et al 2022, found that 1.26% of blood donors in Basra were Anti HBc antibodies positive which is relatively similar to our result.[11] Other studies done in Baghdad and Babylon revealed rates of 0.7% and 0.5% respectively which are less than reported in this study.[12,13] Different rates of AntiHBc had been recorded in nearby countries including Turkey, Iran

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and Saudi Arabia: 8.5%, 2.6, 2.91% respectively.[14-16] Different studies revealed different results which could be affected by the type of kits used and the techniques applied. there is a debate regarding the use of anti HBc tests to screen blood donors for OBI in blood transfusion centers attributed to the false positive Anti HBc results among blood donors from 8-10%).[17,18] Application of this test may reveal high percentage of Anti HBc with deferral of many blood donors which will have an impact on reducing blood donors reserve.[19,20] When Anti-HBs antibodies were tested for all donors with positive Anti-HBc, we discovered that 80/100 donors (80%) had positive results with titers of 10-100 iu/Ml (15 donors) and more than100 iu/Ml (65 donors), in contrast the remaining 20 donors (20%) had negative results with titers of less than 10 iu/Ml. This shows that the majority of cases with Anti HBs >10 have previously been exposed to the virus and recovered, resulting in the acquisition of both Anti HBc and Anti HBs antibodies. However, the deferral of these donors is dubious, and additional research is necessary to determine whether or not we might use their blood, particularly if their PCR results are negative for cases with Anti HBs >100 iu/Ml. Just 20% of Anti-HBc positive donors, even in the case of negative PCR results, had negative Anti-HBs antibodies, placing them in the category of OBI.The Anti HBc positive donors were tested for HBV – DNA detection by real time PCR but no one was found positive. The Routine test in Duhok-transfusion center does not include nucleic acid test (NAT) although, some transfusion centers use it as a screening method.[21] Using NAT as the sole test for OBI identification is controversial as it has produced negative results in certain donors who tested positive for Anti-HBc, even though liver biopsy samples contain HBV-DNA instead of blood.[22,23] As a result, the cost-effectiveness and if the area under inquiry is endemic for HBV with a high frequency of anti-HBc determine whether to apply anti-HBc, NAT, or both tests. utilizing both tests together is more beneficial compared to using just one of them.Most of the cases of Anti-HBc positive donors were from 30-49 years old. Two studies done in India and Nigeria released results that total AntiHBc was correlated with increasing age and found to be more in group of blood donors who were older than 35 years in comparison to those younger than 35 years which could be emphasized by the fact that HBV vaccine was not available routinely 35 years ago.[24,25] Since 98% of donors were male, no significant difference was detected regarding sex. In addition, there is no significant correlation between donors for the first time and repeated donors regarding the serological markers and molecular test.To ensure the safety of blood transfusion it is recommended to check donors for HBsAg and simultaneously for Anti-HBc to exempt those with suspected OBI and if applicable to run NAT to prove occult infection although these measures may withstand deferral of many donors from reserve.[26]

Conclusion:

The use of PCR was conclusive in determining the rate of OBI among donors in Duhok transfusion center which revealed negative results. Detection of protective titer of AntiHBs among most of AntiHBc positive donors mandates urgent intervention to apply new strategy to how to deal with this situation since the policy in Duhok and other centers is to discard these donors from blood donors reserve with future consequences.

Conflict of interest

We the authors declare no conflict of interest.

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