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Prevalence of Hepatitis B Core Antibodies and Occult Hepatitis B Infection among Blood Donors in Erbil Governorate, Iraq

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ABSTRACT

The Hepatitis B Virus (HBV) remains a considerable risk to blood transfusion safety, especially through occult hepatitis B infection (OBI), defined by undetectable Hepatitis B surface antigen (HBsAg) yet the presence of HBV DNA in the bloodstream. Identifying and investigating the prevalence of OBI is essential as these infections can get past normal screening tests, which can lead to accidental transmission through transfusion. This study aimed to evaluate the prevalence of total hepatitis B core antibody (HBcAb) and identify OBI among blood donors in Erbil Governorate, Iraq. A total of 31,631 blood donors were tested for total HBcAb between September 2024 and January 2025, using the Liaison XL chemiluminescence immunoassay machine. Out of these 31,631 blood donors, 388 (1.23%) showed positive results for the total HBcAb. Among the positive cases, 65 samples were randomly chosen to detect OBI by viral load detection using quantitative real-time polymerase chain reaction. All samples were negative for HBsAg during routine screenings. Occult OBI was detected within 17 (26.15%) of the HBcAb-positive, HBsAg-negative blood donors. Despite the application of HBcAb screening, the absence of molecular testing may continue to provide an opportunity for HBV transmission. Incorporating HBV DNA testing for positive cases may enhance the safety of blood transfusions.

Index Terms: Blood Donors, Hepatitis B Core Antibody, Occult Hepatitis B, Quantitative Polymerase Chain Reaction, Erbil

1. INTRODUCTION

The Hepatitis B virus (HBV) infection is a significant worldwide health concern. In 2022, around 254 million individuals were chronically infected with HBV, accounting for 83% of the 1.3 million viral hepatitis deaths in that year [1]. Blood and blood derivatives are important elements used to preserve life. The safety of blood and its components is crucial, as individuals are frequently transfused when in a compromised health condition. The HBV, as a transfusion-transmissible disease, continues to pose a risk in donated blood despite advancements in blood donation precautions [2]. HBV transmission through transfusion poses a challenge to guaranteeing transfusion safety, particularly in regions with high HBV prevalence [3].

The extensive use of sensitive tests for Hepatitis B surface antigen (HBsAg) screening has significantly decreased the occurrence of transfusion-transmitted HBV. However, it has been established that persons lacking detectable HBsAg could transmit HBV. Nevertheless, these diagnostic methodologies are susceptible to overlooking occult hepatitis B infection (OBI), characterized by the detection of replication-competent HBV DNA in liver tissue or blood of patients who have tested negative for HBsAg employing chemiluminescent

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immunoassay or enzyme-linked immunosorbent assay [4], with the existence of other viral markers, such as Hepatitis B core antibody (HBcAb) [5].

The OBI was identified in blood donors with a frequency that ranges from >1% to 16%, based on the endemicity of HBV infection, the screening nucleic acid testing (NAT) employed, and the confirmatory methods utilized [6]. Despite the considerable risk of transfusion-transmitted infection with HBV associated with OBI donations, screening for OBI is challenging because of the occasional presence of extremely small viral loads and mutations within the HBV genome [7]. If detected, virus loads in the blood of persons with OBI are typically low (below 200 IU/mL) [8], while the lowest infectious dosage of OBI donor plasma through transfusion was detected as 3 IU [9]. Although the increasing quantity of research on occult HBV infections, the global incidence patterns of such infections remain unclear, and their clinical significance has been contentious [10].

Considering the established HBcAb screening standards in Iraq, particularly in Erbil Governorate, the true frequency of OBI within blood donors remains poorly examined because of the absence of routine molecular analysis. The present research seeks to address this deficiency by evaluating the incidence of HBcAb and the detection of HBV DNA within blood donors in the area.

2. METHODOLOGY

2.1. Study Population

This cross-sectional study was conducted between September 2024 and January 2025, during which a total of 31,631 blood donor candidates visited the Blood Bank in Erbil Governorate, Kurdistan Region, Iraq. Blood samples were collected from all blood donors in gel tubes (with silica-based clot activators), centrifuged at 4000 rpm for 15 min, and sent for serological analysis. A whole blood sample was put into a citrate phosphate dextrose adenine-1 blood bag and spun at 4000 rpm for 10 min to separate the plasma, which was used for molecular analysis. A questionnaire was used to collect the blood donors' demographics.

2.2. Serological Testing

The screening for detection of HBcAb was done using the Liaison XL, which is a high throughput, fully automated immunoassay analyzer using chemiluminescence technology.

For the results, the cutoff value discriminating between the presence and absence of anti-HBc has an index value of 1. Sample results were interpreted as samples with anti-HBc levels equal to or above an index value of 1 being considered non-reactive in the assay, and samples with anti-HBc levels below an index value of 1 being considered reactive in the assay.

2.3. Molecular Detection

2.3.1. DNA extraction

The DNA extraction from samples was carried out using a DNA-sorb-B nucleic acid extraction kit (AmpliSens Russia). The extraction steps provided by the manufacturer were followed after the optimization. In summary, samples were initially settled at room temperature, followed by the addition of 300 µL of Lysis Solution and 100 µL of sample to microcentrifuge tubes, which were then vortexed and incubated at 65°C for 5 min. Subsequently, 25 µL of Universal Sorbent was introduced, followed by vortexing, 5 min of incubation at room temperature, and centrifugation at 5,000 rpm for 30 s. The samples were subjected to a series of three washing procedures using Washing Solutions 1 (1 time) and 2 (2 times), which involved vortexing, centrifugation at 5,000 rpm for 30 s, and supernatant removal, with repetition of the second washing step for comprehensive cleansing. The pellet was dried out at 65°C, and DNA was eluted using 50 µL of Tris-EDTA (TE) buffer, followed by incubation at 65°C for 5 min and vortexing. Following the final centrifugation at 12,000 rpm for 1 min, the supernatant, which contains pure DNA, was taken and preserved at −80°C for further analysis.

2.3.2. Viral DNA detection and quantification

For the extracted samples, an HBV-Monitor-L polymerase chain reaction (PCR) Kit (AmpliSens®, Russia) was used to quantify viral DNA. Using specialized primers and fluorescent probes, the Conformité Européenne - *in vitro* Diagnostic certified, lyophilized real-time PCR Kit quantifies HBV DNA in human blood plasma by targeting the Precore/Core region of the HBV genome. 50 µL of each of the extracted DNA, TE buffer as a negative reaction control, Positive and Negative Extraction Controls, and Calibrators (C1L and C2L) were added to separate reaction tubes containing lyophilised PCR-mix HBV-Lyo. The tubes were closed and then transferred to the machine.

Amplification was conducted using the Rotor-Gene Q system. The PCR reaction setup includes a starter two-step hold phase, beginning at 50°C for 15 min, proceeding by 95°C for an additional 15 min, for beginning denaturation. This is followed by two cyclic phases: Cycling 1 (5 cycles) comprises denaturation at 95°C for 5 s, annealing at 60°C for 20 s, and extension at 72°C for 15 s, excluding fluorescent

signal detection. Cycling 2 (40 cycles) maintains the exact temperature and timing protocol, although it incorporates fluorescent signal detection during the annealing phase, utilizing Fluorescein Amidite for amplified HBV DNA and 6-Carboxy-4',5'-Dichloro-2',7'-Dimethoxyfluorescein for internal control DNA dyes, facilitating real-time amplification monitoring.

2.3.3. Data interpretation

The Rotor-Gene program was used to analyze data. The device automatically determined Ct values when fluorescence exceeded a threshold. A calibration curve was created using Ct values from C1L and C2L calibrators to quantify HBV DNA content in test samples (IU/mL).

2.4. Statistical Analysis

Statistical analysis was conducted utilizing the Statistical Package for Social Sciences SPSS and (R software version 4.3.2.). Descriptive statistics were employed to present the demographic information. Chi-square tests were utilized to investigate correlations among categorical variables, while Pearson and Spearman correlation tests have been used to assess connections among continuous variables. A $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Sociodemographic Characteristics of Blood Donors

Between September 2024 and January 2025, a total of 31,631 blood donors visited the Erbil Blood Bank in the Kurdistan region of Iraq. The distribution of the blood donors on a monthly basis showed variation with a decrease in donor numbers from 6,553 in September to 5,639 in November, followed by an increase and stability in the next months, with values increasing to 6,573 in December and 6,641 in January 2025, as shown in Fig. 1.

Of the total blood donors, 31,370 (99.18%) were male, whereas 261 (0.82%) were female, demonstrating a predominantly male donor demographic.

3.2. The Prevalence of Total HBcAb among Blood Donors

Among the whole-blood donor population, 388 individuals (1.23%) showed positive for total HBcAb, comprising 382 males and six females. The probability of blood donors being male was 98.5%, with the Chi-square test indicating statistical significance regarding the sex ratio ($P < 0.05$). Chi-square examination of gender distribution over months ($P = 0.9$) revealed no significant temporal change.

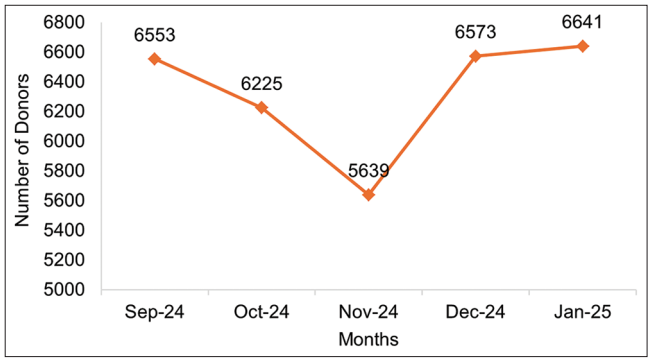

Fig. 1. Monthly distribution of blood donor numbers. The line figure reveals a decrease in donor numbers between September and November, followed by an uptrend and stability in December and January 2025.

Among the randomly selected 65 samples, the prevalence of cases according to their recency showed that the majority of cases were from Erbil city. Followed by Anbar, Ari, Bardarash, and Baghdad, with lower-case numbers from other areas.

The occupation distribution of the 65 cases showed that most HBcAb-positive cases were freelancers, followed by Peshmarga, employees, and police, as shown in Fig. 2.

The respective ages of the 65 HBcAb-positive cases varied from 23 to 59 years, having a mean age of 39.55 years, a standard deviation of 9.46, and a standard error of the mean of 1.17. A large percentage of HBcAb-positive blood donors was between 35 and 45 years of age. The highest rate was detected at the age of 40 years, as shown in Fig. 3.

3.3. Molecular Testing and Occult Hepatitis B (OBI) Detection

Among all positive cases, 65 random samples were subjected to DNA extraction and quantitative PCR (qPCR) analysis. Of them, 48 samples (73.85%) demonstrated undetectable viral loads, showing a negative OBI. Meanwhile, 17 samples exhibited detectable HBV DNA, indicating a total OBI prevalence of 26.15%. The viral load among the OBI-positive cases varied from 0.14 IU/mL (0.784 Copies/mL) to 69.64 IU/mL (570.9 Copies/mL). As shown in Figure 4.

The Spearman correlation coefficient between age and viral load showed $P = 0.282$.

4. DISCUSSION

The current study aimed to evaluate the overall prevalence of HBcAb among the blood donors attending Erbil Blood

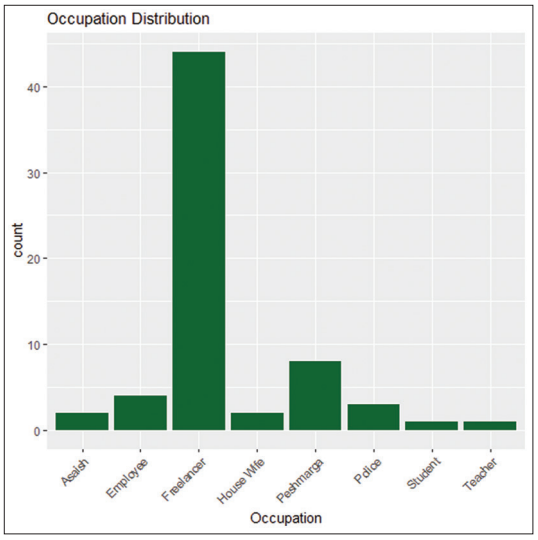

Fig. 2. The distribution of total hepatitis B core antibody-positive cases according to occupation. The figure shows that the highest prevalence was among the freelancers, followed by the Peshmarga.

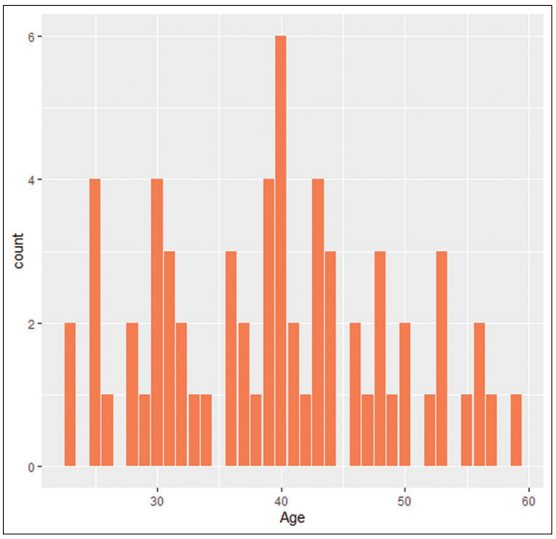

Fig. 3. The distribution of total hepatitis B core antibody-positive cases according to age. The figure shows that among the randomly selected 65 samples, ages varied from 23 to 59 years.

Bank in the Kurdistan Region of Iraq. It also assessed the possibility of OBI infection among this population through molecular analysis.

The results indicated that the prevalence of total HBcAb among the study population was 1.23% (388/31,631), including 382 males with only six females, indicating an imbalanced gender distribution. A study conducted among 438 blood donors in Duhok city, Kurdistan Region, Iraq [11], showed that the total prevalence of HBcAb was 8.2%

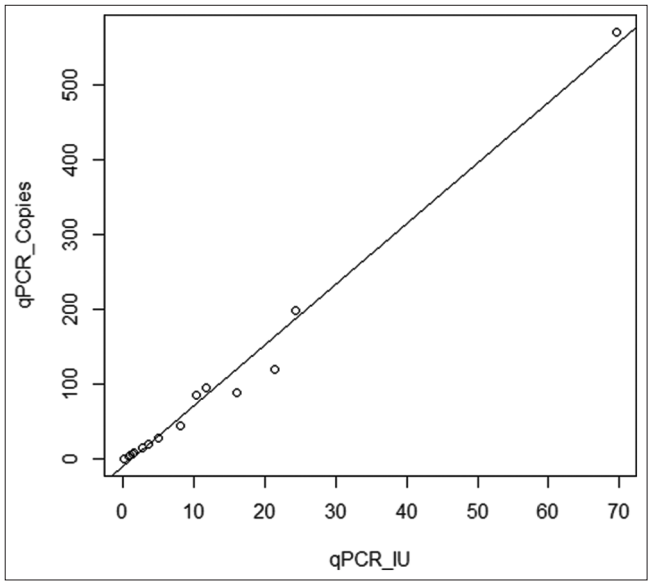

Fig. 4. The hepatitis B virus (HBV) viral load variation among occult hepatitis B infection (OBI) cases. The viral load in quantitative polymerase chain reaction-positive individuals varied from 0.14 IU/mL (0.784 copies/mL) to 69.64 IU/mL (570.9 copies/mL), showing significant diversity in HBV DNA quantities among OBI blood donors.

(36/438) in the study group. Another study in Yemen, including a total of 16,367 blood donors, revealed that the prevalence of HBcAb was 10.8% [12]. During a descriptive study in Khartoum, Sudan, blood samples were collected from 100 random blood donors to assess the prevalence of HBcAb, indicating a prevalence of 51% among the selected blood donors [13]. All the mentioned studies contrast with the findings of our study, showing a much higher prevalence among the same selected population. This can be due to the different exclusion criteria used by different blood banks and the difference in geographical distribution of HBV, as well as differences in the testing methods used.

In our study among the positive HBcAb cases, further molecular analysis on 65 random samples revealed that the percentage of detectable HBV DNA and the total prevalence of OBI among the samples was 26.15% (17/65). A study on 450 blood donors from Basrah Province in Iraq indicated that of the selected blood donors, 21.6% (97/450) were OBI positive [14]. Although some variation exists, our study's results align with this study's findings.

In a study among blood donors in the Aseer Province of Saudi Arabia, 28232 samples were screened for HBV DNA and anti-HBc to assess the prevalence and annual trends of OBI. Among the screened samples, Anti HBc reactive

samples were 3.21% (908/28232), while after matching the markers of OBI from this population, only 16 cases (18%) proved to be OBI positive [15]. In Cameroon, a study conducted on 288 HBsAg-negative blood donors revealed that 58% (167/288) of the blood donors showed positive for anti-HBc, and OBI was confirmed in 4.5% of the blood donors [16]. Furthermore, in a study conducted on 250 blood samples obtained from blood donors in Guinea, the OBI cases showed a prevalence of 15.6% [17]. While all the previous studies reveal a lower OBI prevalence, the higher detected values from our study can be due to a smaller sample size, different geographic areas, or variation in the sensitivity of the used methods.

Furthermore, the viral load detected in the OBI-positive cases in our study varied from 0.14 IU/mL (0.784 Copies/mL) to 69.64 IU/mL (570.9 Copies/mL). A study conducted in Nigeria involving 200 blood donors tested for serological markers, DNA detection, and viral load quantification revealed that among the HBcAb-positive blood donors, the HBV DNA was detected in only three cases (1.5%), showing viral loads of 753.1, 2.193 × 104, and 4.538 × 104 IU/mL [18]. In another study in Ethiopia, 973 HBsAg-negative samples were analyzed for anti-HBc and viral DNA. A total of 144 blood samples (14.8%) tested positive for anti-HBc antibodies, from which four samples (2.8% of anti-HBc-positive samples) were proven to have OBI using DNA testing. The average viral load in the verified OBI samples was 31 IU/mL [19]. The mentioned studies reported higher viral loads in comparison to our results, which can be due to differences in the immune status, genotype of the virus, or sensitivity of the utilized tests.

5. CONCLUSION

This study demonstrates the existence of occult HBV infection between HBsAg-negative blood donors in Erbil, with 26.15% of HBcAb-positive blood donors exhibiting measurable HBV DNA. Majority of the infected people were middle-aged males, and virus loads were predominantly low. These findings emphasize the inadequacies of HBsAg-only screening and recommend the integration of NAT to improve blood safety.

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