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Research Article

Prevalence and Characterization of Hepatitis B and Hepatitis C Infection among Blood Donors in Erbil

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ABSTRACT

Blood transmitting infectious disease still remains a considerable global health problem. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are two of the most commonly transmitted infectious agents. This prospective cross-sectional study was conducted between December, 2017, and February, 2018, at the Directorate of Blood Bank in Erbil Province, Northern Iraq. During that period, a total of 6173 blood donors donated blood; all blood donors were asked a series of questions through a structured questionnaire designed for such purpose. These patients were serologically examined for HBV and HCV. Positive blood samples were further analyzed serologically and confirmed by real-time polymerase chain reaction (RT-PCR). Among 6173 blood donors who were investigated for HBV, 7 (0.11%) and 98 (1.6%) were positive for hepatitis B surface antigen (HBs-Ag) and hepatitis B core Antibody (HBc-Ab), respectively, whereas during screening for HCV, 4 (0.06%) were positive for HCV-Ab. Coinfection (dual infection (HBV and HCV) was positive in 1 patient (0.01%). Among 98 reactive samples, 75.5% were positive for HBs antibody (HBs-Ab), the remaining 24 samples (24.5%) were regarded as occult hepatitis B infection (OBI), since they were positive for HBc-Ab, whereas negative both for HBs-Ag and HBs-Ab. The diagnosis of OBI could be confirmed by RT-PCR in 8 samples, 33% of samples. The overall incidence of HBV and HCV among examined blood donors was 0.5 %, and 0.06%, respectively. Amidst that incidence, 0.39 % were diagnosed as OBI. To prevent viral transmission through blood transfusion is needed to combine a different and sensitive method for HBV detection as well as evolve tests that have high sensitivity and specificity for serological markers. Moreover, a molecular tool that is sensitive enough to detect very low copies of viral DNA must also be developed.

Keywords: *Blood donors, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B, hepatitis C*

INTRODUCTION

Infections with hepatitis B virus (HBV) and hepatitis C viruses (HCV) are major global health problems worldwide: It is predictable that about 350 million persons are chronically infected with HBV, and nearly 200 million people are infected with HCV worldwide. Both hepatitis B and hepatitis C are among common infectious liver diseases worldwide.[1] Viral hepatitis is a systemic disease primarily affecting the liver, which induces acute inflammation of the liver, resulting in a clinical illness characterized by fever, gastrointestinal symptoms such as nausea, vomiting, and jaundice. Regardless of the virus type, identical histopathologic lesions are observed in the liver during acute disease.[2] Both HBV and HCV infections are known for their parenteral transmission; therefore, blood transfusion is a potential route of transmission.[3,4] The risk of infection with transfusion-transmitted viruses has been reduced remarkably since the introduction of serological screening; on the other hand, a zero-risk blood supply remains the ideal aim.[5] The risk of transfusion-transmitted HBV infection has been reduced by screening all blood donations for hepatitis B surface antigen (HBs-Ag) since 1970, screening for HBs-Ag is still regarded as an important tool for blood screening by the World Health Organization (WHO).[6] It was accepted that the disappearance of HBs-Ag indicates the clearance of HBV. Meanwhile, many reports pointed to the occurrence of post-transfusion hepatitis B despite the screening for HBs-Ag, necessitating the use of other markers for viral screening. For this, screening for HBc-Ab was recommended by the German Advisory Committee Blood in March 2005, especially for areas with low HBV prevalence.[7,8] Recently in the major blood bank of Erbil, HBc-Ab screening has also been performed for all blood donors. The donated blood with negative HBs-Ag but positive HBc-Ab will be

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discarded, these groups of blood donors are either infected but have been cured of the diseases, or else the donor is still infected with HBV that can be further confirmed and need to be further analyzed both serologically and by molecular detection using polymerase chain reaction (PCR).[9] The blood donors with HBs-Ag negative serology, HBc-Abs positive or negative and the presence of viral DNA by PCR are known as occult HBV infected occult hepatitis B infection (OBI). OBI is a risky group playing an important role in hepatitis virus transmission by blood transfusion.[10]

Regarding the HBV prevalence in the world could be divided into three areas where the prevalence of chronic HBV infection is high (>8%), intermediate (between 2 and 8%), and low (<2%).[11] Infection with Hepatitis B and C is regarded as a major cause of liver cirrhosis and hepatocellular carcinoma. The WHO estimates that 57% of cases of liver cirrhosis and 78% of primary liver carcinomas result from a hepatitis B or C virus.[12] Approximately 70%–80% of HCV-infected patients fail to clear the virus and develop chronic HCV infection, which is a risk factor for liver diseases such as fibrosis, cirrhosis, and hepatocellular carcinoma.[13,14]

Several studies have recorded the prevalence of HBV and HCV infection in blood donors of different Iraqi cities.[15-17] According to the studies that have been conducted in the past years investigating the seroprevalence of hepatitis B and hepatitis C, the prevalence rate in our region is low which is <2%.[18,19]

Lack of documentary data in Erbil governments and immigration of a significant number of refugees from a neighboring country to Kurdistan region, as well as foreign tourists and the traveling of Kurdish peoples to other countries, necessitate updated research on seroprevalence of infectious agents (HBV and HCV) among blood donors. Nonetheless, the HBV vaccine was added to the expanded program of vaccination in early 2000.[20] There was an absence of data on the success of the vaccination program; therefore, we thought of evaluating the antibody for these viruses in the blood which offer an idea of the level of protection against these infectious agents.

This study was conducted to find out the incidence of HBV and HCV among blood donors in Erbil community. Besides this, we further characterized the HBV infected individuals along with the screening for HBs-Ab, evaluating the level of protection against HBV infection among our community.

MATERIALS AND METHODS

Study Population and Sampling

This is a prospective and cross-sectional study investigating HBV and HCV among blood donors who donated blood in the Directorate of Blood Bank-Erbil, Iraq. All volunteers who visited the blood bank to donate blood from December 2017 to February 2018 were included in the study: Blood donors were selected after an interview through a structured questionnaire designed for such purpose. Blood donors with low or high hemoglobin concentration, high body temperature, having any disease, weight <50 kg, cupping in Past 6 months, operation in the last year, drinking alcohol, uncontrolled blood pressure, history of taking any drugs, fever, and having tattoos on their body were excluded in our study.

In total, 6173 participants donated blood during the specific time and were screened for HBV and HCV. Among those blood donors, 109 were reactive for HBV or HCV. Fifty healthy individuals were also included in this study as a control group. Each blood sample was collected in a 5 ml of a sterile tube, and then the whole blood was centrifuged, plasma was taken and stored at minus 20 freezer. The study was approved by the Ethics Committee at the Hawler Medical University, for dealing with the samples and participant information and a written informed consent was obtained from each blood donor for their participation in the study.

Detection of HBV Serological Markers

ELISA screening test for HBs Ag and anti-HBc

Monolisa™ HBs Ag ULTRA (Bio-Rad, France) assay is a one-step enzyme immunoassay based on the principle of the "sandwich" type using monoclonal antibodies and polyclonal antibodies selected for their capability to bind themselves to the numerous subtypes of HBs Ag.[21] Serologically, the samples were rechecked by Elecsys HBs Ag II by device Cobas e411(Roche, Germany). The Elecsys HBsAg II assay uses monoclonal and polyclonal anti-HBs antibodies (mouse and sheep) for the HBsAg determination. Monolisa™ Anti-HBc PLUS (Bio-Rad, France) is an enzyme immunoassay (indirect ELISA type) for the simultaneous finding of total antibodies to HBV core.[22]

Chemiluminescent immunoassay (CLIA) screening test for anti-HBs

CLIA way was used for detecting antibody of HBs by the Liaison XL (DiaSorin, Italy). Amid numerous enzyme assays that give light-emitting reactions, one of the most successful tests is the improved CLIA regarding a horseradish peroxidase labeled antibody or antigen and a mixture of the chemiluminescent substrate, hydrogen peroxide, and enhancers.[23]

Detection of HCV serological markers

Monolisa™ Anti-HCV PLUS Version 2 or 3 (Bio-Rad, France) is an indirect qualitative enzyme immunoassay for the detection of infection by the HCV based on the detection of anti-HCV antibodies in serum or human plasma.[24]

Detection of HBV DNA (PCR)

The extraction of HBV DNA is done by a kit EZ1 Virus Mini kit (v2.0 Qiagen, Germany) using the EZ1 Advanced XL device (QIAGEN, Germany).[25] The *artus* HBV RG PCR Kit is used in the automated system for the detection of HBV DNA using the PCR on Rotor-Gene Q (QIAGEN, Germany).[26] This is conducted for 24 samples that are negatives for anti-HBs and positives for anti-HBc IgG, and six samples are used as a negative control.

Statistical Analysis

Microsoft Office Excel and Statistical Package for the Social Sciences (SPSS) are used for data entry and analysis.

RESULTS

Among 6173 participants, 109 were reactive for HBV and HCV. Fifty negative samples were also included in this study as a control group for further analysis. Among the 159 participants

who were included in this study as a test and control group, the mean and standard deviation in age for the control group was 35.5 ± 8.6 and that for the test group was 40.9 ± 12. The ages of blood donors both for test and control group were grouped into six groups [Tables 1 and 2]. The minimum age is 19 years, and the maximum age is 73 years for the test group and 22 and 57 years for the control group.

Prevalence of Hepatitis B and Hepatitis C

Serologically, based on detection of HBs-Ag, HBc-Ab, and HCV-Ab, we decided whether the patient has HBV or HCV infection. These tests were carried out for all the participants (6173). Among all examined participants, only 7 patients were reactive for HBs-Ag (0.11%), whereas 98 donors were reactive for HBc-Ab (1.58%). Regarding HCV infection only 4 patients were positive for anti-HCV Ab (0.065%), as described in Figures 1 and 2.

Immune Blood Donors for HBV Infection

Among the sero-reactive blood donors for HBV, we further characterize them through detection of antibody against HBs-Ag (HBs-Ab). Should individuals of HBc-Ab positive blood have HBs-Ab in their blood, this indicates that these participants are cured of HBV infection and means that they have immunity to HBV infection. Among the 98 sero-reactive blood donors, 74 blood donors were positive for HBs-Ab (75.5 %) indicating that they are cured of natural infection.

We also examined 50 healthy volunteers that are free from HBV and HCV infection, among them only 4 blood donors were positive for HBs-Ab, reflecting the successful vaccination and protection against HBV infection [Table 3].

Diagnosing Occult HBV Infection

As we defined OBI before, blood donors with HBs-Ag negative serology, HBc-Abs positive or negative, and the presence of viral DNA by PCR are known as occult HBV infection (OBI). Blood donors with only positive HBc-Ab in the absence of HBs-Ag and HBs-Ab are regarded as OBI.[10] In our study, we further characterize the positive blood donors. Among the samples that are included in our study, 98 samples were positive for HBc-Ab, from which 24 blood donors (24.5%) were negative for HBs-Ag and HBs-Ab (HBc-Ab +ve, HBs-Ag –ve, and HBs-Ab –ve), indicating that these blood donors were defined as having occult hepatitis virus infection [Table 3]. These 24 samples were further investigated for the detection of HBV DNA [Figure 3].

HCV Incidence and Dual Infection

In this study, as we mentioned above, besides screening for HBV infection, screening for HCV was also done for these participants. Among 6173 participants, only four blood donors were positive for HCV-Ab 0.06%. Among HCV reactive donors, one individual was positive both for HBV infection and HCV infection, which means that he has a dual infection [Table 4].

Sero-status in Relation to Socio-demographic Characteristics

Among the HBV and HCV positive blood donors, the highest frequency of 38 (35%) was found among those aged between 26 and 35 years (young age groups). Blood donors with blood Group O were found to have the highest frequency for

Table 1: Demographic distribution of the HBV and/or BCV positive samples (positive blood donors)

Demographic distribution	Frequency (*n*=%)
	Test group
Age groups (year)	
Under 25	4 (3.7)
2–35	38 (35)
36–45	33 (30)
46–55	22 (20)
56–65	7 (6.4)
Above 66	5 (4.6)
Blood groups	
O	39 (36)
A	36 (33)
B	25 (23)
AB	9 (8)
Occupations	
Private sector employee	73 (67)
Public sector employee	30 (27.5)
Unemployed	6 (5.5)
Student	-
Total	109 (100)

Table 2: Demographic distribution of the samples control group (negative blood donors)

Demographic distribution	Frequency (*n*=%)
	Control group
Age groups (year)	
Under 25	6 (12)
26–35	25 (50)
36–45	10 (20)
46–55	8 (16)
56–65	1 (2)
Above 66	-
Blood groups	
O	17 (34)
A	18 (36)
B	10 (20)
AB	5 (10)
Occupations	
Private sector employee	34 (68)
Public sector employee	13 (26)
Unemployed	1 (2)
Student	2 (4)
Total	50 (100)

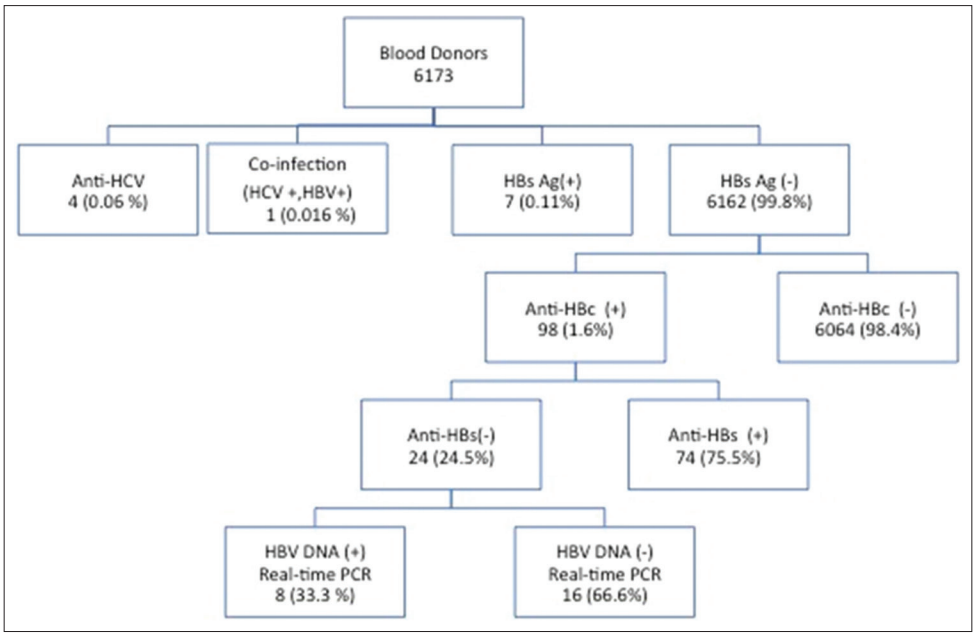

Figure 1: Serological and molecular characterization of hepatitis B virus and hepatitis C virus infection among blood donors in the major blood bank of Erbil city. HBs Ab – hepatitis B surface antibodies; HBc Ab – hepatitis B core antibodies: PCR – a polymerase chain reaction

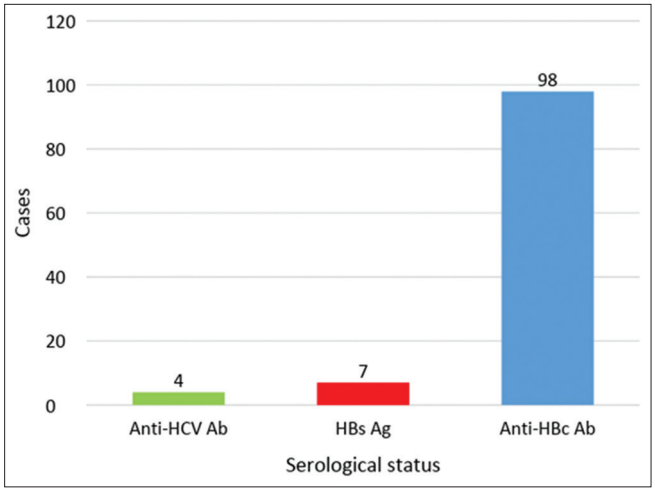

Figure 2: Incidence of both hepatitis B virus and hepatitis C virus among blood donors

its immunoreactivity among test group 39 (36%) [Table 1], whereas those with blood Group A are among those with the highest frequency among control group 18 (36%) [Table 2]. Most of the reactive individuals for HBV positivity were among the private sector employees [Table 1]. None of these parameters were statistically significant.

Molecular Detection of the HBV by Real-time Quantitative PCR (RT qPCR)

For further characterization of serum samples from HBs-Ag negative, HBs-Ab negative, and HBc-Ab positive blood donors were processed for HBV-DNA detection using molecular amplification using the RT PCR. This test was done using artus® HBV RG* PCR Kit 24, V1 (Qiagen, Germany). After extracting the nucleic acid of the HBV by EZ1®Virus Mini Kit v2.0 (Qiagen, Germany), we ran the purified DNA of HBV by Rotor-Gene Q (Qiagen, Germany). Twenty-four samples were selected (Anti-HBc IgG positive and negative for anti-HBs), and six control samples (negative control) were also included in the study. Out of these samples, 8 of the samples (33%) were positive, showing the DNA in their blood, confirming the diagnosis of occult hepatitis B [Figure 3].

DISCUSSION

Hepatitis B and C viruses are responsible for causing about 80% of liver cancer cases worldwide, and it could be the major cause of morbidity and mortality.[27] Regarding hepatitis prevalence, the world is divided into three areas; low prevalence area, which means <2%, an intermediate area 2–8% and high prevalence area more than 8%.[28,29] Most countries on the earth are still viewed as intermediate to high endemicity for HBV infection.[28,29] HCV follows the same division regarding prevalence such as low prevalence (<1.5%), moderate prevalence (1.5%–3.5%), and high prevalence area, which is more than 3.5%.[30]

The current study characterizes and defines the frequency of HBV and HCV (6173) blood donors. For HBV detection, two serological markers were used (HBs-Ag and Hbc-Ab), among those, 98 donors were reactive for HBc-Ab (1.58%), whereas only 7 people were positive for HBs-Ag (0.11%). For screening of HCV, HCV-Ab was used and only 4 blood donors proved positive for anti-HCV-Ab (0.065%), as described in Figures 1 and 2.

The prevalence of reactive blood donors in the current study for HBV is 1.7% (105 out of 6173) from which 1.5% is reactive against HBc-Ab and 0.11% is reactive against HBs-Ag, whereas for HCV is 0.06%. They are in agreement on a national level with these results by Alhilfi *et al.,* who screened 36620 blood donors in Missan Governorate in Iraq,

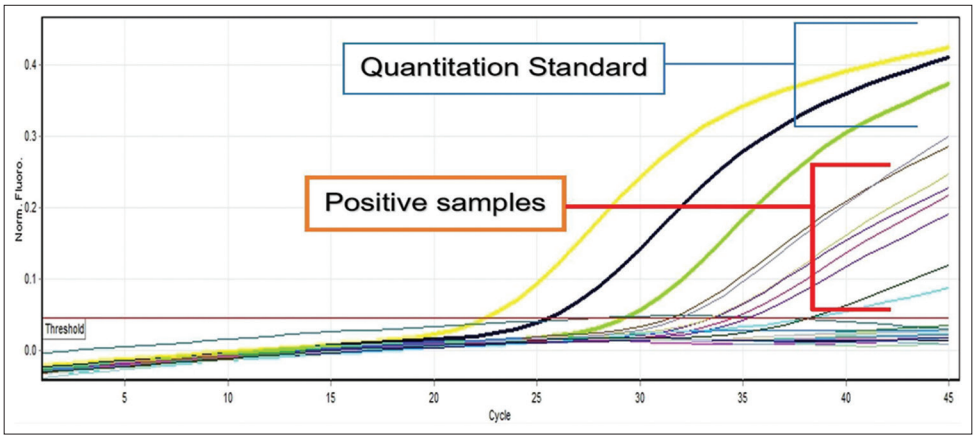

Figure 3: Quantitation data for cycling A. Green for hepatitis B virus by real-time quantitative polymerase chain reaction

Table 3: Distribution of HBs-Ab among HBc-Ab positive individuals and control group

Serology (HBc IgG)	Serology (HBs antibody)			*P*-value
	Positive (*n*=)	Negative (*n*=)	Total (*n*=)	
Positive	74	24	98	<0.05
Negative	6	55	61	
Total	80	79	159	

Hbs Ab: Hepatitis B surface antibodies

Table 4: The incidence and percentage of HCV reactive donors and dual infection

Serology (HBs antigen)	Serology (HCV-Ab)		
	Positive	Negative	Total
	n= %	*n*=%	*n*=%
Positive	1 (0.6)	7 (4.4)	8 (5.0)
Negative	3 (1.9)	148 (93.1)	151 (95.0)
Total	4 (2.5)	155 (97.5)	159 (100.0)

HCV-Ab: Hepatitis B surface antibodies

they found 0.12% positivity for HBs-Ag and 0.09% positivity for HCV-Ab.[31] Similar results were also found by Al-Rubaye *et al.,* who screened 69915 blood donors in Basra in 2013, from which they found 125 reactive individuals for HBs-Ag (0.2%), and 1475 reactive individuals for HBc-Ab (2.1%) and HCV-Ab positivity in 0.1%.[17]

On an international level, similar results to ours were also found in a neighboring country by Boustani *et al.,* which was done in Ilam city in Iran between 2009 and 2013: They screened 72,527 blood donors, they found HBs-Ag in 102 (0.14%), and HCV-Ab in 27 (0.037%).[32] Moreover, another study conducted in Southwestern Iran, by Sajjadi *et al.,* they Screened 180,304 blood donors and found HBV in 0.13% and HCV in 0.06%.[33]

On the other hand, our data were much lower than that of other related studies performed in other centers. Nationally, Hussein *et al.* performed a study in Duhok at 2004 found HBs-Ag in 62/7900 (0.78%), and HCV-Ab in 16/7900 (0.2%).[15]

In addition, internationally, our result was lower than those found in India in a study conducted by Gulia, they found HBV in 2.54%.[34] In Libya, a study carried out by Qowaider *et al.,* they examined 78,987 blood donors in different regions in the Northeast in Libya, and they found HBV in 172 blood donors (0.21%), and HCV in 197 blood donors (0.24%).[35] Furthermore, in Turkey, Hope *et al.,* examined 53,985 blood donors, among them 2.1% were positive for HBV and 0.3% for HCV.[36]

Besides the blood screening, we further characterized the reactive blood donors (positive samples) among 105 positive samples for HBc Ab (1.7%), 98 samples were HBc-Ab positive but negative for HBs-Ag (HBs-Ag −ve/HBc-Ab +ve), further characterization was done for these group by investigating for HBs-Ab, among these 74 (75.5%) blood donors were HBs-Ab positive (HBc-Ab +ve, HBs-Ag –ve, and HBs Ab +ve) which indicated that they are cured of natural infection [Figure 1 and Table 3] whereas the remaining 24 blood donors (24.5 %) were HBc-Ab positive but negative both for HBs-Ag and HBs-Ab. Individuals with negative HBs-Ag and HBs-Ab but positive HBc-Ab are defined as occult HBV infection (OBI), this is because HBc-Ab is regarded as a pathognomonic marker of HBV infection, particularly when HBs-Ag and HBs-Ab are negative.

We performed HBV-DNA detection assay solely on samples of blood donors who tested positive for HBc-Ab but negative for both HBs-Ag and HBs-Ab (24 samples), including six control samples as well. Among 24 samples we could confirm the presence of HCV DNA only in 8 (33%) samples, using RT PCR, indicating the overall incidence of OBI by DNA detection to be about (0.13 %; 8 out of 6173) [Figures 1 and 3]. Based on the reaction graph, most of the positive sample has very low copy numbers since the reaction was arrived at between the 35 and 40 cycles [Figure 3].

Meanwhile, the remaining 16 (66 %) samples were HBc-Ab positive but failed to be confirmed by HBV-DNA

detection. In most of the areas, these groups of blood donors are still regarded as OBI, and their blood will be discarded to prevent virus transmission.[37]

Internationally, many studies have been published throughout the world showing the frequency of OBI within HBs-Ag-negative donors, the results are varied: For example in the United Kingdom it was 0.56%;[38] in the United State of America it was 0.8%;[39] in Iran it was 2.1%;[40] and in Brazil it was 3.1%.[41]

On a national level, a similar study performed in Erbil City of Iraq shows OBI in 0.09% which is close to our finding (0.13%).[42] Discarding the infected blood on the basis of HBc-Ab detection, especially in areas where the prevalence of HBc-Ab is high (more than 10%), will negatively affect the blood supply. In the present study, we found that the incidence of HBc-Ab in the Erbil city was low (1.56%, 98 out of 6173) [Figure 1]. Further detection of HBs-Ab saved 78 of our samples (75.5%) since they were positive for HBs-Ab. On the other hand, DNA confirms the OBI only in 8 samples (33%). In high prevalence area based on HBc-Ab detection such as Korea, Greece, and Ghana, they discarded only blood that is positive for HBV-DNA, consequently saving more blood units.[43,44] Meanwhile, in many places, especially in low prevalence countries, blood donor samples were still considered as OBI based on HBC-Ab positivity, and all such donated blood was discarded to prevent transfusion-transmitted viral infection.

Despite the above, the failure of detecting HBV-DNA might be due to a mutation in viral structure, and the false serological result might be due to the imperfection of the assays used for such purpose.[37]

Many researchers argued in favor of the implementation of HBc-Ab or HBV DNA alone or combination for the screening of donated blood.[45,46] In a large study screening, 6.5 million participants using three markers (HBs-Ag, HBc-Ab, and PCR for HBV DNA), prioritizing the use of HBc-Ab along with the DNA nucleic acid testing was confirmed for the detection of a low level of HBV DNA positive donors.[46]

CONCLUSION

The overall incidences of HBV and HCV among examined blood donors were 0.5% and 0.06%, respectively. Among that, 0.39% were diagnosed as OBI. The highest avoidance of HBV blood transmission can be attained by combining a different, more sensitive, method for HBV detection with the evolving of tests that have high sensitivity and specificity for HBs Ag and HBc Ab detection. Further, developing a molecular tool that is sensitive enough to detect a very low copy of viral DNA would conserve many pints of blood.

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CONFLICTS OF INTEREST

There are no conflicts of interests to be declared.

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