



E-ISSN: 2706-9575  
P-ISSN: 2706-9567  
[www.medicinpaper.net/](http://www.medicinpaper.net/)  
IJARM 2019; 1(2): 62-67  
Received: 01-05-2019  
Accepted: 03-06-2019

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## Risk factors for hepatitis b virus among blood donors in Baghdad, Iraq

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DOI: <https://doi.org/10.22271/27069567.2019.v1.i2b.17>

### Abstract

Despite the implementation of effective vaccination programs, hepatitis B remains an important cause of morbid it and mortality worldwide. Understanding the epidemiology of the disease is essential in developing programs to prevent and treat this global infection. The lack of knowledge about HBV modes of transmission, its consequences, and its preventive measures is a major cause of increasing prevalence of HBV.

Preventive strategies for HBV infection include healthy blood transfusion services and vaccination against HBV. The agenda of every national blood program should be focused on the implementation of effective quality systems, as well as the development and implementation of quality standards, effective documentation systems, training of all staff and regular quality assessment to ensure that all donated blood is screened for transfusion-transmissible infections.

Globally, however, there are significant variations in the extent to which donated blood is screened, the screening strategies adopted and the overall quality and effectiveness of the blood screening process. As a result, in many countries, the recipients of blood and blood products remain at unacceptable risk of acquiring life-threatening infections that could easily be prevented. There is a need of a public awareness programs especially in rural areas and people at high risk to decrease the burden of HBV infection. Each country should establish voluntary blood donor programs which provide donor information and education.

**Keywords:** Blood donors, hepatitis B virus

### Introduction

The system in place for blood safety involves first and foremost on the collection of blood from a low-risk blood donor, followed by quality assured transfusion transmissible infections (TTIs) serological screening apart from the molecular testing as an additional layer of safety and quality practices during collection, processing, and storage of blood and blood components<sup>[1]</sup>.

Hepatitis B virus is 50–100 times more infectious than HIV; and it is the etiologic agent of hepatitis B. HBV is a double-stranded DNA virus of a complex structure that causes infection of the liver. The virus belongs to the Hepadnaviridae family and is the most common cause of chronic liver disease; hepatocellular carcinoma and necrotizing vasculitis. HBV can cause both acute and chronic infections; and during the acute phase of infection, symptoms are not experienced by most people. Nevertheless, certain individuals develop acute illness with symptoms that last several weeks, including yellowing of the skin and eyes (jaundice), nausea, dark urine, extreme fatigue, abdominal pain, and vomiting<sup>[2]</sup>.

Additionally, in individuals with acute hepatitis, a small subset can develop life-threatening acute liver failure whereas, in certain individuals, HBV establishes a chronic liver infection that progresses to cirrhosis or cancer of the liver<sup>[3]</sup>.

Blood transfusion is a life-saving intervention that has an essential role in patient management within health care systems. Unfortunately, blood transfusion is not without risks and may lead to the transmissions of infectious agents from donor to recipient, human immunodeficiency virus (HIV), syphilis-causing *Treponema pallidum* and hepatitis B virus (HBV). Many cases of HBV infections in adult populations were found to be associated with blood transfusions, since HBV is infective through blood and body-fluid, including vertical transmission<sup>[4]</sup>.

The hepatitis B surface antigen (HBsAg) in serum is the first zero marker to indicate active HBV infection, either acute or chronic. Since 1982 there was available a hepatitis B vaccine, highly effective in the prevention of HBV transmission, with a consequence of a remarkable

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Reduction in the prevalence and incidence of HBV infection. Despite this, the World Health Organization (WHO) has estimated that there are still 360 million chronically HBV infected people and 5.7 million HBV-related cases worldwide, spread with a high variability across the countries (e.g. in the difference between low and high-income countries). It has been estimated that infections with HBV was responsible for about 59% of hepatocellular carcinoma cases in developing countries [5].

According to the WHO's 2017 global hepatitis report (the latest and first such report by the WHO), 257 million people were living with chronic HBV infection in 2015, with African and Western Pacific regions accounting for the highest-burden.

Laboratory diagnosis of HBV includes detection of markers such as HBsAg, HBsAb, HBcAb, HBeAg, and HBeAb in the serum. Detection of HBsAg in the serum is indicative of HBV infection and this marker is the most frequently used in testing for HBV infection. HBsAg is detected within 10 weeks in the serum following exposure to the virus and its persistent presence for longer than 6 months may depict chronic infection [6].

Additionally, new HBV infection in certain individuals evolves into chronic infection, whereas there's a spontaneous clearance of the virus in others, with the risk of developing chronic infection being highest in children. As such, the focus of prevention of HBV infection is on children below five years of age, and children five years of age who test positive for HBsAg have chronic infection [7].

Blood donor selection is targeted on identifying donors at low risk of infection while donor deferral criteria are used to distinguish those at high risk of infection, based on the epidemiology of the (HBV). In practice, this is done utilizing the uniform donor history questionnaire (UDHQ) and consent proforma, which acts as a checklist covering the spectrum of the questions that are required to screen out donors who have been exposed to the risk factors for hepatitis. Therefore blood donor selection process has to keep evolving in order to meet the variation in the epidemiology of (HBV) coupled with the differences owing to educational, cultural, and socio-economic diversity of the donor population [8].

However just like the innate immunity barrier of the human immune system, the donors who pass the selection process donate blood and the blood thus becomes part of the quarantine blood inventory. Only those units which test negative during (HBV) testing will then be taken into the ready to issue stock. Therefore in order to have the safest possible blood inventory; the process of the selection of a low-risk blood donor is the most crucial step. This step involves a dialogue between the trained medical staff and the volunteer blood donor, and this is where the knowledge of the prospective blood donor with regard to hepatitis B assume importance of significance for blood safety [9].

## Background

Infection with the hepatitis B virus (HBV) is a serious global health problem, with two billion people infected worldwide and 350 million suffering from chronic HBV infection. Of these, 75% are Asians.

Among the many transmission routes, transfusion is the one that could be prevented. The first major success in enhancing transfusion safety came with the implementation of hepatitis B surface antigen (HBsAg) detection in the early

1970 s. However, studies have demonstrated that transmission by HBsAg-negative blood components can still occur in the acute phase of infection during the seronegative window period, or during chronic stages of infection with undetectable HBsAg [10].

With the development of sensitive assays to detect HBV-DNA, it has been shown that healthy HBsAg-negative donors who have antibodies to HBV core antigen (anti-HBc) may harbor an occult HBV infection and maintain HBV-DNA sequences in their liver and blood, thus representing potential sources of HBV transmission [11].

The serological pattern defined as isolated anti-HBc (HBsAg-negative, hepatitis B surface antibody (anti-HBs)-negative, and anti-HBc-positive) is observed in 10–20% of individuals from areas of low endemicity for HBV.

The significance of this serological pattern is unclear. It may reflect past infection with HBV, after which anti-HBs either did not develop or decreased to an undetectable level. Also, this serological pattern can be observed in the window phase of a resolving case of acute hepatitis B. Finally it may represent occult chronic HBV infection, with levels of the HBsAg below the limits of detection [12].

Detection of HBV-DNA without detectable HBsAg is defined as occult HBV infection. The frequency of detection of occult HBV infection depends on the relative sensitivity of both HBsAg and HBV-DNA assays. It also depends on the prevalence of HBV infection in the population. The prevalence of occult HBV infection is higher in areas in which HBV infection itself is more frequent. Iran is in the low endemic area for HBV. The prevalence of HBsAg in Iran ranges from 1.7% to over 5% in the different provinces. In the Central Province of Iran, the prevalence of HBV is estimated to be 0.5%. It is estimated that 7500–15 000 chronic HBV individuals live in this province, which has a population of 1.5 million [13].

## The prevalence of HBV in blood donors

An epidemiology in 1992 reported that the prevalence of HBsAg was 75% in the Chinese general population. The investigation also found that the positive rate of HBsAg in infants of 1–4 years old is comparable with that in adults, which indicated that perinatal infection and early childhood infection contribute significantly to the HBV chronic infection.

In China, the Chinese government implemented universal HBV vaccination of neonates in 1992. The neonates were vaccinated within 24 hours after birth and then were injected with vaccines in 1 month and 6 months. Since 2002, children were included to routine HBV vaccination for free (only a small amount of service fee was charged); the routine HBV immunization of neonates was completely free of charge since 2005 [14].

The HBV vaccination rate in children increased from 30% in 1992 to 90% in 2005 [13], yet the rate was 20% lower in rural than in urban areas [14]. As a consequence of these administrative measures, the prevalence of HBV remarkably declined in the general population. As reported by a national seroepidemiological survey of HBV infection [15].

## HBV DNA-positive rate in HBsAg-negative blood donors

Later in 2015, the NAT was applied in all the blood services across the country. Since then, a substantial number of HBsAg/ DNA+ blood specimens have been discovered by the NAT [16].

Most of these blood specimens are identified as OBI, except for some specimens which are the HBV WP infection. For example, using the Roche Cobas Ampli Screen system, Wang *et al.* identified eight HBV DNA+ specimens from 28,800 HBsAg ELISA-negative voluntary blood donors (28%, mini-pool of 24 donation, limit of detection [LOD] 30–60 IU/ml) who were recruited in Dongguan Blood Center of Guangdong Province during the period of August 2006–August 2007. These eight donors with HBV DNA+ were followed up<sup>[17]</sup>.

One donor was identified to be ‘window period’ infection because of the seroconversion of HBsAg 2 weeks after

donation, while the other seven donors appeared to be OBI since their HBsAg remained negative. Zheng X *et al.* conducted NAT on 165,371 HBsAg-negative blood specimens from voluntary donors at Shenzhen City of Guangdong Province<sup>[18]</sup>.

The positive rates of HBV DNA in HBsAg-negative donors reported by some blood centers or stations are shown in Table 1. The HBsAg/DNA+ reported in the different areas of country varies as great as over 10-fold, which may be explained by the divergent prevalence of HBV across geographic region, as well as the different sensitivities of ELISA and NA

**Table 1:** Prevalence of HBsAg in Chinese blood donors

| Blood centre/ Blood bank | Time of investigation | Number of donors | Number of HBsAg-positive case | Positive rate (%) | References |
|--------------------------|-----------------------|------------------|-------------------------------|-------------------|------------|
| Van                      | 1999-2009             | 263 299          | 3057                          | 1.16              | [13]       |
| Zhengzhou                | 2014                  | 96 820           | 484                           | 0.50              | [14]       |
| Guiyang                  | 2014                  | 77 971           | 546                           | 0.70              | [15]       |
| Chongqing                | 2008-2012             | 551 133          | 6039                          | 1.10              | [16]       |
| Beijing                  | 2007-2011             | 1 065 177        | 3830                          | 0.36              | [17]       |
| Nanjing                  | 2015-2016             | 66 742           | 225                           | 0.34              | [18]       |
| Xuzhou                   | 2003-2012             | 570 809          | 3512                          | 0.62              | [19]       |
| Hainan                   | 2011-2012             | 80 547           | 1084                          | 1.35              | [20]       |
| Urumqi                   | 2011                  | 14 696           | 76                            | 0.52              | [21]       |
| Beihai                   | 2005-2009             | 46 121           | 226                           | 0.49              | [22]       |
| Nanchang                 | 2012                  | 64 400           | 862                           | 1.34              | [23]       |
| Zhuhai                   | 2010-2015             | 149 310          | 2331                          | 1.56              | [24]       |
| Jiangshan                | 2011-2014             | 19 368           | 135                           | 0.70              | [25]       |
| Shiyan                   | 2010-2014             | 211 639          | 1087                          | 0.51              | [26]       |

**Table 2:** HBV DNA-positive rates in HBsAg-negative blood donors in some regions of China

| NA, not available. | NA, not available. | NA, not available. | NA, not available. | NA, not available. | NA, not available. | NA, not available. | NA, not available. | NA, not available. |
|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Xiamen             | 2011-2014          | 130 659'           | MP6                | 23                 | 113                | 0.09               | NA                 | [37]               |
| Dalian             | 2010-2013          | 158 232            | MP6                | 23                 | 69                 | 0.04               | 6/34'              | [38]               |
| Arian              | 2010-2011          | 93 613             | MP6                | 23                 | 60                 | 0.06               | NA                 | [139]              |
| Urumqi             | 2015-2016          | 57 644'            | MP6                | 23                 | 61                 | 0.11               | NA                 | [40]               |
| Shenyang           | NA                 | 105152'            | MP8                | 63                 | 15                 | 0.01               | NA                 | [41]               |
| Tianjin            | 2011               | 53 431             | ID                 | 10.4               | 25                 | 0.05               | NA                 | [42]               |
| Guangzhou          | 2011               | 199 631            | ID                 | 10.4               | 104                | 0.05               | NA                 | [43]               |
| Shanghai           | 2011-2012          | 250 376            | MP6                | 38                 | 197                | 0.08               | 3/14               | ( <sup>44</sup> )  |
| Fuzhou             | 2011-2013          | 102 866            | ID                 | 10.4               | 66                 | 0.06               | 7/3                | [45]               |
| Hongkong           | 2005-2006          | 3044'              | ID                 | 3.7                | 4                  | 0.13               | NA                 | [46]               |
| Hongkong           | 2006-2008          | 9967               | ID                 | 3.7                | 11                 | 0.11               | NA                 | [46]               |
| Shenzhen           | 2006-2008          | 41 301             | MP8                | 5.0                | 2                  | 0.004              | NA                 | [47]               |

NA, not available. 'Represented HBsAg-negative donors, others represented all donors. Best format of ID and MPX represented individual donation and mini-pool (where X is the pool size used), respectively. `One additional genotype D was reported.

**The HBV screening strategy in blood donors**

The first serological marker that can be detected after HBV infection is HBsAg, whose titer ascends rapidly at the early phase of infection and then gradually declines. HBsAg becomes negative in most adults who are infected and then clear the virus. The first antibody that can be detected after infection is anti-HBc, including IgG and IgM. To minimize transfusion transmission of HBV, the HBsAg has been officially included in the items for blood donation screening. At present, most of the serological tests for HBsAg are detected by ELISA. However, cases of HBV WP and OBI infection presented low level of circulating viral DNA but negative for HBsAg<sup>[19]</sup>.

In recent years, the Chinese government requests the additional test by HBV NAT on all the blood donations to further reduce the risk of transfusion-transmitted HBV infection. While anti-HBc is the most reliable marker of

OBI, in some countries or regions such as the USA, Canada, Germany and Japan, anti-HBc is also tested to exclude the HBV carriers with chronic low viremia and undetectable HBsAg. Nevertheless, currently in China, it is not feasible to apply anti-HBc in routine screening of blood donation, because of the high prevalence of anti-HBc in blood donors<sup>[20]</sup>.

The positive rate of anti-HBc is 23.4% in HBsAg-negative donor. This rate remains as high as 21.4% in blood donors who were born after 1992 and might be vaccinated by HBV vaccines. Alternatively, HBV NAT seems a feasible way to exclude HBsAg/DNA+ cases. It should be noted that the circulating HBV DNA is usually very low in OBI and WP cases, which requires highly sensitive HBV DNA to identify. Spreafico *et al.* demonstrated that MP-6 NAT with a sensitivity of 22.2 IU/ml failed to detect 50% of previous

donations from Italian blood donors subsequently identified with OBI (ID-NAT, 3.7 IU/ml) [21].

Similarly, our recent study identified nine HBV DNA+ cases by Ultrio plus NAT (3.4 IU/ml) in 1732 donors who previously qualified Ultrio NAT (10.4 IU/ml). These two studies and others establish that the viral load in OBI is often very low, and in the absence of anti-HBc testing, a very sensitive NAT assay is required to detect OBI. In addition, a case report involved a transmission from an ID-NAT-negative donation in the acute window period. Thus in China, a country with high prevalence of HBV, it is of great necessity to conduct HBsAg and highly sensitive HBV DNA tests to reduce transfusion transmission of HBV [22].

## Results and Discussion

Blood transfusion services are an integral part of health care system, which potentially saves lots of lives every day. Blood and blood products must be free from HIV, hepatitis viruses and other threatening infections and transfused safely to the needy persons which is the basic requirement of each country of the world. According to World Health Organization (WHO) guideline, at minimum, all the blood and blood products for transfusion should be tested for HIV, Hepatitis B & C, and Syphilis. Replacement donation is encouraged by WHO. In present study, 45.9% were voluntary and 54.1% were replacement donors. This finding is comparable with the study from Karnataka and from Haryana which reported proportion of voluntary donors as 58% and 31.4% respectively. However, studies done by Sehgal S *et al.* and Patel PA *et al.* from western Ahmedabad reported voluntary blood donors as 77.6% & 95.56% respectively.

In our study, males (95.6%) outnumbered females (4.4%). Occult hepatitis B infection (OBI) is one of the most challenging topics in the field of viral hepatitis. OBI is defined by the presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) in patients with serological markers of previous infection (anti-HBc and/or anti-HBs positive) or in patients without serological markers (anti-HBc and/or anti-HBs negative). The prevalence of OBI is quite variable depending on the level of endemic disease in different parts of the world, the different assays utilized in the studies, and the different populations studied. Occult HBV may impact in several different clinical contexts, including the transmission of the infection by blood transfusion or organ transplantation and its acute reactivation when an immunosuppressive status occurs. Occult HBV infection in blood donors is considered a potential threat for the safety of the blood supply however conclusive studies on this issue are lacking [23].

Although the incidence of transfusion-transmitted hepatitis B has been steadily reduced over the last four decades, HBV still remains the most frequent transfusion-transmitted viral infection. There is a high variability of infection with HBV across the countries, with high level in prevalence and incidence in developing world such as in Brazil (1.6%–7.7%), in Egypt (19.6%), and from various areas of India (2%–10%). Nascimento *et al.*, however, in their Brazilian multicenter serosurvey, reported a low seroprevalences of HBV among first-time voluntary blood donors, a population usually expected to have a higher prevalence of viral hepatitis infection than repeat blood donors. However little data are available on the seroprevalence of, and risk factors

for HBV infection in Latin American countries including Brazil [24].

In addition, in many developing countries, the relative contributions of various routes of HBV infection have not been defined in population-based studies. Due to a lack of universal and appropriate blood screening in these countries, the risk of post-transfusion HBV infection is still unknown [25].

Moreover, in low socio-economic settings, horizontal transmissions of HBV through contact with infected family member have also been reported. El Beltagy *et al.* did not find a significant association with history of exposure to high-risk procedure or behavior while in literature parenteral routes are implicated as the most likely factors for HBV transmission that include unsterilized needles and syringes in health-care settings. In their study, Akhtar *et al.* found that dental care provider and injections are risk factors. In the general population, history of repeated blood transfusions, history of injections, including re-use of contaminated syringes, contaminated surgical instruments, and blood products; number of pregnancies; hemodialysis; tooth extraction; dental procedures, needle prick and surgical procedures for health care workers; unsafe surgery are the main risk factors [26].

On the other hand, a recent study conducted in Egypt showed that HBV transmission is community rather than iatrogenic-acquired. Behavioral risks as intravenous drug use, needle stick injuries, tattooing, and multiple sexual partners have been identified as common modes of HBV transmission in the developed world. Nascimento *et al.* however found that anti-HBc was associated with lifetime number of sexual partners among men, but not among women and there was no relationship between sexual behavior and the seroprevalence of HBsAg in either gender [27].

HBV is a common public health problem especially in developing countries and is associated with serious consequences such as liver cirrhosis and hepatocellular carcinoma. In the Middle East, the prevalence of HBV ranges around 3% in Iraq, Iran, and Syria to about 7% in Yemen and some regions in Saudi Arabia. The prevalence of HBV was studied thoroughly in Iraq. The prevalence varied in this country according to the geographical regions. In studies from the middle part of Iraq investigating the prevalence of HBsAg positivity in the cities of Babylon, the seroprevalence was found to be approximately 0.7%. The same results were found in Kurdistan region, Northern Iraq. However, in a study conducted in Kerbala, southern Iraq, the prevalence rate was 3.5% [28].

An urgent public health plan is needed for surveillance and infection prevention. HBV can be transmitted vertically from mother to newborn baby during delivery. Besides, it can be transmitted sexually, through blood and blood products transfusion and exposure to contaminated blood via needles. There is a variation in the mode of transmission of the virus from a community to another in accordance with norms, traditions, and social factors. In a study conducted in China recruiting more than 8000 subjects, it was found that male gender, old age, and history of surgical operations were associated with high risk of HBV positivity [29].

In another study conducted in Iran, marriage and old age were associated with high risk of acquiring HBV infection. In Turkey, dialysis, family history of HBV and sexual

contact with HBV positive subjects were found as the risk factors for acquiring HBV infection. No studies have been conducted to determine the risk factor of HBV infection in Iraq. In this study, dental surgery was found as a predictive factor for HBV transmission in the community<sup>[30]</sup>.

### Conclusion

The prevalence of HBV blood donors is about 2%, ranged from 22% to 43%. AntiHbC test has been applied in some countries to excluded obi. However, to further reduce the risk of transfusion transmission of HBV, anti-HbC tests in blood screening cannot be applied because the high anti-HbC prevalence in blood donors would lead to unacceptably high donor deferral. Instead, it is of great necessity to apply highly sensitive NAT to test HBV DNA in blood donors.

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