

## Original Article

## Prevalence of Occult Hepatitis B Infection among HBsAg Negative Blood Donors in Golestan Province

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#### Key words

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**Background and Aims:** Occult hepatitis B virus infection (OBI) is known as an important source of hepatitis B virus (HBV) infection. It is categorized as Hepatitis B surface antigen (HBsAg) not being present and low DNA viral load in serum. In this study, an attempt was made to investigate the outbreak of anti-HBc and OBI among the HBsAg-negative donors in Golestan province.

**Materials and Methods:** The present cross-sectional experiment was conducted on 3500 voluntary blood donors in Golestan province to examine the presence of human immunodeficiency viruses Ag-Ab, HBsAg, and hepatitis C virus Ab. Then, samples with negative results for the mentioned tests were screened for total HBc antibody (IgM-IgG) through ELISA technique. Afterward, HBV-DNA extraction and R-T PCR assay were conducted for all HBsAg negative samples by using Real ART HBV LC PCR kit on a Light Cycler instrument.

**Results:** The study participants included 3255 (93%) male and 245 (7%) female. In general, 385 (11%) out of 3500 samples were anti-HBc positive. HBV-DNA results for every sample with either positive or negative anti-HBc were found to be negative.

**Conclusions:** As the area under study has a high rate of anti-HBc outbreak (11%) without the presence of HBV-DNA, anti-HBc screening can cause blood donor deferrals and limit blood supply; therefore, the HBsAg test with high analytical sensitivity is recommended for HBV screening in this area. Regarding the cost analyses and also the status of HBV endemicity, HBsAg test along with ID-NAT is preferable, if possible, for improving blood safety.

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## **Introduction**

Hepatitis B virus (HBV) infection as a main health-threatening factor can result in hepatic cirrhosis, primary liver cancer, and chronic hepatitis [1, 2]. The estimations presented by World Health Organization (WHO) indicate that HBV has affected 450 million individuals around the world [3]. Generally, HBsAg is a sign of HBV infection and the removal of this antigen simply demonstrates HBV disappearance [4].

Almost 35% of people in the world are infected with HBV and 75% of the chronic people are in Asia [5]. Southeast Asia is one of the high endemicity areas in which more than 8% of the people are chronic HBV carriers. The Middle East is a region with a moderate endemicity to HBV out of which 2% to 7% of the populations are carriers of chronic HBV [6]. The introduction of HBsAg in the early 1970s was the first successful attempt to promote transfusion safety [7].

Nevertheless, as reported in the early 1978 [8], HBV infection is likely to be transferred through HBsAg-negative blood products in the critical stage of the infection throughout either the seronegative window period or chronic phases with unnoticeable HBsAg called occult Hepatitis B infection (OBI) [4].

The presence of HBV-DNA (less than 200 IU/mL) in the liver or serum of HBsAg negative patients with or without anti-HBc positivity is the most important characteristic of the OBI. Furthermore, 20% of the subjects show no symptom of HBV serological marker [9]. In these individuals, this antigen fails to

be identified through conventional serological techniques even if there is a virus in peripheral blood and liver [10]. Therefore, HBV-DNA in either liver tissue or serum of the blood donors with OBI is the main cause of HBV infection even if there is no noticeable HBsAg with/without anti-HBc or anti-HBs antibodies [11]; therefore, anti-HBc and HBV-DNA are highly significant measures to decrease post transfusion hepatitis [12].

Recently, the frequency of HBsAg in Iran has been estimated to be in a range of 1.7% to 5% and the HBV infection epidemiology has shifted from vertical to horizontal transmission. Approximately, 72% of the subjects with hepatocellular carcinoma (HCC) are infected with HBV in Iran, and more to the point, 46% of them are carriers of hepatitis B (HBcAb positive). These findings suggest that hepatitis B is the most prevalent source of liver cancer and cirrhosis in Iran [13]. The outbreak of anti-HBc in Iranian blood donors has been reported to range between 2.1-11.5% [14-20].

The Golestan province in the north of Iran has a high outbreak of HBV [21]. The spread of anti-HBc and HBsAg in the general population of this province was reported to be 36.9% and 5.1%, respectively [22]. Therefore for increasing blood safety, we decided to conduct this study and identify the outbreak of OBI and anti-HBc in HBsAg-negative donors in the Golestan province.

## Materials and Methods

### Experimental design

The current cross-sectional study investigated 3500 voluntary blood donors from October to November 2016 in the Golestan province of Iran. Demographics collected by a questionnaire were completed by blood donors. The present study followed the Declaration of Helsinki for Human Research. Ethics approval was obtained from the local Research Ethics Committee of High Institute for Research and Education in Transfusion Medicine (Number: IR.TMI.REC.1396.013). All the subjects were asked to sign the written knowledgeable approval before the study.

### Serological tests

All 3500 subjects were examined for HBs-Ag (Siemens, Germany), HCV Ab (Monalisa V.3, BIORAD, Germany), and human immunodeficiency viruses (HIV) Ab (HIV Ag-Ab, BIORAD, USA) based on the manufacturer's instructions. All blood donor serum samples having negative results for the mentioned tests, were enrolled in this study and stored at  $-80^{\circ}\text{C}$ . The serum samples testing was conducted by the enzyme linked immunosorbent assay method using the monoclonal antibody against total HBc Ab (IgM-IgG) (DiaPro, Milan, Italy) in compliance with the recommendations provided by the manufacturer. Finally, the positive anti-HBc results were retested.

### Molecular analysis

For molecular evaluation, HBV-DNA was extracted by the QIAamp DSP Virus Kit

(Hilden, Germany) in compliance with the manufacturer's recommendations. For the HBV-DNA removal, in-house real-time quantitative polymerase chain reaction (PCR) was used on the Eppendorf Mastercycler thermal cycler (Hamburg, Germany). The assay sensitivity for HBV-DNA was measured to be 30 IU/ml [22].

For the prevention of false positive and negative results, negative and positive control plasma samples were used in each run. Afterwards, real-time PCR assay was carried out on all HBsAg negative samples by using Real ART HBV LC PCR kit on a Light Cycler instrument-complying with protocols recommended by the manufacturers to validate the DNA extraction and inhibition in PCR reaction.

### Statistical analysis

Data analysis was conducted with Chi-Square in SPSS software (v.13.5). In this examination, the results were noteworthy when  $P < 0.05$ .

## Results

The participants included 3255 (93%) male and 245 (7%) female. Their mean age was  $35.03 \pm 10.59$  (range 18 to 65). In general, 385 (11%) out of 3500 samples were anti-HBc positive. Also, out of 385 samples with positive anti-HBc, 14.85 % were first blood donors and 85.2 % of them were regular blood donors (Table 1). HBV-DNA results were negative for all anti-HBc-positive or anti-HBc-negative samples.

**Table1.** Demographic and serological markers of the blood donors

Characteristics	No. (%)
<b>Gender</b>	
Female	245 (7)
Male	3255 (93)
<b>Material status</b>	
Single	805 (23)
Married	2695 (77)
<b>Level of education</b>	
Illiterate	35 (1)
Diploma or lower	2415 (69)
Higher diploma	875 (25)
Bachelor degree or higher	175 (5)
<b>Age group (yr)</b>	
18-25	560 (16)
26-35	1365 (39)
36-45	1015 (29)
46-55	455 (13)
56-65	105 (3)
<b>Jobs</b>	
Housekeeper	245 (7)
Student	420 (12)
Self-employment	1820 (52)
Employee	840 (24)
Laborer	175 (5)
<b>Serological markers</b>	
Anti-HBc-positive	385 (11)
First-blood donors	57 (15)
Regular blood donors	328 (85)

## Discussion

HBV has been proven to be one of the main risk factors causing chronic and acute hepatitis, HCC, and cirrhosis in the developing countries. This virus has affected nearly 400 million people in the world and 75% of this population lives in Asia. Each year, one million people, mainly with HCC and cirrhosis, die from a liver disease associated with HBV [5]. Accordingly, investigating the outbreak of OBI among healthy blood donors seems to be a highly valuable step towards the

estimation of HBV through blood transfusion. Moreover, promotion of a set of strategies associated with pre-donation screening and post-transfusion follow-up of recipients to decrease HBV transmission rate is highly mandatory.

The residual risk of HBV infection in endemic regions is mostly due to the chronic HBV carrier donors with occult hepatitis and undetectable HBsAg level [23]. Several mechanisms have been hypothesized for

OBI infection, including peripheral blood mononuclear cells infected with HBV, inefficient immune response, coinfection with other hepatotropic viruses, insertion of a virus into the host cell chromosomes, and HBV genes mutations [24, 25]. The anti-HBc outbreak among donors was estimated to be 11% in this study. In other studies performed on blood donors in Tehran, Zahedan, and Mashhad, this rate was 9.98 %, 9.6%, and 8.5%, respectively and 78.4%, 74.3% and 82% of the patients had positive anti-HBs respectively. These studies show that approximately 78% of the blood donors contain both antibodies and are recovered from HBV infection. About less than 22% of them that are called “isolated anti-HBc” may be placed in one of these categories: a) false anti-HBc positivity due to poor specificity of the test; b) the loss of anti-HBs antibody followed by recovery from the infection; c) late-stage chronic HBV infection with the loss of detectable HBsAg. Several studies have also been performed to estimate the outbreak of anti-HBc and OBI in various regions of Iran and different reports have been published on the prevalence of OBI among Iranian HBsAg negative individuals. Some of these results are consistent with those of ours [16, 19, 20]: none of the HBc Ab positive donors have HBV-DNA in their serum. For example, in an analysis conducted on 531 blood donors in the central part of Iran (Markazi province) with low HBsAg outbreak [19], the authors concluded that lack of OBI in blood donors was due to the correlation between the OBI and HBV epidemiology in the respective

region. In another study in southeastern part of Iran (Sistan and Baluchestan province), which is a high HBsAg prevalence area, HBV-DNA positive was observed in neither the HBsAg-negative nor anti-HBc-positive donors.

Furthermore, the broad range of OBI (0.006%-22%) has been reported among blood donors in different parts of the world. For instance, in European countries like Poland, Italy, Spain, and Germany, OBI pervasiveness rates of 0.006%, 0.22%, 0.05%, and 0.0006%, have been reported respectively. All in all, these data imply that the prevalence of OBI is distinctive in every country [11].

OBI pervasiveness in multiple findings, on tests of anti- HBc positive as a distinctive indicator of HBV, has been reported to be 0% to >20%. Also, findings from India and Pakistan have indicated an average of 20.6% OBI in anti-HBc-only blood donor the anti-HBc pervasiveness being around 20% [26]. The results of the study conducted in Turkey has shown the absolute isolated rate of anti-HBc positivity in the analysis province being 2.5% (225/9107) and HBV-DNA positivity and in the HBsAg negative assembly was 0.011% (1/9100) [27].

On the whole, there are several reasons why HBV-DNA fails to be revealed in the blood samples of donors such as the high analytical sensitivity of the HBsAg test applied for blood donors screening. As well as the molecular methods and the analytical sensitivity of HBV NAT. On the other hand, HBV-DNA may be intermittently detected in blood donors serum regardless of HBV DNA replication in the liver [20].

Previous studies have shown that the rate of HBV transmission in pre-seroconversion window periods is higher (81%) compared to occult carriers (21%). Also, the infectivity of HBV is associated with the number of transferred blood components, HBV-DNA viral burden, the presence of anti-HBs in either donors or recipients, and immune factors affecting the susceptibility recipients to infection. On the other hand, the screening of anti-HBc faces difficulties due to false reaction and the scarcity of recombinant confirmatory tests, immunological tolerance of babies born from HBeAg-positive women to HBcAg, and deletion of core regions. In our study, as a high prevalence area, there was no HBV-DNA in HBs Ag negative individuals. Therefore, based on the above studies, occult hepatitis transmissibility from donors with high anti-HBs positivity and undetectable HBsAg level is very low in immune competent individuals. In OBI patients, the HBV replication is very low thereby the liver diseases cannot be detected. Accordingly, the immunological system fails to respond to HBV. Indeed, increased serum level of interleukin -10 may prevent an immune reaction to HBV while increased level of interleukin -17A can highly stimulate hepatocyte survival [28]. Replication of the virus occurs by using a reverse transcriptional system without the function of reading readjustment, which means its mutation to be 10 times more likely than other DNA viruses [29]. The S region mutations can be due to the decreased HBV surface proteins expression. Consequently, mutations in both pre S1/ pre

S2 are mostly found in patients with occult HBV, leading to the imperceptibility of HBsAg [30] due to the post-transcriptional impact of the mutation upon the expression of HBsAg [31]. However, the trace-back (HBV detection in the receiver) and look-back (HBV detection in the blood donor component) strategies can be suitable for detecting receivers facing transfusion-transmitted HBV infection. Moreover, applying pathogen-reduction technologies for blood products and HBV vaccination for both donor and recipient populations can terminate the hazard of transfusion-transmitted HBV, especially in the immunosuppressed recipients.

## **Conclusion**

Regarding the fact that anti-HBc has a high spread among blood donors in the Golestan province without the presence of HBV-DNA, anti-HBc screening can cause blood donor deferrals and limit blood supply. Therefore, reducing transmitted-HBV risk regarding the HBV transmissibility in preseroconversion window periods is higher than occult carriers. Also cost-effective tests and the status of HBV endemicity, the high analytical sensitivity HBsAg test, and the HBsAg test in combination with NAT to detect the HBV-DNA is recommended. On the other hand, to reduce the discrepancy between the different reports in Iran on the OBI incidence ranging from 0.0-1.54%, with different molecular methods (e.g., qualitative PCR, nePCR, and Real-Time PCR), a new designing or a unique high sensitive procedure to detect the HBV-DNA seems to be necessary. However, the

epidemiological and geographical parameters are dissimilar in different studies.

### Conflict of interest

The Authors declare no conflict of interest to disclose.

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