



Original Article

Prevalence of Antibodies to Hepatitis B Core Antigen in Hepatitis B Surface Antigen Negative Healthy Blood Donors

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ABSTRACT

The screening of HBsAg has been the cornerstone in HBV research in various states and has greatly decreased but not eradicated TAHBV. Anti-HBc was supposed to be a good indicator of latent HBV infection in the window after HBsAg disappeared. **Objective:** To determine the prevalence of hepatitis B core antibodies in hepatitis B negative surface antigen healthy blood donors. **Methods:** This Cross-sectional study was held in the Medical Ward 7 of Jinnah Postgraduate Medical Center, Karachi from July 17, 2020 - January 16, 2021. A total of 147 healthy blood donors of both sexes, aged 17 to 65 years, who submitted an application for blood donation, were selected. The venous blood (5 ml) was gathered using aseptic technique. For 5 mints; Sera was centrifuged at 3000 rpm and separated. For the qualitative and quantitative detection of anti-HBc IgM, an ELISA test by DIA was performed. **Results:** Of the 147 patients, 81 (55.10%) were male, 66 (44.90%) were females with a M: F ratio of 1.3: 1. In this study; the patients age range was 17-65 years with 41.45 ± 8.97 years of mean age. The pervasiveness of anti-hepatitis B antibodies in healthy donors of blood who have negative surface antigen of hepatitis B virus was 6.12%. **Conclusions:** It was found that the pervasiveness of anti-hepatitis B antibodies in healthy donors of blood who have negative surface antigen of hepatitis B virus was 6.12%.

INTRODUCTION

Hepatitis B virus (HBV) infection result in chronic, acute and occasionally latent infection. HBV has varying rates of endemism around the globe [1, 2]. The incidence of latent hepatitis B depends on the different endemic HBV infection in the peoples studied, the different samples (liver or serum samples), and the different tests performed to detect HBV DNA [3]. Transfusion-related hepatitis B virus (TAHBV) infection remains a serious issue in under-developed regions, even with the implementation of compulsory HBsAg detection tests by ELISA [4]. Blood transfusions are common when this virus is transmitted. The close interpersonal contact with body fluids and blood, sexual contact and intravenous drug user causes transmission from mother to child are other modes of transmission. HBsAg is a blood marker for HBV infection

diagnosis [5]. Current HBV infections or previous HBV infections are routinely tested in blood banks for HBsAg [6]. The (HBsAg) screening in the donors of blood has substantially condensed the post-transfusion hepatitis B incidence. In HBsAg negative patients, HBV DNA is identified in the blood or patients' liver tissue with or without HBV antibodies called occult HBV infection [7]. Another important factor in preventing PTH in the past has been the adoption of antibody testing called anti-hepatitis B (anti-HBc). It may also helpful in preventing infection of hepatitis B virus (HBV) spread by various negative blood HBsAg-donors [8]. Anti-HBc was supposed to be a good indicator of latent HBV infection in the window afterwards the HBsAg disappeared. The recent infection is supposed if first class of IgM showed HBc, while during infection in later

stages anti-HBc IgG appears and indicates previous HBV infection [9, 10]. A study was conducted by Karimi et al. Anti-HBc antibodies were found in 4.9% of HBsAg negative blood donors [11]. Anti-HBc was found in 17.2% of HBsAg negative healthy blood donors [12]. The purpose of this study was to determine the prevalence of hepatitis B core antibodies in hepatitis B negative surface antigen healthy blood donors.

METHODS

A total of 147 healthy blood donors of both sexes, aged 17 to 65 years, who submitted an application for blood donation were selected by non-probability convenient sampling technique. The following simple formula was used to calculate the appropriate sample size for a prevalence study; $n = Z^2 P(1 - P)/d^2$. Where n is the sample size, Z is the confidence level statistic. A solid phase enzyme-linked immunosorbent assay (ELISA) based on the "sandwich" principle using microtiter plates has been developed for the detection of hepatitis B surface antigen (HBsAg). The detection level was less than or equal to 5-10 ng HBsAg/ml. HBsAg-positive patients with a history of HCV, syphilis, or HIV were omitted. The analysis was conducted after CPSP approval and the Ethics Review Committee of Jinnah Graduate Medical Center in Karachi. All participants gave the informed consent after a detailed clarification of the benefits, and samples were collected who agreed to participate. The demographic features like age, sex, and marital status were recorded. The venous blood (5 ml) was gathered using aseptic technique. At room temperature; blood was permitted to retract and clot. For 5 minutes; Sera was centrifuged at 3000 rpm and separated. For the quantitative and qualitative determination of anti-HBc IgM, ELISA was performed with DIA PRO HBc IgM test kits (Diagnostics Bioprobes, Milan, Italy) following the manufacturer's instructions. Conferring to the manufacturer's user manual, the specificity and sensitivity of the test method are 99% and 98% correspondingly. Additional serological tests were accomplished with enzyme immunoassay to detect other HBV markers (HBsAg; Anti HBs) and HCV antibodies. Its lower levels were evaluated by the calorimetric method of Frankel and Reitman. All data collected was recorded on pre-designed structured forms. Information about each participant is confidential and available only to an authorized person. Data were analyzed by means of SPSS 20.0. The variables which are continuous expressed as mean \pm SD. Qualitative variables such as gender, blood donor type, and presence / absence of anti-HBc antibodies are presented as frequency and percentage. Modifiers such as age, sex, and blood donor type were managed by stratification. A chi-square post-stratification test was used to determine the relationship of these modifiers with the presence/absence

of anti-HBc antibodies. Less than 0.05 P-value was taken as significant.

RESULTS

In this study; the patients age range was 17-65 years with 41.45 ± 8.97 years of mean age. Eighty-two (55.78%) patients were between 17 and 40 years of age (Table 1).

Age (in years)	No. of Patients
17-40	82(55.78%)
41-65	65(44.22%)
Total	147(100.0%)
Mean \pm SD	41.45 \pm 8.97 years

Table 1: Distribution of patients according to Age (n=147)

Of the 147 patients, 81 (55.10%) were male and 66 (44.90%) were females with M: F ratio 1.3: 1 as shown in figure 1.

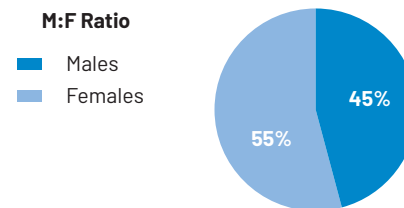


Figure 1: Gender distribution of patients

Patient distribution by blood donor type is shown in Table 2.

Type of Blood Donors	Frequency (%)
A	39(26.53%)
B	60(40.82%)
O	36(24.49%)
AB	12(8.16%)

Table 2: Distribution of patients according to type of blood donors (n=147)

In our study, the pervasiveness of hepatitis B antibodies in healthy blood donors with a negative hepatitis B surface antigen was found in 09 (6.12%) patients (Figure 2).

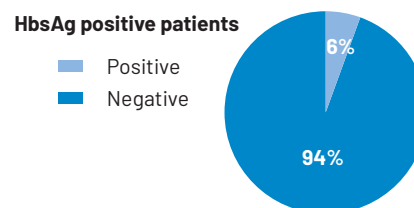


Figure 2: Prevalence of patients with hepatitis B antibodies in healthy blood donors with a negative hepatitis B surface antigen.

The stratification of the core anti-Hep B antibody by age and sex is shown in Tables 3 and 4, respectively and is statistically significant with P-value of 0.989.

Age (years)	Anti Hep B Core Antibody		p-value
	Present	Absent	
17-40	05	77	0.989
41-65	04	61	

Table 3: Stratification of anti Hep B core antibody with respect to age groups.

Gender	Anti Hep B Core Antibody		p-value
	Present	Absent	
Male	05	76	0.977
Female	04	62	

Table 4: Stratification of anti Hep B core antibody with respect to gender

DISCUSSION

Even after hepatitis B surface antigen (HBsAg) recognition by enzyme-linked immunosorbent assay became necessary, transfusion-related hepatitis B virus (TAHBV) infection remained a severe issue in underdeveloped nations (ELISA) [13]. Most under-developed nations have high rates of hepatitis B virus (HBV) infection, particularly in rural regions. The high costs of management, treatment and prevention add to this burden. Blood transfusions are a typical way for this virus to spread [14]. Other means of transmission are through sexual activity, intravenous drug user (IVDU), close interpersonal contact (vertical contact) and risky conventional procedures such ear piercing, tattooing, traditional uvulectomy and circumcision. A diagnostic indicator of HBV infection is the existence of HBsAg in the blood [15, 16]. Blood banks do regularly test for HBsAg to find HBV infection, either present or past. When a patient has neither HBsAg nor HBV antibodies, latent HBV infection is definite as the HBV DNA presence in the blood or liver tissues of the patient [17]. Therefore, the absenteeism of HBsAg in the blood of persons who appear to be in good health points to lack of circulation. Without detectable HBsAg, HBV and anti-HBc antibodies may be infectious [18]. The risk of TAHBV is based on a window phase of the disease that depicts the disease's carrier state. The anti-HBc detection in window phase is a helpful serological indicator of hepatitis B infection at this time [19]. Anti-HBc IgG class appears later and implies prior HBV infection, whereas anti-HBc IgM class occurs first and indicates a recent infection. The mainstay of HBV screening in many countries has been the practice of anti-HBc and HBsAg screening, which has greatly decreased but not completely eradicated TAHBV [20]. In the window phase after HBsAg eradication, anti-HBc has been demonstrated to be a very good indicator of latent infection with HBV. Between 500,000-1.2 million individuals died from infection of HBV and it's increasing each year, making it the ninth most prevalent cause of death worldwide [21]. Cirrhosis, liver failure, or hepatocellular cancer will appear in 15% to 40% of infected people. In Nigeria, HBV infection is regarded as hyperendemic; the HBsAg prevalence in the adult people ranges from 6% to 27% [22]. Nigeria which is an emerging state with a high poverty level, cannot pay to analyse the DNA of all blood units drawn in spite of the fact that this is the only way to attain 100% results [23]. In our investigation, 09 (6.12%) patients had anti-hepatitis B

antibodies, which were common in healthy blood donors with a negative hepatitis B surface antigen. Karimi et al exhibited that 4.9% of HBsAg-negative blood donors tested positive for anti-HBc. Abdelaziz et al and others stated that 17.2% of healthy donors of blood who tested negative for HBsAg also have anti-HBc [24]. Gish, and Locarnini found a 2.1% incidence but no HBV DNA was detected. Anti-HBc as the only marker in a serological pattern is not unusual [25]. There are significant discrepancies between the findings of similar studies conducted in different countries on the pervasiveness of HBV-DNA in HBsAg- and anti-HBc-positive blood donors [26]. Turkey and Greece have a frequency of 0%, Saudi Arabia of 1.25%, Germany of 1.59%, Italy of 4.86%, India of 7.5%, Egypt of 17.2%, Japan of 38%, and Sudan of 90.5% [27-30]. The first study was conducted in the Tripoli region (500 blood donors) in 2014, the anti-HBc rate was 143(9.8%), and the second study was carried out in 2015 (1,256 donors) from the Northwest region of Libya [31]. (Including Tripoli and its vicinity), the anti-HBc frequency was 10.5%. However, our discovery was quite low compared to another study of 200 blood donors at the blood bank of Tripoli Central Hospital in 2009; This "frequency" difference may be due to the small sample size [32]. In contrast, the anti-HBc rate in this study is high compared to that found in a 2015 study (979 blood donors) in the north-central region of Libya (Misurata and neighboring cities).

CONCLUSIONS

In this study, it was found that the pervasiveness of anti-hepatitis B antibodies in healthy donors of blood who have negative surface antigen of hepatitis B virus was 6.12%. Therefore, we recommend routine testing of anti-Hep B core antibodies in healthy blood donors negative for hepatitis B surface antigen to detect hepatitis B infection in donors of blood.

Conflicts of Interest

The authors declare no conflict of interest

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