Research Article

Seroprevalence and risk factors of hepatitis B virus among blood donors in district Charsadda Khyber Pakhtunkhwa Pakistan

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Abstract

Hepatitis B Virus (HBV) is a serious worldwide public health issue both in underdeveloped and developed countries. About two billion individuals were infected by HBV globally, of which 400 million were chronic HBV carrier. The aim of this retrospective study was to investigate the seroprevalence and some possible risk factors of hepatitis B virus infection among male blood donors in district Charsadda, Khyber Pakhtunkhwa Pakistan. A total of 460 male blood donors with age range 15-55 years were screened for HBsAg by Immunochromatographic technique (ICT) and 3rd generation enzyme-linked immunosorbent assay (ELISA). Out of 460 samples, 11(2.39%) were found positive for HBsAg by ICT, and 13(2.82%) were HBsAg positive by ELISA. The mean seroprevalence of HBV from both ICT and ELISA was 2.60%. The highest seroprevalence (46.15%) was observed among the donor groups with age range of 21-30 years followed by (30.76%) among 15-20 years, (15.38%) among 31-40 years and the lowest seroprevalence (7.69%) among 41-55 years. Blood transfusion (30.76%) was the most apparent influencing risk factor for HBV followed by dental treatment (23.07%), Sexual partner positive for HBV infection (15.38%), surgery and shave by the barbers (7.69%) for each and unknown reason (15.38%).

Keywords: Hepatitis B virus; ELISA; ICT; Risk factors; Blood donors

Introduction

Hepatitis B virus (HBV) is a minute, enclosed DNA virus identified in 1947 and was placed in family Hepadnaviridae and Genus Orthohepadna [1, 2]. It is a serious worldwide public health issue both in third

world countries and developed countries [3]. About 2 billion individuals are infected with HBV globally, of which 400 million were chronic HBV carrier [4-6]. Every year approximately 1 to 2 million people die due to HBV-related diseases such as fibrosis,

cirrhosis, chronic hepatitis, and hepatocellular carcinoma [7, 8]. Some important ways for the transmission of HBV are the utilization of unsterilized medical instruments, blood transfusion, sharing of individual items, offering needles to drug addicts, barber risk, reuse of contaminated needles and syringes without sterilization for therapeutic injections, vertical transmission, furthermore by unsafe sexual interaction with HBV infected patients [9-12]. The prevalence of HBV infection among the developing countries of Asia, the Pacific Islands and Africa is very high as compared to developed countries like Australia, Western Europe, and the USA, where the prevalence of HBV is very low [13]. In Russia, Japan, and Eastern Europe about 2-8% of their population are infected with HBV. It is estimated by world health organization (WHO), that about 0.6 million deaths of the people occur annually due to HBV infection. As per 2010 report of WHO, it was observed that 0.12 billion individuals were infected with HBV in China followed by India with 0.04 billion and Indonesia with twelve million infected individuals [14]. In Pakistan, HBV is also a prominent public health issue, and its rate of infection is rising rapidly [15]. The reason for high infection rate might be due to deficiency of suitable public health services, poor financial condition or lack of awareness about the transmission of major sexually transmitted diseases (STDs) i.e. HBV, HCV, and HIV [16]. According to different research studies performed in the various regions of Pakistan, showed that the prevalence rate of HBV is 2-10% among healthy blood donors, 5-9% among medical services staff, 3.16% among the pregnant women, 3.6-18.66% among the general population, 10-20% of patients with temporary diagnosis of hepatitis and 3.16-10.4% among professional blood donors [17].

Blood donors, who are volunteers for blood donation, are usually considered as a healthier part of public community. All the blood banks in public and private hospitals have standard selection criteria for blood donation that helps to identify, and consequently only healthy donors are allowed to donate blood [18]. The percentage of HBsAg positive blood donors and risk factors associated with HBsAg positive situation among the healthy individuals may reflect the extent of HBV infection in the general population. Thus the current study was conducted to evaluate the prevalence and risk factors associated with HBsAg positivity among blood donors of district Charsadda, Khyber Pakhtunkhwa, Pakistan.

Materials and methods

Study design and sample collection

This retrospective study was conducted at the Department of Pathology, Hayatabad Medical Complex (HMC), Peshawar, from 3rd September 2015 to 30th August 2016. A total of 460 blood samples were collected from volunteer blood donors of district Charsadda and tested for hepatitis B surface antigen (HBsAg). Risk factors were evaluated carefully, and detailed medical history of each blood donor was recorded on structured questioner (Performa) and consent form. All the blood donors included in the study were male with age range 15-55 years. About 5 mL blood sample was collected from each blood donor with a disposable syringe under aseptic conditions and allowed to clot. Serum was separated from each blood sample in a centrifuge machine at 4000 rpm for 5 minutes and was transferred to sterilized test tubes for further qualitative and quantitative screening tests. The initial qualitative tests were carried out by chromatographic immunoassay (Acon, USA) for the detection of HBsAg in blood serum. All the positive and negative samples on ICT were further screened on 3rd Generation ELISA Technique (EASE BN-96 TMB, Taiwan) to evaluate the

specificity and sensitivity of ICT and ELISA assays.

Detection of hepatitis B surface antigen (HBsAg) by ICT

We used ICT strips (Acon, USA) according to maker's instructions for the detection of HBsAg. The test strip was removed from the foil pouch and was kept on a clean, dry surface. Then 100 μ L serum sample was dispensed in the strip. After 15 minutes, the results were interpreted according to the appearance of color bands. The control was also run to check the validity of the strip. Both purplish red test bands and purplish red control band appeared on the membrane of the strip which showed a positive result. One red line appears in the layer of the strip in the control region (C). The appearance of no red line in the test area indicated a negative result [19].

Detection of hepatitis B surface antigen (HBsAg) by ELISA

All the positive and negative samples on ICT were further screened on third generation ELISA (EASE BN-96 TMB, Taiwan) according to maker's instructions. Three wells covered with anti-HBs were taken and placed in a holder. 50 μ L of the positive control, negative control and specimens were added to their respective wells. Then 50 μ L of HRP-conjugate was added to each well except the blank and were mixed by tapping the plate gently. Covered the plate with glue slip and was incubated at 37°C for one hour. After incubation, the glue slip was removed from each well and washed five times with diluted buffer. 50 μ L of chromogen solution A and 50 μ L of chromogen solution B were dispensed into each well including the blank and were mixed by tapping the plate gently for 15 seconds. The plate was then incubated at 37°C in the dark for 15 minutes without

shaking. 50 μ L of Stop solution was added to stop the reaction. The absorbance of controls and specimens were determined within 15 minutes by spectrophotometer. The enzymatic reaction between the chromogen solutions and HRP-conjugate produced a blue color in positive control well and HBsAg positive sample wells before the addition of stop solution. After addition of stop solution, the blue color in positive control well and HBsAg positive wells changed to yellow color; Negative samples have clear water like appearance before and after addition of stop solution. Absorbance values of Specimen equal to or greater than the cut-off value i.e. (2.00) were considered HBsAg positive and Specimen with absorbance values less than the cut-off value were considered HBsAg negative [20].

Results

A total of 460 male blood donors with age range 15-55 years were tested for HBsAg by Immunochromatographic technique (ICT) and 3rd generation enzyme-linked immunosorbent assay (ELISA). Out of 460 blood samples, 11(2.39%) were found positive for HBsAg by ICT, and 13(2.82%) by ELISA. The mean seroprevalence of HBV from both ICT and ELISA was (2.60%), (Table 1). The highest seroprevalence (46.15%) was found among participants within the age range of 21-30 years followed by (30.76%) among 15-20 years, (15.38%) among 31-40 years and lowest seroprevalence (7.69%) among 41-55 years (Figure 1). Blood transfusion (30.76%) was the most apparent influencing risk factor for HBV followed by dental treatment (23.07%), Sexual partner positive for HBV infection (15.38%), surgery and shave by the barbers (7.69%) for each and unknown reason (15.38%) (Figure 2).

Table 1. Frequency distribution and percentages of HBV by ICT and ELISA among blood donors of district Charsadda, KP, Pakistan

Parameters	Total Number of samples	Total Negative Samples	Total Positive Samples	Positive Percentage
HBsAg by ICT	460	449	11	2.39%
HBsAg by ELISA	460	447	13	2.82%
Mean Seroprevalence by ICT & ELISA	460	448	12	2.60%

Figure 1. Percentages of the different age groups of blood donors from district Charsadda, KP Pakistan. The blue color in the figure showed positive samples and red color showed the positive percentage of different age groups

Figure 2. Different risk factors of HBV and their percentages among blood donors of district Charsadda, KP, Pakistan

Discussion

The purpose of the current study was to investigate the seroprevalence of HBV infection and its possible risk factors associated with HBsAg positivity through ICT and 3rd generation ELISA among healthy blood donors of district Charsadda, Khyber Pakhtunkhwa Pakistan. All the blood donors included in this study were males because the majority of females in Pakistan do not donate blood due to traditions, especially in Khyber Pakhtunkhwa. The seroprevalence of HBV in our study was (2.60%) which was quite similar to the prevalence of HBV among blood donors in Peshawar (2.68%) [21] and Bahawalpur (2.69%) [22]. While it was lower than the prevalence reported from some other regions of the country including (6.2%) in Interior Sindh [22], (5.86%) in Rawalpindi [23] and (5.81%) in Thatta [24]. Where it was higher when compared with data reported from some other cities of the country including Lahore (1.52%) [25] and Abbottabad (1.55%) [26].

Overall 2.60% prevalence of HBV indicated that Charsadda falls into the intermediate range of HBV infective region of the country. The low prevalence of HBV infection might be due to public awareness, adopting the sensitive techniques like ELISA for screening and implementation of strict standard criteria for blood donor's deferral in the case of possible risk factors.

Our results indicated that ICT used for detection of HBsAg was less sensitive as compared to ELISA because two additional samples were detected by ELISA (Table 1). This might be due to the short incubation period of ICT because characteristically short incubation tests do not detect low concentration of HBsAg in the serum. Therefore the probability of false negative results in a nutshell incubation test i.e. ICT is higher than that of ELISA using longer incubation periods and multiple antigens. It was observed in our study that ELISA

Method is more specific and sensitive than ICT for routine screening of blood donors. The similar results were also reported by Khan *et al.* [27], Abdelbagi *et al.* [28] and Mustafa *et al.* [29].

In this study, risk factors associated with HBV were evaluated by a detailed medical history of each blood donor, recorded by the structured questioner (Performa) and consent form. Blood transfusion (30.76%) was the most deceptive influencing risk factor for HBV active blood donors followed by dental treatment (23.07%), Sexual partner positive for HBV infection (15.38%), surgery and shave by the barbers (7.69%) for each and unknown reason (15.38%). These results were supported by other study conducted in Abbottabad which revealed the different risk factors; blood transfusion of hepatitis B positive individuals was 32 (91.4%), dental treatment was 21 (60%) and surgical procedure was 12 (34.28%) [30].

Conclusion

In this study we found 2.60 % seroprevalence of HBV among blood donors from district Charsadda, Khyber Pakhtunkhwa, Pakistan. The highest seroprevalence (46.15%) was among the age group of 21-30 years, while the lowest seroprevalence (7.69%) was among the age group of 41-55 years. Blood transfusion (30.76%) was the most apparent influencing risk factor for HBV, while surgery (7.69%) and shave by the barber (7.69%) were the lowest risk factors. It was concluded that ELISA is more specific and sensitive method than ICT for routine screening of blood donors.

Recommendations

Awareness regarding control measures in health-care settings including proper sterilization techniques of medical instruments and to educate barbers about the significance of sterilization of their instruments might reduce the problem of HBV infection in this and similar settings.

There is also an urgent need to increase relevant guidelines for counseling and management of HBs Ag-positive blood donors.

It is necessary to implement the strict standard selection criteria to defer and discourage the blood donors with certain high-risk factors. Vaccination for HBV should be adopted at national level. ELISA assay should be adopted in routine blood screening to prevent the transmission of HBV infection and to ensure the safe blood transfusion

Authors' contributions

Conceived and designed the experiments: M Israr & F Ali, Performed the Experiments: M Israr, F Ali & N Bahadar, Analyzed the Data: M Israr, N Bahadar & M Muhammad, Contributed reagents/ materials/ analysis tools: M Israr, N Bahadar & M Muhammad, Wrote the paper: M Israr.

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