Epidemiology of antibody to hepatitis B core antigen screening among blood donors in Eastern Saudi Arabia

Need to replace the test by HBV DNA testing

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ABSTRACT

Objective: Prevention of the residual risk of transfusion transmitted hepatitis B virus (HBV) infection is mostly relied on serological screening of blood donors for antibody to hepatitis B core antigen (HBc), to detect donors in window period of HBV infection. This study was carried out to determine the prevalence of anti-HBc antibody among blood donors and its impact on rejection of collected blood units.

Methods: Blood bank records of all the blood donors who donated blood at blood bank of King Fahad Hospital, Al-Hofuf, Kingdom of Saudi Arabia, during the period of 2000 to 2003 were reviewed. All the collected blood units were screened for hepatitis B surface antigen (HBsAg), anti-HBc, hepatitis C virus (HCV), human immunodeficiency virus (HIV) 1 and 2, HIV p24, human T-cell lymphotropic virus (HTLV) I/II, venereal disease research laboratory (VDRL) and malaria. All the HBsAg negative with anti-HBc positive units were checked for anti-HBsAg antibodies.

Results: Of 26606 blood donors screened, 514 (1.9%) were HBsAg positive, 853 (3.2%) were isolated anti-HBc positive and 2687 (10.1%) were both anti-HBc and anti-HBsAg positive. The blood units, which were anti-HBc and anti-HBsAg positive, were utilized and the isolated anti-HBc positive blood units were rejected. There was a significant (odds ratio of 1.653, 95% confidence interval 1.298-2.105, $p<0.0001$) decline in anti-HBc positivity during the study period.

Conclusion: Isolated anti-HBc positivity as a marker for window period of HBV infection leads to high rejection rate of collected blood units without completely covering the residual risk of HBV transmission by transfusion. Policy for checking the collected blood unit by 3 tests for anti-HBc, anti-HBsAg and HBsAg should be reconsidered in favor of HBV-DNA testing by polymerase chain reaction, to possibly achieve the zero risk goal of transfusion transmitted HBV infection.

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Worldwide, safe blood transfusion (BT) still remains a major concern and despite of all the efforts there is residual risk of transfusion transmitted hepatitis B virus (HBV) infection.[1] Anti-hepatitis B core antigen (HBc) antibody, a sensitive marker of HBV infection, was introduced as surrogate marker for non-A and non-B in blood donors, prior to the availability of test for detection of hepatitis C virus (HCV). Anti-HBc appears in acute phase of infection and can persist in most cases through out life.[2,3] Screening for anti-HBc is useful in detecting the window phase of infection in

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hepatitis B surface antigen (HBsAg) negative donors.[4,5] Other situations where isolated anti-HBc is positive in HBsAg negative blood donors can be; low level of anti HBsAg, the HBsAg escape mutants which cannot be detected by routine assay methods, and false positive anti-HBc.[6-8] Anti-HBsAg positivity is a marker of recovery from infection and immunity but presence of these antibodies are not always indicative of absence of persistent low level of viremia, undetected by HBsAg assay.[8] Although anti-HBc remains an important marker of window period of HBV infection in the blood donors, the false positive results of this marker with negative HBV-DNA have been observed.[1,7]

High anti-HBc positivity among blood donors from the high HBV endemic areas results in a high rate of rejection of collected blood units leading to blood shortage. In Al-Hasa region, 3.2% of HBsAg positivity among blood donors has been reported.[9] The present study was undertaken in Al-Hasa region to determine the prevalence of anti-HBc among blood donors and its impact on the rejection rate of collected blood units.

Methods. This retrospective study was conducted at the Department of Laboratory and Blood Bank, King Fahad Hospital and Tertiary care Center, Al-Hofuf, Kingdom of Saudi Arabia (KSA). Blood bank records of blood donors who donated blood during 2000 to 2003 were reviewed. Donors were 17-55 years of age with body weight of >55 kg. Donors were selected after completing the physical examination and questionnaire. Exclusion criteria were donors aged <17 years or >55 years, body weight <55 kg, hemoglobin <13 gm, history of jaundice, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, uncontrolled diabetes, moderate-severe hypertension, history of recent fever and visit to a malaria endemic area within one year. These donors were predominantly male native Saudi while non-Saudi donors were encouraged to donate for their families.

All the blood donations were screened for HBsAg, anti-HBc, HCV, human immunodeficiency virus (HIV) 1 and 2, HIV p24, human T-cell lymphotropic virus (HTLV) I/II, venereal disease research laboratory (VDRL) and malaria. Hepatitis B surface antigen was tested by enzyme-linked immunosorbent assay (ELISA) using Murex version 3 kits (Murex Biotech Ltd, Dartfort, United Kingdom). All the anti-HBc positive blood units were rechecked for confirmation using total antibodies kits from the same source (DiaSorin, Saluggia, Italy). The confirmed anti-HBc samples were tested for anti-HBsAg using ELISA (DiaSorin, Saluggia, Italy).

Results. During 4 years of study period, plasma samples from 26606 blood donors were tested. Of these, 514 (1.9%) were HBsAg positive. Out of 3540 (13.4%) anti-HBc positive donors, 2687 (10.1%) were anti-HBsAg positive. Isolated anti-HBc positivity was observed among 853 (3.2%) donors (**Table 1**). None of the isolated anti-HBc positive donors had the history of jaundice. The blood units which were HBsAg positive (1.9%) or isolated anti-HBc positive (3.2%) were rejected while donors with anti-HBc and anti-HBsAg positive blood units were utilized.

Hepatitis B surface antigen positivity was higher (368/514, 71.5%) among donors who were more than 40 years of age. No significant (p>0.291) difference in HBsAg positivity was observed during 4 years of study period. There was a significant (odds ratio 1.653, 95% confidence interval

Table 1 - Anti-hepatitis B core antigen positivity among blood donors during 2000-2003.

Hepatitis B virus serology	**2000 N=6742**	**2001 N=6132**	**2002 N=7001**	**2003 N=6724**	**Total N=26606**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**N (%)**
HBsAg positive	131 (1.9)*	125 (2.0)	145 (2)	113 (1.6)	**514 (1.9)**
Anti-hepatitis B core antigen positive	1068 (15.8)	967 (15.7)	767 (10.9)	738 (10.9)	**3540 (13.3)**
Anti-hepatitis B core antigen + Anti HBs positive	793 (11.7)	620 (10.1)	664 (9.5)	610 (9)	**2687 (10.1)**
Anti-HBc positive + Anti-HBsAg negative	275 (4)†	347 (5.6)	103 (1.47)	128 (1.9)	**853 (3.2)**

*HBsAg positivity decline (positivity comparison in 2000 with 2003) p>0.291. †Anti-HBc positivity decline (positivity comparison in 2000 with 2003) OR: 1.653, 95% CI 1.298-2.105, p<0.0001.
HBSAG - hepatitis B surface antigen, HBc - hepatitis B core antigen

1.298-2.105, $p<0.0001$) decline in anti-HBc positivity from 2000 to 2003.

Discussion. Hepatitis B virus infection presents a higher residual risk of transmission by BT. The infectious HBV blood units collected from blood donors were rejected by HBsAg screening.[8,10] The HBV transmissions can also occur by transfusion of HBsAg negative units if the donor is in serologically negative window period. Studies from a high endemic areas have projected 0.2-1 post-transfusions HBV infections for every 1000 transfused blood units.[11] To overcome this risk the donor screening for anti-HBc, to detect the window period of HBV infection has been advocated in the KSA by Ministry of Health. According to the criteria imposed by the Ministry of Health, HBsAg positive blood units must be rejected and the anti-HBc positive blood units need further verification for anti-HBsAg. Anti-HBc and anti-HBsAg positive units of blood were utilized and the isolated anti-HBc positive units were rejected. In the present study, 514 (1.9%) of the collected blood units were rejected due to HBsAg positivity and additional 853 (3.2%) blood units were rejected due to isolated anti-HBc positivity.

Anti-HBc positive blood donors with titer of $\geq$1:10 or >20 IU/ml have a higher positivity of HBV-DNA as detected by polymerase chain reaction (PCR).[12-14] In the present study, none of the anti-HBc positive sample was false-positive on rechecking by the same kit; however the titer of anti-HBc was not determined. There was a significant (odds ratio 1.653, 95% confidence interval 1.298-2.105, $p=0.0001$) decline in anti-HBc positivity from 2000-2003. This could be due to a better awareness on HBV among donors, improvement of health care facilities and a better health education. However, there was no significant decline in HBsAg positivity during the study period as this was more common among donor population aged >40 years due to old infection. Anti-HBsAg positivity is a marker of recovery from HBV infection and immunity. It is claimed that HBsAg negative but anti-HBc and anti-HBsAg positive units of blood are safe for transfusion.[8] Low level of viremia is detectable only by ultra sensitive test nucleic acid test (NAT), which has been described in anti-HBc and anti-HBsAg positive donors suggesting the residual risk involved in accepting these donors.[15,16] Hepatitis B virus DNA positivity has been reported even among donors with high titer of anti-HBsAg; however, other studies have not shown any correlation of HBV-DNA positivity with the level of anti-HBsAg.[17] In such cases the viral load is generally very low, mostly below 10-100 IU/ml and at such level NAT in mini pools is ineffective. Anti-HBsAg or anti-HBc positive donors with such a low level of viral load are infectious in immunosuppressed patients.[18]

Recently, a high (4-38%) positivity of HBV-DNA among HBsAg negative, anti-HBsAg negative and isolated anti-HBc positive donors have been reported.[12,17-20] Earlier study reported that in the Riyadh region up to 30% of blood donors might disqualify if they were screened for anti-HBc.[21] Report from Eastern Province of KSA described a steady decreased from 15.3% to 9.9% in anti-HBc positivity among blood donors during the years 1998 to 2001.[22] In the Western region of KSA anti-HBc positivity of 21.4% has been reported, out of these, 1.2% were positive for HBV-DNA by PCR.[23] This suggests that screening blood units for isolated anti-HBc leads to a higher rejection rate of collected blood units, although they can be negative for HBV-DNA by PCR. In KSA, the screening policy for the blood units needs reconsideration and potential for discontinuation of HBsAg, anti-HBc and anti-HBsAg testing, in favor of HBV-DNA testing by PCR, should be reviewed in the light of cost-effectivity of testing by 3 enzyme immunoassays versus one PCR test. Other possible option could be all isolated anti-HBc positive units of blood should be tested for HBV-DNA by PCR to reduce the rejection rate of collected blood units. This option sounds expensive as all the collected units of blood will have to be screened first by enzyme immunoassay for HBV markers (HBsAg, Anti-HBc and Anti-HBsAg) followed by PCR confirmation. Nucleic acid testing of blood donors in KSA for lesser prevalent infections such as HIV and HCV has been recently suggested to minimize the risk of transfusion associated with infections due to these viruses. [24]

In KSA, HBV immunization was included in the expanded immunization program in 1989. The first blood donor from this generation will be available to the blood banks in 2006 (when he reach the age of 17 years), hence after 2006 onwards the high safety level of BT is expected. Until such time this generation of blood donors is available, only HBV-DNA screening by PCR of all the collected units of blood should be considered a safe practice towards possibly achieving the zero risk goal of HBV transmission through BT. However, prospective studies are needed with NAT testing to show the magnitude of incremental benefits, cost effectiveness and as a replacement for other tests.

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