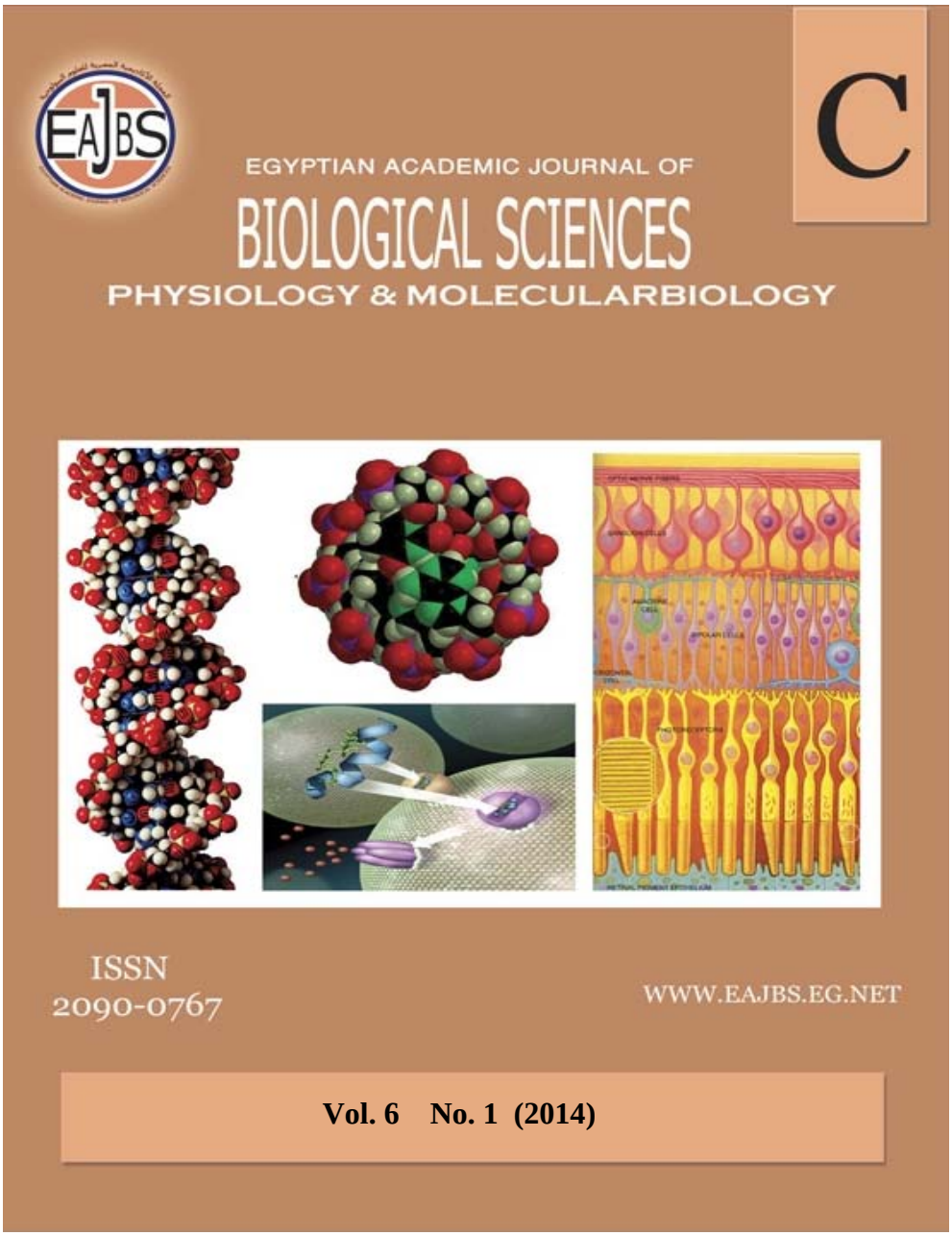

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Hepatitis B Vaccine Reduced the Prevalence of Antibodies to Hepatitis B Core Antigen in Blood Donors in Aseer Region, Saudi Arabia

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ABSTRACT

Viral hepatitis is one of the major problem worldwide. The detection of hepatitis B virus (HBV) surface antigen (HBsAg), antibodies to HBV core antigen (HBc-Ab), and HBV-DNA in donated blood in Aseer Region (Southern part of Kingdom of Saudi Arabia) to detect prevention of the residual risk of transfusion transmitted HBV, donors in window period of HBV infection and the prevalence of HBV infection was attempted. The study was conducted on random blood samples collected from healthy blood donor volunteers, who were referred to blood transfusion centers found at Aseer region, during the period from March 2012 to January 2013. 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, human T-cell lymphotropic virus (HTLV) I/II, venereal disease research laboratory (VDRL), and malaria. All donated blood samples were checked for HBV-DNA, HCV-RNA, and HIV-RNA by nucleic acid test (NAT) technology. Of 6698 (19 females (0.28%) and 6679 males (99.72)) blood donors screened, with median age of 28 (female) and 30 years (males), 69 (1.03%) were HBsAg positive of them 64 (0.96) were positive to HBV-DNA, 411 (6.14%) were anti-HBc positive of them 73 (1.09%) were positive to HBV-DNA. Cases positive to both HBsAg and HBc-Ab were 68 (1.02%) of them 63 (0.94%) were positive to HBV-DNA. All HBsAg and HBc-Ab positive cases were shown to be among male volunteers. In conclusion, prevalence of HBsAg in Asser region is very low. The rate of HBc-Ab in units of blood donation is relatively high. The presence of HBV-DNA in HBc-Ab positive donations makes it risky for use. Vaccination program against HBV decreased the rate of HBV transmission.

INTRODUCTION

Blood transfusion and blood products therapies are well established medical practices. However, collected blood sometimes associated with a risk of infectious pathogen transmission (Hoofnagle 1990; Schreiber *et al*. 1996; Whitsett *et al*. 2012). Transmission of hepatitis B virus (HBV) infection through donated blood is more common than hepatitis C virus (HCV) infection (1:60000 versus 1:103000) (Schreiber *et al*. 1996; Chen and Morgan 2006; Lok and McMahon 2009). Transfusion of blood contaminated with hepatitis B surface antigen (HBsAg) usually followed by post-transfusion infection with HBV. Blood that is free of HBsAg but has high-titer antibodies against hepatitis B core antigen (anti-HBc) in the absence of antibodies against hepatitis B surface antigen (anti-HBs) can also transmit HBV infection (Hoofnagle *et al*. 1978; Mosley *et al*. 1995). The safety of blood and blood products is one of the major issues in the area of transfusion medicine, so serologic screening, as well as the nucleic acid amplification testing (NAT), for HBV, HCV, human immonodeficiency virus (HIV), human T-lymphotropic virus types I/II (HTLV-I/II), and syphilis are the main methods used to reduce the frequency of post-transfusion infections. These different screening policies effectively decreased transmission rates (Alter *et al*. 1972; Donahue *et al*. 1992; Busch 1998; Kleinman 2000; Munoz Bertran *et al*. 2010; Vermeulen 2010; Meena *et al*. 2011). In 1986, screening for anti-HBc was implemented in the United States to reduce HBV transmission and as a surrogate marker for HCV (Stevens *et al*. 1984; Mosley *et al*. 1996). However, a small proportion of donors with anti-HBc in the absence of HBsAg has circulating HBV-DNA and may has a risk of infectivity (Hoofnagle *et al*. 1978; Stevens *et al*. 1984; Mosley *et al*. 1995; Mosley *et al*. 1996; El-Zayadi *et al*. 2008; Hollinger 2008; Raimondo *et al*. 2008). Blood that is collected during the early window period of HBV infection is highly infectious, but this risk declines as anti-HBs develops (Satake *et al*. 2007; Yoshikawa *et al*. 2007; Raimondo *et al*. 2008; Tabuchi *et al*. 2008; Bremer *et al*. 2009; Manzini *et al*. 2009; Phikulsod *et al*. 2009). The infectious blood units which come from the serologically negative window period can be overcome by using NAT for detecting viral nucleic acids. Nucleic acid amplification testing for HBV, HIV, and HCV has already been implemented in many blood banks around the world including Saudi Arabia (El Ekiaby *et al*. 2010; Niemz *et al*. 2011; Strobl 2011; Jain *et al*. 2012; Roth *et al*. 2012). The estimated residual risk of HBV infection from donations to the American Red Cross ranges from 1 in 280,000 to 1 in 357,000 donations (Zou *et al*. 2009). After the introduction of nucleic acid testing for screening in minipools (pools of 6-16 donations), the estimated yield of HBV infection ranges from 1 in 830,000 to 1 in 2 million donations (Stramer *et al*. 2011). Using NAT, investigators have discovered that among individuals with past hepatitis B infection, around 0.5-25%, retained viral DNA in their blood or blood cells; this is termed as occult HBV infection (OHB) (Brechot *et al*. 2001; Allain 2004; El-Zayadi *et al*. 2008; Hu 2002). Even among individuals positive for anti-HBs, 0.5-15% are still tested positive for serum HBV-DNA, though at a very low titer (Matsumoto *et al*. 2001; Noborg *et al*. 2000). In low prevalence countries, like the USA and Japan, blood donors are screened for both HBsAg and antibody to anti-HBc (Busch 1998; Allain *et al*. 1999). Individuals positive for either are disqualified because of ongoing infections or possible OHB. The strategy of combined HBsAg and anti-HBc screening virtually eliminates blood-transmitted HBV, with the rare exception of donations in the early phase of the window period when all serological markers are still negative (Schreiber *et al*. 1996; Matsumoto *et al*. 2001). Such practice may be only in countries where the overall HBV infection rate is low (less than 1%), but not applied in many Asian countries where HBV is intermediately or highly endemic

where about 16-90% of adults may have either past or ongoing hepatitis B infections (Chen 1993; Alter 2003). Under this condition, combined HBsAg and anti-HBc screening strategy would disqualify most volunteer blood donors. Therefore, in several Asian countries blood donors are screened only for ongoing infections by HBsAg, but not for past infections by anti-HBc. This strategy avoids unnecessary waste of donations but bears residual risk of HBV transmission, especially those caused by donors in the window period or with OHB. The incidence of acquiring HBV infection from these HBsAg-negative and anti-HBc-positive donors with OHB is likely higher than in non-endemic areas. Blood banking services in Saudi Arabia are hospital-based, and most government hospitals derive blood from relatives and friends of patients (replacement donors), and rather infrequently, from volunteer donors. Occasionally, blood donor drives are conducted to obtain blood from security and educational institutions. Individuals who are required by law to donate blood before they are given a driver's license or national identity card (statutory donors) augment this pool (Ankra-Badu *et al*. 2001). A volunteer donor pool has been found to be the safest source of blood worldwide (Eastlund 1998; Leikola 1998) and is endorsed by the WHO (1997). In Saudi Arabia, anti-HCV test was introduced in 1994 and anti-HBc and anti-HTLV I/II tests were introduced in 1996 (Ankra-Badu *et al*. 2001).

The aim of this work was to study the prevalence of HBsAg and HBV-DNA in positive HBcAb donations among blood donors referred to blood banks at Aseer region, KSA, as an indicator for the efficacy and efficiency of hepatitis B vaccination program.

MATERIALS AND METHODS

Study Population

The study was conducted on random blood samples collected from healthy blood donor volunteers, who were referred to blood transfusion centers found at Aseer region (Southern part of KSA), during the period from March 2012 to January 2013. According to routine practice, volunteer blood donors were interviewed (history of intravenous drug abuse, jaundice, admission to fever hospital, and history of HBV vaccination) and medically examined before donation. Those with high risk behaviors including intravenous drugs abusers, history of promiscuous sexual relationships, homosexuals, homeless, or those with any medical problem especially jaundice or hospitalization at fever hospitals, bleeding disorders necessitating component transfusion, pregnancy, or recent delivery less than 12 weeks were rejected.

Screening of Serological Markers

All blood specimens were tested on sequential basis for routine serological tests after singing of informed consent. The routine serological tests according to predefined protocol of blood banking safety requirements by Saudi Ministry of Health comprised HBsAg, anti-HBc antibodies (Abs), anti-HCV-Abs, anti-HIV-1/2-Abs, anti-HTLV-I/II-Abs, Malaria, and Treponema Abs, as well as, nucleic acid test (NAT) technology for HCV-RNA, HBV-DNA, and HIV-RNA. Detection of HBsAg was done using Murex HBsAg Version 3 (DiaSorin, UK). Detection of antibodies to hepatitis B core antigen (anti-HBc) was done using Murex anti-HBc (total) kit (DiaSorin) and positive samples were confirmed using Monolisa™ Anti-HBc PLUS kit (Bio-Rad).

Nucleic Acid Test (NAT)

All samples were tested for the presence of HBV, HCV, and HIV nucleic acids by NAT using Roche COBAS® TaqScreen MPX Test which is a qualitative multiplex test that enables simultaneous screening of HIV-1 Group M and Group O RNA, HIV-2 RNA, HCV RNA, and HBV-DNA in pooled and individual plasma donations.

Statistical Analysis

The biochemical data recorded were expressed as mean±SD and statistical and correlation analyses were undertaken using the one-way ANOVA followed by a post-

hoc LSD (Least Significant Difference) test. A P value < 0.05 was statistically significant. A statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

RESULTS

The study was conducted on 6698 random blood samples collected from healthy blood donor volunteers, who were referred to blood transfusion centers found at Aseer region (Southern part of KSA), during the period from March 2012 to January 2013.

The studied samples included 6698 randomly selected blood donations from donors with a median age of 30 years. Donors of ages between 21 and 30 years constituted the largest proportion (50.52%, $P \leq 0.001$) with a median age of 26 years (Table 1). Table 2 lists the distribution of the nationalities of the study participants. Blood donors were mostly Saudi nationals (95.67%). Non-Saudi donors were Afghani, Bengali, Egyptians, Eritrean, Indians, Filipinos, Jordanians, Lebanese, Pakistanis, Palestinians, Sudanese, Turkish, and Yemenis. The higher non-Saudi proportion was Yemenis (1.49%), followed by Egyptians (1.17%), Pakistani (0.37%), Sudanese (0.34%), Indians (0.25%), Jordanians (0.19%), and then Syrian (0.18%).

Table 1: Age and number distribution of accepted blood donors for donation.

Age range	n (%)	Age (median)
18-20	408 (6.09137)	20
21-30	3384 (50.5225)	26
31-40	2021 (30.1732)	35
41-50	725 (10.8241)	45
51-60	160 (2.38877)	55
All	**6698 (100)**	**30**

Table 2: Distribution of nationalities among accepted blood donors for donation.

Nationalities	Number	%
Afghani	2	0.02985966
Bengali	3	0.044789489
Egyptian	79	1.179456554
Erytrian	3	0.044789489
Indian	17	0.253807107
Jordon	13	0.194087787
Lebanese	1	0.01492983
Pakistani	25	0.373245745
Philipine	4	0.059719319
Palastine	4	0.059719319
Saudi	6408	95.67034936
Sudanese	23	0.343386085
Syrian	12	0.179157958
Tyrkey	4	0.059719319
Yemani	100	1.49298298
Total	**6698**	100

All donors showed normal blood pressure, pulse rate, hemoglobin level, and temperature and were voluntary non-remunerated blood donors and were qualified by a questionnaire authorized by MOH. Blood donations from 19 females (0.28%) and 6679 males (99.72%) with a median age of 28 and 30 years, respectively, were tested for the presence of HBsAg and antibodies to HBc-Ag. Screening of the blood donors for the presence of HBsAg resulted in the presence of 69 (1.03%) positive cases, 64 (0.96%) of these cases were positive to HBV-DNA. Cases positive to both HBsAg and HBc-Ab were 68 (1.02%) of them 63 (0.94%) were positive to HBV-DNA.

Antibodies to HBc-Ag was found in 411 (6.14%) donations. There were 68 cases positive for HBsAg and HBc-Ab. Cases positive to HBc-Ab and positive to HBV-DNA in the same time were 73 (Table 3). All positive cases for HBsAg and HBc-Ab were shown to be among male volunteers, while female volunteers were negative for all tested markers.

Table 3: Age and number of blood volunteers positive to HBsAg, HBV-DNA and HBc-Ab.

Marker positive	N (%)	Age (mean ± SD)
HBsAg	69 (1.03)	37.55 ± 9.40
HBsAg + HBV-DNA	64 (0.96)	38.09 ± 9.23
HBc-Ab	411 (6.14)	36.97 ± 9.40
HBc-Ab + HBV-DNA	73 (1.09)	38.11 ± 8.74
HBsAg + HBc-Ab	68 (1.02)	37.40 ± 9.38
HBc-Ab + HBsAg + HBV-DNA	63 (0.94)	37.94 ± 9.22

DISCUSSION

The trend of blood transfusion in medicine saves millions of lives but it might cause unwanted reactions such as hepatitis B virus (HBV) infection. The risk of HBV transmission by transfusion is higher than HCV and HIV (Schreiber *et al*. 1996; Comanor and Holland 2006). In the time that screening of blood units for HBsAg may reduce the rate of HBV transmission, HBsAg negative blood units perhaps still transmit HBV, this may happen due to the seronegative window period or during occult HBV infection (Candotti and Allain 2009). The term occult HBV infection is defined as the presence of HBV-DNA in individuals with undetectable HBsAg outside of the window period (Torbenson and Thomas 2002; Candotti and Allain 2009).

The age range 21-30 years old constituted the largest population among blood donors. In addition, majority of the donors were males and there were very little number of female donors during the period of this study. The percentage of non-Saudi donors was low. A similar study conducted on blood donors in Saudi Arabia by El-Hazmi (El-Hazmi 2004) also showed that the largest group of donors was those at age range 20-29 years old and female donors were as low as 1.2% at the year 2000 and declined to reach 0.7% by the year 2002. Also El-Hamzi (El-Hazmi 2004) showed that the percentage of non-Saudi donors declined from 17.2% at the year 2000 to reach 14.8 by the year 2002. Also Ankra-Badu *et al*. (Ankra-Badu *et al*. 2001) previously showed that the proportion of Saudi blood donors increased with the decrease in the non-Saudis blood donors.

In the present study, we have screened blood donors for the presence of HBsAg, anti-HBc, and HBV-DNA. We found large number of anti-HBc carrier with or without HBsAg positivity. According to De Villa and coworkers (de Villa *et al*. 2003), HBcAb positivity with HBsAg (−) status can reflect a number of situations: (1) it may indicate a false-positive result, so in the present study positive cases were confirmed using different detection kit; (2) it may represent past and currently healed infection, and this is why we in the present study screened units for HBsAg positivity; and (3) it may constitute the sole marker of occult HBV infection, which is thus potentially transmissible, as has been demonstrated by contagion occurring through blood transfusion from donors who are only HBcAb (+) (Hoofnagle *et al*. 1978).

In the present study, it was found that HBsAg positive cases were low while HBc-Ab positive cases were relatively high. Similar work done by Panhotra and coworkers (Panhotra *et al*. 2005) on blood donors showed that 1.9% were HBsAg positive alone, 3.2% were anti-HBc positive alone, and 10.1% were both anti-HBc and anti-HBsAg positive. In the current study, we found only one case (0.02%) positive for

HBsAg alone which means that the presence of HBsAg infection alone in Aseer region is low. It was previously shown that there is a decline in hepatitis B viral infection in South-Western Saudi Arabia and it was attributed to the effectiveness and efficacy of the integration of hepatitis B vaccination into the extended program of immunization in KSA. The significant decline of HBV markers among unvaccinated Saudi adults indicated an indirect effect of other factors (for example, health education and socio-economic progress) on the prevalence and transmission of HBV. In areas of high endemicity, the epidemiological characteristics HBV are modified significantly by the combination of HBV vaccination and other complimentary control strategies (Ayoola *et al*. 2003).

Occurrence of HBsAg positivity among subpopulation was the lowest in 50-60 years old group, while it was zero in young population (group 18-20 years old). Vaccination against HBV was introduced in 1989 for all infants at birth and in 1990 for school children (Al-Faleh 2003). This may be the most important factor responsible for the decline in HBV infection (Al-Faleh 2003).

A study done by El-Hazmi (El-Hazmi 1989) was conducted on male and female population in different provinces of Saudi Arabia. The overall prevalence of hepatitis B surface antigen (HBsAg) was high (16.7%) and no significant difference was encountered between the rate in males and females. Different regions of Saudi Arabia showed a significantly variable prevalence of HBsAg. The eastern province had a prevalence of about 9% compared to the southwestern province where the prevalence was 25% in Jizan. The antibodies anti-HBs and anti-HBc were encountered in 30-67% of the individuals in different provinces, suggesting that a significant number of Saudis were already immune to HBsAg before they reached adulthood.

The presence of HBV-DNA in HBsAg positive samples was in nearly all cases except one. This indicates to how much these blood units are highly infective. Also, HBV-DNA was found in high percentage (17.76%) of HBc-Ab positive cases. This indicated that positive blood units for HBc-Ab should be discarded as it carries high possibility of infectivity. Current infection showing HBsAg and HBc-Ab in the same time was high (16.55%). In the time where HBc-Ab positive cases with HBV-DNA negative is more than those cases with HBV-DNA positive, it still not secure to use these units in blood transfusion. The virus may be found in polymorhonuclear cells or other places other than serum or plasma used for the detection of HBV-DNA (Catterall *et al*. 1994).

The real prevalence of occult HBV infection is still unknown (Khamesipour *et al*. 2011). The data generated by various studies are not comparable mainly due to differences in the methodologies used; however, detection of HBV-DNA using a NAT method is the most sensitive test currently used, but there is no valid standard assay to detect occult HBV infection (Raimondo *et al*. 2007; Raimondo *et al*. 2010). It seems that a complementary test is needed to precisely diagnose HBV infection in HBsAg negative blood donors. However, each country needs to develop its own strategy to screen blood samples based on HBV prevalence, detection of infection by different serologic/NAT screening methods, and cost effectiveness of the screening method to assure that safe blood is used (Kuhns and Busch 2006; Shang *et al*. 2009; Altunay *et al*. 2010). In some countries, like Saudi Arabia, both HBsAg and anti-HBc screening tests are routinely used to reduce the rate of HBV transmission. Such screening is not suitable for countries with a high HBV infection rate, because using such criteria may cause most of the donated blood to be discarded (Kleinman and Busch 2001; Wang *et al*. 2002). An important tool for HBV detection is to use NAT which is expensive and not available everywhere. In KSA, the strategy of screening blood donation for HBV infection depends mainly on screening for HBsAg, HBc-Ab, and

HBV-DNA. This largely goes side by side with vaccination against HBV to decrease rate and transmission of HBV.

CONCLUSIONS

Prevalence of HBsAg in Asser region is very low. The rate of HBc-Ab in units of blood donation is relatively high. The presence of HBV-DNA in HBc-Ab positive donations makes it risky for use. Vaccination program against HBV decreased the rate of HBV transmission.

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