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Sero-prevalence of Hepatitis B Virus infections among blood banking donors in Makkah city, Saudi Arabia: an institutional-based cross-sectional study

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ABSTRACT

Introduction: Worldwide about 350 million people are infected with chronic hepatitis B (HBV) putting HBV infection among the most serious infectious disease problems. Blood transfusion is vital in saving lives but is associated with transfusion-transmittable infections e.g. HBV which can lead to adverse consequences.

Objective: To determine HBV infection prevalence between donors of blood banking in Makkah, Saudi Arabia.

Methods: An institutional-based cross-sectional study was conducted among 2024 blood donors in two hospitals blood banks. Data were collected using laboratory blood screening tools. Data were analyzed using the SPSS statistical program. Statistical significance was considered at $p < 0.05$.

Results: Our study was conducted on 4024 blood donor's data. For Saudi donors, statistical analysis of HBV results has showed that 1% of donors were +ve for HBsAg and 6.8% of donors were +ve for anti-HBc. The results of NAT have showed that 1.1% of donors were positive and all were males. There is a high significance in age between 30-39 and 40-49 for NAT. The overall prevalence of HBsAg was 1% in Saudi donors. For non-Saudi donors, HBV results showed that 0.5% and 12.8% of donors were positive for HBsAg test and Anti-HBc test, respectively. The results of the Anti-HBc test were highly significant for the age range 30-39. The statistical analysis of NAT results showed that 0.8% of donors were positive and all were males. The overall prevalence of the HBsAg test among non-Saudi donors was 0.5%.

Conclusion: The HBsAg and anti-HBc rates among blood donors have declined significantly. NAT is an important tool for viral detection which is expensive and not available everywhere. The total HBV prevalence between donors in Makkah was very low which may be due to recommended safety regulations followed in Makkah as its religious importance and application of an effective vaccine program.

1. Introduction

Globally, HBV infection is considered a major health-related problem. Worldwide, it is stated that about 350 million people are infected with chronic HBV which causes hepatitis [1]. Hepatitis is considered to be a serious disease which causes high rates of morbidity and mortality in the world. After chickenpox, viral hepatitis is ranked second as a viral disease by the Saudi Ministry of Health at 2007. Over the year, there are about 9000 new diagnosed cases with percentages of 52% HBV, 16% HAV and 32% HCV [2].

The safety of blood and blood products is one of the important issues in the area of transfusion medicine. According to the blood banking safety protocol established in advance by the Saudi Health Ministry, the routine serological tests include anti-HCV antibodies (Abs), anti-HIV-1/2-Abs, anti-HTLV-I/II HBsAg, anti-HBc Abs, Treponema Abs, Malaria and the nucleic acid amplification test (NAT) mechanism for HIV RNA, HBV DNA and HCV RNA. These main methods are applied to minimize the recurrence of post-transfusion infections.

The transmission rates are decreased effectively by using different screening policies [3,4]. The HBV Abs raised against core region (anti-HBc), in the absence of both the hepatitis B surface antibody/antigen (anti-HBs, HBsAg), is a chronic HBV infection evidence, which stays detectable for long time [5]. Ordinarily, detection of anti-HBc and HBsAg is used in the diagnosis of HBV infection [6].

Many recent studies were done for the determination of the HBV prevalence among blood donors in different regions of Saudi Arabia

[7,8,9], but to the best of our knowledge the last research study for Makkah was done by Wafa et al., 2015 [10].

The current study aimed to determine the prevalence of HBV infection between donors of blood banking in Makkah, Saudi Arabia.

2. Materials and Methods

2.1 Study Site

King Abdul-Aziz hospital and Security Forces Hospital - Makkah (SFHM) in Makkah which is in the west region of Saudi Arabia

2.2 Study Population

An institutional-based cross-sectional study was conducted among 4024 blood donors in two hospitals blood banks (2821 donors from King Abdul-Aziz and 1203 donors from SFHM). Data were collected using laboratory blood screening tools. The selected donors had an age range of 17 - 67 years with average 31.64 ± 8.55 year, and 3997 were males and 27 were females. According to physical examinations and clinical histories, all blood-donating subjects were healthy and they satisfied the donation criteria.

2.3 Screening of Serological Markers

All blood units were tested on a sequential basis for routine serological tests after giving informed consent. The mandatory serological tests were conducted according to predefined protocol of the blood banking safety requirements by Saudi Ministry of Health and comprised HBsAg, anti-HBc antibodies (Abs), anti-HCV-Abs, anti-HIV-1/2-Abs, anti-HTLV-I/II-Abs, Malaria and Treponema Abs. All the donated blood units were serologically screened by an enzyme-

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linked immunosorbent assay (ELISA) (ARCHITECT plus analyzer, Abbott, Abbott Park, Illinois, U.S.A) for HBsAg, anti-HBc, and anti-HCV according to the manufacturer’s instructions.

2.4 Nucleic Acid Test (NAT)

All samples were tested for the presence of HBV, HCV and HIV nucleic acids by NAT using Roche COBAS@Taq Screen MPX Test (Roche, Basel, Switzerland) 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.

The prevalence of confirmed-positive test results of these viruses was evaluated among different gender, ages and nationalities.

2.5 Data Analysis

A statistical analysis was performed using the Statistical Package for the Social Sciences for Windows (SPSS, version 20.0, Chicago, IL, USA). Percentages and means were calculated and compared between sero-positive and sero-negative hepatitis B markers among different gender and nationalities. According to the HBV markers seroprevalence between participants, we compared the distribution of study factors using a t-test for the continuous variables and chi-squared test for the categorical variables. P values ≤ 0.05 were considered significant for differences in means.

3. Results

3.1 Analysis of the study population

Our study was conducted on 4024 blood donor’s data collected from the blood bank of two hospitals (King Abdel-Aziz and SFHM). In the King Abdel Aziz hospital, the number of blood donors was 2821 of which 1539 were Saudi and 1282 were non-Saudi. 1531 of the Saudi donors were males and 8 were females while in the non-Saudi donors 1280 were males and 2 were female.

In SFHM hospital, the number of blood samples was 1203 of which 1135 were Saudi and 68 were non-Saudi. 1120 of the Saudi donors were males and 15 were females while in non-Saudi donors, 66 were males and 2 were female. Participating blood donors were mostly Saudi donors (66.5%) while participating non-Saudi donors were (33.5%) (Table 1).

Table 1: Numbers and distribution of participated blood donors in the two hospitals

	King Abdel Aziz Hospital	SFHM Hospital	Total	%
Number of donors	2821	1203	4024	100%
Male Saudi Donors	1531	1120	2674	66.5%
Female Saudi Donors	8	15		
Male Non-Saudi donors	1280	66	1350	33.5%
Female Non-Saudi donors	2	2		

The period of the study was between Jan. 2016 to Dec. 2017. The age range of participating blood donors was between 17 and 67 years and the average age was 31.64 ± 8.55 years.

Table 2 shows the distribution of age groups for the participants and Table 3: show the analysis of serological HBV markers among the study participants.

Table 2: Age grouping of the participated donors age.

Age range	N	(%)
<20	135	3.4
20-29	1704	42.3
30-39	1476	36.7
40-49	544	13.5
50-59	150	3.7
>60	15	0.4
Total	4024	100

Table 3: Statistical analysis of HBV and HCV test results among age groups of Saudi and Non-Saudi donors

age classification	Saudi donors			Non-Saudi donors		
	HBsAg	Anti HBc	NAT	HBsAg	Anti HBc	NAT
< 20	0 (0.0%)	1 (0.04%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
20 -29	4 (0.1%)	20 (0.75%)	4 (0.1%)	4 (0.3%)	51 (3.8%)	6 (0.4%)
30 -39	10 (0.4%)	67 (2.5%)	10 (0.4%)	3 (0.2%)	66 (4.9%)	4 (0.3%)
40 -49	10 (0.4%)	65 (2.4%)	10 (0.4%)	0 (0.0%)	43 (3.2%)	1 (0.1%)
50 -59	3 (0.1%)	25 (0.9%)	5 (0.2%)	0 (0.0%)	7 (0.5%)	0 (0.0%)
> 60	0 (0.0%)	4 (0.1%)	0 (0.0%)	0 (0.0%)	5 (0.4%)	0 (0.0%)
TOTAL	27 (1%)	182 (6.8%)	29 (1.1%)	7 (0.5%)	173 (12.8%)	11(0.8%)
p value	<0.0001	<0.0001	<0.0001	0.897	<0.0001	0.985

3.2 Prevalence of HBV among Saudi donors

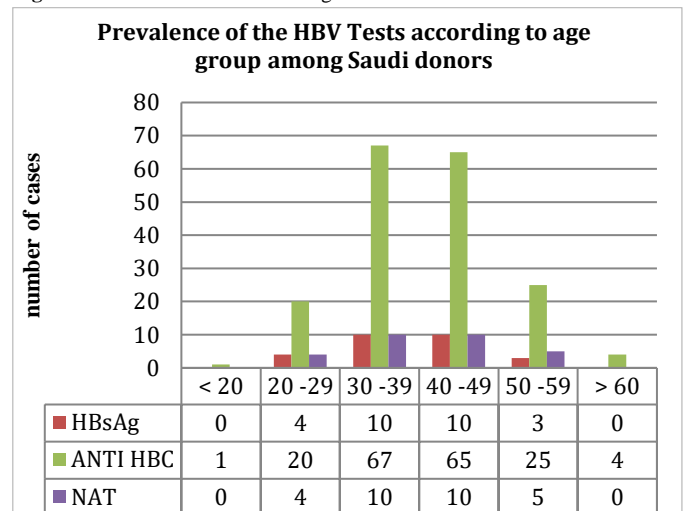
Statistical analysis of HBV results showed that 27 out of 2674 (1%) donors were positive for **HBsAg** serological test. Also, it was found that 182 out of 2674 (6.8%) donors were +ve for **Anti-HBc** test. The molecular diagnostic test results showed that 29 out of 2674 (1.1%) donors were positive for **NAT** test. All the positive donors for the three tests were males and with high significance in the ages 39 – 49 years (p<0.0001) (Table 4).

Table 4: Statistical analysis of HBV and HCV test results among age groups of Saudi donors

age classification	HBsAg	Anti HBc	NAT
< 20	0 (0.0%)	1 (0.04%)	0 (0.0%)
20 -29	4 (0.1%)	20 (0.75%)	4 (0.1%)
30 -39	10 (0.4%)	67 (2.5%)	10 (0.4%)
40 -49	10 (0.4%)	65 (2.4%)	10 (0.4%)
50 -59	3 (0.1%)	25 (0.9%)	5 (0.2%)
> 60	0 (0.0%)	4 (0.1%)	0 (0.0%)
TOTAL	27 (1%)	182 (6.8%)	29 (1.1%)
p value	<0.0001	<0.0001	<0.0001

The overall HBsAg prevalence between Saudi donors was 1%. The results also showed that the units of blood with anti-HBc positive and HBsAg negative results had a prevalence of 6.8% between Saudi donors (Table 3, Figure 1).

Figure 1: Prevalence of HBV among Saudi donors



3.3 Prevalence of HBV among Non-Saudi donors

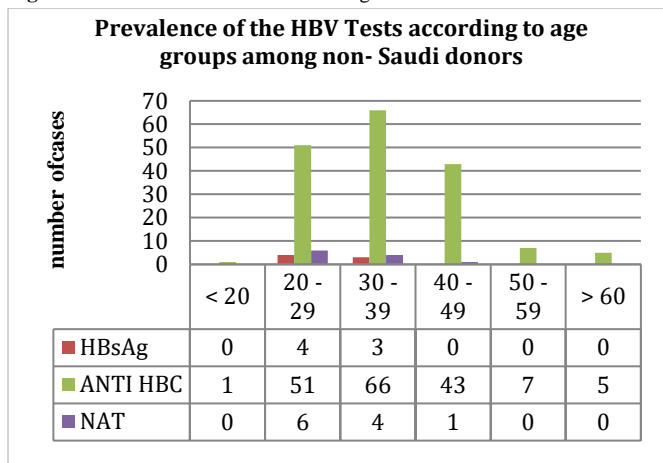
Statistical analysis of HBV results has showed that 7 out of 1350 (0.5%) donors were positive for **HBsAg** test while 173 out of 2674 (12.8%) donors were +ve for **Anti- HBc** test. On the other hand, the NAT test results have showed that 11 out of 2674 (0.8%) donors were positive.

All the positive donors for the three tests were males with no significance except for **Anti HBc** results which showed high significance for the age range 30-39 years (Table 3).

The overall prevalence of the HBsAg test for non-Saudi-donating subjects was 0.5%. The results also showed that the units of blood with anti-HBc positive and HBsAg negative results had higher prevalence

among non- Saudi donors (12.8%) than that of Saudi donors (6.8%) (Table 3, Figure 2).

Figure 2: Prevalence of HBV tests among non- Saudi donors



4. Discussion

Hepatitis transmitted during blood transfusion is a major public health problem all over the world, especially in districts where practices of blood screening are at low level and the prevalence of parentally transmitted infections between blood donors is increasing [11,12]. The trend of blood transfusion in medicine saves millions of lives but it might cause unwanted reactions such as HCV and HBV virus infection.

The objective of a previous study was to evaluate HCV and HBV infections seroprevalence between voluntary blood donors. To get the safest blood for patients, proper screening of blood and proper donor selection should be followed [13,14].

HBsAg discovery was a master penetration in lowering the incidence of post transfusion hepatitis. After HBV infection, HBV DNA is the first marker to appear in the blood, followed by the appearance of markers HBsAg, DNA polymerase and hepatitis B 'e' antigen (HBeAg). After that, the antibodies to each of core antigens (HBcAb), e antigens (HBeAg) and surface antigens (HBsAg) can be detected. ELISA screening for HBsAg of donated blood is the common detecting method for HBV infection. However, blood screening to detect this viral marker does not totally negate the transmission risk of HBV [15] because through the serological response of the host to infection, HBsAg cannot be detected in blood during a phase called the 'window period' although existing HBV infection represents a disease carrier state [15,16].

A previous study conducted by El Beltagy et al., 2008 [7] found a significant association between blood transfusion and prevalence of anti-HBc due to visiting the barber's shop, a family history of Jaundice, tattooing, ear piercing or previous surgery. Generally, their results were unequal to a study done in the Tabuk region, Saudi Arabia, in which the only significant results were with the family history of HBV infection, while no significant association appeared concerning all other risk factors [7].

Another probability is that the donors' cohort aged from 16 to 21 years with no HBV markers were born in years following vaccination for HBV so that they lost their anti-HBs. During the age progress between vaccinated individuals, it is reported that anti-HBs have been declining [17,18]. Moreover, 99.4% of all donors had no HBV markers suggesting a susceptibility for HBV. Furthermore, the donor cohorts aged from 17 to 20 years, who were born after the start of an HBV vaccination program, showed an anti-HBs low rate (99.4%). On the other hand most of them had no HBV markers (98.5%) indicating susceptibility. This suggested that immunization had a limited impact on the low infection rate of HBV between study subjects. HBsAg positive occurrence between subpopulation was zero in the young population (group 18-20 years), while it was the lowest in 50-60 years group.

In a study done in Aseer district, KSA, prevalence of HBcAb was elevated between donors older than 21 years while only one case was HBsAg positive which indicates that the infection with HBsAg alone is low in this district. In the past, it was stated that there is a decline in HBV infection in South-Western Saudi Arabia due to the efficacy and

effectiveness of the integration of HBV vaccination into the extended immunization program (EPI) in Saudi Arabia [19].

The aim of the current study was to determine HBV infection seroprevalence between donors of blood in Makkah, Saudi Arabia by targeting two large hospitals for the study. Our finding that was almost all the donors participating in our study were male and with low female participation like previous studies such as that of Ayoola 2003 [19] which was related to Saudi culture.

The highest numbers of blood donors were aged between 20 to 29 years. Also, the percentage of non-Saudi donors was low which is agrees with a study done by El-Hazmi 2004 in Saudi Arabia [20]. He stated that the major donors were aged from 20 to 29 years and female donors were low, namely, 1.2% in year 2000 reducing to 0.7% at year 2002.

Our results have shown that the prevalence of the HBsAg marker between Saudi blood donors has declined significantly to 1 % and non-Saudi donors to 0.5% which is less than in the southern region (Prevalence was 25% in Jizan), less than Asser region (3%) [7]. This finding can be explained due to adherence to the recommended safety regulations in Makkah because of its religious importance.

Our results have also shown that the occurrence of HBsAg positivity between donors was the lowest in the age range 50 to 60 years, and in the young population between 20 to 29 years old (0.1%) with high significance ($p < 0.0001$). This finding could be related to the effectiveness of the HBV vaccine which was introduced in 1989 for all infants at birth and in 1990 for school children [ALFALEH 21]. This may be the most important factor responsible for the decline in HBV infection.

Our study sample was almost entirely male similar to previous studies [22,23] and non-Saudi donors have also declined from 17.2% to 14.8% between 2000 and 2002 [20]. On the other hand, Ankra-Badu et al. 2001 [24] showed that the proportion of Saudi donor's increased while that of non-Saudi donors decreased.

The current research showed that the prevalence of anti-HBc was significantly decreased from 6.7% to 0.2% with that obtained in the study done in Makkah by Wafa et al., 2015. This finding can be explained due to the improvement in people's knowledge about hepatitis risk through the Ministry of Health educational programs, and the recommended use of disposables at barber's shop.

The NAT technique is a valuable tool for the detection of HBV, but is expensive and is not available everywhere. In KSA, screening strategy of 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 its rate of transmission. The use of NAT techniques in blood donors led to demonstrating HBV infection in low endemicity regions such as Hong Kong and Japan [25].

Detection of infection by variable serologic and NAT methods, and the cost effectiveness of screening to assure blood safety is recommended [26,27] by comparison with post transfusion risk of HBV infection in countries that are using NAT for screening HBV in blood-donating individuals [28].

The current study has showed that the NAT test percentage between non-Saudi donors was 0.8% and between Saudi donors was 1.1%, which is in consistent with the study of Wafa et al., 2015 where molecular DNA-HBV prevalence was 0.72%. This means that combining serological testing with viral nucleic acid will identify more infected individuals. The use of NAT, which is expensive and not available everywhere, is considered to be an important tool that conferred further safety to blood transfusion.

5. Conclusions

1. The best way to get safe blood for patients is by effective blood screening.
2. Almost all donors in the current research study were male like previous studies which is a consequence of how females participate in Saudi culture.
3. Largest group of donors was aged from 20 to 29 years, and almost all showed the lowest prevalence of HBV due to the effectiveness of the HBV vaccination program initiated by Saudi Arabia in 1990.
4. In our study, the HBsAg rate between Saudi blood donors has declined significantly to 1 % and between non-Saudi donors to 0.5% which is less than in the southern region (Prevalence was 25% in Jizan). This finding can be explained due to adherence

to recommended safety regulations in Makkah because of its religious importance and improvements in people's knowledge about the risk of hepatitis via Ministry of Health educational programs, the use of disposables at barber shops and a decreasing culture of tattooing.

5. In our study, the percentage of positive NAT test among non-Saudi donors was 0.8% and among Saudi donors it was 1.1%, which means that NAT is an important tool for HBV detection, but is expensive and not available everywhere.

6. Funding and Ethical Approval

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval for the study was acquired from the committee of research ethics (vice deanship of research at faculty of public health and health informatics/Umm Al-Qura University/Saudi Arabia).

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