Prevalence of HBV, HCV, HIV-1, 2 and HTLV-I/II infections among blood donors in a teaching hospital in the Central region of Saudi Arabia

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ABSTRACT

Objective: Several infectious diseases are transmissible by blood transfusion, especially viral infections. The most common blood-transmitted viruses are hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). These viruses cause fatal, chronic and life-threatening disorders. The prevalence of these viruses varies by nationality and geography. The purpose of this study was to establish the current prevalence of hepatitis viruses (B and C) and human retroviruses (HIV-1, 2 and human T-lymphotropic virus type I and II, HTLV-I /II) among blood donors at King Khalid University Hospital (KKUH), Riyadh, Kingdom of Saudi Arabia (KSA).

Methods: Serological markers of HBV, HCV, HIV 1, 2 and HTLV-I/II were studied in 24173 (23952 males and 221 females), 20423 Saudi and 3750 non-Saudi blood donors, using commercially available kits, over a period of 3 years from January 2000 to December 2002 at KKUH, Riyadh, KSA. The prevalence of confirmed-positive test results of these viruses was evaluated among different gender, ages and nationalities.

Results: During the study period, prevalence rates of HBV and HCV infections were 1.5% and 0.4%, and zero for retroviral infections. The prevalence was not significantly higher in male than in female donors. Hepatitis B surface antigen (HBsAg) and anti-HCV positivity tend to increase with increase in age. The prevalence of HBsAg and anti-HCV positivity was significantly more prevalent among non-Saudi compared to Saudi donors.

Conclusion: This study highlights the prevalence rates of HBV and HCV among different groups. The prevalence varies from one group to another, being the lowest among Saudi and young donors. Therefore, extensive recruitment of Saudi and young donors should help ensure a long-term increase in the blood supply without jeopardizing safety.

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In recent years, there has been increased public concern on the safety of blood transfusion (BT) with respect to transfusion-transmitted infections mainly include hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV-1, 2) and human T-lymphotropic virus I / II (HTLV-I/II). In developing countries, the risk of transfusion-transmitted infectious diseases can be minimized by appropriate selection of donors, promoting altruistic voluntary repeat donation, improving serologic screening, and by reducing the number of BT in accordance with appropriate standards of medical practice.[1] In developed countries, where screening for infectious diseases is universal, there is still a potential risk of transmitting viral infections during the serologic window period early after infection when antibodies are still not detectable.[2,3] Hepatitis B virus and HCV are

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blood-borne hepatotropic viruses and they are the major causes of chronic liver diseases worldwide, particularly cirrhosis and hepatocellular carcinoma. Although the incidence of HBV infection has been markedly reduced after mass vaccination programs, the prevalence of chronic HBV infection worldwide has been estimated as 6.6% (2.8% in developed countries and 7.6% in developing countries) giving a total of more than 260 million cases, most of them from the Asia-Pacific region.[4] Hepatitis C virus infection remains a worldwide public health concern, it is infecting about 3% of the world's populations; the World Health Organization (WHO) recently estimated that approximately 170 million persons worldwide may be infected.[5] Transfusion of HTLV-I infected blood products may be associated with the development of HTLV-I associated myelopathy/tropical spastic paraparesis (TSP) within one month to 4 years after transfusion.[6,7] Screening of blood donations for anti-HTLV-I/II and exclusion of HTLV-I seropositive blood donors is not only effective in preventing transfusion-transmitted HTLV-I infection, but also in preventing transfusion-associated myelopathy.[6,7] Human T-lymphotropic virus II has not been associated with any disease, despite reports of HTLV-II infection of patients with granular lymphocyte leukemia and TSP like diseases. Serological studies have shown that HTLV-I infection is endemic in Southern Japan, the Caribbean region and some parts of Central and South America, Africa, Asia and Australia.[8] Worldwide, WHO estimates that approximately 13 million acquired immunodeficiency syndrome cases (with approximately two thirds in sub-Saharn Africa) had occurred by 1999. Human immunodeficiency virus-1 is the most prevalent HIV type throughout the world; HIV-2 has been found primarily in west Africa.[9] Few current epidemiologic reports on the prevalence of HBV, HCV, HIV and HTLV infections in blood donors in the Kingdom of Saudi Arabia (KSA) have been published. The aim of the present study was to determine the current prevalence of HBsAg, anti-HCV, anti-HIV and anti-HTLV among blood donors at KKUH, Riyadh, KSA and to compare them among different age groups and nationalities of blood donors.

Methods. This is a retrospective study in which the target population consisted of all subjects who donated blood at King Khalid University Hospital [KKUH], Riyadh, KSA from January 2000 to December 2002. Blood donors were volunteers, unpaid and in many cases were relatives or friends of patients who were having medical or surgical treatment. All the donors were screened thoroughly based on the history, physical and hematological examinations before donating blood. A total of 24173 blood donors were tested from different ages and nationalities, these included mainly Saudis, Egyptians, Sudanese, Syrians, Yemenis, Palestinians, Pakistanis, Indians and others. The age groups of the studied donors were as follows: <20, 20-29, 30-39, 40-49 and ≥ 50 years. Many of them were first time male donors.

Serological tests. Serum samples were tested for viral markers by commercially available enzyme-linked immuno-sorbent assay (ELISA), for HBV (Hepanostika HBsAg Uni-Form II, Organon Teknika), HCV (HCV EIA 4.0: United Bio-medical Incorporation-UBI, United States of America), HIV-1, 2 (Vironostika HIV Uni-Form II Ag/Ab, Organon Teknika) and HTLV-I/II (Vironostika HTLV-I/II, Biomerieux). Repeatedly reactive specimens were confirmed by the following tests: the neutralization assay (Hepanostika HBsAg Uni-Form II Confirmatory, Organon Teknika) for reactive HBsAg, samples repeatedly reactive for anti-HCV were further tested by LiaTek (LiaTek HCV III, Organon Teknika), anti HIV-I/2 repeatedly reactive samples were confirmed by (INNO-LIA HIV Confirmation, Innogenetics) and anti HTLV-I /II repeatedly reactive samples were confirmed by (INNO-LIA HTLV I / II, Innogenetics). Serological tests were performed according to manufacturer's instructions.

Statistical analysis. The Chi-square (x^2) test and Fisher's exact test were used to compare the prevalence rates of HBsAg and anti-HCV positivity in relation to the demographic characteristics of blood donors. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were used to estimate relative risk, where appropriate. Mantel-Haenszel Chi-square (MHX^2) test was used to summarize the relationship between positivity for HBsAg and anti-HCV with age. A test for linear trend was conducted to determine whether positivity tended to change with age. A $p<0.05$ was considered significant.

Results. During January 2000 to December 2002, 24173 blood donors were enrolled in this study. As shown in **Figure 1**, most donors were males 23952 (99.1%) and Saudis 20423 (84.5%). There was a statistically significant decline in the percentage of female blood donors from 1.2% in the year 2000 to 0.7% in the year 2002 ($p=0.0026$). The percentage of non-Saudi blood donors decline significantly from 17.2% in the year 2000 to a lowest 14.8% in 2002 ($p<0.001$). The age group 20-29 years, which included 12268 (50.8%) of the donors was the largest group, the smallest group was that of 50 years old and above with 625 donors (2.6%) (**Table 1**). The overall results of confirmed viral markers tested in each year and their percentage are presented in **Table 2**. Among the 24173 blood donors, 370 were found to be positive for HBsAg, giving an overall prevalence of HBsAg of 1.5%. The prevalence was not significantly higher in male than in female donors (1.5% versus 0.5%, $p=0.2702$) (**Table 3**). Hepatitis B surface antigen positivity tended to increase with increase in age (test of linear trend of proportions $p=0.0057$). Among Saudi donors, the prevalence of HBsAg did not differ

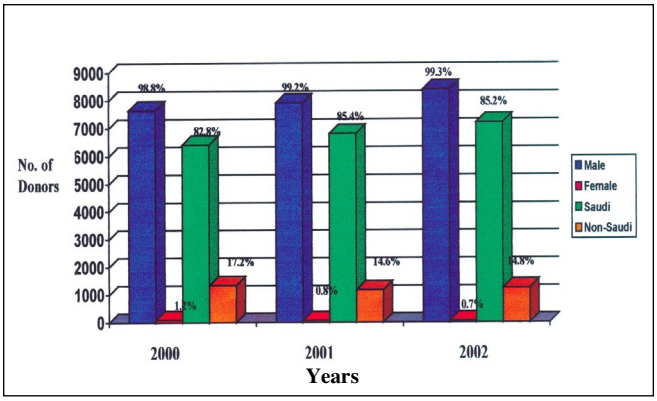

Figure 1 - Distribution of blood donors according to sex and nationality.

Table 1 - Distribution of blood donors according to age.

Age (years)	N of donors	(%)
<20	1466	(6)
20-29	12268	(50.8)
30-39	6973	(28.8)
40-49	2841	(11.8)
≥50	625	(2.6)
Total	**24173**	**(100)**

significantly between the different age groups (p=0.1251). However, a test of linear trend of proportions was significant (p=0.0281), thus indicating that HBsAg positivity tended to increase with age. The older age group (≥50 years) was at a significantly higher risk of being HBsAg positive (OR=2.6, 95% CI=1.1-6.14) compared to the younger age group (<20 years). Among non-Saudis, the prevalence of HBV infection did not differ significantly between the different age groups (p=0.8559), as well as a test of linear trend of proportions was not significant (p=0.4149) and no specific age group was at a significantly higher risk of being HBsAg positive relative to the younger age group (<20 years). In addition, there was no significant correlation between age and HBsAg positivity among non-Saudi (MHX^2 test p=0.9983, OR=1.08, 95% CI=0.48-2.49) in contrast to Saudi donors, where there was a significant correlation between age and HBsAg positivity (p=0.0055, MHX^2 test, OR=1.56, 95% CI=1.14-2.22) across all age groups (**Table 4**). Prevalence of HBsAg positivity was significantly higher among non-Saudis (2.0%) compared to Saudis (1.4%) (p=0.0198). The estimated risk of being HBsAg positive was 1.37% (OR=1.05-1.78) times higher for a non-Saudi compared to a Saudi. Among non-Saudis, HBsAg positivity was the highest among Yemenis (5.7%), followed by Pakistanis (3.3%), Palestinians (2.6%), Sudanese (1.7%), and Syrians (1.4%). Indians were the only ethnic group without prevalence of HBsAg positivity in 346 blood donors. Egyptians had a very low prevalence rate (0.4%) of HBsAg positivity. Overall, among non-Saudis, the prevalence of HBsAg

Table 2 - The prevalence of HBV, HCV, HIV and HTLV among blood donors.

Year	N of donors	HBsAg		Anti-HCV		Anti-HIV		Anti-HTLV	
		Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)
2000	7744	135	(1.7)	37	(0.5)	0	-	0	-
2001	7974	106	(1.3)	36	(0.4)	0	-	0	-
2002	8455	129	(1.5)	30	(0.3)	0	-	0	-
Total	**24173**	**370**	**(1.5)**	**103**	**(0.4)**	**0**	-	**0**	-

HBV - hepatitis B virus, HCV - hepatitis C virus, HIV - human immunodeficiency virus, HTLV - human T-lymphotropic virus, HBsAg - hepatitis B surface antigen,

Table 3 - The prevalence of hepatitis B and hepatitis C among blood donors according to sex and age.

Groups	HBsAg					Anti-HCV*			
	Positive	(%)	Negative	(%)	OR (95% CI)	Positive	(%)	Negative	(%)
Sex									
Male	369	(1.5)	23583	(98.5)	3.44 (0.48-24.6)	103	(0.4)	23797	(99.6)
Female	1	(0.5)	220	(99.5)	1.00	0		221	(100)
p-value	0.2702					0.3867			
Total	**370**		**23803**			**103**		**24018**	
Age (years)									
<20	16	(1.1)	1450	(98.9)	1.00	0	(0.2)	1463	(100)
20- 29	174	(1.4)	12094	(98.6)	1.30 (0.76-2.26)	20	(0.6)	12235	(99.8)
30- 39	114	(1.6)	6859	(98.4)	1.51 (0.87-2.65)	42	(1.1)	6903	(99.4)
40- 49	50	(1.8)	2791	(98.2)	1.62 (0.90-2.98)	31	(1.6)	2804	(98.9)
≥50	16	(2.6)	609	(97.4)	2.38 (1.12-5.04)	10		613	(98.4)
p-value	<0.001					<0.001			
Total	**370**		**23803**			**103**		**24018**	

*results for 52 blood donors were indeterminate and excluded from statistical analysis. HBsAg - hepatitis B surface antigen, HCV - hepatitis C virus, OR - odds ratio, CI - confidence interval

Table 4 - The prevalence of hepatitis B in Saudi and non-Saudi blood donors according to age groups.

Age groups (years)	N of Saudi donors	Positive cases		OR (95% CI)	N of non-Saudi donors	Positive cases		OR (95% CI)
		n	(%)			n	(%)	
<20	1363	14	(1)	1.00	103	2	(1.9)	1.00
20- 29	11094	152	(1.4)	1.34 (0.75-2.42)	1174	22	(1.9)	0.96 (0.23-8.58)
30- 39	5500	88	(1.6)	1.57 (0.87-2.89)	1473	26	(1.8)	0.91 (0.22-8.0)
40- 49	2048	31	(1.5)	1.48 (0.76-2.93)	793	19	(2.4)	1.24 (0.29-11.13)
≥50	418	11	(2.6)	2.60 (1.1-6.14)	207	5	(2.4)	1.25 (0.2-13.33)
p-value		0.1251				0.8559		

OR - odds ratio, CI - confidence interval

positivity differed significantly between the various nationalities ($p<0.001$) (**Table 5**). Infection with HCV was detected in 103 (0.4%) of 24121 donors, 52 samples were indeterminate and were therefore excluded from statistical analysis. The prevalence was not significantly higher in male than in female donors (0.4 versus 0%; $p=0.3867$). Anti-HCV positivity was correlated with age ($MHX^2=102.77$, $p<0.001$, OR=5.18, 95% CI=3.91-8.39) (**Table 3**). Among Saudi donors, results from the MHX^2 test indicated that anti-HCV positivity was significantly correlated with age ($MHX^2=37.3$, $p<0.001$, OR=4.41, 95% CI=2.78-8.72). In the 4 age groups 20-29 years to ≥ 50 years, a test for linear trend of proportions was significant ($p<0.001$), thus indicating that anti-HCV positivity tended to increase with increased age. Also, among non-Saudis, the MHX^2 test indicated statistically significant correlation between age and anti-HCV positivity ($MHX^2=17.2$, $p<0.001$, OR=2.8, 95% CI=1.69 - 4.88). In addition, a test for linear trend of proportions across the four age groups was significant ($p=0.00125$), thus indicating that prevalence of HCV infection tended to increase with age (**Table 6**). Anti-HCV positivity was significantly more prevalent among non-Saudis (1.6%) compared to Saudis (0.2%), ($p<0.001$). Non-Saudis had a significantly higher estimated risk (OR=7.43, 95% CI=4.94 - 11.2) of being anti-HCV positive compared to Saudis. Among non-Saudis, Egyptians (8.1%) and Pakistanis (3.6%) had the highest prevalence rates for anti-HCV positivity. On the other hand, anti-HCV positivity was non-prevalent among 402 Yemeni and 345 Indian blood donors included in the study (**Table 5**). None of our donors had a confirmed positive result for HIV or HTLV, only 8 were indeterminate to HIV and 7 to HTLV.

Discussion. The prevalence of hepatitis B during this study on blood donors was 1.5% and hepatitis C was 0.4%. None of the donors had a confirmed positive result for retroviral infections. The prevalence rates of hepatitis B and C were higher among non-Saudis and older age group than among Saudis and young age group. Comparisons of the prevalence of blood-borne viruses among different sex blood donors may not be valid because of high percentage of male blood donors, this is due to low hemoglobin in female and the fact that women are less willing to donate blood. The high ratio of male to female blood donors in KSA was similar to other countries.[10] The prevalence of HBsAg in our blood donors studied was 1.5%, 1.4% among Saudis and 2% among non-Saudis. These results were lower than previously reported, as

Table 5 - The prevalence of hepatitis B and hepatitis C among blood donors according to nationality.

Nationality	HBsAg			Anti-HCV*		
	N examined	Positive	(%)	N examined	Positive	(%)
Saudi	20423	296	(1.4)	20390	44	(0.2)
Non-Saudi	3750	74	(2)	3731	59	(1.6)
p-value		0.0198			<0.001	
Egyptians	494	2	(0.4)	482	39	(8.1)
Sudanese	596	10	(1.7)	594	4	(0.7)
Syrians	590	8	(1.4)	588	2	(0.3)
Yemenis	403	23	(5.7)	402	0	(0)
Indians	346	0	(0)	345	0	(0)
Pakistanis	276	9	(3.3)	276	10	(3.6)
Palestinians	270	7	(2.6)	270	1	(0.4)
Others	775	15	(1.9)	774	3	(0.4)
p-value		<0.001			<0.001	

*results for 52 blood donors were indeterminate and excluded from statistical analysis.
HBsAg - hepatitis B surface antigen, HCV - hepatitis C virus

Table 6 - The prevalence of hepatitis C in Saudi and non-Saudi blood donors according to age groups.

Age Groups (Years)	N of Saudi donors	Positive cases N	(%)	N of non-Saudi donors	Positive cases N	(%)
<20	1360	0	(0)	103	0	(0)
20-29	11083	11	(0.1)	1172	9	(0.8)
30 – 39	5485	18	(0.3)	1460	24	(1.6)
40 – 49	2044	10	(0.5)	791	21	(2.7)
≥50	418	5	(1.2)	205	5	(2.4)
p-value		<0.001			0.0094	

HBsAg was detected in 4% in Saudi donors in Riyadh,[11,12] Qaseem,[13] and 3.3% in Al-Baha.[14] While the prevalence rate of anti-HCV in the blood donors tested was 0.4%, 0.2% among Saudi donors and with higher rate in non-Saudi donors (1.6%). In comparison with earlier reports, there was an overall decrease in the prevalence of anti-HCV, in which the prevalence of HCV antibody among Saudi blood donors ranges between 1-1.5% in the Riyadh area,[11,12,15] 1.2% in Al-Baha,[14] 1% in Qaseem,[13] 1.2-1.7% in Dammam[16,17] and 1.7% in Jeddah.[18] The differences in the prevalence between our study and other studies may be attributed to differences in the sensitivities of the assays used, the criteria of positivity, types of donors as well as in the degree to which individuals with risk factors for blood-borne viral infections may have been excluded. In most of the earlier studies, an earlier generation of anti-HCV ELISA (which was less sensitive and less specific) was used. However, in our study a fourth generation ELISA and LiaTek III were used, which were more sensitive and more specific. As the prevalence of anti-HCV in our study corresponded well with the study using same criteria of positivity (0.48% for entire donors and 0.33% for Saudi donors)[19] (As we have defined HBsAg and HCV antibodies sero-prevalences by reactivity in both screening and confirmatory tests). It is well known that donors who are found to be positive for markers of infections are asked not to donate blood again, so the prevalence in repeat donors is lower than that in first-time donors,[20,21] and this is obvious by revisions presented by Soldan et al in UK,[21] using new donor prevalence rate (HBsAg of 0.03% in new donors rather than 0.005% in donors; HCV 0.04% in new donors rather than 0.016% in donors and HIV 4.13 per 100,000 in new blood donors rather than 0.73 per 100,000 donors). Differences in infection rates between voluntary and replacement blood donors have been observed.[22,23] Actually, in our study as well as the previous studies carried out in KSA, the blood donors were not classified according to the types of donors (first-time donors versus repeat donors and replacement versus volunteer blood donors). In general, the prevalence rates of hepatitis B and C were lower among young donors than older donors. This confirm the results reported earlier by other investigators,[13,24,25] this may be explained on the basis of increased exposure with age and on the fact that a high awareness of blood-borne viral infections has developed and a comprehensive vaccination program against hepatitis B has been implemented in KSA. It should be noted that the carrier rate of HBV was higher than the carrier rate of HCV in this study and in other studies.[11-14,23,26,27] These data suggested that the mode of transmission and the efficiency of transmission of HBV may be different from that of HCV. The prevalence of hepatitis B and C among different nationalities in KSA as shown in **Table 5** was lower than it is in their native countries. The prevalence of hepatitis B among blood donors was 3.8% in Syria,[26] 9.8% in Yemen,[27], 1.2% in Egypt[28] and 1.2-1.7% in India.[22] Likewise, the prevalence of HCV in blood donors was ranging between 1.3 and 1.8% in India,[22,29] 0.95% in Syria,[26] 2% in Yemen,[27] 1.2% in Libya[30] and high in Egypt (13.6%).[28] This was probably due to the mandatory screening of all expatriates prior to granting residency in KSA. The prevalence of HCV among Saudi donors was shown to be relatively low (0.2%), this was in an agreement with other studies carried in USA (0.29%),[31] Central America (0.19%),[32] Germany (0.1%),[33] Australia (0.29%),[34] Singapore (0.37%)[35] and Iran (0.09%).[36] This can be explained by an introduction of newer generation of anti-HCV testing in BT service has contributed to control and reduction of transmission of HCV as this virus is primarily parenterally transmitted. Human immunodeficiency virus infection is a major health problem in sub-Saharan Africa where the

prevalence of HIV among blood donors ranges between 2-20% in Kenya[37] and 5.9% in Ethiopia.[38] However, our results showed no confirmed HIV in the analyzed donors. Thus, in our study the prevalence of HIV in KSA was recorded as 0% among blood donors and other studies have reported the same results,[14,39] this can be explained on the basis that KSA is an Islamic country where religious culture and traditions are practiced, as Islamic rules prohibit extramarital sexual activities and drug abuse, in addition to screening of expatriates workers entering the kingdom and increased educational awareness have contributed to the success of HIV control in KSA. None of our donors had a HTLV positive result with confirmatory testing. Seven of them were indeterminate and this was in an agreement with other studies in Jeddah[40,41] and Riyadh.[42] As the result, HTLV-I/II seem to be non-endemic in Middle Eastern countries including KSA.[8]

In summary, this study has shown that prevalence of hepatitis B and C (1.5% and 0.4%) has reduced in KSA. Further educational programs should target both public and hospital personnel to increase awareness concerning these pathogens. It should be noted that the prevalence of hepatitis B and hepatitis C markers was lower among young donors than among older donors, hence, young people should be encouraged to donate blood to help ensure a long-term increase in the blood supply without jeopardizing safety. The prevalence of hepatitis B and hepatitis C was higher among non-Saudi compared to the Saudi blood donors, hence, non-Saudis had a significantly higher estimated risk of being HBsAg and anti-HCV positive compared to Saudis. Therefore, Saudis should be encouraged to donate blood. Among expatriates, HBV, HCV, HIV testing were mainly carried out for issuing residency permit which is mandatory for working in KSA, but the screening has remained inadequate and incomplete. We feel that a strong need exists for seeking the collaboration of private and public sector laboratories to provide definitive right results.

Finally, implementation of more sensitive tests (such as nucleic acid amplification testing [NAT] for HIV, HBV and HCV) that detect infection earlier (reduce the window period) will further decrease risks of transfusion-transmitted viral infections. A further study can be carried out performing NAT on seronegative blood donor samples to determine the risk of transfusion-transmitted infections associated with window periods.

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