JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Seroprevalence rates of transfusion-transmitted infections among blood donors in Jordan

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Abstract

Introduction: Statistics on the prevalence of donor screening for hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (anti-HCV) in Jordan are outdated. There are no studies on the prevalence of anti-HIV I/II, anti-human T-cell lymphotropic virus type I and II (anti-HTLV-I/II), or anti-syphilis. Data are also lacking on the prevalence and significance of using anti-HBc screening in Jordan. This study aimed to evaluate the prevalence of transfusion-transmissible infections (TTI) among donors at King Hussein Cancer Center and compare it with neighboring countries and to evaluate the significance of screening for anti-HB core total (anti-HBc) antibodies.

Methodology: A retrospective analysis covering the period from 2009 to 2013 was conducted on records of healthy donors. The number of donors was 10,101, 12,694, 13,387, 14,256, and 12,495, respectively. Donors were screened for HBsAg, anti-HBc, anti-HIV I/II, anti-HCV, and anti-HTLV-I/II using ELISA technique, while syphilis antibodies were detected using rapid chromatographic immunoassay.

Results: Among 62,933 donors, the prevalence of HBsAg was 0.52%, of anti-HBc was 6.04%, and of anti-HCV was 0.16%. None of the donors were positive for anti-HIV I/II, anti-HTLV I/II, or anti-TP.

Conclusions: This study demonstrates that the seroprevalence for HBsAg, anti-HBc, and anti-HCV in Jordan was low compared to neighboring countries. None of the donors were confirmed positive for anti-HIV I/II, anti-HTLV I/II, or anti-TP during the studied period. This study demonstrates the importance of screening for anti-HBc to improve blood and platelet safety and stresses the need to complement it with an algorithm that qualifies reentry of anti-HBc false-positive donors.

Key words: donors; transfusion-transmitted disease; anti-HBcore total antibody.

J Infect Dev Ctries 2016; 10(4):377-383. doi:10.3855/jidc.8160

(Received and Accepted 25 January 2016)

Introduction

It is crucial for centers collecting blood and blood products to ensure the safety of these products so as not to harm patients subjected to transfusions. For example, Jayaraman *et al.* found that the median overall risks of becoming infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) from a blood transfusion in sub-Saharan Africa were 1, 4.3, and 2.5 infections per 1,000 units, respectively, which systematically quantified the risks of transfusion-transmitted infections across sub-Saharan Africa [1]. Hence, they postulated that transfusions alone might be responsible for 28,595 HBV infections, 16,625 HCV infections, and 6,650 HIV infections every year if the annual transfusion requirements predicted by the World Health Organization (WHO) were met [1].

A review article by Kaur *et al.* showed that the risk of transmitting HBV virus was 1:63,000 of transfused units in the United States [2]. Moreover, in 2005, the estimated number of HCV-infected people in the world exceeded 185 million [3].

According to the latest statistics published by the WHO, Jordan's total population in 2013 reached 7,274,000 [4], and with the recent influx of huge numbers of refugees from neighboring countries, it is important to establish baseline/benchmark information on the prevalence rates of transfusion-transmissible infection (TTI) markers in Jordan.

Previous studies reporting on the prevalence of HBV and other potentially TTIs in Jordan are either outdated (published results appeared during 1987 – 2002) or were limited to a specific socioeconomic class [5,6] or gender [7].

The Jordanian Ministry of Health (JMOH) reported that the incidence of HIV infection among the Jordanian population in 2013 was 0.4 per 100,000, while the incidence of clinical HBV infection among Jordanians ranged from 0.2 per 100,000 in 2009 to 0 per 100,000 in 2013. These figures were based on transfusion-transmissible infectious disease notifications received

by the JMOH [8]. The aim of this study was to evaluate the prevalence rates of TTI markers among donors in Jordan, since there is very little or incomplete data in this field, and to compare our data with published data from neighboring countries. Another aim was to assess the percentage of donors who, despite testing negative for HBsAg, might still pose a risk of transmitting HBV infection due to previous/occult exposure to HBV by being positive for anti-HB core total as was published by Hoofnagle *et al*. [9] along with several other studies [10-12]. These studies demonstrated the importance of screening for anti-HBc in order to detect chronically infected donors who are healthy, asymptomatic, and do not realize that they were previously infected with HBV. Hence, they are considered carriers for HBV and could transmit this virus to their families and patients when they donate blood and/or blood products.

To achieve the aims of this study, we analyzed data from healthy volunteer blood and aphaeresis donors to find estimates of prevalence data for all six TTI markers: anti-HIV I/II, anti-hepatitis C virus antibodies (anti-HCV), HBsAg, anti-human T-cell lymphotropic virus type I (anti-HTLV-I), anti-HTLV-II, and anti-hepatitis B core total antibodies (anti-HBc), with a particular focus on the anti-HBc marker, in order to detect possible healthy HBV carriers among the donor population.

Methodology

At King Hussein Cancer Center (KHCC), the College of American Pathologists' guidelines are followed in screening blood donors [13]. Hence, serological tests using enzyme-linked immunosorbent assay (ELISA) technique were performed to screen blood and platelet donors for anti-HIV I/II, anti-HCV, HBsAg, anti-HTLV I/II [13], and anti-HBc. Anti-syphilis antibodies are detected by using rapid chromatographic immunoassay. In addition, nucleic acid testing for HIV, HCV, and HBV units that tested negative by ELISA was performed.

Subjects studied

A retrospective cross-sectional study was done on 62,933 healthy donors at KHCC between 2009 and 2013. There were 46,517 blood donors and 16,416 apheresis-platelet donors. The donors were adults ranging in age from 18–60 years of age.

All blood and platelet donors were either volunteers or family replacement Jordanian citizens and were interviewed according to the JMO Health blood donor rules and regulations.

A five-milliliter blood sample was collected from each healthy donor, using a red top/plain blood collection tube, taken at the time of blood/platelet donation. The sample was kept at room temperature until fully clotted. Blood samples were spun at 2,000 relative centrifugal force for 10 minutes at room temperature. Serum was collected thereafter to perform the infectious diseases screens.

HBsAg screening

Screening for HBsAg was performed using either Murex HBsAg Version-3 ELISA kit (DiaSorin S.p.A., Dartford, UK) or Bio-Rad Monalisa HBsAg sandwich ELISA kit (Bio-Rad, Marnes-la-Coquette, France). Testing procedure and interpretation of the results were done according to the manufacturer's instructions. Samples giving equivocal (gray zone) readings were confirmed by repeating the sample in duplicate using the same kit and testing it using another methodology, such as the chemiluminescent microparticle immunoassay (CMIA) on Abbott Architect i1000 analyzer, ARCHITECT HBsAg Qualitative assay kit (Abbott Ireland, Sligo, Ireland), or using the microparticle enzyme immunoassay technique on Abbott AxSYM Immunoassay System, AxSYM HBsAg (V2) (Abbott Park, IL USA). Repeatedly positive samples were considered positive for HBsAg.

Anti-HBc antibody screening

Murex anti-HBc (total) ELISA kit (DiaSorin S.p.A., Dartford, UK) or Bio-Rad Monolisa Anti-HBc PLUS assay kit (Bio-Rad, Marnes-la-Coquette, France) were used to screen for anti-HBc. Testing procedure and interpretation of the results were done according to the manufacturer's instructions. Samples giving equivocal (gray zone) readings were confirmed by repeating the sample in duplicate using the same kit and testing it using another methodology such as the CMIA on Abbott Architect i1000 analyzer, ARCHITECT Anti-HBcII assay kit (ABBOTT Wiesbaden, Germany), or using the microparticle enzyme immunoassay technique on Abbott AxSYM Immunoassay System, AxSYM CORE (ABBOTT, Wiesbaden, Germany). Repeatedly positive samples were considered positive for anti-HBc.

Anti-HCV antibody screening

Screening for anti-HCV antibody was performed using either Murex anti-HCV (version 4.0), (DiaSorin S.p.A., Dartford, UK) or DIAsource anti-HCV V 4.0 (DIA-source ImmunoAssays SA, Nivelles, Belgium) ELISA kits. Testing procedure and interpretation of the

results were done according to the manufacturer's instructions. Samples giving equivocal (gray zone) readings were confirmed by repeating the sample in duplicate using the same kit and testing it using another methodology such as the CMIA on Abbott Architect i1000 analyzer, ARCHITECT Anti-HCV assay kit (ABBOTT, Wiesbaden, Germany), or using the microparticle enzyme immunoassay technique on Abbott AxSYM Immunoassay System, AxSYM HCV version 3.0 (ABBOTT, Wiesbaden, Germany), or by PCR.

Anti-HIV I/II antibody and antigen screening

Screening for anti-HIV I/II antibody and antigen was performed using either Murex Ag/Ab combination assay (Murex HIV 1.2.0 kit, Murex Biotech, Dartford, UK) or Bio-Rad Genscreen ULTRA HIV-1/2 Ag-Ab (Bio-Rad, Marnes-la-Coquette, France) sandwich ELISA for the detection of HIV antigen and antibodies combo ELISA kit. The testing procedure and interpretation of the results were done according to the manufacturer's instructions. Samples giving equivocal (gray zone) readings or preliminary HIV reactive samples were sent to the JMOH Central Public Health Laboratory (CPHL), Amman, Jordan for confirmation according to the JMOH's guidelines rules and regulations.

Anti-syphilis antibody screening

The One Step Ultra Syphilis Test Device (ABON, ABON Biopharm, London, UK), a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to *Treponema pallidum* (TP) in serum to aid in the diagnosis of syphilis, was used for screening of donors. Repeatedly positive samples were considered preliminarily positive for syphilis. To confirm preliminary syphilis-positive samples, KHCC follows the JMOH rules and regulations by sending the preliminary reactive syphilis samples to the JMOH CPHL, Amman, Jordan for confirmation.

Anti-HTLV I/II antibody screening

Murex anti-HTLV I/II qualitative enzyme immunoassay (ELISA) test kits were used for detecting antibodies against HTLV I/II (DiaSorin S.p.A., Dartford, UK). Testing procedure and interpretation of the results were done according to the manufacturer's instructions. Samples giving equivocal (gray zone) readings were confirmed by repeating the sample in duplicate using the same kit. Repeatedly positive samples were considered positive for HTLV I/II.

Note

All the kits used in the screening process for all tests have a sensitivity and specificity greater than 99.8% according to the manufacturer's claim. Moreover, all initially reactive units for any of the above transfusion-transmitted pathogens were discarded immediately prior to getting the confirmatory result. Once the result was confirmed, the blood bank supervisor notified and followed up with the donor and the appropriate infectious disease authorities according to KHCC policies.

Statistical methods of analysis

Descriptive analysis was used for the prevalence rates. Only confirmed results were included in the analysis in this study. Comparisons of seroprevalence rates throughout 2009–2013 were carried out using Chi-square or Fisher's exact test. The same statistical tests were used for subgroup analyses. A significance criterion of $p \leq 0.05$ was used in the analysis. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, USA). The prevalence was calculated based on the number of donations tested and number of donations with positive results in confirmatory screening tests [14].

Results

The total number of healthy Jordanian donors at KHCC from 2009–2013 was 46,517 blood donors and 16,416 apheresis-platelet donors. The donors were adults ranging in age from 18–60 years and were randomly distributed among both genders.

Table 1 summarizes the prevalence rates and numbers of positive donors. Data shows that the seroprevalence rate of HBsAg among donors was around 0.52%, on average, from 2009–2013 and the seroprevalence of anti-HBc was around 6.04%. On the other hand, the seroprevalence rate of anti-HCV was 0.16% during the studied period. Moreover, there was a significant decrease in the rates of HBsAg and anti-HBc among the donors between 2009 and 2013 with p values of 0.045 and 0.001, respectively. In contrast, the rates for anti-HCV did not show any significant change over the years.

A breakdown of the prevalence rate for HBsAg among blood and platelet donors at KHCC during the studied period was 62/10,101 (0.6%) in 2009, 79/12,694 (0.6%) in 2010, 78/13,387 (0.6%) in 2011, 63/14,256 (0.4%) in 2012, and 51/12,495 (0.4%) 2013. The prevalence rate for anti-HBc it was 731/10,101 (7.2%) in 2009, 922/12,694 (7.3%) in 2010, 846/13,387 (6.3%) in 2011, 762/14,256 (5.3%) in 2012,

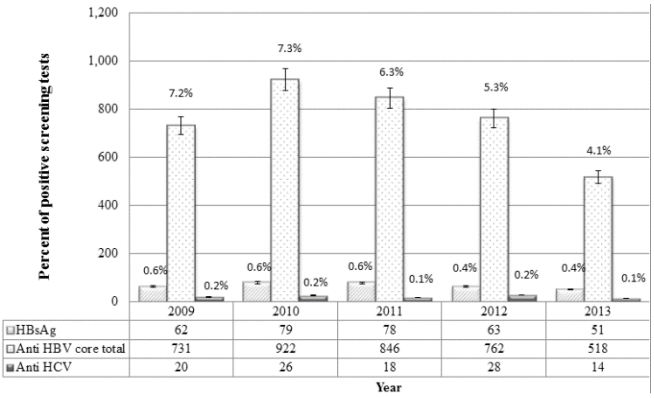
and 518/12,495 (4.1%) 2013. On the other hand, the prevalence rate for anti-HCV was 20/10,101 (0.2%) in 2009, 26/12,694 (0.2%) in 2010, 18/13,387 (0.1%) in 2011, 28/14,256 (0.2%) in 2012, and 14/12,495 (0.1%) 2013. There were no positive cases for anti-HTLV I/II or anti-TP (Figure 1 and Table 1).

A cross-sectional/representative analysis on the total number of blood and platelet donors between 2012 and 2013 was done. It demonstrated that the seroprevalence rate for anti-HBc in donors who were also negative for HBsAg was 92.5%. This percentage dropped to 90.5% in 2013; hence, the average percentage in 2012 and 2013 was 91.5% in anti-HBc-positive who also tested negative for HBsAg. None of the HBsAg-positive donors were negative for anti-HBc, while the average percentage of donors who tested positive for both HBsAg and anti-HBc was 8.5% (data not shown).

Moreover, this data showed that none of the donors were double positive for anti-HCV antibodies and HBsAg between 2012 and 2013. However, there were two donors in 2012 who tested positive for both anti-HCV and anti-HBc in 2012, and no cases tested positive for both anti-HCV and anti-HBc in 2013 (data not shown).

During the study period, there were four donors who tested repeatedly positive for anti-HIV I/II by the ELISA technique. However, when the samples were tested again using confirmatory western blot analysis at the MOH laboratories, they were confirmed negative. Likewise, there were three repeatedly positive donors using the ABON-rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to Treponema pallidum (TP) test, which were confirmed negative when repeated using a confirmatory Treponema pallidum particle agglutination assay (TPHA) test performed at the MOH laboratories.

Figure 1. The prevalence rates and numbers of positive infectious disease markers among KHCC donors over five years (January 2009–December 2013) (N = 62,933). None of the donors were confirmed positive for anti-HIV I/II, anti-HTLV I/II, or anti-TP during the studied period.



Discussion

This is the first cross-sectional study which explores the seroprevalence rates of six TTI markers among healthy Jordanian blood and aphaeresis platelet donors. Previous studies reporting on the prevalence of HBV and other potentially transfusion transmittable infections in Jordan are either outdated (published results appeared during 1987–2002) or were limited to a specific socioeconomic class or gender [5-7]. In this study, we used data on healthy blood and aphaeresis donors to shed light on the prevalence rates of TTI markers in Jordan in more details. Furthermore, we sought to expand the scope of previous findings by including estimates of prevalence data for all six TTI markers, with a particular focus on the anti-HBc marker, in order to detect possible healthy HBV carriers among the donor population.

In this study, we did a retrospective analysis of 46,517 blood and 16,416 platelet donors, a total of 62,933 Jordanian donors, from both genders who donated blood/platelets at KHCC blood bank between

Table 1. Seroprevalence rates and number of blood and platelet positive donors at King Hussein Cancer Center from 2009–2013 (N = 62,933).

	Year						
Infectious disease marker	**2009**	**2010**	**2011**	**2012**	**2013**	**Average**	**P value**
HBsAg	62 (0.6%)	79 (0.6%)	78 (0.6%)	63 (0.4%)	51 (0.4%)	0.52%	0.045
Anti-HBc	731 (7.2%)	922 (7.3%)	846 (6.3%)	762 (5.3%)	518 (4.1%)	6.04%	0.001*
Anti-HCV	20 (0.2%)	26 (0.2%)	18 (0.1%)	28 (0.2%)	14 (0.1%)	0.16%	0.242
Anti-HIV I/II	0%	0%	0%	0%	0%	0%	NA#
Anti-HTLV I/II	0%	0%	0%	0%	0%	0%	NA#
Total antibodies to TP	0%	0%	0%	0%	0%	0%	NA#
Total number of donors per year	10,101	12,694	13,387	14,256	12,495	-----	------

* Significant; # NA: Not applicable.

the years 2009 and 2013. Our results showed that the average seroprevalence of HBsAg in apparently healthy donors was around 0.52%. The only available peer-reviewed data on the Jordanian population was published in 1985 and demonstrated that the prevalence rate of HBsAg ranged from 5.7%–12.8% among Jordanian village inhabitants [5,6], which is significantly higher compared to the current study. This discrepancy might be due to the fact that our study population belongs to a higher socioeconomic status because many of our donors were national citizens from the capital Amman, while those in Toukan's study were from rural areas. In addition, the general socioeconomic status of the Jordanian population has tremendously improved over time, as the current study is more than 25 years more recent than the previous one. The improvement in the socioeconomic status of the Jordanian population is evident in the lower incidence of infectious diseases and infant mortality rates [4,8]. The positive effect of the enhancement in the socioeconomic status [15,16] in Jordan was also seen in our study where the prevalence rates in HBsAg and anti-HBc markers among healthy donors showed a significant decrease from 2009 to 2013. In addition, it is possible that the lower prevalence rates in the HBsAg marker might reflect the fact that some of the donors in this study were healthcare providers, which means that they were previously vaccinated against HBV, thus providing a selection bias.

Another important aspect in our study is the fact that the seroprevalence of HBsAg among donors at KHCC is the lowest in the region. For example, in Tabuk, northwestern Saudi Arabia, the seroprevalence of HBsAg-positive donors was found to be 3.0% when a total of 3,192 healthy donors who were screened over the period from 1 June, 2005 to 31 May, 2006 [17]. Moreover, a six-month study conducted between 2007 and 2008 in a blood bank in Alexandria found that, out of 3,420 donors, 1.4% were positive for HBsAg [18]. Furthermore, blood donors in Nyala, South Darfur State of western Sudan had 6.25% reactive donors for HBsAg from May to July 2007 [19]. Interestingly, we found that the average prevalence rate of anti-HBc positive donors among the Jordanian population at KHCC was around 6.04%, which is also less than that of neighboring countries. For example, in Tabuk, Saudi Arabia, the prevalence rate of anti-HBc was found to be 18.7% [17].

Moreover, we found that an average of 91.5% of the total positive donors for HBV markers in this study were positive for anti-HBc only and negative for HBsAg. This means that these donors were not aware that they were carriers for HBV and were considered healthy asymptomatic blood/platelet donors, according to the WHO blood donor criteria, when they completed the blood-bank questionnaire. In addition, the data demonstrated that there was a much lower percentage of the presumably healthy/asymptomatic donors who were positive for both HBsAg and anti-HBc markers (an average percentage of 8.5%).

Though anti-HBc reactivity alone does not necessarily indicate a high likelihood of infection transmission, especially in the absence of other markers of HBV disease, and screening for anti-HBc is considered an added precaution for a safer blood product [20], this study recommends that blood banks screening for anti-HBc must either design or adopt previously published algorithms [20,21], which would enable these banks to re-qualify anti-HBc positive donors for blood donation, thus reducing donor loss whilst trying to ensure the safety of blood and blood products [22].

As for HCV infection among donors, we found that the average prevalence rate of anti-HCV among donors at KHCC was 0.16% compared to 0.14% from a study by Alyousef *et al*., in which the authors screened a total of 130,586 Jordanian donors at a large military medical center in Jordan [23]. Our slightly higher rate might be due to the smaller population studied at KHCC compared to the Alyousef study or may be due to the varying sensitivity of ELISA kits used for screening purposes in the two studies. The seroprevalence rates of anti-HCV markers was much lower than that of Saudi Arabia, where the prevalence rate of anti-HCV was reported to be 0.41% among donors screened from January 2004 to December 2009 [24]. Additionally, the rate of anti-HCV among blood donors in Sudan was 0.65% [19] and 0.3% among blood donors from the National Blood Transfusion Center in Baghdad, Iraq, from 2006–2009 [25]. In Crete, the prevalence of anti-HCV in donors was 0.49% in females and 0.37% in males [26]. On the other hand, the seroprevalence rate of anti-HCV positive individuals in Israel was reported to be 1.96% in 2001–2010, while the infection rate reached more than 4% among immigrants from the former USSR [27]. Finally, our data analysis showed that all donors who presented for donations at KHCC from 2009–2013 were negative for anti-HIV I/II, anti-HTLV I/II, or anti-TP antibodies.

Conclusions

Our results demonstrated, for the first time, that the Jordanian population of blood and aphaeresis donors at KHCC has the lowest seroprevalence of HBsAg, anti-

HBc, and anti-HCV antibodies compared to its neighboring countries. None of the donors presented with anti-HIV I/II, anti-HTLV I/II, or anti-TP antibodies during the study period. This is an important finding in that it is the first study that sheds light on the prevalence of HTLV I/II in the Jordanian donor population. It also highlights the almost absent HIV and syphilis prevalence among the same group and provides a baseline or benchmark for future epidemiological studies in this area.

While these results reflect a general overview of the seroprevalence of TTI markers among presumably healthy blood and platelet donors in Jordan, it cannot be used to make exact statements of the overall seroprevalence rates among the entire Jordanian population because the studied group was restricted to apparently healthy blood and aphaeresis donors at KHCC only, which encompass a selection bias compared to the Jordanian population. Furthermore, this study is unique because it is the first of its kind that covers this panel of TTI markers among blood and aphaeresis donors in Jordan, which has important implications for understanding the burden of HIV I&II, HBV, HCV, HTLV I/II, and syphilis that might contaminate the blood products and provides a benchmark for the current status of the seroprevalence rates of the TTI markers among the healthy Jordanian population.

Acknowledgements

The authors wish to thank Ms. Dalia Al-Rimawi and Ms. Ayat Taqash from the Biostatistics Unit, Research Office team at KHCC for performing the statistical analysis of the data.

This work was approved by King Hussein Cancer Center IRB committee (IRB# 12 KHCC 47).

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Conflict of interests: No conflict of interests is declared.