JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Prevalence of serological markers and nucleic acid for blood-borne viral infections in blood donors in Al-Baha, Saudi Arabia

Hatem H AlMutairi[1], Mohammad M AlAhmari[2], Badr H Al-Zahran[3], Ibrahim S Abbas[4], Juma`an S Al Ghamdi[5], Yahia A Raja`a[6], Talal A Sallam[7].

[1] *Central Laboratory and Blood Bank, Riyadh, Saudi Arabia*
[2] *Khamis Mushait General Hospital, Khamis Mushait, Saudi Arabia*
[3] *Central Laboratory and Blood Bank, Al-Baha, Saudi Arabia*
[4] *Health Affairs Office, Al-Baha, Saudi Arabia*
[5] *Primary Health Care Centre, Bani Farwa, Al-Baha, Saudi Arabia*
[6] *Faculty of Public Health and Health Informatics, Umm Al-Qura University, Makkah, Saudi Arabia*
[7] *Department of Medical Microbiology, Faculty of Medicine, Al-Baha University Al-Baha, Saudi Arabia*

Abstract

Introduction: Data on blood-borne viral infections in some regions in Saudi Arabia remain scarce. This study investigates the prevalence of serological markers and nucleic acid for blood-borne viruses among blood donors in Al-Baha, Kingdom of Saudi Arabia.

Methodology: In this cross-sectional study, 2,807 donors who donated blood between January 2009 and November 2011 were investigated for blood-borne viral serological markers including HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV, and anti-HTLVI/III in addition to viral nucleic acid.

Results: All donors were males between 16 to 66 years of age (mean: 31.5 ± 9.3 years). Viral nucleic acid and/or serological markers were detected in a total of 36 (1.3%) donors; of them, 26 (72.2%) had nucleic acid concomitant with serological markers, 6 (16.7%) had only viral nucleic acid, while 4 (11.1%) had only serological markers. Of all donors, 22 (0.8%) had HBsAg, 227 (8.0%) had anti-HBc, 157 (5.0%) had anti-HBs, 2,577 (91.8%) had no HBV markers, 2 (0.07%) had anti-HIV, 1 (0.04%) had anti-HCV, and 1 (0.04%) had anti-HTLVI/II. The donors who were born during HBV vaccination era showed no HBsAg (0.0%; $p = 0.052$), lower rates of anti-HBc (1.5%; $p < 0.001$) and anti-HBs (0.7%; $p < 0.001$), while the majority had no HBV markers (98.5%; $p < 0.001$).

Conclusions: Combined viral nucleic acid and serological testing of donated blood enhances blood safety. The absence of HBV markers among donors suggests susceptibility or declined anti-HBs levels. Thus, HBV revaccination or a vaccine boost among adolescents and adults might be indispensable.

Key words: HBV; HCV; HIV; Al-Baha; KSA; blood donors.

J Infect Dev Ctries 2016; 10(6):619-625. doi:10.3855/jidc.6666

(Received 30 January 2015 – Accepted 11 October 2015)

Introduction

Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are the main blood-transmissible viruses. HBV is a major global health problem, with 240 million carriers of hepatitis B surface antigen (HBsAg) reported worldwide [1]. Infection with HBV results in chronic hepatitis in 90% of newborns, in 29%–40% of children, and in 5%–10% of adults [2], and is responsible annually for 780,000 deaths globally due to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [1], ranking as the tenth principal cause of death worldwide [3,4]. Control programs including mass immunization as well as screening of donated blood has made major progress in the decline of global HBV prevalence.

Hepatitis C virus chronically infects an estimated 180 million people worldwide [5] and is a leading cause of cirrhosis and HCC around the globe, with 350,000 annual deaths [6].

With respect to human immunodeficiency virus (HIV)/ acquired immune deficiency syndrome (AIDS), around 34 million people are currently infected with HIV and nearly 30 million people have died due to AIDS [7-9]. Apart from screening of blood donors and health education, vaccine-based control measures of HIV spread remain unachievable. Human T-lymphotropic virus I and II (HTLV-I/II) are other transfusion-transmitted human retroviruses. HTLV-I is associated with adult T-cell leukemia/lymphoma and tropical spastic paraparesis myelopathy (TSP/HAM),

and HTLV-II has been reported to be associated with neurological disorders [10].

In Saudi Arabia, the mass immunization program against HBV launched in 1990 has resulted in a major drop in HBsAg prevalence [11-13]. However, in the year 2007, 9,000 newly diagnosed viral hepatitis cases were reported by the Saudi Ministry of Health, ranking viral hepatitis as the second-most common viral disease after chickenpox [14], with about 50% of the cases were due to HBV and over 30% due to HCV [15]. In Saudi Arabia HIV rates of 1.5 cases per 100,000, Saudis, and 13.2 per 100,000 non-Saudi residents [16], and a prevalence of 0.0% to 0.4% of anti-HTLV-I/II been reported [17,18]. Control of blood-borne viruses has relied for a long time on screening donated blood using highly sensitive serological assays. However, the inadequate sensitivity of these assays to detect these viruses during the window periods of the infections poses a major threat of infected blood units getting into the blood supply undetected. A number of countries have established, along with serological screening, parallel nucleic acid testing of donated blood [19,20].

In Saudi blood banks, 47% of donated blood is from the relatives, friends, or colleagues of patients, and voluntary donation constitutes 40% [21] of donated blood. A stringent screening policy has been implemented with an ultimate aim to achieve further reduction of blood-transmissible viral infections.

Al-Baha region in the Kingdom of Saudi Arabia (KSA) lies in the south-west of the KSA, between the holy city of Makkah and Aseer region. It has a population of 406724 people [22]. Reports of blood-transmitted viral infections from Al-Baha region remain scarce. Therefore, this study examined the prevalence of molecular and serological markers for blood-borne viral infections based on nucleic acid and comprehensive serological testing in the blood bank in Al-Baha, KSA.

Methodology

In this cross-sectional study, 2,807 blood donors were retrospectively investigated for hepatitis B and C viruses, human retroviruses including HIV-1 and -2, and human T-lymphotropic virus type I and II (HTLV-I/II) for the period from January 2009 to November 2011. Records of blood donors who were consented for the use of their anonymous data prior to blood donation were recruited in this study. Those who did not provide consent were excluded from this research. With written consent, blood bank records were used as a source of information.

The donors were selected for donation based on a personal questionnaire, physical examination, and normal pre-donation investigations including hemoglobin (Hb) level, blood pressure, body weight, and temperature. Whole blood was collected in a plain tube for serum testing and in EDTA when plasma was required for nucleic acid testing. Serum or plasma was separated and tested immediately. Enzyme-linked immunosorbent assays (ELISAs) (BioRad, Marnes-la-Coquette, France) were used for detection of HBsAg, total anti-HBc, anti-HBs, HBeAg, anti-HBe, and HIVAg-Ab, according to the manufacturer's instructions. The HBsAg-positive results were confirmed by Monolisa HBsAg neutralization confirmatory assay (BioRad, Marnes-la-Coquette, France). Anti-HCV positive results were performed using Murex anti-HCV ELISA (version 4.0) (Abbot, Kyalami, South Africa). Screening for HTLV-I/II was carried out using Murex HTLV I+II ELISA (Abbot, Kyalami, South Africa). Anti-HCV-positive results were confirmed by third-generation INNO-LIA HCV score (INNOGENETICS, Ghent, Belgium). HIVAb-Ag-positive results were confirmed by INNO-LIA HIV I/II score (INNOGENETICS, Ghent, Belgium)

Nucleic acid amplification was performed using real-time polymerase chain reaction (PCR). This is a qualitative multiplex Cobas TaqMan MPX PCR test (Roche, Basel, Switzerland) that allows simultaneous detection of HBV-DNA and RNA of both HCV and HIV without specifying which of the three viruses was detected. The assay has a reproducibility of 71.7% to 98.0% for HIV-1, 61.7% to 81.4% for HCV, and 95.0% to 98.3% for HBV. The assay can detect HBV, HIV, and HCV nucleic acid with 100% sensitivity. A total of six internal controls (five positive and one negative) must be included in each run of the assay in order for the run to be valid.

Testing was carried out on individual plasma samples according to the manufacturer's instructions. The testing policy of the blood bank included screening all donors for HBsAg, anti-HBc, anti-HBs, HBeAg, anti-HBe, anti-HCV, HIVAb-Ag, and HTLV-I/II antibodies. Donors who were positive or weak-positive for HBsAg, anti-HCV, and HIVAb-Ag were further confirmed by HBsAg neutralization, third-generation line immune assay utilising 6 HCV antigens incorporated to nylon, and HIV-1 and 2 synthetic and recombinant nylon-coated peptides. All donors were simultaneously tested for HBV, HCV, and HIV nucleic acids. Statistical analysis was conducted using SPSS version 20.0 (SPSS Inc., Chicago, USA).

Results

All donors (n = 2,807) were males between 16 and 66 years of age (mean: 31.5 ± 9.3 years). Nucleic acid to either of the three viruses HBV, HCV, or HIV and/or serological markers were detected in 36 (1.3%) of all donors. Of these 36 donors, 26 (72.2%) had nucleic acid concomitant with HBsAg and/or anti-HBc serological markers, 6 (16.7%) had only viral nucleic acid, while 4 (11.1%) had only serological markers that encompassed confirmed anti-HCV in 1 donor, confirmed anti-HIV in 2 donors, and anti-HTLV-I/II in 1 donor. Of all donors (n = 2,807), 22 (0.8%) were HBsAg confirmed positive, 227 (8.1%) were anti-HBc positive, 157 (5.6%) were anti-HBs positive (154 [98.1%] had concomitant anti-HBc and 3 [1.9%] had isolated anti-HBs), 2,577 (91.8%) had no HBV markers, 2 (0.07%) had anti-HIV, 1 (0.04%) had anti-HCV, and 1 (0.04%) had anti-HTLV-I/II (Table 1). Of the HBsAg confirmed positive cases (n = 22) 1 (4.5%) was HBeAg positive while 21 (95.5%) were anti-HBe positive. Of 2,807 donors, 4 (0.14%) were nucleic acid positive but HBsAg negative with either anti-HBc plus anti-HBs (1 donor), with an isolated anti-HBc (2 donors), or with an isolated anti-HBs (1 donor) (Table 1). These donors were serologically negative to HCV, HIV, and HTLV-I/II.

With respect to progress of age, the HBsAg prevalence did not differ significantly ($p > 0.05$), but the prevalence of anti-HBc and anti-HBs significantly increased ($p < 0.001$), while rates of donors with no markers significantly decreased ($p < 0.001$) (Table 2). Only 1 of 138 (0.7%) non-Saudi national donors was HBsAg, anti-HBc, and anti-HBe positive, while 137 were negative for all markers.

Compared to the older donors, the cohort of donors between 16 and 21 years of age, who were likely to have been born after the inclusion of HBV vaccine to the Expanded Program on Immunization (EPI) in Saudi Arabia, showed lower prevalence of HBsAg (0/407 [0.0%]; -$p = 0.052$), significantly lower rates of anti-HBc (6/407 [1.5%]; -$p < 0.001$) and anti-HBs (3/407

Table 1. Serological markers and viral nucleic acid detected among blood donors tested during the period from January 2009 to November 2011.

Markers	Positive/total (%)
HBsAg	22/2,807 (0.8)
Total anti-HBc	227/2,807 (8.0)
Anti-HBs	157/2,807 (5.0)
HBeAg+ of HBsAg +	1/22 (4.5)
Anti-HBe+ of HBsAg +	21/22 (95.5)
Absence of HBV markers	2,577/2,807 (91.8)
Anti-HCV	1/2,807 (0.04)
Anti-HIV	2/2,807 (0.07)
Anti-HTLVI/ II	1/2,807 (0.04)
Viral nucleic acid*	32/2,807 (1.1)
HBsAg+/anti-HBc+ nucleic acid+	22/2,807 (0.8)
HBsAg-/anti-HBc+ anti-HBs+ nucleic acid+ **	1/2,807 (0.04)
HBsAg-/anti-HBc+/ nucleic acid+**	2/2,807 (0.07)
HBsAg-/anti-HBc-/ nucleic acid+**	1/2,807 (0.04)

*Nucleic acid to either HBV, HCV, or HIV as the assay did not specify which of the three viral nucleic acids was detected; **Donors with a likely occult HBV infection.

Table 2. HBV serological markers among different age groups of blood donors tested during the period from January 2009 to November 2011.

Age groups	*Markers*			
	HBsAg n (%)	**Anti-HBc n (%)**	**Anti-HBs n (%)**	**No markers n (%)**
16–25	1/920 (0.1)	32/920 (3.5)	25/920 (2.7)	888/920 (96.5)
26–35	12/1,017 (1.2)	64/1,017 (6.3)	38/1,017(3.7)	953/1,017 (93.7)
36–45	7/616 (1.1)	75/616 (12.2)	51/616 (8.3)	541/616 (87.8)
46–55	2/230 (0.9)	47/230 (20.4)	36/230 (15.7)	182/230 (79.1)
> 55	0/24 (0.0)	9/24 (37.5)	7/24 (29.2)	13/24 (54.2)
Total	**22/2,807 (0.8)**	**227/2,807 (8.1)**	**157/2,807 (5.6)**	**2,577/2,807 (91.8)**
p	**> 0.05**	**< 0.001**	**< 0.001**	**< 0.001**

Table 3. Comparison of HBV serological markers in a cohort (16–21 years of age) of blood donors born after full implementation EPI with older blood donors.

Age groups	Marker			No markers n (%)
	HBsAg n (%)	Anti-HBc n (%)	Anti-HBs n (%)	
16–21	0/407 (0.0)	6/407(1.5)	3/407 (0.7)	401/407 (98.5)
> 21	22/2,400 (0.9)	221/2,400 (9.2)	154/2,400 (6.4)	2,176/2,400 (90.7)
Total	**22/2,807 (0.8)**	**227/2,807 (8.1)**	**157/2,807 (5.6)**	**2,577/2,807 (91.8)**
p	**> 0.05**	**< 0.001**	**< 0.001**	**< 0.001**

[0.7%]; $p < 0.001$), but a significantly higher proportion (401/407 [98.5%]; $p < 0.001$) of them had no HBV markers (Table 3).

Discussion

In this study, the viral nucleic acid detection assay identified six more infected blood donors (16.7%) as compared to serological screening alone. This nucleic acid detection assay seems to bridge the gaps of serological window periods characterized by low-level serological viral markers, which are usually undetectable by conventional serological assays. According to the manufacturer, the Cobas TaqMan MPX PCR assay can detect HBV, HIV, and HCV nucleic acid with 100% sensitivity. However, four donors had only serological markers, one to HTLV-I/II and three serologically confirmed to HCV and HIV, despite being negative for nucleic acid. This is unlikely to be due to a lower sensitivity of the nucleic acid assay compared to ELISA. One straightforward explanation could be that these two HIV-positive donors were under highly active antiretroviral therapy. However, it is unreasonable that individuals with a known HIV positive status would attempt to donate blood. The situation in these two donors remains difficult to explain. However, the anti-HCV positive/nucleic acid negative donor may have had either fluctuating HCV-RNA or spontaneously cleared HCV viremia. This clearly demonstrates the high value of the combined serological and nucleic acid screening of blood donors. Based on these, other countries have established, along with serological screening, parallel nucleic acid testing of donated blood, conferring more safety for blood supply [19,20]. Despite the high cost of this screening combination, it remains cost-effective given the extra safety that it confers to blood transfusion.

This study detected lower HBsAg prevalence (0.8%) and lower prevalence of HBV infections (8.0%) among blood donors than had been previously reported in the southern region of the KSA [13,23], although one recent study in the Aseer province reported an HBsAg prevalence of as low as 1.03% [24]. Additionally, the cohort of donors between 16 and 21 years of age, whose birth corresponds with the HBV vaccine inclusion to EPI, showed no HBsAg and a low prevalence of HBV infections. This may suggest a role of HBV vaccination that was included to the EPI in Saudi Arabia in 1990. A role of vaccine in decline of HBV infections has also been reported in other parts of southern Saudi Arabia [24]. However, only 5.6% of all donors had anti-HBs, and the majority of them (98.1%) had concomitant anti-HBc, suggesting acquisition of anti-HBs through past infection rather than through vaccination, while the remaining 1.9% who had isolated anti-HBs likely had been vaccinated. Moreover, 91.8% of all donors had no HBV markers, suggesting susceptibility to HBV. Furthermore, the cohort of donors between 16 and21 years of age, who were born after the inclusion of HBV vaccination in EPI, showed a low rate (0.7%) of anti-HBs, while the majority (98.5%) of them had no HBV markers suggesting susceptibility. This suggest a limited role of immunization on the low HBV infection rate seen among our donors. The explanation of the paradox of a high rate of susceptibility concurring with a low infection rate is that infections among donors may be caused by a strain of HBV replicating at low level, *i.e.*, casing inactive HBV infections. The HBeAg negativity among the donors supports this. We have previously reported subjects with no HBV markers in two independent reports of blood donors [25] and in the general population [26] in Yemen, and as a result, we proposed immunizing adults as an adjunct to the ongoing expanded infant immunization program, a recommendation that seems valid for the situation revealed by the current study. One more possibility is that the cohort of donors between 16 and 21 years of age with no HBV markers and who were born during HBV vaccination had lost their anti-HBs over the years following vaccination. A decline of anti-HBs with the progress of age among vaccinated individuals has been repeatedly reported [27-29]. Such individuals have been demonstrated to have immunological memory that developed after primary immunization [30,31]. This immunological memory could still be boosted by natural exposure, which may explain the low HBV infection rate among our donors. Further boosting of

such donors by an additional vaccine dose is required to establish whether these donors were susceptible or only had declined anti-HBs.

The HBsAg prevalence revealed by the current study seems to be higher among donors over 21 years of age. This, together with the age-dependent increase of prevalence of anti-HBc and, consequently, the decrease in rates of donors who lacked HBV markers, is consistent with horizontal transmission of infection acquired during adult life rather than perinatal transmission, which usually results in high levels of viral replication during the immuno-tolerant phase. A low HBeAg rate has been reported among HBsAg-positive Saudi mothers, suggesting the absence of a major role of vertical transmission of HBV [32]. This further suggests that infections with HBeAg- negative HBV that replicates inactively also exist in the Saudi community. We have previously reported apparently healthy blood donors from Yemen with HBeAg negative and low viral load (< 400 copies/mL), demonstrating an inactive form of HBV infection [33]. Carriers infected with HBeAg-negative inactive HBV have also been reported elsewhere [34]. Similarly, the rate of recovery with a subsequent development of anti-HBs that significantly increased with age is consistent with adulthood-acquired HBV infection, which usually results in recovery in the majority of infected individuals.

One limitation in most of the studies among blood donors is that they only included males. Previous studies have shown that males have a 1.8 greater likelihood [35] of HBV infection and that HBV occurs at a higher incidence in males than in females [15]. Therefore, studies among blood donors may not accurately reflect the situation in the general community but rather may overrate HBV carrier rate and prevalence. Furthermore, studies among blood donors may result in a selection bias that may lead to a lower carrier rate and prevalence than that existing in the community at large. Therefore, the low prevalence of HBV revealed by the current study could be due to a selection bias exerted by the method of blood donor recruitment, which, in turn, contributes further to the safety of blood supply, as the blood donor community is becoming less infected. A community-based survey is needed to establish the actual epidemiology of HBV in the region of Al-Baha.

Occult hepatitis B among blood donors in the middle east remains to be adequately evaluated. In Egypt, occult hepatitis B was found 1.6% of donors [36]. In the current study, the four HBsAg-negative donors who had viral nucleic acid with either anti-HBc plus anti-HBs, an isolated anti-HBs, or an isolated anti-HBc may suggest occult HBV. However, the nucleic acid assay used does not indicate which of HBV, HCV, or HIV was positive. Although these four donors were serologically negative for HCV and HIV infections, there remains a possibility that they could have early HCV or HIV infections that have not yet seroconverted. Thus, the status of these four donors with regard to occult HBV remains elusive. However, occult hepatitis remains to be evaluated in our blood bank.

The low prevalence of HIV, HCV, and HTLV-I/II reported by this study demonstrates that the three viruses are less common among the Saudi general population, assuming that blood donors, to some extent, represent the general population. Other studies have also shown a prevalence of 0.41 [12] and 0.7 [13] among blood donors elsewhere in the southern part of the country, as determined by third-generation ELISA. However, our study reports a far lower prevalence of HCV antibodies (0.04%) compared to other regions of Saudi Arabia. This lower prevalence could be attributed to the more specific confirmatory testing to which our donors were subjected. Again, before a final conclusion that Al-Baha is a low HCV endemicity region can be made, a comprehensive, community-based survey is needed.

This study reports a low prevalence of retroviruses: 0.07% for HIV and 0.04% for HTLV-I/II. This is consistent with the reports that Saudi Arabia is a country of low HIV prevalence in the world. The stringent blood screening policy consisting of a combination of serological and nucleic acid in testing Saudi blood donors further prevents the spread of HIV through blood transfusion. The low anti-HTLV-I/II prevalence in this study is in accordance with previous reports from other parts of the Saudi Kingdom where either zero [37] or as low as 0.04% [18] prevalences were reported. This suggests that HTLV-I/II is not a major threat to donated blood in Saudi Arabia.

The exclusion of 138/2,807 (4.9%) non-Saudi nationals, the majority of whom (99.3%) were negative to HBV and negative to HCV, HIV, and HTLV-I/II did not significantly influence the prevalence of the various markers reported in this study. However, their inclusion may better reflect the real epidemiological profile in the blood donor community.

Conclusions

Combined viral nucleic acid and serological testing identified more infected donors and thus conferred further safety to blood transfusion. Absence of HBV markers in a high rate of donors who were born after the

HBV vaccine was included in EPI indicates either susceptibility or declined anti-HBs levels. This suggests a need for either revaccination or a booster vaccine dose for adolescents and adults. The paradox of the association of a high rate of apparently susceptible donors to HBV with a low rate of HBV infections merits further investigation.

Acknowledgements

The authors would like to acknowledge Saleh Sefar Al-Ghamdi, the head director of the blood bank; Mr. Khalufa Al-Khathami, head director of central laboratories and blood banks in Al-Baha region; and the blood bank personnel, especially doctor Rangole MM, for facilitating this study.

References

1. World Health Organization (WHO) Hepatitis B. Fact sheet N°204 (Updated March 2015). Available: http://www.who.int/mediacentre/factsheets/fs204/en. Accessed 28 August 2015.
2. Ott JJ, Stevens GA, Groeger J, Wiersma ST (2012) Global epidemiology of hepatitis B virus infection: New estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 30: 2212-2219.
3. Chu CM, Liau YF (1997) Natural history of chronic hepatitis B virus infection: an immunopathological study. J Gastroenterol Hepatol 12: 218-222.
4. Ganem D, Prince AM (2004) Hepatitis B virus infection — natural history and clinical consequences. N Engl J Med 350: 1118-1129.
5. Gupta E, Baipai M, Choudhary A (2014) Hepatitis C virus: Screening, diagnosis, and interpretation of laboratory assays. Asian J Transfus Sci 8: 19-25.
6. Papatheodoridis G, Hatzakis A (2012) Public health issues of hepatitis C virus infection. Best Pract Res Clin Gastroenterol 26: 371-380.
7. Joint United Nations Programme on HIV/AIDS (UNAIDS) (2012) Report on the Global AIDS Epidemic. Global Report. Available: http://www.unaids.org/sites/default/files/media_asset/20121120_UNAIDS_Global_Report_2012_with_annexes_en_1.pdf. Accessed 10 October 2014.
8. Joint United Nations Programme on HIV/AIDS (2012) Together We Will End AIDS. Available: http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2012/JC2296_UNAIDS_TogetherReport_2012_en.pdf. Accessed 10 October 2014.
9. UNAIDS World AIDS Day Report (2012) Results. Available: http://www.unaids.org/sites/default/files/media_asset/JC2434_WorldAIDSday_results_en_1.pdf. Accessed 10 October 2014.
10. Orland JR, Engstrom J, Fridey J, Sacher RA, Smith JW, Nass C, Garratty G, Newman B, Smith D, Wang B, Loughlin K, Murphy EL (2003) HTLV Outcomes Study Prevalence and clinical features of HTLV neurologic disease in the HTLV Outcomes Study. Neurology 61: 1588-1594.
11. Al-Faleh FZ, Al-Jeffri M, Ramia S, Al-Rashed R, Arif M, Rezeig M, Al-Toraif I, Bakhsh M, Mishkkhas A, Makki O, Al-Freihi H, Mirdad S, AlJuma A, Yasin T, Al-Swailem A, Ayoola A (1999) Seropepidemiology of hepatitis B virus infection in Saudi children 8 years after mass hepatitis B vaccination programme. J Infect 38: 167-170.
12. Mohammed Abdullah S (2013) Prevalence of hepatitis B and C in donated blood from the Jazan region of Saudi Arabia. Malays J Med Sci 20: 41-46.
13. Abdo AA, Sanai FM, Al-Faleh FZ (2012) Epidemiology of viral hepatitis in Saudi Arabia: are we off the hook? Saudi J Gastroenterol 18: 349-357.
14. Aljarbou AN (2013) The emergent concern of hepatitis B globally with special attention to Kingdom of Saudi Arabia. Int J Health Sci 7: 333-340.
15. Memish ZM, Knawy BA, El-Saed A (2010) Incidence trends of viral hepatitis A, B, and C seropositivity over eight years of surveillance in Saudi Arabia. Int J Infect Dis 14: 115-120.
16. Mazroa MAAI, Kabbash IA, FelembanSM, Stephens GM, Al-Hakeem RF, Zumla AI, Ziad A. Memish (2012) HIV case notification rates in the kingdom of Saudi Arabia over the past decade (2000–2009). PLoS One 7
17. Bernvil SS, Andrews V, Coulter N (1997) International forum: Saudi Arabia. Donor screening for HTLV-I in Saudi Arabia: is it cost effective? Transfus Sci 18: 45-47.
18. Taha MA, Bashawri LA, Ahmed MS, Ahmed MA (2003) Prevalence of antibodies to human T- lymphotropic viruses types I and II among healthy blood donors. Saudi Med J 24: 637-640.
19. Moiz B, Moatter T, Shaikh U, Adil S, Ali N, Mahar F, Shamsuddin N, Khurshid M (2014) Estimating window period blood donations for human immunodeficiency virus Type 1, hepatitis C virus, and hepatitis B virus by nucleic acid amplification testing in Southern Pakistan. Transfusion 54: 1652-1659.
20. Shyamala V (2014) Transfusion transmitted infections in thalassaemics: need for reappraisal of blood screening strategy in India. Transfus Med 24: 79-88.
21. Saudi Arabian Blood Services (2011) Are we on the right track? Available: https: http://www.transfusionmedicine.ca/sites/transfusionmedicine/files/events/Saudi%20Arabian%20Blood%20Services.pdf. Accessed 10 October 2014.
22. Abdul Salam A, Elsegaey I, Khraif R, Al-Mutairi A (2014) Population distribution and household conditions in Saudi Arabia: reflections from the 2010 Census. Springerplus; 3:530. Available:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4174546/pdf/40064_2014_Article_1229.pdf530. Accessed 14May 2016.
23. El Beltagy KE, Al Balawi IA, Almuneef M, Memish ZA (2008) Prevalence of hepatitis B virus markers among blood donors in a tertiary hospital in Tabuk, northwestern Saudi Arabia. Int J Infect Dis 12: 495-499.
24. Ibrahim EH, Bin Dajem SM, Heijan AA, Hadish HF, Zahar YA, Alshehri A, Kilany M, Mostafa OMS (2014) Hepatitis B vaccine reduced the prevalence of antibodies to hepatitis B core antigen in blood donors in Aseer region, Saudi Arabia. Egypt Acad J Biol Sci 6: 13-22.
25. Sallam TA, Cuevas LE, Tong CYW (2003) Increase in susceptibility of young adults to hepatitis B infection in the Republic of Yemen. Trans R Soc Trop Med Hyg 97: 302-304.
26. Sallam TA, Raja'a YA, Bahaj S, Al-Shami AM, Lu M, Roggendorf M, Tong CY (2012) Hepatitis B virus carrier rate, prevalence and susceptibility and impact of immunization program among households in the city of Taiz, Yemen. Vaccine 30: 5564-5568.

27. Sallam TA, Alghshm HM, Ablohom AA, Alarosi MS, Almotawakel RE, Farea NH, Mosleh AA (2005) Immune response to Hepatitis B vaccine among children in Yemen. Saudi Med J 26: 281-284.
28. Bagheri-Jamebozorgi M, Keshavarz J, Nemati M, Mohammadi-Hossainabad S, Rezayati MT, Nejad-Ghaderi M, Jamalizadeh A, Shokri F, Jafarzadeh A (2014) Persistence of anti-HBs antibody and anamnestic response 20 years after primary vaccination with recombinant hepatitis B vaccine at infancy. Hum Vaccin Immunother 10: 3731-3736.
29. Sugunan AP, Bhattacharya H, Bhattacharya D, Mandal A, Ghosal SR, Rao RC, Mandal AK (2014) Impact of hepatitis B immunization among the Nicobarese tribe antibody titres & seroprotection five years after vaccination. Indian J Med Res 139: 427-430.
30. do Livramento A, Schultz J, Batista KZ, Treitinger A, de Cordova CM, Spada C (2014) Immune memory response induced in vitro by recombinant hepatitis B surface antigen challenge 13-18 years after primary vaccination. J Med Virol 86: 1700-1704.
31. Salama II, Sami SM, Salama SI, Foud WA, Abdel Hamid AT, Said ZN (2014) Persistence of protection to hepatitis B vaccine and response to booster dose among children and adolescents in Dakahleya- Egypt. Egypt J Immunol 21: 13-26.
32. Alrowaily MA, Abolfotouh MA, Ferwanah MS (2008) Hepatitis B virus sero-prevalence among pregnant females in Saudi Arabia. Saudi J Gastroenterol 14: 70-72.
33. Sallam TA, Tong CYW (2002) Two distinct types of hepatitis B virus core promoter variants in Yemeni blood donors. J Med Virol 68: 328-334.
34. Deltenre P, Laleman W, Van Gossum M, Lenaerts A, Colle I, Michielsen P, Adler M, Delwaide J, Assene C, Reynaert H, D'Heygere F, Robaeys G, de Galocsy C, Brenard R, Langlet P, Sprengers D, Mairlot MC, Preux C, Lefçbvre V, Orlent H, Henrion J (2012) HBV infection in Belgium: results of the BASL observatory of 1,456 HBsAg carriers. Acta Gastroenterol Belg 75: 35-41.
35. Al-Tawfiq JA, Anani A (2008) Profile of viral hepatitis A, B, and C in a Saudi Arabian hospital. Med Sci Monit 14: CR52-CR56.
36. Said ZN, Sayed MH, Salama II, Aboel-Magd EK, Mahmoud MH, Setouhy ME, Mouftah F, Azzab MB, Goubran H, Bassili A, Esmat GE (2013) Occult hepatitis B virus infection among Egyptian blood donors. World J Hepatol 5: 64-73.
37. Arif M, Ramia S (1998) Seroprevalence of human T-lymphotropic virus type I (HTLV-I) in Saudi Arabia. Ann Trop Med Parasitol 92: 305-309.

Corresponding author
Talal A. Sallam
Professor of Medical Microbiology
Department of Medical Microbiology
Faculty of Medicine
Al-Baha University
P.O. Box 1988
Al-Baha, Kingdom of Saudi Arabia
Phone: +966 559784401
Fax: +966 7 7247272
Email: talalsallam2000@yahoo.com

Conflict of interests: No conflict of interests is declared.