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Seroprevalence of Anti-*Treponemapallidum* Antibodies (Syphilis) In Blood Donors in the Southern Area of Saudi Arabia.

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ABSTRACT

Transfusion-transmissible infectious agents such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis are among the greatest threats to blood safety for the recipient. This study aimed at determining the seroprevalence of transmissible bacteria such as syphilis and viruses as HIV, HBV, HCV and HTLV infections among blood donors at Aseer Region, Kingdom of Saudi Arabia. The study was conducted on random blood samples collected from healthy blood donor volunteers, who were referred to Blood Transfusion Centers found at Aseer region, during the period March 2012 to January 2013. All the collected blood units were screened for syphilis antibodies, hepatitis B surface antigen (HBsAg), anti-hepatitis B core antibody (HBc-Ab), hepatitis C virus (HCV), human immunodeficiency virus (HIV) 1 and 2 and human T-cell lymphotropic virus (HTLV) I/II. All donated blood units were checked for HBV-DNA, HCV-RNA and HIV-RNA by nucleic acid test (NAT) technology. A7267 donors (26 females (0.36%) and 7241 males (99.64%)) were accepted for donation with median age of 28 (female) and 30 years (males). Screening resulted in two (0.028%) positive cases for anti-*Treponemapallidum* antibodies, two (0.028%) positive cases for HIV-Ab but negative for HIV-RNA as confirmed by PCR, 5 (0.069%) positive cases for HCV-Ab with 2 (0.028%) of them positive for HCV-RNA, 71 (0.98%) were HBsAg positive of them 66 (0.91) were positive to HBV-DNA, 449 (6.18%) were anti-HBc positive of them 78 (1.07%) were positive to HBV-DNA. There were no positive samples for HTLV-1/2 antibodies. Prevalence of syphilis, HBsAg, HCV-Ab in Aseer region is very low. The rate of HBc-Ab in units of blood donation is relatively high. The presence of HBV-DNA in HBc-Ab positive donations make it risky for use.

Key words: syphilis; blood transfusion; blood donors; Human immunodeficiency virus (HIV); Hepatitis B Virus (HBV); Hepatitis C Virus (HCV); Seroprevalence; Saudi Arabia

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INTRODUCTION

Transfusion of human blood is an essential procedure that can save human lives. However, the microbial agents that are transmissible by blood transfusion can cause morbidity and mortality in recipients. In order to be transmissible by blood, the infectious agent or infection usually has the following characteristics: presence in the blood for long periods, stability in blood stored at 4°C or lower, long incubation period before the appearance of clinical signs, asymptomatic phase or only mild symptoms in the blood donor, hence not identifiable during the blood donor selection process [1].

Syphilis is a sexually transmitted disease caused by the spirochete *Treponemapallidum*. The disease is transmitted by sexual contact or congenitally from mother to the unborn baby. Syphilis may also be transmitted via blood and blood products, and intravenous drug use[2-3]. Syphilis is rare in developed countries but much more common in developing countries where prevalence can reach 25% amongst blood donors[4-5]. If not treated, syphilis can cause serious effects such as damage to the aorta, brain, eyes, and bones[6], these effects may be fatal in some cases. Syphilis has also acquired a new potential for morbidity and mortality through association with increased risk for HIV infection. Coinfection with both syphilis and HIV occurs frequently due to common risk factors. These two diseases interact with each other making both diagnosis and treatment more complicated [3]. This will make it increasingly difficult to get safe blood because of this blood borne infection.

Syphilis is still a public health problem worldwide. In the latest report of the WHO about the prevalence of sexually transmitted infections (STIs), an estimate of 10.6 million new cases of syphilis were reported in adults aged 15-49[7]. Most of these cases were from developing countries. Moreover, the Centers for Disease Control and Prevention reported that 20 % of STIs in the United States were syphilis [8]. The disease progresses through four distinct stages; primary, secondary, latent, and tertiary. The main cases of transfusion-transmitted syphilis were reported to occur when donors were in the primary or secondary stage of the disease[9-13]. *T. pallidum* may be found in the blood stream, but levels are variable, and bacteremia is often short-lived even in recent contamination. Moreover, the treponemes are relatively fragile and sensitive to cold; storage below +20°C for more than 72 hours destroys the organism and reduces dramatically the infection risk. Although clearly potentially infectious, the risk of transmission through the transfusion of blood and blood components stored below +20°C is very low [10, 14]. However, it was observed that many cases were associated with appearance of a sore on the blood donor few days after the donation. Thus, syphilis can be transmitted from donors who are clinically and biologically negative. It is clear that medical selection and mainly information and questioning are essential to identify those who have been exposed to the infection two months prior to donation of their blood[15].

Very little systematic information is available on the profile of positive blood donors including differences between donors with recent versus past infection. The exclusion of donors with past and treated infection is still a matter of discussion. Abusive exclusion reduces the blood supply and could be problematic in developing countries.

The transfusion risk of syphilis is closely related to risk factors in the blood donor, in particular the sexual behaviors, the disease being primarily transmitted by sexual route. The rates of infection are highest amongst homosexual men[16]. Recent syphilis infections have been shown to be associated with younger age, male-male sex, two or more sex partners, past syphilis treatment, past syphilis history, HIV seropositivity. Risk factors usually associated with transfusion transmitted syphilis also include more than one sexual partner, intravenous drug use, and skin scarification (tattooing, blood rituals).

It is just as high in females as in males, in the different age groups and in voluntary donor as well as family donors. The family blood donation and remunerated blood donation, mostly found in developing countries is statistically associated with higher prevalence of the disease [5, 17]. The donors who have been positive for syphilis during the previous donation are less likely to donate again, whereas donors who were negative for the presence of syphilis in the past would be more likely to donate again.

The tests based on enzyme-linked immunoassays are the more specific and are usually used to confirm the results of simpler screening tests for syphilis. According to WHO, blood banks may choose Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), or enzyme immunoassay (EIA).

The aim of the present study was to estimate the prevalence of Syphilis and other transfusion-transmitted infectious agents among blood donors in the southern region of Saudi Arabia. The study was conducted over a period of one year to reflect trends in the infections of interests among the general population.

MATERIALS AND METHODS

Samples collection

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

Serological Screening of Donated Blood

All blood specimens were tested on sequential basis for routine serological tests after signing of informed consent. The routine serological tests according to predefined protocol of blood banking safety requirements by Saudi Ministry of Health comprised hepatitis B virus surface antigen (HBsAg), anti-HBcore antibodies (HBc-Ab), anti-hepatitis C virus antibodies (HCV-Ab), anti-human immunodeficiency virus-1/2 antibodies (HIV-1/2 Ab), anti-human T-lymphotropic virus type I and II antibodies (HTLV-I/II-Abs), malaria, and syphilis-Ab, as well as nucleic acid test (NAT) technology for HBV-DNA, HCV-RNA and HIV-RNA.

Detection of anti-*Treponemapallidum* antibodies was done using ICE* Syphilis kit (DiaSorin, UK), the enzyme immunoassay for the detection of antibodies against *Treponemapallidum*. Anti-*Treponemapallidum* antibodies positive unit was retested using DiaMed-ID Micro Typing System [DiaMed (GB) LTD.]. Detection of HBc-Ab was done using Murex anti-HBc (total) kit (DiaSorin) and positive samples were confirmed using Monolisa™ Anti-HBcPLUSULTRA kit (BIO-RAD, France). Detection of HIV-1/2 Ab was done using enzyme immunoassay for improved detection of seroconversion HIV types 1 (HIV-1, HIV-2 group O) and detection of anti-HIV-2 antibodies using Murex HIV Ag/Ab Combination kit (DiaSorin, UK). Confirmatory test for HIV-1/2-Ab was done using Genscreen™ ULTRA HIV Ag-Ab kit (BIO-RAD, France). Detection of HCV-Ab was done using an enzyme immunoassay for the detection of antibodies to HCV in human serum or plasma (Murex; version 4.0, DiaSorin). Confirmatory test for HCV-Ab positive samples was done using Monolisa™ ULTRA HCV Ag-Ab kit (BIO-RAD, France). Detection of HBsAg was done using an enzyme immunoassay for the detection of the presence of hepatitis B surface antigen in serum and plasma samples using Murex HBsAg Version 3 (DiaSorin, UK). Confirmatory test for HBsAg was done using Monolisa™ HBs Ag ULTRA kit (BIO-RAD, France). Detection of HTLV-I/II was done using a qualitative enzyme immunoassay for the detection of antibodies against human HTLV-I and HTLV-II in serum and plasma (DiaSorin, UK).

Nucleic Acid Test (NAT)

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

Statistical Analysis

The biochemical data recorded were expressed as mean±SD and statistical and correlation analyses were undertaken using the one-way ANOVA followed by a post-hoc LSD (Least Significant Difference) test. A *P* value < 0.05 was statistically significant. A statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

RESULTS

Depending on donor selection criteria, voluntary non-remunerated 7267 blood donors (26 females (0.36%, median age of 28) and 7241 males (99.64%, median age of 30) were selected to donate their blood. Donors of ages between 21 and 30 years constituted the largest proportion (50.52%, *P* ≤ 0.001) with a median age of 26 years (**Table 1**). As shown in **Table 2**, the nationality distribution of the donors was from 15 countries. The majority of donors were Saudis (95.13%) followed by Yemenis (1.58%) and then Egyptians (1.3%).

Table 1: Age ranges of accepted volunteers for donation.

Age range	Number	Median
18-20	429	20
21-30	3676	26
31-40	2203	35
41-50	786	45
51-60	173	55
Total	7267	30

Table 2: The nationality distribution of the accepted blood donors for donation.

Nationality	Number	(%)
Afghani	2	0.0275
Bengali	5	0.0688
Egyptian	96	1.3210
Erytrian	3	0.0413
Indian	22	0.3027
Jordan	20	0.2752
Lebanese	1	0.0138
Pakistani	26	0.3578
Philipine	5	0.0688
Palastine	7	0.0963
Saudi	6913	95.1287
Sudanese	27	0.3715
Syrian	21	0.2889
Tyrkey	4	0.0550
Yemani	115	1.5825
Total	7267	100

Table 3: Serological marker after screening of accepted donors for donation.

Marker	Syphilis	HBsAg	HBcAb	HCV	HIV	HTLV	NAT
Syphilis	2	0	2	0	0	0	0
HIV	0	1	1	0	2	0	0
HCV	0	0	1	5	0	0	2
HBsAg	0	71	70	0	1	0	66
HBcAb	0	69	449	0	1	0	78
HTLV	0	0	0	0	0	0	0

Table 4: Other markers associated with NAT positive samples.

Marker	HCV	HBcAb	HIV	HBsAg
HCV	2	1	0	0
HBV	0	65	0	1
HIV	0	0	0	0
HBcAb	1	78	0	65

Serological screening of samples resulted in positivity to many different markers (Table 3). Two (0.028%) positive cases to anti-*Treponemapallidum* antibodies, one is 33 years old and the second is 36 years old both with positive markers for HBcAb. Two (0.028%) positive cases to HIV-1/2, one is 22 years old with positive markers for HBsAg and HBcAb and the second is 33 years old with no other associated markers. Detection of HIV-RNA by PCR showed that there were no RNA for HIV in samples confirmed positive by ELISA. There were 5 (0.069%) positive cases to HCV-Ab, 2 of them (0.028%, 36 and 45 years) were positive for HCV-RNA as confirmed by PCR. Only one (0.014%) case, in addition to positivity to HCV-Ab, showed positivity to HBcAb. Detection of HBsAg resulted in 71 positive cases. There were 70 cases positive to HBcAb and one case positive for HIV. Cases positive for HBV-DNA by PCR were 66. Cases positive for HBcAb and HBV-PCR were 65 and negative for HBV-DNA by PCR were 5. There were no coinfection with either HCV or HTLV viruses. Screening of samples for HBcAb resulted in 449 positive cases. Of these cases there were 69 positive to HBsAg and one positive for HIV. Cases positive for HBcAb and HBV-DNA were 78 and negative for HBV-DNA by PCR were 371. There were no HBcAb cases companied with HCV, syphilis and HTLV markers. No positive samples for HTLV-1/2 antibodies were found.

Screening for HBV, HCV and HIV nucleic acid by NAT resulted in 88 positive cases. Cases positive for NAT and associated with other positive markers were as follow; 66 cases were positive to HBsAg, one case was positive to HCV-Ab and 79 cases positive to HBcAb. Mixed infections were one positive case with positive markers for HCV-Ab and one HBcAb with HIV-ab and 63 positive cases with positive markers for HBsAg and HBcAb (Table 4).

DISCUSSION

Strategies for blood safety were proposed and modified during the years until the adoption in 1987 by the WHO of a common international strategy. The general recommendations focus on the control of the bacterial dissemination of the disease through blood transfusion by the selection of low risk blood donors and the screening of the disease by efficient lab tests[1].

Blood donors with high-risk sexual behavior and other risk factors may be infected withsyphilis and compromise the safety of blood used for transfusion. The medical selection of the blood donors consists of information of the donor, the finding of the risk factors in the behaviors and the medical history using a questionnaire, and the physical examination in order to find clinical signs of the infection. Donor deferral follows identification of any risk.Medical selection is crucial because it could permit to defer more than half of infecteddonors, especially the ones in the early period of infection where laboratory tests are notefficient [18-19].

The prevalence of syphilis is still very low in developed countries and the veryrare cases of recipient contamination raised the question of whether syphilis screening was still necessary for blood donors. On the other hand, in developing countries the prevalence of positive serologic tests for syphilis can reach 25%. The prevalence is however very variable from one area to another and from a country to another[15].In the present study 0.028 % of blood donors were positive for syphilis. A higher prevalence of syphilis among blood donors (0.36%) was recently reported in China [20].

Data in most of the reported studies revealed that the prevalence of syphilis is low relying on the observation that syphilis bacteria die quickly during normal storage conditions for blood. However, the survival time of *T. pallidum* in banked donor blood stored at 4°C was found to be 72-120 hours in a recent report [21]. Therefore, regardless of blood banking temperature, *T. pallidum* and other transfusion transmissible infections should be screened for prior to transfusion.

The problem with this disease, first of all, is its high prevalence in blood donors in various areas of the developing countries. In Africa, The recent prevalence was 3.7% in Congo[17], 7.9% in Ghana [22-23], 9.1% in Cameroon [4, 24].The prevalence was 12.7% among Tanzanian donors [25], and 15% among Sudanese donors [26]. The prevalence of Syphilis was also 8% in 1997 [27] and 7.5% in 2003[22].

In other parts of the world, the prevalence of syphilis is generally lower than that in Africa but still significant. Seroprevalence of Syphilis was 2.3% in the population of blood donors in Georgia[28].Antibodies to syphilis were found in 3% of immigrant sex workers in Madrid[29]. In India, syphilis was detected in 0.11%in male replacement donors [30].

In Saudi Arabia, infection with *T. pallidum* seemed to be more common in men than in women (1.6:1) and predominated in the age group 20-39 years [31].In a recent five-year surveillance study for sexually transmitted infections(STIs) in Saudi Arabia, it was reported thatAmong 39049 STIs, syphilis infections were 3385 (8.7%)[32]. The present study reports a low prevalence of syphilis among blood donors. Whether this reflects lower prevalence of the disease in the general population needs further investigations.

Blood transfusion, an integral part of medicine and surgery, also carries the risk of infections like Hepatitis B and C, HIV and Syphilis, malaria and infrequently toxoplasmosis, brucellosis and viral infections like CMV, Epstein Barr Virus and Herpes[33]. Due to danger, WHO [7]has recommended pre-transfusion blood test for Syphilis as mandatory. This infection is capable of causing significant mortality, morbidity along with a financial burden for both the affected person and the country.

With every one unit of blood transfusion there is 1% chance of transfusion related complications including transfusion transmitted infections[33]. Keeping in mind the grave consequences of these infections and to restrain the transmission to a minimum, it is very important to remain vigilant about the possible spread of these diseases through blood transfusion.

Of 4,468,570 donations, 12,145 (0.27%) were sexually transmitted syphilis positive in United States blood donors[34].

From the total of 6361 consecutive blood donors in Northwest Ethiopia, the seroprevalence of syphilis was 1.3%[35].A high seroprevalence of syphilis in South Western Sudan (23.5%) was detected using ELISA with higher frequencies than Immuno-chromatography test andhemagglutination assay. This study revealed that ELISA was more accurate to evaluate the safety of blood donation in comparison to ICT and TPHA. Strict selection of blood donors and comprehensive screening of blood using standard methods are highly recommended to ensure the safety of blood transfusion for recipients in Heglig area[36].

The seroprevalence of syphilis is low among voluntary blood donors in North-eastern, Nigeria, where only the male donors had syphilis (1.2%)[37].

The prevalence of syphilis in South Korea was found to be 0.2%. Although has been consistent decreasing trend since 1977 ($p < 0.0001$), there is no statistical difference between 1995 and 2000 ($p = 0.6992$)[38].

However, There was apparent increase in syphilis among men who have sex with men in Beijing in 2004, 2005, and 2006 [39].

The challenges and the perspectives of the disease during transfusion are related to improvement clinical selection of blood donor (identifying the precise risk factors) and to the development of tools for treatment of red blood cell concentrates. Prevention of the spread of syphilis is primarily by education and developpement of effective and treatment programmes.Eliminating high risk sexual behaviors is very effective in helping prevent Syphilis[15].

CONCLUSION

Prevalence of anti-Treponemapallidum antibodies as well as HBsAg in blood donors in Asser region is very low. The rate of HBcAb in units of blood donation is relatively high. The presence of HBV-DNA in HBcAb positive donations makes it risky for use. The screening of anti-Treponemapallidum antibodies in blood donors will greatly eliminate parental transmission of syphilis.

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