HIV SCREENING OF BLOOD DONORS IN THE CENTRAL BLOOD BANK OF MADINA, AL-MUNAWARA, SAUDI ARABIA.

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BSTRACT

The study was designed to investigate and exclude the risk of human immunodeficiency virus (HIV) infection among blood donors, which might be transmitted to the patients. The study included 11,815 blood donors. All samples were screened throughout the study period of two years. HIV-1 and HIV-2 were determined by using enzyme immunoassay and subsequently confirmed by HIV Western blot assay. World Health Organization (WHO) protocol for transfusion services were strictly followed in the current study conducted in the Central Blood Bank, MOH, Madina Al-Munawara, Saudi Arabia. All sera were tested by enzyme immunoassay for improved detection of seroconversion to HIV types 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies the Murex HIV Ag/Ab combination was used to detect the reactive HIV core antigen. In addition, IgG, IgM, and IgA were estimated as the envelope glycoproteins of HIV-1 and HIV-2. The potentially infectious samples of serum, EDTA plasma, or citrate plasma were identified by using BEPIII DADE Behring autoanlyzer. In the present study, Western blot assay was performed using INNO-LIA™ HIV I/II Score to confirm the presence of antibodies and to differentiate between HIV-1 and HIV-2 infection. The reactive samples were retested if found repeatedly reactive. Only two volunteers were proven to be reactive for HIV-1 infection. In one case, the bands at spg120, gp41 rate 3+ and the band at p24 rate 1+ were observed. In the second case results remained indeterminate. However, after follow up the donor was proven to be HIV-1 positive. Based on the results of present study, it is emphasized that HIV screening for healthy blood donors is essential to detect the positive cases, which would lower the risk of transmission of HIV.

Key words: blood donors, HIV-1 and HIV-2, enzyme immunoassay, Western blot assay

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INTRODUCTION

It is well documented that autoimmune deficiency syndrome (AIDS) is a disease caused by a blood-born infectious agent that may easily be propagated by blood transfusion (Galel et al., 1995; CDC, 2013). A reduced ratio of CD4+ to CD8+ T cells has been used to be as an indicator to identify AIDS patients and the probable carriers of AIDS pathogen (Al-Anazi 2009;
Blood transfusion is essential in acute medical services and is considered life-saver in many situations. Recently, the blood donation criteria were improved to ensure blood quality and safety which resulted in shortage of blood in the blood banks. In Saudi Arabia, blood donation is believed to be a part of moral obligations associated with religious implications hence comparatively lesser shortage is observed (Strong et al., 2005; Abdul-Gader et al., 2011; Abolfotouh et al., 2014; Ibrahim, 2015). Nevertheless, blood transfusions might also be a quick and easy route for the transmission of infectious agents such as HIV, HBV, HCV, gastrointestinal opportunistic infections, and malaria (Otani et al., 1992; Franceschi et al. 1995; Leon et al. 2003; Zia and Besa 2012). It is worth noteworthy that in developing countries HIV and hepatitis transmission through blood transfusion is a major concern, where the economic constraints seriously limit safety in blood supply chain (Wake and Cutting 1998; Alrajhi et al. 2006; van Hulst et al., 2010; WHO, 2010). Blood transfusion may account for the transmission of up to 15% of blood-borne infection, therefore, blood donations are always suggested to be screened for antibody to HIV (Ward et al., 1988; Hu et al. 1991; Shrestha 1996; Office of AIDS 2014; CDC, 2015).

No doubt, the safety has been improved by using excellent serologic tests, and nucleic acid based tests now available to avoid transfusion of transmissible diseases (Strong et al., 2005; Abdullah, 2013; Memish et al., 2015). However, an important safety factor is the use of young volunteers, who are unpaid blood donors (Holland 2000). The diagnosis of HIV infection is normally made indirectly via virus-specific antibodies virtually found in all the HIV infected individuals. The individuals, who persistently fail to have detectable antibodies against HIV despite the presence of HIV infection, are extraordinarily rare and have little or no role in clinical practice (Connick 2005; Wafa and Fageeh 2010).

Besides using indirect diagnosis a direct diagnosis of HIV infection is possible by using cell culture facilities for infectious viruses or viral antigens (p24 antigen ELISA), or viral nucleic acid (viral genome based nucleic acid testing ‘NAT’). NAT is an accurate and relatively simple method, which does not require any high security laboratory (Kassutto et al. 2005). In all cases the test must be sensitive to detect low-avidity antibodies of a primary infection as well as all different HIV types such as HIV-1, HIV-2 and the subtypes: HIV-1-N, HIV-1-O, HIV-1-M (UNAIDS / WHO 1992; UNAIDS/WHO, 1997; KSA-MOH, 2014). A positive screening test is further confirmed by using Western blot or immunofluorescence assay (IFT or IFA), and is not only cost effective but more specific ELISAs, however, the situation in the relatively poor countries might differ (Tamashiro et al., 1993; WHO, 2010; RCN and ANSTB, 2011).

The present study was therefore conducted in the Central Blood Bank, Madina Al-Munawra, Saudi Arabia, where World Health Organization (WHO) protocols are followed for HIV screening (Gibbs and Britten, 1992; Gibbs and Corcoran 1994; WHO, 2010). The blood donors included were volunteers (unpaid), and high-risk donors were eliminated and more sensitive and selective HIV tests were used. The official WHO guidelines for blood administration were followed and any unnecessary transfusions and promotions of voluntary donation were avoided as a public responsibility. However, the improved guidelines were added for better information for patient’s blood management (WHO, 2010; RCN and A&NSBT 2011; Uhl 2011; CDC 2015).

MATERIALS AND METHODS

The present study was conducted over a period of two years in the Central Blood Bank,
Madina Al-Munawra, Saudi Arabia. A total of 11,815 Saudi volunteer blood donors were included in the study. All participants were informed about the goals of present project and an informed consent was obtained as approved and recommended by the Ethical Committee for Research headed the Director General, Hospital Laboratories and Blood Banks, MOH, Riyadh, Saudi Arabia. For safe blood transfusion services, the WHO standards were followed (Gibbs and Britten, 1992), which are approved and adopted by the MOH, Saudi Arabia as basic protocols for such studies (Gibbs and Corcoran 1994; KSA-MOH, 2014). Highly sensitive assay methods, which are well known to detect anti HIV seroconversion and respond to both IgM and IgG were used. The core antigen was typically detectable during a short period prior to antibody seroconversion.

All sera were tested by enzyme immunoassay for improved detection of seroconversion to HIV types 1 (HIV-1, HIV-1 group O) and detection of anti HIV-2 antibodies using the Murex HIV Ag/Ab combination. It was designed to detect reactive HIV core antigen in addition to IgG, IgM, and IgA to the envelope glycoproteins of HIV-1 and HIV-2 (Gendler and Pascuccio 2007). Consequently, the potentially infectious samples of serum, EDTA plasma, or citrate plasma could be identified using the BEPIII DADE Behring fully automated ELISA processor.

The reactive samples were retested and if persistently reactive than Western blot was performed using the INNO-LIA™ HIV I/II Score to confirm the presence of antibodies against the HIV -1 and HIV-2. The method could also differentiate between HIV-1 and HIV-2 infections. The assay principle depended on the presence of recombinant proteins from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O was coated as discrete lines on a nylon strip with plastic backing. Five HIV-1 antigens were applied: sgp120 and gp41, which could detect the specific antibodies to HIV-1 and p31, p24 and p17. It could also cross-react with antibodies to HIV-2. HIV-1 group O peptides present in the HIV-1 sgp120 band. The antigens gp36 and sgp105 were applied to detect antibodies to HIV-2 (Micropathology, 2010; Innogenetics, 2011).

In addition to the HIV antigens mentioned, four control lines were coated on each strip, i.e., the anti-streptavidine line, cut off line +/- (human IgG), 1+positive control line (human IgG) and one strong 3+positive control line which was also the sample addition control line. Samples tested were incubated in a test trough together with the multiple antigen-coated test strips. HIV antibody if present in the sample, would bind to the individual HIV antigen lines on the strip.

RESULTS

Among the 11,815 tested blood samples, 31 were found reactive for the HIV combined immunoassay. After retesting the same samples in duplicate; 4 samples were still found reactive for HIV. However, Western blot test confirmed just 2 samples out of the 4 reactive samples to be reactive (positive). The other 2 non-Western blot reactive cases were considered false positive.

The two reactive cases to Western blot test were as follows (Table 1): Case-1 was reactive for HIV-1, sgp120 and gp41 showed band rate 3+, and p24 gave band rate 1+. Case-2 was considered indeterminate for HIV-1 because it showed bands sgp120 and gp41 rate+/-.

Case-2 was followed up every 3 months and was finally proven to be positive by Western blot as another band rate 1+ appeared at p31. The rating was done by the INNO-LIA™ HIV I/II Score.
Table 1: Results for western blot assay for two reactive cases.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>gp36</th>
<th>sgp105</th>
<th>p17</th>
<th>p24</th>
<th>p31</th>
<th>Gp41</th>
<th>sgp120</th>
<th>Strip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-1</td>
<td>Negativ e</td>
<td>Negativ e</td>
<td>1+</td>
<td>Negativ e</td>
<td>3+</td>
<td>3 +</td>
<td>Negativ e</td>
<td></td>
</tr>
<tr>
<td>Case-2</td>
<td>Negativ e</td>
<td>Negativ e</td>
<td>Negativ e</td>
<td>1 +</td>
<td>+/-</td>
<td>+/-</td>
<td>Negativ e</td>
<td></td>
</tr>
</tbody>
</table>

The donors identification cards were given to the Infection Control Department to inform them and to start follow up consultation and the PCR confirmation testing and treatment.

DISCUSSION

The Saudi government has reported its nationwide statistics of HIV reactive cases. As a result, the report issued in 2004 revealed 262 HIV positive cases. Saudi Ministry of Health (MOH) has strict policy on communicable diseases and to avoid the residual risk in such situations. The observations of Saudi MOH are well in agreement with the findings elsewhere (Khattak et al., 2002). The risk of viral transfusion transmission of HIV, HCV, HBV, and HTLV is globally reduced due to improvements in serological assays; however, it is also adversely affected by the cost and availability of blood units in the blood banks. Our findings are substantiated by the earlier reports on: increase in prices per test, result quality and to reduce the cost HIV treatment (Panhotra et al. 2005; Granich et al., 2009; Barlet 2011; Engleman 2011; Wu and Zaman, 2012). Screening for HIV in tuberculosis patients was found to be higher; however, such results were reported to be underutilized (Alrajhi et al. 2002; Al-rajhi et al., 2006).

In the present study, two cases were detected positive during screening for HIV-1 and HIV-2 among the total of 11,815 healthy blood donors in Central Blood Bank, Medina Al-Munawara in 24-months period of the project. In Saudi Arabia the observed trend in blood donation is not much affected due to religious believe; nonetheless, the decrease in blood units in blood banks was associated with high cost of assay procedures and quality criteria set for improving the safety parameters (Abolfotouh et al., 2014). In general, it has been observed that people in developing countries know little about the blood donation, which in turn caused increase in price of blood units. Hence, a public awareness campaign is suggested to combat the misconceptions regarding the blood donation. It would lead to motivate the youth for blood donation voluntarily (Thaver et al., 2014).

In fact, in developing countries blood donation is generally not safe because it has been commercialized. There are paid professional blood donors available, who belong to poor class and suffering from communicable disease due to mal-practices as per reports published from India and Thailand (Wiwanitkit 2005). It has also been described that not all the blood donors are properly monitored or tested for HIV, hepatitis, syphilis, and other diseases that might also be transmitted by blood transfusion (Hu et al., 1991; Wiwanitkit 2005; Fernandes et al. 2010; Thaver et al., 2014). The possible effect of residual risk due to technology in blood donation and screening was recently highlighted (Barlet 2011; Skinner-Thompson, 2015). It is worth mentioning that HIV antibody detection is mandatory for all countries since 1997 and p24 antigen screening is also recommended (Gendler and Pascuccio 2007). Regrettably, some Asian
countries adopted WHO recommendation for blood donation and transfusion but such facilities are available only in limited number of blood banks. The sharp increase in the price of different tests or treatment cost, are matters of high concern and a part of limitations (Constantine, 2001; Sanders et al., 2010). For example, in a big and thickly populated country like India only five cities have such a facility. In Pakistan the program of safe blood and blood products was initiated in cooperation with German Agency for International Cooperation (GIZ) providing both technical and financial support (SBTP, 2012). On the other side, Blood Banks in Japan and Singapore are reported to check their blood supply and blood donations routinely (Otani et al. 1992; Gibbs and Corcoran 1994).

Saudi program regarding safety and quality in blood banks is progressing faster than many other countries. Otherwise, in developing countries, usually the primary reasons for inadequate blood testing were found to be the cost of test kits and reagents and the unreliability of supplies (Constantine, 2001). Blood safety might be improved by proper quality assurance, more appropriate use of transfusions and the provision of alternatives such as saline and colloids (Gibbs and Corcoran 1994). To avoid the risk of transfusion transmission of HIV, HBV, and HCV, the application of microbial inactivation technology to blood and blood components was suggested (CDCP 1989; Schwartz. 1995; Jayaraman et al., 2010).

Earlier screening reports described advanced screening test to be 100% effective to detect HIV infection in all cases, however, some false-negative results were found (Preiser et al. 2000). Similarly, HIV-1 p24 antigen testing is concerned for early detection of HIV infection in blood donors. In a previous report from Saudi Arabia, four interactively positive cases out of 24,654 blood donors were found, which were found to be negative in other highly sensitive tests. As regards p24 serological test is concerned, it was recommended not to be used in such studies (Alamawi et al. 2003). In our current study, among 11,815 samples screened, 29 samples out of the 31 reactive samples for HIV, showed false positive results in combined immunoassay for the HIV-I/II, and only 2 samples were proven to be HIV-1 reactive. Therefore, emphasis has been placed on the utmost sensitivity of testing procedures; because any failure to identify a positive sample correctly could have grave consequences. Needless to say, the costs of new tests play a vital role to achieve the goals on each blood donation centre in the developing countries (Constantine, 2001; Constantine and Zink, 2005). Presently, available HIV screening tests have a specificity of at least 99.5 %. This high sensitivity however, caused somewhat lower specificity as described earlier (Beckwith et al. 2005; Fernandes et al., 2010; WHO and Red Cross, 2010).

It is worth mentioning that worldwide, different organizations developed different sets of criteria for interpretation of HIV Western blot results. For instance, the American Red Cross demands at least three bands (for each group: one GAG, one POL and one ENV band). On the other side, the US Food and Drug Administration (FDA) demands the p24, the p34, as well as the gp41 or gp120/160 bands (CDCP, 1989). According to WHO recommendations, however, a Western blot may be judged positive if two ENV bands are found. In Germany, the DIN norm 58969 part 41 applies: A serum sample is considered HIV positive, if it reacts with at least one viral glycoprotein and one of the other HIV proteins. All other virus-specific band patterns are regarded as questionable (Deutsches Institut für Normung 2000; WHO, 2011; Office of AIDS, 2014; Skinner-Thompson, 2015).

Earlier, transmission of HIV infection in children in Saudi Arabia was attributed to the lack of knowledge among patients, parents, and health care providers and early diagnosis, treatment, and follow up was suggested (Alam and Masalmeh, 2004; Kordy et al. 2006; Al-
Anazi, 2009). As part of an effort to monitor the safety of global blood transfusion services, the government of Saudi Arabia plays a vital role in screening of the HIV among the blood donors and even among their citizens by including the HIV screening tests among the pre-marital investigations (El-Hazmi, 2004). However, such a screening is recommended mandatory for some other countries as a part of health policy (Sweat et al., 2000; WHO and Red Cross, 2010; Burns 2011; KSA-MOH, 2014). Our results are of great significance for blood donation by young people in a society and are in full agreement with the other recent reports (Abdel-Gader et al., 2011; Abolfotouh et al., 2014; Thaver et al., 2014; Memish et al., 2015). It is concluded that the recruitment of more Saudi young donors and proper screening protocols of blood might help to ensure a long-term increase in the blood supply without jeopardizing the safety.

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