PREVALENCE OF TRANSFUSION TRANSMITTED INFECTIONS IN ASYMPTOMATIC BLOOD DONORS: IS SYPHILIS ALARMING?

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ABSTRACT
Background and Objective: Blood transfusion is a life saving procedure but always carries a potential risk of infections. By proper screening techniques the risk can be reduced. The present study was designed to evaluate the prevalence of infectious disease markers and frequency in males and females of various age groups among healthy blood donors at the blood bank of a tertiary care hospital in Lahore. This was an observational study, conducted at Ittefaq Hospital, Lahore from Jan. 2011 to Dec. 2013.

Methods: A total of 29522 (26688 males, 2834 females) apparently healthy blood donors were screened for Anti HCV, HBs Ag, HIV and syphilis by Chemiluminescent Immunoassay (CMIA) technology on Architect Ci4100 (Abbott Diagnostic, USA).

Results: Our study population mostly constitute directed donors as compared with voluntary donors. Individually, HBsAg and Anti HCV were positive in 368 (1.24%) and 743 (2.51%) subjects respectively whereas co-infection of HBsAg and anti HCV was found in 9 (0.03%) cases. HIV was detected in 20 (0.07%), and syphilis was positive in 531 (1.79%) subjects referred to donate blood. The prevalence of TTIs was higher among male donors than females. HIV was only detected in male donors and frequency of syphilis was also higher in male donors as compared with female donors. Transfusion transmitted infections were mostly seen in age group between 20 – 30 years.

Conclusion: In HCV and HBV endemic countries like Pakistan, critical screening of blood donors is of prime importance because they serve as an asymptomatic reservoir and a potential source of transmission of these infections. Syphilis is detected in significantly high number among healthy donors. So most sensitive methods should be adopted to screen syphilis. More studies are needed to find the prevalence of syphilis in public sector hospitals.

Key words: Blood Transfusion, Infections, blood donors, Syphilis.

INTRODUCTION
Transfusion medicine being relatively emerging field, risk for transfer of infections from blood transfusion was considered unavoidable in the past, however by the advent of new screening techniques the risk is greatly reduced.¹

Blood transfusion is a life saving procedure, but always carries a potential risk of transmissible diseases.²³ Hepatitis C, Hepatitis B, Human Immunodeficiency Virus (HIV), Malaria and syphilis are the major infections acquired through blood donations worldwide especially in endemic countries like Pakistan.⁴

Globally 130 – 150 million people have chronic hepatitis C infection ⁵ and due to the acute or chronic consequences of hepatitis B more than 780 000 people die every year.⁶

The overall prevalence of hepatitis B (HBV) and hepatitis C (HCV) was 2.5% and 4.9% respectively in general populations of Pakistan, reflecting a population pool of about 13 million of chronic hepatitis B and C carriers.⁷ The major risk factors include injudicious use of injectables, intravenous drug abusers, unsafe dental procedures and substandard barber practices.

Human immune deficiency virus (HIV), having the ability to cause latent infection for relatively longer periods is also potential candidate to be transmitted through transfused blood,⁸ the highest number of HIV patients (22.9 million) is reported from sub-Saharan Africa.⁹ According to United Nations Programme on HIV (UNAIDS) there are about 68000 (41000–130000) people living with HIV in our country and the prevalence rate is 0.1%.¹⁰

The World Health Organization estimated that approximately 12 million new cases of syphilis are reported each year in the world with more than 90 percent from developing countries.¹¹

Screening tests vary among different regions. Screening techniques for blood donors have travelled from simple qualitative to ELISA based quantitative to chemiluminescence assay to nucleic acid amplification.
techniques (NAT). With the advent of new techniques in the diagnostic field more and more sensitive instruments are available for blood screening. CMIA (Chemiluminescence Microparticle immunoassay) is among one of the most sensitive technique used for serological testing, it uses light to indicate the presence of antibodies and/or antigen. The objective of the present study is to evaluate the prevalence of infectious disease markers hepatitis B, C HIV and syphilis among healthy blood donors performed by Chemiluminescent Immunoassay (CMIA) at the blood bank of a tertiary care hospital. The study will provide baseline data for the prevalence of syphilis in blood donors of developing countries like Pakistan.

METHODOLOGY
Participants of the study were blood donors covering the period from January 2011 to December 2013 at the blood bank of Ittefaq hospital, which is a 450 bedded tertiary care public sector, teaching hospital in Lahore, Pakistan. It is an observational study. A total of 29,522 blood donors during 3 years (9673, 9860 and 9989 during the year 2011, 2012 and 2013 respectively) were screened for transfusion transmitted infection markers. Donors age ranged 18 – 50 years, with their informed consent were included in the study. Donors who were physically fit and fulfilling the donor selection criteria as mentioned in our donor query form were selected. Guidelines on assessing donor suitability for blood donation was developed in accordance with the WHO guidelines, which included demographic data, detailed present and past history, blood donation history and medical history. Physical examination of pallor, jaundice, cyanosis, dyspnoea or intoxication from alcohol or drugs. Examination of any tattoos or injection marks were noted. Measurement of vital signs like pulse, blood pressure and temperature were noted. Baseline hemoglobin level was also measured before selection of a donor.

Five ml of Blood sample was collected from median cubital vein taking aseptic measures. Samples were poured into gel vial and allowed to clot at room temperature. Samples were centrifuged at 9000rpm for ten minutes and serum was taken for further testing. Screening tests were performed by Chemiluminescence Microparticle immunoassay (CMIA) technique. The tests were carried out on a fully automated and state of the art instrument Architect Ci4100 (Abbott Diagnostic, USA). The quality control of the instrument was maintained with the help of commercially available low and high value known controls. The instrument was maintained on daily bases according to manufacturers’ guide lines. For samples giving a positive marker, the analysis was confirmed on the same instrument as well as on latent flow one step chromatographic devices (Alere Medical, Japan). Data was entered on SPSS version 11 for Statistical analysis and frequencies and percentages of various characteristics were determined. Percentages of transfusion transmitted infections in both genders and different age groups were also determined. Graph was plotted on excel sheet.

The research project is approved by the institutional ethical committee. Authors state that they have no conflict of interest.

RESULTS
The total number of donors who were found positive for transfusion transmissible infections (TTIs) were 1662 (5.76%). Seropositivity found in the donors was 368 (1.24%) for HBs Ag, 743 (2.51%) for Anti HCV, 20 (0.07%) for HIV, 531 (1.79%) for syphilis, co infection of HBsAg and anti HCV was positive in 9 (0.03%), anti HCV and syphilis in 27 (0.09%) and HBsAg and syphilis in 4 (0.02%) (Table 1, Fig. 1). Most of the patients were males 26688 (90.4%) with a mean age of 27.8

<table>
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<tr>
<th>Serological Marker</th>
<th>Seropositivity (No)</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>HBs Ag</td>
<td>368</td>
<td>1.24</td>
</tr>
<tr>
<td>Anti HCV</td>
<td>743</td>
<td>2.51</td>
</tr>
<tr>
<td>HIV</td>
<td>20</td>
<td>0.07</td>
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<tr>
<td>HBs Ag + Anti HCV</td>
<td>9</td>
<td>0.03</td>
</tr>
<tr>
<td>Syphilis</td>
<td>531</td>
<td>1.79</td>
</tr>
<tr>
<td>Anti HCV + syphilis</td>
<td>27</td>
<td>0.09</td>
</tr>
<tr>
<td>HBs Ag + syphilis</td>
<td>4</td>
<td>0.02</td>
</tr>
<tr>
<td>HIV + Syphilis</td>
<td>0</td>
<td>0</td>
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Table 1: Seroprevalance of TTIs in blood donors in 2011-2013 (n = 29,522).

Fig. 1: Transfusion Transmitted Infections.
years (17 – 50) and there were 25684 (87%) replacement donors/directed donors as compared with voluntary donors who constituted 3838 (13%).

Out of 368 HBsAg positive donors, males were 63% and females were 37%. 743 apparently healthy donors were anti HCV positive and this HCV positive population consisted of 74% males and 26% females. HIV was detected only in male donors and similarly syphilis was in high prevalence among (males 93%) than females (7%) (Table 2). Maximum male donors presented in age group of 20 – 30 years (Table 3). The age of female donors ranged from 18 – 40 years. Maximum female donors were also presented in age group of 20 – 30 years. The prevalence of various TTIs in different age groups of female population is given in (Table 4).

DISSCUSSION

According to WHO, about 1.5 million units of blood products are transfused annually in Pakistan.14 Prevalence of TTI in developed countries is reduced significantly after the advent of advanced screening techniques.

There is a wide variation in the prevalence of TTI in Pakistan as seen by different studies. According to PMRC 2007 – 2009 it was estimated that there was a high prevalence of HCV in Punjab (6.7%) while it was low in Khyber Pakhtunkhwa (1.1%). HBV prevalence was high in Baluchistan (4.3%) and low in Khyber Pakhtunkhwa (1.3%). Although the figures in Punjab were 2.4% while in Lahore it was 1.42 this might be due to people’s education status, knowledge about vaccination and donors selection criteria.

In the present study, the seroprevalance of hepatitis C was 2.51% and of hepatitis B was 1.24%. A study by Attaullah S15 reported a seroprevalance of HBV 2.68% and HCV as 2.46%, the seroprevalance of HCV was in concordance with our study but prevalence of HBV was high, this may be due to lack of knowledge about vaccination.

The seroprevalence of HBV in blood donors ranged from 1.46% to 8.4%, while for HCV it ranged from 0.27% to 8.68% in various studies.16 In a study from Sindh17 the prevalence of HCV in healthy blood donors was 3.45% and 1.82% for HBV. The high prevalence is due to lack of awareness about the vaccination in rural areas and might be due to the use of different generation of ELISA test kits, having different sensitivities and specificities. Our study was conducted in a private sector hospital and people were aware about the vaccination of Hepatitis B, but it was only a one centre observation.

A study by H. Nazar18 reported the prevalence of HCV and HBV as 2.06% and 1.71% respectively which was very near to our observation. Co infection of anti HCV in our study was 0.03% while it was 0.09% by H. Nazar, and 0.07% by Tunio SA.17

The trend of HCV seroprevalence in India with almost similar socioeconomic and climatic conditions to Pakistan was relatively decreased from past years.9 Another recent study in India reported the prevalence of hepatitis B surface antigen (HBs Ag) 1.2%, hepatitis C virus (HCV) 0.9%. And trends of these infections were high in replacement donors.19 In our study the percentage of replacement donors were also more as compared to voluntary donors. The prevalence of HBs Ag was in concordance with our study but prevalence of HCV was very low as reported in other studies from India as well.

The prevalence of HBV infection was lower in developed countries like United States and Western Europe (0.1 – 0.5%) and was reported to be higher, 5 – 15% in South East Asia.20 HBV transmission was also showing a decreasing trend in Pakistan, it cannot be relied upon, because the donors were screened only for the HBsAg and not for antibody to the Hepatitis B core

<table>
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<tr>
<th>Table 2: Frequency of Male and Female in TTI.</th>
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<tr>
<td>Gender</td>
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<tr>
<td>Males</td>
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<td>Females</td>
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<th>Table 3: Frequency of TTI’s in different age groups of Male donors.</th>
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<tr>
<td>Age (Years)</td>
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<tr>
<td>17 – 20</td>
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<td>20 – 30</td>
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<td>40 – 50</td>
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<th>Table 4: Frequency of TTI’s in different age groups of Female donors.</th>
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<td>Age (Years)</td>
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<td>18 – 20</td>
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<td>20 – 30</td>
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<td>30 – 40</td>
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antigen and other sensitive markers to the screening protocol.\textsuperscript{21}

In Pakistan Human immunodeficiency virus (HIV) and syphilis prevalence in blood donors was 0.05% and 0.5%, respectively. WHO report states that the viral dose in HIV transmission will lead to death on an average, after 2 years in children and after three to 5 years in adults, through one HIV positive transfusion.\textsuperscript{22}

In the current study the prevalence of HIV in blood donors was 0.07% and prevalence of syphilis was very high 1.79%. Co-infection of syphilis with HCV and HBV were also seen in 0.09% and 0.02 % respectively but no co-infection with HIV is seen. Attaullah S\textsuperscript{5} reported the prevalence of 0.06% for HIV in Peshawar which was same as our observation, but prevalence of syphilis is very low 0.43% as compared to our study. Irfan M\textsuperscript{23} reported the prevalence of HIV as 0.10% in replacement donors in Karachi.

Prevalence of HIV was 0.06% in Bangladesh like our study while prevalence of syphilis is low 0.15%.\textsuperscript{6,24} A study by Negi G reported the prevalence of HIV and syphilis was 0.2% and 0.3% respectively.\textsuperscript{19}

One of the reasons for varying results in prevalence of TTIs is the test methodology used. Variety of screening techniques ranging from one step horizontal device to manual, semi automated and fully automated instruments are being used. In our study the infections are mostly seen in young age group, the reason may be that the younger population of our country are usually healthy and they are more eagerly involved in blood donation as compared to elderly. The results are also significant to be considered in the context that they will aid in the awareness to the high prevalence of TTIs in the healthy carriers of younger age group population in the country.

The technique and instrument used in this study are most sensitive and latest ones being used in the developed countries of the world. So some difference also arose due the fact that the technique and instrument used here are neither in common practice in Pakistan nor in the other developing countries. A working hypothesis can be devised that in the absence of sensitive and specific techniques, TTIs in blood donors are being under reported. So the reason for high prevalence of syphilis in our study might be due to more numbers of replacement donors and improved detection methods. This MIA technique used here though most sensitive but not superior to NAT. But NAT technique is much more costly and not available everywhere. Also the instruments used for serological testing accommodate serology section of the laboratory as well as blood bank screening and no extra instrument is required which is a cost effective factor.

It is concluded the blood transfusion services in our country are not fully organized. Only few centers are following internationally recommended standards; secondly they are not consistently regulated by an authority. True burden of Sexually transmitted infections is unknown in our country and mostly go unnoticed due to unavailability of strict donor selection criteria and methodology of screening devices, so more studies are needed to find out the exact burden. Most important, the sensitive methods of screening like nucleic acid amplification techniques (NAT) should be implemented where possible.

**Conflicts of Interest:** None.

**Author’s Contribution**

All the authors contributed equally in the study.

**ACKNOWLEDGEMENT**

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**REFERENCES**