Original Article

Seroprevalence of Transfusion Transmitted Infections by Using 4th Generation Enzyme- Linked Immunosorbent Assay kit: A 3 Year Study in a Tertiary Health Care Centre of Delhi

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ABSTRACT

Background: One of the greatest challenges of the blood transfusion services is to prevent the transmission of Transfusion transmitted infections (TTI), because these unsafe blood transfusions leads to increase morbidity and mortality and eventually leads to economic burden on the society. The objective of our study was to find out the seroprevalence of TTI among blood donors at our blood bank by using 4th generation ELISA kit and to compare our study with other studies conducted at national and state level.

Method: 2401 units of blood collected during 3 years period were screened for 5 infections. HIV, Hepatitis B and Hepatitis C infections were screened by using 4th generation ELISA kit. Test for syphilis was done by Rapid plasma reagin card test and test for malaria parasite was done by Advantage Mal card test.

Results: A total of 2401 units were collected during the period of three years. 98.25% donors were male. 91.09% donors were replacement donors. A total of 71 blood donors (2.95%) were tested positive for any one of the TTI. Out of these 71 cases, 69 were males and only 2 were females. The overall prevalence of HIV, HBV, HCV and syphilis in our study were 0.33%, 1.7%, 0.74% and 0.16% respectively. None of our donors was tested positive for malaria.

Conclusion: In order to minimize the risk of TTI, voluntary donors should be encouraged by means of educating general people about benefits of blood donation and motivating them by conducting regular blood camps.

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Introduction

Although blood transfusion (BT) is an integral part of medical and surgical therapy which can saves millions of lives worldwide each year and also reduces morbidity, however it has a life threatening hazards as well, which may vary from only trivial to potentially life threatening complications. Thus proper selection of donors and meticulous testing of all donated blood is essential to reduce the morbidity and mortality associated with blood transfusion.[1,2,3] The infectious agents must have the following characteristics in order to be transmitted by blood. These include "presence in the blood for long periods, stability in blood stored at 4° C or lower temperature, long incubation period before the appearance of clinical signs and asymptomatic phase or only mild symptoms in the blood donors hence not identifiable during the blood donor selection process".[4] Human immunodeficiency virus (HIV), Hepatitis B virus (Hep B), Hepatitis C virus (Hep C), syphilis and malaria is responsible for majority of transfusion transmitted infections (TTI), however infrequently other infections like Cytomegalovirus (CMV), Epstein bar Virus (EBV), Herpes virus, Human T-cell leukotropic virus (HTLV), Toxoplasmosis, Brucellosis and Chagas disease etc. may also be transmitted through BT.[1] The World Health Organization (WHO) recommended mandatory screening of all donated bloods for HIV-1 & HIV-2, Hep B, Hep C and syphilis. [5] They also recommended screening of other infections like malaria, chagas disease or HTLV based on local epidemiological evidence. [5] In India, according to guidelines of National AIDS Control Organization (NACO), all donated blood must be tested for HIV-1 & HIV-2, Hep B, Hep C, syphilis and malaria.[6] There is always 1% chance of transfusion associated problems including TTI with every unit of blood transfused.[7] Thus one of the greatest challenge of the transfusion services is to prevent the transmission of these TTI, because these unsafe blood transfusions leads to increase morbidity and mortality and eventually leads to economic burden on the society.[8] According to WHO "the minimum evaluated sensitivity and specificity level of all assay used for blood screening should be as high as possible and preferably not less than 99.5%". The NACO recommended use of 3rd or 4th generation ELISA kit, which is 100% sensitive for testing donated blood for HIV-1 & HIV-2.[9]

The present study was carried out to find out the seroprevalence of TTI among blood donors at our blood bank attached to newly establish medical college by using 4th generation ELISA kit, since inception of our blood bank in March, 2012. This is essential for monitoring the safety of blood supply. We also compare our study with other studies conducted at national level as well as with studies conducted at Delhi and adjoining states.

Material and Method

The present study was conducted at our newly established blood bank attached to our Medical college. The 3 years data was collected between the periods 20th March, 2012 to 20th March, 2015.

The blood was collected in our blood bank both from replacement donors and voluntary donors. The first step of blood donation starts with filling of a registration form by the donors which include age, sex, address, contact number, occupation, history of previous donation along with medical history like history of previous major or minor surgery, blood transfusion, hospitalization, history of any febrile illness in the recent past, weight loss, uncontrolled diarrhea, recent jaundice, liver disease, lung disease, cardiovascular disease, malignancy, epilepsy, malaria, dog bite and intake of alcohol or any contraindicated drugs etc. Pre-donation counseling of the donor was the next step which includes explanation of the procedure of the blood donation, post-donation care and the final outcome of the donation in the form of result of TTI screening test. Blood grouping and hemoglobin (Hb) estimation was done. All the donors were then screened by blood transfusion officer (BTO) as per the strict donor selection criteria led down by NACO. Height, weight, pulse, blood pressure and temperature were recorded. Thorough inspection was done for any marks of drug abuse, infection or skin lesion at the site of venepuncture. This screening procedure was helpful in excluding professional donors.

All the donors between the age group of 18 to 60 years, weight more than 45 kg, Hb level of more than 12gm%, without any history of hepatitis, jaundice or any major surgery in past one year and with normal pulse and blood pressure were considered fit for blood donation. Blood was then collected by taking all aseptic precautions as per surgical operative procedure (SOP) of our blood bank after obtaining a written informed consent from the donor.

As per NACO guidelines, all 2401 units of blood collected during 3 years period were screened for 5 infections, i.e HIV, Hep B, Hep C, Syphilis and malaria. HIV, Hep B and Hep C infections were screened by using 4th generation ELISA kit. HIV screening was done by using Erba sure HIV Gen4 ELISA kit (Transasia, India) with reported sensitivity and specificity of 99.8% and 99.7% respectively. The screening of Hep B was done by Hepalisa ultra kit (J.Mitra, India) with reported sensitivity of 100% and specificity of 99.92%. The Hep C was screened by using Monolisa HCV

Ag-Ab ultra kit (Bio-Rad, France) with reported sensitivity and specificity of 100% and 99.83% respectively. Test for syphilis was done by Rapid plasma reagin (RPR) card test (Tulip, India) and the test for malaria was done by Advantage Mal card test (J. Mitra, India). Manufacturer's instructions were strictly followed for performing all these tests. Before labeling a test as seropositive, the samples were repeated in duplicate. The donated blood found positive for any TTI were discarded according to SOP of our blood bank.

Results

A total of 2401 units were collected during the period of three years. The majority of the donors were male (98.25%) with female donors constituting only 1.75% of total donation. Out of 2401 donors, 2187 (91.09%) were replacement donors (RD) and 214 (8.91%) were voluntary donors (VD). **(Table-1)** A total of 71 blood donors (2.95%) were tested positive for any one of the TTI. Out of these 71 cases, 69 were males and only 2 were females. Out of these 71 positive cases, 67 cases (3.06%) were positive in RD and only 4 cases were positive in VD (1.86%). The overall prevalence of HIV, HBV, HCV and syphilis in our study were 0.33%, 1.7%, 0.74% and 0.16% respectively. None of our donor was tested positive for malaria.**(Table-2)** Out of 71 positive cases, 41 cases were positive for HBV, 18 cases for HCV, 8 cases for HIV and 4 cases for syphilis. **(Table-3)** The age wise distribution of positive cases showed that majority of positive cases (63.28%) were seen in age group of 18-30 years, followed by 30.98% cases in age group of 31-40 years**.(Table-3)** Blood group distribution of positive cases showed that majority of positive cases (32 cases) belongs to blood group B, followed by 21 cases of blood group O. **(Table-4)**

Table 1: Sex distribution and Type of donors.

	Voluntary Donors	Replacement Donors	Total Donors
Male	192	2167	2359 (98.25%)
Females	22	20	42 (1.75%)
Total	**214 (8.91%)**	**2187 (91.09%)**	**2401 (100%)**

Table 2: Sex distribution of seropositive markers.

TTI	Male	Females	No. of cases
HIV	08	00	08 (0.33%)
HBsAg	40	01	41 (1.7%)
HCV	17	01	18 (0.74%)
Syphilis	04	00	04 (0.16%)
Malaria	00	00	00 (0.00%)
Total	**69**	**02**	**71 (2.95%)**

Table 3: Age wise distribution of transfusion transmitted infections.

Age (Years)	HIV	HBsAg	HCV	Syphilis	Malaria	Total
18-30	05	23	15	02	00	45 (63.38%)
31-40	02	16	02	02	00	22 (30.98%)
41-50	01	02	01	00	00	4 (5.63%)
51-60	00	00	00	00	00	00 (0.00%)
Total	**8 (11.26%)**	**41 (57.74%)**	**18 (25.35%)**	**4 (5.63%)**	**00 (0.00%)**	**71 (100%)**

Table 4: Blood group distribution of seropositive markers.

TTI	A	B	AB	O	Total
HIV	02	02	01	03	08
HBsAg	07	19	04	11	41
HCV	03	10	00	05	18
Syphilis	01	01	00	02	04
Malaria	00	00	00	00	00
Total	**13 (18.30%)**	**32 (45.07%)**	**05 (7.04%)**	**21 (29.57%)**	**71 (100%)**

Table-5: comparison of prevalence rate of TTI from various parts of India.

Author	Duration of study	Place	HIV (%)	HBV (%)	HCV (%)	Syphilis (%)	Overall TTIs (%)
Patel PA et al.[8]	2005-2011	Ahmadabad, Gujrat	0.08	0.30	0.09	0.06	0.53
Ahmed Z et al.[23]	2008-2011	Mangalore, Karnataka	0.1	0.5	0.08	0.07	0.82
Sastry JM et al.[12]	2008-2013	Pune, Maharashtra	0.28	1.23	0.41	0.008	1.56
Raut MM et al[3]	2005-2011	Akola, Maharashtra	0.53	1.6	0.14	0.03	2.30
Karmakar PR et al.[17]	2008-2011	Kolkata, WB	0.6	1.41	0.59	0.23	2.79
Our study	**2012-2015**	**Delhi**	**0.33**	**1.7**	**0.74**	**0.16**	**2.95**
Chandra T et al.[24]	2001-2007	Lucknow, UP	0.23	1.96	0.85	0.01	3.05
Pahuja S et al.[14]	2002-2005	Delhi	0.56	2.23	0.66	-	3.45
Kaur G et al.[21]	2001-2005	Chandigarh	0.6	1.7	0.8	0.7	3.8
Arora D et al.[1]	2002-2006	Hisar, Haryana	0.3	1.7	1.0	0.9	4.0
Kulkarni N[13]	2005-2009	Bellary, Karnataka	0.91	3.28	0.35	0.04	4.58
Gupta R et al.[18]	2003-2008	Delhi	0.35	1.66	0.65	2.8	5.46
Sinha SK et al.[20]	2007-2008	Kolkata, WB	0.64	2.27	1.62	1.31	5.8

Discussion

The main objective of blood transfusion services is not only to provide safe and adequate blood supply at all levels but also to eliminate or reduce the risk of TTI associated with BT, because BT is a significant route of transmission of these infections. HIV and hepatitis are two deadly infections which are transmitted by BT. It is reported that on an average one HIV positive transfusion leads to death after 2 years in children and after 3-5 years in adults.[1] With prevalence of 0.3%, an estimated 2.4 million people are living with HIV in our country.[10] Despite the availability of safe and effective vaccine against hepatitis B since 1982, India is still placed in the Intermediate zone of prevalence of Hepatitis B by WHO (prevalence rate of 2-7%) with an estimated 40 million HBsAg carriers.[11]

In our study out of 2401 blood donors, 98.25% were males and only 1.75% female donors. Our findings were comparable to the study done by various authors from different parts of India, who found more than 95% male donors in their study. These include Arora et al, Haryana (96.2%), Mangalore (97.5%) , Raut MM et al, Wardha (97.51%) , Patel PA et al, Ahmadabad (95.48%) , Pallavi P et al, Mysore (97.84%), Sastry JM et al, Pune (96.6%), Kulkarni N, Bellary (98%) and Pahuja S et al, Delhi (97.24%). [1,2,3,8,11-14] The less number of female donors in our study may partly be due to social and cultural habit in our country but mainly due to the fact that majority of female donors are declared unfit during pre donation screening because of very high incidence of anemia during child bearing age in India. [2]

The majority of our donors were RD (91.09%) as compared to VD (8.91%). The predominance of RD was also reported by Pahuja S et al. (99.48%), Kakkar N et al (94.7%) and Singh B et al (82.4%). [14-16] Contrary to our study, the majority of VD was reported by Raut MM et al (87.13%), Patel PA et al. (95.58%) and Karmakar PR et al (100%). [3,8,17] In this study out of 2401 blood donations, 71 blood was found to be positive for any one TTI. Thus the overall prevalence of TTI in our study was 2.95%. This is much less than that reported by Arora D et al, Haryana (4%), Pahuja S et al, Delhi (3.45%), Kulkarni N, Bellary (4.58%),Gupta R et al, Delhi (5.46%), Singh B et al, Delhi (5.61%), Sinha SK et al, Burdwan, WB (5.8%) and Kaur G et al, Chandigarh (3.8%), [1,14,15,18-21] but overall prevalence of TTI in our study was higher than that reported by Fernandez H et al, Mangalore (0.6%) Raut MM et al , wardha (2.30%), Patel PA et al, Ahmadabad (0.53%), Pallavi P et al, Mysore (2.22%), Fernandez H et al, Sastry JM et al, Pune (1.56%), Karmakar PR et al, Kolkata (2.79%) and Adhikari L et al, Sikkim (1.63%). [2,3,8,11,12,17,22] This may be because majority of donors in their study were VD.

Out of 71 positive cases, HIV was positive in 8 cases, HBsAg was positive in 41 cases, HCV in 18 cases and syphilis in 4 cases. Thus the overall prevalence of HIV, HBsAg, HCV and syphilis in our study was 0.33%, 1.74%, 0.74% and 0.16% respectively. None of our case was positive for malaria parasite. The prevalence of HIV was 0.33%, which was similar to the prevalence of HIV (0.3%) in our country. [10] The prevalence of HIV in this study was much less than that reported by Pallavi P et al (0.44%), Pahuja S et al (0.56%), Kulkarni N (0.9%), Singh B et al

(0.54%), Karmakar PA et al (0.6%), Singh B et al (0.8%), Sinha SK et al (0.64%) and Kaur G et al (0.6%), [11,14-17,19-21] but it was much higher than Fernandes H et al (0.06%), Patel PA et al (0.08%), Ahmed Z et al (0.1%) and Chandra T et al (0.23%). [2,8,23,24] The prevalence of HIV close to 0.33% were reported by Arora D et al (0.3%), Sastry JM et al (0.28%), Gupta R et al (0.35%)and Adhikari L et al (0.32%). [1,12,18,22] The prevalence rate of HBsAg in present study was 1.7%, which was less than the lower limit of prevalence rate of 2-7% as laid down by WHO for India. Our finding was less than that reported by Kulkarni N (3.28%), Pahuja S et al (2.23%) and Sinha SK et al (2.27%), [13.14,20] but it was more than that reported by Fernandes H et al (0.34%), Patel PA et al (0.30%), Sastry JM et al (0.28%) and Adhikari L et al (0.78%). [2,8,12,22] The prevalence rate of HBsAg close to our finding of 1.7% was also reported by Arora D et al (1.7%), Raut MM et al (1.6%), Gupta R et al (1.68%), Singh B et al (1.8%), Kaur G et al (1.7%) and Chandra T et al (1.96%).[1,3,18,19,21,24] The 0.74% prevalence of HCV in our study was comparable to study by Pahuja S et al (0.66%), Gupta R et al (0.65%), Kaur G et al (0.8%) and Chandra T et al (0.85%).[14,18,21,24] Arora D et al (1%) and Sinha SK et al (1.62%) reported higher prevalence of HCV as compared to our study, [1,20] while Fernandes H et al(0.06%), Patel PA et al (0.09%), Pallavi P et al (0.23%), Sastry JM et al (0.41%),Kulkarni N (0.35%)and Adhikari L et al (0.27%) reported lower prevalence of HCV than our study. [2,8,11-13,22] The overall prevalence of syphilis in our study was 0.16%, which was comparable to study done by Fernandes H et al (0.11%), Pallavi P et al (0.28%), KarmakarPR et al (0.23%) and Adhikari L et al (0.27%). [2,11,17,22] Arora D et al (0.9%) , Singh B et al (2.6%), Gupta R et al (2.8%), Sinha SK et al (1.3%) and Kaur G et al (0.7%) reported higher prevalence of syphilis than our study, [1,16,18,20,21] while Raut MM et al (0.03%), Patel PA et al (0.06%), Sastry JM et al (0.008%), Kulkarni N (0.04%), Chandra T et al (0.01%) and Khageshan AP et al (0.04%) reported lesser prevalence of syphilis as compared to our study. [3,8,12,13,24,25] None of our donors were found positive for malaria parasite, which was comparable to the study done by Patel PA et al, Pallavi P et al, Sastry JM et al, Kulkarni N, and Ahmed Z et al. [8,11,12,21,23] This may be due to the presence of typical fever with rigor in patients of malaria, therefore these patients were usually eliminated during pre donation screening. Fernandes H reported one positive case of malaria in their study.[2]

Out of 71 positive cases of TTI, 67 cases were found in RD (3.06%, 67/2187), while only 4 cases were positive in VD (1.86%, 4/214). Similar finding of high prevalence of TTI in RD was also reported by Arora D et al, Pahuja S et al and Singh B et al .[1,14,16] RD are usually one time donor who donate blood in emergency for their family members, close relatives or friends, thus because of compulsion to donate blood in these situations, information regarding past illnesses or high risk behavior may be concealed by the donors.[17] In our country due to lack of awareness and motivation, RD still constitute the largest group of blood donors.[11] VD donates blood at regular intervals and they are usually young motivated college students and employee of various institutions. Thus in order to reduce the risk of TTI, there is need to promote VD by proper education, motivation and providing accurate information about the advantages of blood donation by conducting regular blood donation camps. .[11,13] Age wise distribution of positive cases in this study showed that 63.38% of positive cases fall in the age group between 18-30 years, which was similar to the study conducted by Arora D et al (69.95% between age group 18-31 years), Patel PA et al (42.79% between age group18-30 years) and Ahmed Z et al (59.55 between age group 18-35%).[1,8,23] The younger age group (18-40 years) is most sexually active group of the community, therefore they are more prone for developing TTIs, however this is of much concern as well because this age group is also the most productive and economically viable group of the society.[1,8,23] Blood group profile of positive cases showed that 32 cases (45.07%) were of blood group 'B', followed by 21 cases (29.57%) of blood group 'O', 13 cases (18.3%) of blood group 'A' and 5 cases of blood group 'AB'. This may be explained by the fact that the most common blood group of the donors in our study was blood group B followed by blood group O. When we compare our study with previous studies from Delhi and adjoining states, there is definite decrease in overall prevalence of TTIs over last one and half decade, with decrease in prevalence of HIV and syphilis. The prevalence of hepatitis is almost similar as compared to previous studies.[1,14,16,18,19]

The increasing seropositivity of TTIs among RD is of major concern for BTS. By practicing the deferral of high risk donors by donor self exclusion and use of nucleic acid amplification test (NAT) for screening the blood, the developed countries more or less achieve the decreased risk of TTI. .[11,21] However in underdeveloped countries like ours, because of inability to use NAT for blood screening due to its high cost and lack of awareness about voluntary blood donation, TTI is still a major concern for BTS. Thus in order to minimize the risk of TTI, VD should be encouraged by means of educating general people

about benefits of blood donation and motivating them by conducting regular blood camps along with use of NAT for blood screening, which can detect very low levels of viral DNA or RNA in the donated blood.

Conclusion

During three years period, 2401 donors were tested for various TTIs, comprising of 98.25% male donors and 1.75% female donors. Most of the donors were RD (91.09%) with only 8.91% VD. The seropositivity was highest for HBsAg (1.7%), followed by HCV (0.74%), HIV (0.33%) and syphilis (0.16%). None of our donors were tested positive for MP. Out of 71 positive cases, 67 cases were seen on RD with only 4 positive cases among VD. One of positive outcome of our study was definite decrease in prevalence of TTI in Delhi over the past one and half decade. The TTI can be minimized by encouraging voluntary non-renumerated blood donors by proper education, motivation and counseling of general population along with judicious use of blood only in cases of absolute emergency and use of advanced technique like NAT for pre transfusion screening of blood.

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