Original Article:

A study on patterns of co-infections among blood donors at the blood bank of a tertiary care referral teaching hospital in South India

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ABSTRACT

Background: Blood serves as a vehicle for transmission of blood-borne pathogens including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), malaria parasite (MP) and syphilis. Safe blood and blood products should be transfused to all patients in need for blood transfusion.

Material and Methods: All blood donors attending to the blood bank during the period January 2009 to December 2014 were screened for hepatitis B surface antigen (HBsAg), anti HCV antibody, anti HIV-1, 2 antibodies and HIV p24 antigen by using the appropriate enzyme-linked immunosorbent assay (ELISA) method and further confirmed using an ELISA kit from a different manufacturer. Malarial antigen testing was done by rapid diagnostic device, which is based on immunochromatographic technique. The rapid plasma reagin (RPR) test was used for estimation of syphilis infection and further confirmed by Treponema pallidum haemagglutination assay (TPHA).

Results: Of the 41,785 donors who were screened during the study period, 20 (0.05%) were reactive for different combination of infections. The various combination of infections seen were as follows; HBV+HCV and HBV+HIV (6/20) each, HIV+HCV (3/20), HIV + syphilis (2/20) and HBV+HIV+HCV, HBV+MP, HBV+syphilis (1/20 each); and HIV+Syphilis constituted for 10% (2/20).

Conclusion: A properly conducted donor screening, notification and counseling of permanently deferred donors will help in reducing these co-infection rates.

Key words: *Blood donors, Co-infections, Transfusion transmissible infections*

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INTRODUCTION

Timely transfusion of blood saves millions of lives, but unsafe transfusion practices lead millions of people to risk of transfusion transmissible infections (TTIs).[1] Morbidity and mortality resulting from the transfusion of infected blood have far-reaching consequences, not only for the recipients themselves, but also for their families, their communities and the wider society.[2,3] Only continuous improvement and implementation of proper donor selection, sensitive screening tests, and effective inactivation procedures can ensure the elimination, or at least reduction, of the risk of acquiring TTIs.[4] TTIs can exist as asymptomatic disease in the hosts, so donors must be screened for high-risk behaviour related diseases. Evaluation of data on the prevalence of TTIs namely human immune deficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), malaria parasite (MP) and syphilis among blood and blood component donors permits an assessment of the occurrence of infections in the blood donor population and consequently the safety of the collected donations.

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HIV, HBV and HCV co-infection has emerged as a leading cause of morbidity throughout the world in the last two decades.[5,6] Because of the significant burden and clinical impact of HBV in HIV-infected individuals, understanding the epidemiologic characteristics of HBV infection in HIV infected populations is crucial. The prevalence of HBV infection among HIV-infected persons varies markedly, from 5% -30% in different regions of the world.[7]

Knowledge about burden of these diseases among healthy blood donors can provide useful information on the behaviour pattern of the general population in our region. The present study was undertaken to know the co-infection rate of HIV, HBV, HCV, MP and syphilis among blood donors of our blood bank.

MATERIAL AND METHODS

A retrospective study was conducted over the period January 2009 to December 2014 at the department of Transfusion Medicine of our tertiary care referral teaching hospital in Tirupati, Andhra Pradesh. All voluntary or replacement blood donors who were eligible to donate blood and blood components as per the Drugs & Cosmetics act,1940 and rules,1945[8] who donated their blood at our blood bank during the study period were included in the study. Voluntary donations were obtained either at the blood bank or at voluntary blood donation camps. Replacement donors were either relatives or friends of patients. Data retrieved from blood bank records included the demographic characteristics of donors such as age, gender, residence, and the serological results of HIV, HBV, HCV, MP and syphilis.

Sample collection and laboratory testing

Five mL of peripheral venous blood samples in acid citrate dextrose (ACD) and 5 mL of plain blood samples were collected from the donors after obtaining written informed consent and phlebotomy. ACD sample was used for testing for MP and plain samples were centrifuged, sera separated and analyzed for different TTIs such as HIV, HBV, HCV and syphilis as per the standard operating procedures followed in the blood bank.[9] Samples were analyzed for HIV p24 antigen and antibodies to HIV-1 and 2 (Microlisa HIV Ag & Ab, J.Mitra & Co. Pvt. Ltd, New Delhi, India), HBsAg (Hepalisa, J.Mitra & Co. Pvt. Ltd, New Delhi, India), and anti HCV antibodies (Microlisa HCV Ab, J.Mitra & Co. Pvt. Ltd, New Delhi, India), by enzyme linked immunosorbent assay (ELISA) method strictly following the manufacturer's instructions. Any serum found reactive by the first assay was retested using a second assay by different manufacturer's ELISA kits for HIV, HCV and HBV (Erba Sure HIV gen4 for HIV p24, anti HIV-1 and 2, Erbalisa Hepatitis C for HCV Ab and Erba lisa Hepatitis B for HBsAg, Transasia bio-medicals LTD, Daman, India). Testing for syphilis was done by rapid plasma regain (RPR) method (RPR TEST, Span Diagnostics Ltd, Gujarat, India). The donors who were reactive for RPR were further confirmed by TPHA (Plasmatec TPHA, Plasmatec, Bridport, Dorset, DT6 5BU, UK). Malarial antigen testing was done by rapid diagnostic device, which is a pan malaria test based on detection of malaria parasite-specific lactate dehydrogenase (pLDH) (Pan Malaria Card, J.Mitra & Co. Pvt. Ltd, New Delhi, India) as per the manufacturer's instructions. The validity of the test is assured as per the given criterion and the results were computed.

Statistical analysis

Data were analyzed using Chi-square test for trend to compare infection rates in consecutive 5 years. Statistical analysis was carried out using SPSS version 16 (SPSS Inc,Chicago,USA). A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 41,785 donors were screened during the 5 year period from January 2009 to

December 2014. Of these 40576 (97.1%) were males (Table1). Among 41785 donors, 25992 (62%) were voluntary and 15793 (38%) were replacement donors. Out of all donors screened 20 (0.05%), all men, were reactive for different combinations of infections (Table 2). Most of the donors were in the age group of 20-30 years and the fewest number were found in above 40 years age group (Table 3). There was no statistically significant difference between the age groups (p=0.750). Among the donors reactive for different combinations of co-infections 12 (60%) were voluntary and 6 (20%) were replacement donors (Table 4). The distribution of voluntary and replacement blood donors was similar (p=0.411). Out of 20 reactive blood donors, 16 (80%) belonged to rural areas while 4 (20%) were from urban population. Even though a higher prevalence of co-infections were observed in rural population, there was no statistically significant difference between the two groups (p=0.374) A comparison of data of the present study with other published studies is depicted in Table 5.[10-16]

DISCUSSION

Blood components, like all other medications in India, are regulated by the Drugs and Cosmetics act and rules.[8] The act requires manufacturers of medication to verify the suitability of every raw material. For biologic pharmaceuticals, the donor is the key ingredient whose suitability must be scrutinized. The first step in the assessment of blood safety of any blood transfusion service is the evaluation of the seroreactivity of the donated blood for TTIs. Screening for HIV, HBV, HCV, MP and syphilis are the mandatory tests that are carried out routinely. Although blood transfusion is a life-saving measure, it still has the risks resulting from the transfusion of infected blood. It is difficult to know when a particular donor is in the window period. The whole blood or components should not be issued for transfusion, till the mandatory tests are completed and reported as non-reactive. One of the most important aspects of laboratory practice is the safe disposal of waste. After segregating and pasting biohazard label on all blood units reactive for TTIs, their respective components and pilot tubes, they were subjected for autoclaving at a temperature of 121 °C for 30 minutes at 15 Pound-force per square inch (PSI). After autoclaving all units were disposed off as per hospital biomedical waste management.[17]

Table 1: Gender-wise distribution of donors

Type of donor	No.	%
Male	40576	97.1
Females	1209	2.9
Total	41785	100

Table 2: Burden of co-infections among blood donors

Co-infection	No. (%)
HBV+HCV	6 (30)
HBV+HIV	6 (30)
HBV+HIV+HCV	1 (5)
HIV+HCV	3 (15)
HBV+MP	1 (5)
HBV+syphilis	1 (5)
HIV+syphilis	2 (10)
Total	20 (100)

HBV=hepatitis B virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MP=malaria parasite

With every unit of blood, there is 1% chance of a transfusion associated problem including TTIs.[18] Among the HIV infected individuals, HBV and HCV co-infections are more prevalent due to overlapping transmission routes.[19] They share similar routes of transmission, namely through blood and blood products, sharing of needles to inject drugs, and sexual activity resulting in co-infection with the two viruses as a common event.[21] Studies[21-23] have reported that co-infection with HBV was a well documented cause of liver-related complications in individuals with HIV infection

Table 3: Age distribution of donors with co-infections

Age (years)	Co-infection							
	HBV+ HCV	HBV+ MP	HBV+ syphilis	HIV+ HBV	HIV+HBC +HCV	HIV +HCV	HIV+ syphilis	Total
20-30	4	1	1	3	1	1	1	12
31-40	2	0	0	3	0	2	0	7
41-50	0	0	0	0	0	0	1	1
Total	6	1	1	6	1	3	2	20

HBV=hepatitis B virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MP=malaria parasite

$\chi^2 = 13.26$, p=0.750

Table 4: Donor category-wise distribution of co-infections

Co-infection	Type of donor		
	Voluntary	Replacement	Total (%)
HBV+HCV	2	4	6 (30)
HBV+MP	0	1	1 (5)
HBV+syphilis	1	0	1 (5)
HIV+HBV	5	1	6 (30)
HIV+HBV+HCV	1	0	1 (5)
HIV+HCV	2	1	3 (15)
HIV+syphilis	1	1	2 (10)
Total	12	8	20 (100)

HBV=hepatitis B virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MP=malaria parasite

$\chi^2 = 4.63$, p=0.411

and is associated with an increased risk of mortality.

Seroprevalence of co-infections in our study was 0.05%. This is figure higher compared to the observation from another study,[16] where seroprevalence of co-infections was observed to be 0.01% among all the blood donors. In this study,[16] maximum seropositivity was observed in the age group of 30-40 years, whereas in our study maximum seropositivity was observed in 20-30 years age group. One of the studies,[11] reported HIV and HCV co-infection in the age group 21-50 years; HBV and HCV co-infection in the age group 31-40 years and syphilis and HCV in the age group 21-30 years. In our study we observed co-infection of HIV and HCV as well as HBV and HIV in the age group 20-40 years, and no HCV reactive donor tested positive for syphilis (Table 3).

In the present study, of the 20 co-infected blood donors, 12 were voluntary, seroreactive for 2 or more infections, and there was no statistically significant difference in their distribution (p=0.411). This is in contrast with some studies,[10,15] where a higher proportion of replacement donors were observed. This high prevalence in voluntary donors might be due to broadened National AIDS Control Organization (NACO) definition (2009) of voluntary donors that includes family replacement donors also under voluntary donor category.[24]

Among HIV co-infected donors, one donor tested seropositive for for HBV, HCV, syphilis each and one tested positive for both HBV and HCV. In a study,[16] only one HIV seroreactive donor was found to have associated HCV infection.

Table 5: Comparison of burden of co-infection in blood donors in other published studies with present study

Study	Place and year of the study	No. of donors co-infected (n/N):(%)	HBV+ HCV No. (%)	HBV+ HIV No. (%)	HBV+ HIV+ HCV No. (%)	HIV+ HCV No. (%)	HBV+ MP No. (%)	HBV+ SYP No. (%)	HIV+ SYP No. (%)	HIV+ SYP+ HCV No. (%)	HCV+ HBV+ SYP No. (%)	HCV+ SYP No. (%)	HIV+ HBV+ SYP No. (%)
Kaur et al[18]	Chandigarh, India, 2001-2005	23/42439 (0.05)	2	5	-	2	-	5	4	-	-	4	1
Ymele et al[11]	Cameroon, Central Africa, Jan-Jun, 2008	(1.1) 51/4644	10	36	2	3	-	-	-	-	-	-	
Shrestha et al[17*]	Kathmandu, Nepal, Mar-Sep, 2008	(5.8) 8/139	1	-	-	5 (5)	-	-	-	-	-	2	
Aenan et al[37†]	Benu, Nigeria, Jul-Dec, 2009	(40.7) (137/337)	-	-	-	-	40.7 (137)	-	-	-	-	-	-
Kaur et al[34]	Amritsar, Punjab, India, 2001-2011	(0.04) 21/56915	14.3 (3)	0	0	19 (4)	0	33.4 (7)	9.5 (2)	0	4.8 (1)	19 (4)	-
Chavan et al[35]	Maharashtra, India, 2004-2013	(0.02) 9/38,237	33.3	-	-	55.6	-	-	-	11.1	-	-	-
Dogra et al[36]	Jammu, India 2010-2011	0.01 (6/50446)	2	-	-	1	-	1	-	-	-	2	-
Present study	Tirupati, Andhra Pradesh, India 2009-2014	(0.05) 20/41785	6	6	1	3	1	1	2	-	-	-	-

* co-infection rate of HIV, HBV and syphilis among HCV seropositive identified blood donors

† prevalence of Plasmodia and hepatitis B virus co-infection in potential blood donors

n=number positive; N=number tested; HBV=hepatitis B virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; MP=malaria parasite; SYP=syphilis

Among the five HBV co-infected donors, one donor tested positive for HIV, HCV, MP and syphilis, one was reactive for both HIV and HCV. In another study,[25] the prevalence of HBV co-infection in HIV infected blood donors was (42/417) (10.1%).[25] Occult HBV infection may originate from recovered infections with persistent low level viral replication, from escape mutants blocking export of antigen, or from a reduced HBV replication after co-infection with HCV infection.[26,27]

Among the three HCV co-infected donors, there was one seroreactive case for each HBV, HIV and one tested positive for HBV and HIV. In a study,[11] among 139 HCV seroreactive cases, 8 had co-infections; the total co-infection rate was 5.73%. Co-infection rate of HIV among HCV was 3.6%, HBV among HCV was 0.71% and syphilis among HCV was 1.4%. None of the HCV seropositive samples were positive to more than one infection.[11]

There are reports indicating the association between HIV and malaria in most endemic regions of malaria.[28,29] But in our study we did not find any of the donors co-infected with HIV and MP. In a study[13] from Nigeria, (n=337) a high (40.7%) occurrence of infection with MP and HBV was observed. The authors attributed this to the fact that malaria is already a looming endemic problem in Nigeria, including Benue State. We also observed one donor seroreactive for both MP and HBV and this could reflect high endemicity of HBV in our region.

Serologic screening for syphilis has been justified in part as a surrogate marker for infections caused by other pathogens such as HIV. There were significantly higher frequencies of HIV, HCV, HBsAg, and human T-lymphotrophic virus (HTLV) confirmed-positive donations among those with positive syphilis test results, although the sensitivity of syphilis test positivity in these groups was low. More than 3 million repeat donors were reactive for both syphilis and HIV (anti-HIV and HIV RNA). Among these, 225 donors were seroconverted for syphilis but not for anti-HIV or HIV RNA and 83 donors for HIV (anti-HIV or HIV RNA) but not for syphilis and only 1 converted for both syphilis and HIV, concluding syphilis reactivity may exclude HIV co-infection in high risk donors.[30] In our study we observed 2 donors reactive for both RPR and HIV.

Many factors will influence the co-infections which include similar routes of transmission and epidemiological similarities, risk factors associated with other sexually transmitted diseases such as syphilis. Compared to individuals infected with a single pathogen, co-infected individuals will have more risk towards morbidity and mortality of the disease. A study[31] from India showed that one third of HIV deaths are associated with HCV either directly or indirectly.[31] So the occurrence of these infections should be monitored carefully to ensure safer and more reliable blood transfusion practices. A properly conducted donor screening, notification and counseling of permanently deferred donors will help in reducing these co-infections rates.

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