

Research Article

A Study on Serosurveillance of Blood Donors

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Abstract: This study was undertaken to find out the seroprevalence of transfusion transmissible infections among voluntary and replacement blood donors over a period of two years. A total number of 16,872 donor's blood units were screened using standard blood tests for transfusion transmissible infections. Replacement donors constituted 91.6% and remaining 8.4% were voluntary donors. The seroprevalence of HIV and HBsAg was 0.17% and 1.58% respectively in total donors. The seroprevalence of HCV and syphilis was 0.09% and 0.01% respectively. No donors were found positive for malaria parasites. Our study showed that the seroprevalence of transfusion transmissible infections (TTI) was more in replacement donors compared to voluntary donors, which stress upon the need for implementing programs to achieve 100% voluntary blood donation. With the implementation of strict donor selection criteria, use of sensitive screening tests, and establishment of strict guidelines for blood transfusion, it may be possible to reduce the incidence of TTI in Indian setup.

Keywords: voluntary, transfusion, donors, infection

INTRODUCTION

Blood transfusion involves transfer of biological material from man to man. Many infectious diseases are likely to be transmitted by blood transfusion. Preventing transmission of these infectious diseases through blood transfusion presents one of the greatest challenges of transfusion medicine [1]. This study is undertaken to find out the seroprevalence of transfusion transmissible infections among voluntary and replacement blood donors. The evaluation of the data of the prevalence of the transfusion transmitted infections (TTIS) like Human immunodeficiency virus (HIV), Hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and malaria, among blood donors permits an assessment of the acquisition of the infections in the blood donor population and consequently the safety of the collected donations. It also gives an idea for the epidemiology of these infections in the community [2]. Voluntary non remunerated blood donation is the source of the safest blood supply to the transfusion service. In the Indian setup where voluntary donations are fewer and poorly structured, safety of blood could still be compromised [3].

METHODOLOGY

The present study was carried out in Blood bank of J.J.M Medical College, Davangere from July 2009 to June 2011. The blood bank of J.J.M. Medical College is a licensed blood bank with average annual collection of 8000 units of blood from healthy blood donors from in and around Davangere. The blood units were collected from voluntary and replacement donors. A voluntary donor is one who donates voluntarily and is not paid for it. A replacement donor is non-remunerated donor who donates blood for a particular patient admitted in hospital. The donors were selected by detailed history and physical examination according to the criteria laid down in the standard operating procedure of our blood bank. The selected donors were subjected to phlebotomy. During the study, blood units were screened for HIV, HBsAg, HCV, syphilis and malaria.

Sample collection for screening of TTIS: Two ml of blood samples were collected in labelled pilot tubes (two ml each in plane and EDTA tubes) from tubing of the blood bag at the time of collection of blood from donor. The sample from plane tube was further centrifuged at 3500 rpm for 5 minutes to obtain clear non hemolysed serum. These samples

were tested for HIV, HBsAg, HCV, syphilis. Malaria was screened by thick and thin blood smears examination using EDTA blood.

Inclusion criteria: Healthy voluntary and replacement donors.

Exclusion criteria: Blood donors who are unfit to donate blood according to standard blood donor's criteria.

1) Screening test for HIV by ELISA: SD HIV 1/2 ELISA – 3.0 third generation indirect ELISA kit (Biostandard diagnostic Pvt. Ltd. India) was used. Test Procedure was followed as given in the user manual. The enzyme substrate reaction is read by ELISA reader for absorbance at the wavelength of 450nm.

Interpretation of results:

a) Test validation: The individual values of the absorbance for the control sera are used to calculate the mean value.

If $0.010 \leq \text{absorbance (Neg)} \leq 0.200$

$0.010 \leq \text{absorbance (Pos)} \leq 1.000$

If these specifications are not met the test to be repeated.

b) Evaluation: Calculate the mean absorbance of the negative controls, then calculate the cut off value by adding 0.300.

Cut off value = mean absorbance (neg) + 0.300

Test results:

-Absorbance (sample) < cut off value = negative

-Absorbance (sample) > cut off value = positive

Test specimen with absorbance value within 10% below the cutoff should be considered suspect and were repeated in duplicate.

2) Screening test for hepatitis B virus (HBsAg) by ELISA :SD HBsAg ELISA 3.0 – Enzyme immunoassay for detecting HBsAg (Biostandard diagnostics Pvt. Ltd, India).

Tests were performed by following the procedure as given in the user manual of the reagent kit. Colorimetric reading was performed by using a spectrophotometer at 450nm. SD HBsAg ELISA 3.0 is double sandwich ELISA for the qualitative detection of HBsAg with high degree of sensitivity and specificity.

Interpretation of results:

A) Test validation

The individual values of the absorbance for the control sera are used to calculate the mean value if

- $0.005 \leq A \text{ (neg)} \leq 0.200$

$A \text{ (pos)} \geq 1.000$

Both absorbance values of the positive Controls must comply with specification. If these specifications are not met, the test is to be repeated.

b) Evaluation: Calculate the mean absorbance of the negative controls then calculate the cut- off value by adding 0.050

$A \text{ (neg)} + 0.050 = \text{cut off value}$

Test results

$A \text{ (sample)} \leq \text{cut off: HBs Ag negative}$

$A \text{ (sample)} \geq \text{cut off value: HBsAg positive.}$

Samples with a test result which is equal to or greater than the cut-off value should first be tested in duplicate. If in the retest the mean absorbance is again equal or greater than the cut-off, such samples were verified using a confirmatory test.

3) Screening test for Hepatitis C Virus. ELISA: SD HCV ELISA 3.0 is an indirect sandwich Elisa for the qualitative detection of antibodies against HCV.

Tests were performed by following the procedure as given in the user manual of the reagent kit. Colorimeter reading was performed by using a spectrophotometer at 450nm.

Interpretation of results:

a) Test validation

The individual values of the absorbance for the control sera are used to calculate the mean value if

- $0.010 \leq A \text{ (neg)} \leq 0.200$

$A \text{ (pos)} \geq 1.000$

Both absorbance values of the positive control must comply with the specification.

b) Evaluation: Calculate the mean absorbance of the negative controls then calculate the cutoff value by adding 0.400

$A \text{ (neg)} + 0.400 = \text{cut off value.}$

Based on the criteria of the test, the samples are classified as follows :

Test results:

-A (sample) = < cutoff = anti HCV neg.

-A (sample) >= cutoff = anti HCV positive.

4). Screening test for syphilis

This is performed using Rapid Plasma Reagin test kit (BEACON diagnostic Pvt. Ltd). RPR antigen suspension is a carbon containing cardiolipin antigen, which detects “reagin” antibody present in serum of persons with syphilis. When a specimen contains antibody, flocculation occurs due to coagulation of the carbon particles of the RPR antigen which appear as black clumps against the white background of the card.

Test procedure as per manufacturer’s instructions was followed.

Interpretation of result:

Positive result: Black aggregates (carbon) which may be deposited at the periphery. The liquid appearing before 4 minutes of rotation. Read the results under strong source light with a hand lens. Test results showing slight but definite clumping is reported as reactive or positive.

Negative result: Complete absence of black aggregates with uniform grayish background at the end of 4th minute rotation

Screening test for malaria: Both thick and thin smears were stained with Leishman stain and subjected to microscopic examination. After screening the smear on 40X object for protozoan, 100X oil immersion objective is used to detect plasmodium species. At least 100 oil immersion fields of thick film and 200 oil immersion fields of thin film using 100 x objectives are examined before labeling the blood unit as negative for parasite.

Statistical analysis:

Prevalence of each transfusion transmissible infection was compared between voluntary and replacement donors by statistical analysis. Z test for proportion was used to compare between two donor categories.

RESULTS

During the study total 16,872 donors blood units were screened for HIV, HBsAg, HCV, Syphilis and Malaria. The donor age ranged from 18-60 yrs, majority (76.2%) in the age group of 18-35 yrs. Out of the 16,872 blood donors, 15,456 were replacement donors and remaining 1,416 were voluntary donors [Table 1].

Table 1: Showing Type of blood donors

Type of donor	No. of screened blood	Percentage
Voluntary	01,416	8.4%
Replacement	15,456	91.6%
Total	16,872	100%

Out of the total 16,872 donors, males constituted 16,451 and only 471 donors were females [Table 2].

Table 2: Sex wise distribution of blood donors

Sex	No of screened blood	Percentage
Male	16,451	97.5%
Female	0471	2.5%
Total	16,872	100%

Out of the total 16872 screened blood units 312 units were seropositive for transfusion transmissible infections (TTI), giving prevalence rate of 1.85%. Out of this 305 were replacement donors and remaining 7 were voluntary donors.

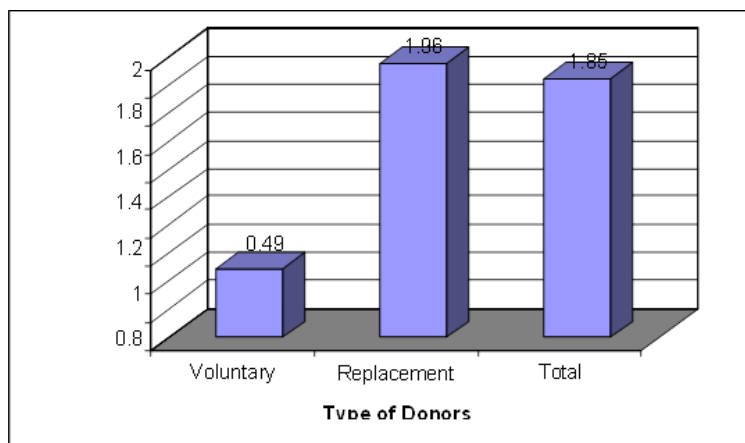


Fig. 1: Seropositivity in different types of donors / total donors

Table 3: Seroprevalence of TTI

Donors	No.	HIV n (%)	HbsAg n (%)	HCV n (%)	Syphilis n (%)
Voluntary	1416	00 (0%)	07 (0.49%)	00 (0%)	00 (0%)
Replacement	15456	28 (0.18)	260 (1.68)	15 (0.10%)	02 (0.02%)
Total	16872	28 (0.17)	267 (1.58%)	15 (0.09%)	02 (0.01%)

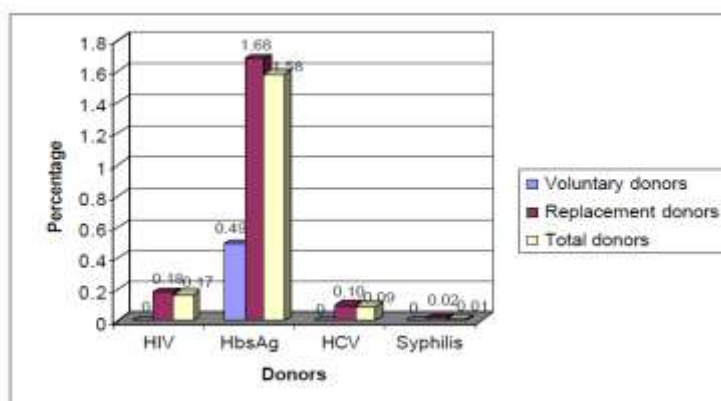


Fig. 2: Seroprevalence of TTI

The overall seroprevalence for HIV, HBsAg, HCV and syphilis was 0.17, 1.58, 0.09 and 0.01 respectively. No blood donors were positive for malaria parasites.

Table 4: Seroprevalence of HIV in different donor category

Donor category	No of screened blood units	No. of seropositive units	Percentage
Voluntary	1,416	00	00
Replacement	15,456	28	0.18%
Total	16,872	28	0.17%

(Z = 0.09, p = 0.92)

Out of total 16,872 blood units screened 28 (0.17%) units were seropositive for HIV and all of the seropositive were replacement donors. The percentage seropositivity in replacement donors is 0.18% [Table 4].

Out of the total 28 seropositive HIV donors majority (20) were in the age group 18 to 35 years cases. All of the seropositive HIV donors were males. Out of the total 28 seropositive HIV donors 18 were married and 10 were unmarried.

Table 5: Seroprevalence of HBsAg in different donor categories.

Donor category	No of screened blood units	No. of seropositive units	Percentage
Voluntary	01,416	07	0.49%
Replacement	15,456	260	1.68%
Total	16,872	267	1.58%

(Z=0.46, p=0.65)

Out of the total 267 HBsAg positive donors 7 were voluntary and 260 were replacement donors [Table 5], majority (223) were in the age group of 18-35 years. Out of the total 267 seropositive donors for

HBsAg, 264 were males and 3 were female donors. Out of the total 267, HBsAg positive donors 192 were married and remaining 75 were unmarried.

Table 6: Seroprevalence of HCV in different donor categories.

Donor category	No of screened blood units	No. of seropositive units	Percentage
Voluntary	01,416	00	00%
Replacement	15,456	15	0.10%
Total	16,872	15	0.09%

(z=0.17, p= 0.87)

Out of the total 16,872 screened blood units 15 (0.09%) were seropositive for HCV [Table 6]. All the seropositive units were from replacement donors. None of the blood units from voluntary donors were positive for HCV. The seroprevalence in replacement donors was 0.10%. Out of the 15 HCV positive donors 12 (80%) were married and 3(20%) were unmarried. Out of 15 donors positive for HCV, nine were from urban area and remaining six were from rural area.

Table 7: Seroprevalence of Syphilis in different donor categories.

Donor category	No of screened blood units	No. of seropositive units	Percentage
Voluntary	01,416	00	00%
Replacement	15,456	02	0.02%
Total	16,872	02	0.01%

(z = 0.07, p = 0.84%)

Among the total 16,872 donors only 2 donors were positive for syphilis with a seroprevalence of 0.01%. Out of total 16,872 donors screened, 2 (0.01%) [Table 7] units were seropositive for rapid plasma

reagin test for syphilis. Two seropositive units were from replacement donors. None of the blood units from voluntary donors show seropositivity to RPR test for syphilis. However the difference was statistically not significant. Both the positive donors were males and one was married and other was unmarried. One positive donor for syphilis was from urban and other one from rural area.

Of the total 16,872 screened blood donors, none of the blood units were positive for malaria parasites. Out of 16,872 total screened blood units, there was no co- infection of HIV with HBsAg, HCV or syphilis. There was no co- infection of HBsAg with HCV either.

DISCUSSION

The risk of transfusion transmissible infections (TTI) has declined dramatically in developed nations over the past two decades, primarily because of extraordinary success in preventing HIV and other established transfusion transmitted viruses from entering the blood supply. But same may not hold good for the developing countries. The National Policy for Blood Transfusion Services in our country is of recent origin and the transfusion services are hospital based and fragmented [4].

In the present study replacement donors, constituting 91.6% and only 8.4% were voluntary

donors. This is comparable to study done by Kakkar *et al* (94.7%) [3], Srikrishna *et al* (98.5%) [1] and Singh *et al* (84.5%) [5].

In contrast predominance of voluntary donors was noted by Bhattacharya *et al* (94.6%) [6] and Pallavi *et al* (64.78%) [7]. It is shown that replacement donors constitute the largest group of blood donors in India reflecting lack of awareness among the general population, the presence of misconceptions and fears associated with donating blood, the lack of health education and the indifference attitude of the health sector.

Seroprevalence of HIV

The sexual contact is a major mode of HIV transmission; however blood donation is also important mode of infection [8]. In India, according to the latest estimates the National adult HIV prevalence is 0.34% in general population and in blood donors 0.28% [9]. In the various Indian studies, the seroprevalence of HIV among blood donors varies from 0.16% to 0.8% [Table 8]. In our study, the seroprevalence for HIV was 0.17% in total donors. The seroprevalence in replacement donors was 0.18%. No voluntary donors were positive for HIV. However, the difference in seroprevalence among voluntary and replacement donors was statistically not significant.

Table 8: Comparison of HIV seroprevalence among donors in different studies

Authors (yrs)	Voluntary	Replacement	Total
Srikrishna <i>et al</i> (1999) [1]	00%	0.44%	0.44%
Garg <i>et al</i> (2001) [8]	0.28%	0.46%	0.44%
Kakkar <i>et al</i> (2004) [3]	00%	0.2%	0.2%
Matee <i>et al</i> (2006) [10]	2.0	4.5%	3.8%
Bhattacharya <i>et al</i> (2007) [6]	-	-	0.35%
Arora <i>et al</i> (2010) [11]	00%	0.3	0.3
Farnandes <i>et al</i> (2010) [12]	-	-	0.06
Kaur <i>et al</i> (2010) [13]	-	-	0.6%
Pallavi <i>et al</i> (2011)[7]	-	-	0.44%
Present study	00%	0.18%	0.17%

The seroprevalence of HIV in our study in total donor was 0.17% and seroprevalence in replacement donors was 0.18% which is comparable to the study by Kakkar *et al* [3]. In our study no voluntary donor was found to be positive for HIV, which is similar to the finding of Srikrishna *et al* [1], Kakkar *et al* [3] and Arora *et al* [11]. In our study seroprevalence of HIV is slightly less compared to national data (0.28%). This can be attributed to strict donors selection criteria. Since no voluntary donors blood units show seropositivity to

HIV in our study, suggest the need for implementing programs to achieve 100% voluntary donations.

In present study, we followed the strategy according to which the seroreactive donors were referred to VCTC for counseling and further confirmatory testing.

Seroprevalence of HBV

Hepatitis B virus is the most important causative agent of transfusion associated hepatitis. India

has been placed in the intermediate zone for prevalence of hepatitis B by WHO (2-7%). In previous Indian studies by Srikrishna *et al* [1], Sonawane *et al* [14] and Singh *et al* (2004) [15] observed the seroprevalence of HBsAg among the blood donors was 1.86%, 4.07% and 1.8% respectively. They concluded that voluntary donors are comparatively safe donors.

In the present study out of the total 16,872 screened blood units 267 were seropositive for HBsAg with 07 being voluntary donors and 260 being replacement donors, giving the seroprevalence of 0.49% and 1.58% among voluntary and replacement donors respectively. However the difference in seroprevalence among voluntary and replacement donors was statistically not significant. The overall seroprevalence HBsAg in our study (1.58%) correlated well with the studies of Bhattacharya *et al* [6] and Arora *et al* [11]. The difference in the seroprevalence of HBsAg among voluntary and replacement donors in the present study suggests the need for the concrete and non remunerated voluntary blood donor's base in India.

The seropositive HBsAg donors in this study were given post test counseling and enquired about the past history of jaundice; they were advised to undergo liver function tests and serology marker for HBeAg to know the status of their infectivity. Also they have been advised about screening of their family members for HBsAg and immunization.

Seroprevalence of HCV

In HCV infection 75-80% reported to progress to chronic hepatitis of which 10- 20% may progress to cirrhosis and hepatocellular carcinoma [16]. Worldwide it is estimated that 3% of the population have been infected with HCV which means there are 170 million chronic carriers. The prevalence of HCV antibodies in blood donors in developed countries ranges from 0.4% to 2 % [5].

The wide variations of HCV seroprevalence in different studies in India might be due to the use of different generation of ELISA test kits, having different sensitivities and specificities [7]. The prevalence estimated to be 1.5% in India [17]. Srikrishna *et al* [1] and Singh *et al* [5] observed the seroprevalence of HCV as 1.02% and 0.5% respectively among the blood donors. Bhattacharya *et al* [6] noted a statistically significant increase in seroprevalence of HCV among blood donors over a period of 2 years. The observed seroprevalence in 2005 was 0.35%.

Singh *et al* [5], Bagga *et al* [18] and Matee *et al* [10] noted that the seroprevalence of HCV in voluntary donors was less than that in replacement donors, suggesting that the voluntary donors are safe donors.

In the present study of the total 16,872 screened blood units, 15 units were seropositive for HCV. All the 15 seropositive blood units were from replacement donors. None of the screened blood units from voluntary donors were seropositive for HCV. The seroprevalence HCV among total donors in the present study was 0.09%. The seroprevalence among replacement donors was 0.10%, while among the voluntary blood donors the seroprevalence is zero. However the difference in seroprevalence among voluntary and replacement donors was statistically not significant. The observed seroprevalence of HCV of 0.09% in the present study is comparable with that observed by Farnandes *et al* [12]. Since all the seropositive blood units were from replacement donors and none of screened blood units from voluntary donors showed seropositivity, the study suggests the need for collection of blood from voluntary donors.

Seroprevalence of syphilis

In India, constant decline in the prevalence of syphilis is observed. The prevalence is decreased from 10.4% in 1977-85 to 2.5% in 1995-96.

Srikrishna *et al* [1], Sonawane *et al* [14] and Singh *et al* [15] in their studies noted the seroprevalence of syphilis among the blood donors as 1.6%, 0.87% and 0.26% respectively. Singh *et al* [5] and Matee *et al* [10] observed a statistically significant difference among voluntary and replacement donors suggesting that voluntary donors are safe donors.

In the present study out of the total 16,872 screened blood donors only two donors blood units showed seroreactivity for syphilis giving the seroprevalence of 0.01%. The seroprevalence in replacement donors was 0.02%. No voluntary donors found positive for syphilis. However the difference was statistically not significant.

The seroprevalence of syphilis in our study was 0.01%, which is low compared to other studies. This finding of our present study might be due to the declining trends in the prevalence of syphilis in general population due to improved access to health care, improved diagnostic means, treatment modalities and general health awareness among the population especially due to the emergence of HIV.

Prevalence of malaria

Though globally malaria constitutes a big health problem in general population, the prevalence of malaria among the blood donors is low and ranges from 0% to 0.05%. In a study by Srikrina *et al* [1], out of the total 8,617 screened blood units none of the units were positive for malaria.

Similar finding was noted by Sonawane *et al* [14]. None of the donors in our study were positive for malaria, this finding is same as that observed by Srikrishna *et al* [1], Sonawane *et al* [14], Farnandez *et al* [12] and Pallavi *et al* [7]. This observed zero percent malaria positivity rates may be the result of use of less sensitive technique for screening that is peripheral blood smear which requires presence of at least 100 parasites/ μ l of blood to be detected microscopically. These points to the need of more sensitive technique for screening of malaria to avoid post transfusion malaria.

CONCLUSION

Our study showed that the seroprevalence of transfusion transmissible infections (TTI) was more in replacement donors compared to voluntary donors. These results suggest that voluntary blood donor's services are needed to ensure the safety of blood units. All blood should be tested for TTI with reduction in unnecessary blood transfusion, thus ensuring safe blood supply to the recipients. It has been established that the incidence of TTI decreased considerably after mandatory testing of blood units for HIV, HbsAg, HCV, Syphilis, and Malaria. However, the risk of TTI cannot be eliminated completely even after mandatory testing of blood units because of risk associated with donations during window period. With the implementation of strict donor selection criteria, use of sensitive screening tests, and establishment of strict guidelines for blood transfusion, it may be possible to reduce the incidence of TTI in Indian scenario.

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