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Original Article

Pathology Section

Seropositivity of Transfusion Transmitted Infections among Blood Donors in Hilly Region of Karnataka, India

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ABSTRACT

Introduction: One of the major problem associated with blood transfusions are Transfusion Transmitted Infections (TTIs). Accurate estimates of risk of TTIs are essential for monitoring the safety of blood supply. National AIDS Control Organisation (NACO) guidelines state that all donor's blood samples should mandatorily be tested for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Syphilis and Malaria.

Aim: To estimate the seropositivity of HIV, Hepatitis B surface Antigen (HBsAg), HCV, Syphilis and Malaria among blood donors for tertiary care hospital of hilly region in Karnataka, India.

Materials and Methods: This cross-sectional study was done from January 2012 to March 2018 in District Hospital Blood bank, Department of Pathology, Kodagu Institute of Medical Sciences, Madikeri, Karnataka, India. The blood donors were screened with detailed history and physical examination according to Standard Operating Procedures (SOP) of Blood bank as per Guidelines for Blood donor selection and referral. Donor blood sample was subjected to screening investigations for TTIs diseases: HIV, HBV, HCV, Syphilis and Malaria. Data collected was tabulated in Microsoft Excel and results were expressed as percentage.

Results: The study included total 9599 blood donors. The total number of male donors was 8985 and female donors were 614. There were 74 seropositive donors of which 71 (96%) were males and 3 (4.0%) were females. The total seropositivity rate of TTIs was 0.77% among which the incidence of seropositivity was higher in males as compared to females. There were 55 donors (0.58%) positive for HBV, eight donors (0.08%) positive for Syphilis, five donors (0.05%) positive for HIV, four donors (0.04%) positive for Malaria and two donors (0.02%) positive for HCV.

Conclusion: Screening and better selection of donors by testing for TTIs are necessary to improve blood safety in the blood transfusion centre of hospital.

Keywords: Blood transfusion, Screening, Seroprevalence

INTRODUCTION

Transfusion of blood and blood products is an inherent component of health care. Blood transfusion which is timely, adequate and safe is life-saving procedure. Unsafe blood transfusion has risks of lethal complications and also increases the chances of TTIs [1]. There is a high risk of morbidity and mortality because of unsafe blood transfusions. There is always a concern regarding blood safety as infectious diseases transmission through blood donation is possible. These TTIs during blood donation include HIV, Hepatitis B, Hepatitis C, Syphilis, Malaria, Toxoplasmosis, Brucellosis and few viral infections like Herpes virus, Cytomegalovirus, Ebstein Barr Virus [1].

There is 1% chance of transfusion-associated problems including TTIs with every unit of blood. HIV and hepatitis are the most lethal amongst all the infections. In India, prevalence of TTIs in population is still unknown due to following reasons: unavailability of screening tests, lack of understanding, unavailability of surveillance systems and limited access to health facilities [2].

In late 1940's, evidence of TTI was first observed during blood transfusion and till 1970; blood banks were testing only hepatitis and syphilis inspite of being aware of other infections [3]. Preventing transmission of these infectious diseases through blood transfusion is one of the greatest challenges of transfusion medicine [4]. Blood donated by family/replacement donors carries a higher risk of TTIs as compared with blood donated by voluntary non-renumerated donors. Paid blood donors have the highest incidence and prevalence of TTIs [5]. The safest donors are the voluntary blood donors as they have the passion to donate blood to an unknown patient along with good health seeking behaviour. It has been recommended by World Health Organisation (WHO) that pre-transfusion testing for HBV, HIV, HCV and Syphilis is mandatory [6]. The whole blood or its blood components from any unit which tests positive should be discarded as per the NACO guidelines [7]. Evaluation of the data of the seropositivity gives an information about the epidemiology of these infections in the community [8]. Voluntary blood donation without any renumeration is the source of the safest blood supply to the transfusion system [9]. Voluntary donations are fewer in India and are poorly organised, hence safety of blood could be compromised [10].

Hence, this study was undertaken to find out the seropositivity of HIV, HBsAg, HCV, Syphilis and Malaria among blood donors for Kodagu Institute of Medical Sciences District Hospital Blood Bank, Madikeri, Karnataka, India.

MATERIALS AND METHODS

This cross-sectional study was done from January 2012 to March 2018 in District Hospital Blood Bank, Department of Pathology, Kodagu Institute of Medical Sciences, Madikeri, Karnataka, India, for a period of six years and three months. Institutional Ethical Committee Clearance was taken (Reference: KOIMS/IEC/04/2018-19). Universal sampling was followed to include all the blood donors who donated blood to the blood bank. The present blood bank received blood only through voluntary donations with no replacement donations. They were screened with detailed history and physical examination according to standard operating procedures of Blood Bank as per Guidelines for Blood Donor Selection and Referral [11]. Donors were provided a unique donor number and their name, age, gender, date of birth, profession, marital status and phone numbers were noted. Each donor before blood donation was required to give written informed consent.

Questionnaire was given to the donors which was prepared in the Institute Blood bank as per annexure of Guidelines for Blood Donor Selection and Blood Donor Referral [11]. The questionnaire was in English and local vernacular Kannada language for the convenience of the patient which included information about donors general health, previous blood donation or transfusion history, history of ailments like allergy, renal diseases, endocrine diseases, heart diseases, high/low blood pressure, sexually transmitted diseases, current or past febrile illness, weight loss, infections like tuberculosis and malaria, unusual or excessive bleeding, drug history, tattoo piercing, dental treatment, sexual history and risk behaviours [11]. Inspection was made for any marks of drug abuse or any skin lesion at the venipuncture site. During blood collection proper sterilisation procedures were followed and blood units were stored in refrigerator at 2-6°C.

Inclusion criteria: Physically fit donors of 18-55 years of age who donated blood at blood bank with haemoglobin more than 12.5 gm/dL for both gender and weighing more than 50 kg were included.

Exclusion criteria: Weight less than 50 kg, age less than 18 years, anemic, apparently unhealthy or malnourished donors, donor with history of jaundice or asthma, high risk behaviour individuals like history of unsafe sexual intercourse or drug abuse and donors with past history of syphilis, malaria, HBV, HCV or HIV excluded. Donors with history of dental treatment were deferred for six months, donors with tattoo piercing were deferred for 12 months and those who had unusual or excessive bleeding were permanently deferred as per Guidelines for Blood Donor Selection and Blood Donor Referral, Government of India [11]. Care was taken to eliminate professional and paid donors by taking history and clinical examination during the study period. Pilot tubes were taken for screening of TTIs.

Sera were separated and tested by protocol whereby samples seropositive by the first test were further tested by the second and third tests to confirm the seropositive result. Samples were tested for HIV, HBsAg and HCV by the third generation Enzyme-Linked Immunosorbent Assay (ELISA) assay with automated ELISA washer and reader (Robonik ELISA, Robonik India Pvt., Ltd.,). HBV testing was done by Merilisa HBsAg (Meril Diagnostics, India) and HCV by Erba Lisa HCV Gen 3 (Transasia Biomedicals, India). HIV screening was performed by third generation Merilisa HIV 1-2 Gen 3 (Meril Diagnostics, India) and followed by Individual Donor–Nucleic Acid Testing (ID-NAT) at the State referral laboratory of each donor sample. Controls, procedure and cut-off values for positive and negative results calculated as per kit manufacturer's literature. Syphilis was tested by Aspen Syphilis card test which is a quick chromatographic immunoassay for detection of antibodies to *Treponema Pallidum*. Malaria was detected using Rapid Antigen testing by card method for *Plasmodium* spp (*P.falciparum/P.vivax/P.malariae/P.ovale*) malaria parasite using Advantage Mal Card (J Mitra and Co. Pvt., Ltd., New Delhi). All the seropositive samples were tested in duplicate before labelling it positive. Seroprevalence of TTI was calculated by dividing the number of seropositive cases by the total number of blood donations annually. Seropositive units were discarded as per biomedical waste management regulations [12].

STATISTICAL ANALYSIS

The data collected was entered into Microsoft Excel. Categorical data was expressed in terms of rates, ratios and percentage.

RESULTS

There were total of 10,588 donors who were willing for blood donation out of which 9599 donors were fit for donation and 959 donors were excluded based on exclusion criteria. All donors were voluntary donors. The total number of male donors was 8985 and female donors were 614. There were total of 5567 out of 9599 (57.9%) donors who had previous history of blood donations. There were 74 seropositive donors of which 71 (96%) were males and 3 (4.0%) were females. Maximum TTIs were seen in the age group of 26-35 years (37.83%) followed by 18-25 years (33.78%) as shown in [Table/Fig-1]. The year wise distribution depicted in [Table/Fig-2] shows that maximum TTIs were detected in year 2012 and lowest in the year 2017. The total seropositivity rate of TTIs was 0.77% (74/9599) among which the incidence of seropositivity was higher in males as compared to females. There were 55 donors positive for HBV, eight donors positive for Syphilis, five donors positive for HIV, four donors positive for Malaria and two donors positive for HCV as depicted in [Table/Fig-3]. There was no single case of co-infection of any TTI in this study. Seropositive donors for any TTI were informed and advised to consult physicians in the hospital. Reactive blood donors were interviewed to understand regarding their lifestyle. During interview, donors were enquired about previous blood transfusion history, previous history of jaundice, sexual history and high risk behaviours to understand the cause of TTI reactivity. It was elicited that most donors seropositive for HIV and Syphilis had high risk sexual behaviour and most donors positive for HBV, HCV and Malaria revealed no significant history.

Age range (years)	Total seropositive cases (n)	Percentage (%)
18-25	25	33.78
26-35	28	37.83
36-45	17	22.98
>45	4	5.41

[Table/Fig-1]: Age wise distribution of seropositivity of TTIs (n=74).

DISCUSSION

Screening of blood for TTIs provides data of prevalence of these infections in healthy people along with ensuring safe blood transfusion to patients [13]. A good epidemiological data of disease prevalence in population helps the government in making policies [14]. Safety of blood supply and calculation of effectiveness of current screening procedures is possible as described by Busch MP et al., [15]. Prevalence of asymptomatic carriers of TTIs and blood donations received during window period pose major challenges for blood safety.

HBV is a major source of percutaneously transmitted hepatitis and is associated with a protracted carrier state and chronic liver disease. The seropositivity of HBV was highest among TTI in most of the studies [16-18]. The seropositivity rates of HBV (0.58%) and

Year	Total no. of donors	Male donors (n)	Female donors (n)	HBV	HCV	Syphilis	Malaria	HIV	Total TTIs	Percentage (%)
2012	1428	1383	45	13	1	0	0	0	14	0.98
2013	1429	1386	43	10	1	1	0	0	12	0.83
2014	1451	1387	64	10	0	1	0	2	13	0.89
2015	1424	1348	76	5	0	0	0	2	7	0.49
2016	1104	1013	91	8	0	1	1	0	10	0.90
2017	1427	1293	134	4	0	2	0	0	6	0.42
2018	1336	1175	161	5	0	3	3	1	12	0.89
Total	9599	8985	614	55	2	8	4	5	74	0.77

[Table/Fig-2]: Year wise distribution of seropositivity of TTIs.

Transfusion transmitted infections (TTIs)	Number of donors (n)	Percentage (%)
Hepatitis B	55	0.58
Syphilis	8	0.08
HIV	5	0.05
Malaria	4	0.04
Hepatitis C	2	0.02
Total	74	0.77%

[Table/Fig-3]: Seropositivity of TTIs in donors (N=9599).

Syphilis (0.08%) in this study were higher and those for HIV, Malaria and HCV were lower. Percentage seropositivity of HBV in an Indian study was shown to be 1.55% in 1996 which came down to 0.99% in 2002 [19]. Seroprevalence of HBV ranged from 1.86% to 4% in other Indian studies [20-22].

The transmission of HIV through blood transfusion and the consequent emergence of transfusion associated Acquired Immunodeficiency Syndrome (AIDS) epidemic have arguably transformed the areas of transfusion medicine over past several decades. HIV-1 and HIV-2 are the aetiological agents. Seroprevalence of HIV in India has been reported between 0.2 to 1% [20]. After the first, second and third generation ELISA, fourth generation ELISA was introduced with the hope of reducing the window period to 15-18 days and is used now-a-days as a screening test for HIV which detects both p24 antigen and antibody to HIV [23]. Though, NAT is not a mandatory screening test for TTIs as per Drug and Cosmetic Act [24] but, it is used in some centres in India as in this centre to reduce the residual risk of borderline and seronegative donations. Implementation of viral NAT system has greatly reduced the residual risk of viral transmission by reducing the time for effective detection to 5-11 days. NACO reported that seroprevalence of HIV among blood donors in India was 0.27% in the year 2013 [25]. There has been a significant reduction in prevalence of HIV in India due to implementation of NAT and chemiluminescence assay [25]. In India, blood donors constitute the third main source of HIV. The seroprevalence of HIV infection in the general population is 0.3% [26]. In this study, the HIV seroprevalence was 0.05%. The reported seroprevalence in present study is far less than other studies from various parts of India [20,27].

HCV is transmitted primarily through blood exposure. About 20%-40% of HCV cases are acute infections while majority of patient's progress to chronicity. The long term significance of subsequent disease due to cirrhosis and hepatocellular carcinoma is greater in HCV infected individuals than patients with HBV infection. Seroprevalence of HCV ranges between 0.4 and 1.09% in different Indian studies [20]. In this study, the seroprevalence for Hepatitis C was 0.02% which is less as compared to the study done by Bagga PK and Singh SP [28].

The risk of transfusion-transmitted syphilis is particularly high in developing countries with limited blood supplies where the blood is collected from family donors and transfused within hours [29]. Presence of syphilis points towards donor indulgence in "high-risk" behaviour and consequent higher risk of exposure to infections like HIV and hepatitis [20].

Malaria is the most commonly recognised parasitic complication of transfusion even though its incidence is rare. Malarial parasites survive for at least a week in components stored at room temperature or at 4°C [30]. Asymptomatic carriers are generally the source of transfusion transmitted malaria [20]. The gold standard test for diagnosis has been peripheral smear examination of thick and thin smear. This process needs necessary technical skills and technicians along with being a labour intensive procedure. Hence, there is evolution of numerous rapid antigen detection tests for whole blood to identify the malaria parasite antigen. Semi-immune malaria high risk blood donors can be detected using ELISA. Specific IgG antibody against *P.falciparum, P.vivax, P.ovale* and *P.malariae* can be detected using Malaria antibody tests [31,32].

In the various Indian studies, blood donors were evaluated for seroprevalence of HIV, HBV, HCV, Syphilis and Malaria. The seroprevalence of HIV ranges between 0.3% and 0.44%, HBV ranges between 0.99% and 3.44%, HCV ranges between 0.285% and 1.2%, Syphilis ranges between 0.11% and 1.6%, and Malaria ranges between 0% and 0.05%. In these studies, it has been observed that voluntary blood donation is more safe and advocated, as compared to replacement donation, as high incidence of TTIs are observed in replacement donors [4,22,29].

According to Fernandes H et al., prevalence of TTIs in total donors was 0.6%, the prevalence of Hepatitis B was highest (0.34%) then followed by Syphilis (0.11%) which is similar to present study. The prevalence in their study was HIV (0.06%), HCV (0.06%) and Malaria (0.01%). Prevalence of TTIs was more in male replacement donors [33]. In a study done Leena MS and Mohd S, there were a total of 6939 blood donors, out of these donors, 94 (1.35%) were positive for sero-markers of TTIs [26]. Regarding different TTIs, highest numbers of blood donors (0.71%) were having Hepatitis B followed by HIV (0.27%), HCV (0.14%), Malaria (0.129%) and Syphilis (0.10%). Various studies reported that seroprevalence of Hepatitis B among blood donors was higher than HIV, HCV and Syphilis [4,13,26,34,35].

[Table/Fig-4] shows comparison of different studies of TTI done by Mandal R and Mondal K, Leena MS and Mohd S, Makroo RN et al., Kaur G et al., NACO and Sethi B et al., [16,26,27,36-38]. Most of these studies show highest seropositivity with HBV as compared to others. The difference in the values of seropositivity in different studies may be due to the difference in prevalence of TTI in different areas, the effectiveness in selection of donors and variable proportion of voluntary and replacement blood donations in different studies. There was gradual increase in female donors from 2012 to 2018 as shown in [Table/Fig-2]. The seroprevalence of HBV has reduced drastically from over the years as shown in the present study. However in contrast, the incidence of syphilis has increased over the years. The overall incidence of TTIs was higher in most the years except in 2015 and 2017 where there was a sharp decline. In the present study, maximum TTIs were seen in the age group of 26-35 years (37.83%) which is similar to study done by Mandal R and Mondal K [16].

Sl No.	Author	HBV	HIV	HCV	Syphilis	Malaria
1	Kaur G et al., [36]	1.7	0.6	0.8	0.7	0
2	Makroo RN et al., [27]	1.18	0.24	9.87	0.43	0
3	Mandal R and Mondal K, [16]	1.24	0.42	0.62	0.65	0.004
4	NACO [37]	1.09	0.19	0.28	0.04	0.039
5	Leena MS and Mohd S, [26]	0.71	0.27	0.14	0.10	0.12
6	Sethi B et al., [38]	0.63	0.19	0.20	0.02	0
7	Present study	0.58	0.05	0.02	0.08	0.04

[Table/Fig-4]: Comparison of seropositivity (in percentages) of blood donors in different studies [16,27,28,37-38].

Professional or replacement donors often conceal medical history which poses a great threat to the safety of blood supply. Present study shows limited number of female donors. Hence, efforts should be made to encourage donation by female donors. Non-renumerated donors who are even termed as 'Safe blood donors' also contribute to complications of TTIs during blood transfusion. Voluntary donation should be encouraged by motivating the people as there is higher seropositivity detected among replacement donors. TTIs cannot be reduced to zero due to following reasons: deficiency of tests which are 100% sensitive, presence of novel infectious agents, adopting tests like DNA hybridisation and Polymerase Chain Reaction (PCR) for all donors is difficult and failure to detect those donors during window period. Increased rate of testing will cause deferral of safe donors due to increased false positivity results and additional expenditure of testing the patient.

Limitation(s)

The actual prevalence of TTIs may have been underestimated due to presence of window period in HCV, HBV and HIV. The study must have been more comprehensive in analysing the associated risk factors in donors for designing preventive measures for TTI.

CONCLUSION(S)

Seroprevalence of TTIs in present study was low which indicates low level of TTIs in the community. Sexually active blood donors have higher seroprevalence of TTIs. This study recommends the following steps rigorous donor screening, accurate screening tests and prudent use of blood and blood products for safe blood transfusion practice in the hospital.

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