

Syphilis in blood donors: Pre-transfusion serological screening by Rapid Plasma Reagin (RPR) Test at the blood bank of a Teaching Medical Institute in North Gujarat, India

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Abstract

Introduction: Apart from sexual transmission, blood transfusion is the other common route of transmission for Syphilis.

Aims & Objectives: To study prevalence of seroreactivity of Syphilis in blood donors of our geographical area in relations to their different demographic variables and co-infection with other TTI (Transfusion Transmissible Infections).

Materials & Methods: Records of all blood donations and TTI testing at the blood bank of our teaching medical institute from April 2012 to March 2016 were reviewed retrospectively. Data of all blood donors who were seroreactive for Syphilis were analysed in relations to their different demographic variables and results were interpreted accordingly.

Results: Out of 6633 registered blood donors, 6360 were accepted including 6246 males (98.2%) and 114 females (1.8%) with 5597 Voluntary (88%) and 763 Replacement (12%) donors. Out of 6360, total 48 donors (0.75%) were found seroreactive for Syphilis including 45 males (0.72%) and 3 females (2.63%) with 39 Voluntary (0.70%) and 9 Replacement (1.18%) donors. Prevalence of Syphilis is comparatively high among age group of 46-55 years. Co-infection of Syphilis with HBV was 2.08%, while any co-infection with HIV, HCV and Malaria was not found.

Conclusion: Seroprevalence of Syphilis in blood donors in present study is 0.75%. Even though trend of syphilis is declining, screening of blood donors should be continued to avoid the transmission of undiagnosed and untreated syphilis. Considering the limitations of RPR test, more sensitive and specific tests like Recombinant antigen based EIA and Rapid ImmunoChromatographic Strip (ICS) should be used if available and feasible.

Keywords: Blood donor, Pre-transfusion, RPR, Syphilis, Seroprevalence.

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Introduction

Sexually Transmitted Infections (STI) include various Bacterial (Syphilis, Chlamydia, Gonorrhoea), Viral (Genital Herpes, Human Papilloma Virus [HPV], Human Immunodeficiency Virus [HIV], Hepatitis B & C Virus [HBV, HCV]), Fungal (Candidiasis) and Protozoal (Trichomoniasis) infections. Among them, Syphilis is one of the few which can also be transmitted by blood transfusion^{1,2} and poses a major public health risk to blood recipients worldwide.^{3,4} Vertical transmission (mother to baby) and direct inoculation are some other routes of transmission as well. It is caused by *Treponema Pallidum* (TP) which belongs to spirochete group of bacteria. According to World Health Organization (WHO) 2016 updates, each year around 5.6 million people acquire new infections with Syphilis which leave profound impact on their sexual, reproductive and overall health.⁵ Syphilis is known to cause still births, infertility, serious neonatal infections as well as persistent

disabilities of joints, brain, heart, eyes etc. in long run.⁶ Being a sexually transmitted infection, its presence shows donor's 'high risk' behaviour and consequently higher risk of exposure to other STI and TTI like HIV, HBV and HCV⁷. So pre-transfusion testing of Syphilis along with other TTI (HIV, HBV, HCV and Malaria) in blood unit collected from each and every donor was recommended by WHO⁸ & NACO⁹ and made mandatory by Government of India (GOI).¹⁰ Testing for Syphilis can be done by Treponemal and Non-Treponemal assays. Treponemal assays like *Treponema Pallidum* Haemagglutination Assay (TPHA) and Enzyme ImmunoAssay (EIA) which detect treponemal antibodies are generally used for confirmatory diagnostic tests while Non-Treponemal assays such as Venereal Diseases Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) which detect antibodies (Reagins) to cardiolipin or lipoidal antigen which rise significantly in active infection due to the cellular damage are used for screening purpose as in blood donors. In the United States (US) and certain

European countries, Non-Treponemal Assays are most commonly used for screening because of the advantage of being Simple, fast, cheap and more sensitive.¹¹ Syphilis is prevalent in both volunteer and replacement blood donors in some developed as well as developing countries and some other parts of India also.^{12-14,22-31} But very scanty published data available regarding seroprevalence of syphilis in blood donors in rural geographical area around our institute. So this study is conducted to know the prevalence of seroreactivity of syphilis in blood donors of our region and its co-infection with other TTI. It can help in revealing the magnitude of Syphilis infection in apparently healthy looking blood donors and in making plans and policies for management of safe blood supply to the recipients.

Materials and Methods

This retrospective study was conducted in the hospital based blood bank of our teaching medical institute. Records of all blood donors including voluntary and replacement blood donations as well as results of their TTI testing from April 2012 to March 2016 were reviewed. According to records, mandatory pre-transfusion serological screening of collected blood units for Syphilis along with HIV, HBV, HCV and Malaria was done. Serological screening for HIV, HBV and HCV was carried out by Enzyme Linked Immunosorbent Assay (ELISA) while by Peripheral Smear Microscopy for Malaria.

Interpretation:

- | <i>Observation</i> | <i>Interpretation</i> |
|--------------------------------------------------------|-----------------------|
| 1. Black floccules or clumps against white back ground | Reactive/Positive |
| 2. Uniform greyish suspension with no floccules | Nonreactive/Negative |

Syphilis screening was done by RPR Carbon Antigen Test (IMMUNOPAK- Reckon Diagnostics P.Ltd., India).¹⁷

Principle: RPR (Rapid Plasma Reagin) is a qualitative slide test based on the detection of 'Regains' (non-treponemal antibodies against cardiolipin or lipoidal antigen) in donor's serum. A suspension of modified cardiolipin coated on microparticulate carbon is used as an antigen against the 'Reagin'. The antigen reacts with 'Reagin' in the sample to form black clumps or floccules indicating a Reactive (Positive) test.

Sample: Two ml of blood sample was collected in labelled EDTA vacutainer from the tubing of blood bag during taping from each blood donor. The samples were centrifuged at 3500 rpm for 5 minutes to obtain clear non-haemolysed serum.

Procedure

1. Reagent and sample were allowed to attain room temperature before use.
2. One drop of sample serum, positive control & negative control were placed into separate reaction circles of the test kit.
3. One drop of RPR Carbon reagent was added to each of this.
4. Reagent and sample were mixed in the respective circles & spreaded uniformly over the circle.
5. Stop-watch started & the circle card was rotated gently on a mechanical rotor at 100rpm.
6. Test results were observed at 6 minutes & check for presence of flocculation or black clump under a strong source of light.

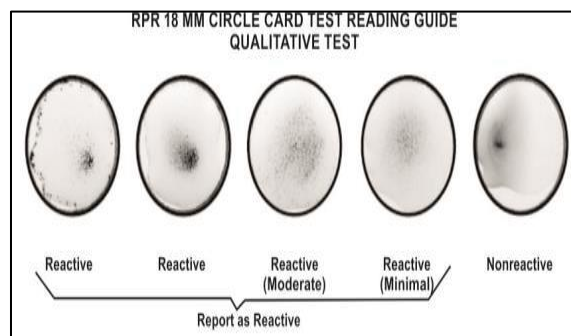


Fig. 1: Interpretation of RPR test for Syphilis screening in Blood Donors

The results were read immediately after 6 minutes and reported as Reactive or Non-reactive against the respective donor's identification number and entered in TTI register. Reactive blood units were discarded and the donors were called & referred to STI Clinic of our institute for counselling, confirmatory testing and treatment. Data of all blood donors who were seroreactive for Syphilis were analysed in relations to their different demographic variables and results were interpreted accordingly.

Seroprevalence was defined as the number of Syphilis reactive donors in the total donor population and was calculated accordingly & expressed in percentage (%).

Results

During the four year period from April-2012 to March-2016, total 6633 blood donors were registered in the blood bank of our teaching medical institute. Out of which, 273 were deferred for various reasons while 6360 were accepted for donation. Accepted donors include 6246 males (98.2%) and 114 females (1.8%) with 5597 Voluntary (88%) and 763 Replacement (12%) donors as shown in Table 1. Out of 6360, total 48 donors (0.75%) were found seroreactive for Syphilis including 45 males (0.72%) and 3 females (2.63%) with 39 Voluntary (0.70%) and 9 Replacement (1.18%) donors. Prevalence of Syphilis is comparatively high among age group of 46-55 years (0.98%). Gender, Donor type and Age group wise distribution of seroreactivity of syphilis is shown in Table 2. Only 1 donor found reactive both for Syphilis & HBV showing 2.08% of co-infection rate with HBV. We did not find any co-infection with HIV, HCV and Malaria (Table 3).

Table 1: Year wise Distribution of Blood Donors with Seroreactivity for Syphilis

Financial Year	Accepted Donors					Seroreactive Donors For Syphilis						
	Total	V	R	Male	Female	Voluntary			Replacement			Total (V+R)
						Male	Female	Total	Male	Female	Total	
2012-13	695	495	211	686	9	7	1	8	2	0	2	10
2013-14	1282	1196	95	1229	53	4	0	4	0	0	0	4
2014-15	1875	1628	265	1840	35	18	1	19	4	1	5	24
2015-16	2508	2278	192	2491	17	8	0	8	2	0	2	10
TOTAL	6360	5597	763	6246	114	37	2	39	8	1	9	48

V- Voluntary, R- Replacement

Table 2: Gender, Donor type & Age group wise Seroreactivity of Syphilis

	Blood Donors Tested	Donors Seroreactive for Syphilis		
		Number	%	% of Total
Gender				
Male	6246	45	0.72	0.70
Female	114	3	2.63	0.05
Total	6360	48	---	0.75
Type of Blood Donation				
Voluntary	5597	39	0.70	0.61
Replacement	763	9	1.18	0.14
Total	6360	48	---	0.75
Age Group (in years)				
18-25	930	5	0.54	---
26-35	2559	17	0.66	
36-45	1595	14	0.87	
46-55	1020	10	0.98	
56-65	256	2	0.78	
Total	6360	48	0.75	

Table 3: Co-infection of Syphilis with other TTI

Other TTI	Syphilis Reactive Total (n)=48	% of Co-infection
HIV Reactive	0	0
HBV Reactive	1	2.08
HCV Reactive	0	0
Malaria Reactive	0	0
Total	1	2.08

Discussion

The absence or decrease of transfusion-transmitted syphilis in many developed countries leads to question the rationale for continuing syphilis testing of blood donors.^{12,19} However, in some poorly developed and developing countries, it is still highly prevalent.^{4,13,14,32,33} Seroprevalence rate of Syphilis among blood donors ranged from 0% to 12.7% across the world^{3,4,13,14,19-21,32-35} (Table 4). In India it ranges from 0.03% to 2.6% in different states^{11,15,22-31,36,37} (Table 5). This range of variation may be due to geographical locations, lifestyle of respective population, donor selection criteria, age range of donors, sample sizes, study period, variable sensitivity & specificity of test kits and different socio-cultural practices like sexual behaviour, marriage practices etc. across the world.¹⁸ Seroprevalence of Syphilis in our study was 0.75% which was quite nearer to some international^{19,20,21} and Indian studies.^{22,23} In India, Higher prevalence of Syphilis was noted in Delhi and Chhattisgarh,^{24,25} while lower in Jharkhand, Andhra Pradesh, Telangana and Madhya Pradesh.^{27,29,30,31} Countries like Tanzania and Ghana showed very high prevalence^{32,33} while lower in Sri Lanka, Egypt, Israel.^{3,34,35} Multiple sexual partners and growing incidence of intravenous drug abuse can be the reasons behind high prevalence while strict donor selection criteria, increased public awareness, self-exclusion of high risk groups from blood donation etc. behind lower prevalence.

In present study, majority donors were male compared to females, also noted by many researchers.¹⁹⁻³¹ Similarly males (45/6246) outnumbered females (3/114) in seroreactivity for Syphilis, findings similar to other studies. While Tserenpuntsag et al reported higher seroprevalence in females.¹⁴ Voluntary donors were more compared to replacement donors. Some showed similar pattern^{26,37} while others reported opposite trend with higher percentage of replacement donors.^{1,2, 27-31} On the contrary, seroreactivity of Syphilis was higher in replacement donors compared to voluntary donors which was supported by finding of some authors.^{4,15,36} Seroprevalence of Syphilis was least in 18-25 year age group and then increased up to 55 years, followed by decline in age group of 56-65 years which were similar to findings of Karmakar et al.²⁶ Although Syphilis is known for increasing susceptibility to other TTI, We didn't

found any co-infection with HIV, HCV or Malaria except HBV (2.08%) in our study. Kumar et al reported Syphilis co-infection rate with HBV (3.7%) and with HIV (2.9%),²⁵ while Makroo et al found 3% for HIV, 18.7% for HCV.¹⁶ The lower co-infection rate with other TTI could be due to limitation of screening tests to detect them in window period or either they were true negative!

Seeing past literature of last 35 years, hardly few cases of transfusion transmitted Syphilis were reported all over the world.¹¹ According to WHO and NACO, Syphilis causing organism *T. Pallidum* being very fragile and heat sensitive, cannot survive for more than 3-5 days below +20°C.^{8,9} It's a fact that most of the blood product (Whole Blood, RBC, Cryoprecipitate, Fresh Frozen Plasma etc.) except platelets (+22°C) being stored below this temperature, transfusion transmitted syphilis is a rarity now a days, but still can occur. Some condition like Exchange transfusion for sickle cell disease, cardiac surgery and massive haemorrhage requires fresh blood (approx. less than 2-3 days old) for transfusion creating a potential risk for transmitting syphilis. So pre-transfusion serological testing of all collected blood units for Syphilis should be continued in blood banks. Further it should be kept in mind that the RPR Carbon antigen test is basically a screening test having some advantages as well as limitations.

Advantage: It is cheap, easy, fast and can detect active infection.

Limitations:¹⁷

1. **False Negativity:** In incubating primary and late syphilis, in the presence of high titres of antibody (prozone phenomenon) in secondary syphilis,
2. **False Positivity:** Concomitant conditions or diseases like Mononucleosis, Leprosy, Malaria, Lupus erythematosus, Vaccinia, Viral pneumonia, Collagen diseases, Measles, Rubella, HIV, Pregnancy, Narcotic addiction and autoimmune diseases.

With introduction of More sensitive and specific tests like Recombinant antigen based EIA and Rapid Immunochromatographic Strip, it is now possible to diagnose and treat syphilis reactive person in a single visit with lower chances of false positivity or negativity.

Table 4: Comparison of Seroprevalence Rate of Syphilis in Blood donors of Present Study to other International Studies^{3,4,13,14,19-21,32-35}

Studies from various Countries	Country	Study Period	Total Donors	Syphilis Reactive Donors	Prevalence (%)
Nada et al ³⁴	Egypt	1996-2011	149381	0	0
Matee et al ³²	Tanzania	1999	300	38	12.7
Li et al ¹⁹	China	2000-2010	4366283	20521	0.47
Adjei et al ³³	Ghana	2003	536	40	7.5
Tessema et al ¹³	Ethiopia	2003-2007	6361	83	1.3
Tserenpuntsag et al ¹⁴	Mongolia	2004-2005	2250	44	2
Vera et al ³⁵	Israel	2005-2009	605549	283	0.04
Damulak et al ²⁰	Nigeria	2007-2010	9500	86	0.9
Tiwari et al ²¹	Nepal	2008	21716	106	0.49
Morawakage ³	Sri Lanka	2010-2012	66087	37	0.05
Nazir et al ⁴	Pakistan	2012	14352	449	3.1
Present Study	India	2012-2016	6360	48	0.75

Table 5: Comparison of Seroprevalence Rate of Syphilis in Blood donors of Present Study to other Studies in various States of India ^{11,15,22-31,36,37}

Studies from India	Zones of India	States in India	Study Period	Total Donors	Syphilis Reactive Donors	Prevalence (%)
Singh et al ²⁴	NORTH	Delhi	2000-2002	76089	1978	2.6
Arora et al ²²		Haryana	2002-2006	5849	54	0.92
Lamba et al ²³		Panjab	2011-2015	22645	201	0.88
Adhikari et al ¹⁵	EAST	Sikkim	2001-2008	3735	10	0.27
Sunderam et al ²⁷		Jharkhand	2008-2012	63803	19	0.03
Karmakar et al ²⁶		West Bengal	2009-2011	24320	56	0.23
Sabharwal et al ³⁶	WEST	Rajasthan	2007-2010	21399	111	0.51
Patel et al ³⁷		Gujarat	2007-2013	15368	22	0.14
Patil et al ²⁸		Maharashtra	2008-2014	5152854	2709	0.11
Bhavani et al ²⁹	SOUTH	Andhra Pradesh	2004-2009	8067	7	0.08
Fatima et al ³⁰		Telangana	2010-2016	55291	22	0.03
Das et al ¹¹		Karnataka	2013	10000	45	0.45
Kumar et al ²⁵	CENTRAL	Chhattisgarh	2011-2013	12680	134	1.05
Yadav et al ³¹		Madhya Pradesh	2012-2013	4007	2	0.04
Present Study	WEST	Gujarat	2012-2016	6360	48	0.75

Conclusion

Seroprevalence of Syphilis in blood donors of our rural region in present study is 0.75% with 2.08% of co-infection rate with other TTI. Special attention should be given for increasing awareness and education regarding STI among blood donors and high risk populations of rural areas, which consequently improve public health and increase blood safety and quality. Even though the prevalence of syphilis is on the decline due to improved donor selection, uniform serologic testing of blood units and increased trend of transfusion of refrigerated blood, screening of blood donors should be continued to

avoid the transmission of undiagnosed and untreated syphilis. Considering the limitations of false positivity and negativity of RPR test, more sensitive and specific tests like recombinant antigen based EIA and Rapid ImmunoChromatographic Strip (ICS) should be used if available and feasible.

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