

## SEROPREVALENCE OF SYPHILIS AMONG PATIENTS ATTENDING SEXUALLY TRANSMITTED INFECTION (STI) CLINIC AT A TERTIARY CARE HOSPITAL

R. Radhika<sup>1</sup>, V. Dillirani<sup>2</sup>, V. Rengashini<sup>3</sup>

Received : 16/03/2026  
Received in revised form : 08/05/2026  
Accepted : 23/05/2026

Keywords:  
STI, Syphilis, RPR, TPHA, NACO.

Corresponding Author:  
Dr. R. Radhika,  
Email: radhadr06@gmail.com

DOI: 10.47009/jamp.2026.8.3.81

Source of Support: Nil,  
Conflict of Interest: None declared

Int J Acad Med Pharm  
2026; 8 (3); 447-454



<sup>1</sup>Post graduate student, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India.

<sup>2</sup>Professor & Head, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India.

<sup>3</sup>Assistant Professor, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India.

### ABSTRACT

**Background:** Syphilis is a sexually transmitted disease caused by the Spirochete *Treponema pallidum*. Clinical features in adults progress through different stages primary, secondary, latent and tertiary syphilis, with different signs and presentations. The seroprevalence of syphilis in India shows different rates of occurrence in different population groups, ranging from 21.9% among long distance truck drivers to 5.4% among STD clinic attendees. The aim of this study is to estimate the Seroprevalence of Syphilis among patients attending STI clinic at a tertiary care hospital using Rapid Plasma Reagin (RPR) as screening test and *Treponema Pallidum* Hemagglutination Assay (TPHA) treponemal tests as confirmatory test. **Materials and Methods:** A prospective study was conducted over 6 months (August 2025-January 2026) at Government Stanley Medical College, Chennai. A total of 868 consecutive blood samples from patients attending STI clinic were collected. The serum samples were tested as per National STI prevention and control program guidelines issued by National AIDS Control Organization (NACO) by non-treponemal test, RPR (Rapid Plasma Reagin) and treponemal test, TPHA (*Treponema Pallidum* Haemagglutination Assay) was performed by both qualitative and quantitative method to confirm RPR reactive samples. **Results:** Among 868 patients attending STI clinic, 58 were reactive for Syphilis by RPR method. Out of 58 RPR reactive cases, 47 were positive for syphilis by TPHA method. The seroprevalence of Syphilis among patients attending STI clinic was 5.4%. **Conclusion:** This study highlights a seroprevalence of 5.4% of syphilis among STI clinic attendees, indicating that syphilis remains a significant public health concern in India. Young adults, particularly males aged 21–30 years, are the most affected group. RPR is a useful screening tool; however, due to biological false positivity, confirmatory testing with Treponemal test like TPHA is essential. Early diagnosis and treatment are crucial to prevent complications and transmission. Strengthening STI surveillance, ensuring adherence to NACO guidelines, and increasing awareness about safe sexual practices are necessary to reduce disease burden.

## INTRODUCTION

Sexually Transmitted Infections (STIs) are major public health problems in all populations and socioeconomic groups worldwide. About 6% of Indian population is reported to be having STIs.<sup>[1]</sup> STIs can be grouped into ulcerative and non-ulcerative based on the type of lesions and presenting symptoms. Genital Ulcer Diseases (GUD) often represent a diagnostic dilemma, especially in developing countries, like India. The annual global

incidence of GUD exceeds 20 million cases.<sup>[2]</sup> The common causes of GUD are Herpes Simplex Virus, *Treponema pallidum*, *Haemophilus ducreyi*, *Chlamydia trachomatis* and *Klebsiella granulomatis*. Syphilis is a sexually transmitted disease caused by the Spirochete *Treponema pallidum*.<sup>[1]</sup> It affects 12 million people annually worldwide.<sup>[3]</sup> This infection may progress to chronic infection with various adverse systemic outcomes if not treated in early stages.

Clinical features in adults progress through different stages beginning with Primary Syphilis: The primary stage starts after 21 days (range of 10 – 90 days) following syphilis infection. Primary syphilis is characterized by a painless, indurated ulcer (known as hard/primary chancre) in the genital/oral/anal area resulting from direct sexual contact with a person with syphilis. The chancre has obvious edges, and the lymph nodes in the groin may also appear swollen.<sup>[1]</sup> Within 3 to 6 weeks, the chancre heals spontaneously (without treatment). Venereal syphilis is acquired by sexual contact. However, it can also be transmitted by non-venereal modes such as direct contact, blood transmission or trans-placental transmission. If transmitted by direct contact, the primary chancre is extragenital, usually on the fingers. If transmitted by blood transmission, the primary chancre doesn't occur. Dissemination of the organism occurs during this primary stage; once the organism has reached a sufficient number (usually within 4 to 10 weeks), clinical manifestations of secondary syphilis become apparent.<sup>[4]</sup>

**Secondary Syphilis:** The secondary stage is characterized by the development of non-itchy skin rashes over the body and generalized lymphadenopathy, arising 1 to 6 months after primary syphilis often associated with fever and muscle pain. This stage is a highly infectious state, again because large numbers of spirochetes are present. The stage lasts for 2 to 6 weeks. This stage may be followed by a latent stage for a few years. The stage is characterized with no signs and symptoms. It may be early latent syphilis (occurs within first year after infection) and late latent syphilis (occurs after the first year of infection). The spirochetes may circulate in blood during this phase leading to infection of all the organs in the body.

**Tertiary Syphilis:** The stage occurs after several years of infection and can be manifested as neurosyphilis (when brain/spinal cord is affected), cardiovascular syphilis (when heart and aorta is affected) or late benign syphilis (when the skin is primarily involved). The complications can be developed in 40% of people with latent infection in the absence of treatment. Tertiary syphilis takes 1 to 10 years to develop, but it can take up to 50 years.

**Congenital syphilis** is transmitted from a mother to an unborn fetus during any stage of infection but is most often associated with early syphilis. The unborn fetus may develop an asymptomatic infection or symptomatic infection. Clinical signs known as Hutchinson's triad (deafness, blindness, notched peg-shaped teeth) may occur. Additionally, poor bone formation may result, such as "saber shin" bowing of the tibia and the "bulldog" appearance of a deformed maxilla. Finally, neurosyphilis or neonatal death can occur.<sup>[4]</sup>

The seroprevalence of syphilis in India shows different rates of occurrence in different population groups, ranging from 21.9% among long distance truck drivers to 5.4% among STD clinic attendees. According to NACO, the RPR seropositivity was

reported to be 1.3%, 0.3% and 1.9% among males, females, and transgender/hijra population respectively in Designated STI/RTI Clinic (DSRC) in 2022-23. While the overall RPR positivity was reported to be 0.7%, the trend for RPR positivity at DSRC continues to rise in 2022-23 when compared with test positivity reported in 2020-21 (0.49%) and 2021-22 (0.60%).<sup>[5]</sup>

The serological tests for syphilis include Non-treponemal tests such as Rapid Plasma Reagin(RPR), Venereal Disease Research Laboratory (VDRL), Unheated Serum Reagin (USR), Toluidine Red Unheated Serum Test(TRUST) and Treponemal tests like T.pallidum immobilization test(TPI), FTA-ABS (Fluorescent treponemal antibody absorption test), TPHA (T.pallidum hemagglutination test), TPPA(T.pallidum particulate agglutination test), MHA-TP(microhemagglutination assay for antibodies), Chemiluminescence, TP-ELISA. Treponemal tests detect antibodies to T.pallidum proteins while nontreponemal tests detect antibodies against lipoidal antigens, damaged host cells and possibly from treponemas.

Nontreponemal tests are suitable for screening or diagnosis in conjunction with medical history and physical examination when antibody titers are important to determine recent exposure to infection, a presumptive diagnosis or to determine response to treatment. The sensitivity of RPR in primary, secondary, tertiary syphilis are 86%, 100%,73% respectively and specificity is 98%.<sup>[6]</sup> Quantitative RPR/VDRL test with a titre  $\geq 1:8$  is considered as significant titre and is suggestive of syphilis.<sup>[1]</sup>

The nontreponemal tests can be false positive in other conditions like HIV, autoimmune conditions, vaccinations, injecting drug use. So, Treponemal tests are done to confirm results of reactive nontreponemal tests. The FTA-ABS test is highly specific and sensitive; however, it may produce variable results due to variations in equipment, reagents, and interpretation. The sensitivity of TPHA ranges from 82-100% and the specificity is 99%. TPHA is widely used in laboratories, is simpler and cheaper than FTA-ABS.<sup>[7]</sup>

Some of the TPHA positive cases with RPR titre  $<1:8$  may be either because of biological false positivity or representative of early/ latent/ late syphilis cases or treated cases of syphilis. Biological False Positive (BFP) reactions are defined as positive reaction in a cardiolipin test and a negative reaction in a confirmatory treponemal test. BFP reactions can be observed in various acute and chronic conditions in the absence of syphilis. Examples of BFP reactions are; infections, injuries, inflammation and early HIV infection, SLE, collagen vascular diseases, leprosy, malaria relapsing fever, hepatitis, Infectious mononucleosis, tropical eosinophilia etc.<sup>[1]</sup> Despite India's STI/RTI control strategy under the National Health Mission and NACO achieving a significant decline in bacterial STIs, Syphilis persists as a major public health challenge.

This study aims on serological diagnosis of Syphilis by both Rapid plasma Reagin (RPR) non-treponemal test as screening test and Treponema Pallidum Hemagglutination Assay (TPHA) treponemal tests as confirmatory tests for early detection and management to prevent complications and spread of infection.

The aim of this study is to estimate the Seroprevalence of Syphilis among patients attending STI clinic at a tertiary care hospital using Rapid Plasma Reagin (RPR) as screening test and Treponema Pallidum Hemagglutination Assay (TPHA) treponemal tests as confirmatory test.

## MATERIALS AND METHODS

Study was conducted after obtaining consent from Institutional Ethical Committee.

As per the inclusion criteria, 868 consecutive blood samples from patients attending STI clinic during study period were included.

Blood samples collected were centrifuged and the serum samples were tested as per National STI prevention and control program guidelines issued by National AIDS Control Organization (NACO) by non-treponemal test, RPR (Rapid Plasma Reagin) and it was performed as screening test according to manufacturer's instructions. A volume of 0.05 ml of unheated serum was placed onto test circle of RPR card using a pipette. The serum sample was evenly spread over the circle with a disposable plastic stirrer. The RPR card test antigen was gently shaken and one drop of antigen was added to each serum sample on the card. The card was then placed on the mechanical rotator and rotated at the recommended speed for 8 minutes specified by the kit manufacturer. Following rotation, the card was removed from the rotator and the results were observed immediately with naked eye under bright light. Reactive samples were indicated by macroscopically visible black clumps against white background on card while non-reactive samples appeared to have no clumps or slight roughness (1) and the samples were further subjected to serial dilutions (1:2, 1:4, 1:8, 1:16 and so on) to determine the highest dilution with visible clumps, which was reported as RPR titre.

Then treponemal test, TPHA (Treponema Pallidum Haemagglutination Assay) were performed by both qualitative and quantitative methods to confirm RPR reactive samples. It was done in microtiter plate.

**Reagent preparation:** All the reagents were supplied in a ready-to-use format. Test and control cells were thoroughly suspended prior to use. All the reagents were allowed to reach room temperature before use.

**Qualitative testing:** Each sample required three microwells. 190µl of diluent was added to well 1 to which 10µl of sample was added and mixed. 25µl of this solution was transferred from well 1 to wells 2 and 3. Further 75µl of control cells were added to well 2 and 75µl of test cells were added to well 3.

Plate was covered and incubated for 45-60 minutes in an area which was away from heat, direct sunlight and vibration.

**Quantitative testing:** Each sample required 9 well of a microtitration plate. 190µl of diluent was added to well 1 and 25µl to wells 4 through to 9. A dilution of 1/20 was made by adding 10µl of serum to well 1 and then 25µl of 1/20 dilution was transferred to wells 2, 3 and 4. A dilution of 1/40 was then mixed in well 4 and 25µl of it was transferred to well 5. This step was repeated until the serial dilution was completed with discarding 25µl from the last dilution well. Further 75µl of test cells was added to wells 3, 4, 5, 6, 7, 8, 9 and 75µl control cells was added to well 2. Final specimen dilution range after addition of cells was 1:80 to 1:10240. Plate was covered and incubated for 45-60 minutes in an area away from heat, direct sunlight and vibration. If antibodies against Treponema pallidum were present in the serum, agglutination of the sensitized red blood cells occurs, producing smooth matt overing a well while in negative test, there was clear button type deposits of RBCs indicating no agglutination (1).

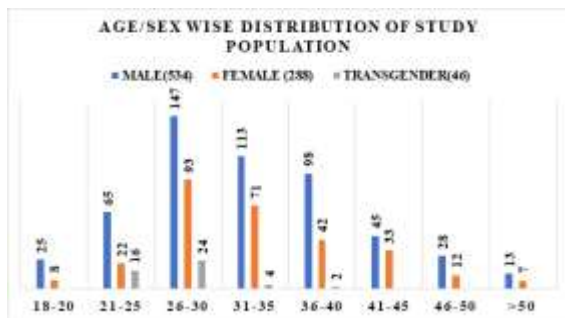
**Quality control (QC):** Quality control procedures were performed for both the Rapid Plasma Reagin (RPR) test and Treponema pallidum Hemagglutination Assay (TPHA) using kit-provided controls as well as in-house known positive and negative serum samples.

For the RPR test, reactive and non-reactive control sera supplied with the kit were included with each batch of testing to ensure proper reagent performance and accuracy of test interpretation. In addition, previously confirmed in-house positive and negative serum samples were periodically tested for internal quality assurance.

For the TPHA test, manufacturer-provided positive and negative controls were processed according to the kit instructions with every test run. Known in-house serum samples previously confirmed by standard serological methods were also used to monitor consistency and reliability of the assay results. All tests were performed strictly according to the manufacturer's instructions, and results were considered valid only when quality control results were satisfactory.

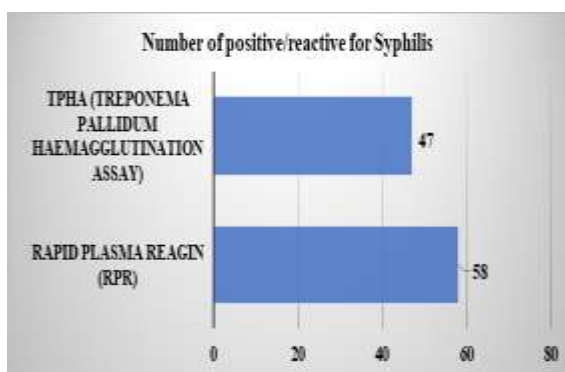
## RESULTS

In this study a total of 868 patients attending STI clinic above the age of 18 years were enrolled.



**Chart 1: Age/sex wise distribution of study population**

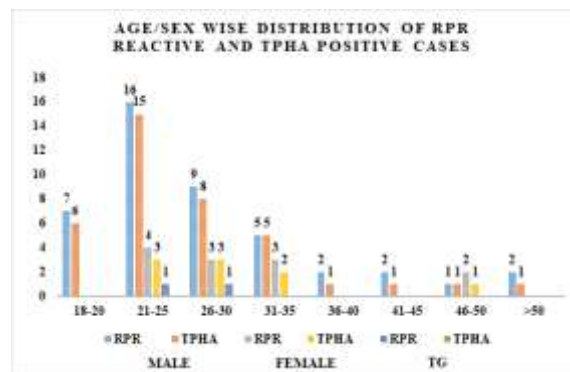
Our study revealed that the maximum number of patients attending STI clinic were from the age group of 26-30 years-264(30.4%), followed by 31-35 years - 188 (21.6%) and 36-40 years 142 (16.3%). Majority of patients 534 (61.5%) were male, compared to female 288 (33.2%).



**Chart 2: RPR and TPCHA tests to diagnose syphilis for patients attending STI clinic**

Out of the 868 patients tested, 58 were reactive for Syphilis by RPR method. Out of 58 RPR reactive

cases, 47(81%) were found to be positive and 11(18.9%) were negative by TPCHA method. The seroprevalence of Syphilis among patients attending STI clinic were 5.4%.



**Chart 3: Age/Sex Wise Distribution of RPR Reactive and TPCHA Positive Cases**

In our study, the maximum number of RPR and TPCHA positive cases were from the age group of 21-25 years 21(36.2%) and 18(38.3%), followed by 26-30 years 13(22.4%) and 11 (23.4%) respectively. 44(75.9%) male, 12(20.7%) female and 2(3.4%) transgenders were RPR positive and 38(80.9%) male and 9(19.1%) were TPCHA Positive. Male: Female: Transgender ratio of RPR and TPCHA positivity was 3.6: 1: 0.1 and 4.2: 1 respectively. Infection was more common in males, suggesting high risk sexual behaviour.

Biological False Positive cases 11 (18.9%) were more in males 6(54.5%) than females 3(27.2%) and majority of cases were from the age group of 21-25 years 3(27.2%) followed by 26-30 years 2(18.1%).

**Table 1: Age/sex wise distribution of study population (n=868)**

AGE IN YEARS	MALE (534) 61.5%	FEMALE (288)33.2%	TRANSGENDER (46) 5.3%	TOTAL (868)
18-20	25(4.7%)	8(2.8%)	-	33(3.8%)
21-25	65(12.2%)	22(7.6%)	16(34.7%)	103(12%)
26-30	147(27.5%)	93(32.2%)	24(52.2%)	264(30.4%)
31-35	113(21.2%)	71(24.6%)	4(8.7%)	188(21.6%)
36-40	98(18.3%)	42(14.5%)	2(4.3%)	142(16.3%)
41-45	45(8.4%)	33(11.4%)	-	78(9%)
46-50	28(5.2%)	12(4.2%)	-	40(4.6%)
>50	13(2.4%)	7(2.4%)	-	20(2.3%)

**Table 2: Socio-demographic and behavioural characteristics of study participants (n = 868)**

Characteristics	Category	Number (n=868)	Percentage (%)
Occupation	Skilled	274	31.6
	Unskilled	346	39.8
	Unemployed	248	28.6
Education Level	Illiterate	412	47.5
	Literate	456	52.5
Marital Status	Married	472	54.4
	Unmarried	299	34.4
	Divorced	69	8
	Widowed	28	3.2
Sexual Orientation	Heterosexual	691	79.6
	Homosexual	177	20.4
Number of Sexual Partners	Single	492	56.7
	Multiple	376	43.3
Type of Sexual Practice	Oral	114	13.1
	Anal	92	10.6

	Vaginal	662	76.2
Condom Use (Last Exposure/ Consistent Use)	Yes	259	29.8
	No	609	70.1

Our study included participants from various sociodemographic levels having variable sexual orientation, numbers and types of sexual practices. Majority of participants were unskilled workers (39.8%) and literate (52.5%). Most were married (54.4%) and significant proportion (56.7%) had

single sexual partners. Heterosexual behavior predominated (79.6%), though a fair proportion (20.4%) reported homosexual exposure. Vaginal sexual exposure (76.2%) was the most common route and low condom usage (70.1% non-users) were prevalent.

**Table 3: RPR and TPHA to diagnose syphilis for patients attending STI clinic**

NAME OF THE TEST	NUMBER REACTIVE/ POSITIVE FOR SYPHILIS
Rapid Plasma Reagin (RPR)	58(6.7%)
TPHA (Treponema Pallidum Haemagglutination Assay)	47(5.4%)

**Table 4: AGE/SEX WISE DISTRIBUTION OF RPR REACTIVE (n=58) AND TPHA POSITIVE CASES (n=47)**

AGE IN YEARS	MALE		FEMALE		TRANSGENDE R		TOTAL	
	RPR - 44(75.9%)	TPHA- 38(80.9%)	RPR- 12(20.7%)	TPHA- 9(19.1%)	RPR- 2(3.4%)	TP HA	RPR- 58(6.7%)	TPHA- 47(5.4%)
18-20	7(15.9%)	6(15.8%)	-	-	-	-	7(12.1%)	6(12.8%)
21-25	16(36.3%)	15(39.4%)	4(33.3%)	3(33.3%)	1(50%)	0	21(36.2%)	18(38.3%)
26-30	9(20.4%)	8(21%)	3(25%)	3(33.3%)	1(50%)	0	13(22.4%)	11(23.4%)
31-35	5(11.4%)	5(13.1%)	3(25%)	2(22.2%)	-	-	8(13.8%)	7(14.9%)
36-40	2(4.5%)	1(2.6%)	-	-	-	-	2(3.4%)	1(2.1%)
41-45	2(4.5%)	1(2.6%)	-	-	-	-	2(3.4%)	1(2.1%)
46-50	1(2.3%)	1(2.6%)	2(16.7%)	1(11.1%)	-	-	3(5.2%)	2(2.2%)
>50	2(4.5%)	1(2.6%)	-	-	-	-	2(3.4%)	1(2.1%)

**Table 5: socio-demographic and behavioural characteristics of RPR reactive (n=58) cases**

Characteristics	Category	RPR reactive (n=58)	Percentage (%)
Occupation	Skilled	25	43.1
	Unskilled	21	36.2
	Unemployed	12	20.7
Education Level	Illiterate	36	62.1
	Literate	22	38
Marital Status	Married	28	48.3
	Unmarried	18	31
	Divorced	8	13.8
	Widowed	3	5.2
Sexual Orientation	Heterosexual	39	67.2
	Homosexual	19	32.7
Number of Sexual Partners	Single	36	62.1
	Multiple	22	37.9
Type of Sexual Practice	Oral	8	13.8
	Anal	11	19
	Vaginal	39	67.2
Condom Use (Last Exposure/Consistent Use)	Yes	19	32.7
	No	39	67.2

Our study revealed a maximum RPR positivity among skilled workers (43.1%) and illiterate (62.1%). Most of them were married (48.3%) and a significant proportion (62.1%) had single sexual partners. Heterosexual behavior predominated

(67.2%), though a small proportion reported homosexual exposure (32.7%). Vaginal sexual exposure (67.2%) was the most common route and low condom usage (67.2% non-users) were prevalent.

**Table 6: Distribution of Clinical Symptoms Among RPR Reactive (N=58) Cases**

Clinical Symptoms	MALE (n=44)	FEMALE (n=12)	TRANSGENDER (n=2)	TOTAL (n=58)	Percentage (%)
Painless Genital ulcer	13	-	-	13	22.4
Painless Inguinal lymphadenopathy	9	-	-	9	15.5
Skin rashes	14	5	-	19	32.7
Condylomatalata	4	1	-	5	8.6
Generalized lymphadenopathy	9	2	-	11	19
Asymptomatic with Past history of Genital ulcer (<1 year)	14	5	2	21	36.2
Asymptomatic with Past history of Genital ulcer (>1 year)	3	2	-	5	8.6

Majority of cases were asymptomatic, with a past history of painless genital ulcer (<1 year) (36.2%) followed by skin rashes (32.7%) and painless genital ulcer (22.4%) at present.

**Table 7: Gender wise distribution in various stages of syphilis**

STAGES OF SYPHILIS	FEMALE (n=12)	MALE (N= 44)		TRANSGENDER (n=2)	TOTAL (n=58)	PERCENTAGE (%)
		HETERO-SEXUAL	HOMO-SEXUAL			
PRIMARY SYPHILIS	0	8	5	0	13	22.4
SECONDARY SYPHILIS	5	9	5	0	19	32.7
EARLY LATENT SYPHILIS	5	8	6	2	21	36.2
LATE LATENT SYPHILIS	2	3	0	0	5	8.6

In our study, majority of cases were in early latent syphilis stage (36.2%) followed by secondary syphilis stage (32.7%).

**Table 8: Sex wise distribution of RPR reactive cases (n=58)**

TITRE	MALE (44)	FEMALE (12)	TRANSGENDER (2)	TOTAL (58)
1:2	9(20.4%)	2(16.7%)	2(100%)	13(22.4%)
1:4	11(25%)	1(8.3%)	-	12(20.7%)
1:8	13(29.4%)	6(50%)	-	19(32.7%)
1:16	7(15.9%)	2(16.7%)	-	9(15.5%)
1:32	2(4.5%)	1(8.3%)	-	3(5.2%)
1:64	2(4.5%)	-	-	2(3.4%)

The most commonly reported titers were 1:8 (32.7%) followed by 1:2(22.4%) by RPR test in our study.

**Table 9: Sex wise distribution of quantitative results of TPHA (n=47)**

TITRE	MALE (38)	FEMALE (9)	TOTAL (47)
1:80	2 (4.2%)	-	2(4.2%)
1:160	4(8.5%)	1(2.1%)	5(10.6%)
1:320	2(4.2%)	1(2.1%)	3(6.3%)
1:640	5(10.6%)	2(4.2%)	7(14.8%)
1:1280	9(19.1%)	2(4.2%)	11(23.4%)
1:2560	11(23.4%)	3(6.4%)	14(29.8%)
1:5120	3(6.4%)	-	3(6.4%)
1:10240	2(4.2%)	-	2(4.2%)

The most commonly reported TPHA titers were 1:2560 (29.8%) followed by 1:280(23.4%).

**Table 10: Titre wise distribution of biological false positive cases (n=11)**

TITRE	MALE(6)	FEMALE(3)	TRANSGENDER(2)	TOTAL (11)
1:2	4(36.3%)	2(18.2%)	2(18.2%)	8(72.7%)
1:4	2(18.2%)	1(9.1%)	-	3(27.3%)

Biological False Positive Cases were noted to happen in 11(18.9%) cases in the dilutions 1:2(72.7%) and 1:4(27.3%).

**Table 11: Association of RPR reactive cases status with HIV**

HIV STATUS	MALE		FEMALE		TRANSGENDER		TOTAL
	RPR REACTIVE(n=44)	RPR NEGATIVE (n=490)	RPR REACTIVE(n=12)	RPR NEGATIVE (n=276)	RPR REACTIVE(n=2)	RPR NEGATIVE (n=44)	
POSITIVE	4	5	1	1	0	0	11
NEGATIVE	40	485	11	275	2	44	857

The RPR reactive cases were tested for HIV and 5 cases were found to have HIV co-infection. Among these 5 cases, 2 were newly detected HIV cases and 3 were known cases on antiretroviral therapy. The stages of presentations of the 5 HIV co-infected cases were primary syphilis (1 case), Secondary syphilis (2 cases) and early latent syphilis (2 cases).

## DISCUSSION

Sexually Transmitted Infections (STIs) are major public health problems in all populations and socioeconomic groups worldwide. Nontreponemal tests are suitable for screening or diagnosis in conjunction with medical history and physical

examination. The nontreponemal tests can be false positive in conditions like HIV etc. So, Treponemal tests are done to confirm the results of reactive nontreponemal tests.

This study aims on the serological diagnosis of Syphilis by both Rapid plasma Reagin (RPR), a nontreponemal test as screening test and Treponema Pallidum Hemagglutination Assay (TPHA), a treponemal test as confirmatory tests. A total of 868 consecutive blood samples from patients attending STI clinic were collected and analyzed.

Our study included participants from patients attending STI clinic. This study revealed, the maximum number of participants were from the age group of 26-30 years 264(30.4%) and majority of patients were males 534 (61.5%) (Table 1, Chart 1). This is in concordance with the study conducted by Susmitha Maity et al in which majority of the patients attending STI clinic were males (53.9%) and from age group 25-29 years (24.1%).<sup>[8]</sup>

Majority of participants were unskilled workers (39.8%) and literate (52.5%). Most were married (54.4%) and significant proportion had single sexual partners (56.7%). Heterosexual behavior predominated (79.6%), though a small proportion reported homosexual exposure (20.4%). Vaginal sexual exposure (76.2%) was most common route and low condom usage (70.1% non-users) were reported (Table 2).

In this study, 58(6.7%) patients attending STI clinic were reactive for Syphilis by RPR method. Out of 58 RPR reactive cases, 47(81%) were positive for syphilis and 11(18.9%) were negative by TPHA method (Table 3, Chart 2) compared to 81.6% in study of Arti et al.<sup>[9]</sup> The seroprevalence of Syphilis among patients attending STI clinic were 5.4%. This is in concordance with the study conducted by Schneider et al in which seroprevalence of syphilis among STI clinic attendees were 5.4%.<sup>[10]</sup> Another study conducted by Clarissa et al showed a seroprevalence of syphilis among STI clinic as 9.6%, which was higher than our study.<sup>[3]</sup>

The maximum number of RPR and TPHA positive cases were from the age group of 21-25 years 21(36.2%) and 18(38.3%) respectively. 44(75.9%) male, 12(20.7%) female and 2(3.4%) transgenders were RPR positive and 38(80.9%) male and 9(19.1%) female were TPHA Positive (Table 4, Chart 3). This is in concordance with the study conducted by Shah et al in which majority of the patients showed positive for Syphilis were male (53.9%).<sup>[11]</sup>

Among seropositive cases, 43.1% were skilled workers and 62.1% were illiterate. Most were married (48.3%) and significant proportion had single sexual partners (62.1%). Heterosexual behavior predominated (67.2%), though a small proportion reported homosexual exposure (32.7%). Vaginal sexual exposure (67.2%) was the most common route and low condom usage (67.2% non-users) were prevalent indicating increased transmission risk (Table 5).

In this study, majority of cases had past history of painless genital ulcer(<1year) (36.2%) followed by skin rashes (32.7%) and painless genital ulcer (22.4%) (Table 6). Majority of cases were in early latent syphilis stage followed by secondary syphilis stage (Table 7) and majority of cases have dilution 1:8 (32.7%) by RPR (Table 8). A study conducted by Shruti et al showed majority of cases have dilution 1:16 by RPR.<sup>[12]</sup>

Most commonly reported titer in this study was 1:2560(29.8%) followed by 1:280(23.4%) dilution by quantitative test of TPHA (Table 9) and Biological False Positive Cases in this study were 11 (18.9%) noted in dilutions 1:2(72.7%) and 1:4(27.3%) dilutions (Table 10) compared to 12.4% in study conducted by Arti et al.<sup>[9]</sup>

Among 58 RPR reactive cases, 5 cases had HIV infection. 2 were newly detected HIV patient and 3 were on antiretroviral therapy. Out of 5 HIV coinfecting cases 1 had primary syphilis, 2 had secondary syphilis and 2 had early latent syphilis (Table 11).

## CONCLUSION

The present study highlights that syphilis continues to remain an important public health problem among sexually active populations attending STI clinics. A higher burden was observed among males and individuals in the younger age group, emphasizing the need for focused awareness and preventive interventions in these populations.

The study demonstrated the importance of combining nontreponemal and treponemal tests for accurate diagnosis. While RPR served as an effective screening test, confirmation by TPHA was essential to avoid false positive results and improve diagnostic reliability.

Behavioral and sociodemographic factors such as unsafe sexual practices, inconsistent condom usage, and HIV co-infection were found to contribute significantly to the transmission of syphilis. These findings stress the importance of integrated STI screening, counseling, partner notification, and early treatment services. Strengthening public health measures including routine screening, sexual health education, promotion of consistent condom use, and adherence to national STI control guidelines is essential for reducing the burden of syphilis and other sexually transmitted infections in the community.

## REFERENCES

1. Laboratory manual for diagnosis of Sexually Transmitted and Reproductive Tract Infections, NACO
2. Genital ulcer disease: how worrisome is it today? A status report from new delhi, India ,sumathimuralidhar, richatalwar, deepa anil kumar, joginderkumar, manjubala, nilofar khan, v.ramesh
3. Evaluation of serological tests for the diagnosis of syphilis Clarrisa J.Lyngdoh, Mndira Ramudamu, Manika Agarwal, Shikha verma, Abhijit prasad, 2024.

4. Bailey & Scott's DIAGNOSTIC MICROBIOLOGY Fifteenth Edition
5. National technical guidelines on Sexually Transmitted Infections and Reproductive Tract Infections 2024, NACO
6. Lum B, Sergent SR, Rapid Plasma Reagin, Starpearls 2025 Jan.
7. Laboratory diagnostic tools for syphilis: current status and future prospects 2020
8. Syphilis seroprevalence among patients attending a Sexually Transmitted Disease Clinic in West Bengal, India Susmita Maity, Somesh Chandra Bhunia, Subrata Biswas and Malay kumar Saha 2011
9. Comparative study of serological test RPR & VDRL for diagnosis of Syphilis at tertiary care hospital. Arti B. Ninama and Mihir R. Dedun 2018
10. Schneider, J.A., Lakshmi, V., Dandona, R., et al. (2010) :Population based seroprevalence of HSV-2 and Syphilis in Andhra Pradesh state of India. BMC Infect. Dis., 10, 59.
11. Shah BJ, Karia DR, Pawara CL. Syphilis: is it making resurgence? Indian J Sex Transm Dis AIDS 2015;36:178-81
12. Syphilis: is it back with a bang? Shruti Kamat, Aditi Vaghasia, Dharmender J, Kajal G. Kansara, Bela J. Shah 2023.