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Original Research Article

SEROPREVALENCE OF SYPHILIS AND BIOLOGICAL FALSE POSITIVE REACTIONS AMONG PATIENTS ATTENDING A TERTIARY CARE CENTRE

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ABSTRACT

Syphilis screening conventionally relies on the sensitive but non-specific Rapid Plasma Reagin (RPR) assay, with reactive results requiring confirmation by a specific treponemal test such as the Treponema Pallidum Haemagglutination Assay (TPHA) to differentiate true infection from biological false-positive (BFP) reactions. This descriptive cross-sectional study analysed 20 RPR-reactive serum samples referred to the Department of Microbiology, Government Medical College, Kallakurichi, for confirmatory TPHA testing. Demographic details (age, sex, referring ward), RPR titre, and TPHA result were recorded for each sample, and data were analysed using Python (Pandas). Of the 20 RPR-reactive samples, 11 (55%) were TPHA-reactive (true-positive), eight (40%) were TPHA non-reactive (biological false-positive), and one (5%) was equivocal. The dermatology/sexually transmitted infection ward accounted for the majority of referrals (14/20, 70%), followed by the obstetrics-antenatal ward (2/20, 10%). The median RPR titre was higher among true-positives (1:8) than biological false-positives (1:3, range 1:2-1:4); all four samples with the lowest titre (1:2) were biological false-positive. Among true-positive cases, seven of ten (70%) were female, whereas among biological false-positive cases, five of eight (62.5%) were male. Forty percent of RPR-reactive samples in this referral-based study were biological false-positive, and false-positivity was associated with a lower RPR titre. These findings reinforce the need for mandatory treponemal confirmation before diagnosing syphilis or initiating treatment. The small sample size and absence of a screening denominator warrant caution in generalising these findings, and a larger, prospective study is recommended.

Keywords: Syphilis; Rapid Plasma Reagin; Treponema Pallidum Haemagglutination Assay; Biological False-Positive; Seroprevalence; Cross-Sectional Studies.

INTRODUCTION

Syphilis is a sexually transmitted, systemic infection caused by the spirochaete Treponema pallidum, transmitted through sexual contact, blood transfusion, and from mother to foetus.[1] Although curable with antibiotics, it remains a major cause of reproductive morbidity and foetal wastage in resource-poor countries.[2] Several tertiary-care surveillance studies across India report a rise in syphilis cases detected over the last decade, underscoring its continuing morbidity and economic burden.[9,11] The World Health Organization's global strategy on sexually transmitted infections estimated approximately 7.1 million new syphilis infections worldwide in 2020, part of an estimated 374 million new infections with the four major curable sexually transmitted infections, with a disproportionate burden in low- and middle-income countries where confirmatory testing infrastructure remains limited.[6]

Serological testing remains the cornerstone of both screening and confirmation. Screening relies on non-treponemal tests such as the RPR or Venereal Disease Research Laboratory (VDRL) assay, which measure antibody titre against the cardiolipin-lecithin-cholesterol antigen. Confirmation requires a specific treponemal test such as the TPHA. Non-treponemal tests are moderately sensitive and specific, and commonly yield BFP reactions in pregnancy, autoimmune disorders, viral infections, and older patients. Reactivity on a non-treponemal test with non-reactivity on a treponemal test defines a BFP reaction. Such patients merit careful counselling, since they often undergo unwarranted anxiety and treatment, including unnecessary antibiotic use, particularly during antenatal screening.[5] Recognising BFP promptly is clinically important, as it prevents unnecessary partner notification, psychological distress, and antibiotic exposure, particularly in antenatal patients, where the differential diagnosis also extends to autoimmune and viral aetiologies.

Data on the proportion of RPR-reactive sera that prove to be BFP remain limited, particularly from tertiary-care centres in the Kallakurichi region. This study aimed to characterise a RPR-reactive referral population and describe the factors associated with a BFP outcome.

MATERIALS AND METHODS

This descriptive, laboratory-based cross-sectional study was conducted at the Department of Microbiology, Government Medical College, Kallakurichi, Tamil Nadu, India. The study comprised 20 serum samples previously reactive on RPR testing, referred from the dermatology/sexually transmitted infection (STI), obstetrics and antenatal (OBG/OBG-ANC), chest medicine, ENT, and neonatal units for confirmatory TPHA testing.

The master dataset comprised only RPR-reactive samples; a denominator of total samples screened (RPR-reactive plus non-reactive) by each department was unavailable. Hence, overall or department-wise seroprevalence of syphilis by RPR could not be calculated, and all percentages reported represent proportions of the 20 RPR-reactive samples rather than of all samples screened in each department. This is noted as a limitation of the study.

All samples were tested for RPR reactivity by card test with serial dilution to determine titre, and RPR-reactive samples were further tested by TPHA. Samples reactive for both RPR and TPHA were classified as true-positive; those reactive for RPR but non-reactive for TPHA were classified as BFP. One sample with an equivocal TPHA result was excluded from calculation of the proportion of BFP cases.

De-identified data (sample code, age, sex, referring ward, residential locality, RPR titre, TPHA result) were extracted from the master chart. One neonatal sample had age recorded in days of life rather than years and was excluded from analyses by age category. Descriptive statistics were used given the limited sample size; no inferential statistics were applied because of small cell sizes. Analysis was performed using Python (Pandas) and Matplotlib.

Patient data were extracted from routine laboratory records without identifying information; residential locality is recorded only as a village name and is not reported at the individual level. The study was approved by the Institutional Ethics Committee, Government Medical College, Kallakurichi (Proposal No. GMCK/IEC/23/2025, approved 27 October 2025).

RESULTS

Twenty RPR-reactive serum samples were referred for confirmatory TPHA testing. Patient age ranged from eight to 70 years (median 19 years); there were ten females (50%), nine males (45%), and one neonate (5%) (Table 1).

Of these, 11 samples (55%) were TPHA-reactive (true-positive), eight (40%) were TPHA non-reactive (BFP), and one (5%) was equivocal (Table 1, Figure 1A). RPR titre ranged from 1:2 to 1:32 (Table 1, Figure 1B).

The dermatology/STI ward accounted for the largest share of referrals (70%), followed by the obstetrics-antenatal (10%) and obstetrics (5%) wards (Table 1). Across age groups (n = 19, excluding the neonatal sample), true-positives were distributed fairly evenly between the 18-25 and 26-40 year groups (four each), while a higher proportion of BFP cases occurred in the 26-40 year group (50%, n = 4). The single sample aged under 18 years was BFP (Table 1). The eight BFP samples were drawn from three referring wards (dermatology/STI, obstetrics, and ENT), whereas true-positive samples spanned four wards, including dermatology/STI, obstetrics-antenatal, chest medicine, and the neonatal unit.

By sex, females comprised 63.6% (7/11) of true-positives and 37.5% (3/8) of BFP cases, while males comprised 27.3% (3/11) of true-positives and 62.5% (5/8) of BFP cases. The one neonatal sample was true-positive (sex unspecified).

Table 1: Demographic profile, RPR titre, and TPHA outcome distribution of the study population (N = 20)

Characteristic	Category	n (%)	True positive, n	BFP, n	Equivocal, n
Age (years)†	< 18	1 (5.3)	0	1	0
	18-25	6 (31.6)	4	2	0
	26-40	8 (42.1)	4	4	0

	> 40	4 (21.1)	2	1	1
Sex	Female	10 (50.0)	7	3	0
	Male	9 (45.0)	3	5	0
	Not recorded (neonate)	1 (5.0)	1	0	0
Referring ward/dept.	Dermatology / STI	14 (70.0)	7	6	1
	Obstetrics-antenatal (OBG-ANC)	2 (10.0)	2	0	0
	Obstetrics (OBG)	1 (5.0)	0	1	0
	Chest medicine	1 (5.0)	1	0	0
	ENT	1 (5.0)	0	1	0
	Neonatal unit	1 (5.0)	1	0	0
RPR titre	1:2	5 (25.0)	1	4	0
	1:4	5 (25.0)	3	2	0
	1:8	5 (25.0)	3	1	1
	1:16	3 (15.0)	2	1	0
	1:32	2 (10.0)	2	0	0
Total	-	20 (100.0)	11	8	1

† Age-group percentages calculated on n = 19, as one sample in the neonatal unit recorded age in days.

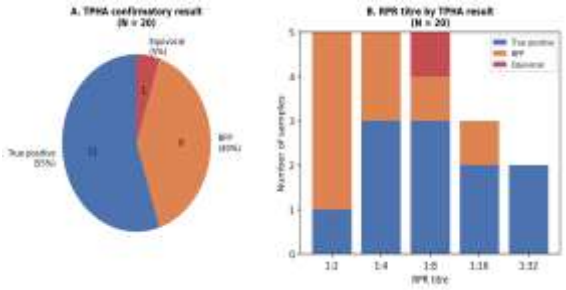

Figure 1: TPHA confirmatory test results (A) and RPR titre distribution by TPHA result (B) among RPR-reactive samples (N = 20)

DISCUSSION

In this referral-based study of 20 RPR-reactive samples, 55% were true-positive and 40% were BFP, comparable to other reports from tertiary-care hospitals in India.[1,3,12]

Most cases (70%) were referred from the dermatology/STI ward, reflecting this centre's preference for RPR testing in patients with suspected sexually transmitted infection, in contrast to population-based screening such as antenatal or blood-donor surveillance.[3,8,9]

A BFP result was more common among samples with lower RPR titre: four of the five samples with the lowest titre (1:2) were BFP, whereas both samples with the highest titre (1:32) were true-positive (Table 1). This is consistent with the general observation that BFP reactions tend to show a lower titre than true infections, although individual patients still require evaluation by a specific treponemal test.[1,4] Reporting RPR titre alongside the qualitative result may help ordering clinicians anticipate the likelihood of a false-positive while confirmatory TPHA testing is pending, particularly for the low-titre reactive samples that make up the bulk of ambiguous referrals. Given the high proportion of BFP among low-titre samples, laboratories may consider explicitly flagging low-titre RPR-reactive reports as provisional pending TPHA confirmation, which could reduce premature clinical action and patient anxiety while confirmatory results are awaited.

Similar referral-based series from other Indian tertiary-care centres have reported BFP proportions broadly in keeping with the present findings, although differences in referral source and underlying population risk profile limit direct comparison.[8,9,10]

Only two antenatal samples were included, both true-positive; this small number precludes meaningful comment on the prevalence of pregnancy-associated BFP reactions described in larger series.[5,6]

This analysis has several limitations. The overall sample size is small (n = 20). Only reactive samples were analysed; the absence of a denominator of total samples screened precludes estimation of overall or department-wise seroprevalence by RPR. This is a single-centre, retrospective analysis without adjustment for confounders. Finally, information on pregnancy status, HIV, or other conditions associated with BFP was unavailable in the master chart and could not be analysed as predictors of BFP. These limitations should be addressed in a larger, prospective study with an adequate denominator.

CONCLUSION

In this study of 20 RPR-reactive samples, 40% proved biological false-positive rather than true syphilis infection; among true-positives, half were aged 26-40 years and 63.6% were female. These findings reinforce that a positive non-treponemal test should not be interpreted in isolation, and specific treponemal confirmation (e.g., TPHA) is mandatory before informing patients or initiating treatment. A larger, adequately powered study is needed to generate local seroprevalence estimates and further evaluate the association between titre and BFP.

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REFERENCES

1. Srivastava P, Bhargava A, Bansal S, Khunger N, Saxena AK. Trends of syphilis seroprevalence at a tertiary care center in New Delhi, India: A 10-year analysis. Indian J Sex Transm Dis. 2023;44:20-3.
2. Centers for Disease Control and Prevention. CDC laboratory recommendations for syphilis testing, United States, 2024. Atlanta: CDC; 2024.
3. Paudel P, Arumugam M, Sinha P. Seroprevalence of syphilis among blood donors in a tertiary care hospital in and around Koodapakkam, Puducherry, India. Indian J Pathol Oncol. 2024;11(3):243-7.
4. World Health Organization. WHO guideline on syphilis screening and treatment for pregnant women. Geneva: WHO; 2017.
5. Department of AIDS Control, Ministry of Health and Family Welfare, Government of India. National Guidelines on Prevention, Management and Control of Reproductive Tract Infections and Sexually Transmitted Infections. New Delhi: MoHFW; 2014.
6. World Health Organization. Global Health Sector Strategy on Sexually Transmitted Infections, 2022-2030. Geneva: WHO; 2022.
7. Centers for Disease Control and Prevention. Sexually Transmitted Infections Treatment Guidelines, 2021: Syphilis. MMWR Recomm Rep. 2021.
8. Kumar A, Peter CV, et al. Upward trends of syphilis in the non-pregnant adults: a six-year report on clinical and epidemiological profile of syphilis from a tertiary care center, India. Front Public Health. 2022;10:908591.
9. Sethi S, Mewara A, Hallur V, Prasad A, Sharma K, Raj A. Rising trends of syphilis in a tertiary care center in North India. Indian J Sex Transm Dis AIDS. 2015;36(2):140-3.
10. Jain A, Mendiratta V, Chander R. Current status of acquired syphilis: A hospital-based 5-year study. Indian J Sex Transm Dis AIDS. 2012;33(1):32-4.
11. Sharma A, Rawat D, Bhalla P. Trend of syphilis in a tertiary care hospital, New Delhi: 2001-2009. Indian J Public Health. 2013;57(2):117-8.
12. Ninama AB, Dedun MR. Comparative study of serological tests RPR and TPHA for diagnosis of syphilis at a tertiary care hospital. Int J Curr Microbiol Appl Sci. 2018;7(9):3771-5.