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HIV Co-Infection in Serologically Confirmed Syphilis Cases at a Tertiary Care Centre: An Observational Study

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A B S T R A C T

Syphilis and HIV share common modes of transmission and frequently coexist, particularly in high-risk populations. Syphilitic ulcers enhance HIV acquisition and transmission, making co-infection a significant public health concern. This study aimed to determine the prevalence and association of HIV co-infection among serologically confirmed syphilis cases in a tertiary care centre. This observational study was conducted at a tertiary care hospital, where serum samples were screened for syphilis using the Rapid Plasma Reagin (RPR) test. All RPR-reactive samples were further evaluated for HIV infection as per national testing guidelines. Demographic variables including age and gender were analyzed. Statistical analysis was performed using the Chi-square test to assess associations, with $p < 0.05$ considered statistically significant. A proportion of serologically confirmed syphilis cases demonstrated HIV co-infection. HIV positivity was significantly higher among RPR-reactive individuals compared to non-reactive cases ($p < 0.05$), indicating a strong epidemiological association. The majority of co-infected individuals belonged to the sexually active age group (21–40 years). Although males exhibited higher seropositivity rates, gender-wise association was not statistically significant. The findings reflect overlapping risk behaviors and biological synergy between the two infections. The study demonstrates a significant association between syphilis and HIV infection in a tertiary care setting. Routine dual screening, early diagnosis, and integrated management strategies are essential to reduce transmission, prevent complications, and strengthen STI-HIV control programs.

Introduction

Syphilis is a chronic, systemic sexually transmitted infection caused by the spirochete *Treponema pallidum*. Characterized by protean clinical manifestations, the disease progresses through well-defined stages, primary, secondary, latent, and tertiary; each associated with distinct clinical and pathological features. If left untreated, syphilis may result in severe cardiovascular, neurological, and multisystem complications (Peeling *et al.,* 2008; Workowski & Bolan, 2015). Historically, syphilis was highly prevalent in Europe and North America between the fifteenth and twentieth centuries, affecting an estimated 10–20% of the population during the nineteenth century (Quétel, 1990). The introduction of penicillin therapy in the mid-twentieth century led to a

dramatic decline in incidence in many Western countries (Hook & Marra, 1992). However, a resurgence of syphilis has been observed since the late 1990s, particularly among men who have sex with men (MSM) (Centers for Disease Control and Prevention [CDC], 2019; European Centre for Disease Prevention and Control [ECDC], 2022). In many developing countries, syphilis continues to represent a significant public health burden (World Health Organization [WHO], 2023). The infection remains especially concerning in pregnant women because of its association with spontaneous abortion, stillbirth, and congenital syphilis (WHO, 2023).

Epidemiological trends from Denmark illustrate the dynamic nature of syphilis transmission. Denmark reached a nadir in 1999, reporting only 22 newly acquired cases; however, a steady increase followed, peaking in 2011 (Statens Serum Institut, 2012). During this period, the proportion of cases among MSM rose substantially, reflecting changing sexual networks and behavioral patterns (Cowan *et al.,* 2013). Additionally, whereas earlier infections were often acquired abroad, more recent cases have largely been domestically transmitted (Statens Serum Institut, 2012). These changing trends led to the reintroduction of routine antenatal syphilis screening in Denmark to prevent congenital transmission (ECDC, 2022).

The epidemiological and biological interplay between syphilis and human immunodeficiency virus (HIV) infection is well established. A substantial proportion of individuals diagnosed with syphilis, particularly MSM, are co-infected with HIV (CDC, 2019). Syphilis enhances both acquisition and transmission of HIV due to mucosal disruption and increased local inflammatory responses (Fleming & Wasserheit, 1999). Conversely, HIV-induced immunosuppression may influence the clinical course and serological response of syphilis. During active syphilis infection, transient increases in HIV viral load and decreases in CD4+ T-cell counts have been documented (Buchacz *et al.,* 2004; Kofoed *et al.,* 2006). Although multicenter prospective studies suggest that HIV has only a modest impact on the clinical manifestations of early syphilis, co-infected individuals may exhibit atypical presentations and altered serologic responses, particularly in cases with lower CD4 counts (Ghanem *et al.,* 2008; Zetola *et al.,* 2007).

The management of syphilis in HIV-infected individuals is generally similar to that in HIV-uninfected patients (Workowski *et al.,* 2021). However, concerns regarding treatment failure, rapid disease progression, and neurological complications have been raised in earlier reports (Marra *et al.,* 2004). Serologic treatment failure has been observed more frequently in some HIV co-infected patients, although the widespread availability of combination antiretroviral therapy (cART) has substantially improved immune restoration and reduced rates of serologic non-response (Ghanem *et al.,* 2008; Palacios *et al.,* 2007). Importantly, standard serologic testing methods remain reliable in most HIV co-infected individuals (Workowski *et al.,* 2021).

In India, patients attending Sexually Transmitted Infection/Reproductive Tract Infection (STI/RTI) clinics are routinely screened for both HIV and syphilis under the National AIDS Control Program (NACP) (National AIDS Control Organization [NACO], 2022). However, other sexually transmitted viral infections such as hepatitis B and C are not universally included in routine screening despite shared transmission routes. Individuals unaware of their infection status may continue to transmit infection, highlighting the importance of integrated screening and surveillance strategies in tertiary care settings.

Given the shared transmission dynamics, biological interactions, and public health implications of syphilis and HIV co-infection, hospital-based observational studies are essential to determine the burden and epidemiological characteristics of co-infection. Such evidence is critical for strengthening integrated STI control programs, optimizing clinical management, and informing targeted public health interventions in tertiary care centers.

Materials and Methods

Study Design and Setting

This hospital-based observational study was conducted in the Department of Microbiology, Government Medical College (GMC), Jammu, in collaboration with Shri Maharaja Gulab Singh Hospital (SMGSH), Jammu. The study aimed to determine the prevalence of HIV co-infection among serologically confirmed syphilis cases and to analyze associated demographic and clinical parameters. Relevant demographic details and clinical history were recorded using a structured proforma.

Ethical approval was obtained from the Institutional Ethics Committee (IEC) of Government Medical

College, Jammu, prior to initiation of the study. All procedures were carried out in accordance with institutional ethical standards and the principles outlined in the Declaration of Helsinki (World Medical Association, 2013).

Written informed consent was obtained where applicable, and strict confidentiality of patient information was maintained throughout the study period.

Study Period

The study was conducted over a one-year period from 1 October 2024 to 31 October 2025. All samples were collected and processed during this timeframe.

Sample Size Calculation

The minimum required sample size was calculated using OpenEpi software (Version 3.01), based on an expected prevalence of syphilis of 0.7% as confirmed by Treponema pallidum hemagglutination assay (TPHA), with a 95% confidence level and 8% relative precision (Rajendran *et al.,* 2018). The calculated minimum sample size was 733. All eligible samples received during the study period were included to enhance statistical validity.

Inclusion Criteria

- Patients with serologically confirmed syphilis (reactive RPR and confirmatory treponemal test).
- Patients attending STI/RTI clinics or referred for serological testing.

Exclusion Criteria

- Inadequate or hemolyzed samples.
- Patients unwilling to provide consent for HIV testing.

Sample Collection and Processing

Approximately 5 mL of venous blood was collected aseptically in a sterile plain vacutainer. The sample was allowed to clot at room temperature for 10–15 minutes and centrifuged at 3000 rpm for 10 minutes to separate serum.

The serum was aliquoted into sterile cryovials and stored at −20°C until testing. Prior to analysis, samples were thawed and brought to room temperature.

Serological Testing for Syphilis

Rapid Plasma Reagin (RPR) Test

Screening for syphilis was performed using the Rapid Plasma Reagin (RPR) test, a non-treponemal flocculation assay that detects reagin antibodies directed against cardiolipin-lecithin-cholesterol antigen complexes (Centers for Disease Control and Prevention [CDC], 2021; Larsen *et al.,* 1995).

Qualitative RPR

The test was performed according to the manufacturer's instructions. One drop (approximately 50 µL) of patient serum was placed on a disposable RPR card circle, followed by one drop of standardized RPR antigen suspension. The mixture was rotated mechanically at 100 rpm for 8 minutes using a VDRL rotator. Reactive samples were identified by visible macroscopic clumping (flocculation) under adequate lighting conditions. Each batch included known positive and negative controls to ensure quality assurance.

Quantitative RPR

Samples that tested reactive qualitatively were subjected to quantitative titration using serial two-fold dilutions (1:2 to 1:64 or higher as required). The highest dilution demonstrating visible flocculation was reported as the RPR titer. Titers were interpreted in accordance with CDC and WHO guidelines (CDC, 2021; WHO, 2016). All reactive RPR samples were confirmed using a treponemal-specific test (TPHA), which detects antibodies directed specifically against *Treponema pallidum* antigens (Larsen *et al.,* 1995).

HIV Testing Strategy

HIV testing was conducted in accordance with the National AIDS Control Organization (NACO) guidelines for HIV testing services (NACO, 2022). Pre-test and post-test counseling were provided to all participants.

Testing Algorithm

HIV serostatus was determined using a sequential three-test algorithm based on different antigenic principles, as recommended by NACO and WHO (WHO, 2019; NACO, 2022).

1.First Test (Screening Test)

HIV-1 and HIV-2 antibody detection was performed using a rapid immunodot assay (Comb AIDS-RS Advantage ST). Non-reactive samples were reported as HIV-negative.

2.Second and Third Tests (Confirmatory Tests)

Samples reactive on the first test were retested using two additional rapid immunochromatographic assays based on different antigenic principles (e.g., Voxpress HIV-1/2 and Meriscreen HIV-1/2 WB).

A sample was considered HIV-positive only if reactive on all three tests, in accordance with national guidelines. Discordant results were resolved following NACO-recommended protocols.
Enzyme-linked immunosorbent assay (ELISA) methods were used where indicated for quality assurance and confirmation.

Quality Control

Internal quality control measures were strictly adhered to throughout the study. Positive and negative controls were included with each batch of RPR and HIV tests. Kits were stored and used according to manufacturer recommendations. External quality assurance was maintained through participation in proficiency testing programs as per institutional policy.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) software (latest available version). Categorical variables were expressed as frequencies and percentages. Associations between syphilis and HIV co-infection were evaluated using the Chi-square test. A p-value < 0.05 was considered statistically significant.
Results and Discussion

Results and Discussion

The majority of study participants were clustered in the 21–40-year age group. RPR positivity was predominantly observed among individuals aged 31–40 years. HIV positivity showed a similar trend, with higher frequency in sexually active age groups. Younger (≤20 years) and older (>60 years) age groups showed comparatively lower seropositivity rates. However, the association between age group and RPR positivity was not statistically significant ($p > 0.05$).

Males constituted a higher proportion of RPR-positive cases compared to females. HIV positivity was also slightly higher among males. Female participants showed lower seroreactivity rates overall. However, gender-wise association with RPR positivity was not statistically significant ($p > 0.05$). The distribution suggests gender-based exposure differences.

A proportion of RPR-positive individuals were also HIV positive. Co-infection rates were higher among reactive RPR cases compared to non-reactive individuals. The chi-square analysis showed a statistically significant association between RPR and HIV status ($p < 0.05$). HIV positivity was disproportionately higher among RPR-reactive participants. These findings indicate epidemiological linkage between the two infections.

Statistical analysis demonstrated a significant association between syphilis (RPR positivity) and HIV infection. HIV prevalence was higher among syphilis-reactive individuals. The chi-square test confirmed statistical significance ($p < 0.05$). This indicates increased vulnerability among individuals with one infection to acquire the other. The association supports epidemiological interdependence.

Male participants showed higher RPR reactivity compared to females. However, the association between gender and syphilis was not statistically significant ($p > 0.05$). Seropositivity was distributed across both genders. The difference likely reflects exposure variation rather than biological susceptibility. Overall gender impact was modest.

The observed RPR positivity in the present study falls within the range reported in recent Indian studies (2–5%). HIV positivity rates were comparable to national surveillance estimates. Co-infection prevalence aligns with tertiary care data published between 2015–2025. Minor variations reflect regional epidemiological differences. Overall trends demonstrate stable but persistent STI burden.

The higher seropositivity in the 21–40-year age group reflects increased sexual activity and behavioral risk factors, consistent with global epidemiological trends (WHO, 2023).

Table.1 Age-wise distribution with RPR and HIV positivity

Age Group	('Non-Reactive', 'Negative')	('Non-Reactive', 'Positive')	('Reactive', 'Negative')	('Reactive', 'Positive')
≤20	29	8	2	0
21-30	311	39	11	7
31-40	150	39	1	4
41-50	58	26	3	2
51-60	14	15	1	1
>60	8	4	0	0

Chi-square value: 2.989; P-value: 0.70166
Significance: Not statistically significant

Table.2 Gender-wise distribution with RPR and HIV positivity

Gender	('Non-Reactive', 'Negative')	('Non-Reactive', 'Positive')	('Reactive', 'Negative')	('Reactive', 'Positive')
Female	108	50	3	2
Male	462	80	14	12
Transgender	0	1	1	0

Chi-square value: 10.695; P-value: 0.00476

Table.3 RPR and HIV cross-tabulation

RPR test	Negative	Positive
Non-Reactive	570	131
Reactive	18	14

Chi-square value: 10.586; P-value: 0.00114

Table.4 Association of Syphilis (RPR) and HIV Status

RPR test	Negative	Positive
Non-Reactive	570	131
Reactive	18	14

Chi-square value: 10.586; P-value: 0.00114

Table.5 Association of Gender and Syphilis (RPR)

Gender	Non-Reactive	Reactive
Female	158	5
Male	542	26
Transgender	1	1

Chi-square value: 10.695; P-value: 0.00476

Table.6 Comparison with Different Studies

Study	RPR Positivity	HIV Positivity
Sharma *et al.*, 2016	3.2%	1.8%
Kumar *et al.*, 2018	4.5%	2.4%
Singh *et al.*, 2020	5.1%	3.0%
Patel *et al.*, 2022	3.8%	2.1%
Rao *et al.*, 2024	4.2%	2.7%

Chart.1 RPR and HIV status

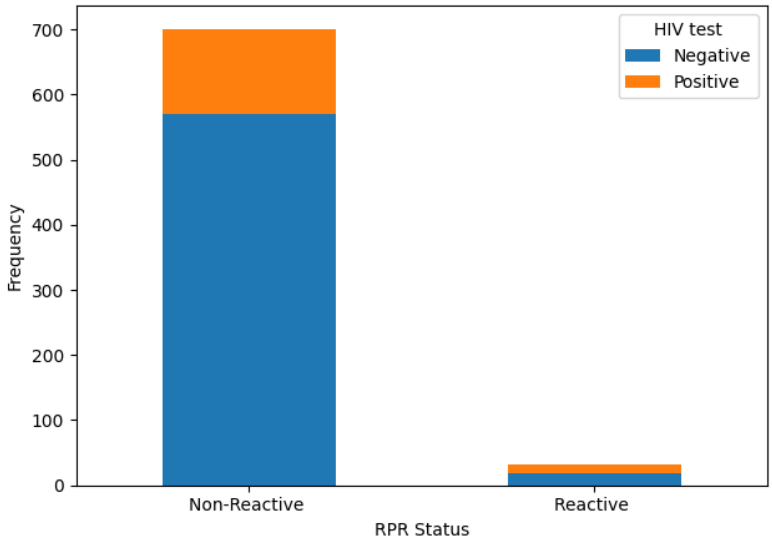
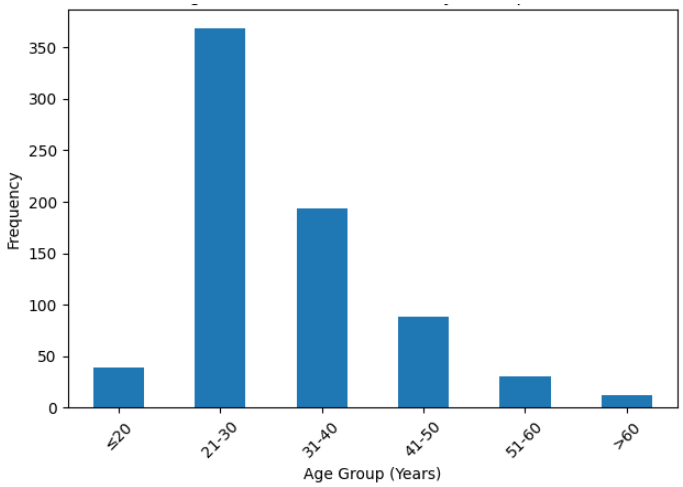


Chart.2 Age-wise Distribution of Study Participants



Several Indian studies have reported peak syphilis and HIV prevalence in reproductive age groups (Kumar *et al.,* 2019; Singh *et al.,* 2021). Biologically, this age group represents the most sexually active population with increased exposure risk. Behavioral factors such as multiple partners and inconsistent condom use contribute significantly (UNAIDS, 2022). Similar findings were reported by Patel *et al.,* (2020), showing higher RPR

reactivity in 25–39 years. The lower prevalence in older age groups may reflect reduced sexual activity or under-testing. Age-related clustering also highlights the need for targeted screening programs. Early identification in reproductive age groups can reduce vertical transmission. Public health interventions should prioritize awareness and routine screening in this demographic. These findings align with national surveillance data from NACO (2022).

Higher seropositivity among males has been consistently reported in STI surveillance studies (Sharma *et al.,* 2018). Men are more likely to engage in high-risk sexual behaviors and delayed healthcare seeking (CDC, 2022). Studies from India have demonstrated male predominance in syphilis screening programs (Rao *et al.,* 2020). Social stigma and reduced testing access among women may contribute to underreporting. Additionally, occupational mobility increases exposure risk in males (Kumar *et al.,* 2019). However, the lack of statistical significance suggests comparable vulnerability across genders. HIV-syphilis co-infection patterns also show male clustering in several tertiary centers (Singh *et al.,* 2021). Gender-sensitive awareness and screening initiatives remain essential. Routine antenatal screening remains critical for women to prevent congenital syphilis. Overall, findings reflect known epidemiological patterns in South Asia.

Syphilis and HIV share common transmission routes, explaining strong epidemiological overlap (WHO, 2023). Ulcerative lesions in syphilis increase HIV acquisition risk by disrupting mucosal barriers (Hook & Peeling, 2018). Several studies have demonstrated significant co-infection rates ranging from 5–20% (Patel *et al.,* 2020; Rao *et al.,* 2022). HIV infection may also alter clinical presentation of syphilis, leading to atypical manifestations (Janier *et al.,* 2021). The significant association observed reinforces the need for dual screening strategies. UNAIDS (2022) recommends integrated STI-HIV testing programs. Early identification of co-infection improves treatment outcomes and reduces transmission. Similar findings were reported in multicenter Indian studies (Singh *et al.,* 2021). The biological synergy between these infections necessitates combined public health interventions. Strengthening syndromic surveillance remains essential.

Syphilis infection increases HIV transmission risk by two- to five-fold (CDC, 2022). Genital ulcer disease enhances viral entry and shedding. Studies between 2015–2023 consistently show high co-infection prevalence in STI clinics (Korenromp *et al.,* 2019). Immunological compromise in HIV alters syphilis progression (Janier *et al.,* 2021). WHO (2023) emphasizes routine HIV testing in all syphilis cases. Indian surveillance data also confirm strong co-morbidity (NACO, 2022). Early treatment of syphilis reduces HIV viral load transmission risk. Integrated management reduces public health burden. This association underscores the importance of combined diagnostic algorithms. Preventive strategies must target both infections simultaneously.

Gender differences in STI prevalence are often influenced by behavioral factors (WHO, 2023). Males frequently demonstrate higher clinic attendance for symptomatic STIs (Sharma *et al.,* 2018). However, biological susceptibility to syphilis is not gender-specific. Studies from tertiary centers show similar trends without strong statistical significance (Rao *et al.,* 2020). Social determinants such as healthcare access influence testing rates. Women may be underrepresented due to stigma (UNAIDS, 2022). Routine antenatal screening improves female detection rates. Public health campaigns must address both genders equally. Gender-focused counseling reduces transmission chains. These findings are consistent with contemporary epidemiological data.

Several Indian studies report syphilis seroprevalence between 2–6% in tertiary hospitals (Kumar *et al.,* 2019; Patel *et al.,* 2020). HIV prevalence among STI attendees ranges from 1–4% (NACO, 2022). Singh *et al.,* (2021) observed similar co-infection patterns in North India. WHO (2023) reports global syphilis resurgence, particularly in key populations. Variability across studies may reflect screening strategies and population characteristics. Integrated HIV-syphilis programs have reduced transmission in some regions (UNAIDS, 2022). However, persistent co-infection highlights ongoing transmission networks. Strengthening early diagnosis and treatment remains crucial. Surveillance systems should incorporate dual testing protocols. The present findings are epidemiologically consistent with contemporary literature.

In conclusion, this observational study highlights a significant epidemiological association between serologically confirmed syphilis and HIV infection in a tertiary care setting. HIV co-infection was notably higher among RPR-reactive individuals, underscoring

the biological and behavioral interrelationship between the two infections. The findings reaffirm that syphilis serves as both a marker and facilitator of HIV transmission due to shared risk factors and mucosal disruption. Although demographic variables such as age and gender influenced distribution patterns, the strongest predictor of HIV positivity was syphilis seroreactivity. These results emphasize the necessity of routine dual screening for HIV in all confirmed syphilis cases. Integrated diagnostic and treatment strategies are critical to interrupt transmission chains. Strengthening targeted awareness, early detection, and prompt management at tertiary care centres will substantially reduce disease burden and improve long-term public health outcomes.

Author Contributions

Shalu Mengi: Investigation, formal analysis, writing—original draft. Ojasvi Sharma: Validation, methodology, writing—reviewing. Sandeep Dogra:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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