

Research Article

Syphilis Serology among HIV-Seroreactive Patients

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Abstract: Sexually transmitted diseases (STD's), including Syphilis, are associated with an increased risk of Human immunodeficiency virus (HIV) infection. Though Rapid Plasma Reagin test (RPR) is a screening test to diagnose syphilis, a negative RPR may not rule out syphilis in HIV sero-reactive patients. For laboratory confirmation, one specific treponemal test, namely Treponema Pallidum Haemagglutination Assay (TPHA) or Fluorescent Treponemal Antibody Absorption test (FTA-ABS) should be done along with RPR. The aim of present study was to diagnose syphilis among HIV-reactive Integrated counseling and testing centre (ICTC) attendees visiting Government Hospital, Khammam using both RPR and TPHA. The present study was conducted at Dept. Of Microbiology, Mamata Medical College, Khammam. Blood was collected from 200 HIV-reactive ICTC attendees over a period of 10 months (January to October 2013). Sera were separated and stored at -20^oc. All the sera were screened for syphilis by RPR and also with TPHA immunochromatographic cassette test. Out of 200 HIV positive cases, 56 (28%) were reactive to RPR and 18 (9%) were positive to TPHA. Among 18 TPHA positive sera only 14 were reactive to RPR. 42 out of 56 RPR reactive sera were biological false positives (BFPs). Seroprevalance of syphilis was higher in the age group of 30-40yrs and in females. 15 among 18 syphilis positive cases had a CD₄ count below 250 cells/ mm³. The specificity of non-treponemal serological test may be compromised in a HIV-infected with biological false positives. Both treponemal and non-treponemal tests for syphilis are accurate in the majority of patients with syphilis and HIV co-infection.

Keywords: Sexually transmitted diseases, Syphilis, HIV, Rapid Plasma Reagin test (RPR), Treponema Pallidum Haemagglutination Assay (TPHA)

INTRODUCTION

An emerging epidemic of human immunodeficiency virus (HIV) infection in India has made sexually transmitted infection (STI) control as one of the strategies imperative and probably the most important one to decrease HIV transmission. Epidemiological studies demonstrate that STIs, including syphilis, are associated with an increased risk for HIV infection among both homosexual and heterosexual persons [1, 2]. Sexual behaviors that increase the risk of acquiring STD's further increase the risk of acquiring HIV. Genital lesions and inflammation in syphilis are considered as catalyst for acquiring or transmitting HIV infection [3]. Recent data suggest that in the presence of other STI's, individuals are three to five times more likely to acquire HIV if exposed to the virus through sexual contact [4].

Concurrent infection with *Treponema pallidum* and HIV presents a serious health problem. HIV alters the natural history of syphilis and response to therapy. Incidence of neurosyphilis is increased among the HIV infected persons, even when treated in recommended complete dosage [5].

Atypical clinical presentations of syphilis are more common in HIV patients which makes diagnosis of *Treponema pallidum* (*T. pallidum*) infection more complicated and difficult. However, serological tests appear to be accurate and reliable for diagnosis of *T.pallidum* and the evaluation of treatment response in HIV patients. Serological screening investigations e.g.Venereal Diseases Research Laboratory test (VDRL) and Rapid Plasma Reagin (RPR) test is mainstay of diagnosis for syphilis. The clinician should seek confirmatory Investigation for syphilis in HIV patients [3].

Unusual serological responses have been reported in HIV patients with syphilis, namely, higher than expected serologic VDRL/RPR titers. But false positive results have been reported in HIV-infected patients with syphilis and specific tests like Fluorescent Treponemal Antibody Absorption test (FTA-ABS) or Treponema pallidum Haemagglutination Assay (TPHA) would be beneficial.

Even though syphilis continues to be a major problem in India, the true incidence will never be known, because of inadequate reporting. Serological

surveys continue to be the best source of information on the prevalence of Syphilis.^[6] Hence, the present study was designed to determine the seroprevalence of syphilis in HIV –seroreactive patients and to correlate with age, sex and CD₄ count.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Mamata Medical College at Khammam. Sera were collected from 200 HIV- reactive ICTC (Integrated Counseling and Testing Centre) attendees visiting Government Hospital, Khammam over a period of 10 months (January 2013 to October 2013). Sera were separated and stored at -20^oC. Their consents were taken to perform RPR and TPHA on the same sera samples which were collected for HIV testing. The HIV tests and interpretation were done as per National AIDS Control Organisation (NACO) guidelines. Confidentiality of the results was maintained.

A total of 200 patients were enrolled in this study including 114 females and 86 males. The Rapid Plasma Reagin (RPR) Card test (Figure 1) was performed using carbon antigen from Tulip diagnostics

and TPHA test was performed by using Syphicheck immunochromatographic cassette test from Qualpro diagnostics (Figure 2).

RESULTS

Among 200 HIV-Seroreactive samples tested for Syphilis serology, 56(28%) were reactive for RPR and 18(9%) were TPHA positive. A total of 14samples were reactive for both RPR and TPHA. Out of these 14 RPR reactive samples, 8 had a titer of more than 1:64, 2 had 1:8 titer and 4 had 1:4 titer. A total of 4 samples were positive with TPHA but non-reactive to RPR (Figure 1). 42 sera were biological false positives.

RPR showed a sensitivity and specificity of 77.7o% and76.92% respectively with 25% positive predictive value (PPV) and 97.22% negative predictive value (NPV).

Seroprevalence was higher in the age group of 31-40yrs with female preponderance (Table 1). Among 18 TPHA seropositive patients 15 had a CD₄ count below 250 cells/ mm³ (Table 2).

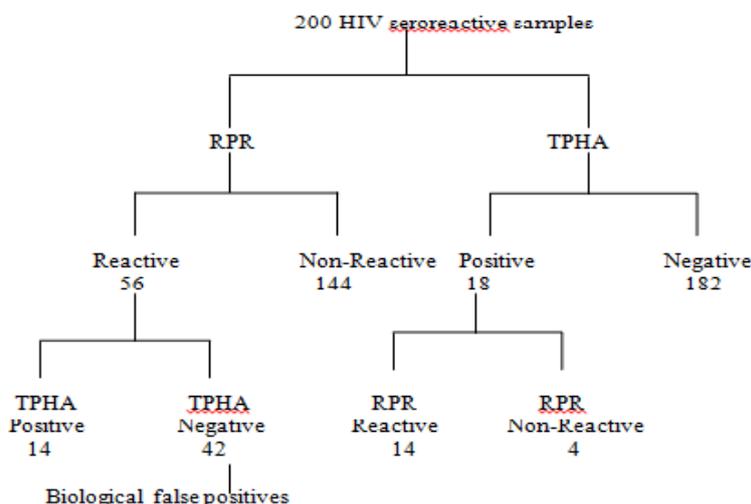
Table-1: Correlation of RPR and TPHA among HIV positive patients

Age (years)	Total Patients	RPR(56)			TPHA(18)		
		Male	Female	Total	Male	Female	Total
21-30	42	4	2	6	1	1	2
31-40	98	15	24	39	4	7	11
41-50	33	6	4	10	1	2	3
51-60	27	0	1	1	0	2	2
Total	200	25	31	56	6	12	18

Table 2: CD₄ counts among TPHA positive patients

TPHA positive patients (18)	CD ₄ count (Cells/mm ³)
1	>500
2	250-500
15	<250

Flow chart depicting RPR and TPHA positive and negative results among HIV-seroreactive samples



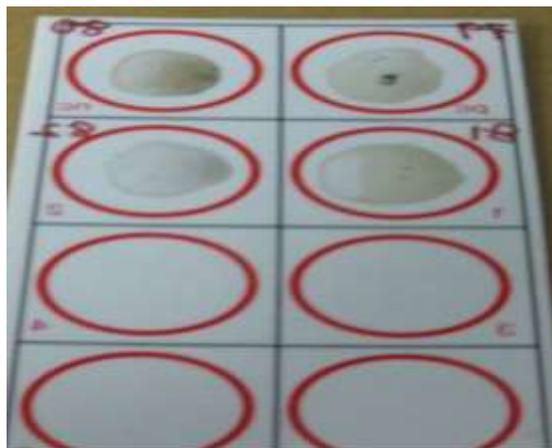


Fig. 1: Rapid Plasma Reagin Test



Fig. 2: Treponema Pallidum Haemagglutination Assay

DISCUSSION

The clinical manifestations, serological responses, efficacy of treatment and occurrence of complication of syphilis may be altered in patients co-infected with HIV. Serological tests for Syphilis may be difficult to interpret in HIV seropositive patients because of atypical responses such as delayed responses to both treponemal and non-treponemal test.

Irrespective of signs and symptoms, all HIV-positive patients should have baseline RPR screening and follow-up at 3 months, to rule out the possibility of false negative results, as seroconversion generally takes about 4-6 weeks after infection. In the course of reactive RPR, the diagnosis of Syphilis should be confirmed by the specific treponemal test, namely, FTA-ABS or TPHA [7].

Present study showed that 18 were reactive by TPHA test while 56 were reactive by RPR. A total of 14 samples were reactive to both TPHA and RPR. 4 samples were TPHA positive and RPR non-reactive. A negative RPR test may not rule out syphilis in patients with HIV infection, while the sensitivity of this serologic test in diagnosing secondary syphilis is generally high. A rare case report of seronegative

secondary syphilis in patients with HIV infection suggest that some patients fail to develop normal antibody response to *T. palladium* [8, 9]. Therefore, in all suspected cases of syphilis two serological tests, i.e., one non-treponemal screening test, e.g., RPR and one specific treponemal test, e.g., TPHA should be done for laboratory diagnosis of syphilis. Most of TPHA positive patients had a CD4 count below 250 and were started on antiretroviral therapy.

A total of 42 tested sera were biological false positives (PPV 25%). The specificity of non-treponemal serologic test for syphilis may be compromised in a HIV-infected person [4, 8, 10]. Very high RPR titers of greater than 1:64 have been reported in HIV-infected patients without syphilis. This non-treponemal test detects antibodies against non-specific antigen, i.e., reagin, cardiolipin and lecithin. Many persons with HIV infection have anticardiolipin, antilecithin antibodies and polyclonal gammopathy. Thus in such cases positive RPR test result might be biologically false positive and does not represent syphilis infection.

CONCLUSION

Serologic test for syphilis are the cornerstone in diagnosing untreated syphilis infection, even in a

HIV-infected patient. Unusual serologic responses have been reported in HIV-infected persons with syphilis. Most reports involved higher than expected serologic titers, but false negative serologic result or delayed appearances of seroreactivity have also been reported. Nevertheless, both treponemal and non-treponemal serologic tests for syphilis are accurate in the majority of patients with syphilis and HIV coinfection.

Thus it is recommended that all the patients with newly diagnosed syphilis should be counseled for HIV testing. Similarly serological testing for syphilis in all patients with newly diagnosed HIV infection should be carried out.

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