Original Article

Sero-prevalence and comparative study of diagnostic tests in syphilis in tertiary care teaching hospital, Western India

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ABSTRACT

Background: Syphilis testing may be a useful strategy to provide data for implementation of Sexually transmitted disease (STD) control programme. Serological surveys continue to be the best source of information on the prevalence of syphilis.

Aims: The aim of study was to estimate the seroprevalence and to determine diagnostic performance of treponemal TPHA (*Treponemapallidum* hemagglutination assay) and nontreponemal RPR (Rapid plasma regain) test for syphilis.

Materials and Methods: To assess the prevalence rate Total 13115 serum samples of patients with clinically suspected syphilis infection and antenatal screening were analyzed by using either RPR test or by TPHA in tertiary care hospital, western India during time periods of March 2014 to April 2015. To assess the reactivity of RPR test in comparison with TPHA test, serum samples from 148 patients with high clinical suspection of syphilis infection were investigated by both above mentioned methods.

Results: Among tested 13115 serum samples of patients, overall seroprevalence was 0.930% (13115/122). Highest seroprevalence (6.21%) was seen in STD clinic attendees. Among 148 samples which were tested by both assays, 98 were truly reactive by TPHA while 92 were by RPR. Thus TPHA was found to be more sensitive and specific while comparing the results with RPR test.

Conclusion: Serological surveys need to be continuously done to avoid the adverse consequences from missed diagnosis. TPHA should be performed along with RPR test as no single serological test can act as the marker of acute infection.

Keywords: Rapid plasma reagin test, Syphilis, Seroprevalence, *Treponemapallidum* hemagglutination assay

INTRODUCTION

Syphilis is a curable venereal disease caused by gram negative fragile spiral spirochete *Treponema pallidum* which affects 12 million people each year worldwide [1, 2]. In India, syphilis and chancroid were the main causes of genital ulcer disease (GUD) in 1970s and early 1980s.When human immunodeficiency virus (HIV) infection was identified in late 1980s, then Sexually Transmitted Infection pattern has shifted from bacterial to viral. Constant decline in its prevalence has been observed in recent years but in developing countries like India, syphilis continues to be a major health problem [3]. It is also important intransmission of HIV infection among HIV positive individuals dually infected with syphilis by increasing the amount of HIV viral shedding [2].

Its transmission is only possible by direct inoculation from one person to the other and thus gets transmitted only by sexual contact, blood transfusion with infected blood and by transplacental route from mother to baby. The disease has evolved through primary, secondary and tertiary stages [4].

Treponemapallidum cannot be cultivated in vitro and thus the diagnosis is dependent on clinical signs, direct demonstration of bacilli by Dark ground microscope, Polymerase chain reaction (PCR) and detection of antibodies by serology [5]. Serological tests includes specific tests like *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* Immobilisation test (TPI), Fluorescent treponemal antibody (FTA) test and non-specific tests like Venereal Disease Reference Laboratory (VDRL) test, Rapid Plasma Reagin test (RPR). Antibodies to syphilis infection become detectable by serological tests 3 to 4 weeks after exposure to bacterium *Treponema pallidum*. Non treponemal anti- lipoidal antibodiesare found in

active disease and the levels subside after successful treatment, while Treponema-specific antibodies persist for a prolonged period of time even after infection has been cured. The non-Treponemal tests also called as standard tests for syphilis(STS) such as RPR and VDRL test measures the host response to cardiolipin and lecithin like non-treponemal antigens released from the damaged host cells [6]. These non-treponemal tests are sensitive in early syphilis, but their disadvantages being biological false-positive reaction in patients with acute and chronic diseases which are causing tissue damage, false-negative reactions due to the prozone phenomenon, and lack of sensitivity in the late stage of infection [7].These tests are used for rapid screening of patients in field camps, blood donation camps and for assessing the effectiveness of therapy in syphilis patients.

The treponemal tests such as the *Treponema pallidum* Haemagglutination Assay (TPHA) detect human serum antibodies to *T. pallidum* by principal of indirect hemagglutination. It has high sensitivity for all the stages of disease except in very early primary syphilis. These advantages have made TPHA a standard confirmatory test. But some disadvantages like false positive or negative reaction due to results depends on skill and training of person who is reading the result of test. The TPI requires living pathogenic *T. pallidum*, While FTA-ABS test requires a fluorescence microscope [6]. In addition, both these tests require a high level of specialist expertise. Enzyme Immunoassays (EIA) using specific *T. pallidum* recombinant antigens is also available in various diagnostic laboratories [8]. It has capability to screen large number of samples with good specificity same as TPHA and even with more sensitivity than TPHA.Recently, immunochromatographic Treponemal test is also available for the detection of syphilis antibody in human blood.

Syphilis screening by the non-treponemal RPR test is not regularly followed up by specific treponemal tests in most of the diagnostic laboratories in India. Since the cross reacting serological response by non-venereal treponema and the biological false positivity reactions compromise correct diagnosis of syphilis, so the confirmation of positive non-treponemal test by fluorescent treponemal antibody (FTA) or Treponemapallidumhemagglutination (TPHA) test is recommended [9].

Thus to assess the diagnostic inaccuracy by single syphilis screening test, the aim of present study was to evaluate the detectability of syphilis by commercially available non-treponemal test like RPR in comparison with treponemal test like TPHA. So that appropriate actions can be taken by the responsible authority towards implementation of proper testing algorithm for diagnosis of syphilis.

MATERIALS AND METHODS

In present study, to assess the prevalence rate we have analyzed serum specimens of venous blood of 4233 patients with clinically suspected syphilis infection and of 8882 antenatal patients in tertiary care teaching hospital, western India during time periods of March 2014 to April 2015 by using either RPR(Rapid plasma regain) test or by OMEGA's IMMUTREP TPHA(*Treponema pallidum* hemagglutination assay).This study was also undertaken to assess the reactivity of RPR test in comparison with TPHA test in different patient groups. Thus serum Specimens from 148 patients with high clinical suspicion of syphilis infection by clinician were investigated by both above mentioned methods for comparison. Test procedures were done according to the particular manufacturer's instruction given in Test kit literature. Positive and Negative controls along with in-house controls were also used for both the tests. Aliquots of positive sera samples were stored at –70°C.

For analysis of age related prevalence, age of patients were subdivided into following groups: <20 years, 21-45 years, 46-60 years and >60 years.

The RPR test was performed qualitatively for all clinically suspected cases of syphilis and quantitatively only for titration of reactive samples. For RPR testing 5 ml venous blood sample was collected into a plain vacutainer, allowed to clot for about 10 to15 minutes or centrifuged for 10 minutes at 2000 *g*. A standard RPR test with 18 mm circle card (Span Diagnostics) was carried out, by mixing one drop of serum with one drop of RPR reagent, on a shaker for 8 minutes, and results read in good light. A Reactive sample is indicated by macroscopically visible black clumps against white background on card whereas non-reactive samples appear to have smooth uniform light gray colour. Results were recorded as positive and negative with respect to positive and negative control sera which were included in each test run.

The TPHA test was also performed qualitatively, wherein an even layer of agglutination of cells in a round bottom of micro-titration plate well was interpreted as positive reaction, while non-agglutinated cells in case of absence of antibody form compact button which was interpreted as a negative reaction. Agglutination in the control cell well together with the test cell well indicates the

presence of nonspecific agglutination in the sample, it is considered as the test was invalid.

True positive and True negatives were calculated in RPR in comparison with TPHA.

RESULTS

Among 4233 serum samples of patients with clinically suspected syphilis infection including patients of Sexually transmitted diseases (STD) clinic attendees and from different outdoor patient departments (OPDs) and among 8882 serum samples of antenatal patients for syphilis screening between time periods of March 2014 to April 2015, overall seroprevalence was 0.930% .

Table 1: Seropositivity of syphilis infection in different groups of patients

Patients	Total No. tested	RPR or TPHA positive No.	Sero-prevalence in %
Patients suspected of syphilis in different outdoor patient departments (OPDs)	3412	54	1.58%
STD clinic attendees	821	51	6.21%
Antenatal screening	8882	17	0.19%
Total	13115	122	0.93%

Table 1 indicates that seroprevalence (6.21%) was highest in patients who have attended STD clinics by considering both RPR and TPHA tests than other patient groups.

Most commonly affected age group was 21-45 years (61.47%). Majority of the patients with confirmed syphilis infection in the study were males (68.85%).

Among 148 samples of patients with high clinical suspicion of syphilis which requested for testing by both RPR and TPHA method by clinician, 92 were positive by both above mentioned methods, 43 were negative by both methods, 9 samples were positive by TPHA and negative by RPR and 4 samples were positive by RPR method and negative by TPHA and 1 sample was positive by TPHA and initially negative by RPR but when several dilutions (quantitatively) were tested on titre of 1:128 sample was even positive by RPR method.

The results of comparison of RPR test Vs TPHA are given in Table 2.

Table 2: Comparison of results by RPR and TPHA tests

Results	RPR+ TPHA (N=148)		RPR test (N=148)		TPHA test (N=148)	
	No.	%	No.	%	No.	%
Reactive	92	62.16	96	64.86	101	68.24
Non-reactive	43	29.05	52	35.14	47	31.76

Both RPR and TPHA tests were reactive in 92 patients. 4 cases (4.16%) which were positive by RPR and Negative by TPHA were later found that they all were false positive due to viral and malarial infection. The TPHA test was performed for 52 RPR non-reactive samples, Out of these 9 sera samples were positive by TPHA-6 were late syphilis cases and 3 were fully treated syphilis cases. Thus in present study, TPHA was found to be more sensitive and specific while comparing the results with RPR test (98 were truly reactive by TPHA while 92 were truly reactive by RPR).

DISCUSSION

Though there is effective intervention against syphilis infection through penicillin therapy and some public health initiatives, India continues to be burdened due to this disease so there is need for appropriate diagnostic facilities in each healthcare Settings [9].

In present study, syphilis was commonly reported in various patient groups including STD clinic attendees; in already HIV infected patients, immigrants, in sex workers and in pregnant patients who have attended antenatal clinic. Thus screening of syphilis in different patient groups with appropriate testing algorithm should be emphasized to minimize syphilis related health consequences [10, 11].

In present study, 122 (0.93%) of the 13,115 sera screened for syphilis were reactive. The seropositivity among pregnant women in our study was 0.191% which was lesser than the seropositivity of 1.582% among outdoor patients suspected of syphilis. This low seropositivity rate among Antenatal clinic attendees was found 0.24% in a study conducted at Burkina Faso is comparable with our study [15]. Seropositivity of diagnostic tests was observed highest in STD clinic attendees (6.21%) than other patient groups as also observed by other studies [12,16]. This finding has important implications for STI management in India.

Syphilis was observed highest in adult age group (21-45 years) in present study due to its

transmission during vaginal, anal or oral sex through direct contact with a syphilis sore (chancre) which occurs mainly on the external genitals, vagina, anus or on the lips. Syphilis was more commonly seen in men due to more immigrants were observed in men population.

Diagnosis of syphilis in most of the diagnostic centres in healthcare settings is mainly by clinical symptoms and by serological tests of non-treponemal antibody- VDRL and RPR tests as they are easy to perform and more economic to use. The situation becomes complicated during calculating the prevalence rate of syphilis in a community for instituting preventive measures because RPR positive sera when not confirmed by specific treponemal test (in present study 4.17%) may lead to miscalculation of the disease burden in the community [13]. In this study 95.83% of RPR reactive results were confirmed by TPHA test as compared to only 73.2% of RPR reactive results were confirmed by TPHA test in study of SP Dumre [11]. So it seems unnecessary for patients to take anxiety and drug therapy with penicillin injections when single test is positive. Single test of non-treponemal antibody like RPR and VDRL should not considered as confirmative due to following reasons: It detects only the reaginic antibodies which do not conclusively prove the active stage of the disease, the occurrence of biological false positivity due to physiological conditions and certain acute and chronic infections, biological false negative results in late and latent syphilis. So, confirmation of RPR results should be done by TPHA or other specific treponemal tests in all patients to ensure that appropriate syphilis diagnosis has been made as mentioned in the national STI management guidelines [10].

Even, TPHA as a treponemal antibody test does not satisfy as confirmatory test as it lacks the sensitivity in sera from patients with primary syphilis. In our study, 91.08% of the TPHA positive cases were positive by RPR test as compared to only 80.4% in study of SP Dumre [11]. TPHA demonstrated relatively less false positive results compared to RPR in our study. TPHA test was positive in 17.30% of our RPR negative specimens same as in study of SP Durme [11]. Such TPHA positive cases with RPR negative result were found as treated syphilis cases and some as late syphilis cases which indicates more specificity of TPHA test.

Young *et al* reported that approximately 8 to 10% of patients with various stages of infection gave a false-negative results in VDRL test due to the prozone phenomenon whereas in our study only 1 sample has showed this phenomenon (RPR test was reactive at dilution of 1:128) [14].

So our findings suggest on the use of combination tests to avoid any misdiagnosis of syphilis or single performed test limitations must be well notified to clinicians or the laboratory reports must state that the RPR test alone cannot confirm or exclude syphilis infection.

CONCLUSION

On the basis of results of presented study, we concluded that either of the single tests produces inaccurate results so all patients whose sera are reactive in the RPR test, irrespective of titre, should be routinely confirmed by more sensitive and specific TPHA or any other specific treponemal test. Those patients who found positive in both the tests should be treated. This type of treatment strategy is more beneficial to the patients, so there is a need to focus on formulation of strict policy for the implementation of the existing guidelines throughout the country to prevent misdiagnosis in syphilis with the use of single test.

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