Comparative evaluation of serological tests in the diagnosis of Syphilis

Sita Mahalakshmi B[1], Sreedevi S[2], Jyothi K[3]

[1,2,3]Assistant Professor, Department of Microbiology, Kakatiya Medical College, Warangal,Telangana.

Address for correspondence: Dr. B. Sita Mahalakshmi, Assistant Professor, Department of Microbiology, Kakatiya Medical College, Warangal,Telanagana, India.

Email Id: banoth.lakshmi04@gmail.com

ABSTRACT

Background: Diagnostic and therapeutic approaches in syphilis show wide variation. The use of only one type of serological test is insufficient for diagnosis.

Objective: The aim of the study was to review serologic data to determine diagnostic performance of three different methods of syphilis patients from MGM Hospital, Warangal in urban and rural areas during period of 2012-2013.

Materials & Methods: In total, 144 RPR reactive samples were collected, with 1 in 4 or more titre of antibody by using RPR, TRUST and TPHA assays

Results: 144 test sera were collected from 3 sources namely ICTC, STD and Antenatal OP. 70 sera (48.6%) were taken from ICTC, 62 (43.5%) from STD and 12 (8.3%) from antenatal OP. Table compares the sensitivity and titres of antibody in RPR, TPHA and TRUST. Maximum number of samples (68) are reactive at 1:8 dilutions by RPR, 320 dilutions in TPHA and 64 dilutions in TRUST. The reactivity of all the samples by TPHA is 80 and above, and by TRUST, 32 and above.

Conclusion: Based on our results we think that specific Treponemal tests could contribute to reducing the errors that depend on specificity of the method used. Considering the methodology, rapid results and high sensitivity of RPR tests makes it a good choice as screening test in microbiology laboratories.

Keywords: Syphilis, *Treponema pallidum,* RPR and TPHA

INTRODUCTION

Sexually transmitted diseases are a major public health problem in India. Syphilis is one of the most fascinating diseases of humans. The disease has been of great historical importance not only for the practice of medicine but also because of its effect on many individuals who played important roles in the history of the western world. Syphilis is a sexually transmitted disease caused by the spirochaete Treponema *(T.pallidum)* which affects 12 million people each year and results in significant morbidity and mortality. Despite the availability of relatively sensitive tests and affordable treatment, the disease remains a global health problem[1].

Syphilis was an extremely common infection only a few decades back, with prevalence of 5-10% in various autopsy series in the first half of the twentieth century. In certain groups of low socioeconomic status studied in the prepenicillin era, syphilis effected 25% or more of the population. Widespread use of antibiotics after World War II reduced the incidence of syphilis. Although the spirochetes or their DNA can be consistently detected in lesions by either microscopy (dark field, immunofluorescence) or PCR, the most reliable method for laboratory diagnosis of syphilis, regardless of the stage of infection, is still serology[2]. However, the present international recommendations cannot be applied in some areas of the world either because the recommended diagnostic assays are not available or diagnostic strategies are too expensive[3]. Alertness to the possibility of complications in late syphilis and awareness of the varied clinical manifestations of late syphilis are crucial, if these forms of disease are to be diagnosed and treated properly. Thus it is important for proper diagnosis especially of latent syphilis by choosing appropriate serological tests.

A battery of tests is available for the serodiagnosis of syphilis, each varying in sensitivity and specificity. The present work is undertaken to understand syphilis in the present era, and to evaluate some of the currently available and often used tests for serodiagnosis of syphilis.

Rapid plasma reagin test (RPR) is the routinely used screening test in the laboratory. The sensitivity and specificity of the test are compared with two other serological tests, namely Toluidine red unheated serum test (TRUST) and Treponema pallidum haemagglutination test.

The present study was designed to evaluate the three serological diagnostic tests for syphilis in patients attending Mahatma Gandhi Memorial Hospital and Government Maternity Hospital, Warangal.

Despite the availability of relatively sensitive tests and affordable treatment, the disease remains a global health problem[1].

MATERIALS AND METHODS

Study Design: The present study group consisted of persons attending sexually transmitted disease outpatient department, Mahatma Gandhi Memorial Hospital, ICTC, MGM

Hospital and the women attending antenatal outpatient department, Government Maternity Hospital, Warangal. In total, 144 RPR reactive samples were collected, with 1 in 4 or more titre of antibody. The period of study was from June 2012 to May 2013. All the patients from STD outpatient department were symptomatic. All the patients represented different strata of society from both rural and urban segments.

Among the test samples, 62 were from STD patients , 70 samples were from ICTC, and 12 samples were from antenatal women. The blood samples were collected as per the standard protocol, serum is separated and stored at 4° C till use.

Rapid Plasma Reagin Test:

Serum samples were tested for Treponema pallidum IgG antibodies by Rapid Plasma reagin (RPR) kit ,supplied by Ensure biotec pvt. Ltd., I.D.A., Mallapur, Hyderabad. The technique of R.P.R. is performed as per the standard procedure and manufacturer's directions. It is an in vitro diagnostic test for the detection of human serum IgG directed against cardiolipin antigen, used for the standard tests for syphilis.

TRUST (Toluidine Red Unheated Serum Test):

All the RPR positive samples were tested by TRUST. The kit is manufactured by Span Diagnostics Ltd., Surat, India. The kit is commonly used for rapid serological diagnosis of syphilis.

The technique of TRUST is performed as per standard protocol[4]. It is a macroscopic non-treponemal test for syphilis, utilizing a suspension of toluidine red toner particles coated with synthetic cardiolipin antigen . It is a test which is rapid, simple to perform and easy to interpret , compared to the standard VDRL test.

Treponema Pallidum Haemagglutination Test: (TPHA Test):

All the serum samples were subjected to Treponema pallidum haemagglutination test (TPHA) for comparative study. The kit is manufactured by Fortress Diagnostics Ltd. (Northern Ireland), U.K[5]. In brief, each sample requires three microwells, add 90 µl of Diluent to well 1, add 10 µl of sample to well 1 and mix, transfer 25 µl from well 1 to wells 2 & 3,then add 75 µl of control cells to well 2,Add 75 µl of antigen reagent (or test cells) to well 3, tap the plate gently to mix the contents, cover the plates and incubate for 60 min. at room temperature. Observe for uniform mat of cells over the entire base of microwell.

The highest dilution in which a smooth mat of agglutinated cells are formed, covering the entire bottom of microwell is taken as the antibody titre for that particular test sample.

Quantitative test:

Each sample requires 9 microwells of a microtitre plate, add 190 µl of diluent to well, add 25 µl of diluent to wells 4 to 9. Make a 1/20 dilution by adding 10 µl of serum to well 1. Mix and transfer 25 µl of the 1/20 dilution to wells 3 & 4 . Mix ¼ dilution and transfer 25 µl to well 5. Repeat this step until the doubling dilutions has been completed and discard 25 µl from the last well. Add 75 µl of antigen reagent to all wells 3, 4, 5, 6, 7, 8 & 9, add 75 µl of control cells to well 2. Tap the plate gently and cover the plate, incubate for 60 min. Observe for uniform matting of cells over the entire base of the microwell.

RESULTS

RPR reactivity of Test samples collected

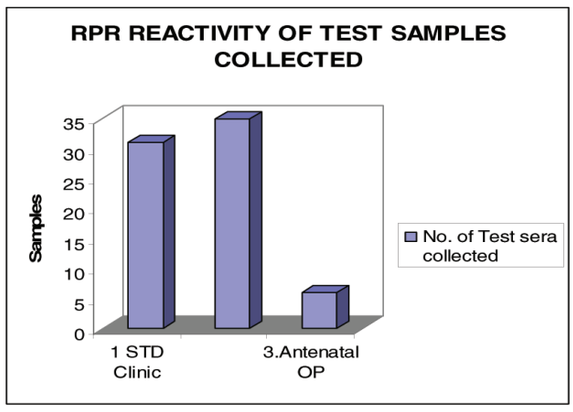
144 test sera were collected from 3 sources namely ICTC, STD and antenatal OPD. 70 sera (48.6%) were taken from ICTC, 62 (43.5%) from STD and 12 (8.3%) from antenatal OPD. Sera studied were between age group of 15-50 years. Most of the cases were from the age group of 30-39 (41.6%) followed by 20-29 age group (37.5%), followed by age group 40 and above (15.2%). Out of 144 cases studied 100 (69.5%) were females and 44 (30.5%) were males.

Most of the cases studied were from rural area (81.95%), only 18.05% cases were from urban area. Out of 144 cases studied 68 (47.2%) had primary education, 34 (23.6%) had secondary education and 26 (18.05%) were illiterate, 16 (11.12%) were having higher education.

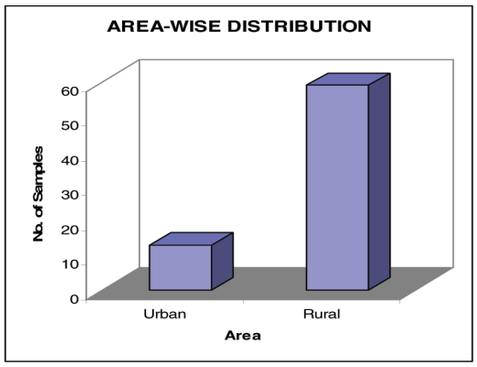
Comparison of RPR reactivity with TPHA and TRUST

Table 1 compares the sensitivity and titres of antibody in RPR, TPHA and TRUST. Maximum number of samples i.e 68 were reactive at 8 dilutions by RPR, 320 dilutions in TPHA and 64 dilutions in TRUST. The reactivity of all the samples by TPHA is 80 and above, and by TRUST, 32 and above.

Graph 1: RPR Reactivity of Test Samples collected



Graph 2: Area wise Distribution of Test samples



Graph 3: Comparison of RPR in relations to Literacy

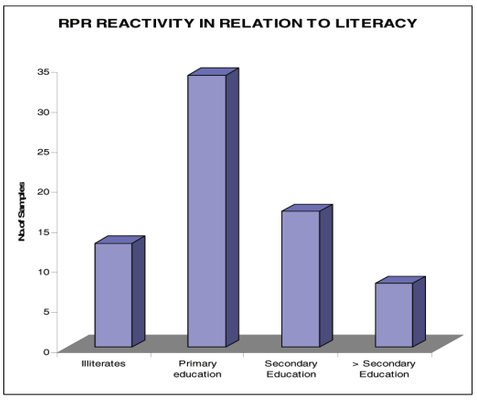


Table 1: Comparison of RPR reactivity with TRUST and TPHA of Test Samples

Test		RPR				TPHA					TRUST					
Titre		4	8	16	32	≤80	160	320	640	1280	≤8	16	32	64	128	156
No. of Sera	No	46	68	26	4	20	26	68	26	4	-	-	46	68	26	4
	%	31.9	47.2	18.05	2.7	13.8	18.05	47.2	18.05	2.7	-	-	31.9	47.2	18.05	2.7
Total					144					144						144

DISCUSSION

Serodiagnosis of syphilis occupies an important place in any diagnostic laboratory. The commonly used screening tests are the non treponemal tests, employing cardiolipin antigen. These tests are non specific. Inspite of this, they are used widely and are preferred by clinicians and diagnosticians because they are affected by antitreponemal therapy. As a result they are useful for following the progression of the disease and response to therapy.

If the titre of the antibody does not fall progressively with treatment, the possibility of a treatment failure should be considered. There should be at least a four fold decrease in an antibody titre after 3 months of antitreponemal therapy. Patients who are treated in the late stage of syphilis or are reinfected may develop titres that remain stable. These are called “chronic persisters” who may maintain positive non treponemal tests for life.

As shown in Table 1, the two non treponemal tests namely RPR and TRUST are compared. There is a significant fourfold increase in the titre of positivity with TRUST compared with RPR test. TRUST carries the same advantage as RPR test mentioned above. RPR test uses burned charcoal particles , due to which there is a possibility of inconsistency in the reactions. TRUST is a CDC approved standard test wherein there is a constant result. The other advantages are room temperature storage, no glass ampoules and cost effectivity. TRUST is a more sensitive and specific alternative for RPR and other more expensive non treponemal tests for syphilis[6,7].The approximate cost for each test in RPR and TRUST are almost the same (Rs 6 to Rs 8). Thus it is felt that TRUST can replace RPR in detecting and quantitating more number of positive sera .

Studies elsewhere (West et al 2002)[8] have compared RPR test with Rapid syphilis immunochromatographic strip test (RST). They felt that RST was easier to use and interpret than RPR test, especially in difficult conditions. They suggested an urgent necessity to develop an appropriate alternative rapid test in place of RPR.

Comparison of RPR with TPHA:

As mentioned in the literature, specific treponemal serological tests detect treponemal antibodies against the antigens of the organism themselves. Once positive, their usefulness is limited because these tests tend to yield positive results throughout the patient’s life. In TPHA, erythrocytes from turkey are coated with Treponemal antigens (antigenic components of pathogenic Treponema pallidum -Nichol’s strain). However TPHA test cannot distinguish between syphilis and other pathogenic treponemal infections like Yaws.

Diagnostic and therapeutic approaches in syphilis show wide variation[9]. The use of only one type of serologic test is

insufficient for diagnosis because each type of test has some limitations[10]. The sensitivity of non-treponemal tests depends on the stage of syphilis[11]. The traditional algorithm used in the diagnosis of the disease includes screening with a non treponemal tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR) and Toluidine Red Un Heated Serum Test (TRUST), with positive results confirmed by a treponemal test, Treponema Pallidum Hemagglutination Assay (TPHA)[1,12]. All non-treponemal serologic tests measure antibodies to cardiolipin. Generally, these tests take the form of flocculation reactions.

The positive result with TPHA can be indicative of an ongoing or a past infection. Thus TPHA cannot be used as interpretative of successful or unsuccessful antitreponemal therapy. Although TPHA is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders[13].

TPHA carries more advantages when compared to the other treponemal tests, and is more laboratory-friendly. TPI test requires live virulent Treponemes which are a laboratory hazard, and it is cumbersome to maintain Nichol's strain . TPHA requires specific treponemal antigens coated on RBC. On the otherhand, FTA-ABS is highly expensive and cumbersome. The reagents have short shelf life and the test requires fluorescent microscope. The results of TPHA can be read with naked eye. However, the FTA-ABS test is advantageous in that it can detect IgM antibody and IgG antibody separately. Thus it can also be used to detect ongoing infection, unlike TPHA[14].

In the present study, RPR and TPHA is compared. Among the 72 positive serum samples, 34 sera gave titres upto 320 (Table VI) with TPHA whereas with RPR, titres more than 32 were not reached. Serum samples give titres upto 1280 with TPHA. Thakar et al (1996)[15] collected serum samples from clinically diagnosed syphilis and screened them with VDRL and TPHA tests. They opined that TPHA was found to be superior to VDRL in their study groups. It is mentioned that a reactive VDRL of more than 1 in 8 should be subjected to a treponemal test to differentiate between true and false positive reactions. In the present study there do not seem to be any false positive reactions including the sera from pregnant women, as all the reactive sera with RPR gave titres of more than 80 with TPHA.

Rajendran et. al 2003[16] interpreted their results of seroprevalence studies of syphilis. They analysed that the prevalence was 2.7% as per RPR positivity and 0.7% as per TPHA.They felt that no single serological test can act as a marker of ongoing infections in an apparently healthy population. Since their study was conducted on clinically healthy subjects, their study cannot be compared with the present study.

D'Errico et. al, 1996[17] compared VDRL and TPHA tests in parallel as per the diagnostic strategy suggested by WHO. They felt that the application of these two tests in parallel produces a statistically significant increase of sensitivity from 47%-98%. The use of these two tests in parallel, according to them is indispensable to screen blood donors,as the use of VDRL alone does not exclude infectivity of a blood sample.

Brozan et al 2006[18] compared 3 syphilis screening strategies namely RPR, immunochromatographic strip (ICS) and TPHA. According to them, the onsite RPR had low sensitivity. In short, the results of the present study indicate a higher detectability of TPHA and TRUST, in higher titres compared to RPR test which could only detect positives upto 32 dilutions and not above.

Comparison of RPR with TRUST:

SA Larsen, DE Pettit, MW Perryman et al 1983[19] Clinical and diagnostic laboratory immunology Vol. VII No.4 P. 658. Compared RPR test with TRUST for serodiagnosis of syphilis. In their observation TRUST gave better and more sensitive results with stored serum samples than RPR.

CONCLUSION

Based on our results we think that specific Treponemal tests could contribute to reducing errors that depend on specificity of the method used. Considering the methodology, rapid results and high sensitivity of RPR tests makes it a good choice as screening test in microbiology laboratories. The limits of screening tests for the diagnosis of syphilis should not be forgotten, i.e. confirmatory tests like TPHA must be done. Serology has the prime importance in the laboratory diagnosis of syphilis, but must be viewed in the context of clinical presentation.

REFERENCES

1. Peeling RW, Hook EW. The pathogenesis of syphilis: the great mimicker, revisited. J Pathol 2006;208:224-32.
2. Tsang RSW, Martin IE, Lau A, Sawatzky P. Serological diagnosis of syphilis: comparison of the Trep-Chek IgG enzyme immunoassay with other screening and confirmatory tests. FEMS Immunol Med Microbiol 2007;51:118-24.
3. Sokolovskiy E, Frigo N, Rotanov S, Savicheva A, Dolia O, Kitajeva N, et al; EESRH Network. Guidelines for the laboratory diagnosis of syphilis in East European countries. J Eur Acad Dermatol Venereol 2009;23:623-32.
4. Wicher K, Horowitz HW, Wicher V. Laboratory methods of diagnosis of syphilis for the beginning of the third millennium. Microbes Infect 1999;1:1035-49.
5. M Kingston et al 2008. UK National Guidelines on the Management of Syphilis 2008.,International Journal of STD & AIDS 19., 2008; 729-740.

6. www.nhdiag.com/syphilis.shtml.

7. www.stdprevention training.org.

8. B West1, G Walraven1, L Morison2, J Brouwers1, R Bailey1. Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa. Sex Transm Infect 2002;78:282-285.

9. Tasbakan MI, Pullukçu H, Senol S, Yamazhan T, Kidak L, Gökengin D. Review of syphilis patient records in Izmir State Venereal Diseases Clinic from 1994 to 2004. Turk J Med Sci 2008;38:181-6.

10. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2010. http://www.cdc.gov/std/treatment/2010/ STD-Treatment-2010-RR5912.pdf.

11. Sambri V, Lautenschlager S. Diagnosis of syphilis: clinical and laboratory problems. J Dtsch Dermatol Ges 2006;12:1058-72.

12. Centers for Disease Control and Prevention. Syphilis testing algorithms using treponemal tests for initial screening – four laboratories, New York City, 2005-2006. MMWR Morb Mortal Wkly Rep 2008;57:872-5.

13. Lesinski,J.,Nawara,A.,Hryniewicka,I.,Lyzwa, T., Kotysiska, J. (1977). Investigations on the specificity of the TPHA reaction in subject with suspected biologically false results of cardiolipintes' and its usefulness as a routine method of serological verification.Przeglqd Dermatologiczny,64,441-446.

14. Koneman, Allen, Janda, Paul C, Schreckenberger C. Winn Jr., 2000: Test Book of Diagnostic Microbiology 5th edition Pg No.956-960.

15. Thakar YS, Chande C, Mahalley AD, Saoji AM.Seroprevalence of syphilis by TPHA test.Indian J Pathol Microbiol. 1996;39(2):135-8.

16. Rajendran P1, Thyagarajan SP, Pramod NP, Joyee AG, Murugavel KG, Balakrishnan P, Hari R, Jeyaseelan L, Kurien T. Serodiagnosis of syphilis in a community: an evaluatory study.Indian J Med Microbiol. 2003 ;21(3):179-83.

17. M. M. D'Errico, M. Mariottini, S. Di Rosa, E. Prospero, M. Raffo, F. Carle. Syphilis and blood donors: Comparison of two different diagnostic strategies. European Journal of Epidemiology,1996; 12 (1)., 77-80.

18. Brown ST, Zaidi A, Larsen SA, Reynolds GH. Serological response to syphilis treatment. A new analysis of old data. JAMA 1985;253:1296-9.

19. Pettit, D E, Larsen, S.A. Harbee PS Feeky JC Pashem, CE, Cruce DD, Hambie, EA, and Perryman, MW, 1983. Toluidine red unheated serum test a non-treponemal test for syphilis . J. Clin. Microbiology 18: 1141-1145.

Please cite this article as: Sita Mahalakshmi B, Sreedevi S, Jyothi K. Comparative evaluation of serological tests in the diagnosis of Syphilis . Perspectives in medical research 2017;5(1):50-54.

Sources of Support: Nil,Conflict of interest:None declared.