ORIGINAL ARTICLE
pISSN 0976 3325 | eISSN 2229 6816
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Comparison of Semi-Quantitative RPR Test and TPHA for Serodiagnosis of Syphilis

Nasiruddin M Shaikh[1], Tanmay K Mehta[1], Parul D Shah[2]

Financial Support: None declared
Conflict of Interest: None declared

How to cite this article:
Shaikh NM, Mehta TK, Shah PD. Comparison of Semi-Quantitative RPR Test and TPHA for Serodiagnosis of Syphilis. Natl J Community Med 2017; 8(9):541-545.

Author's Affiliation:
[1]Assistant Professor; [2]Professor & Head, Dept of Microbiology, Smt. N.H.L. Municipal Medical College, Ahmedabad

Correspondence
Tanmay K Mehta
tanmay.smit@gmail.com

Date of Submission: 27-02-17
Date of Acceptance: 28-09-17
Date of Publication: 30-09-17

ABSTRACT

Introduction: A reactive RPR should always be confirmed with treponemal test like TPHA in suspected syphilis patients to rule out biological false positive cases. It is important to find an alternative to TPHA as it is frequently not available in resource limited health care facilities of developing nations like India.

Aim: Aim of our study is to evaluate semi-quantitative RPR test and TPHA in serological diagnosis of syphilis in resource limited health care facilities.

Methodology: A retrospective cross-sectional study from July 2012 to July 2015 was conducted on 216 suspected cases of syphilis. All cases were tested for qualitative RPR test, semi-quantitative RPR test ant TPHA test. Serum samples that are positive in qualitative RPR test but negative in TPHA were referred to as biologic false-positive (BFP) reactions. Statistical analysis was done by using Fischer's exact test.

Results: We found 30(13.88%) biological false positive (BFP) cases in dilutions 1:8 or below on semi quantitative RPR test (P=0.0039). No BFP case was found in dilutions 1:16 or more. (*P*=0.0177).BFP can occur in any age group. We have noted that female 20(66.66%) were showing more BFP cases in our study (*P*=0.0009).

Conclusions: No biological false positive reaction has been found in above 1:8 dilution of RPR test. Semi-quantitative RPR test results in 1:16 or more dilution is equivalent to TPHA results for diagnosis of syphilis.

Key-words: RPR, TPHA, Syphilis, Biologically False Positive (BFP)

INTRODUCTION

Syphilis is a sexually transmitted disease caused by the bacterium Treponema pallidum subspecies pallidum. After an initial dramatic decline due to the availability of penicillin in the 1940s, rates of infection have increased since the turn of the millennium in many countries attributed to unsafe sexual practices, homosexuality and co-infection with Human Immunodeficiency Virus (HIV). Despite the availability of relatively sensitive tests and affordable treatment, the disease remains a global health problem.[1] 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] Syphilis serological diagnosis is demanded for different purposes like antenatal care, to investigate cases of bad obstetric history and primary infertility and of course to confirm a suspected case of syphilis.

Two-step serological testing is required for the diagnosis of syphilis. A nontreponemal screening test is initially done using cardiolipin-lecithin-cholesterol antigen to detect cross-lipid antibodies produced in response to infection with Treponema pallidum (The rapid plasma reagin [RPR]card test). If Positive, the RPR test result is confirmed by a more specific, treponemal antigen-based test like (Treponema pallidum hemagglutination assay

(TPHA)).The RPR test is a sensitive but nonspecific treponemal screening test for syphilis.[3-7]It is positive in late primary syphilis and stages thereafter.[8] After treatment the RPR test usually soon becomes negative except when treatment is instituted in the late secondary or tertiary stage.[9] In some cases of apparently untreated latent syphilis it may eventually become negative.[9-10] A positive RPR test result indicates active treponemal disease if a biological false-positive reaction can be excluded by a specific treponemal test like TPHA. The TPHA test is highly specific and highly sensitive, practically equal to that of the FTA-ABS test, but more difficult to perform and more expensive than nonspecific treponemal tests.[11-18] In the absence of immune-suppression, a negative specific treponemal test is indicative of no past or present infection.[19]

Serum samples that are positive in nontreponemal tests but not in confirmatory treponemal tests are referred to as biologic false-positive (BFP) reactions. Confirmatory treponemal tests like TPHA are expensive and require more technical expertise compared to nontreponemal test. These tests are frequently not available in resource limited health care facilities and laboratories in developing nations like India. They are performed only in reference laboratories and results may not return for days. This scenario yields a biological false positive (BFP) results in patients without syphilis and may compromise clinical decision-making. This study sought to describe the prevalence of BFP and to evaluate role of economical semi quantitative RPR in detecting them compared to TPHA. Such type of evaluation has not been published yet. Objective of our study was to compare semi-quantitative RPR test and TPHA in serological diagnosis of syphilis.

METHODS

We had carried out a retrospective cross-sectional study after approval from institutional review board at a tertiary care hospital in Ahmedabad from July 2012 to July 2015. Confidentiality of all the data was maintained.

Total 216 suspected cases of syphilis were positive by qualitative RPR (Rapid plasma regain) test. All samples were also tested for semi-quantitative RPR (Rapid plasma regain) test and TPHA (Treponema pallidum Hemagglutination assay) test. All the tests were done according to the manufacturer's instructions with commercially available kits.

Procedure for RPR qualitative test:

Serum samples and reagents were brought to room temperature. With dropper one free falling drop of serum was dispensed onto a circle of white reaction card of RPR test kit. Gently shake the antigen dispensing bottle prior to use. Holding in a vertical position one drop was dispensed on the same circle containing serum. Do not mix. Place the card on mechanical rotator under humidifying cover and rotate card for 8 minutes at 100 rpm. Observe the results under a high intensity lamp or strong day light after 8 minutes by rotating card gently by hand (3-4 to and from motions)

Procedure for RPR semi quantitative test:

Semi quantitative testing is a measure of the amount of a substance present in the positive sample either to guide treatment or to quantify the infection. The samples which tested positive for qualitative RPR were retested using a semis quantitative RPR method.

Place 50 µl of 0.9% saline solution in 2nd, 3rd, 4th and 5th circles of the card by using micropipette. Do not spread the saline solution. Using Micropipette add 50 µl sample to saline in 1st and 2nd circle. Mix sample in saline in 2nd circle by drawing the mixture up and down for 8 times in micropipette. Avoid bubble formation. Aspirate 50 µl from 2nd circle and transfer to 3rd circle. Repeat the same successively upto 5th circle. Aspirate 50 µl from the 5th circle and discard it. Perform as mentioned in qualitative test for each of diluted sample drop. The end point is the highest dilution showing visual black clumps.

Procedure for Treponema Pallidum Hemaglutination Assay (TPHA):

In order to compare the two tests all the qualitative RPR positive samples were retested with TPHA to confirm their positivity. Allow samples and reagents to reach room temperature and ensure that samples and all reagents are fully resuspended before use. Each test requires 4 wells of a microtitre plate. Dispense Diluent into the microtitration plate as follows: 25 µl in rows 1, 3 & 4 and 100 µl in row 2. Dispense 25 µl of each sample into a well in row 1. Mix well and transfer 25 µl from row 1 to row 2. Mix well and transfer 25 µl from row 2 to row 3. Mix well and discard 25 µl from row 3. Transfer 25 µl from row 2 to row 4. Mix well and discard 25 µl from row 4. Add 75 µl of well mixed Control Cells to row 3. Add 75 µl of well mixed Test Cells to row 4. Tap plate gently to mix. The final dilutions in row 3 and 4 are 1/80. Cover and let stand at room temperature for 45 to 60 minutes (alternatively the plates can be left overnight). Examine for agglutination patterns. Agglutinated cells form an even layer over the bottom of the well. Non-agglutinated cells form a compact button in the centre of the well .Kit controls are prediluted and should be added directly into individual wells in row 3 and 4 (no diluents required).

Serum samples that are positive in qualitative RPR test but negative in TPHA were referred to as biologic false-positive (BFP) reactions.

Statistical analysis was done by making 2x2 table and applying Fischer's exact test. We have considered *P* value <0.05 to be significant (calculated at 95% confidence interval).

RESULTS

Out of 216 qualitative RPR positive cases 186 (86.11%) cases were TPHA positive and 30 (13.88%) cases are TPHA negative. 30 (13.88%) biological false positive cases were noted to happen in dilutions 1:8 or below on semi quantitative RPR test. This finding is very statistically significant (*P*=0.0039). No Biological false positive case was found in dilutions 1:16 or more. This finding is statistically significant (*P*=0.0177). Further details are shown in table 1.

135(62.5%) male and 81(37.5%) female were RPR positive. Male: Female ratio of RPR positivity is 1.6:1. Biological False positive cases were more in female 20(66.66%) than male 10(33.33%) in our study. Maximum False positivity in Females were noted in 1:1 dilution 11(36.66%). Further details are shown in table 2.

Table 1 : Results of RPR and TPHA tests in suspected syphilis cases

Dilution for Semi quantitative RPR	RPR positive Cases (%)	TPHA positive Cases (%)	TPHA negative Cases (%)	*P* value*
1:1	88(44.74%)	74(39.78%)	14(46.66%)	1.0000
1:2	39(18.05%)	31(16.66%)	8(26.66%)	0.5494
1:4	29(13.42%)	23(12.36%)	6(20%)	0.1092
1:8	31(14.35%)	29(15.59%)	2(6.66%)	**0.0039**
1:16	17(7.87%)	17(9.13%)	0(0%)	**0.0177**
1:32	5(2.31%)	5(2.68%)	0(0%)	0.3807
1:64	6(2.77%)	6(3.22%)	0(0%)	0.5969
1:128	1(0.46%)	1(0.53%)	0(0%)	1.0000
Total	**216(100%)**	**186(100%)**	**30(100%)**	**-**

*P value for Biological False positivity rate for the dilution less than mentioned in the row.

Table 2 : Gender distribution among results of RPR and TPHA tests in suspected syphilis cases

Dilution	RPR positive cases		TPHA positive cases		TPHA Negative cases	
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
1:1	49(22.68%)	39(18.05%)	46(24.73%)	28(15.05%)	3(10%)	11(36.66%)
1:2	29(13.42%)	10(4.62%)	24(12.9%)	7(3.76%)	5(16.66%)	3(10%)
1:4	15(6.94%)	14(6.48%)	14(7.52%)	9(4.83%)	1(3.33%)	5(16.66%)
1:8	20(9.25%)	11(5.09%)	19(10.21%)	10(5.37%)	1(3.33%)	1(3.33%)
1:16	14(6.48%)	3(1.38%)	14(7.52%)	3(1.61%)	0(0%)	0(0%)
1:32	3(1.38%)	2(0.92%)	3(1.61%)	2(1.07%)	0(0%)	0(0%)
1:64	4(1.85%)	2(0.92%)	4(2.15%)	2(1.07%)	0(0%)	0(0%)
1:128	1(0.46%)	0(0%)	1(0.53%)	0(0%)	0(0%)	0(0%)
Total	135(62.5%)	81(37.5%)	125(67.2%)	61(32.8%)	10(33.33%)	20(66.67%)

Table 3: Age group distribution among results of RPR and TPHA tests in suspected syphilis cases

Age group	RPR positive cases	TPHA positive cases	TPHA negative cases	P value*
10 years or less	3(1.38%)	3(1.61%)	0 (0%)	1.0000
11-20 years	16(7.4%)	15(8.06%)	1(3.33%)	0.4840
21-30 years	91(42.12%)	75(40.32%)	16(53.33%)	0.5580
31-40 years	63(29.16%)	**57**(30.64%)	6(20%)	0.6247
41-50 years	37(17.12%)	31(16.66%)	6(20%)	0.5969
51 years or more	6(2.77%)	5(2.68%)	1(3.33%)	1.0000

*P value for Biological False positivity rate for the age group less than mentioned in the row.

Out of 216 RPR positive cases, 91 (42.12%) cases were between 21-30 years of age group. 53.33% false positive cases were also noted in the same age group. But this is statistically not significant (*P*=0.5580). This indicates that false positive cases can occur in any age group. Further age distribution is as shown in table 3.

DISCUSSION

The prevalence of Biological False Positive (BFP) reactions in the general population should be established before the level of association with any clinical entity can be accurately assessed.[20] Biological false positive reaction rate of 13.88% in our study. This is lower than that observed in study done by Yassa et al, which shows 39% BFP rate.[21] Biological false positives occur because RPR test detects non-treponemal antilipoidal antibodies which are not only produced by syphilis infection but also produced by other viral and bacterial infections (Infectious mononucleosis, Epstein-Barr viral infections, viral hepatitis, herpes simplex infections, chancroid, and lymphogranulomavenereum, tuberculosis, malaria, measles). Chronic biological false positivity may occur in Leprosy, Systemic lupus erythematosus, Rheumatoid arthritis, narcotics addiction (especially methamphetamines) and in some neoplasm. This means that people with these conditions continue to have qualitative RPR positivity for life and if the test is not confirmed they can continue treated repeatedly for syphilis for lifetime. [22]

Male: Female ratio of RPR positivity was 1.6:1. This could be attributed to the fact that young male are more sexually active and the symptoms of syphilis show up early in men, generally within two weeks. Biological False positive cases were more in female 20(66.66%) than male 10(33.33%) in our study. For reasons that remain unclear, BFP results were more common in women than men.[23] Autoimmune diseases like Systemic lupus erythematosus affect approximately 8% of the population, 78% of whom are women.[24] This could also be the reason for higher false positive cases in females. Further studies need to be carried out in this regard. Maximum 53.33% false positive cases were noted in 21-30 years of age group. But this is statistically not significant (P=0.5580). This indicates that false positive cases can occur in any age group.

The Biological False Positive (BFP) results encountered in routine screening of the general population are often difficult or impossible to explain and may provide cause for worry or embarrassment to patients. Of even greater importance, a BFP may be the harbinger of an underlying serious disorder.[20,24] If all qualitative RPR positive cases are considered for treatment without checking for biological false positive reactions it may lead to misdiagnosis and overtreatment.This is of particular importance to patients with autoimmune diseases as there is a greater frequency of penicillin and other hypersensitivity drug reactions in this group. The situation is further complicated by the fact that administration of penicillin has been associated with the onset of symptoms of autoimmune disease.[20,26] Such BFP cases are really unfortunate because they would face the potential side effects of syphilis drugs besides missing the actual infections they were suffering from.

In our study we noticed RPR test false positivity maximum in dilutions 1:8 or below on semi quantitative RPR test. This finding is very statistically significant (P=0.0039). Maximum False positivity in Females was noted in 1:1 dilution 11(36.66%). This finding is very statistically significant (P=0.0073). False positivity decreases as the dilution increases. No Biological false positive case was found in dilutions 1:16 or more. This finding is statistically significant (P=0.0177). Semi-quantitative RPR test results in 1:16 or more dilution were equivalent to TPHA results for diagnosis of syphilis. In resource-limited health care settings or unavailability of TPHA one should do semi-quantitative RPR test after positive qualitative RPR test to increase the confidence in results of RPR test. So unnecessary treatment of syphilis can be avoided in false positive cases and other diagnosis can be searched for. This will enable us to reduce the cost of diagnosis and management of the syphilis.

CONCLUSION

No biological false positive reactions have been found in above 1:8 dilution of RPR test. Semi-quantitative RPR test results in 1:16 or more dilution can be considered equivalent to TPHA results for diagnosis of syphilis in a resource limited health care facility.

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