

Seroprevalence of Syphilis and Biological False Positive Reactions among Different Population Groups attending a Tertiary Care Hospital, Warangal

Sreedevi S¹, A. Mrudula S²

¹ Associate Professor

² Postgraduate Student

Department of Microbiology
Kakatiya Medical College
Warangal
Telangana, India.

CORRESPONDENCE:

¹ Dr. Sreedevi S, MD
(Microbiology)

Associate Professor

Department of Microbiology
Kakatiya Medical College
Warangal

Telangana, India.

E-mail:

Sreedevikonda143@gmail.com

ABSTRACT

Background and Aim: Syphilis is a sexually transmitted infection, remains an important cause of morbidity in developing countries like India. The purpose of this study was to determine the true picture of seroprevalence of syphilis and biological false positive reactions in different patient groups at a tertiary care hospital, Warangal.

Materials and Methods: A total of 4571 serial serum samples obtained over a period of 1 year (August 2017 to July 2018) from different patient groups were screened by RPR (Rapid Plasma Reagin) test both qualitatively and quantitatively. RPR reactive sera were confirmed by *Treponema pallidum* hemagglutination assay (TPHA) .

Result: The overall seroprevalence of syphilis by RPR test was 5.81% and biological false positive rate in test population was 1.09%.

Conclusion: All the sera positive with RPR test must be confirmed as positive by TPHA.

Keywords: Syphilis, RPR, TPHA, Biological false positive

INTRODUCTION

Syphilis was first documented as a disease process in the 1500s but it took until 1905 for the spirochete *T. Pallidum* to be isolated by Schaudinn and Hoffman and was subsequently confirmed as the causative agent of syphilis in 1912.^[1-3] Once the spirochete was identified, laboratories were quick to find new methods to detect the pathogen. In 1906, Landsteiner and Mucha introduced the use of dark field microscopy as a method to directly

detect the presence of *Treponema pallidum* in a chancre lesion.^[4]

August Wasserman subsequently developed the first serologic test for syphilis.^[3,5,6] Venereal Disease Research Laboratory (VDRL) assay, was developed in 1946.^[7] It is based on detecting the body's antibody response to the release of cardiolipin, which is elevated in numerous chronic conditions and infections including syphilis.^[8] The RPR assay, developed in 1957^[9] is a modification of

VDRL that is visualized by the naked eye with the assistance of charcoal particles rather than microscopically as with the VDRL test.^[10]

Treponemal assays, that either directly detect the pathogen or antibodies to the pathogen, have also evolved over the course of the 20th century and now 21st century.^[3,6] There are also several tests that detect treponemal antibodies including *Treponema pallidum* - particle agglutination (TP-PA) assay.^[11] Definitive laboratory diagnosis has always been challenging due to both the wide array of clinical manifestations and the lack of a single optimal test.^[5]

A biological false positive (BFP) test for syphilis is considered to be a reactive non-treponemal test but a nonreactive treponemal test.^[12] It has been documented that the BFP rate may be as high as 50% in few selected populations although it usually ranges between 1% and 2% in a healthy population^[3,12,13] whereas the treponemal antibody tests such as *T. Pallidum* hemagglutination (TPHA) yield <1% false positive results making it highly reliable.^[14] However, many centers for sexually transmitted infections (STIs) in India perform only a single screening assay either VDRL or (rapid plasma reagin) RPR for serodiagnosis of syphilis.^[15]

The purpose of study was undertaken to determine the seroprevalence of syphilis and to assess the biological false positivity rate in different patient groups at a tertiary care Hospital, Warangal.

MATERIALS AND METHODS

After taking permission from ethical committee of the hospital, a prospective study was conducted on 4571 serial serum samples obtained over a period of 1 year i.e from August 2017 to July 2018 at Mahatma Gandhi Memorial Hospital, Warangal from different patient groups like antenatal cases (n=1080), HIV positive cases on anti-retroviral therapy (n=1098), STD clinic cases (n=1298), high risk groups (n=1095) like transgender (n=210), male having sex with male (MSM) (n=362), female sex workers (FSW) (n=523) and were screened by RPR (Rapid Plasma Reagin) test using kit by Beacon diagnostics both qualitatively and quantitatively. RPR reactive sera were confirmed by TPHA (*Treponema pallidum* hemagglutination assay) using IMMUTREP TPHA by omega diagnostics. 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. Place one drop of serum (50 µl) on the slide with disposable serum dropper. After gently mixing

R.P.R. antigen suspension place one drop (15-20 µl) by antigen dropper. Mix well and spread out the liquid on entire area of the circle by using disposable mixing stick. Rock the slide gently for 6 minutes and observe under good light source with a hand lens for appearance of carbon particle clumping is reported as positive.

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 semi 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 in 1st and 2nd circle.

Mix sample with saline in 2nd circle by drawing the mixture up and down for 5 times in the micropipette. Avoid bubble formation. Aspirate 50 µL from 2nd circle and transfer to 3rd circle. Repeat the same successively up to 5th circle. Aspirate 50 µL from the 5th circle and discard it. After gently mixing RPR antigen suspension place one drop (15 to 20 µL) by antigen dropper in each diluted sample drop. Mix well and spread out the liquid on entire area of circle by using disposable mixing stick. Rock the slide gently for 6 minutes and observe under good light source for appearance of carbon particle clumping. The end point is the highest dilution showing visual black clumps.

Procedure for *Treponema Pallidum* Hemagglutination Assay

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 re-suspended 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. Weakly agglutinated cells form a characteristic ring pattern. Agglutination of the Test

Cells but not the Control Cells indicates the presence of specific antibody to *T. Pallidum*.

STATISTICAL ANALYSIS

Chi-square test was used to calculate P value for analysis of statistical significance of the data. $P \leq 0.05$ was considered as statistically significant.

RESULTS

Out of 4571 serial serum samples screened by RPR (Rapid Plasma Reagin) test both qualitatively and quantitatively 266 sera (5.81%) were positive. RPR reactive sera were confirmed by TPHA. With TPHA 216 Sera (4.72%) were reactive. Biological false positivity was found to be 1.09%.

Table 1: Results of RPR test, TPHA test, Biological false positive reactions among different study groups

| Study groups | Total samples tested | RPR positive | TPHA positive | Biological positive reactions | false (BFP) |
|------------------|----------------------|--------------|---------------|-------------------------------|-------------|
| | | | | (n) | (%) |
| ART cases | 1098 | 44 | 22 | 22 | 2 |
| STD clinic | 1298 | 56 | 53 | 3 | 0.23 |
| ANC cases | 1080 | 29 | 11 | 18 | 1.67 |
| High risk groups | | | | | |
| Transgender | 210 | 54 | 52 | 2 | 0.95 |
| MSM | 362 | 11 | 10 | 1 | 0.27 |
| FSW | 523 | 72 | 68 | 4 | 0.76 |
| Total | 4571 | 266 | 216 | 50 | 1.09 |

Overall seroprevalence by RPR test was 5.81%. Seroprevalence of syphilis was high 12.51% among high risk groups i.e among transgenders, male having sex with male, female sex workers.

The seroprevalence among different genders was highly significant ($p < 0.0001$) with 20.07%, 5.3%, 4.1% seropositivity in transgenders, females, males

respectively.

Among these RPR test reactive sera biological false positivity was high among females 1.36% ($p < 0.0001$). All sera with RPR titre $\geq 1:8$ were found to be positive with TPHA test as well. Biological false positive reaction was seen in sera with RPR titre $< 1:8$ ($p < 0.0001$).

Table 2: Seroprevalence of syphilis based on gender

| Gender | total | Seropositive (n) | % | (P < 0.0001) |
|-------------|-------|------------------|-------|--------------|
| Male | 1731 | 71 | 4.1 | |
| Female | 2556 | 138 | 5.3 | |
| Transgender | 284 | 57 | 20.07 | |
| Total | 4571 | 266 | | |

Table 3: Biological false positive reactions based on gender

| Gender | Biological false positive (BFP) reactions | % | (P < 0.0001) |
|-------------|---|------|--------------|
| Male | 13 | 0.75 | |
| Female | 35 | 1.36 | |
| Transgender | 2 | 0.70 | |
| Total | 50 | | |

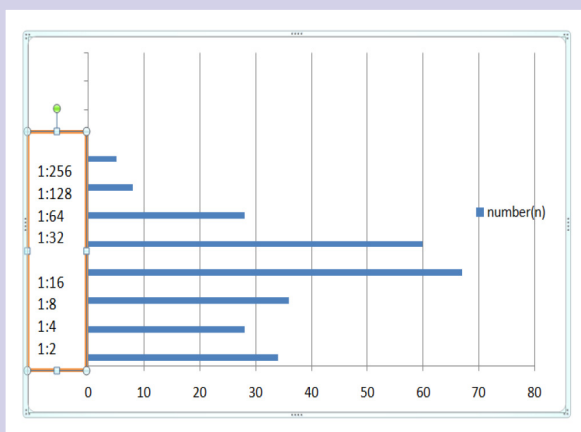


Chart 1: Quantitative analysis of RPR titres.

DISCUSSION

In our study overall seroprevalence of syphilis was 5.81% (266/4571). Among the reactive sera, prevalence of syphilis was high among high risk groups upto 12.5% (137/1095). Transgenders constitute 20.07% (57/284) among positive sera. This higher rate of seroprevalence of syphilis is similar to the study conducted by Subhash Dasarathan et al was 20.07% (17 of 82).^[16] Seroprevalence of syphilis in other groups was 4%, 4.31%, 2.68% among HIV positive cases on anti-retroviral therapy, STD clinic cases, antenatal cases respectively. In India, other studies show the prevalence of syphilis ranges between 2.0-4.8% among women of reproductive age^[17, 18, 19, 20], STD clinics 5.4% to 8.2%.^[21, 22]

Biological false positive reactions were high among females and among RPR test titers <1:8. 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 lymphogranuloma venereum, 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.^[23] For reasons that remain unclear, BFP results were more common in women than men. Autoimmune diseases like Systemic lupus erythematosus affect approximately 8% of the population, 78% of whom are women. This could also be the reason for higher false positive cases in females.^[24, 25]

CONCLUSION

Prevalence of syphilis can be reduced by educating people regarding its modes of transmission and precautions that could be taken to prevent the spread of infection. Early and true positive diagnosis of syphilis is necessary for prompt initiation of treatment and avoiding dreadful complications of syphilis. Therefore, we conclude that all sera positive with RPR test must be confirmed as positive by TPHA.

CONFLICT OF INTEREST:

The authors declared that no conflict of interest.

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Seroprevalence of Syphilis and Biological False Positive Reactions among Different Population Groups

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