Original Research Article

Study of prevalence of biological false positive serological tests for syphilis in pregnancy

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Abstract

Background: A reactive RPR should always be confirmed with Treponemal test like Treponema pallidum Hemagglutination assay (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 find out prevalence of biological false positive serological tests for syphilis in pregnancy and 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 2018 to July 2019 was conducted on all cases were tested for qualitative Rapid plasma reagin (RPR) test, semi-quantitative RPR test and 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 11 (0.39%) biological false positive (BFP) cases in dilutions below 1:8 on semi quantitative RPR test. No BFP case was found in dilutions 1:8 or more.BFP can occur in any age group as being noticed. **Conclusions:** No biological false positive reaction has been found in above 1:8 dilution of RPR test. Semi-quantitative RPR test results in 1:8 or more dilution is equivalent to TPHA results for diagnosis of syphilis.

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

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INTRODUCTION

Syphilis is sexually transmitted diseases caused by the spirochete *Treponema pallidum* subspecies *pallidum*. Syphilis is an important cause of perinatal morbidity and mortality in resource poor setting. Adverse infant or fetal outcomes arise in 50-80% of pregnancies that survive beyond 12 weeks of gestation. Syphilis control is facilitated by the availability of inexpensive and sensitive diagnostic test as well as affordable treatment.[1] For early diagnosis of Syphilis in pregnancy government of India has recommended every antenatal care patient must be screened for syphilis and treated. In pregnancy there are chances to obtain false positive result for non treponemal test like RPR, VDRL that is called Biological false positive reaction (BFPR). This BFPR is defined as positive results in non treponemal tests with negative results in treponemal tests, in absence of syphilis and not caused by technical fault. Cardiolipin antigen being nonspecific may react with the sera of patients suffering from unrelated diseases but not having syphilis. Reagin antibodies are induced against the cardiolipin antigen present in *T. pallidum* or to similar lipid haptens released from the damaged host tissues.[2] A biological false positive reactions may be acute or chronic. The acute reaction which disappears spontaneously in a few weeks to 6 months is seen most frequently after immunization or during pregnancy. The chronic reaction lasts for months or even a lifetime and may be caused by SLE, rheumatoid disease, rheumatic fever etc.[3] For treatment of syphilis we use penicillin and this drug can cause Jarisch Herxheimer reaction in syphilis as well as in Q fever, Bartonellosis, Brucellosis, Trichinellosis, Lymes disease, relapsing

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fever, leptospirosis, African Trapanosomiasis etc. Apart from this reaction penicillin itself causes hypersensitivity reaction in some individual. So it is better to avoid unnecessary use of penicillin and for this it is required to rule out BFP reaction.

MATERIALS AND METHODS

This study was designed as a descriptive retrospective study after approval from institutional review board at PIMS, Udaipur from January 2018 to December 2018. Confidentiality of all the data was maintained. Total 15 suspected cases of syphilis were positive by qualitative RPR (Rapid plasma regain) test. All samples were also tested for semi-quantitative RPR (Rapid plasma reagin) test and TPHA (Treponema pallidum Hemagglutination assay) test. All the tests were done according to the manufacturer's instructions with commercially available kits.

INCLUSION CRITERIA: A Total 2795 non duplicate venous blood was collected from antenatal care patients attending outpatient departments (OPD) and admitted in wards and labor room at PIMS hospital with all aseptic precautions and transported to the Microbiology laboratory. A Performa was used to collect medical and demographic data of the patients.

EXCLUSION CRITERIA: Contaminated, insufficient sample, hyperlipidemic sample and repeated samples from same patients were excluded from this study.

Statistical analysis was done by using Fischer's exact test. P value less than 0.05 was accepted as statistical significance level.

RESULTS

Out of 2795 only 15 samples were RPR positive. These positive samples were taken for quantitative test RPR in dilution showing results 1:2, 1:4, 1:4, 1:16, 1:8, 1:4, 1:4, 1:4, 1:2, 1:16, 1:32, 1:4, 1:2, 1:4, 1:4 respectively. Table 1.Those samples showing quantitative results ≥ 1:8 were further tested with Treponemal test TPHA. All the 4 samples which have quantitative results ≥ 1:8 are also positive in TPHA.11 biological false positive cases were noted to happen in dilutions below 1:8 on semi quantitative RPR test. Biological False Positive results were 0.39 % (11 out of 2795).Fig 1. Out of 15 RPR positive cases, 07 (46%) cases were between 21-30 years of age group. 06(40%) false positive cases were also noted in the same age group. But this is statistically not significant. This indicates that false positive cases can occur in any age group. Table 2.

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

Dilution for Semi quantitative RPR	RPR positive Cases (%)	TPHA positive Cases (%)	TPHA negative Cases (%)
1:2	3 (20%)	0 (0%)	3 (27.3%)
1:4	8 (53.3%)	0 (0%)	8 (72.7%)
1:8	1 (6.7%)	1 (25%)	0 (0%)
1:16	2 (13.3%)	2 (50%)	0 (0%)
1:32	1 (6.7%)	1 (25%)	0 (0%)
Total	15	4	11

Table 2: Age related Semi-Quantitative RPR test Positivity

Dilution RPR	21-30yrs	31-40yrs	41-50yrs
1:2	2	1	0
1:4	4	3	1
1:8	0	1	0
1:16	1	1	0
1:32	0	1	0
Total (15)	**7**	**7**	**1**

DISCUSSION

Syphilis can seriously complicate pregnancy and result in spontaneous abortion, stillbirth, non immune hydrops, intrauterine growth restriction, and perinatal death, as well as serious sequelae in liveborn infected children. While appropriate treatment of pregnant women often prevents such complications, the major deterrent has been inability to identify the infected women and get them to undergo treatment. Screening in the first trimester with non-Treponemal tests such as rapid plasma Reagin (RPR) or Venereal disease research laboratory (VDRL) test combined with confirmation of reactive individuals with Treponemal tests such as the TPHA or fluorescent Treponemal antibody absorption (FTA-ABS) assay is a cost effective strategy. Those at risk should be retested in the third trimester. Treatment during pregnancy should be with penicillin. In determining a penicillin regimen, the clinician must consider the stage of the maternal infection and the HIV status of the mother. Patients who are allergic to penicillin should be desensitised before treatment. Despite appropriate treatment, as many as 14% will have a fetal death or deliver infected infants. Treatment may further be complicated by the Jarich–Herxheimer reaction, a complex allergic response to antigens released from dead micro-organisms, which can cause fetal distress and uterine contractions. Pregnancy has no known effect on the clinical course of syphilis. The cervical changes, such hyperaemia, eversion, and friability, which occur during pregnancy may facilitate the entry and lead to Spirochaetaemia.[4] False positivity rate is around 1% when screening the general population with these tests.[5-8] False reactive results may be more frequent when testing certain patients groups, such as the elderly or the pregnant, or patients with drug addiction, malignancy, autoimmune diseases (for example, systemic

lupus erythematosus), viral diseases (particularly with Epstein–Barr and hepatitis viruses), protozoal or mycoplasma infection.[9,10] In low risk populations, all reactive test results should be confirmed by a treponemal test since over 50% of the non-treponemal tests may be false reactive. False reactive results also occur with approximately 1% of uninfected individuals when screening the general population.[6,8,11] The proportion of biological false positivecases in the present study were 0.39 % (11 out of 2795) which is comparable to 0.47% (61/13008) by Tankhiwale *et al*.[12] ,0.5% by Hossain *et al.*[13.59% by M.F.Smikle *et al.*14] A lower proportion of biological false positives (0.39%) in the present study shows the importance of TPHA in diagnosing syphilis. More false positives would have reported if only the non-specific non-treponemal test had been used (15/2795=0.54%). 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. 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 anti-treponemal therapy. Though TPHA is not a 100% sensitive and specific test, the ease of performing the test in a less-equipped laboratory makes it a better option than more specific treponemal tests and hence is considered more laboratory friendly. No biological false positive reactions have been found in above 1:8 dilution of RPR test. Semiquantitative RPR test results in 1:8 or more dilution can be considered equivalent to TPHA results for diagnosis of syphilis in a resource limited health care facility.

CONCLUSION

Single test of non-treponemal antibody like RPR 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. So, specific Treponemal tests could contribute to reducing errors that depend on specificity of the method. Based on our results we think that the limits of screening tests for the diagnosis of syphilis should not be forgotten and confirmatory tests like TPHA must be done. Serology being the prime importance in the laboratory diagnosis of Syphilis must be viewed in the context of clinical presentations.

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