International Journal of Community Medicine and Public Health
Sahota SK et al. Int J Community Med Public Health. 2021 Mar;8(3):1217-1220
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20210803

Original Research Article

Biological false positive rapid plasma reagin tests in pregnant females in North India

Somanpreet Kaur Sahota*, Barun Kumar Shakya, Deepak Arora, Neerja Jindal

Department of Microbiology, Guru Gobind Singh Medical College, Faridkot, Punjab, India

Received: 10 November 2020
Revised: 04 January 2021
Accepted: 15 January 2021

***Correspondence:**
Dr. Somanpreet Kaur Sahota,
E-mail: sheeta.sahota5@gmail.com

ABSTRACT

Background: Syphilis, a sexually transmitted disease seriously complicate pregnancy and produce harmful results in fetus. Screening of pregnant females in early pregnancy with non-treponemal tests such as RPR and VDRL is a cost effective strategy for diagnosis of syphilis. However, these tests could produce BFP reaction in pregnancy. The prevalence of which may be different in different populations and at different times. The current prevalence of the biological false positive rapid plasma regain tests in pregnant females of North India was there for assessed and being reported.
Methods: A total 500 consecutive pregnant females who presented to our tertiary care center for the first time were screened for syphilis by RPR (a non-treponemal test) and Hi-Quik (a treponemal test) after obtaining their written consent.
Results: Of the 500 females 21 (4.2%) were RPR positive and only one (0.2%) was Hi-Quik positive. Thus the prevalence of biological false positive rapid plasma regain tests in pregnant females of North India was 4% (20/500). In semi quantitative RPR, these tests were positive in ≤1:4 dilution and were not found to be related to any particular age, geographical distribution, literacy status, occupation or period of gestation (p value >0.05) and were transient in nature.
Conclusions: Our study concludes that although RPR is a good, cost effective test for mass screening of pregnant females, but it produces BFP reactions in considerable percentage (4%) in pregnant females of North India.

Keywords: Antenatal screening, BFP reactions, Hi-Quik test, Pregnant females, RPR test, Syphilis

INTRODUCTION

Syphilis, a common sexually transmitted disease, with an estimated worldwide prevalence of 12 million new infections each year.[1] It can seriously complicate pregnancy and may result in spontaneous abortion, stillbirth, non-immune hydrops fetalis, intrauterine growth restriction, perinatal death and serious sequelae in live born infectious children.[2] It is still a challenge to diagnose this complex infection because of the inability of the culture of its causative agent, that is *T. pallidum spp. pallidum.* Antenatal screening of pregnant females provides a good opportunity to detect this infection in early stage of pregnancy. Historically the assays for non-specific antibodies have been used as routine screening tests for diagnosis of syphilis. This is because of their low cost and technical simplicity. However, the antigen utilized in these test is cardiolipin, which is non-specific and thus may cross-react with the sera of the patients who may not have syphilis.[3]

Biological false positive reaction is defined as positive result in non treponemal test and negative result in treponemal test in the absence of syphilis and is not caused by any technical fault.[4] The false positivity rates have been reported to be <1% in general population and

may be more in certain patient groups such as elderly or the pregnant females or the patients with drug addiction, malignancy, autoimmune disease (SLE), viral diseases (Epstein Barr virus, hepatitis, HIV), protozol and mycoplasma infection.[5,6] Lubinski suggested in his study that the percentage of false positive finding varies widely even for the same disease by different authors.[7] In literature these reactions have been described as transient and chronic. Transient are due acute processes and last for less than six months. While chronic reactions persists beyond six months and have been found in patients with autoimmune diseases (rheumatic disorder) and malignancies.[8,9] It has also been reported that BFP reactions comprise a higher proportion of reactions in a low syphilis prevalence population in comparison to high syphilis proportion population.[10]

Although, the exact prevalence of syphilis in India is not known, but rising trends of syphilis from North India have been observed in some studies.[11] Therefore, the present study was undertaken to assess the current prevalence of BFP reactions in RPR test in pregnant females, where this test is most commonly employed for screening of syphilis in pregnancy.

METHODS

This cross-sectional hospital based study was conducted on a total of 500 consecutively pregnant females who visited the antenatal clinic of a tertiary care hospital of North India from 3rd March 2019 to 3rd December 2019.

Inclusion criteria

All the pregnant females who were above the age of 18 years and were attending the antenatal clinic for the first time were included

Exclusion criteria

Females <18 years and who were not resident of North India were excluded from the study.

After taking their informed written consent their demographic profile was recorded in the prescribed proforma. Their blood samples were collected (using all the sterile precautions). Serum was separated and stored at 4°C. Rapid plasma regain (RPR), a slide flocculation test was performed on the quantitative basis on the serum samples using Recon Diagnostic Kit RPR card tests. Hi-Quik immuno chromatographic test (ICA) was employed to confirm the RPR positive test results. The Hi-Quik is a rapid ICA for the qualitative detection of antibodies (IgG and IgM) to *T. pallidum* in serum or plasma. In this test recombinant syphilis antigen is immobilize in the test line region of the test. After specimen is added to the specimen well of the test cassette, it reacts with the syphilis antigen coated particles and this mixture then migrates chromatographically along the length and interact with the immobilize syphilis antigen. The positive result is indicated by a colored line in the test line region and negative result by the appearance of color only in the control line region and no colored line in the test line region.

The study was approved by the Institutional Ethical Committee. The data pertaining to the sociodemographic profile was compiled and analyzed by using SPSS V. 20.0.

RESULTS

The study of sociodemographic profile of 500 females showed that their age was between 18-40 years and mean age was ±25.4. The maximum number of females (40.8%) belonged to age group of 21-25 years, followed by 32.2% in the age group of 26-30 years. Only 3.6% females were more than the age of 35 years. Eighty four percent female of the study group belonged to rural area while only 16% were from urban area. Although most of them (92%) were literate (had primary school education or above), only 25.2% were employed, It was observed that as many as 79% came to our tertiary care hospital only in third trimester of pregnancy (Table 1).

Of these 500 females, 21 (4.2%) were found to be RPR positive. Out of these 21 only 1 (0.2%) was Hi-Quik positive and the test of 20 (4%) were Hi-Quik negative (Table 1). This shows that biological false positive reactions were present in 20 (4%) pregnant females of our study. It was further observed that BFP reactions occurred in dilutions of 1:4 or below on semi quantitative RPR test. The maximum 9 (45%) false positivity was noted in 1:2 dilutions (Table 2).

The study of the 20 females who showed the biological false positive reactions revealed that 35% of these false positive reactions were shown by females each in the age groups of 21-25 and 31-35 years. This was followed by 20% in age group of 26-30 years. However the difference between these age groups were statistically not significant (Table 1, p value: a and b=0.43; b and c=0.087). Out of 20 females showing false positive RPR, 17 (85%) belonged to rural area and 3 (15%) were from urban area, but the difference was statistically insignificant (p value =1). Similarly the differences between the literacy status (p value=0.75) and occupation (p value =0.79) of these females with BFP reactions were also statistically insignificant (Table 1).

Table 1: Sociodemographic profile and results of RPR and Hi-Quik tests of pregnant females (n=500).

Sociodemographic characteristics	Total	RPR positive	Hi-Quik positive	False positive RPR	p value <0.05 significant
Age (years)					
<20[c]	47 (9.4)	2 (9.53)	-	2 (10)	a and c= 0.43
21-25[b]	204 (40.8)	7 (33.34)	-	7 (35)	b and c= 0.87
26-30	161(32.2)	5 (23.8)	1	4 (20)	
31-35[a]	70 (14)	7 (33.34)	-	7 (35)	
36-40	18 (3.6)	0	-	-	
Geographical distribution					
Urban[d]	80 (16)	3 (14.3)	-	3 (15)	d and e=1.
Rural[e]	420 (84)	18 (85.7)	1	17 (85)	
Literacy status					
Illiterate[f]	43 (8)	1 (4.7)	-	1 (5)	f and g=0.75
Literate[g]	457 (92)	20 (95.3)	1	19 (95)	
Occupation					
Unemployed[h]	375 (74.8)	17 (80.9)	1	16 (18)	h and i=0.79
Employed[i]	126 (25.2)	4 ((19.1)	-	4 (20)	
Period of gestation					
1st trimester[j]	25 (5)	1 (4.7)	-	1 (5)	j and k=1.
2nd trimester	80 (45)	1 (4.7)	1		
3rd trimester[k]	395 (79)	19 (90.6)	-	19 (95)	

Figure in parenthesis represent percentage

Table 2: RPR titres of RPR positive RPR and Hi-Quik positive test (n=21).

RPR titre	RPR positive	Hi--Quik positive	False positive RPR
R1	8 (38)	-	8 (40)
R2	9 (42.9)	-	9 (45)
R4	3 (14.3)	-	3 (15)
R16	1 (4.8)	1	-

Figure in parenthesis represent percentage

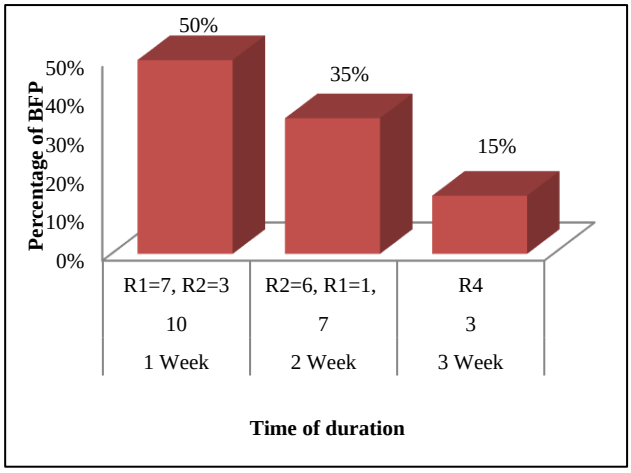

Figure 1: Seroconversion among BFP (n=20).

Of the 20 females who showed BFP RPR tests, affirmative tests for serology for all positive females were performed every week. On follow-up, the expected range for seroconversion was between 1 to 3 weeks. The maximum 10 (50%) number of females (with RPR reactive titre R1 and R2) completely returned to non-reactive state within 2 weeks followed by 3 (15%) (RPR titre R4) within 3 weeks (Figure 1).

DISCUSSION

Timely diagnosis and proper management of the syphilis in the pregnant females are important to prevent adverse outcomes of pregnancy. Primarily, screening with a non-treponemal test such as VDRL or the RPR is cost effective and is usually employed in antenatal clinics in India.[10] Pregnancy is well recognized cause of biological false positive (BFP) reactions in non-treponemal tests.[12,13] However, the percentages of reported false positive findings vary widely in literature.[8] We studied the occurrence of BFP reactions in 500 consecutive pregnant females of our tertiary care hospital and the prevalence of BEP reaction was found to be 4%. This is much less than that observed in studies of Shaikh et al (13.88%) and Yassa et al (39%).[14] This could be because their study populations included all suspected syphilis patients and not only pregnant females.[15] In a similar study from North India BFP reactions were found in 0.27%, while it was 6.93% in the pregnant females of Nigeria.[16]

Out of 20 females who show BFP reactions in our study, 9 (45%) had titre R2 and 8 (40%) had titre R1 (Table 2). The studies conducted by Taiwo et al and Smikle et al had also reported maximum BFP reactions in females having lower titres in RPR test.[9,16] Out of 20 RPR reactive pregnant females, 7 (35%) each were from age group 21-

25 and 31-35 years. No female above the age 35 years were found to be reactive. The difference in the distribution of RPR reactivity between females of 31-35 years and 15-20 was statistically insignificant (p value 0.43). This shows that no specific age group is prone to show BFP reactions. Similar results have been reported by Mehta et al.[17] The other factors studied for BFP reactions were geographical profile, education, occupation and all of these were found to have no statistical significant effect of BFP reaction (Table 1). In 2013 Mehta et al studied their effect of duration of pregnancy (trimester) and it was found to be statistical insignificant. This is in accordance with the result of the present study.[17]

In the present study the biological false positive RPR reactive sera underwent seroconversion after 1 week in 50% females, 2 week in 35% females and 3 week in 15% females (Figure 1). This indicates that BFP reactions were transient in nature. However all BFP reactors should be followed up and categorised as acute (transient) or chronic. Patients with chronic BFP results should be investigated for clinical laboratory indicators of autoimmune or other chronic diseases. These measures would reduce the indiscriminate and inappropriate use of antibiotics and other medicines. Wiwanitkit studied 30 patients who had biological false positive VDRL test and on follow up the seroconversion range was between 9.25 and 10.49 weeks.[19]

Unfortunately, our study had limitations of small sample size collected from the pregnant females visiting the single health care facility only.

CONCLUSION

The results of the present study emphasize the need to continue the use of cost effective RPR tests for screening of syphilis of pregnant females to prevent its adverse effect of pregnancy. However, the females showing positive RPR test should be carefully followed up by either doing confirmatory test or by retesting (RPR test) to rule out BFP rapid plasma reagin tests.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee BFUHS/2K19P-TH/8904

REFERENCES

1. Maves RC, Dean K, Gadea N, Halsey ES, Graf PC, Lescano AG. False-positive rapid plasma reagin testing in patients with acute *Plasmodium vivax* malaria: a case control study. Travel Med Infect Dis. 2014;12(3):268-73.
2. Genç M, Ledger WJ. Syphilis in pregnancy. Sexual Transmit Infect. 2000;76(2):73-9.
3. Kanungo R. Spirochetes. In: Ananthanarayan and Paniker's Textbook of Microbiology. 10th edn. The Orient Blackswan; 2017:377-391.
4. Garner MF. The serodiagnosis of syphilis. Med J Australia. 1966;2(7):328-1.
5. Augenbraun MH, DeHovitz JA, Feldman J, Clarke L, Landesman S, Minkoff HM. Biological false-positive syphilis test results for women infected with human immunodeficiency virus. Clin Infect Dis. 1994;19(6):1040-4.
6. Moore JE, Mohr CF. Biologically false positive serologic tests for syphilis: type, incidence, and cause. J Am Med Assoc. 1952;150(5):467-73.
7. Lubinski HH. False positive serologic reactions. Canadian Med Assoc J. 1947;57(1):33
8. Smikle MF, James OB, Prabhakar P. Biological false positive serological tests for syphilis in the Jamaican population. Sexual Transmit Infect. 1990;66(2):76-8.
9. Stone JH, Amend WJ, Criswell LA. Antiphospholipid antibody syndrome in renal transplantation: occurrence of clinical events in 96 consecutive patients with systemic lupus erythematosus. Am J Kidney Dis. 1999;34(6):1040-7.
10. Geusau A, Kittler H, Hein U, Dangl-Erlach E, Stingl G, Tschachler E. Biological false-positive tests comprise a high proportion of Venereal Disease Research Laboratory reactions in an analysis of 300,000 sera. Int J STD AIDS. 2005;16(11):722-6.
11. Sethi S, Mewara A, Hallur V, Prasad A, Sharma K, Raj A. Rising trends of syphilis in a tertiary care center in North India. Indian J Sexual Transmit Dis AIDS. 2015;36(2):140.
12. Nandwani R, Evans DT. Are you sure it's syphilis? A review of false positive serology. Int J STD AIDS. 1995;6(4):241-8.
13. Brede HD, Willey KD, Kindermann RA, Finlayson MH. Detection of biological false positive syphilis serum reactions. SAMJ. 1974;48(6).
14. Shaikh NM, Mehta TK, Shah PD. Comparison of semi-quantitative RPR Test and TPHA for Serodiagnosis of Syphilis. Nat J Community Med. 2017;8(9):541-5.
15. Yaassa P, Bwalya CB, Hira RS, Kwenda, Kunda M, Sarenje K, et al, Quantitative RPR Test as a Guide for the Diagnosis and Treatment of Syphilis in Zambia. Int Infect Dis. 2013;1(12).
16. Taiwo SS, Adesiji YO, Adekanle DA. Screening for syphilis during pregnancy in Nigeria: a practice that must continue. Sexual Transmit Infect. 2007;83(5):357-8.
17. Mehta KD, Antala S, Mistry M, Goswami Y. Seropositivity of hepatitis B, hepatitis C, syphilis, and HIV in antenatal women in India. J Infect Dev Countries. 2013;7(11):832-7.
18. Wiwanitkit V. Biological false reactive VDRL tests: when to re-test? Southeast Asian J Trop Med Public Health. 2003;33:131-2.

Cite this article as: Sahota SK, Shakya BK, Arora D, Jindal N. Biological false positive rapid plasma reagin tests in pregnant females in North India. Int J Community Med Public Health 2021;8:1217-20.