

Rapid Plasma Reagin Card Test: A Screening Method for Syphilis in Antenatal Patients of Bastar, Chhattisgarh, India

SAHINA HASSAN¹, SATYARAM SATAPATHY², RATNA AGRAWAL³

ABSTRACT

Introduction: Sexually Transmitted Disease (STD) like syphilis is one of the important factor leading to adverse pregnancy outcomes in antenatal mothers. The infant and neonatal mortality rate itself is higher in the tribal region of Chhattisgarh like Bastar due to poor educational status and limited availability of health infrastructures. To prevent the disease related adverse pregnancy outcomes, early diagnosis and treatment of STDs is important by rapid screening method like Rapid Plasma Reagin (RPR) card test, which is comparatively cost effective tool available at clinical settings.

Aim: To detect the incidence of syphilis among antenatal mothers of Bastar, Chhattisgarh, India by using RPR card test.

Materials and Methods: The present cross-sectional study was conducted on 150 blood samples of patients attending Obstetrics and Gynaecology, Outpatient and Inpatient Department (OPD and IPD) from August to October 2021. The samples referred to microbiology laboratory of Late Baliram Kashyap Memorial Government Medical College Jagdalpur, Chhattisgarh, India, were

tested by RPR card test taking all aseptic precautions. The data were collected in Microsoft Excel sheet and results were presented in counts and percentages using descriptive statistics.

Results: Out of 150 samples, 11 (7.33%) were tested positive by RPR card test while rest 139 (92.67%) were found negative. Out of the 11 (7.33%) samples which tested positive, 3 (27.27%) were from Antiretroviral Therapy (ART) centre (concurrent Human Immunodeficiency Virus (HIV) and syphilis positive), 7 (63.64%) were from Obstetrics and Gynaecology OPD and 1 (9.09%) sample was from labour ward. Age of the patients attending antenatal clinic ranged between 11-50 years with maximum patients 103 (68.67%) were in 21-30 years of age group.

Conclusion: The RPR card test is one of the important cost effective, rapid screening tools for STDs like syphilis, especially in the resource poor areas like tribal region of Bastar, Chhattisgarh, India. Comparatively higher percentage of syphilitic infection reported in the study may contribute in improving health infrastructure policies in the affected regions of Bastar, Chhattisgarh, India.

Keywords: Sexually transmitted diseases, Slide flocculation test, Treponemal diseases, Tribal region

INTRODUCTION

One of the sexually transmitted treponemal disease syphilis, affects around 1% or more of the antenatal population worldwide [1]. In India, the estimated prevalence of syphilis is around 6% [2,3]. Although, the accurate data of prevalence of syphilis in Bastar region is not available but, one study reported the prevalence of same among blood donors is around 1.48%, and the actual prevalence of the disease may be higher as blood donors are merely selected population [3,4]. Atleast half of the untreated pregnant women suffer from serious adverse events associated with syphilis, including still birth, neonatal or early infant deaths, prematurity and infants with congenital anomalies [1]. The prevalence of infant and neonatal mortality rate in rural areas of Bastar are 45 and 29/1000 live births [5]. Syphilis related infant and neonatal mortality can be prevented easily if every antenatal patient is screened at an early gestational period. So, every pregnant woman should undergo for a screening test for syphilis at her first antenatal visit and if there is high risk of exposure then they should be retested again at the third trimester and at the time of delivery.

There are a number of serological tests available, which fall under categories of treponemal and non treponemal tests. The non treponemal tests like Venereal Disease Research Laboratory (VDRL), RPR and Unheated Serum Reagin (USR) etc. which detect non specific reagin antibody by using cardiolipin antigen are used for screening purpose while the treponemal tests like Fluorescent Treponemal Antibody (FTA), FTA-absorption (FTA-ABS), *Treponema pallidum* Immobilisation (TPI) etc. which detect specific antibody by using *Treponema pallidum* specific antigen are used for confirmation of diagnosis [6].

The RPR and VDRL a slide flocculation test, both can be used for screening purpose but RPR is preferred over VDRL because: RPR antigen has prolonged shelf life, the sensitivity and specificity of the RPR card test is equal or better than VDRL test, materials required for RPR card test are easy to use, inexpensive and expendable and the test results can be read with naked eyes [7-13].

Owing to the high percentage of infant and neonatal mortality rate in Bastar region, Chhattisgarh, India and inconsistent data regarding the prevalence of syphilis among antenatal population and due to the above mentioned advantages of RPR card test over VDRL test, the present study aimed to detect the incidence of syphilis among antenatal patients of Bastar, Chhattisgarh, India by using RPR card test.

MATERIALS AND METHODS

The present cross-sectional study was conducted on the specimens received in the Department of Microbiology, Late Baliram Kashyap Memorial Government Medical College Jagdalpur, Chhattisgarh, India, after obtaining informed consent from patients and approval from Institutional Ethics Committee (IEC). Convenience sampling was used. The study population comprised of IPD and OPD patients of Department of Obstetrics and Gynaecology for three months i.e. from August to October 2021 of the hospital.

Inclusion criteria: All antenatal patients visiting out patients and in patients Department of Obstetrics and Gynaecology, antenatal patients coming to labour ward and Human Immunodeficiency Virus-Acquired Immunodeficiency Syndrome (HIV-AIDS) clinic of all age group were included in the study.

Exclusion criteria: Non pregnant patients attending outpatients and inpatients Department of Obstetrics and Gynaecology and

HIV-AIDS clinic, women tested negative by urine pregnancy test and using contraceptive pills or other methods of contraception were excluded from the study.

Sample Collection and Preparation

The samples included all identification (patient's full name, date of sample collected and the patient's hospital identification number). Blood sample was drawn by using all aseptic precautions; the required sample was a minimum of 2.5 mL of clotted blood. The specimen was promptly centrifuged and an aliquot of the serum removed. Severely lipemic or homolysed serum was unsuitable for testing. Before testing, the serum was heated and inactivated at 56°C for 30 minutes. Inactivated serum was reheated at 56°C for 10 minutes, it tested more than four hours after the original inactivation.

Rapid Plasma Reagin Antigen Suspension

The RPR antigen suspension is stabilised combination of:

0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 10% choline chloride, 0.012M Ethylenediaminetetraacetic Acid (EDTA), 0.01875% charcoal, 0.01M Na₂HPO₄, 0.01M KH₂PO₄, 0.1% thimerosal in distilled water [14].

Control serum samples:

- 1. Positive control serum:** Prepared from human serum samples containing antibodies to *Treponema pallidum* [14].
- 2. Reactive minimal control serum:** Prepared from human serum samples containing antibodies to *Treponema pallidum*. Serum should be non reactive at 1:2 dilution, but reactive at 1:1 dilution [14].
- 3. Non reactive control serum:** Prepared from human serum samples free of *Treponema pallidum* antibodies [14].

Diluent (control): A 2% solution of human serum in 0.9% saline by diluting a human serum non reactive for syphilis 1:50 in 0.9% saline [14].

Methods

The RPR is 18 mm circle card test (a macroscopic non treponemal slide flocculation test) for the qualitative and semiquantitative detection of plasma regain [14]. The antigen suspension, a lipid complex, is agglutinated when mixed with samples containing reagin of patient affected by syphilis. Patients suffering from syphilis produce antibodies that react with cardiolipin antigen in a slide flocculation test which can be read using a microscope.

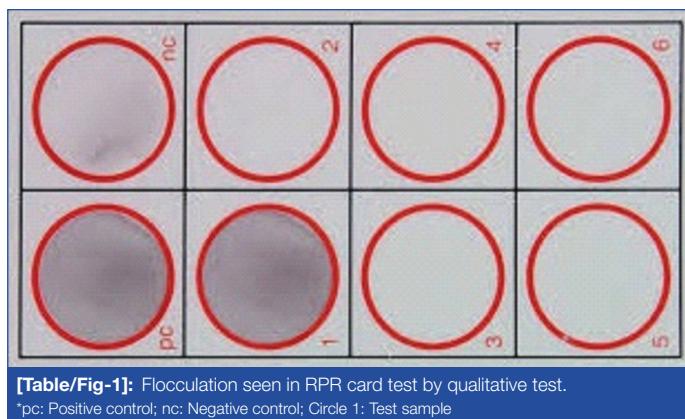
Qualitative Method [14]

One drop (50 µL) each, from the test sample, positive and negative controls was pipetted into separate reaction circles of the 18 mm circle of the RPR test card and was spread smoothly. A drop of diluted antigen suspension was added to the measured volume of sample, positive control and negative control each. The antigen was not spread or moved. The card was gently placed on an automatic rotator and rotated continuously at 180 rotations per minute (rpm) for 5-8 minutes. Following rotation, a brief hand rotation and tilting of the card (3-4 to-and-fro motions) was performed to aid in differentiating non reactive from minimally reactive results.

Interpretation of Results

Reporting of qualitative test was done after seeing the visible flocculation of the RPR card antigen suspension, which appears as black clumps against the white background, indicates a Reactive (R) specimen. Slight but definite clumping indicates a Minimally Reactive (Rm) specimen. In contrast, Non reactive (N) specimens appear to have a uniform grey colour. The flocculation seen in RPR card test by qualitative test is presented in [Table/Fig-1].

Any specimen exhibiting rough reactions has been quantitated using quantitative method as described by Pope V [14, 15].



[Table/Fig-1]: Flocculation seen in RPR card test by qualitative test.
*pc: Positive control; nc: Negative control; Circle 1: Test sample

STATISTICAL ANALYSIS

The data was collected in Microsoft Excel sheet and results were presented as count and percentage.

RESULTS

Out of 150 samples, 11 (7.33%) were tested positive by RPR card test while rest 139 (92.67%) tested negative. The age of the patients attending antenatal clinic ranged between 11-50 years with maximum patients 103 (68.67%) were between 21-30 years with high positivity rate 8 (72.73%); as presented in [Table/Fig-2]. The ward wise distribution of collected samples along with the positive samples by RPR card test is shown in [Table/Fig-3].

S. No.	Age (in years)	Number of samples (N=150) (%)	Number of positive samples (n=11) (%)
1	11-20	18 (12)	-
2	21-30	103 (68.67)	8 (72.73)
3	31-40	23 (15.33)	3 (27.27)
4	41-50	6 (4)	-

[Table/Fig-2]: Age-wise distribution of collected samples (N=150) along with number of positive samples (n=11).

S. No.	Ward	Number of samples (N=150) (%)	Number of positive samples (n=11) (%)
1.	Obstetrics and gynaecology (Outpatient department)	95 (63.33)	7 (63.64)
2.	Labour ward	31 (20.67)	1 (9.09)
3.	Gynaec ward	20 (13.33)	-
4.	Antiretroviral Therapy (ART HIV-AIDS) centre	4 (2.67)	3 (27.27)

[Table/Fig-3]: OPD/IPD wise distribution of samples (N=150) along with number of positive samples (n=11).

DISCUSSION

Syphilis is associated with many adverse pregnancy outcomes and owing to increased infant and neonatal mortality rate in Bastar region, the present study has been conducted to assess the incidence of syphilis among antenatal patients of Bastar using RPR card test. The incidence of syphilis in pregnancy was more (7.33%) in the present study as compared to study conducted by Murawala SM et al., (0.19-6.21%), Bala M et al., (0.3-4.4%) and Barot N et al., (0.5%) [16-18]. The reason behind higher percentage of syphilis in the present study may be because of the study has been conducted in the tribal region of Bastar where education level of people is very poor (53.6%) and their knowledge about STDs is less (59.2%) [5]. Some other studies carried out in India reported the prevalence of syphilis ranging from 1.8-3.4%, which is again lower than the present study [19-21]. The decreasing trend of syphilis in above studies may be due to greater awareness and better education of women about the complications of syphilis related outcomes in pregnancy [19]. The trend of syphilis prevalence reported in different regions of India by various previous studies has been presented in [Table/Fig-4] [16-18,22-28].

S. No.	Author name and place of study	Duration of study	Year of publication	Total samples	Syphilis prevalence
1.	Kumarasamy N et al., [22], Chennai, India.	August 2002 to December 2003	2008	480	11% (Women) 8% (Men)
2.	Jain A et al., [23], Delhi, India.	January 2005 to December 2009	2012	570	7.36%
3.	Khan S et al., [24], Valsad, India.	January 2006 to December 2008	2014	14639	0.61%
4.	Sethi S et al., [25], Chandigarh, India.	January 2006 to December 2011	2015	28920	3.36%
5.	Mehta KD et al., [26], Gujarat, India.	February to May 2010	2013	1038	0.48%
6.	Bala M et al., [17], New Delhi, India.	April to July 2011	2013	10489	0.3-4.4%
7.	Tankhiwale S and Naikwade SR, [27], Maharashtra, India.	June 2012 to March 2013	2014	13008	0.7%
8.	Murawala SM et al., [16], Gujarat, India.	March 2014 to April 2015	2015	13115	0.19-6.21%
9.	Parveen SS and Madhavi S [28], Telangana, India.	January to October 2015	2015	409	0%
10.	Barot N et al., [18], Gujarat, India.	January to December 2018	2019	2258	0.53%
11	Present study	August to October 2021	2022	150	7.33%

[Table/Fig-4]: Trends of syphilis prevalence reported in India in last few years [16-18,22-28].

Maximum number of positive samples 8 (72.73%) in the present study was from age group 21-30 years which was in accordance with the study conducted by Murawala SM et al., (21-45 years) and Barot N et al., (20-30 years) as syphilis is a sexually transmitted disease affecting more to adult population [16,18].

Concomitant HIV and syphilis infection was reported in 3 (27.27%) out of 11 positive samples in the present study which was in corroboration of study conducted by Sethi S et al., where they also had reported rising trend (2.84-6.99%) of concurrent HIV and syphilis infection with increasing years (2006 to 2011) [25]. Reason behind high percentage of HIV infection in antenatal clinic patients in present study may be due to higher prevalence of HIV in antenatal clinic patients of Chhattisgarh state (0.41%) as compared to other states [29].

As syphilis in pregnancy is associated with many adverse outcomes like infant and neonatal mortality, congenital syphilis etc, early diagnosis and treatment of the same is mandatory. The infant and neonatal mortality rate itself was higher in this region, so the early screening of the pregnant women for the disease like syphilis is important because it is easily treatable with penicillin. RPR card test provides the test results very fast and also due to its cost effectiveness, it has been added in essential diagnostic list by Indian Council of Medical Research (ICMR), even at Primary Health Centre (PHC) level [30-32], so it may prove as a useful aid in preventing STD related neonatal and infant mortality and morbidity.

Limitation(s)

There are some limitations of the study, like small sample size, inconsistent data about outcomes of pregnancies and co-existence of other infections etc., which may be addressed further in other studies along with comparing the RPR card test with other available screening methods (like automated RPR card test, VDRL, USR test etc.) for different STDs.

CONCLUSION(S)

The STDs like syphilis leads to increased neonatal and infant mortality and morbidity. Comparatively higher percentage of syphilis infection reported in the study may contribute to the existing data of increased infant and neonatal mortality rate of Bastar District, Chattisgarh, India. RPR card test is an important cost effective and rapid screening tool for the disease like syphilis, which need early diagnosis and treatment to prevent adverse pregnancy outcomes, especially in the resource poor areas like tribal region of Bastar, Chattisgarh, India.

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PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India.
2. Demonstrator, Department of Microbiology, Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India.
3. Assistant Professor, Department of Pharmacology, Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Ratna Agrawal,
Assistant Professor, Department of Pharmacology, Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh, India.
E-mail: ratna.arang@gmail.com

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