

Antenatal screening of syphilis: diagnosed by rapid plasma regain and rapid immunochromatography method

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Abstract

Background: Syphilis is one of the major causes of adverse outcomes in pregnancy in developing countries. Antenatal screening for syphilis aims primarily at timely detecting & treating pregnant women with congenitally transmissible syphilis, there by preventing perinatal deaths. **Objective:** To study prevalence of syphilis in antenatal women by screening them with the Rapid Plasma Reagin (RPR) & rapid immunochromatographic (ICT) method. **Materials and Methods:** A total of 2258 pregnant women attending antenatal care from January to December 2018 were tested for Syphilis by RPR & rapid ICT. **Results:** Out of 2258 women screened during their antenatal visits, 12 (0.53%) were positive by RPR as well as rapid ICT test method. **Conclusion(s):** Early detection of syphilis in pregnancy is the best tool for prevention of adverse pregnancy outcomes. Antenatal screening of syphilis with Rapid plasma regain (RPR) and Rapid immunochromatography (ICT) is sensitive, specific, rapid and cost-effective method.

Key words: Antenatal screening, Syphilis, RPR, Rapid Immunochromatography(ICT).

Introduction

The etiologic agent of syphilis is *Treponema pallidum*. Syphilis infection in pregnancy is common in developing countries. About 5% to 8% of all pregnancies surviving past 12 weeks will have an adverse outcome caused by syphilis, such as spontaneous abortion, perinatal or infant death, or a living infant with syphilis [1].

The disease has been divided into stages based on clinical findings, helping to guide treatment and follow-up. Persons who have syphilis might seek treatment for signs or symptoms of primary syphilis infection (i.e., ulcers or chancre at the infection site), secondary syphilis (i.e., manifestations that include, but are not limited to, skin rash, mucocutaneous lesions, and lymphadenopathy), or tertiary syphilis (i.e. cardiac, gummatous lesions, tabes dorsalis, and general paresis). Latent infections (i.e., those lacking clinical manifestations) are detected by serologic testing [2]. All women should be screened serologically for syphilis early in pregnancy. Pregnant women with reactive treponemal screening tests should have additional quantitative nontreponemal testing, because titers are essential for monitoring treatment response [2].

As *T. pallidum* cannot be grown in culture, so diagnosis is done by various direct and indirect diagnostic methods. Direct methods include the detection of *T. pallidum* by microscopic examination and nucleic acid amplification. Indirect methods include serological test such as treponemal tests (FTA-ABS, TPHA) & non treponemal (RPR & VDRL). The disease may be asymptomatic, and there are problems in diagnosing very early syphilis. Among all serological test RPR is the most sensitive during early stages of syphilis infection. The test measures both immunoglobulin (Ig) G and IgM antiphospholipid antibodies formed by the host in response to lipoidal material released by damaged host cells early in infection and lipid from the cell surfaces of the treponeme itself. It is a macroscopic flocculation tests and uses stabilized suspension of RPR antigen to which charcoal particles are added to aid in the visualization of the test reaction. RPR is also used as quantitative tests that allow for the establishment of a baseline titre to evaluate recent infection and response to treatment.

Rapid ICT method is a qualitative test for detection of antibodies against *Treponema pallidum* antigen. Test is developed with an aim to integrate rapid, simple and technologically appropriate syphilis testing at venues with limited resources [3].

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Material & Methods

Type of study: It is a retrospective conducted to determine the prevalence of antibodies to *Treponema pallidum* among patients attending the antenatal clinic of GMERS Hospital & Medical College, Vadnagar.

Sampling Method: Five-mL blood samples were collected using a sterile plain vacutainer, and the serum was separated by centrifugation into sterile serum storage vials.

Sample collection: Serum specimens from 2258 antenatal women were received during a period of 1 year (January–December 2018). These samples were tested for hepatitis B (HbsAg), hepatitis C, syphilis, and HIV per Strategy III of the National AIDS Control Organization by using different test systems to establish diagnosis of HIV.

Inclusion criteria: All pregnant women of reproductive age group attending Obstetric OPD of GMERS Hospital, Vadnagar were included in this study.

Exclusion criteria: Females Under 18 years and above 45yrs of age were excluded from this study.

Testing method: The RPR syphilis screening test (BEACKON Diagnostics Pvt Ltd) which is a macroscopic non-treponemal flocculation card test for the detection and quantitation of antilipoidal antibodies

present in serum was used for all antenatal women. A standard RPR 18 mm circle qualitative card test was carried out, mixing one drop of serum (50µl) with one drop of RPR reagent(15-20 µl), mixing on a shaker for 8 minutes, and read in the best available light.

Test results showing slight but definite clumps were reported as reactive. No flocculation indicates negative reaction. Positive and negative control sera were included in each day's testing. All reactive samples were tested by quantitative method for titre detection.

All the serum specimens were also tested for syphilis antibody by Rapid immunochromatography (ICT, BEACKON Diagnostics Pvt Ltd). 100 µl of serum was aliquoted into a fresh serum tube. An ICT strip was removed from the foil pouch and added to the tube. This was left for 20 minutes and the results read, according to the manufacturer's guidelines.

Statistical methods: The data was processed with simple statistical analysis using Microsoft Office Excel 2010 software.

Ethical consideration & permission: The necessary approval to conduct this study was obtained from the Institutional Ethics Committee (IEC) of the college before starting the study. In the present study no any scoring system or any surgical procedure were used.

Results

A total of 2258 serum were screened for syphilis antibody from January to December 2018. 12(0.5%) samples were read as positive by RPR and ICT. Among 12 positive samples, 10 samples had titre of $\geq 1:8$ & 2 had titre of 1:2. Among All the positive samples, 11 were from age group 20-30 yrs.

Table No.-1: Prevalence of syphilis in various Age-groups

	Result	Age group (in years)					Total
		18-20	21-25	26-30	30-35	>35	
Syphilis	Non-reactive	475	922	781	60	8	2246
	Reactive	1	6	5	0	0	12
	Total	476	928	786	60	8	2258

Among All the positive samples, 6 cases were from age group 26-30year.

Discussion

Syphilis is a sexually transmitted disease (STD) caused by the bacterium *Treponema pallidum*. Spirochetes can infect the fetus from about 14 weeks' gestation, and the risk of fetal infection increases with gestational age [9]. The manifestations of Congenital Syphilis are influenced by gestational age, stage of maternal Syphilis, maternal treatment, and immunological response of the fetus [10]. Early diagnosis of congenital Syphilis can be done by Antenatal serological screening. Seropositive pregnant women should be considered infected unless an adequate treatment history is documented clearly in the medical records and sequential serologic antibody titers have declined appropriately for the stage of syphilis [2]. In present study, we have screened all females with RPR test and quantitative RPR was performed to monitor prognosis of these patients after treatment.

Original Research Article

The guidelines for serological diagnosis for syphilis, produced by the World Health Organization, recommended use of a cardiolipin test such as the RPR and the TPHA for screening purposes [11]. All RPR positive samples were confirmed with Rapid immunochromatography (ICT) test. In the present study, we found that the ICS test provides accurate, qualitative detection of antibodies to *T. pallidum*. The test appears to be as sensitive as the RPR test, yielding positive test results for all syphilis patients who had reactive RPR tests.

The prevalence rate of syphilis in the present study (0.5%) was similar to the rate reported by Mehta et al [4] & very low compared to the rate reported by Kebede et al [5] (2.9%), and the rate reported by Gupta et al., [6] (1.47%). (Table No.2). In India, available information indicates that the prevalence of maternal syphilis has remained at around 1.5% between 2003 and 2007 [7]. The most common age group involved was 21-25 yrs (Table No.1).

Table No.-2: Comparison of percentage of positive cases of syphilis in various studies

Sr.No	Study	Total sample	Percentage of positivity
1.	Mehta <i>et al.</i> [4]	1038	0.48
2.	Present study	2258	0.5
3.	Gupta et al.[6]	30045	1.47
4.	Kebede et al.[5]	410	2.9

Prevalence rate in the present study (0.48%) is same as Maheta et al. Study(0.5%).

National aids control programme recommends the use of a non-treponemal test – Rapid Plasma Reagin (RPR) test for routine screening for syphilis [8]. Quantitative RPR titres can help evaluate the response to treatment. Rapid plasma reagin (RPR) is the preferred tests for syphilis screening, as it the most sensitive (80-86%) in early syphilis.

RPR test has a limitation of Biological False Positive (BFP) reactions and Prozone phenomena. However, operational requirements for RPR/VDRL testing are not available at most primary care sites. Delay in obtaining test results through referrals can delay or result in missed opportunities for treatment.

Rapid ICT is developed to provide appropriate syphilis screening at point of care. These are simple point of care tests that can be performed outside a laboratory setting with minimal training.Hence, they can address the problem associated the low patient return and can be used in all health care settings to allow immediate treatment.

Conclusion

Syphilis remains a leading cause of perinatal mortality and morbidity in many parts of the world despite widely available and affordable technology for diagnosing and treating infection in pregnant women.

Because of the serious complications of syphilis in pregnancy, the first priority should be to ensure universal antenatal screening.

Antenatal screening of syphilis should be done by combination of RPR and rapid treponemal ICT test for detection of early stage of syphilis & thereby prevention of congenital syphilis.

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Ethical approval: The study was conducted after ethical approval by the Institutional Ethics Committee.

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Contribution from authors

- Dr. Nidhi Barot: Preparation of manuscript, Data collection, Data compiling, literature review, final approval.
- Dr. Hetvi Chawda: Manuscript editing, literature review, final approval.
- Megha Patel: Sample processing, test performance and data collection.

What this study adds to existing knowledge? This study provides awareness on importance congenital syphilis and antenatal screening by serological methods. It also provides method of choice for diagnosis at point of care.

Findings: Nil; **Conflict of Interest:** None initiated
Permission from IRB: Yes

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