Original Article

Utility of immunochromatographic assay as a rapid point of care test for screening of antenatal syphilis

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Abstract

Background and Objectives: Syphilis is one of the most common preventable causes of adverse effects during pregnancy. Antenatal screening prevents the delay between diagnosis and treatment there by reducing the risk of congenital syphilis. The objective of this study was to evaluate the utility of an immunochromatographic assay as a point of care test for antenatal screening of syphilis. **Materials and Methods:** Sera of 200 antenatal mothers were evaluated for serodiagnosis of syphilis by the venereal disease research laboratory (VDRL), *Treponema pallidum* hemagglutination assay (TPHA) and SD BIOLINE Syphilis 3.0 test. The performance of SD BIOLINE Syphilis 3.0 test was compared with VDRL as screening assay and TPHA as a confirmatory test. **Results:** The antenatal prevalence of syphilis was found to be 2% by both VDRL and TPHA. The sensitivity, specificity, positive predictive value, and the negative predictive value of SD BIOLINE Syphilis 3.0 test were 75%, 100%, 100%, and 99.45%, respectively. **Conclusions:** Antenatal screening and treatment of maternal syphilis are cost-effective health interventions even under the low prevalence of infection. SD BIOLINE Syphilis 3.0 test, although having less sensitivity than the existing testing strategy, can have a tremendous impact on the disease burden if used prudently for the screening of antenatal mothers in peripheral health settings.

Key words: Antenatal screening, India, SD BIOLINE Syphilis 3.0 test, syphilis (*Treponema pallidum*)

INTRODUCTION

Syphilis is one of the major causes of sexually transmitted infections (STIs) throughout the world. The World Health Organization (WHO) reports 10.6 million cases of adult syphilis anually.[1] Mother to child transmission of syphilis, carrying a severe impact on pregnancy outcome, can be prevented by the timely management of syphilis in pregnant women. Maternal syphilis causes stillbirths and spontaneous abortion in 80% of cases and survivors are at risk for a range of severe effects and longer-term sequelae.[2] According to the WHO global estimates for congenital syphilis burden based on review of published data from 1997 to 2003 there are 2,036,753 syphilis infections among pregnant women annually, of which 65% result in adverse pregnancy outcomes.[3] Screening for syphilis can prevent complications of syphilis and reduce transmission, which in turn will reduce the occurrence of congenital syphilis and transmission of human immunodeficiency virus (HIV) infection and other STIs. Many countries follow policies of antenatal screening for syphilis during first antenatal visit followed by an early repeat test in the third trimester to reduce adverse outcomes on pregnancy due to syphilis.[4]

Access this article online

Quick Response Code:

Website: www.ijstd.org

DOI: 10.4103/0253-7184.167159

How to cite this article: Kashyap B, Sagar T, Kaur IR. Utility of immunochromatographic assay as a rapid point of care test for screening of antenatal syphilis. Indian J Sex Transm Dis 2015;36:162-5.

Syphilis is routinely diagnosed using nontreponemal and treponemal tests. Antenatal screening is done by a nontrepenomal test (venereal disease research laboratory [VDRL] or rapid plasma reagin [RPR]) based upon reactivity of patients' immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to nonspecific cardiolipin-cholesterol lecithin antigens. These tests are simple, affordable, helpful in mass screening, and monitoring response to treatment; but they lack sensitivity in early and late syphilis and give biological false positive reactions associated with age, pregnancy, drug addiction, malignancy, autoimmune diseases, and numerous other infections.[5] Nontreponemal screening tests are followed by more specific confirmatory trepenomal tests. These include *Treponema pallidum* hemagglutination assay (TPHA), fluorescent treponemal antibody-absorption test (FTA-ABS), micro hemagglutination assay for antibody to *T. pallidum* (MHA-TP), and various enzyme–linked immunosorbent assays. All these tests cannot differentiate past and present infection and can be reactive in treated cases of syphilis in addition to the requirement of laboratory settings and considerable time to obtain the results. There has been recent interest for the implementation of point of care (POC) tests to enable early diagnosis and treatment of maternal syphilis. One of these tests is a solid phase immunochrommatographic assay for qualitative detection of all isotypes (IgG, IgM, immunoglobulin A [IgA]) against *T. pallidum*. This treponemal POC test can be performed in peripheral settings and ensures immediate treatment provision besides being rapid and cost effective. This study was carried out to evaluate the utility of a rapid immunochromatography assay as a POC test in the antenatal screening of syphilis.

MATERIALS AND METHODS

A prospective study was carried out in serology section of Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. Two hundred women aged 20–30 years were recruited in the study on their first antenatal visit or follow-up visits. Previous history of syphilis or any contact history was ruled out. Written and informed consent were obtained from the study participants.

Sera separated from the blood samples of all the cases were stored at 4°C till further processing. The performance of SD BIOLINE Syphilis 3.0 test was compared with VDRL and TPHA test on each serum sample to screen for antenatal syphilis. These tests were performed as per manufacturer's instructions.

TREPOLIPIN kit from Tulip Diagnostics Pvt. Ltd. is a modified VDRL test.

TPHA TEST kit from Plasmatec Part of Lab21 Healthcare Ltd., is based on the principle of agglutination.

SD BIOLINE Syphilis 3.0 test was performed using the SD BIOLINE Syphilis 3.0 (Bio Standard diagnostics Pvt., Ltd.,) which is a solid phase immunochromatographic assay.

The sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of the SD BIOLINE Syphilis 3.0 test was determined using McNemar's test.

RESULTS

The study population included 200 antenatal mothers aged 20–30 years among whom the majority (44%) belonged to 21–23 years age group with the remaining belonging to 24–26 (42%) and 27–30 (14%) age groups. Fifty-two percent mothers presented during the third trimester of their pregnancy with the remaining 32% and 16% in the second and first trimesters, respectively. The antenatal prevalence for syphilis in the study population was found to be 2% using VDRL as a screening assay and TPHA as a confirmatory test with the prevalence of 3.57%, 1.13%, and 0% in the age groups 24–26, 21–23, and 27–30, years respectively.

All the 200 antenatal mothers were screened for syphilis using VDRL, SD BIOLINE Syphilis 3.0 test, and TPHA. Four were reactive by VDRL giving a positivity of 2%. The same four samples that were reactive by VDRL were positive by TPHA also. Of the 200 serum samples, three (1.5%) samples tested positive by SD BIOLINE Syphilis 3.0. All the three samples positive by the SD BIOLINE Syphilis 3.0 test were also positive by the VDRL and TPHA tests.

The sensitivity, specificity, PPV, and NPV of SD BIOLINE Syphilis 3.0 test were 75%, 100%, 100%, and 99.45%, respectively with VDRL as the screening assay and TPHA as the confirmatory test. When the SD BIOLINE Syphilis 3.0 test and the VDRL test were compared, there was no significant difference between the performances of the two tests ($P > 0.05$). The agreement between the two tests was found to be 99%.

DISCUSSION

Syphilis is an important cause of adverse effects during pregnancy, besides being one of the most common preventable sexually transmitted diseases. According to WHO, approximately 2 million

pregnant women are infected with syphilis each year globally and around 1.2 million antenatal mothers with syphilis transmit the infection to their newborn annually.[6,7] The WHO reports adverse pregnancy outcomes caused by untreated maternal syphilis in the range of 730,000 and 1,500,000 each year, of which about 650,000 are fetal and newborn deaths.[8] Syphilis has a myriad of variable clinical presentations and is a great mimic of many other infections and diseases. A WHO antenatal care (ANC) trial conducted in four countries (Argentina, Cuba, Saudi Arabia, and Thailand) estimated the prevalence of syphilis in the range of 0.1–2.2% across these four countries.[9] While antenatal screening and treatment of syphilis are cost-effective health interventions even under low prevalence of infection,[10,11] many countries do not have an efficient universal antenatal screening protocol. Due to the sporadic availability of ANC in resource-limited settings, it becomes crucial to identify infected mothers and provide appropriate treatment during the same encounter. Since ANC services provide the only opportunity to screen for such mothers the availability can be reached by decentralization of syphilis testing or on-site testing which would further decrease the dropout rate, as well as the delay in diagnosis.

Our study was primarily focused on the antenatal mothers between the ages of 20 and 30 years. The highest percentage of antenatal mothers belonged to the age group 20–23 years with the maximum (52%) presenting in the third trimester. A prevalence rate of 2% was found in these pregnant women for syphilis with the highest prevalence of 3.5% in 24–26 years age group. This finding is concordant with a recent report of syphilis seropositivity of 2.2% in antenatal clinic clients.[12] A much lower seroprevalance of 0.3% was reported from an Indian study that evaluated the performance of three treponemal tests in VDRL/RPR nonreactive sera, within the context of a National Reference Laboratory for STIs.[13] Another study on antenatal mothers reported 8.5% active syphilis, 3.3% old or treated cases, 1% biological false positives, and 0.04% primary syphilis on the basis of RPR and TPHA test results.[14]

The nontreponemal tests used predominantly for screening purpose miss cases of late syphilis along with having probabilities of giving biological false positives and false negatives in early syphilis. The false negativity of the nontreponemal tests, attributed to prozone phenomenon, may be exaggerated in HIV/syphilis co-infection and thus become a matter of concern in high HIV prevalence settings.[15] Moreover the interpretation of such tests is subjective open to differences in training and technical factors. Numerous simple, treponema specific, rapid POC diagnostic tests are commercially available (Determine Syphilis TP [Abbott] SD BioLine Syphilis 3.0 ICS [Standard Diagnostics], Visitec Syphilis [Omega Diagnostics], Qualpro Syphicheck-WB Rapid Syphilis Test [Qualpro Diagnostic], etc.); most of which detect IgM, IgG, and IgA antibodies and involve immunochromatographic strips in which one or multiple *T. pallidum* recombinant antigens are applied to nitrocellulose strips as a capture reagent. POC tests for syphilis have the clear benefit of a rapid result facilitating immediate treatment at the initial visit. Though many of these tests are being evaluated in settings where laboratory facilities and trained personnel are not readily available, these are not yet being used widespread. The reported sensitivities and specificities of these tests vary from 84% to 98% and 94–98%, respectively.[16] The WHO Sexually Transmitted Diseases Diagnostic Initiative established the assured criteria to define the characteristics of an ideal rapid and POC test: Affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to those who need them.[17] However, very few studies with these newer rapid treponemal assays have focused on their use among high-priority populations like antenatal mothers.

A WHO-sponsored evaluation trial of rapid syphilis tests reports the sensitivities and specificities ranging from 84% to 98% and 94–98%, respectively besides the high degree of operational ease with these tests.[16] In a recent study, from Brazil on comparative validation of four rapid treponemic tests for the diagnosis of syphilis compared with VDRL as a routine diagnostic and FTA-ABS as a screening assay, the sensitivity varied from 84% to 96%, specificity and PPV were >98% and 90%, respectively with the reproducibility >97%.[18] With VDRL as the screening assay and TPHA as the confirmatory tests, the sensitivity, specificity, PPV, and the NPV SD BIOLINE Syphilis 3.0 test were 75%, 100%, 100%, and 99.45%, respectively. One study reports that an onsite rapid immunochromatographic test for syphilis screening among antenatal mothers resulted in a high proportion of correct diagnosis and treatment (89.4%) compared with the on-site RPR test (63.9%) or off-site RPR/TPHA tests (60.8%).[19] Lower sensitivity of ICT in early primary syphilis could be responsible for the negative SD BIOLINE Syphilis 3.0 test result in one sample of our study that tested reactive by VDRL and TPHA. One recent Indian study reports 100% negative correlation between nonreactive and weakly reactive VDRL test and rapid test with 100%

positive correlation between the two tests in sera with titer >8 whereas 27 false positives in sera with titer 1–8; suggesting that in financially constraints conditions a rapid test can be used to select out true positive cases among the VDRL R1–8 reactive sera and to confirm the true negativity of VDRL weakly reactive sera.[20] In another study, from Brazil that evaluated rapid syphilis kit in 12 antenatal clinics, the POC test detected 62.5% of syphilis cases, 62.5–66.7% of active syphilis cases and 100% cases of syphilis with high titer.[21]

Several rapid syphilis serological tests have been cleared for use in the United States by the Food and Drug Administration as diagnostic, confirmatory, and blood donor screening tests.[22] The predictive value of a nontreponemal test can be increased with the addition of confirmatory treponemal test with equivalent sensitivity but greater specificity to the syphilis serology algorithm. The use of POC tests after appropriate validation can prevent the delay between the diagnosis and treatment of maternal syphilis thus reducing the risk of congenital infection, persistent infection, failure to follow-up, and transmission of other STIs. Moreover, these tests present an opportunity for detecting syphilis in nonclinical settings as well. There being a positive impact of these POC tests on reducing the disease burden in resource-limited settings, improvements in accuracy parameters of such tests are warranted.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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