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Syphilis Antibody Screening in Sudanese Aborted Women

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Abstract

Background: Syphilis is prevalent worldwide. In USA there is an increased incidence of cases of congenital syphilis among pregnant women. Recent studies of pregnant women in Africa had revealed frequency rates of 17% in Cameroon, 8.5% in South Africa, 6.7% in Central Africa, 2.5% in Burkina Faso, 2.4% in Sudan, 3.1% in Djibouti and 3% in morocco[1].
Objective: To perform syphilis antibody screening among Sudanese aborted women.
Materials and methods: Blood specimens were collected from aborted Sudanese women attending Khartoum Teaching Hospital (Sudan). Syphilis antibodies were detected by the Rapid Plasma Reagin (RPR) test; and positive results were confirmed by the *Treponema pallidum* Hemagglutination (TPHA) test.
Results: A total number of 50 aborted women were investigated. The population studied was aged 20 to 40 years. The RPR test was positive in 6 (12%) aborted women and TPHA test was negative in all aborted women investigated.
Conclusion: The RPR test was a practical and sensitive procedure for syphilis screening. The TPHA test was more sensitive than RPR test and it was the test of choice for confirming the diagnosis of syphilis.

Key words: Syphilis screening, RPR test, TPHA test, Sudanese aborted women.

Introduction

Syphilis is a sexually transmitted disease (STD) caused by spirochaetes that are motile, helical bacteria belonging to the order *Spirochaetales* and the family *Spirochaetacease*. Spirochetes may be anaerobic, facultative anaerobic or microaerophilic. The family *Spirochaetacease* has four genera: *Spirochaeta, Crispira, Treponema,* and *Borrelia.* The genus *Treponema* is the causative agent of venereal syphilis. Other species of *Treponema* cause non-venereal treponematoses, e.g. yaws (*T. pertenue*), endemic syphilis (*T. endemicum*) and pinta (*T. carateum*) [1].
Treponema pallidum (*T. pallidum*) that causes syphilis is transmitted from one person to another through direct contact with a syphilitic sore (chancre) during sexual intercourse (vaginal, anal, or oral). The infection can also pass from a mother to her baby during pregnancy. One cannot catch syphilis from a towel, door-knob or toilet seat[2].
All women should be screened for syphilis at the first prenatal visit, and women at high risk for

congenital syphilis are to be screened again at 28 weeks of pregnancy. This is because untreated syphilis in pregnancy can lead to still birth or infant death in up to 40% of cases[3]. Pathogenic treponemes are found in the lesions of those with treponematoses. The Treponemes are highly infectious. Saprophytic treponemes can be found on mucous membranes in the mouth and genital tract, and also in skin ulcers[4].
The antigenic structure of *T. pallidum* is poorly understood .infection with these treponemes leads to the production of three antibodies. On the basis of that, the treponemal antigens may be divided into specific antigens (Reiter treponeme) and non-specific antigen (regain)[1].
Depending upon the antigen used serological tests for syphilis may be divided into non-treponemal tests or standard tests for syphilis (STS) such as Wassermann tests, Khan test, RPR test, and the Venereal Diseases Reference Laboratory (VDRL) test; in addition to those tests employing *T. pallidum* antigens such as *Treponema palladium* Haemoagglutination (TPHA) test, *Treponema palladium* Immobilization (TPI) test, *Treponema palladium* Agglutination (TPA) test, *Treponema pallidum* Immune Adherence (TPIA) test, and Fluorescent Treponemal Antibody (FTA) test[3].
Untreated syphilis can lead to serious complications and during pregnancy it can be fatal to the developing fetus and can lead to still birth or abortion. If the infection occurred in first trimester of pregnancy, 40 % of infants will be affected. All women should be screened for syphilis at the first trimester, and those at high risk for congenital syphilis they should be screened again at 28 weeks. Hence the aim of this study was to screen pregnant Sudanese women for syphilis and to highlight the scope of this problem in Sudan.

Materials and methods

This was a descriptive case study; conducted at Khartoum Teaching Hospital (Sudan) during the period from April to June 2010. The study population was pregnant women at different age groups and with past history of abortion. Clinical and laboratory data collected was analyzed using master sheet and the Statistical Packing for Social Science (SPSS) program. Approval to conduct the study was taken from Al- Neelain University, Khartoum, Sudan. Permission to collect the specimens was granted by the Administration of Khartoum Teaching Hospital (Sudan). Verbal consent was obtained from all study participants.
Sampling technique selected was a non-probability, convenance type. Sample size was 50 blood specimens collected from pregnant women with past history of abortion. A structured, interviewing questionnaire was designed to collect demographical and clinical data, e.g. name, age, past history of abortion, and symptoms suggesting syphilis.
Venous blood samples were collected aseptically on a volunteer basis from all participants included in the study. The blood specimens were left to stand on the bench for 6 hours to separate the serum and then centrifuged in the laboratory. All serum specimens were stored in a deep freeze cabinet at -18º C until tested. Syphilis antibodies were detected by the Rapid Plasma Reagin (RPR) test; and positive results were confirmed by the *T. pallidum* Hemagglutination (TPHA) test.
Rapid Plasma Regain (RPR) test:
Principle: The RPR-carbon is a non- treponemal slide agglutination test for the qualitative and semi quantitative detection of plasma regains human serum. Carbon particles coated with a lipid complex are agglutinated when mixed with serum containing regains.

Procedure: RPR test was performed as per the following qualitative method:
1. The reagent and serum sample were allowed to reach room temperature.
2. 50 ml of sample and one drop of each positive and negative control were placed into separate circles on the slide test.
3. The RPR-carbon reagent was swirled gently before use. Then the dropper assembly was inverted and pressed gently to remove air bubbles from the micropipette.
4. The micropipette was placed in a vertical position and perpendicular to the slide, and one drop (20ml) of this reagent was added next to the sample to be tested.
5. The drops were mixed with a stirrer and spreaded over the entire surface of the circle. Different stirrers were used for each sample.
6. The slide was placed on a mechanical rotator at 80-100 r.p.m for 8 min. The test was read within 8 minutes to avoid false positive results.
Reading the result was made by examining the slide macroscopically for presence or absence of visible agglutination immediately after removing from the rotator.
Positive test: The flocculation of carbon particles (black aggregates) was visible with naked eye. Black aggregates may be deposited at the periphery of the liquid.
Treponema palladium Hemagglutination TPHA) test:
Principle: TPHA is an indirect hemagglutination test for the qualitative and semi-quantitative detection of specific anti-*T. pallidum* antibodies in human serum .Stabilized avian erythrocyte sensitized with an antigen *T. palladium* solution agglutinates in presence of anti-*T. pallidum* antibodies to give a characteristic pattern.
Procedure: TPHA test was performed as per the following qualitative method:
1. First the sample, diluent, control and test cells were brought to room temperature. For each test, a test card with three wells was used.
2. To dilute the serum sample, 10µL of patient's serum were added in the first well (well A). Then 190µL of diluent were added.
3. The content well was mixed using a micropipette.
4. 75µL of "control cells" was added to well B and 75µL of "test cells" were added to well C.
5. Then 25µL of diluted serum were added on each B and C wells.
6. The plate was shaken gently to mix the contents thoroughly.
7. The plate was covered and protected from direct sunlight, heat, and vibration.
8. The plate was incubated for 45-60 minutes at room temperature.
9. Positive and negative controls were run along with the test; and the test results were read and interpreted.
Results and interpretation: The result was read by comparing the agglutination patterns of the test cells with the control cells.
Reactive: Smooth mat of cells covering entire or part of well bottom; or they were surrounded by a red circle.

Results

A total of 50 aborted women, attending Khartoum Teaching Hospital, were enrolled in this study**. T**heir age range was from 20 to 40 years.

All aborted women had no typical symptoms or signs of syphilis. Some were suffering from fever, fatigue, and non-specific symptoms. Out of the 50 aborted women investigated, RPR test was reactive in 6 (12%) women, while the confirmatory TPHA test was negative in all positive RPR samples. In age groups less than 20 years and up to 40 years, two women (4%) were found RPR positive. However, RPR was found negative in women over 40 years (Table I).

Table I: Positive RPR among aborted women according to age incidence

Age groups (years)	No. tested (%)	RPR Test	
		Positive (%)	Negative (%)
Less than 20	5 (10%)	2 (4%)	3 (6%)
20-30	25 (50%)	2 (4%)	23 (46%)
30-40	17 (34%)	2 (4%)	15 (30%)
More than 40	3 (6%)	0 (0%)	3 (6%)
Total	50 (100%)	6 (12%)	44 (88%)

Out of the 50 aborted women, 46 (92%) were educated, while 4 (8%) were illiterate. This may explain the absence of positive reactivity (0%) for TPHA test, since the majority was health educated.

Regarding past history of abortion, the number of previous abortions was found to range from 1-4 times. The reactivity of RPR test was not significant as regard the number of repeated abortions (Table II).

Table II: Positive RPR test among aborted women according to history of previous abortions

Past History of abortions	No. tested (%)	RPR Test	
		Positive (%)	Negative (%)
One time	30 (60%)	4 (8%)	26(52%)
Two times	9 (18%)	2 (4%)	7 (14%)
Three times	7 (14%)	0 (0%)	7(14%)
Four times	3 (6%)	0 (0%)	3(6%)
More than 4 times	1 (2%)	0 (0%)	1 (2%)
Total	50 (100%)	6 (12%)	44 (88%)

Discussion:

In this study 50 aborted Sudanese women attending Khartoum Teaching Hospital were investigated for syphilis antibodies using RPR and TPHA tests. RPR test was positive in 6 (12%) of them and TPHA test was negative in all investigated women.
This finding was not similar to that reported by Gichangi and his coworkers (2004) in Nairobi who found 377 out of 12,414 women (3%) were RPR positive. They also found 4.0% of RPR positive and 1.4% of RPR negative women delivered a stillbirth; and 19% of RPR positive and 10% of RPR negative had low birth weight deliveries; and out of the 200 randomly selected cord-positive women 142 (72%) were TPHA positive[5].
Also our results were similar to the findings of Taiwo and his colleagues in Nigeria (2007) who screened 505 newly registered pregnant women and found (9.9%) of them were positive for RPR test and 15 (2.97%) were positive for TPHA test[6].
However, our result was quite different from the findings of the study of Southwick and his colleagues (2001) in Bolivia who showed that 61 out of 1428 mothers (4.3%) of live born infant and 11 out of 43 mothers (26%) of still born infant were found to have syphilis at delivery, while 76% of the study population had received prenatal care, only 17% had syphilis testing carried out during the pregnancy, 91% of serum sample reactive to RPR Test[7].
Also the finding of Ratnam and his colleagues study (1982) was higher than what we got in our study. They performed this study at the University Teaching Hospital, Lusaka (Zambia) and screened 340 pregnant women admitted to the hospital whose pregnancies ended in either spontaneous abortion or still birth. They found TPHA test result reactive in 42% of women who aborted in the latter half of pregnancy[8].
In the Saudi Arabian study made by Shakoor (2004), 3270 pregnant women were investigated at King Khalid University Hospital and only one woman was found to have syphilis, i.e. a prevalence rate of 0.03%[9]. This result was lower than our study findings.
From this context, it may be recommended that syphilis antibodies screening should be routinely performed to aborted women to discover the aetiology of abortion and to control and eradicate syphilis in the community. All antenatal pregnant women should be screened for syphilis antibodies early in pregnancy. It is the responsibility of both the clinician and the bacteriologist to make sure that syphilis screening programme is properly applied.
Conclusion: The RPR test was a practical and sensitive procedure for syphilis screening. The TPHA test was the test of choice for confirming the diagnosis of syphilis.

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