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Research Article

Sero-Detection of Syphilis Antibodies Among Pregnant Women by Different Serological Tests in Khartoum State, Sudan 2019

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ABSTRACT

Syphilis is a common blood-borne disease in area where the disease is endemic. The aim of the study is to detect the *Treponemapallidum* antibodies among Sudanese pregnant women in antenatal clinics and hospital in Khartoum and Omdurman during November to December 2019, hundreds of blood specimens collected from pregnant women and tested for anti- *Treponemapallidum* antibodies using rapid plasma regain RPR, LAB21 healthcare TPHA and Enzyme-linked Immunoassay ELISA. Among the 100 pregnant women tested, 12(12%) showed positive result for T. palladium antibodies by Syphilis *Treponemapallidum* antibody ELISA (Fortress diagnostic, UK). Ten (10%) of the specimens were serologically identified using TPHA test kit (LAB21heathcare, UK); while only 9(9%) were reactive when tested by rapid plasma regain test device (china). Interestingly, an individual with positive ELISA for *Treponemapallidum* antibodies showed false negative result in the syphilis rapid plasma regain test device, In conclusion, syphilis is an important blood-borne disease among Sudanese pregnant women syphilis RPR and TPHA are cheap and technically easy and thus suitable for routine application; however their low sensitivity, as compared to the standard ELISA, limited their value to be used in syphilis prevention program.

Keywords: Syphilis; Pregnant women; RPR; TPHA; ELISA

INTRODUCTION

Syphilis is a muco-coetaneous sexually transmitted infection caused by the Spirochete, *Treponemapallidum* subspecies pallidum even though the primary way of transmission is through sexual Course, it may also be transmitted from Mother to fetus through pregnancy or at birth, resulting in congenital syphilis [1]. However, the illness can also be transmitted *in-vitro* (via the placenta), via blood transfusion or by unintended inoculation from infectious substance. Since syphilis is almost always transmitted by sexual intercourses, it is thus classified as one of the sexually transmitted diseases (STDs) [2,3]. Syphilis remains a significant cause of preventable before baby's birth death in developing countries, with many Women remaining untested because of this untreated. Syphilis is still a leading cause of perinatal mortality and morbidity worldwide even though there is the existence of the available and low-priced methods for diagnosis and treatment for pregnant women [4]. Syphilis is found worldwide an estimated 12 million cases occurred every year. An estimated 50% of these pregnancies will end in fetal or prenatal death, low birth weight babies or babies born with congenital syphilis. In United States, syphilis is the third most common sexually transmitted disease [5]. Active infection with syphilis in pregnant women has long been recognized as a major cause of death or disability in the infants born to infected women. It is estimated that active syphilis infection in pregnancy causes adverse outcomes in50%-80% of pregnancies surviving past 12 weeks gestation, primarily as spontaneous abortions in the second and early third trimester, stillbirths, and congenital syphilis. Syphilis disease in pregnancy is extremely widespread in a lot of areas of the world. Between women presence antenatal clinics in Africa, estimates of syphilis Sero-reactivity range from 4%-15%. Data from Zambia and Malawi suggest that between 26%-42% of stillbirths and 8% of infant deaths in those countries may be attributable to syphilis alone [6,7]. Prevalence of syphilis around sub-Saharan Africa ranges between 2.5% in Burkina Faso, 8.4% in South Africa and 17.4% in Cameroon [8] and a high prevalence of 42% in Mozambique [9]. In 1997 studies between pregnant women reported by WHO in the

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North and North Eastern regions of Africa showed syphilis disease rates of 3.1% in Djibouti, 3% in Morocco and 2.4% in Sudan [10]. More commonly syphilis is diagnosed using a combination of Treponema and non-Treponema serological tests. Serological tests will provide only related to assuming something beforehand results as the organism is not directly identified [11]. Serological examining and testing so a decision can be made and treating mothers for syphilis during pregnancy can prevent bad pregnancy outcomes and up-and-down transmission connected with maternal infection [12]. In theory, syphilis control in pregnancy is a national health policy in many countries, but in reality, this action that helps a bad situation is often not implemented in poor useful valuable supply countries with high syphilis prevalence because of financial status [13]. The serologic identification of syphilis has for many years been carried out mainly with a two-step approach [14]. Clarifying, serum samples were screened with a flocculation assay using non-Treponema antigens to detect antibodies to cardiolipin(Venereal Disease Research Laboratory [VDRL] second rapid plasma reagin [RPR] test and then, sera reactive in the screening tests were evaluated again to identify specific antibodies to *Treponema palladium* antigens [15]TreponemaHaemagglutination assay [MHA-TP] or fluorescent Treponema antibody absorption test [FTA-ABS]). Since there were limitations of the non-Treponema serologic tests [16] (i.e. their lack of sensitivity in early dark-field positive primary cases and in late syphilis, and the relatively high Incidence of false-positive reactions), the quest for a specific serologic test for syphilis began many years ago and still continues. The use of the immunoenzymatic techniques in the serology of syphilis started in the mid-1970s [17] and, at present, many different enzyme-linked immunosorbent assays (ELISAs) are available and their usefulness as syphilis examining testing so a decision can be made and diagnosis methods has been proved alote. The sensitivity and specificity is almost the same as to Treponema tests like the MHA-TP and FTA-ABS, but as in these traditional tests, the sensitivity of ELISA is suboptimal in primary and congenital disease [18,19]. EIAs reported in the literature have used different reference tests to determine sensitivity and specificity, producing a direct comparison of their performance. A comparative evaluation of 10 EIAs using either wild-type or recombinant *T. pallidum* antigens for anti-Treponema IgM and IgG antibody detection demonstrated sensitivities of 94.7%-99% and specificities 100% [20]. This study assess to performance of syphilis rapid plasma reign and *T. pallidum* Haemagglutination test(TPHA) for detection of syphilis against the standard ELISA, and assess some risk factors associated with *Treponemapallidum* infections such as age, pregnancy period and history of previous abortion.

MATERIALS AND METHODS

Time and Location of Study

The study was conducted from November to December, 2019 as a hospital-based study in antenatal clinics and hospital in Khartoum and Omdurman.

Study Subjects

Apparently healthy pregnant women, attending the antenatal Clinics in the three hospitals Omdurman Maternity Hospital, (bushier Hospital and Khartoum North Teaching Hospital) were considered eligible to be enrolled in this study. A total of 100 pregnant women were recruited from the three teaching hospitals, Irrespective of age, residence. Before questionnaires were administered to any eligible women, the latter was provided with a consent form to sign or thumbprint after the study was explained to them in detail.

Data Collection

After explaining the purpose of the study, data were collected from each subject by interviewing questionnaire. The data included the Demographic information (age and residence), pregnancy period and history of previous abortion.

Specimen Collection and Preparation

Using sterile disposable syringes injection, about 5 ml of blood were drawn from the anticubital vein under aseptic conditions. The blood samples were collected in sterile containers without any additives, and left to clot at room temperature. Every blood sample was, then, centrifuged at1500 rpm for 5 minutes, and every serum was divided in another sterile plain container. Samples were labeled by giving laboratory numbers. Serum samples were kept frozen at -20 ° C without addition of preservatives, until the time of analysis (no more than 3 months).

Laboratory Examination

All the specimens were tested for syphilis using one screening serological test, Namely, Rapid Plasma Regain Test (RPR), were further assayed with *Treponema Palladium*Haemagglutination test (TPHA) as a confirmatory test then compare with ELISA.

Rapid Plasma Reagin Test (RPR)

Samples and reagents were brought to room temperature before use. When commencing the assay, all the specimens and controls were carefully recorded on the spread sheet with the kit. Briefly, [21] 50 ml of each serum sample were added to individual circles of the card. The RPR carbon vial was, gently, resuspended. To each circle, 50 ml of the reactive RPR carbon were added near the sample drop under test. Negative and positive controls were treated similar to test and were included in every batch of the 8 samples. Sterile stirrer was used to mix the components covering all the surface of the circle. The card was, then, rotated in a mechanical rotator at 100 rpm for 8 minutes. A comparison was made between the test and control sera. The sample was considered positive if it showed slight to marked visible aggregates of carbon particles while considered negative when it did not show any visible aggregates.

Treponema Palladium Haemagglutination Test (TPHA)

The lab 21 TPHA kits use preserved avian erythrocytes coated with antigen of *Treponema palladium* (Nicols strain), which will

bind with specific antibody present in patients serum or plasma. The cells are hanging in a medium consist of components to rating non-specific reaction. Positive reactions are show by agglutination of the cells, negative reactions by the setting of the cells to the button or small ring. The test is based on the ability of antibodies in patients serum to cause was done in U shape micro titer plate. All samples and reagents were brought to room temperature before use. One well per sample was used including the positive and negative controls in each Batch of test. The components of the control (reagents3 and 4) were reconstituted with 500 ml distilled water. In a sterile tube, 1/40 dilution of the serum samples the controls in the diluents were prepared. Fifty ml and of the diluted sample and control sera were transferred into the respective wells of the micro titer plate. To ensure the erythrocyte suspension (reagent 1) was homogenous, the container was shacked gently, and then 50 ml were added to each well, making a final dilution of 1/80. The components were mixed by repeated gentle tappings of the micro titer plate edges. The plate was, then, left to stand for one hour at room temperature in a room free from vibrations. Results were recorded, immediately, after incubation. A sample was considered positive when uniform mat of erythrocytes were observed covering the entire base of the well. However, weakly positive samples were obtained when slightly turbid mat with a small erythrocytes deposit was observed in the base of the well. Negative reaction was a compact button of erythrocytes in the base of the well [22].

Syphilis Treponemapallidum antibody ELISA (fortress diagnostic –United Kingdom)

The test is used for detection of anti *T. pallidum* antibodies in patients' blood is based on antigen sandwich enzyme linked immune sorbent assay. The sample is incubated in the micro wells together with recombinant *T. pallidum* antigen conjugate to hurries radish peroxides HRP. The pre coated antigen express the same epitopes as the HRP conjugate antigen but are expressed in different hosts. After washing to remove sample and unbound conjugates chromogen solution, in presence of antigen- antibodies sandwich complex, the colorless chromogenhydrolysed by the bound HRP conjugate to blue colored product, which turns yellow upon addition of the stop solution. The colour is then read photo metrically and is directly proportional to the amount of the antibodies in the sample. Well containing negative sample for anti *T. pallidum* remain colorless. Absorbance was measured at 450nm and calculation negative control by take mean from 3 negative controls. Absorbance negative control=0.025, absorbance positive control=2.449, Off value was calculation as follow: Off value (c. o)=NC+0.18, 0.025+0.18=0.20, Cut off value=0.20 Negative result (s/c.o1) sample giving an absorbance greater than the cut off value.

RESULTS

Atotal of 100 serum specimens of pregnant women were screened at antenatal Clinics in the three hospitals Omdurman Maternity Hospital, (bushier Hospital, and Khartoum North Teaching Hospital) to detect *T. pallidum* antibodies using rapid plasma raging, LAB21 healthcare TPHA test and syphilis antibody ELISA. The result is presented in Table 1.

Table 1: Seroprevalence of *Treponemapallidum* infection among pregnant women.

Variable	Study population	ELISA seroprevalence for ***T. pallidum*** n=%
Age per year		
20-30	10(10%)	1(10%)
31-40	60(60%)	8(13.3%)
41-50	30(30%)	3(10%)
Pregnancy periods		
First trimester	30(30%)	3(10%)
Second trimester	30(30%)	3(10%)
Third trimester	40(40%)	6(15%)
History of abortion		
Non abortion	70(70%)	10(14.3%)
Abortion	30(30%)	2(10%)

The majority of study population was females in third trimester 40%; followed by first trimester 30% and second trimester 30%. Their age ranged between (20-50) years and most of them were non abortion (70%) followed abortion (10%). Antibodies against *T. pallidum* were detected in 12(12%). The highest prevalence was observed among the age group31-40 years, accounting13.4% third trimester showed the highest prevalence. According to their history of abortion, non-abortion females showed the highest prevalence (14.3%). The ELISA seropositive (n=12) and seronegative (n=88) specimens were tested in rapid plasma reign and TPHA for detection of *T. pallidum* antibodies. Comparison of the two tests in seropositive and seronegative sera, as shown in Table 2.

Table 2: Comparisons between RPR and LAB21 healthcare TPHA test for detection of ELISA seropositive and seronegative participants.

RPR test	LAB21 healthcare		TPHA	
	+	-	+	-

ELISA+n=12	9(75%)	3(25%)	10(83.3%)	2(16.7%)
ELISA-n=88	0(0%)	88(100%)	0(0%)	88(100%)

Note: RPR: Rapid Plasma Reagin; TPHA: *TreponemaPallidum* HaemagglutinationTest; ELISA: Enzyme Linked Immune Sorbent Assay; +: Positive; -: Negative; n: Number

Out of the 12 ELISA seropositive sera, the rapid plasma reign detected 9(75%) and 3 specimens showed false negative result. Larger population of ELISA seropositive pregnant women demonstrated positive result in TPHA (n=10, 83.3 %). Compared to ELISA as the gold standard, the sensitivity, specificity and positive and negative predictive values of syphilis rapid plasma reign were 75%(95% CI:42.8-94.51), 100%(96.03-100%), 100%(66.37-100%), and 96.81%(90.96-99.34%) respectively. The performance characteristics of LAB21 healthcare TPHA tests in comparison to the gold standard syphilis antibody ELISA are as follows: sensitivity=83.33%(51.559-97.91%), specificity=100%(95.5-100%),positive predictive value=100%(69.15-100%), and negative predictive value=97.83%(92.37-9974%). Thus, while the sensitivity of LAB21 healthcare TPHA tests was higher than that of rapid plasma reign (83.33%vs 75%) the specificity were similar (100%), shown in Table3.

Table 3: Diagnostic performances of syphilis antibodies ELISA, RPR, and LAB21 healthcare TPHA test for detection of *T. pallidum*.

Serological test	TP	FN	TN	FP	Sensitivity estimated at 95% CI	Specificity estimated at 95% CI	PPV	NPV
ELISA	12	-	88	-	100% 73.54% to 100% 100%	100% 95.89% to 100% 100%	100% 73.54% to 100% 100%	100% 98.89% to 100%
RPR	9	3	88	-	75.0% 4.81% to 94.51%	100% 96.03% to 100% 100%	100% 66.37% to 100% 100%	96.81% 90.96% to 100%
TPHA	10	2	88	-	100% 51.59% to 97.91%	100% 95.98% to 100% 100%	100% 69.15% to 100% 100%	97.83% 92.37% to 99.74%

Note: TP: True Positive; FN: False Negative; TN: True Negative; FP: False Positive; PPV: Positive Predictive Value; NPV: Negative Predictive Value; CI: Confidence Interval

DISCUSSION

Syphilis is asexually transmitted infection (STDs) that represented a major public health problem spreading worldwide. In developing countries, the prevalence of disease is very high in African countries such as Sudan [23]. The diagnosis of syphilis is based upon medicine-based signs of sickness and laboratory result [24]. This study attempted to estimate the seroprevalence of syphilis among pregnant women attending antenatal care services in tri-capital Khartoum hospital. During ANC sessions a standardized questionnaire was administered to collect socio-demographic and behavioral characteristics of pregnant women. Syphilis serology was performed using rapid plasma reagent (RPR) slide test and *Treponemapallidum* Haemagglutination assay (TPHA) and enzyme linked immune sorbent assay (ELISA). The choice of these specific and nonspecific serological test for syphilis, in this study because it widely used as screening test in the poor countries, easy to do/complete, does not need advanced equipment an inexpensive.Then compared to ELISA technique to detect gold standard method. The seroprevalence of syphilis reported in this study was 12% with the most age group 31-40. This finding is in agreement with studies done in Khartoum conduct at the same center which low prevalence [25], also with Northwest Ethiopia which also found the high prevalence to be among the same age group [26], and contrasts with the findings from South Africa, which reported high prevalence of maternal syphilis to be among age group of 35 to 39 years [27]. The third trimester showed the highest infected population, higher than first and second, 15%, 10% and 10% respectively, similar to western Nigeria 2014 [28]. The findings of this study exhibited no significant relationship between the age, pregnancy period and history of previous abortion of the pregnant women and likelihood of being harmed or influenced by syphilis disease. We evaluated the diagnosis performance of the syphilis rapid plasma reagent, which is designed to rapidly detected syphilis. The sensitivity and specificity rapid plasma regent to detect syphilis 75% and 100%, match upon each pair of items in order almost the same performance result were reported in previous study in other population from south west Sudan 2016 [29]. The syphilis rapid plasma reign missed three cases that were serologically detected by syphilis antibody ELISA, the gold standard. Although, syphilis rapid plasma reign showed lower sensitivity than the LAB21 healthcare TPHA test, the

advantage of the former test should be acknowledged as simile and rapid test first-aid screening at health care setting with low facilities. However, the syphilis false negative result by the rapid plasma reign and LAB21healthcare TPHA test in some samples indicate the importance of confirming negative result with a more sensitive test such as ELISA. Several factors could account for these highly varied findings, including, the duration and size of studies, educational background, cultural and traditional practices, sexual partners, access to health Information on Sexual Transmitted Infection (STI) and other health care related programs as reported in more than two/but not a lot of African countries and also the level of public acknowledge about the activities of more than two/but not a lot of services businesses/ government units across the nation [30] and testing should be limited to those laboratories with expertise and, where possible, the direct detection of *T. pallidum* by microscopy and PCR-based tests for direct detection should be obtained from appropriate laboratories. The use of only one type of serological test is not enough for diagnosis; both non-Treponema and Treponema Serological tests should be carried out in all medicine related to and science suspected cases. However, a strict quality control program should be maintained to make sure of reliability and reproducibility of the tests. Although this combination provides infection an excellent screen for all stages of syphilis with the exception of very early primary when the Treponema test may not yet be positive [31].

CONCLUSION

In conclusion, syphilis is an important blood-borne disease among pregnant women. In this study we showed that recomWellTreponema is a highly specific and sensitive method, capable of detecting IgG and IgM antibodies in pregnant serum, syphilis RPR and TPHA are cheap and technically easy and thus suitable for routine application, however their low sensitivity, as compared to the standard ELISA, limited their value to be used in syphilis prevention program.

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