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PREVALENCE OF SYPHILIS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN SOME HOSPITALS WITHIN KADUNA METROPOLIS

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ABSTRACT
Syphilis is a sexually transmitted infection (STI) caused by a spirochete bacterium, *Treponema pallidum*, which are widespread in both Developed and developing countries and constitute a major public health problem. This study was conducted to determine the Sero-prevalence of Syphilis infection among pregnant women in Kaduna, Nigeria. Three hundred (300) pregnant women attending ante-natal care in three selected hospitals were chosen for this study. Blood samples were collected and tested for presence of Syphilis antibodies using rapid diagnostic test kit. The positive samples were confirmed using polymerase chain reaction. Antibodies for Syphilis were detected in 2.7% (8/300) of the pregnant women. Further demographic investigation indicates Syphilis to be significantly associated with polygamy and blood transfusion at 95% confidence level. Therefore, screening for Syphilis during pregnancy is essential to improve ante-natal care and inform clinical management.

Keywords: Sexually transmitted Infection, Prevalence, Syphilis, Pregnant women.

INTRODUCTION

Sexually transmitted diseases (STDs) are group of infectious diseases in which their primary mode of transmission is through sexual contact. Sexually transmitted diseases are among the major causes of illnesses in both developed and developing countries (Ageru and Abiso, 2018). Sexually transmitted disease share similar mode of transmission routes and risk factors. The prevalence rate of syphilis is highly variable, being influenced by sexual behavior, demographic aspects, life style and access to health services (Santos *et al*., 2017) Sexually transmitted disease have been associated with conditions such as spontaneous abortion, miscarriages, stillbirth, prematurity, natural loss of the product of conception, low birth weight, preterm labor, and postpartum/postnatal endometritis. Cervical cancers and chronic pelvic infection are also observed in women during pregnancy which causes longtime morbidities (Osazuwa and Ifueko, 2017). Women are more vulnerable to sexual transmitted disease than men, in both cultural, biological and socioeconomic terms. Most STD's have no symptoms in women, and their consequences can be serious, sometimes even fatal for both themselves and their offspring (Lima and Viana, 2009).

Syphilis is a sexually transmitted disease caused by a spirochete bacterium, *Treponema pallidum*, which can be passed to the mother during sexual contact and subsequently to child during pregnancy (vertical transmission) (Sriniwasan *et al*., 2017). The risk of transmission to child is related to the maternal *Treponema* load hence early maternal infection carries a much greater risk of transmission than late infection. Almost all babies born to mothers with untreated primary or secondary infection will be infected, but the likelihood of transmission and severity of infection will fall with subsequent pregnancies as the mother *Treponema* load will fall with time. (Ageru and Abiso, 2016). Syphilis can progress to many stages if left untreated like secondary, primary, tertiary, and latent syphilis which can be characterized by painless sores in the mouth, genitals, rectum, or skin, body rash, headache, fever, fatigue, and lymphadenopathy (disease of the lymph node). Progression to tertiary syphilis can result in damage in the nervous system, eyes, heart and brain (Sriniwasan *et al*., 2017). Syphilis can be easily treated with penicillin the disease almost disappeared in Western countries before it surprisingly reemerged in the late 1990 (Kirsten, 2015). In Western countries, syphilis is now mainly encountered among men who have sex with men, and in developing countries the disease represents an extensive problem, and above all syphilis is concern especially to pregnant women due to the increased risk of congenital malformation of the newborn, stillbirth, spontaneous abortion and miscarriage (Kirsten, 2015). Approximately 12 million new cases of syphilis are detected each year and more than 2 million occur in pregnant women. The risk of contracting syphilis through sexual contact with a person that has primary or secondary syphilis is 30–50% (Fissehatsion *et al., 2017*). Early diagnosis and treatment of pregnant women who tested positive with syphilis have been shown to be effective in reducing still-birth, neonatal death and congenital infection by more than 55 percent (Ageru and Abiso, 2016).

MATERIALS AND METHODS

Subjects

The study consists of 300 pregnant women who attended antenatal clinics in some hospitals within Kaduna metropolis between Septembers to November 2019. Ethical approvals were obtained from Ministry of Health and Human Services Kaduna state, Nigeria and Barau Dikko teaching hospital Kaduna state university. A consent form was used to invite the pregnant women to participate into the study. A structure questionnaire was used to obtain information on socio-demographic factors (Age, Trimester and Educational status).

Sample collection

2ml of blood samples were collected through the vein into the EDTA container from each of the 300 pregnant women using a disposable sterile needle. The blood samples was centrifuged at 500/rpm for 5minutes to obtain serum.

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Screening for Syphilis Antibody
Syphilis Rapid Test Strip (Houston Texas laboratory, USA) was used to detect antibodies produced against *Treponema pallidum*. All the tests were carried out according to manufacturer's instructions.

Molecular Confirmation of Syphilis

DNA Extraction
Extraction of genomic DNA from clinical specimens was carried out using Bioneer Genomic DNA kit K-3032 (Bioneer, Korean), following the manufacturer's instruction. 20µl of Proteinase K was added to a clean 1.5ml tube, 200µl of serum was applied to the tube containing proteinase K, 200µl of Binding buffer (GB) was added to the sample and mixed immediately by vortex mixer, the mixture was Incubated at 60^0c for 10min. 400µl of absolute ethanol was added and mixed well by pipetting. The lysate was carefully transferred into the upper reservoir of the Binding column tube without wetting the rim. The tube was closed and centrifuged at 8,000rpm for 1min. The solution was discarded from the collection tube and the collection tube was reused. 500µl of Washing buffer 1 (W1) was added without wetting the rim and the tube was closed and centrifuged at 8,000 rpm for 1min. The solution was discarded from the collection tube and the collection tube was reused. 500µl of Washing buffer 2 (W2) was added carefully without wetting the rim. The tube was closed and centrifuged at 8,000rpm for 1m The solution was discarded from the collection tube and the collection tube was reuse, the tube was Centrifuged once more at 12,000rpm for 1min to completely remove ethanol, and checked that there no droplet clinging to the bottom of Binding column tube, the Binding column tube was transferred to a new 1.5 ml tube for elution, and 200µl of Elution buffer was added onto Binding column tube, and wait for at least 1min at RT (15~25^0c) until elution buffer was completely absorbed into the glass fiber of Binding column tube, Centrifuged at 8,000 rpm for 1 min to eluted.

PRIMERS
Primer pairs and sequences for the amplification of Syphilis DNA polymerase.

Target gene	Primer sequence	Expected amplicon	Source
tpp47	F-5'GACAATGCTCACTGAGGATAGT3' R-5'ACGCACAGAACCGAATTCCTTG3'	658	Martin *et al.*, 2009

DNA Amplification
PCR assays was performed in a volume of 20µl containing 2µl of DNA (template) extracted from serum, 16µl of water, 1µl of forward primer and 1µl of reverse primer were all added to the premix. Each run contained a positive control, as well as a no-template (water) negative control. Amplification of Syphilis was performed by Conventional PCR, and 1microliter of the first-round PCR product was used as the template for the second-step PCR. PCR conditions of the target genes (tpp47) of Syphilis was as follows: 95°C for15 min, followed by 40 cycles at 95°C for 40s, 65°C for 1 min, and 72°C for 1 min, followed by 72°C for 5 min.

Gel Electrophoresis
1.5g of powder gel was transferred into 100ml of buffer (Tris acetate EDTA). Solution was heated in a microwave until agarose gel was completely dissolved and was allowed to cool for 15-30min at room temperature. Gel casting tray was prepared by sealing ends of gel chamber with tape. Appropriate number of combs were placed in gel tray to create wells. 5ul of ethidium bromide was added to cooled gel and poured into gel tray and was allow to solidify. Combs were removed and placed in electrophoresis chamber and covered with buffer (Tris acetate EDTA). DNA was loaded and standard (Ladder) onto gel and Electrophoresis at a given Voltage for at least 1 hour. DNA strands were visualized using UV light box.

Data Analysis
The data obtained from the questionnaire and the results of the laboratory analysis was analyzed using SPSS 23.0 software (2018). The Pearson chi-square test was applied to assess the relationships between the demographic data and clinical information with syphilis infection and P value <0.05 was considered significant at 95% confidence interval.

RESULTS AND DISCUSSION
Out of the 300 specimens tested, Syphilis was detected in 8 pregnant women giving a prevalence of 2.7%. This indicate that syphilis is endemic in the region of the study and this may be due to individual behavior and practice in the community. Agarose gel electrophoresis Photo of the reaction products of tpp47 gene was represented in (**Figure 1)**. Lane M represent molecular markers: (2000, 1000 and 500bp), lane 1 and 2 represent Negative result. The gene for Syphilis was not detected in all the two (2) samples that shows seropositivity in the syphilis test strip tested among the pregnant women. The reason for a false positive result is the presence of another disease that produces antibodies (IgG and IgM) similar to the ones produced during a syphilis infection. The development of molecular techniques such as PCR that was used in this research gives a more accurate confirmation of positive or negative strip result (Olubukola & Adesina, 2010).

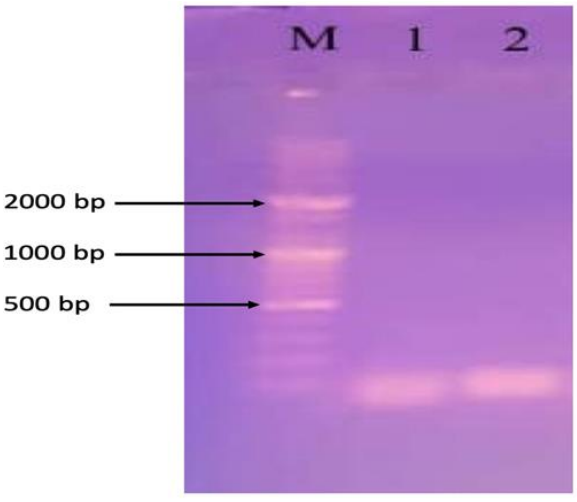

Figure 1: Agarose gel electrophoresis Photo of the reaction products of tpp47 gene.

KEY
Lane M: PCR markers: (2000, 1000 and 500bp)
Lane 1 and 2: Negative result

The distribution of the Syphilis infection among pregnant women according to age group was analysed and the result shown in **Table 1**. There was no significant association (P=0.115 for Syphilis) between the age of participants and the infections. For Syphilis infection, women in age group 21-25 years had the highest prevalence of 4.2% (3/70) while women in both age groups 36-40 and >40 years had the lowest prevalence of 0.0%, indicating that those in the group 21-25 years are sexually active, therefore they have a high risk of contracting the disease (Isa *et al.*, 2014). This suggests that women who are at the peak of their reproductive years are more prone to syphilis infection as earlier suggested by Olokoba, *et al* (2009).

Table 1: Distribution of Syphilis among Study Subjects by Age

Age group (Years)	Total No. Of Specimens	Syphilis	
		No. Positive (%)	No. Negative (%)
16-20	53	1(1.9)	52(98.1)
21-25	70	3(4.2)	67(95.7)
26-30	89	2(2.2)	87(97.7)
31-35	68	2(2.9)	66(97.0)
36-40	17	0(0.0)	17(100)
>41	3	0(0.0)	3(100)
Total	300	8(2.7)	292(97.3)

The distribution of Syphilis infection according to gestational age (Trimester) was analysed and the result shown in **Table 2**. There was no significant association (P=0.063 for Syphilis infection) between the gestational age and presence of the Syphilis infections. For Syphilis infection, women in their second trimester had the highest prevalence of 4.2% (5/120) while those in their last trimester had the lowest prevalence of 1.3% (1/75). Vertical transmission of Syphilis infection is thought to be a major mode of transmission in endemic areas (Wright, 2006, Olokoba *et al*., 2019). Therefore, at whatever stage a pregnant woman presents herself for ante-natal care, her syphilis status should be confirmed for effective prevention of transmission.

Table 2: Distribution of Syphilis by Trimester

Trimester	Total No. of Specimen	Syphilis	
		No. Positive (%)	No. Negative (%)
1-3	105	2(1.9)	103(98.1)
4-6	120	5(4.2)	115(95.8)
7-9	75	1(1.3)	74(98.7)
Total	300	8(2.7)	292(97.3)

The distribution of Syphilis according to educational status was analysed and the result showed that there was no significant association (P=0.071 for Syphilis infection) between the women's educational status and presence of Syphilis infection. For Syphilis infection, women who had only Qur'anic education had the highest prevalence (100%: 4/4) while those with Tertiary education had the lowest prevalence (0.0%: 0/82) **(Table 3).** This suggests that formal western education among women is not a main risk factor. The highest prevalence observed among those who lacked formal western education was probably because those who have formal education are knowledgeable about transmission routes for syphilis and so are (deterrent) preventing them self from engaging in unprotected sexual intercourse and other means of transmission. This agrees with the work of Isa *et al*., (2014) who also reported that there was no statistical significant difference observed between the educational status and the prevalence of the Syphilis infection. Whereby P=0.5939 and have a highest prevalence among those with no formal education. This agrees with the work of Olowe *et al*., (2014) who also reported that those with no formal education have the highest prevalence. An increase in the level of education of women will generally improve their socioeconomic status and might thus lead to a reduction in the prevalence of this disease (Olowe *et al*., 2014).

Table 3: Distribution of Syphilis by educational status

Educational status	Total No. of Specimen	Syphilis	
		No. Positive (%)	No. Negative (%)
Primary	99	1(1.0)	97(97.9)
Secondary	115	3(2.6)	112(97.4)
Tertiary	82	0(0.0)	78(95.1)
None	4	4(100)	0(0.0)
Total	300	8(2.7)	292(97.3)

The distribution of Syphilis according to family type was analysed and the result shown in **Table 4**. There was a significant difference (P<0.012) in family type for Syphilis infection. The highest prevalence of syphilis was obtained for women who were in polygamous marriages (4.9%: 5/102) as against women in monogamous relationships (1.5%: 3/198). This may be because of the multiple sexual relationship in polygamous families as well as large population and person to person contact in polygamous homes (Otegbayo *et al*., 2008).

Table 4: Distribution of Syphilis by Family type

Family type	Total No. of Specimen	Syphilis	
		No. Positive (%)	No. Negative (%)
Monogamy	198	3(1.5)	195(98.5)
Polygamy	102	5(4.9)	97(95.1)
Total	300	8(2.7)	292(97.3)

The result of the distribution of Syphilis according to history of blood transfusion is shown in **Table 5**. There was significant difference

(P < 0.028) between history of blood transfusion and presence of Syphilis infection. The prevalence of Syphilis among women who have had history of blood transfusion was 0.0% (0/88) as against 3.7% (8/212) among those with no history of blood transfusion. This may be due to transfusion of improperly screened blood, or seroconversion after blood transfusion. This is in contrast to Buseri *et al*. (2010) that found a high number of seropositive women among those that have not been exposed to blood transfusion.

Table 5: Prevalence of Syphilis by history of blood transfusion

Blood transfusion	Total No. of specimen	Syphilis	
		No. Positive %	No. Negative %
Yes	88	0(0.0)	88(100)
No	212	8(3.7)	204(96.3)
Total	300	8(2.7)	292(97.3)

Conclusion

This study shows 2.7% prevalence of Syphilis among pregnant women attending ante natal care within Kaduna metropolis. There was a significant association between polygamy marriage and history of blood transfusion. The introduction of PCR in routine screening of pregnant women in Nigeria is essential to minimize the risk of vertical transmission (Mother to child) of Syphilis infection.

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