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Seroprevalence of *Treponema pallidum* Infection among Cohorts of Pregnant Women Attending a Selected Hospital at Northern Nigeria

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Authors' contributions

Authors SPEJ and KAL designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MU, JOO, NAK and SAO managed the analyses of the study and performed the statistical analysis. Authors AAK and FSO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Syphilis is a multifaceted disease with serious implications for the pregnant women and the foetus. *Treponema pallidum*, the causative agent of syphilis has been a public health challenge for centuries. Sexually transmitted infections (STIs) among pregnant women are wide spread in the developing countries, and constitute a major public health problem in sub-Saharan Africa. Information regarding the prevalence of syphilis in pregnant women is scanty from the north-west zone of Nigeria.
Aims: Evaluation of seroprevalence of *Treponema pallidum* infection among pregnant women attending a selected hospital at Northern Nigeria was carried out, with view to assess the socio-demographic data and predisposing factors of syphilis among the study population.
Methods: Exactly 200 pregnant women, who attended antenatal clinic of the selected hospital at Northern Nigeria, from July to September, 2015 were screened for syphilis using syphilis rapid

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immunochromatographic test for *in-vitro* diagnostic technique. Patients' demographic data and predisposing factors of syphilis were assessed using a structured questionnaire.
Results: The result showed that 2.0% of the 200 pregnant women screened were positive. Based on age, individuals who were less than or equal 20 years had the highest prevalence of 4.1% followed by those who aged 21-30 with the prevalence of 1.1%. Family type and place of the study subjects were significantly associated with the disease (Fisher's exact test = 0.014 and 0.008 respectively). Therefore associated risk factors were age, family type and place of residence. While, educational status, employment status, gestational age and blood transfusion were not significantly associated with syphilis in the study area. While seroprevalence of 4(5.7%) was recorded in polygamous individual; no positive case was recorded among those practicing monogamy. Urban dwellers were found to have a prevalence rate of 6.6% with no infection detected among the rural dwellers.
Conclusion: The overall prevalence of syphilis among the study population was 2.0%. The disease is more prevalent among middle aged, rural dwelling and polygamous pregnant women. Hence in a bid to control syphilis infection, both partners should be evaluated and treated. This study has further provided information on the prevalence of *Treponema pallidum* infection among pregnant women at Northern Nigeria.

Keywords: Pregnancy; seroprevalence; syphilis; Treponema pallidum; Northern Nigeria; cohort.

1. INTRODUCTION

Syphilis is a sexually transmitted disease caused by the *Treponema pallidum* spirochaete [1]. The aetiological agent *Treponema pallidum* subsp. Pallidum (*T. pallidum*) is a spirochaete which resembles a Gram-negative bacterium but however, differs with regard to paucity of surface exposed outer membrane protein capable of eliciting a host response and has demonstrated the ability to evade the host immune system effectively by the process of antigenic variation [2].

Treponema pallidum is a motile helical bacterium with a central protoplasmic cylinder, cytoplasmic membrane, peptidoglycan and outer membrane, which resembles a Gram-negative bacterium. It is typically thin, with 6 to 14 spirals and tapered ends. The bacterium size ranges from a length of 6 µm to 20 µm and a width of 0.10 µm to 0.18 µm, which means light microscopy is inadequate for its visualization, however, it can be viewed using dark-field microscopy [3].

Globally, around 340 million cases of curable new sexually transmitted infections (STI) occur every year. Given that *T. pallidum* is a sexually transmitted pathogen affecting individuals worldwide, with an estimated 12 million new cases annually, importance is placed on the raid diagnosis, management and treatment of the aetiological agent. Syphilis remains a major cause of reproductive morbidity and poor pregnancy outcomes in developing countries [4].

The health problem caused by syphilis in adults is serious in their own rights, it is known that the genital sores caused by syphilis in adults also make it easier to transmit and acquire HIV infection sexually [5]. Untreated maternal infection leads to adverse pregnancy outcomes including early foetal loss, still birth, prematurity, low birth weight, neonatal and infant death [6].

Syphilis is a multistage disease that has no geographical boundaries but of which a disproportionate amount of up to 90% occurs in developing countries [7]. The World Health Organization (WHO) estimated that the majority of 12 million annual new cases occur in Africa, South and Southeast Asia, Latin America and the Caribbean [6]. *Treponema pallidum* is predominantly a sexually transmitted pathogen but the risk of vertical transmission remains a concern due to its devastating effects [8]. Congenital syphilis can be of particular concern in developing countries where prenatal diagnosis and treatment of pregnant women may be lacking [3].

The transmission of *T. pallidum* relies on intimate contact with the human host, which can occur during sexual intercourse, transplacentally to the foetus (congenital syphilis), during a blood transfusion or during an organ transplant. The latter two are rare routes of infection. The most common sexual route of transmission appears to be limited to the primary and secondary stages of disease and relies on contact with the lesions formed at these stages [8, 9].

The clinical causes of syphilis are divided into four stages; primary, secondary, and tertiary stages in which characteristic manifestations

occur and a latent stage, which is asymptomatic but seropositive. The latent stage occurs with the resolution of the resolution of the secondary stage. Identifying the stage of disease is important because it affects duration of treatment [10].

The detection of *Treponema pallidum* can be broadly discussed under three topics, namely: microscopy, serology and molecular techniques. Due to the inability of the spirochaete to be cultured *in-vitro* on routine bacteriological media or in rabbit epithelial cell monolayers for more than a few generations, detection has been mostly reliant on serological techniques and microscopy. Examples of treponemal tests include: treponemal enzyme immunoassay (EIA), *T. pallidum* haemagglutination assay (TPHA), *T. pallidum* particle agglutination (TPPA), fluorescent treponemal antibody absorption test (FTA-abs), *T. pallidum* recombinant antigen line immunoassay and rapid point of care (POC) syphilis tests based on rapid plasma reagin (RPR) and immunochromotographic based strips (ICT). The ICT has a sensitivity range of 93.7% to 100% and specificity ranging from 94.1% to 100% [11]. Molecular methods are not commonly used in the detection of *T. pallidum* in a clinical setting but can be considered a complimentary technique to be used in combination with conventional dark-field microscopy or serology [12]. Some of these molecular methods used for detection of the pathogen include the use of polymerase chain reaction (PCR) and Real-time PCR assays [13].

Benzathine penicillin G is preferentially used for the treatment of syphilis and is the only treatment recommended by the Centres for Disease Control and Prevention (CDC) although second-line oral antibiotics, such as tetracyclines, macrolides and cephalosporins have been used alternatively. Benzathine penicillin G is administered intramuscularly (IM) as a single dose, which ideally eliminates the risk of re-infection due to noncompliance with dosage requirements while additionally providing a long lasting treponemicidal effect at 2.4 million units. Penicillin is currently the only treatment option available for infected pregnant women, who might require penicillin desensitization prior to treatment in the case of known allergies to the antibiotic [14].

Untreated syphilis in pregnancy is associated with adverse clinical outcomes for the infant. Most syphilis infections occur in sub-Saharan Africa (SSA), where coverage of antenatal screening for syphilis is in adequate. Recently introduced point of care syphilis tests have high accuracy and demonstrate potential to increase coverage of antenatal screening. Syphilis constitutes a major public health problem in many parts of the world [1], including both developed countries as well as developing countries, Nigeria inclusive. Information regarding the prevalence of syphilis in pregnant Nigerian women is scanty from the north-west zone of Nigeria [15]. Every year, at least half a million infants are born with congenital syphilis. In addition, maternal syphilis causes another half million stillbirth and miscarriages annually [6]. Increases in syphilis among pregnant woman have been reported in various cities and areas including Nigeria. Therefore, this study aimed to determine the prevalence of *Treponema pallidum* among pregnant women attending a selected hospital in Northern Nigeria, with view to identify the risk factors associated with *Treponema pallidum* infection.

2. MATERIALS AND METHODS

2.1 Ethical Considerations and Administration of Questionnaire

Ethical clearance (MOH/ADM/744/VOL.I/298) was obtained from the concerned authorities. Apparently healthy pregnant women present at the antenatal clinic of the selected hospital who gave their consent were considered eligible and enrolled in this study. However, a structured questionnaire was administered to each subject prior to blood sample collection after informed consent was sought. The questionnaire was composed of variables on age, residence, gestational age, marital status etc. in order to obtain the risk factors associated with the prevalence of *Treponema pallidum*.

2.2 Sample Size

Sample size was determined based on the prevalence reported from the previous study by Ogbulie et al. [16] in Nigeria using equation:

$$n = \frac{Z^2 pq}{d^2}$$

where;

n = Minimum number of samples required (sample size)

Z = Standard normal deviate at 95% confidence interval = 1.96

p = Prevalence of syphilis as reported by Ogbulie et al. [16] previously = 15.33% = 0.1533

d = Degree of confidence at = 0.05

q = 1-p = 1 - 0.1533 = 0.8467

$$n = \frac{1.96^2 \times 0.1533 \times 0.8467}{0.05^2} = \frac{3.8416 \times 0.12979911}{0.0025} = 199.4545043904 \approx 200$$

Therefore, minimum sample size for the study is 200.

2.3 Sample Collection

A total of 200 pregnant women were recruited from the hospital within the age bracket ≤ 20-50 for the purpose of this study. Venous blood sample was collected from each of the volunteered pregnant women in heparinized anticoagulated containers. The analysis was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Patient's consent form was filled during the collection of sample to obtain information on demographic data such as age and sex.

2.4 Serologic Immunochromatograpic Test

A rapid test for the diagnosis of syphilis to detect antibodies (IgG and IgM) to *Treponema pallidum* in serum of plasma was applied. The test strips, specimen, buffer and controls were allowed to equilibrate to room temperature (15-30ºC) prior to testing. Each of the collected blood samples was then centrifuged at 1500 rpm for 5 minutes to obtain the blood serum.

The syphilis ultra-rapid test strip (Clinotech Diagnostics®, Canada) was performed using a serum. After which the test strip from the sealed foil pouch was removed and used as soon as possible. Best results were obtained from the performed assay within one hour. The dropper was held vertically and transferred two drops of serum or plasma (approximately 50µm) unto the specimen part of the test strip. One drop of buffer (approximately 40µl) was placed on the sample and the timer started. The setup was kept for red lines to appear. The results were read after 10 minutes and recorded. Two (2) red lines which appeared on the control line and on the determinant (test) line of the test strip indicated a positive result, while a single red line on the control line indicated a negative result. While, in the case where there is no red line appears on the control and the determinant lines, the result was termed as invalid as adopted by Umar et al. [17].

2.5 Statistical Analysis

Processing and analysis of data to be obtained were presented on tables and charts. All statistical analysis was carried out using SPSS.19 version a computer software program. Chi-square tests were used to assess the association between the various groups. Statistical significance was considered below *P* = 0.05.

3. RESULTS AND DISCUSSION

Out of the 200 samples analyzed, 4(2.0%) were positive for the infection indicating a prevalence of 2.0%. Fig. 1 presents the prevalence of the disease in relation to age. Individuals who were less than or equal 20 years of age had the highest prevalence of 4.1% followed by those who were aged 21-30 years with the prevalence of 1.1% (Fig. 1).

This study reveals that age's ≤20 has the highest prevalence of *T. pallidum*. This is followed by age's 21-30 1 (1.1%). The reason could be that age's ≤20-30 are the sexually active members of the community and are at risk of becoming vulnerable behaviorally to this infection. Physical trauma to the vagina cavity also occurs frequently and could facilitate the infection [6]. This was found to be statistically significant. This finding is in agreement with some previous reports Brillman et al. [18] stated that most new cases of syphilis in both men and women of age 15-39 years, with the highest infection rate in persons of age 20-29 years. However, the result of this study which was done only in pregnant women is in conformity with CDC [5] reports, which reported some significant ($P<0.05$) relationship between the age groups and the occurrence of the disease.

Table 1 depicts the prevalence of the disease in relation to employment and education status. Though both parameters showed no significant association with the infection ($p > 0.05$), all the positive cases were detected among unemployed individuals 4(3.6%) while the prevalence increased slightly as level of

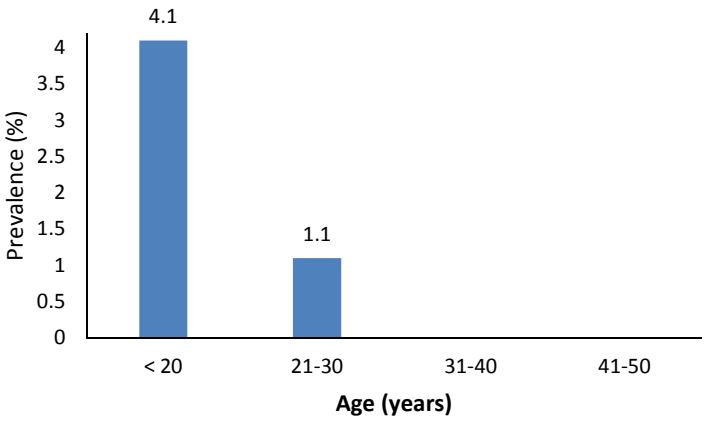

Fig. 1. Prevalence of *Treponema pallidum* infection in relation to age among cohorts of pregnant women

Table 1. Prevalence of *Treponema pallidum* infection based on demographic factors among cohorts of pregnant women

Parameter	No. examined	No. positive (%)	χ^2	*P*	Fisher
Employment status					
Employed	88	0 (0.0)	3.207	0.073	0.132
Unemployed	112	4 (3.6)			
Total	200	4 (2.0)			
Educational status					
Illiterate	20	0 (0.0)	1.368	0.713	
Primary	40	1 (2.5)			
Secondary	110	3 (2.7)			
Tertiary	30	0 (0.0)			
Total	200	4 (2.0)			

** = Fisher's exact test*

education increases. Illiterates had 0.0% prevalence, primary 1(2.5%) and those with secondary education had prevalence of 2.7%.

The common STD/STIs in Nigeria are gonorrhea, *Trichomonas vaginalis*, chlamydia and syphilis [19]. Studies have shown that pregnant women may have syphilis [19,20]. From this study, the overall prevalence of *Treponema pallidum* (syphilis) infection among a cohort of pregnant women attending selected hospital at Northern Nigeria was found to be 2.0%. The result obtained is higher than the prevalence of 0.3% that was found in Enugu [21], 1.3% in Enugu by Ozumba *et al*., [22] and 0.49% found in pregnant Italian women [23]. The result however, is lower than the prevalence rate of 10% reported by Ojo and Oyetunji, [24] in Osogbo south western Nigeria lower also than the 18.3% found in antenatal care attendees in Mozambique [21] and the 5% found in the 4, 452 pregnant Afghanistan women receiving antenatal care in three government maternity hospitals in Kabul [23]. Alo et al. [25] found a prevalence rate of 1.7% which is closely related to the prevalence rate in this study. The differences in the seroprevalence of *Treponema pallidum* (syphilis) infection in the different population of pregnant women within and outside Nigeria might be a reflection in the variation in sexual practices and sexual behavior of the communities where the studies were carried out. It may also be due to the geographical variation, differences in accessibility to treatment of STIs, cultural practices, and differences in the laboratory techniques employed to detect *Treponema pallidum* (syphilis) infection. The much higher seroprevalence rate found for syphilis from southern Africa (Zambia, Malawi and Mozambique) might be due to higher prevalence rate of infection in that part of Africa.

Marital status showed no significant association with the disease (p = 0.773). All the positive

cases were found in married subjects 4(2.0%). On the other hand, family type and place of residence of the study subjects were significantly associated with the disease (Fisher's exact test = 0.014 and 0.008 respectively). While the infection was 4(5.7%) in polygamous individuals, no positive case was recorded among those practicing monogamy. Urban dwellers were found to have prevalence of 6.6% with no infection detected among the rural residents (Table 2).

It was observed from the study that the highest occurrence rate of *T. pallidum* infection was 4(3.6%) recorded among the unemployed pregnant women while 0 (0.0%) has recorded among the employed pregnant women. Although according to these study, employment status is not statistically significant to the transmission of the infection. Thus, the difference could be traced to the level of awareness of the disease among the employed being higher when compared with the unemployed, which could expose them to the risk of the infection.

The highest occurrence rate of *Treponema pallidum* infection 3(2.7%) was also recorded among pregnant women who attended only secondary schools, followed by 1(2.5%) for pregnant women that attended only primary school. These could be due to the fact that the pregnant women within the secondary school level are within the adolescent ages characterized by high youthful exuberance, attractions for the opposite sex and intense desire for sex which not control could leads to sexual promiscuity among multiple partners and therefore exposing them to the risk of being infected this disease. This agrees with the findings of Alo [25] who reported highest seroprevalence of *Treponema pallidum* among pregnant women at Ebonyi state, Nigeria.

Table 2. Prevalence of *Treponema pallidum* infection based on associated risk factors

	No. examined	No. positive (%)	χ^2	*P*	Fisher
Marital status					
Married	196	4 (2.0)	0.083	0.773	1.000
Single	4	0 (0.0)			
Total	200	4 (2.0)			
Family type					
Monogamous	130	0 (0.0)	7.580	0.006**	0.014*
Polygamous	70	4 (5.7)			
Total	200	4 (2.0)			
Residence					
Urban	61	4 (6.6)	9.301	0.002**	0.008**
Rural	139	0 (0.0)			
Total	200	4 (2.0)			

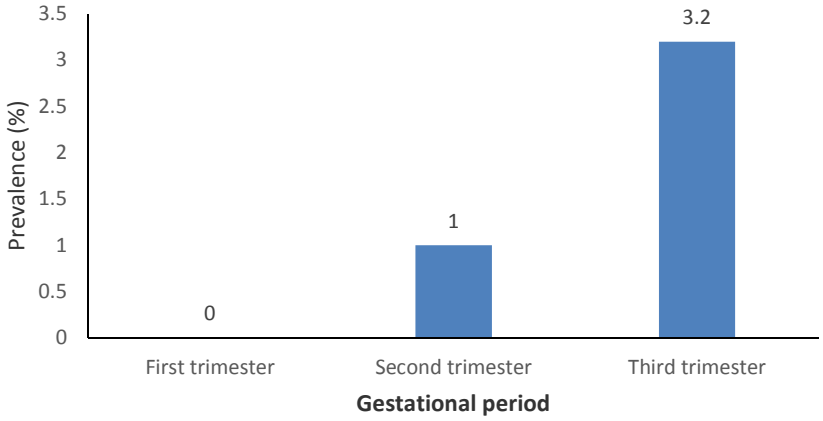

Fig. 2. Prevalence of *Treponema pallidum* infection based on gestation period

Table 3. Prevalence of *T. pallidum* infection based on blood transfusion among a cohort of pregnant women

Blood transfusion	No. examined	No. positive (%)	χ^2	*P*
Yes	4	0 (0.0)	0.083	0.773
No	196	4 (2.0)		
Total	200	4 (2.0)		

Fisher's exact test = 1.000

Based on marital status the highest occurrence of *Treponema pallidum* infection was recorded among the married pregnant women with 4 (2.0%) while the singles recorded a prevalence rate of 0(0.0%). These could be due to the sexual activeness of the married pregnant women leading to sexual promiscuity among the partners, exposing them to risk of being infected with the disease. It was also observed from study that the highest occurrence rate of *Treponema pallidum* infection 4(6.6%) was recorded among the urban dwellers while 0 (0.0%) was found among rural dwellers. The reason could be as a result of the socialization interactions that are higher in the urban areas than the rural areas resulting in high risk for syphilis infection. This higher level of socialization can predispose the urban dwellers to syphilis infection. Abdelbagi [26] reported similar findings.

No positive case was detected among the study subjects whose pregnancies were in first trimester. Study subjects whose pregnancies were in second and third trimesters had prevalence of 1.0% and 3.2% respectively. Table 3 revealed that blood transfusion is not significantly associated with the disease ($p > 0.5$) (Fig. 2).

The result as well revealed that the highest occurrence rate of *Treponema pallidum* 3(3.2%) was found among women within their third trimester followed by 1(1.0%) found among women within their mid trimester which exhibit no significant relationship with the infection as according to this study. This agrees with the result obtained by Abdeelbagi et al. [26], which recorded 41% and 55% prevalence in second and third trimester respectively. This probably could lead to mother to child transfusion.

With respect to family type the highest occurrence rate of *Treponema pallidum* infection 4(5.7%) was recorded among the polygamous and was found to be significantly associated with the infection. This implies that the syphilis is prevalent when there are multiple partners. More so, sexual promiscuities among the partners can expose them to the risk of being infected.

A prevalence rate of 4 (2.0%) was recorded for pregnant that have no history of blood transfusion while 0(0.0%) was recorded for pregnant women that has experience of blood transfusion. This shows that blood transfusion is not statistically significant to syphilis transmission as according to this studies which does not agrees with previous literature, where Ogbuile et al. [16], reported prevalence of 15.3%, and Adeolu et al. [27], who explains that blood transfusion is a risk factor for syphilis infection. This result could possibly be due to low rate and carefulness in blood transfusion.

4. CONCLUSION

In conclusion, the overall seroprevalence of *Treponema pallidum* was 2.0%. Highest prevalence was recorded among patients of age below 20 years. The infection is more rampant among unemployed patients, and those who have secondary school education. Married women living in polygamous settings are more vulnerable to *Treponema pallidum* infection. Patients living in rural areas at their third trimester of pregnancy recorded highest seroprevalence of *Treponema pallidum* infection. It is advisable for pregnant women to be screened for *Treponema pallidum* (syphilis) because the disease is treatable, and it would help eliminate the adverse effects of untreated *Treponema pallidum* (syphilis).

It is also recommended that sexually transmitted disease program should be re-enforced to assess syphilis training and treatment to eliminate the scourge of the disease. Further study using more confirmatory tests, with large sample size, are recommended for proper diagnosis, treatment and management of syphilis among pregnant women. Also it is recommended that the clinical facilities for diagnosis and treatment of syphilis should be made available and accessible to all antenatal care units. It is also recommended that policies and regulations that favour syphilis eradication should not only be

put in place but also be sign into law ensuring that all authorities concern play their own roles effectively.

CONSENT

All authors declare that 'written informed consent was obtained from the patients for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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