SEROPREVALENCE OF *TREPONEMA PALLIDUM* INFECTION AMONG WOMEN WITH HISTORY OF MISCARRIAGE AND STILLBIRTH IN A FERTILITY HOSPITAL IN NNEWI, NIGERIA

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ABSTRACT

Background: Untreated sexually transmitted infections caused by spirochetes in pregnant females are associated with miscarriage and still-birth.

Aim: This study was aimed at screening, confirming and investigating the seroprevalence of *Treponema pallidum* infection among women with history of miscarriage and still-birth in a private fertility hospital in Nnewi, Nigeria. Also, to assess the association between pregnancy outcome in relation to *T. pallidum* infection and determine the association between risk factor variables for *T. pallidum* infection among women with history of miscarriage and still birth in the study group.

Methodology: This cross-sectional study involved 150 pregnant in- and outpatients of aged 20 to 49 years, randomly and who gave consent to participate. *Treponema pallidum* antibody was screened with serum using VDRL (Venereal Disease Research Laboratory) agglutination test and confirmed with T*reponema palladium* hemagglutination ELISA kit spectrophotometric method. Risk-factor variables investigated. Data analyzed using chi-square analysis with level of significance set at <0.05.

Results: Seropositive *T. palladium* was 2.0 %, higher among the age range of between 30 – 34 (0.7%) years old. All results from VDRL screening were confirmed positive by TPHA test. Risk factors like marriage, gestational age, marital background (polygamy), blood transfusion, were important in disease transmission as 3(2.1%), 1 (1.3%), 1 (14.3%), 1 (8.3%), 2 (2.5%) and 1 (10%) of the participants. There was a significant association (X^2 = 15.224; p= 0.002) between presence of the disease and gestational age (p<0.05).

Conclusion: *T. palladium* infection was present in pregnant women in the study group, though the 30-34 years old groups were more affected. Gestational age, especially 3rd month, seemed to be important in encouraging occurrence of the disease in the group studied.

Key words: seroprevalence, *Treponema pallidum*, women, stillbirth, miscarriage, Nnewi.

Introduction

Syphilis is a sexually transmitted disease (STD) caused by the bacterium *Treponema pallidum*. Syphilis can cause serious health effects without adequate treatment **[1].** *Treponema pallidum* is able to cross the placenta of women and result in fatal infections such as still-birth, miscarriages and childhood diseases **[2].** It can be symptomatic and asymptomatic, but it is easy to treat if detected early but may lead to grave consequences or death if left untreated. Recently, the number of syphilis cases in the United States has slightly increased. **[3]** reported a devastating surge in congenital syphilis. They noted that during 2019–2020 the rate of primary and secondary syphilis increased 24% among women aged 15–44 years. In 2020, there was an increase of **16%** from 2019with 5,726 cases of syphilis (all stages) diagnosed among pregnant women **[4].** The three main causes of three-quarters of neonatal death in Nigeria (birth asphyxia, pre-term birth complications, and infections) are easily preventable by healthy practices and simple case-management **[5]**, but awareness about their importance and likely curable causes is low, especially in underdeveloped countries.

A pregnant syphilitic woman can transmit *Treponema pallidum* to the foetus through the placenta from gestation period of 4 weeks, as risk of foetal infection increases with gestational age **[6]**. Some of the infected foetus die or is miscarried, while some die in-utero. Failure to ensure maternal screening in routine antenatal care may lead to congenital syphilis. **[7]** stated that the disease can cause Hutching's teeth, which affect the baby may result to neurosyphilis with a lot of complications like damage to womb, salpingitis, neonatal death and stillbirth.

There is a paucity of literature on reported stillbirths and miscarriages in syphilis-infected women in Nigeria. Reported prevalence of stillbirth according to **[8]** among mothers in rural communities in Anambra Central Senatorial Districts of Anambra state Nigeria who gave birth between January 2012 and December 2016 was 313 cases (74 cases of stillbirth; 38.07 per 1000 total births in 2016), highest in 2012, while *Treponema pallidum* sero-reactivity of 1.7% was found in Nigerian women attending gynaecology clinic in Ilorin **[9]**.The major causes of neonatal deaths in tertiary hospitals in South-west Nigeria include birth asphyxia (46.6%), prematurity (23.1%), and sepsis (17.8%) **[10]**.

In a review of stillbirth in countries of low and middle income, the population attributable fraction was greater than 50% for five risk factors associated with stillbirth, of which two factors, syphilis and chorio-amnionitis, were infection-related **[11]**. **[12]** noted that information regarding syphilis infection in Nigerian women shows a wide geographical variation in seroprevalence. A 5-year multicentre, retrospective descriptive study by **[13]** stated that of all stillbirths delivered in the south- eastern Nigerian hospitals from January 2013 to December 2017, the overall SBR was 56.1 per 1000 deliveries, higher than 42.9 per 1000 deliveries previously reported in Nigeria.

Late or lack of prenatal care is the main challenge for preventing congenital syphilis and its sequels, and even in those receiving care, early detection and treatment of maternal syphilis occurs often too late in pregnancy to prevent its in vitro and after birth adverse effects. Therefore, it is necessary that health departments, in partnership with prenatal care

providers and other local organizations should work together to address the barriers, factors that affect the outcome and effects in women **[14].**

Syphilis can be symptomatic or asymptomatic, and is considered a sexually transmitted disease not too common due to broad spectrum antibiotic use. It however can cross the placenta and infect the foetus with grave consequences like mental disorders, cognitive disorders, and death **[15]**. It is the source of major health-cases in neonates and is 100% preventable. Unfortunately, most screenings in Nigeria are carried out just before child-birth, and if detected slate or left untreated in pregnancy, leads to adverse outcomes among more than half of the women with active disease, including early foetal loss, stillbirth, prematurity, low birth weight, neonatal and infant death and congenital disease.

There is a paucity of literature on the association of syphilis with miscarriage and still - birth in Nigeria due to several undocumented cases and beliefs of spiritual involvement. According to **[16]**, Nigeria was ranked second position worldwide with an estimated 313, 700 stillbirths in 2015. **[17]** stated that stillbirths are one of the most neglected tragedies in today's global health system; with approximately 2.6 million stillbirths occurring each year with 98 recorded in low and middle-income countries. Nigeria accounts for 12% of this 2.6 million **[18]**.

[9] noted the importance of routine STD screening in pregnant women especially among young and illiterate women in Nigeria. The Federal Ministry of Health Nigeria (FMoH) recently reviewed the situation of maternal, new-born, and child health (IMNCH) strategy aimed to address gaps in health care, and from zonal, state and local government authority levels. Strategies were rolled out hoped at helping to bring to recognition the massive burden of new- born death in Nigeria, especially with regards to the causes in each geographical area. Their main objective was to provide a more comprehensive understanding of new born survival and health in Nigeria as well as to analyse data of relevance by state to present a concrete step to accelerate action to save new born lives in Nigeria, **[19]**. Fortunately, if causes of new-born death are discovered early, as the infection is treatable, death is preventable especially when the risk factors are association with miscarriage or still birth. Test type and methodology is a very important determining factor for seropositivity. **[20]** suggested adjusting reported maternal syphilis seropositivity by test type to ensure accuracy. Hence, there is a need to study the seroprevalence of *Treponema pallidum* infection among women with history of miscarriage and stillbirth attending ante-natal care in Nnewi, a sub-urban city in Anambra state using standard methods.

Methodology

The study area is a private fertility hospital situated at Nnewi in Anambra state, South-eastern Nigeria. The study was a cross - sectional research. The study population consisted of one hundred and fifty (150) in- and out-patients of child-bearing age of age-range of 20 – 49 years old, attending antenatal care in a private fertility hospital in Nnewi, Anambra State, Nigeria. Consecutive random selection was used for subject recruitment. Selection was based on age and pregnancy status. Only women who were attending antenatal care, and had been confirmed pregnant in the hospital by laboratory and radiological scan tests, as well as by clinicians' symptomatic assessment were selected. The sample size was calculated using sample size formula by **[21]**. The prevalence of syphilis in pregnant women

in Anambra state is 0.08 % **[22]**. Sample size is approximately 122.9 that is 123 samples, but a total sample of 150 was used for the study. The ethical approval for the research was obtained from the Ethical Review Committee of Faculty of Health Science and Technology, College of Health Science, Nnamdi Azikiwe University, Awka, Nnewi campus. Informed consent was signed by participants. The hospital's name was also not mentioned to maintain confidentiality as agreed on. Inclusion criteria were age as well as laboratory and clinical proven pregnancy status (any trimester) obtained from the hospital records.

Five (5) millilitre syringe (BD, India) was used to collect whole blood from the cubital fossa and sent immediately to the laboratory. Serum was extracted after blood was left to clot, labelled, stored at 4° C and assays carried out after two hours of collection. Samples were collected according to the method described by **[23]**. Additional demographic information was also obtained from the subjects. The serum samples and test reagents were allowed to equilibrate to room temperature before use.

Screening for *T. pallidum* was screened using qualitative VDRL agglutinating test (Venereal Disease Research Laboratory) (Acumen Diagnostic, USA), according to methods described by **[24]**. Confirmation was done using quantitative ELISA method with *Treponema pallidum* passive haemagglutination test kit (TPHA) (Linear chemicals, Spain) as described by **[25]**. The absorbance was measured with a spectrophotometer plate reader (Agilent technologies, USA) calibrated and read at 450nm. The cut-off value was calculated and interpretations done as instructed by the manufacturers. In - built internal positive and negative controls were used for both tests. Tests were performed and evaluated according to manufacturer's instructions.

Data analysis: Data was presented as frequencies, percentages, while student's T-test analysis and chi-square test was used to investigate association of risk-factors with presence or absence of *T. pallidum*. The level of significance was set at ≤ 0.05.

Results

Out of a total of 150 pregnant women tested for *T. pallidum* infection with a mean age bracket of 37.6± 5.1 years old, only 3 were seropositive (2.0%). The highest positive cases were from age- range of 35 – 39 years which had 2 (1.3%), followed by the 30 – 34 years which had 1 (0.7%). No participant tested positive in the other age brackets (see table 1).

Most of the women 110 (73.3%) had a history of miscarriage, 28 (18.7%) had both miscarriage and still-birth, and 12 (8.0 %) having had stillbirth alone. There was a higher sera-positivity with *T. pallidum* antibody in those with stillbirth alone (8.3%), more than those with miscarriages and stillbirth 1(3.6%), and miscarriages alone 1(0.9%); out of a total of 3 (2.0%) participants who were syphilis seropositive. Table 2 presents the association between pregnancy outcome and *T. pallidum* infection and showed no significant association X^2 = (3.476; p = 0.175), though positive cases were higher among those with miscarriage 1(8.3%).

Table 3 presents an association table between presence or absence of *T. pallidum* infection and risk factor variables among women with history of miscarriage and still birth in the study participants. There was a significant association among the three gestational age variables and syphilis status only (X^2 =15.224; p=002) p<0.005 with those in 3rd trimester having highest positive status 1 (14.3). No significant association was observed in between other variables and syphilis status, however, highest

prevalence was observed among the variables as stated; among those who had received blood transfusion in the past 1(10.0%) (X^2 3.499=; p=0.061); had pregnancy outcome of miscarriage only 1(8.3%) (X^2 =0.234 ; p=0.063); were from polygamous marital background 3(5.6%) (X^2 =3.398; p =0.183); attained tertiary educational status 3(3.7%) (X^2 =2.041; p =0.564); were from Anambra state origin 3(3.3%) (X^2= 1.531;p=0.997); resided in urban area 3(2.3%) (X^2= 0.391;p=0.532); had symptoms of syphilis 3(2.1%) (X^2= 0.150;p=0.697); were not on any antibiotics 3(2.1%) (X^2= 0.195;p=0.659); were married 3(2.1%) (X^2= 0.171;p=0.679); and employed 3(2.1%) (X^2= 0.128;p=0.721) , p>0.05.

Table 1 Seroprevalence of *T. pallidum* infection in relation to age among women with history of miscarriage and still-birth

Age (years)	No tested for *T. pallidum* (n)	percentage (%)	No positive for *T. pallidum* (n)	Percentage (%)
20 – 24	1	0.7	0	0.0
25 – 29	1	0.7	0	0.0
30 – 34	46	30.7	1	0.7
35 – 39	48	32	2	1.3
40 – 44	39	26	0	0.0
45 - 49	15	10	0	0.0
Total	150	100	3	2.0

X^2 = (2.300; P = 0.806) (P > 0.05, not significant)

Table 2: association between pregnancy outcome in the pregnant w omen in relation to *T. pallidum* infection.

Type of Pregnancy Outcome	Number who said YES (n)	Percentage (%)	Number sero-positive (n)	Percentage sero-positive (%)	Number sero - negative (n)	Percentage sero-negative (%)	X^2	P-value
Miscarriages only	110	73.3	1	(0.9)	109	(99. 1)	3.476	0.175
Still birth only	12	8.0	1	(8.3)	11	(91.7)		
Miscarriages and stillbirth	28	18.7	1	(3.6)	27	(96.4)		
TOTAL	150	100.0	3	(2.0)	147	(98.0)		

Key:
n = number
% = percentage
X^2 = chi-square
p = p- value at 0.05 level of significance.

Table 3: Association between presence or absence of *T. pallidum* infection and risk factor variables among women with history of miscarriage and still birth in the study participants.

Risk factor variables	Number who said YES (n)	Percentage (%)	%Sera-positive cases (n)	Percentage sera-negative cases (n)	X^2	p-value
Marital status						
Married	142	94.7	3 (2.1)8	139 (97.7)	0.171	0.679
Single	8	5.3	0 (0.0)	8 (100.0)		
Gestational age						
1st trimester	77	50.7	1 (1.3)	76 (98.7)	**15.224**	**0.002**
2nd trimester	55	36.7	0 (0.0)	55 (100.0)		
3rd trimester	7	4.7	1 (14.3)	6 (85.7)		
Full term still birth	12	8.0	1 (8.3)	11 (91.7)		
Employment status						
Employed	144	96	3 (2.1)	141 (98.0)	0.128	0.721
Unemployed	6	4	0 (0.0)	6 (100.0)		
Educational status						
None	4	2.7	0 (0.0)	4 (100.0)	2.041	0.564
Primary	12	8	0 (0.0)	12 (100.0)		
Secondary	53	35.3	0 (0.0)	53 (100.0)		
Tertiary	81	54	3 (3.7)	78 (96.3)		
Marital background						
Monogamy	110	73.3	1 (0.9)	109 (99.1)	3.398	0.183
Polygamy	36	24	2 (5.6)	34 (94.4)		
Single	4	2.7	0 (0.0)	4 (100.0)		
State of origin						
Anambra	99	66	3 (3.3)	93 (94.0)	1.531	0.997
Imo	11	7.3	0 (0.0)	11 (100.0)		
Abia	5	3.3	0 (0.0)	5 (100.0)		
Enugu	20	13.3	0 (0.0)	20 (100.0)		
Delta	7	4.7	0 (0.0)	7 (100.0)		
Edo	3	2	0 (0.0)	3 (100.0)		
Rivers	2	1.3	0 (0.0)	2 (100.0)		
Jos	1	0.7	0 (0.0)	1 (100.0)		
Ilorin	1	0.7	0 (0.0)	1 (100.0)		
Kaduna	1	0.7	0 (0.0)	1 (100.0)		
Residence						
Urban	133	88.7	3 (2.3)	130 (97.7)	0.391	0.532
Rural	17	11.3	0 (0.0)	17 (100.0)		
Blood transfusion						
Yes	10	6.7	1 (10.0)	9 (90.0)	3.499	0.061
No	140	93.3	2 (1.4)	138 (98.6)		
Pregnancy outcome						
Miscarriages only	110	73.3	1 (0.9)	109 (99.1)	0.234	0.063
Still birth only	12	8	1 (8.3)	11 (91.7)		
Miscarriages and stillbirth	28	18.7	1 (3.6)	27 (96.4)		
Presence of symptoms						
No	7	4.7	0 (0.0)	7 (100.0)	0.150	0.697
Yes	143	95.3	3 (2.1)	140 (97.9)		
On antibiotics						
No	141	94	3 (2.1)	138 (97.9)	0.195	0.659
Yes	9	6	0 (0.0)	9 (100.0)		

Discussion

The total number of sera-positive syphilis in pregnant women in this study was (2.0%). This means the disease still exists in the study area and is high. This could be associated with individual's immunity, sexual and life-style habits of the subject, geographical variation, level of adherence to pre-natal care by the individual as well as intervention care in the hospital. Awareness, presence and management of sexually transmitted diseases (STDs) practice like HIV infection, antibiotic use by the individuals in the area, and time of intervention are also possible contributors.

This is however much higher than the national average for syphilis in pregnant women in Nigeria (0.3%) recorded by **[26]**. **[27]** in a hospital-based cross-sectional study in ante-natal clinic of the Federal Medical Centre, Yola, North-Eastern Nigeria, confirmed a seroprevalence of 0.4% out of 231 pregnant women, lower than (2.0%) obtained in this research. **[28]** obtained a seroprevalence of 2.97% in Oshogbo, South-western Nigeria, similar but slightly higher than that obtained in the present study.

Reports from this study showed that more of the women (73.3%) experienced more of miscarriage than miscarriage and still-birth 28(18.7%) and stillbirth alone 12(8.0 %) in table 2. Reasons for higher rate of miscarriage in pregnancy outcomes could be because they may have been exposed to risk- factors that resulted to the outcome. Individual awareness, gestational age at study time, duration and stage of the infection, virulence, concentration of the spirochete bacteria, treatment received and foetal immunological status, interventions by ante-natal cares, time-lag before reporting heath issues in pregnancy, drugs taken, stress, hormonal states, other concomitant infections, likely geographical life-styles and habits in women in the study area could be contributory. **[29]** also noted that the clinical manifestations of congenital syphilis were influenced by most of the listed factors in their study. Higher sera-positivity with *T. pallidum* antibody 1(8.3%) observed in those with stillbirth alone in those with miscarriages and stillbirth 1(3. 6%) and miscarriages alone 1(0.9%) ($p>0.05$) could be because of compounding factors. The infection may have occurred at an early gestational stage, may have been severe, causing maternal fever and other systemic reactions that might have been intolerable to the foetus and resulted to death of the foetus, or a direct foetal infection or placental damage of organs like liver may have occurred **[6]**. Early gestational infection has been found to cause foetal death at later stages of pregnancy. Also, maternal infection of the genital tract as well as infection on other body parts have been found to cause preterm labour as the effects on the foetus becomes intolerable **[26]**. In placental infections, spirochetes have been found to cross into the foetus from 14 weeks, reducing blood flow to the foetus, resulting to foetal death **[6]**. **[16]** in a previous Nigerian study noted that age, household wealth, higher birth order, facility delivery, Caesarean delivery, rural residence, and contraceptive use are cardinal risk factors for stillbirth in Nigeria. In another systematic review by **[30]**, studies associated the cause(s) of stillbirth in low and medium-income countries with poverty, lack of education, maternal age (>35 or <20 years), parity (1, ≥ 5), lack of antenatal care, low birth weight and previous stillbirth. Besides, **[31]** confirmed the relationship between untreated early syphilis in pregnancy and stillbirth, neonatal death or infant disorders. In a prospective hospital study in Enugu state, South-east Nigeria, the prevalence of still - birth was 40.3 per 1000 births in pregnant

women with syphilis. Maternal age, marital status, educational levels and booking status affected the prevalence of still birth in a study by [32] (Nwoga *et al.,* 2021).

All seropositive were from polygamous homes 3(5.6%) suggesting an association between multiple sexual habit associated with polygamy and transmission of syphilis in pregnant women. The possibility of one sexual partner in the marriage circle carrying the disease will be a sure factor for transmission to others. **[33]** and **[34]** also noted this in their study as a risk- factor for syphilis in pregnant women in a China. All the positive subjects attained tertiary educational level maybe because by the time an individual attains tertiary level of education, there is a very high chance of having been exposed to sexual contact or to have come in contact with a positive case by cohabitation in school environment. As the bacteria can be latent and run a chronic course, such infected individual may remain asymptomatic until their pregnancy status demanded that they run a test and hence the late detection. Pregnancy also lowers immunity, allowing most infections to be established.

All subjects that tested seropositive were from Anambra state (3.3%) because the study site was situated in a city in Anambra state. Recruited subjects were attending the anti-natal clinic as of the time of study. Reasons for high prevalence in those who have lived in urban areas (2.3%) observed in the present study is unclear but it could be that the effects of social life-style, increased travel, immigration and possibly higher promiscuity observed in urban dwellers due to social exposures affected their health dynamics. All positive subjects in this study were married, (5.6%). Marital disharmony could create chances of infidelity increasing disease contamination risk for female subjects in this research. This is contradictory to findings by **[34]** who found the odds of infection ten times higher in divorced than in married women.

All positive cases in the study were employed (2.1%). Being employed may give an added advantage of having enough financial backing to encourage free-will living and promiscuity. Though being educated and employed have long since been considered protective factors against risk behaviors and sexually transmitted infections **[36]**, **[35]** found racial differences, geographical differences as well as individual choices as factors that accounted to deviations from expected norms. **[37]** however did not find school enrolment and academic skills to be significantly associated with sexually transmitted infections, in line with findings in this research. Generally speaking, variation in socio-demographic variables could also differ demographically. Variations in socio-demographic variables, sexual practices, community behaviour, inaccessibility of treatment of STD, and cultural practices are amongst risk factors which **[38]** noted to be associated with syphilis, agreeing with findings in this research. All seropositive cases (2.1%) showed symptoms of varying degree and none of them (2.1%) were on antimicrobial regimen as of the time of study. Non-antibiotic usage also increases chances of seropositivity since if one does not use antibiotics empirically recommended at times and is infected, this will increase chances of testing positive to syphilis. Reasons for high positive status associated, though non-significantly with individuals that had symptoms of syphilis 3(2.1%) (X^2= 0.150; p=0.697); and were not on any antibiotics 3(2.1%) (X^2= 0.195; p=0.659), could vary from individual life styles, geographical settings and habits.

The highest number of positive-case in the study

was observed among those at gestational period of 3rd trimester 1 (14.3), with significant association among gestational age and syphilis status in the study (X^2 =15.224; p=0.002) p<0.005. This was followed with those who had received blood transfusion in the past 1(10.0%) (X^2=3.499; p=0.061) and had pregnancy outcome of miscarriage only 1(8.3%) (X^2 =0.234; p=0.063). Significant association found between gestational age and positive status for syphilis (p=0.002) could be because vertical transmission of syphilis to foetus from the mother is largely dependent on duration of the infection in the mother. Pregnancy has no known effect on the clinical course of the infection. Effect therefore could probably depend on the time and duration of infection, cervical changes in pregnancy and immune status. Time lapse between conception may have been enough to establish antigenic response for a positive reaction at 3rd trimester in the infected subjects. A self-programmed weakening of the immune system or immunosuppression which occurs in pregnancy leaves both the mother and the foetus susceptible to infectious diseases **[39]**, though scarce data on congenital syphilis in Africa suggests that 1-3% of neonates under 6 months of age are seropositive and may have signs of congenital syphilis **[40]**. Again, clinical manifestation and result is dependent on gestational age causing miscarriage, premature birth, foetal growth restriction and low birth weight, problems with the placental cord, stillbirth, neonatal birth or serious lifelong health conditions **[41]**. Cervical changes in pregnancy like hyperaemia, eversion, and friability that occur in pregnancy may have facilitated entry and infection **[42]**.

Finally, syphilis antibodies were observed in people with history of transfusion in this research (10%). Transfusion is a potentially hazardous process. In unscreened blood, non- inactivated blood or blood in which syphilis is still in a window phase, the risk of contamination remains high if transfused. **[43]** found certain risk factors like maternal age, husband's occupation, late antenatal care, illiteracy, unemployment, habitual drug use, husband's habitual drug use, husband's extramarital relation, and unscreened blood transfusion associated with a high prevalence of syphilis. More so, syphilis is mostly transmitted through blood contact and blood transfusion **[12]**.

Conclusion: In conclusion, syphilis remains a significant gynaecological and public problem with neglected consequence in general. VDRL screening serological test and a confirmatory TPHA test or its equivalent gave the same result, and 3rd month of pregnancy was associated with higher risk of contacting the infection in the area.

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