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Original Article / Özgün Araştırma

A syphilis screening survey on HIV-positive and HIV-negative pregnant women, with placental histopathological evaluation of reactive cases, in a hospital in Uyo, Southern Nigeria

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Abstract

Objectives: Veneral Disease Research Laboratory (VDRL) test is an important screening tool for syphilis in pregnancy (because of adverse pregnancy outcomes, particularly congenital syphilis {CS}) and can be combined with the placental histopathological examination in the diagnosis of CS. Thus, we aimed to compare VDRL test results from HIV-positive/HIV-negative pregnant women with relevant historical data and perinatal/placental histopathological findings.

Methods: A prospective hospital-based cross-sectional survey of VDRL tests for two study groups (HIV-positive {tests} and HIV-negative {controls} pregnant women) at ante-natal care (ANC)/delivery. A convenient sampling method was used. Relevant (maternal/fetal) historical data were extracted from their case notes, and placentas of reactive VDRL cases were histopathologically examined.

Results: We surveyed 145 pregnant women (49 tests and 96 controls). The VDRL tests, for both groups, were not strongly associated with maternal age (p-value = 0.097), booking for ANC (p-value = 0.770), gravidity (p-value = 0.331), and HIV/AIDS stage (for tests only). Notably, one subject from the tests had a reactive VDRL test, and she was in HIV/AIDS clinical stage 2, her baby had microcephaly (fetal head circumference of 31 cm), low birth weight (of 2.4 kilograms), and intermediate APGAR score. Histopathological examination of her placenta showed avascular villi, large hypercellular villi, and obliterative vasculopathy with an onion-skinning pattern in the placental disk.

Conclusions: The obstetrical, fetal, and placental histopathological features of the reactive VDRL case were consistent with CS and syphilitic placentitis. Hence placental histopathological evaluation can play a critical role in the diagnosis of maternal/congenital syphilis.

Keywords: VDRL test, HIV-positive, pregnant women, Syphilis, Placenta, Histopathology

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Güney Nijerya, Uyo'daki bir hastanede reaktif vakaların plasental histopatolojik değerlendirmesiyle HIV pozitif ve HIV negatif hamile kadınlar üzerinde bir sifiliz tarama araştırması

Öz

Amaç: Zührevi Hastalık Araştırma Laboratuvarı (VDRL) testi gebelikte sifiliz için önemli bir tarama aracıdır (olumsuz gebelik sonuçları nedeniyle, özellikle konjenital sifiliz {CS}) ve KS tanısında plasental histopatolojik inceleme ile birleştirilebilir. Bu nedenle, HIV pozitif/HIV negatif gebe kadınlardan alınan VDRL test sonuçlarını ilgili tarihsel veriler ve perinatal/plasental histopatolojik bulgularla karşılaştırmayı amaçladık.

Yöntemler: Doğum öncesi bakımda (DÖB)/doğumda iki çalışma grubu (HIV-pozitif {testler} ve HIV-negatif {kontroller} hamile kadınlar) için VDRL testlerinin ileriye dönük hastane tabanlı kesitsel araştırması. uygun örnekleme yöntemi kullanılmıştır. İlgili (maternal/fetal) tarihsel veriler vaka notlarından çıkarıldı ve reaktif VDRL vakalarının plasentaları histopatolojik olarak incelendi.

Bulgular: 145 gebeyi inceledik (49 test ve 96 kontrol). Her iki grup için de VDRL testleri, anne yaşı (p-değeri = 0.097), DÖB (p-değeri = 0.770), gravidite (p-değeri = 0.331) ve HIV/AIDS evresi (için yalnızca testler). Özellikle, testlerden bir deneğin reaktif VDRL testi vardı ve HIV/AIDS klinik evre 2'deydi, bebeğinde mikrosefali (fetal baş çevresi 31 cm), düşük doğum ağırlığı (2,4 kilogram) ve orta düzeyde APGAR skoru vardı. . Plasentasının histopatolojik incelemesinde avasküler villus, büyük hiperselüler villus ve plasental diskte soğan zarı desenli obliteratif vaskülopati görüldü.

Sonuç: Reaktif VDRL olgusunun obstetrik, fetal ve plasental histopatolojik özellikleri CS ve sifilitik plasentit ile uyumluydu. Bu nedenle plasental histopatolojik değerlendirme, maternal/konjenital sifiliz tanısında kritik bir rol oynayabilir.

Anahtar kelimeler: VDRL testi, HIV pozitif, gebe, Frengi, Plasenta, Histopatoloji.

INTRODUCTION

Syphilis is an infectious disease caused by the spirochaete bacterium called Treponema pallidum subspecies pallidum (T. pallidum)[1–3]. T. pallidum can be transmitted either through sexual intercourse or vertical transmission (transplacentally) during pregnancy to the fetus in-utero[1,2]. T. pallidum, also called “the stealth pathogen”, is a difficult to culture, highly mobile, 6 - 20µm long delicate spiral-like bacterial organism with a hard, uniform, tight and deep helix (with a small genome of only 1,041 open reading frame); with the ability to disseminate early while avoiding its host’s immunity post-infection because it lacks surface proteins (peptidoglycans) linked to lipopolysaccharides[1,4].

Syphilis is a complex systemic multi-organ disease spanning four distinct stages/phases in its natural history lasting 10 to 20 years, namely: exposure/incubation period/primary, secondary, latent, and tertiary stages; and transplacental transmission can occur in any phase/trimester[1,3]. The pathogenetic hallmark of syphilis is the initiation of the host’s chronic (granulomatous) inflammatory response to the replicating T. pallidum[3]. Notably, T. pallidum belongs to the TORCH (comprising of Toxoplasma gondii; Others {i.e., Syphilis, Malaria, Zika virus, Varicella-zoster, Parvovirus B19}; Rubella virus; Cytomegalovirus; and Herpes simplex virus) pathogens which cause congenital disease (i.e., Congenital syphilis in this context) through transplacental transmission (after bypassing the robust placental antimicrobial defense mechanisms)[4–6]. Congenital syphilis (CS) is the type of syphilis diagnosed in the offspring of mothers with non-treated or poorly treated syphilis[2]. CS can be categorized as early (features appear ≤ 2 years) and late (features appear > 2 years) types[7].

Syphilis has a worldwide prevalence, particularly in Africa (especially sub-Saharan Africa {SSA}), southeast Asia, western Europe, Russia, and China[1]. The prevalence of syphilis in adult women in Africa, the Americas, Europe, and South-East Asia is 2.95%, 0.74%, 0.11%, and

0.39% respectively[8]. The World Health Organization (WHO) estimates that about 1.5 million cases of syphilis in pregnancy occur annually worldwide[9]. The cumulative prevalence of syphilis among pregnant women in SSA is 2.9%[10]. In Gondar, Ethiopia the prevalence of syphilis in pregnancy is 1.9%[11]. In Akwa Ibom State, southern Nigeria, the prevalence of syphilis in pregnancy is 2.63% in urban areas and 1.32% in rural areas[12]. Notably, the incidence of syphilis has been on the rise in recent times, particularly in those with HIV infection, despite the public health measures being put forward for its control[1]. Importantly, the occurrence of genital ulcers in syphilis makes HIV co-infection possible, even in pregnancy[13]. The prevalence of HIV-syphilis co-infection in a study done in 17 sites (within Brazil, South Africa, Argentina, and the United States), the Republic of Congo, Tanzania, and Ethiopia (Gondar) were 10%, 0.73%, and 0.66% respectively[13–15]. Tragically, maternal syphilis is strongly associated with intrauterine and intrapartum/post-partum transmission of HIV from mother to child in HIV-syphilis co-infection cases[13]. Also, syphilis in pregnancy is a leading cause of adverse pregnancy outcomes such as spontaneous abortion/miscarriages, stillbirths, neonatal deaths, prematurity, low birth weight (LBW), intrauterine growth restriction (IUGR), and CS, particularly in developing nations (especially SSA) and HIV-syphilis co-infection[3,6,7,13,16–18]. The hallmarks of CS are chronic stress involution of the thymus, stillbirth/pregnancy loss (accounting for about 150,000 stillbirths and 60,000 neonatal deaths globally), placentomegaly, LBW, fetal hepatosplenomegaly, elevated peak systolic velocity in the middle cerebral artery, anemia, ascites, developmental delay, saddle nose deformity, rhinitis, dental deformity, chorioretinitis, rash, dilated bowel, skin thickening, periostitis, meningitis, non-immune hydrops fetalis, bone marrow suppression, metaphyseal abnormalities, osteochondritis, bone fractures and demineralization[3,4,7,19,20]. The risk factors for CS are the immunological response of the fetus, gestational age (GA), stage of maternal syphilis, maternal treatment of syphilis, maternal age, history of genital ulcer and abnormal vaginal discharge (or in present pregnancy), genital itching, lower abdominal pain, multiple pregnancies, history of miscarriage/stillbirth, marital status/number of sexual partners, absence of antenatal care (ANC) and occupation[7,15,18,19].

A cardinal aspect of the control of the spread of syphilis, particularly in pregnant women, is the availability of accurate and efficient diagnostic modalities because prompt diagnosis is important for a better prognosis, lowering of transmission rates, minimization of the late-stage complications and adverse pregnancy/fetal outcomes[1, 3, 5, 21]. The diagnosis of syphilis (maternal and CS) is based on findings in the maternal's clinical history, physical examination (of mother and child), and laboratory investigations (of mother and child)[1,2,7]. Presently, the laboratory diagnosis of syphilis relies heavily on serologic evaluations for antibodies against T. pallidum using both non-Treponemal (NTTs) and Treponemal tests (TTs)[1,2,17]. The NTTs are the Veneral Disease Research Laboratory (VDRL), Rapid plasma reagin (RPR), and Toluidine red unheated serum (TRUST) tests[1,17,19]. These NTTs evaluate levels of anti-lipid immunoglobulin M or G (i.e., IgM or IgG) antibodies against lipoidal substances produced by infected/damaged host cells or T. pallidum cardiolipin/lecithin/cholesterol; thus, are usually used as first-line screening tests[1]. The NTTs, particularly VDRL, can be carried out cheaply, easily, rapidly, and effectively/efficiently either through titration techniques or immunochromatographic/rapid test strips and is thus optimal for syphilis screening of pregnant women (and their babies), even in HIV-syphilis co-infection scenarios, during their ANC and delivery/post-nataly,

particularly in SSA[1,22]. Likewise, the TTs are the Fluorescent treponemal antibody absorbed (FTA-ABS), T. pallidum particle agglutination (TPPA), T. pallidum hemagglutination assay (TPHA), Microhemagglutination assay for T. pallidum (MHA-TP), Enzyme immunoassays (EIA), Chemiluminescence immunoassay (CIA) tests, Chemiluminescence microparticle immunoassay (CMIA) tests[1,19,23]. These TTs directly detect antibodies against T. pallidum proteins with higher sensitivity and specificity; thus, are usually used as confirmatory tests[1]. The other diagnostic modalities for syphilis include Dark field microscopy (DFM); Direct fluorescent antibody staining for T. pallidum (DFA-TP); culture (namely: in vivo Rabbit infectivity test {RIT} and in vitro cell culture system {Sf1Ep}); morphological/tissue techniques (encompassing skin and placental histopathological, histochemical {with Warthin-Starry and Steiner silver stains} and immunohistochemical evaluations); and molecular biology or Nucleic acid amplification techniques (NAAT), like T. pallidum whole nucleotide emergence sequence which supports the use of polymerase chain reaction (PCR)[1,24]. Interestingly, maternal-derived treponemal and non-treponemal IgG antibodies can be transplacentally transferred to the fetus in-utero, thus confounding the serological screening for CS (which mostly presents asymptomatically at birth), hence underscoring the importance of histopathological evaluation of the placenta to confirm the diagnosis[1,7,25–28]. This forms the rationale for advocacy for use of the acronym SCORTCH (comprising of Syphilis, Cytomegalovirus, Others, Rubella virus, Toxoplasmosis, Chickenpox, and Herpes simplex virus), rather than TORCH, to give syphilis foremost attention, amongst the infections that cause congenital infections, with an encompassing broad-based diagnostic approach (including placental histopathological evaluation)[24]. Characteristically, the histopathological placental features of syphilis (i.e., syphilitic placentitis) are placentomegaly, amniotic fluid infection (AFI), umbilical lesions (particularly necrotizing funisitis), and disk lesions such as proliferative fetal vascular changes, endarteritis, perivasculitis of stem vessels, enlarged hypercellular villi (immature oedematous villi or relative villous immaturity) with/without intervillositis, acute villitis, chronic villitis (lymphoplasmacyticvillitis), avascular villi, intravillous hemosiderin, plasma cells in the basal decidua, and erythroblastosis (villi with increased fetal erythroblasts)[20,23,25–29]. Tragically, the commonest mechanism of death in CS is placental infection and reduced fetal blood supply[7].

Notably, parenteral benzathine penicillin G is the treatment of choice for syphilis (syphilotherapy) in pregnancy given its high effectiveness in lessening adverse pregnancy outcomes, though sometimes treatment failure occurs in HIV-syphilis co-infection[3,7,17]. Sometimes, this syphilotherapy may be complicated by the Jarich-Herxheimer reaction[4].

Regrettably, there are no studies on HIV-syphilis co-infection as well as associated histopathological placental studies in our environment to evaluate syphilitic placentitis as well as CS. Thus, in this survey, we aimed to compare the VDRL test results from the HIV-positive and HIV-negative pregnant women given their age, booking status, and gravidity, as well as to associate VDRL Test reactivity to the clinical stage of HIV/AIDS. Also, we aimed to evaluate the perinatal and placental histopathological findings in reactive VDRL test cases. Furthermore, we will evaluate our findings in light of existing literature.

METHODS

Study design

This study was a prospective cross-sectional hospital-based survey of the ANC venereal disease research laboratory (VDRL) tests done for two

study groups (namely the tests (cases) and controls) at the labor ward.

Study location

This study was conducted at a University Teaching Hospital's Departments of Obstetrics and Gynaecology (O&G) and Histopathology. This hospital is a 500-bed tertiary healthcare facility in the South-South region of Nigeria.

Sampling technique

A convenient sampling technique was used. In the labor ward, for six consecutive months, from December 2015 to May 2016, all consecutive HIV-positive pregnant women (as tests) along with one or two consecutive HIV-negative pregnant women (as controls) were included in this study.

Inclusion Criteria: All consenting HIV-positive (tests) and HIV-negative (controls) pregnant women, who came for delivery, within the study period.

Exclusion Criteria: All non-consenting HIV-positive and HIV-negative pregnant women within the study period.

Data collection

The case notes of all consenting pregnant women (tests or controls) were accessed by the investigators during the delivery period of each index subject. VDRL test data (and the placentas of reactive cases) and all other relevant data were recorded in a pro forma for each participant.

Handling of the placenta (for reactive cases)

Following the delivery of the placenta, its collection, fixation, grossing (macroscopy), Tissue processing, Embedding, Microtomy, Staining (with Haematoxylin and Eosin {H&E} staining), and Microscopy (with CX22 Olympus light

microscope) were performed following standard histological techniques, at the Histopathology laboratory, UUTH, and the placental-birthweight ratio (PBWR) will be calculated using Panti AA et al formulae (placental weight ÷ birthweight × 100%)[30–32].

Procedure for Syphilis (VDRL Test) and HIV screening at Antenatal Clinic of UUTH, Uyo: Blood samples were routinely collected from the study participants during their ANC at the antenatal clinic side laboratory (for booked clients) and the labor ward (for unbooked clients), of the O&G department, for testing (i.e., screening and confirmation). These blood samples (as whole blood) were applied on the FIRST RESPONSE® HIV 1+2/SYPHILIS Combo Card Test {by Premier Medical Corporation Private Limited, India} for screening, using a plastic dropper, and interpreted according to the manufacturer's instruction. Positive/reactive cases are confirmed using STANDARD Q® Rapid Test {by SD Biosensor, Republic of Korea} for HIV/Syphilis Combo or with Uni-Gold™ HIV {by Trinity Biotech PLC, Ireland} for HIV alone.

Ethical Approval

Ethical approval for this study was obtained from UUTH Health Research Ethics Committee (UUTH/AD/S/96/VOL.XII/115) as a part of a larger study on placental pathology in HIV-positive pregnant women. Patient confidentiality was protected, and informed consent was obtained.

Data Analysis

Data generated/collected in this study were written in a "Patient Case Report" form and transferred into Microsoft Office Excel 2013 and SPSS version 25.0 statistical software for statistical analysis. We used cross-tabulation and Pearson's chi-square test to test for statistical differences between the variables of both groups. Statistical significance was set at a P-value of ≤ 0.05. The results were reported as text, tables, graphs, gross photographs, and photomicrographs.

RESULTS

We surveyed a total of 145 pregnant women in this study (49 tests and 96 controls). The tests' age range, mean, median, and mode age were 21 - 38 years, 30.23 ± 4.32, 30, and 29 years respectively, while the controls' age range, mean, median, and mode age were 19 - 41 years, 29 ± 4.36, 28.5 and 28 years respectively (Table 1).

We found that 79.17% of the tests were aged less than 35 years while 84.21% of the controls were aged 35 years and above (Table 1). Also, 87.76% of the tests were booked for ANC while 94.79% of the controls were booked for ANC; this was not statistically significant, with a p-value of 0.77 (Table 1). Furthermore, 91.66% of the tests were multigravida while 77.65% of the controls were multigravida; this was not statistically significant, with a p-value of 0.061 (Table 1). Notably, 2.08% of the tests had a reactive VDRL test result while 0% of the controls had a reactive VDRL test result; this was not statistically significant, with a p-value of 0.162 (Table 1).

Table I: Frequency distribution table showing maternal age, booking status, gravidity, and VDRL Test among the tests and controls.

Status	Test group (n = 49)		Control group (n = 96)		Statistical significance	
Age group						
Status	Frequency	Percentage	Frequency	Percentage		
<35 years	38	79.17	80	84.21		
≥35 years	10	20.83	15	15.79		
Total	48	100	95	100		
Booking						
Status	Frequency	Percentage	Frequency	Percentage	Chi-square	P-value
Booked	43	87.76	91	94.79	2.291	0.770
Unbooked	6	12.24	5	5.21		
Total	49	100	96	100		
Gravidity						
Status	Frequency	Percentage	Frequency	Percentage	Chi-square	P-value
Primigravida	4	8.33	21	22.34	7.387	0.061
2 - 3	24	50	50	53.19		
4 - 5	16	33.33	16	17.02		
≥ 6	4	8.33	7	7.44		
Total	48	100	94	100		
VDRL Test						
Status	Frequency	Percentage	Frequency	Percentage	Chi-square	P-value
Reactive	1	2.08	0	0	1.951	0.162
Not reactive	47	97.92	93	100		
Total	48	100	93	100		

We found that the association of the VDRL test with maternal age, for both groups, was not statistically significant, with a p-value of 0.097 (Table 2). Also, the association of the VDRL test with booking status, for both groups, was not statistically significant, with a p-value of 0.770 (Table 2). Furthermore, the association of the VDRL test with gravidity, for both groups, was not statistically significant, with a p-value of 0.331 (Table 2). Also, we found that, for the test group alone, the reactivity of VDRL test was associated with only the clinical stage 2 of HIV (Figure 1).

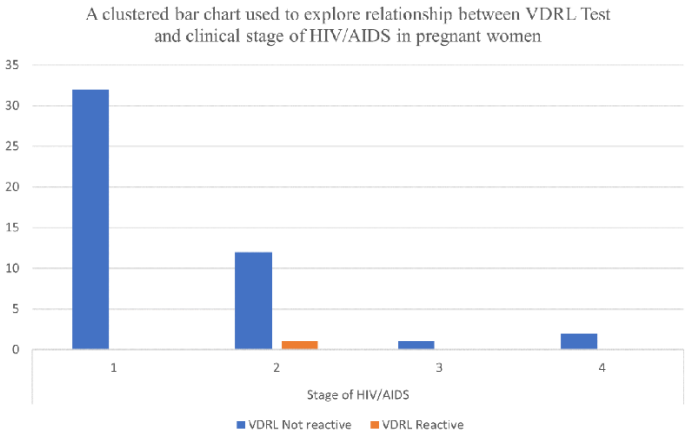

Figure 1: A clustered bar chart demonstrating the relationship between the clinical stage of HIV/AIDS and VDRL test outcome.

Table II: Frequency distribution table showing the association of the VDRL Test with maternal age, booking status, and gravidity for both the tests and controls.

VDRL Test association with maternal age				
VDRL Test	<35 years	≥35 years	Chi-Square	P-value
Reactive	0	1	4.673	0.097
Not reactive	115	24		
Total	115	25		
VDRL Test association with booking status				
VDRL Test	Booked	Unbooked	Chi-Square	P-value
Reactive	1	0	0.085	0.770
Not reactive	129	11		
Total	130	11		

VDRL Test association with gravidity						
VDRL Test	Primigravida	2 - 3	4 - 5	≥6	Chi-Square	P value
Reactive	0	0	1	0	3.574	0.311
Not reactive	25	74	30	11		
Total	25	74	31	11		

Importantly, the sole reactive VDRL test case was a 36-year-old HIV-positive married woman (in a monogamous relationship), who is a teacher with a Bachelor of Science {B.Sc.} degree, who

was receiving ANC at the O&G department of UUTH, Uyo, for her fourth pregnancy, being Gravida 4 Para zero, with three miscarriages/terminations of pregnancy, zero alive {G4P0+3(0A)}. Her last menstrual period (L.M.P) was the 2nd of March 2015 and her expected date of delivery (E.D.D) was the 9th of December 2015. Her HIV diagnosis is of 10 years duration with nine years of highly active antiretroviral therapy [HAART] and she was compliant with her treatment regimen. Her HAART comprises Tenofovir, Lamivudine, and Efavirenz combination. She booked for ante-natal care during the second trimester of pregnancy and her booking height, weight, and body mass index (BMI) were 1.74 meters (m), 67 kilograms (Kg), and 22.13 kg/m2 respectively. She had past obstetrics history of previous ante-natal admissions because of anemia in pregnancy and three previous first-trimester miscarriages/terminations of pregnancy. Her serial pack cell volume (PCV) in this pregnancy were 21% (on the 10th of November), 24% (on the 24th of November), 31% (on the 30th of November), and 30% (on the 8th of December) few days before her delivery. She was in clinical stage 2 of HIV/AIDS (as of December 2015): note that HIV/AIDS stage 2 patients are usually asymptomatic (i.e., in clinical latency or chronic HIV infection stage)[33] (Figure 1). Notably, the CD4 count was not done during this pregnancy. Notably, her VDRL tests, at booking, were reactive, both in the initial screening and confirmatory tests; consequently, she received syphilotherapy with parenteral benzathine penicillin. All other routine investigations, such as urinalysis, random blood glucose, liver function test, and abdominopelvic ultrasound scan, carried out did not show any abnormality. She had an uneventful pregnancy and eventually presented at the ante-natal ward for induction of labor at term due to the absence of initiation of spontaneous labor at term. Her 2.4 kilograms male baby was delivered through emergency cesarean section (secondary to failed induction in a retroviral disease patient) at an estimated gestational age (EGA) of 40 weeks plus one day, with an APGAR score of 4 at the first minute, 5 at five minutes and 5 at 10 minutes. Her baby had a head circumference (HC) of 31 cm (normal range of 33 to 35cm at term)[34], a chest circumference of 32 cm (normal range of 30 to 33cm at term)[34], and crown-heel length of 48 cm(normal range of 47.53 to 552.61cm at term)[34]. He was immediately placed on nevirapine syrup. However, the mother's case note did not indicate whether he was screened with the VDRL test, whether the diagnosis of CS was made, and/or if treatment was given.

Furthermore, her placental delivery was spontaneous and complete, with clear amniotic fluid (liquor). Subsequently, a histopathological evaluation of her placenta (fetal membrane, umbilical cord, and disk) was done. Grossly, her placental weight was 400 grams before fixation in 10% neutral buffered formalin (and 400 grams after 48 hours of fixation), giving a PBWR of 16.67% (with an assessment of large placenta with small for gestational age infant). The fetal membrane was complete, transparent, and greyish, with its point of rupture being 20 cm from its marginal insertion to the placental disk. The umbilical cord measured 11 cm in length, and 1.2 cm in diameter, having 3 patent blood vessels (visualized in the transverse section), focal hematoma, excess torsion, and eccentric insertion to the disk. The placental disk was discoid shaped, measuring 20.0 x 10.5 x 1.0 cm in its widest dimensions, having a transparent greyish white fetal surface, patent connecting vessels, and complete soft to firm reddish-brown maternal surface with about 50 ml of clotted blood diffusely adherent to it, and a cut surface that is spongy, reddish-brown, and interspersed by several septa-like whitish foci (Figure 2). Microscopically: the fetal membrane showed squamous metaplasia of amnion as well as subamniotic hematoma of the fetal membrane; the umbilical cord showed focal edema and

hematoma; the placental disk showed avascular villi, large hypercellular villi, villous vasculopathy (obliterative vasculopathy with onion-skinning pattern) and calcification of the disk (Figure 3, 4 and 5).

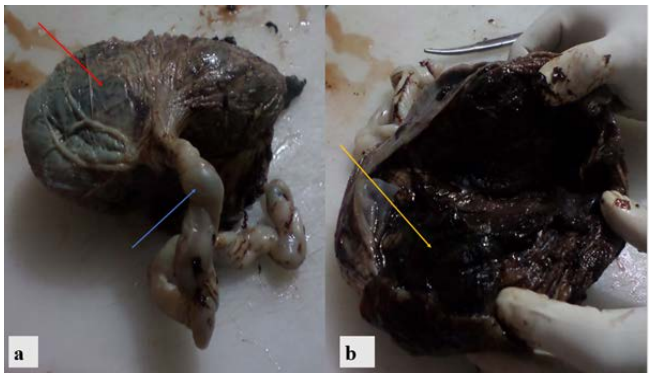

Figure 2: (**a–b**) Gross photographs of the placenta of the 36-year-old HIV-positive multigravida who had a reactive VDRL Test result. (**a**) This is the placenta, after 48 hours of fixation, displaying the fetal surface of the placental disk [red arrow] and the tortuous umbilical cord with focal hematomas [blue arrow]. (**b**) This is the maternal surface of the placenta with diffusely adherent blood clots [yellow arrow].

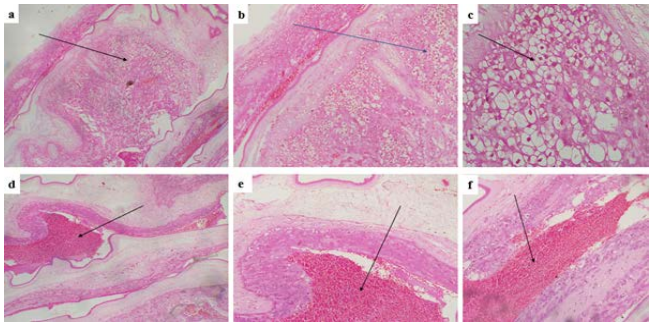

Figure 3: (**a–f**) Photomicrographs of the fetal membrane of the placenta of the 36-year-old HIV-positive multigravida who had a reactive VDRL Test result; with (**a**) to (c) displaying Squamous metaplasia of amnion and (**d**) to (**f**) displaying Subamniotic hematoma of fetal membrane. (**a**) Shows a focal area composed of sheets of squamous cells with clear cell changes [black arrow] within the amniotic layer of the fetal membrane [H&E stain, x 40]. (**b**) It also shows these sheets of squamous cells with clear cell changes [blue arrow] within the amniotic layer of the fetal membrane [H&E stain, x 100]. (**c**) It also shows these sheets of squamous cells with clear cell changes [black arrow] within the amniotic layer of the fetal membrane [H&E stain, x 400]. (**d**) Shows a focal accumulation of blood [black arrow] just beneath the amniotic layer of the fetal membrane [H&E stain, x 40]. (**e**) Also, shows a focal accumulation of blood [black arrow] just beneath the amniotic layer of the fetal membrane [H&E stain, x 100]. (**f**) Shows another area of focal accumulation of blood [black arrow] just beneath the amniotic layer of the fetal membrane [H&E stain, x 100].

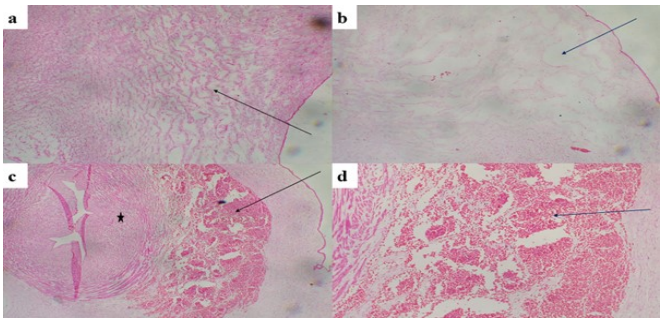

Figure 4: (**a-d**) Photomicrographs of the umbilical cord of the placenta of the 36-year-old HIV-positive multigravida who had a reactive VDRL Test result; (**a**) to (b) shows Umbilical edema and (**c**) to (**d**) shows Umbilical hematoma. (**a**) Shows a focal accumulation of fluid within the Wharton's jelly [black arrow] [H&E stain, x 40]. (**b**) Shows another area with a focal accumulation of fluid within the Wharton's jelly [blue arrow] [H&E stain, x 40]. (**c**) Shows a focal accumulation of blood within the Wharton's jelly [black arrow] just adjacent to a thick-walled umbilical artery [black star] [H&E stain, x 40].(**d**) Shows a focal accumulation of blood within the Wharton's jelly [blue arrow] just adjacent to a thick-walled umbilical artery [H&E stain, x 100].

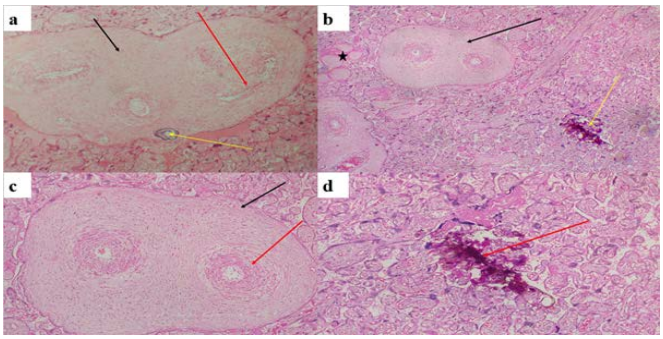

Figure 5: (**a-d**) Photomicrographs of the placental disk of the placenta of the 36-year-old HIV-positive multigravida who had a reactive VDRL Test result, showing some features of Syphilitic placentitis, namely: avascular villi, large hypercellular villi, villous vasculopathy (obliterative vasculopathy with onion-skinning pattern) and calcification of the disk. (**a**) Shows a large villous [black arrow] with three blood vessels with markedly diminished lumen due to villous vasculopathy [red arrow] as well as a focus of calcification [yellow arrow] [H&E stain, x 40]. (**b**) Shows another large villous [black arrow] with two blood vessels with markedly diminished lumen due to villous vasculopathy, a focus with three avascular villi [black star] as well as an adjacent focus of calcification [yellow arrow]

[H&E stain, x 40].(**c**) Shows the same large villous [black arrow] with two blood vessels with markedly diminished lumen due to villous vasculopathy [red arrow] [H&E stain, x 100].(**d**) Shows the same focus of calcification [red arrow] surrounded by numerous small-sized villi [H&E stain, x 100].

DISCUSSION

In this study, we aimed to compare the VDRL test results from the HIV-positive and HIV-negative pregnant women given their age, booking status, and gravidity, as well as to associate VDRL test reactivity to HIV/AIDS stage and to evaluate the perinatal/placental histopathological findings in the reactive VDRL test cases. In summary, we found that majority of the tests and controls were aged less than 35 years, booked for ANC, and multigravida. Also, the VDRL test results, for both the tests and controls, were not strongly associated with maternal age, booking for ANC, gravidity, and HIV/AIDS stage (in the tests only). Furthermore, we found that only one subject from the tests had a reactive VDRL test result, and historical data were extracted from her case note in addition to a histopathological examination of her placenta; these showed features consistent with CS and syphilitic placentitis.

We found that majority of the tests and controls were aged less than 35 years, accounting for 79.17% and 84.21% of the cases respectively. Similarly, the VDRL test results, for both the tests and controls, were not strongly associated with maternal age, with a p-value of 0.097. Notably, the study by Padovani C et al shows that most cases of maternal syphilis are aged less than 35 years[18].

Also, we found that majority of the tests and controls were booked for ANC, accounting for 87.76% and 94.79% of the cases respectively. This was not statistically significant, with a p-value of 0.77. Likewise, the VDRL test results, for both the tests and controls, were not strongly associated with booking for ANC, with a p-value of 0.770. Notably, studies showed that most cases of maternal syphilis are booked for ANC[11,18].

Furthermore, the VDRL test results, for both the tests and controls, were not strongly associated with gravidity, with a p-value of 0.331. However, the study by Padovani C et al has shown that maternal syphilis has been found more in multigravida women[18].

Interestingly, the VDRL test results for the tests were not strongly associated with HIV/AIDS stages, given that the only reactive case was in HIV/AIDS stage 2. However, this finding was not reported in the studies reviewed for this study.

Importantly, we found that the VDRL test results of the tests and controls were 2.08% (1/48) and 0% (0/93) of the cases respectively. This was not statistically significant, with a p-value of 0.162. This finding showed a low reactivity rate in our environment. This is consistent with the prevalence rate in AkwaIbom reported by Opone CA et. al[12]. Indeed, this finding is consistent with several studies on HIV-syphilis co-infection in pregnancy which found low prevalence rates[13–15].

Interestingly, historical data from the reactive VDRL subject's case note showed several vital obstetrical data.

She was 36-year-old at booking for ANC. This finding was not consistent with Duby J et. al., and Wahab AA et. al.'s case reports, with their patient's ages being 28, 29, and 21 years respectively[23,35]. Also, studies by Biadgo B et. al., and Padovani C et. al., found maternal syphilis more in those aged less than 35 years[15,18]. The reason for these differences is unknown.

She was in asymptomatic HIV/AIDS clinical stage 2. This finding was not reported in any of the studies reviewed.

She was booked for ANC. This is consistent with Duby J et. al., and Wahab AA et. al.'s case reports, whose patients booked for ANC[23,35]. Also, Yitbarek and Ayele, and Padovani C et. al., in their

studies found that most maternal syphilis cases were booked for ANC[11,18].

She is a multigravida. This is consistent with the study by Padovani C et al, which found that most maternal syphilis cases were multigravida[18]. Also, Duby J et. al, and Wahab AA et. al.'s case reports, had multigravida women[23,35].

She had a previous history of miscarriages {G4P0+3(0A)}. This finding is consistent with two studies that found a history of miscarriages in cases of maternal syphilis[18,35]. This reflects persistent maternal syphilis, given that miscarriage is a strongly associated adverse pregnancy outcome[7].

She had a normal BMI of 22.13 kg/m2 (normal range of 18.6 to 25.0)[36] at the booking. Notably, none of the studies reviewed for this study surveyed BMI in HIV-syphilis co-infection. This normal BMI is consistent with her being compliant with HAART.

She had an average anemic range third trimester PCV (normal range of 29.16 to 36.92)[37], this finding is consistent with the second case in Wahab AA et. al.'s study, which had mild anemia[35].

She received syphilotherapy once a maternal syphilis diagnosis was made. This finding is consistent with several studies[7,17,21,35].

She had an emergency cesarean section secondary to failed induction of labor at term. This finding is consistent with the study by Padovani C et. al., where 57.04% of 306 cases of maternal syphilis were delivered through cesarean section[18].

Notably, historical data, from the case note of the reactive VDRL subject, showed that the fetus had LBW, being 2.4 kilograms (normal range of ≥ 2.5 to 3.9)[34] at 40 weeks plus one day EGA. This finding is not consistent with other studies by Duby J et. al., with a birth weight of 1,710 grams at 31 weeks GA; Padovani C et. al., with ≥ 2,500 grams birthweight (86.30%) at ≥ 37 weeks GA (82.96%) in 306 cases of maternal syphilis; and Wahab AA et. al., case series with birthweights of 4 kilograms at 38 weeks GA and 1.48 kilograms at 31 weeks GA respectively[18,23,35]. These differences show the non-specific presentation of CS in terms of birth weight and GA, though most of these cases were preterm. However, De Santis M. et. al., in their study noted that though CS commonly presents asymptomatically, LBW may be the only notable feature most of the time[7].

Furthermore, the baby had intermediate APGAR scores of 4 at the first minute, 5 at five minutes, and 5 at 10 minutes (with APGAR scores at five minutes being categorized as normal {7-10}, intermediate {4-6}, and low {0-3} respectively), given its use in monitoring the baby's adjustments to extra-uterine life by measuring Appearance, Pulse, Grimace, Activity, and Respiration. Thus, implying intermediate adjustment of the index fetus to extrauterine life. This finding partially agrees with the finding in the case report by Duby J et al., who found a change in APGAR scores from low {1} to intermediate {5} to normal {7} at first, five, and 10 minutes respectively[23]. In contrast, Padovani C et al., in their survey of 306 maternal syphilis cases found that 98.88% of the babies have normal APGAR scores at five minutes {≥ 7}[18]. This difference could be explained by their larger sample size.

Notably, the fetal anthropometric measurement of 31cm HC (normal range of 33 to 35cm at

term)[34] shows that there is microcephaly. This finding was not reported by notable studies reviewed[4,20,35]. This could be either because anthropometric parameters in these studies were within normal ranges, hence considered non-essential, or they were not measured. Indeed, these fetal findings of LBW, intermediate APGAR scores, and microcephaly, though largely consistent with the asymptomatic presentation of CS, agree with the studies which found that majority of CS have asymptomatic presentation

while the symptomatic ones have subtle/non-specific presentation[7,26,35].

Notably, histopathological examination of the placenta from the reactive VDRL subject showed a net placental weight of 400 grams (average range of 385.36 to 765.66 grams) with PBWR of 16.67% (normal range of 20.59±3.92), however, an assessment of large placenta with small for gestational age infant was made because of the relatively big size of the placenta compared to the LBW. This assessment is consistent with some studies which report the occurrence of placentomegaly with LBW in CS[4,20].

Also, the fetal membrane displayed squamous metaplasia of amnion as well as subamniotic hematoma of the fetal membrane microscopically. Notably, studies reviewed did not report fetal membrane features in syphilis. Thus, this calls for further research to explore the range of placental fetal membrane lesions found in syphilis in pregnancy. However, it is of note that squamous metaplasia generally occurs as a sequela of chronic irritation/inflammation of an epithelial surface, in this case, syphilis may be the cause. Furthermore, the subamniotic hematoma could be because of complications (i.e., vascular rupture) of the vasculopathy caused by syphilis. Similarly, the amniotic fluid (liquor) of our study was clear, however, this is inconsistent with the finding of amniotic fluid infection by Kittipornpechdee N et al in their study[20]. The reason for this difference is unknown, hence needs more research.

Likewise, the umbilical cord showed focal hematoma and excess torsion, with focal edema and hematoma microscopically. These findings contrasted the findings in the majority of the studies reviewed wherein no pathology was found in the umbilical cord[23,25–27]. These focal hematomas and excess torsion could be a sign of excessive movement in-utero due to fetal distress secondary to the opportunistic infection inflammatory state fostered by the HIV-syphilis co-infection. Interestingly, most of these studies found necrotizing funisitis of the umbilical cord (secondary to T. pallidum infection of the umbilical cord artery) to be pathognomonic of CS, but this was absent in our study[27–29].

Importantly, our placental disk findings of avascular villi, large hypercellular villi, villous vasculopathy (obliterative vasculopathy with onion-skinning pattern), and calcification of the disk microscopically, are in agreement with most of the studies reviewed[20,23,25,26,28]. However, we did not find acute or chronic villitis, erythroblastosis, intervillositis, and mural thrombus in the chorionic plate vessel as variably reported in these studies[23,25,26,28]. The reason for this pattern could be that syphilis shows variable combinations of placental lesions per case. Notably, none of these studies reported calcification of disk, this could be because our case was an HIV-syphilis co-infection, in contradistinction to the studies reviewed, which have only syphilis infection. Thus, generally, the placental weight and disk features in our study are consistent with syphilitic placentitis, hence supporting a diagnosis of CS in our study[20,25,26,28].

The major limitation of this study is its small sample size; hence, we were unable to generate robust data that can be well extrapolated to the general population. Secondly, for histopathological evaluation of the placental tissue, we could not carry out histochemical (with silver stains) and immunohistochemical staining to identify T. pallidum. Thirdly, we could not carry out PCR studies on the placental tissue to identify T. pallidum. Fourthly, the trimester of ANC booking at which VDRL was done was not accessible. Fifthly, the VDRL test status, as well as the syphilotherapy history of the VDRL reactive case's baby and male partner (husband), was not accessible.

Finally, the next step for further research is to conduct a more robust multi-center HIV-syphilis co-infection in pregnancy screening survey (with NTTs and TTs) with fetoplacental association (using histopathological, histochemical,

immunohistochemical, and molecular biological studies) to generate more robust data that avail better extrapolation because of better control of HIV-syphilis co-infection in pregnancy (and its adverse outcomes).

CONCLUSIONS

In conclusion, the VDRL test (syphilis screening) done for HIV-positive and HIV-negative pregnant women during their ANC was found not to be strongly associated with their age, booking status, gravidity, and HIV/AIDS clinical stage. However, the only reactive VDRL case showed obstetrical, fetal, and placental histopathological features consistent with congenital syphilis and syphilitic placentitis. Thus, showing the critical role placental histopathological evaluation may play in the diagnosis of cases of maternal/congenital syphilis.

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Ethics Committee Approval: Ethical approval for this study was obtained from UUTH Health Research Ethics Committee (UUTH/AD/S/96/VOL.XII/115) as a part of a larger study on placental pathology in HIV-positive pregnant women. Patient confidentiality was protected, and informed consent was obtained.

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