

Screening for Syphilis among Third Trimester Pregnant Women in a Low Resource Setting: A Missed Opportunity?

ABSTRACT

Syphilis accounts for significant morbidity and mortality especially in the sub-Saharan Africa, where it remains a public health problem. Studies have demonstrated its deleterious effects on pregnancy and its association with HIV/AIDS. The aim of this study was to determine the prevalence of syphilis, its associated factors, and its relationship with HIV, among third trimester pregnant women in the Buea Health District (BHD), Cameroon. This was a cross-sectional study involving 250 third trimester pregnant women. Serum obtained from 5 ml of venous blood from each participant was tested for syphilis using VDRL antigen and *Treponema pallidum* hemagglutination assay (TPHA). The data on some socio-demographic, clinical characteristics and the results of tests were collected using a pretest questionnaire and analyzed using the statistical software Epi-info version 7. P-values less than 0.05 were considered statistically significant. The mean age of the participants was 26.9 (SD 5.2) years. The prevalence of syphilis in Buea Health District was 6.4%. Risk factors identified to have a significant association with syphilis were; ~~number of sex partners~~ ($p=0.02$) and ~~high level of education~~ ($p=0.02$). Of those infected with HIV, 26.3% were also infected with syphilis but this association was not statistically significant ($p=0.19$). The prevalence of syphilis in BHD is still quite high. Apart from screening during the first trimester, sensitization, screening, treatment and proper follow-up should also be encouraged in the third semester to prevent adverse foetal outcomes.

24 **Key words:** prevalence, syphilis, third trimester, associated factors, HIV,Buea

25 **1. INTRODUCTION**

26 There are estimated twelve million new cases of syphilis infection each year, quite an
27 alarming number for a curable disease.A quarter of these occur in the sub-Saharan Africa
28 (SSA) [1, 2].

29 Though syphilis was thought to be under control in many parts of the world, there has been a
30 rising incidence in recent years, more so in low and middle income countries(LMICs), where
31 it is a major cause for unfavorable pregnancy outcomes [3]. In the SSA, studies have
32 demonstrated very high prevalence of syphilis in pregnancy ranging from 3-20%, and 1-3%
33 congenital/neonatal syphilis [4-6]. Buve et al found that there were about 30,000 syphilis
34 related deaths in the year 2004[7, 8].

35 Amidst these high prevalence, it is worth noting that only less than 30% of pregnant women
36 in SSA are screened for syphilis despite the fact that it is highly recommended[3]. This has
37 resulted in poor perinatal outcomes like: spontaneous abortions, still births, congenital
38 syphilis, intrauterine growth restriction, serious neonatal infections or perinatal death[1, 2, 4,
39 9]. Furthermore, some studies have brought forth evidence of increase prevalence of syphilis
40 among those infected with HIV [10], with the prevalence of co-infection varying between
41 2.1-3.3% in Nigeria rendering the diagnosis and management of these conditions difficult
42 [11, 12].

43 In Cameroon, there is limited data on the epidemiology of syphilis, especially at regional
44 levels. In a bid to reduce the prevalence of the disease, screening for syphilis is recommended
45 at the first ANC (antenatal care) visit by the Ministry of Public Health and those who test
46 positive for syphilis are treated. The implementation of this policy still raises concern because
47 third trimester screening for syphilis is not done to diagnose cases of reinfection or those who

were not screened during early pregnancy. The aim of the study was to determine the prevalence of syphilis, its associated factors, and its relationship with HIV, among third trimester pregnant women with the goal of reducing morbidity and mortality associated with these infections in Buea Health District (BHD), Cameroon.

2. MATERIALS AND METHODS

2.1 Study Design

This was a hospital-based cross-sectional analytic study.

2.1.1 Study Setting

This study was carried out in three antenatal care centres: Buea Regional Hospital, Muea Integrated Health Centre and Prevention Maternelle Infantile (PMI) centre, These centres provide more than 80 % of ANC to clients in the BHD. Buea is the capital of the South West region of Cameroon. Participants were enrolled over a period of 12weeks, from December 15th, 2014 to March 15th, 2015.

Buea was chosen because it is rapidly becoming an urban setting, with a fast-growing population and increasing number of learning institutions which attract people across the nation. Furthermore studies on the prevalence of syphilis and HIV co-infection in pregnancy, to the best of our knowledge, have not been done in this region, though the regional HIV prevalence was higher than the national prevalence of 5.7% and 4.3% respectively [13].

2.1.3 Sample Size Calculation

The minimum sample size (n), was obtained from the formula that calculates the sample size needed when estimating the proportion of a study population with a particular attribute, which in this case, is the prevalence of syphilis among pregnant women in the third trimester as shown:

$$n = z^2(p)(q)/d^2$$

72 [14]. $z=1.96$ is the standard normal variate for 95% confidence; $p = 0.15$ pre-study estimate
73 for the prevalence of syphilis in the study population; $q = 1-p$; $d = 0.05$ which is the tolerated
74 sampling error. Using the prevalence of 15% [15], the minimum sample size was calculated
75 to be 195 participants; however 250 participants were enrolled to increase the validity of the
76 study.

77 **2.1.3 Study population and sampling**

78 After pregnant women attending ANC in the above-mentioned health facilities were
79 identified, those in the third trimester who gave written consent to participate in the study was
80 done by consecutive convenience sampling method. Pregnant women on treatment for
81 syphilis or antibiotics for another disease were excluded from the study.

82 Ethical clearance (N^o. 2014-08-0320) was obtained from the Institutional Review Board of
83 the Faculty of Health Sciences, University of Buea and administrative approval from the
84 Regional Delegate of Health, South West Region. During the period of enrollment, potential
85 participants were approached, assembled in a waiting area, informed of the study with its
86 aims and the expected procedures. The measures to ensure confidentiality were also
87 explained.

88 **2.2 Data collection and procedure**

89 Data was collected using an interviewer-administered pretested questionnaire. Information
90 was obtained on: socio-demographic characteristics and clinical data, risk factors for
91 infection with syphilis (such as level of education, number of sexual partners, marital status)
92 and past medical history. Each participant was privately assisted to complete the
93 questionnaire

94 Five milliliters of venous blood was collected from each consenting participant by a trained
95 laboratory technician using the standard procedure. The collected samples were separately

transferred into different plain test tubes and the samples were centrifuged where available or allowed to settle for 10 minutes so that the red blood cells could sediment.

Laboratory methods involved two separate analyses per given sample; one with

Venereal Disease Research laboratory (VDRL) (Omega Diagnostic laboratories, Hillfoots Business Village, Scotland) and another with *Treponema pallidum* hemagglutination assay (TPHA) (Reckon Diagnostic laboratories, Vadodara, India).

I. With the VDRL reagent, **50µl** of serum was collected from the test tube using a micropipette which was then placed onto a glass slide, against a white background, and **16µl** of VDRL antigen (reagent) was added onto the slide sample, stirred for 10minutes s and observed for flocculation. No visible reaction implied a negative result while flocculation implied a positive result.

II. TPHA strip was then used to conduct another test using the same sample collected from the participant. A dropper was used to collect a sample of serum from the test tube, and placed unto the test strip and a buffer was added. A single red line indicated a functional test strip but a negative result, while a double red line implied a positive result.

2.3 Data Management

All data was entered into Microsoft Excel spread sheet for subsequent analysis. Each participant's data was also entered into an Epi InfoTM version 7 data entry form to crosscheck for any errors.

Important variables that were measured were recorded in a tabular form and analyzed.

~~The prevalence of syphilis among third trimester pregnant women in BHD was calculated by simple proportion. it was calculated by dividing the number of those positive for syphilis~~

using each test by the total number of participants, multiplied by 100. (This paragraph is unnecessary)

To describe the relationship between the prevalence of syphilis among third trimester pregnant women with certain some socio-demographic characteristics, the predictor variables; age, marital status, level of education, use of condoms and number of sexual partners were considered and the outcome variable was a positive test for syphilis. These factors were first categorized into three the subgroups: age (11-20 years, 21-30 years and above 30 years), marital status (single or married), level of education (less educated i.e no formal education and primary, and educated), use of condoms (used consistently or did not use condoms), number of sexual partners (1 or >1 partners). In each subgroup the proportion of individuals positive for syphilis was then were calculated. The statistical significance of the differences in proportions was assessed for using the Chi-square test or Fisher's exact test were applicable. P-values <0.05 were considered statistically significant.

The relationship between HIV and the prevalence of syphilis in pregnancy was assessed as follows; the participants were grouped into those who were HIV negative and those who were HIV positive. The proportion of those positive for syphilis in both groups was calculated. The test for statistical significance was then assessed using the Chi-square or Fisher's exact test.

3. RESULTS

The prevalence of syphilis the third trimester of pregnancy in BHD was 6.4%. The mean age of the participants was 26.9 (SD 5.2) years. Risk factors associated with syphilis were: number of sexual partners ($p=0.02$) and high level of education ($p=0.02$). There was a 26.3% prevalence of syphilis and HIV co-infection.

Socio-demographic Characteristics of Study Population

Of the 250 participants, most 130 of them were married (52.0%), most had secondary education [150 (52.0 60.0%)] and those who were employed [skilled 95(38.0%) and unskilled 51(20.4%)] made up the majority of these participants. Table 1 summarizes other socio-demographic characteristics of the participants.

Table 1. Socio-demographic characteristics of 250 participants

FACTOR	FREQUENCY (N=250)	PERCENTAGE
Maternal age group (years)		
11-20	35	14.0%
21-30	165	66.0%
30+	50	20.0%
Occupation		
Skilled	95	38.0%
Unskilled	51	20.4%
Unemployed	104	41.6%
Level of education		
Tertiary	75	30.0%
secondary	130	52.0%
Primary	42	16.8%
None	3	1.2%
Marital status		
Married	158	63.2%
Single	92	36.8%
Region of Origin		
Southwest	94	37.6%
Northwest	128	51.2%

149	Others	28	11.2%
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Clinical characteristics of participants

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153 The gestational age (GA) ranged from 28 to 41 weeks. The mean GA was 32.2 ± 3.8 weeks.
 154 Table 2 shows that most, 113 (45.2%) of the participants were in the GA range of 28-
 155 30weeks. Also, most 128 (51.2%) had attended ANC more than twice. Knowledge of syphilis
 156 was divided into those who had heard about the disease and those who had not, which were
 157 170 (68.0%) and 80(32.0%) respectively. The former was then divided into those who knew
 158 how the infection could be acquired and those who did not; we had 142(83.93%) and
 159 28(16.07%) respectively. Of all the participants, 219(87.6%) had one partner, 126 (50.4%)
 160 occasionally used condoms during sexual intercourse and 245(98.0%) knew their HIV status
 161 of which 19 (7.8%) of them were HIV positive.

162 **Table 2. Clinical characteristics of 250 Participants**

163

FACTOR	FREQUENCY	PERCENTAGE
Gestational age(weeks)		
28-30	113	45.2%
31-33	40	16.0%
34-36	60	24.0%
37-39	26	10.4%
≥40	11	4.4%
N° of partners		
1	219	87.6%
>1	31	12.4%
ANC attendance		
Once	33	13.2%
Twice-thrice	89	35.6%
Four and above	128	51.2%
Use of condoms		
Used	126	50.4%
Did not use	124	49.6%

HIV status	N=245 in category	
Positive	19	7.8%
Negative	226	92.2%
Knowledge of syphilis		
Have not heard	80	32.0%
Have heard	170	68.0%
Know how it is acquired	142 of 170	83.9%

Prevalence of syphilis

As shown in Table 3. The prevalence of syphilis using both VDRL and TPHA was highest at 10.0% (5 of 50) in the age group >30years and least at 4.8% (8 of 165) in the age group 21-30years. It was highest at 8.4% (8 on 95) among those with skilled jobs and greater at 6.7% (14 of 205) among the educated participants. Prevalence was higher at 6.5% (6) among those who were single, 3.4% (3 of 89).

Table 3. Prevalence of syphilis-based socio-demographic characteristics

FACTOR	TOTAL(N=250)	TOTAL (%)	VDRL/TPHA POSITIVE	PERCENTAGE (%)
Maternal age group (years)				
11-20	35	14.0%	3	8.50%
21-30	165	66.0%	8	4.8%%
30 ⁺	50	20.0%	5	10.0%
Occupation				
Skilled	95	38.0%	8	8.4%
Unskilled	51	20.4%	2	4.4%
Unemployed	104	41.6%	6	5.7%
Marital Status				
Married	92	36.8%	6	6.5%
Single	158	63.2%	10	6.3%
Level of education				
Educated	205	82.0%	14	4.6 6.8%
Less educated	45	18.%	2	6.7 4.4%

Prevalence of syphilis based on clinical characteristics

The prevalence of syphilis was 8.75% (7 of 80) among those who had never heard of syphilis and 5.3% (9 on 170) among their counterparts. The prevalence of syphilis was highest at 9.1% (3 of 33) among those who attended ANC only once and least among those who had attended twice or thrice. The prevalence was highest at 8.3 (5 of 63) in GA group of 34-36years.

Using VDRL and TPHA combined, 14 of 219 (6.3%) and 2 of 31 (6.4%) tested positive among those with one partner and among those with more than one partner in the past year respectively. For those who used condoms, 4.7 4.8%(6) and those who did not use condoms, 8.4 8.9%(10) tested positive to both VDRL and TPHA. For both VDRL and TPHA, 26.3%(5) of the HIV positive participants and 4.8%(11) of those who were HIV negative tested positive. Using VDRL and TPHA combined, 14 of 219 (6.4%) and 2 of 31 (6.5%) tested positive among those with one sexual partner and among those with more than one partner in the past year respectively.

Table 4. Prevalence of syphilis based on clinical characteristics

FACTOR		TOTAL N=250	TOTAL (%)	VDRL/TPHA POSITIVE	VDRL/TPHA +VE (%)
Gestational age					
28-30		113	45.2%	7	6.1%
31-33		40	16.0%	3	7.5%
34-36		60	24.0%	5	8.3%
37-39		26	10.4%	1	3.8%
N° of partners					
1		219	87.6%	14	6.3 6.4%
>1		31	12.4%	2	6.4 6.5%
ANC attendance					

Once	33	13.2%	3	9.1%
Twice-thrice	89	34.4%	3	3.4%
≥ Fourth times	128	51.2%	10	7.8%
Use of condoms				
Use	126	50.4%	6	4.7 4.8%
Do not use	124	49.6%	10	8.1 8.9%
HIV status N^x=245				
Positive	19	7.8%	5	26.3%
Negative	226	92.2%	11	4.9%
Knowledge of syphilis				
Had knowledge	170	68.0%	9	5.3%
No knowledge	80	32.0%	7	8.75 8.8%

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190 **N^x=245 (Number of participants who consented to disclose their HIV status)**

191 **Factors Associated with syphilis**

192 Maternal age was divided into two categories: 11-25, and 26-40 years. The prevalence of
 193 syphilis using VDRL and TPHA tests were 6.9% and 5.9% respectively. The difference
 194 however, was not statistically significant with **p=0.70**.

195 The number of partners was grouped into those who had one and those who had more than
 196 one partner within the past year. Of the 250 participants, 219(87.5%) had one partner, while
 197 31(12.5%) had two or more partners. ~~Fourteen of 219 (6.4%) and 2 of 31 (6.5%) tested~~
 198 ~~positive for VDRL and TPHA ,among those with one partner and among those with more~~
 199 ~~than one partner in the past year respectively.~~ Fourteen of 219 (6.4%) women with one
 200 partner and two of 31 (6.5%) women with more than one partner tested positive for VDRL
 201 and TPHA. There was ~~a~~ no statistically difference between the groups in terms of
 202 positivity($X^2=0.14$; $p=0.695$).

For the level of education, the 250 participants were divided into the less educated (none and primary education) and the educated (secondary and above). Of the 250 participants, 18%(45) were less educated and 82.0%(205) were educated. With both tests, 4.4%(2 of 45) of the less educated and 6.8%(14 of 205) of the educated tested positive. There was **a** not statistically significant association with syphilis, **p=0.02** ($X^2=0.35$; $p=0.554$).

Marital status of the 250 participants was as follows: 63.2%(158) were married and 36.8%(92) were single. Of those who were single 6.5%(6) tested positive and 6.3%(10) of those who were married also tested positive . There was no statistically significant difference in the occurrence of syphilis between married and single women **p=0.29** and **p=0.60** respectively. ($p=0.952$).

Of the 250 participants , 50.4%(126) used condoms while 49.6%(124) did not. For those who used condoms, **4.7** 4.8% (6) and those who did not use condoms, **8.4** 8.9%(10) tested positive for syphilis with both tests. There was no significant statistical difference in the occurrence of syphilis between the two groups, **p=0.70** ($X^2=1.14$; $p=0.290$).

A total of 245 participants knew their HIV status, of this 19(7.79%) were positive. Screening using VDRL and TPHA, 26.3%(5) of the HIV positive and 4.8%(11) of HIV negative participants tested positive for syphilis respectively. There was **no** a significant **association** difference between the HIV positive and the negative groups in the occurrence of syphilis ($X^2=13.2$; $p<0.001$). ~~However, there was a statistically significant association between HIV and syphilis using the TPHA test; $p=0.004$ and $p<0.001$.~~ Those who were HIV positive were **1.5** 5 times more likely to be infected with syphilis compared to those who were HIV negative. **RR=1.5** for TPHA

Table 5 is unnecessary.

~~Table 5. Factors associated with syphilis in the third trimester of pregnancy~~

FACTOR	TOTAL (N=250)	TOTAL %	VDRL/TPHA POSITIVE	VDRL/TPHA +VE (%)	P-VALUE
Maternal age					
11-25	115	46%	8	6.9%	0.70
26-40	135	54%	8	5.9%	
N° of partners					
1	219	87.6%	14	6.3%	0.02
>1	31	12.4%	2	6.4%	
Level of education					
Less educated	45	18.0%	2	4.4%	0.02
Educated	205	82.0%	14	6.8%	
Marital status					
Single	92	36.8%	6	6.5%	0.6
Married	158	63.2%	10	6.3%	
Use of condoms					
Use	126	50.4%	6	4.7%	0.7
Do not use	124	49.6%	10	8.1%	
HIV status	N*=245				
Positive	19	7.6%	5	26.3%	0.19
Negative	225	92.4%	11	4.8%	

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228 N*: Number of participants who consented to disclose their HIV status.

229 4. DISCUSSION AND CONCLUSION

230 Prevalence of syphilis

231 The prevalence of syphilis among third trimester pregnant women at the BHD of 6.4% was
 232 high especially considering the fact that these women screened negative at the booking visit.
 233 However, 13.2% (33) presented for their booking visit in their third trimester. This

prevalence was higher than 2.9-5.0 % reported by some authors[16-18] and lower than 7.7-17% reported in other countries,[3, 6, 15, 19-23]. This discrepancy could be due to differences in the ~~geographical~~ geographical location, study population or study setting, bearing in mind that BHD is located in a semi-urban cosmopolitan setting with many young people who generally are more sexually active. Their presence in this locality is influenced by the university and several other tertiary learning institutions with the majority (66.0%) of the participants being in the age group of 21-30 years. Furthermore, some of these studies were carried out not only more than 15 years ago [6,15] but also in early pregnancy [6]. Combined with the effective screening methods for syphilis and treatment during pregnancy recommended by WHO to achieve the sustainable development goals, this decrease in the prevalence is understood.

Socio-demographic characteristics

Having more than one sexual partner and high level of education were significantly associated with syphilis in these individuals. This concurs with Araujo *et al* [19] who also found multiple partners to be associated with syphilis. Shimelis *et al* also reported that the level of education was associated with syphilis [24], however in their study they found it to be associated with a low educational level. This discrepancy could be due to the fact that a majority (82%) of our study participants were educated and most (66.0%) were in the sexually active age.

Clinical characteristics

Maternal age, marital status and use of condoms were not ~~found~~ statistically significant risk factors for syphilis in this study. The prevalence of HIV and syphilis co-infection in the study population was only 0.02%, although 26.3% of those with HIV had syphilis with a 95% CI:16.5%-32.3%. The difference in the prevalence of syphilis among participants with HIV

infection was quite high compared to those who were HIV negative, which was also reported by Msuya *et al* [21].

The prevalence of treponemal antibodies by TPHA test was 11.2%. These results are similar to, but slightly higher than the 10% prevalence Shahrook *et al* reported in Mongolia[20], but less than 13% recorded by Msuya *et al* [21]. Prevalence of syphilis based on TPHA antibodies are usually higher because there are not necessarily suggestive of active infections. That notwithstanding, there was a statistically significant association between HIV and syphilis using the TPHA test. This is not an unusual finding as documented by Kwiek *et al* [16] because syphilitic lesions increase the risk of HIV infections.

Study Limitations

The study highlighted the importance of screening for syphilis in the third trimester of pregnancy because it permits the detection of cases of reinfection and those who missed to be screened in early pregnancy. It however had some limitations. Being a cross-sectional hospital based study, it might not necessarily represent the prevalence of syphilis in the general population. This short coming was reduced by choosing high volume medical facilities that provided ANC to the majority clients in the Buea Health District.

The study revealed a high prevalence of syphilis in the third trimester, a very disturbing finding for a curable disease, hence not screening for syphilis during this period of gestation can be considered a missed opportunity. Syphilis was associated with the number of sexual partners and level of education. Therefore sensitization, screening, treatment and proper follow-up for syphilis even during third trimester of pregnancy should be encouraged to prevent adverse maternal and foetal outcomes.

CONSENT

281 As per international standard, patient's written consent was obtained and preserved by
282 principal investigator.

283 **ETHICAL APPROVAL**

284 Ethical clearance was obtained from the Institutional Review Board of the Faculty of Health
285 Sciences, University of Buea and administrative approval from the Regional Delegate of
286 Health, South West Region, Cameroon.

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