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DETERMINATION OF *T. vaginalis* AND OTHER ASSOCIATED STIs AMONG PREGNANT WOMEN IN ABA NORTH, ABIA STATE NIGERIA

VICTOR S. NJOM [a*] AND ANITA CHIKODI CHIBUKO [a]

[a] Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, P.M.B. 1660, Enugu, Nigeria.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Trichomoniasis is a global public health problem affecting human genitals especially women with severe complications in pregnancy. It is poorly reported. A cross sectional survey of pregnant women in Aba North local council, Abia State southeast Nigeria was undertaken using specimen microscopy and structured questionnaire. Of 286 women investigated the overall prevalence of *T. vaginalis* alone in the population was 2.1%. *T. vaginalis* disease proportion was associated with age ($p < 0.05$). Age range of 25-29 years had highest prevalence (4.8%) while 30-34 years had the least infection (1.05%). Pregnant mothers < 18 years and those within 35 years and above had no *T. vaginalis* infection. There was no association between trimester and disease proportion ($p > 0.05$). Of other STIs observed, *Candida* infection was highest (35.3%) while *Chlamydia* sp had the least prevalence (0.35%). Pregnant mothers within age of 25–29 years had more *Candida* infection (63.9%) than age group of 40 years and above. *E. coli*, Bacteria vaginosis, *Staph aureus*, and *Chlamydia* sp, had prevalences of 11.6%, 6.32%, 15.79%, and 1.05% respectively. Proportion of *T. vaginalis*, *Candida*, and *E. coli* infections in relation to age were statistically significant ($p < 0.05$). Proportions of observed STIs were significantly ($p<0.05$) associated with marital status. *Candida* sp and *Staph aureus* infections were more prevalent among traders and least among housewives. *T. vaginalis* and other STIs were not associated with level of education ($p>0.05$). There was significant ($p<0.05$) relationship between number of sex partners and sexually transmitted infections. Use of contraceptive such as condom was associated with proportion of infection ($p < 0.05$). *T. vaginalis* and other STIs are important public health threat in Aba hence routine screening and education of women about safe sex are highly recommended.

Keywords: Pregnancy; trimester; trichomoniasis; microscopy; Candida.

1. INTRODUCTION

Trichomoniasis, is one of the most common non-viral sexually transmitted disease worldwide [1]. It is caused by the flagellate protozoan parasite-*Trichomonas vaginalis* and humans are the only natural host for the parasite [2-4]. Trichomoniasis occurs in males and females (though males rarely exhibit any symptoms). The disease is characterized in female patients by frothy-greenish yellow foul smelling vaginal discharge accompanied with vulvo-vaginal irritation, dysuria and lower abdominal pains and a condition known as strawberry cervix, an inflammatory reaction that mimics the cervical

**Corresponding author: Email: victor.njom@esut.edu.ng, njomvic@yahoo.co.uk;*

tenderness associated with pelvic inflammatory disease (PID).

The pH of the vagina is a critical factor influencing the establishment and symptoms of the infection in women. If the normal acidity of the vagina is shifted from semi-acidic pH (3.8-4.2) to a much more basic form (5.0- 6.0) the vagina becomes conducive for *T. vaginalis* growth [5]. Globally, it is estimated that 5–10 % (120 million) of women in the general population suffers from trichomoniasis annually [6]. The rate of infection differs in women population [7] and it has been estimated that 10–50 % of *T. vaginalis* infections in women are asymptomatic, though asymptomatic infection is also considered a public health concern [8]. The prevalence of the disease varies in different countries, and the range of infection rate depends on health care, socio-economic and cultural status [9]. Despite the reported decrease of *T. vaginalis* infection in the last decades; the prevalence of infection in some underdeveloped countries is still high.

In sub-Saharan Africa, including Nigeria, trichomoniasis has neither been the focus of intensive study nor of active control programs and this neglect is likely a function of the relatively mild nature of the disease [5]. Several studies have suggested that pregnant women infected with *T. vaginalis* may be at increased risk of an adverse outcome such as premature rupture of membranes, premature labour, low birth weight, and post-abortion or post-hysterectomy infection and other complications [10]. Other effects of trichomoniasis and reproductive tract infection on pregnancy are preterm delivery, vertical transmission to new born baby and cervical neoplasia [11]. More recently, association of trichomoniasis in women with adverse outcomes of pregnancy and increased risk for human immunodeficiency virus (HIV) infection necessitated a need for increased control efforts. Little information is available regarding the burden and prevalence of *T. vaginalis* and other associated sexually transmitted infections among pregnant women in Aba North local government Area of Abia State, hence this current study intend to investigate the prevalence of *T. vaginalis* and other associated STIs among pregnant women attending antenatal clinic in selected health centers in Aba North, Abia State.

2. METHODS

2.1 Study Design

The study was a facility based cross sectional and descriptive study involving only pregnant women attending antenatal consultation and who had symptoms or not and who had not taken antibiotics treatment 14 days before specimen collection. Data collection was in two phases: 1) laboratory investigation to determine occurrence of *Trichomonas vaginalis* and other microbial fauna of the vagina. 2) A cross-sectional survey using a structured pretested questionnaire to collect socio-demographic information, number of previous pregnancy, miscarriages, previous STIs history, sexual history, STIs symptoms and others from the study subjects. Sample size was determined as was previously described by Oyeyemi et al (2015): Mean prevalence from similar previous studies across Nigeria at 12% ± 5 and 5% precisions was used to calculate the population size. A total of 286 pregnant mothers who consented to participate in the study were recruited. These pregnant mothers consulting at Aba general hospital constituted the study subject. Samples were collected from consented individuals for a period of two months (October 15th to December 14th 2021). Collected samples were taken to diagnostic laboratory Aba daily for analysis. Diagnostic technique was pre test to validate its suitability for the study. Pregnant women who tested positive to either of the STIs were counseled and referred for treatment.

2.2 Collection of Urine and Vaginal Secretion Samples

Those who accepted to participate were recruited and were thoroughly educated on how to use the two specimen collection instruments to collect urine and vaginal secretion respectively. The participants were educated on how to do a self swab collection by inserting the swab stick into the vagina with legs spread apart at about 2-2.5cm deep and stick rubbed on the walls of the vagina in a circular motion for a period of about 5-10 seconds after which the stick was put back into the swab stick tube and tightly covered to avoid contamination. Consented subjects were also issued with clean sterile universal urine capped bottles and told to pass mid-stream of their urine into the bottle. The urine bottle was collected and labeled with such information as name and laboratory number. The urine samples were there after transferred into an ice pack and conveyed to the laboratory for analysis. The swab sticks were also collected from the subjects and were labeled with subject name and lab number for identification. Precaution was taken to ensure that the lab number and name on urine bottle, swab stick and questionnaire for a particular participant were correctly maintained throughout the study period. Daily the swab sticks were taken to diagnostic laboratory for analysis.

2.3 Culture and Microscopy for Identification of *T. vaginalis* and Other STIs

A wet smear (wet mount) of each High Vaginal Secretion (HVS) sample, was made soon after arriving the laboratory, in a drop of 96% physiological saline on a clean glass slide and later covered with a cover slip and examined with X20 under a microscope. Observation of a quick jerky motion of the protozoa confirms the presence of T. vaginalis. Samples with T. vaginalis were recorded against the subject name and lab number. Oxoid *Trichomonas* broth medium enriched with sterile bovine serum was prepared as described by Shen et al (2021). The culture was incubated at 36^0C for 48hours and wet mount preparations from it was examined for the presence of *Trichomonas vaginalis* and for any other STIs.

For urine culture, the urine sample was centrifuged at 3000 rpm and thereafter, the sediment was inoculated on a CLED agar medium and then incubated at 37°C for a minimum period of 48hours. At the end of the incubation period, the culture was observed for possible growth for *T. vaginalis* and any other STIs. Samples with growth were recorded.

2.4 Data Analysis

Data obtained were subjected to analysis using SPSS for windows version 20. Prevalence was expressed as percentage. Group average was compared using a one way Analysis of Variance, while Chi-square was used to compare infection proportions and associations among the age groups and other variables such as trimester. Also proportional differences in response to questionnaire interview were tested using Fisher's test, while p value <0.05 was taken to be significant.

3. RESULTS

3.1 Distribution of *Trichomonas vaginalis* and Infections According to Age Group and Age of Pregnancy

The occurrence of *Trichomonas vaginalis* and other vaginal STIs in pregnant women was studied in Aba north LGA of Abia state. A total of 286 pregnant women consented and were recruited into the study and the results are hereby presented in tables. The over all prevalence of *T. vaginalis* alone in the study area was 2.1%. *Trichomonas* infection was age related. There was statistically significant association, ($p < 0.05$) between the disease prevalence and age groups. Pregnant mothers within age range of 25-29 years had highest prevalence of4.8% followed by 18-24 years (1.6%), 30 - 34 years (1.05%) respectively, while pregnant mothers less than 18 years in age and those within 35years and above had no infection, (Table 1). In terms of age of pregnancy, prevalence of *Trichomonas* was highest (2.68%) in mothers within the second trimester of their pregnancy while those in first and third trimesters had 2.63% and 1.02% respectively (Table 1). There was no significant association ($p > 0.05$) between trimester and proportion of infection despite the fact that pregnant mothers in their second trimester had more infection.

Table 2 showed the prevalence of *Trichomonas vaginalis* and other observed STIs according to age group and age of pregnancy. Apart from *T. vaginalis*, other observed microbes that are sexually transmitted include *Candida* sp, *E. coli*, Bacteria vaginosis, *Staphylococcus aureus*, *Chlamydia* sp, and *Neisseria gonorrhea* (Table 2). *Candida* infection had an over all prevalence of 35.3%, followed by *E. coli*, *Staph aureus*, Bacteria vaginosis, *Neisseria gonorrhea* and *Chlamydia* sp with over all prevalence of 15.4%, 12.9%, 2.8%, 1.7% and 0.35% respectively. Pregnant women within the age range < 18 years that participated in the study were all infected (100%) with *Candida* sp while mothers within 25 – 29 years had more *Candida* infection (63.9%)than other older women and was followed by mothers within age group of 30 – 34 years. Least *Candida* infection was observed amongst age group of 40 years and above. Similarly *E. coli*, Bacteria vaginosis, *Staph aureus*, and *Chlamydia* sp, had highest prevalence among pregnant women within 30 – 34 years of age with prevalence of 11.6%, 6.32%, 15.79%, and 1.05% respectively (Table 2). Proportion of *T. vaginalis*, *Candida*, and *E. coli* infections in relation to age group were statistically significant ($p < 0.05$) while Bacteria vaginosis, *Chlamydia* and gonorrhea infections were not associated with age group ($p > 0.05$). For age of pregnancy, those in the 2nd trimester had more *Candida* and *E coli* infections (48.2% and 18.8%), least microbial infection recorded was among pregnant mothers within their 3rd trimester. Highest gonorrhea infection was observed among women in their 1st trimester, while *Staph aureus* infection was detected more from women in their 3rd trimester.

3.2 Distribution of *T. vaginalis* and Other Vaginal ST Infections According to Marital Status

In Table 3, marriage status was considered in comparing the distribution of observed STIs. Proportion of observed STIs was significantly ($p < 0.05$) associated with marriage status. Over all, single mothers were more infected than married expectant mothers. Single mothers were more infected with *Candida* (69.2%) than the married mothers though their sample size were not uniform. Only two (0.73%)

of the married pregnant mothers were infected with *T. vaginalis*, while *E. coli*, *Staph. aureus*, Bacteria vaginosis, and *N. gonorrhea* infected 39 (14.3%), 32 (11.7%), 8 (2.9%), and 1 (1.8%) married pregnant mothers respectively. No married pregnant woman had *Chlamydia* infection. On the other hand 9 (69%) and 4 (30.77%) of single mothers were infected with Candida sp and *T. vaginalis* respectively. *E coli*, *Staph*, gonorrhea, and *Chlamydia* infections were detected in 5 (38.5%), 5 (38.5%), 4 (30.8%), and 1(7.70%), pregnant single mothers. Bacteria vaginosis was not detected in any of the pregnant single women.

Table 1. Distribution of *Trichomonas vaginalis* and infections according to age group and Age of pregnancy

Age group	No sampled	Number infected	Prevalence (%)	p value
<18	2	0	0.00	
18 – 24	62	1	1.61	
25 – 29	83	4	4.81	0.041[a]
30 – 34	95	1	1.05	
35 – 39	36	0	0.00	
40>	8	0	0.00	
Total	286	6	2.10	
Trimester				
1st	76	2	2.63	0.0910
2nd	112	3	2.68	
3rd	98	1	1.02	
Total	286	6	2.10	

**p value with superscript indicates significance at 95% confidence*

Table 2. Trichomonas infection and other STIs according to age group and age of pregnancy

	No	*T. vaginalis*	*Candida* sp	*E. coli*	Bacteria vaginosis	*Staph. aureus*	*Chlamydia*	*Gonorrhea*
Age range								
<18	2	0 (0.0%)	2(100%)	1(50%)	0 (0.0%)	1 (50%)	0 (0.0%)	1 (50%)
18-24	62	1 (1.61%)	16(25.8%)	21(33.9%)	0(0.0%)	6(9.7%)	0(0.0%)	3 (4.84%)
25-29	83	4 (4.81%)	53(63.9%)	9(10.4%)	0 (0.0%)	6(9.7%)	0 (0.0%)	1 (1.20%)
30-34	95	1 (1.61%)	22(23.2%)	11(11.6%)	6(6.32%)	15(15.79%)	1(1.05%)	0 (0.0%)
35-39	36	0 (0.0%)	7(19.4%)	2(5.61%)	0(0.0%)	7(19.4%)	0(0.0%)	0(0.0%)
40>	8	0 (0.0%)	1(12.5%)	0(0.0%)	2(25%)	2(25%)	0(0.0%)	0(0.0%)
p value		0.041	0.0001	0.0112	0.4002	0.0710	0.3210	0.3100
Over all	286	6 (2.1%)	101(35.3%)	44(15.4%)	8 (2.8%)	37(12.9%)	1(0.35%)	5(1.75%)
Trimester								
1st	76	2(2.6%)	19(25%)	14(18.4%)	0(0.0%)	7(9.2%)	0(0.0%)	3(3.94%)
2nd	112	3(2.7%)	54(48.2%)	21(18.8%)	2(1.9%)	11(9.8%)	1(0.89%)	2(1.79%)
3rd	98	1(1.0%)	28(28.6)	9(9.2%)	6(6.1%)	19(19.4%)	0(0.0%)	0(0.0%)
p value		0.0910	0.0001	0.0211	0.4111	0.0310	0.7100	0.0270
Over all	286	6 (2.1%)	101(35.3%)	44(15.4%)	8 (2.8%)	37 (12.9%)	1(0.35%)	5(1.75%)

Table 3. Distribution of *T. vaginalis* and other vaginal ST infections according to marital status

STIs (Lab results)	Marrital status	
	Married (n = 273)	Single (n =13)
Trichomonas vaginalis	2(0.73%)	4(30.77%)
Candida sp	92(33.7)	9(69.2%)
E. coli	39(14.3%)	5(38.5%)
Bacterial vaginosis	8(2.9%)	0(0.0%)
Staph aureus	32(11.7%)	5 (38.5%)
Chlamydia	0(0.0%)	1(7.70%)
Gonorrhea	1(1.8%)	4(30.8%)
p value	0.0001	0.0021

P<0.05 is significant

Table 4. Distribution of *T. vaginalis* and other vaginal STIs according to social status

Occupational Status	No Examined	*T. vaginalis*	*Candida* Sp	*E. coli*	Bacteria vaginosis	*Staph aureus*	*Chlamydia*	Gonorrhea
Civil servant	39	1(2.6%)	21(53.8%)	7(17.9%)	0(0.0%)	9(23.1%)	0(0.0%)	0(0.0%)
Trader	112	5(4.5%)	38(33.9%)	15(13.4%)	1(0.9%)	18(16.1)	1(0.89%)	1(0.9%)
Professional	28	0(0.0%)	13(46.4%)	2(7.1%)	4(14.3%)	3(10.7%)	0(0.0%)	1 (3.6%)
House wife	43	0(0.0%)	8(18.6%)	3(7.0%)	1(2.3%)	2(4.7%)	0(0.0%)	0(0.0%)
Others	64	0(0.0%)	21(32.8%)	17(26.6%)	2(3.1%)	5(7.8%)	0(0.0%)	3(4.7%)
p value		0.7721	0.0211[a]	0.0612	0.413	0.0221[b]	0.9700	0.0614
Total	286	6(2.1%)	101(35.3%)	44(15.4%)	8(2.8%)	37(12.9)	1(0.35%)	5(1.7%)

Table 5. Questionnaire responses on risk factors of sexually transmitted infections in pregnant women

Risk factors	Number interviewed	Number with STIs	Prevalence (%)	P value
Education				0.071
Primary	102	73	71.6	
Secondary	96	43	44.8	
Tertiary	88	26	29.5	
Sexual partners				0.003[b]
Single	278	180	64.7	
Multiple	8	8	100	
Condom use				0.0271[c]
Yes	96	21	21.9	
No	190	104	54.7	

3.3 Distribution of *T. vaginalis* and Other Vaginal STIs According to Social Status

With regard to association of *T. vaginalis* and other observed microbial infection with social status, 112 (39.2%) of the study participants were traders, while 43 (15.0%), 39 (13.64%), and 28 (9.8%) were housewives, civil servants, and professionals respectively (Table 4). Others that refused to clearly declare their occupational status made up the remaining 64 (22.38%). Proportion of *T. vaginalis* infection was not significantly related to occupation ($p > 0.05$). There was no association between *E. coli*, Bacteria vaginosis, *Chlamydia* and gonorrhea with occupation of the pregnant women except in candida and *Staph. aureus* infections where $p < 0.05$ (Table 4). *Candida* sp and *Staph aureus* infections were more prevalent among traders and least among housewives. *Chlamydia* was the least prevalent infection amongst the groups as only 1(0.89%) of the traders had the infection.

3.4 Distribution of *T. vaginalis* and Other Vaginal STIs According to Risk Factors

Analysis of participants' response to questionnaire indicated that there is no significant ($p > 0.05$) association between proportion of infection and level of education attained. Though pregnant women that attained primary education only, had highest prevalence (71.6%) of STIs followed by those whose highest education level was secondary school (44.8%) and least infection was amongst those who had tertiary level of education (29.5%) (Table 5). Pregnant women who responded that they had sex with multiple partners were all infected with one or more STIs indicating a prevalence of 100%. Majority of those who responded that they had sex with single partner were married women but despite that 64.7% of them had STIs. There was significant ($p < 0.05$) relationship between number of sex partners and sexually transmitted infections (Table 5). Use of contraceptive such as condom was associated with proportion of infection ($p < 0.05$). Those who agreed that they used condom had a lower prevalence (21.9%) of STIs when compared to those who said no to use of condom (54.7%).

4. DISCUSSION

Trichomonas vaginalis infection and other vaginal STIs of pregnant women were studied in Aba north Abia state and the result was quite revealing. The study observed a low prevalence of 2.1% for *Trichomonas vaginalis*. The 2.1% prevalence of *T. vaginalis* recorded in this study was lower than most previous reports in Nigeria. For example, prevalences of 4.7, 5.2, 11.0, 12.3 and 17.7% have been reported in Ilorin, Calabar, Maiduguri, Abakaliki and Uyo [12-16], respectively. The prevalence observed was also lower than that observedin Turkey (12.3%) [17] and the Australian aboriginal population (8.4%) [18], and also lower than the prevalences of 24.7%, 34.0% and 49.2% reported in Tanzania [19], Kenya [20] and South Africa [21], respectively. However, the result was similar to the prevalence of 2.4% recorded in

Tehran [22], The current prevalence despite been low still suggests that maternal *T. vaginalis* infection is an important public health threat in Aba and entire Nigerian urban populations. The observed prevalence of *T. vaginalis* could be attributed to many factors. Individual vaginal hygiene, public health education and other important health information given to pregnant mothers during antenatal clinics could be responsible for improved hygiene and sexual lifestyle, which possibly translated to the observed low prevalence. Despite the low prevalence of *T vaginalis* infection observed, the occurrence of the infection amongst the studied population in the present study once more flags the perception that the diseases has not received similar attention as other STIs such as HIV/AIDS, syphilis, gonorrhea, *Staphylococcus* etc hence the control measures has not been adequately implemented [23,24].

Our study also found that *T. vaginalis* infection was age related. This collaborated previous work [25] that reported association between *T. vaginalis* and age groups of studied pregnant women. The natural method of transmission of the disease is not fully understood but age is an important epidemiological factor. The disease is more prevalent in sexually active young women within the age of 20 – 35 years especially in rural and poor resource settings [26]. This study could not establish an association between age of pregnancy and proportion of infection ($p>0.05$) despite high prevalence of the disease amongst women within second and first trimesters. The current prevalence reported here agreed with the reports of [15,27] who observed that *T. vaginalis* were found more in women within first and second trimesters of their pregnancy but contradicts the reports of [26] which showed association between infection and gestation age indicating that *T. vaginalis* were found more in women at latter trimester of their pregnancy. Despite the general belief that sexual activities in women generally decline during pregnancy, most early-stage first trimester women who are pregnant for the first time may engage in more sexual acts than others in later trimesters because they are sometimes unaware of their pregnancy, resulting in the probable higher prevalence of *T. vaginalis* observed in women within first and second trimester. The high prevalence also observed in the early trimester women could have serious implications for pregnancy outcomes and neonatal health [26].

The transmission of *T. vaginalis* are favored by certain physiological changes that occur during pregnancy, including pelvic vascularity and oestrogenic activity on the vaginal epithelium which causes growth, maturation and exfoliation of the squamous cells and an increase in glycogen deposits in vaginal epithelial cells. The effects of which favour parasite multiplication and transmission. Preterm labour, low birth weight and increased rates of neonatal death are the major adverse effects of maternal trichomoniasis.

For other STIs observed: Chlamydia, and Bacteria vaginosis were low in infection proportion and were not associated with age of pregnancy. *Candida* sp was associated with age group and age of pregnancy. The result is in contrast to previous study that recorded increase in proportion of infection among young pregnant women.

The present study showed that, number of sexual partners, and condom use were risk factors for STIs while educational status was not associated with proportion of infection contrary to previous reports [6], which observed that they were risk factors for *T. vaginalis* transmission despite higher prevalence rates in those with lower level of education. Therefore, proper counseling and education on sexual behaviour and genital hygiene is necessary for control and prevention of *T. vaginalis* and other STIs during pregnancy.

Marital status and number of sexual partners were observed to be associated with *T vaginalis* and other sexually transmitted infections ($p<0.05$). Pregnant women who were married and maintain single sexual partners were less infected when compared with unmarried single mothers. This finding agrees with previous reports of [8,28,29] who in their separately observed that proportion of infection among sex workers were higher when compared with married subjects who affirmed that they had only one sexual partner. Number of male sexual partners is epidemiologically important because *T. vaginalis* is less common in men; it is readily passed between sexual partners during penile–vaginal sex, even when the infected partner is asymptomatic. The result supports previous knowledge that sexual promiscuity and unprotected sex are serious epidemiological factors in the transmission of *T vaginalis* and other STIs.

5. CONCLUSION

This study observed *T. vaginalis* infection in the studied population though the prevalence was low (2.1%). The study also showed that Candida sp, E. coli, Staphylococcus *aureus*, Bacteria vaginosis, *N. gonorrhae* and *Chlamydia*sp were other sexually transmitted infections (STIs) found in the studied population. *T. vaginalis* infection was age related and the disease was more prevalent in sexually active young women within the age of 25-29 years. This

study could not establish an association between age of pregnancy and proportion of infection ($p>0.05$) despite high prevalence of the disease amongst women within second and first trimesters. Marital status and number of sexual partners were observed to be associated with *T vaginalis* and other sexually transmitted infections ($p<0.05$). It was observed that number of sexual partners, and condom use were risk factors for STIs while educational status was not associated with proportion of infection.

It is therefore recommended that priority should be given to routine screening of pregnant women for *T. vaginalis* during antenatal consultations to avoid probable adverse effects on the foetus. Retesting for *T. vaginalis* is recommended for all sexually active women Concurrent treatment of all sex partners is critical for symptomatic relief, microbiologic cure, and prevention of ongoing transmission and reinfections. Emphasis should be placed on the education of women about safe sex and the need to know their partners' STIs status.

ETHICAL APPROVAL AND CONSENT

This study involved human subjects, therefore study protocol was reviewed and ethical clearance was obtained from the state Ministry of health, and Aba North local council health authority. The consent of the participants was sought after explaining the importance of the research to them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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