

CONCOMITANT MALARIAL INFECTION AND PATHOGENIC MICROBIOTA IN THE REPRODUCTIVE TRACT OF PREGNANT WOMEN IN ORLU, IMO STATE, NIGERIA

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Abstract – To determine the prevalence and types of concomitant malaria and bacterial/mycotic Reproductive Tract Infections within the study area. Cervico-vaginal swabs were collected from 200 participating women using dry cotton and Dacron by placing them in the vaginal cavity for 10 seconds, removed and immediately rolled onto a slide for diagnosis of BV, *C. trachomatis*, *N. gonorrhoea*, *T. vaginalis* and other RTIs by PCR. The swabs were stored at -20 °C for a few hours pending extraction which was conducted within 24 hours. For Malaria diagnosis and microscopy, thick blood films were stained using 10% Giemsa. A total of 18 attendees (9.05%) had Bacterial Vaginosis, 8 (4%) had Trichomoniasis while 8 (4%) and 4 (2%) had Gonorrhoea and Syphilis respectively. 82(41%) had malaria infection only while 96 (48%) had no malaria infection. 22(11%) had malaria and co-infection of reproductive tract infections (RTIs). There was no significant difference in concomitant malaria infections and RTIs between pregnant women and non pregnant ones. $P>0.001$. Pregnancy was found not to affect prevalence of Reproductive Tract Infections. The findings highlight the importance of an integrated approach to malaria and reproductive tract infections control during pregnancy.

INTRODUCTION

Malaria and Reproductive Tract Infections are both prevalent in most parts of sub-Saharan Africa. The concurrent occurrence of malaria and Reproductive Tract Infections is of vital public health importance especially in pregnant women.

Investigating women for RTIs during Ante Natal Clinic (ANC) visits and giving suitable care has been an issue of public health challenge, especially in developing countries. Because of limited resources, a significant burden of RTIs in pregnancy is not detected and treated (Petifor *et al.*, 2000).

A systematic appraisal of previous studies, reported considerable prevalence of malaria and RTIs in pregnant women attending antenatal clinics in sub-Saharan Africa. It also showed the dearth of data on coinfection of malaria and RTIs during pregnancy. (Chico *et al.*, 2012).

Studies of pregnant women in southern African countries have reported that 0-14.6% of pregnant women have syphilis; 0.5-18.6% have gonorrhoea and 1.7-17.5% have Chlamydia; while the prevalence of trichomoniasis and BV range between 2.4-32.2% and 4.3- 37.6% respectively.

(Latif *et al.*, 1999; Mayaud *et al.*, 1995) The prevalence of malaria and RTIs among the pregnant women identified was further reviewed for predictors of infections.

In some of the studies, risk factors for individual infections were found, whereas in others, predictors of infection were identified for different concomitance of infections e.g. Chlamydia and gonorrhoea; (Latif *et al.*, 1999). BV, trichomoniasis and candidiasis (Kurewa *et al.*, 2010); syphilis, trichomoniasis and gonorrhoea as well as gonorrhoea, syphilis and HIV (Hokororo *et al.*, 2015)

Risk factors for coinfection of RTIs and malaria

identified in some studies showed that syphilis was associated with the following factors: HIV infection, history of stillbirth and past RTIs, genital ulcers, vaginal discharge, genital warts and marital status (Potter *et al.*, 2006; Pham *et al.*, 2005).

Similarly, infection with trichomoniasis was associated with older age, lower education having more than one lifetime partner previous pregnancies and having a partner who takes alcohol (Yahya-Malima *et al.*, 2008; Swai *et al.*, 2006).

Gonorrhea infection was associated with having multiple sexual partners while its concurrent infection with Chlamydia was associated with having had a new sexual partner in the past three months, being separated from one's partner for up to 3 months, lower level of education, being single or in a polygamous marriage and having more than one sexual partner over the previous year (Mayaud *et al.*, 1995; Menendez *et al.*, 2010).

Comparing one area to another, location and/or type of settlement was found to be a predisposing factor for RTIs. For instance rural versus semi / urban (Manyahi *et al.*, 2011)

In a retrospective cohort study of 73 pregnant women syphilis, it was discovered that 34% had co infection of maternal malaria, 18% had placental malaria and 48% had malaria infection earlier in pregnancy.

The importance of RTIs treatment during pregnancy has been highlighted in past studies. (Gray *et al.*, 2001; McGregor *et al.*, 1990). In a clinical trial involving pregnant Kenyan women, those that received a single dose of ceftriaxone antibiotics between 28 and 32 weeks had a significant increase in mean birth weight of 3.21kg compared to 3.06kg for the ones that did not get (Temmerman *et al.*, 1995).

A cohort study in the US (Ryan *et al.*, 1990) found an increased incidence of Premature Rupture Of Membrane (PROM) in pregnant women with untreated Chlamydia compared to those who received treatment.

Another study in the US showed a decreased risk of PROM in successfully treated Chlamydia positive patients compared to patients who were treated but had either recurrent or persistent Chlamydia infection at the end of pregnancy (Cohen *et al.*, 1990).

In another study, treatment of syphilis was shown to be helpful, especially if carried out in the first trimester compared to when such treatment was delayed to the third trimester (Watson-Jines *et al.*, 2002).

Similarly, treatment of pregnant women with BV with clindamycin, metronidazole and erythromycin resulted to a lower incidence of PD and PROM (Hauth *et al.*, 1995).

In the light of the above findings, this research aims to investigate the prevalence and types of Reproductive Tract Infections RTIs in concomitance with malaria, a common infection among pregnant women in the study area and the imperative of an integrated approach to their control in pregnancy.

MATERIALS AND METHODS

Study Areas

This study was conducted in Orlu municipality, Imo State, South East Nigeria. It lies between coordinates 5° 45' 0" N 7° 7' 0" E, and is located in the rain forest belt of Nigeria. The ambience temperature is about 27 °C. The climatic condition is warm and humid with heavy rainfall distribution. The rainy season starts in April, reaches its peak in August and diminishes in November. The dry season starts in December and ends in March.

Orlu is the second largest city in Imo State in Nigeria, right in the heart of Igboland. It has an estimated population of about 100,000. Majority of the population are Christians and belong to the Igbo tribe. The town has a mixed economy of which civil service and trading are prominent.

Study design

This is a prospective group study of antenatal attendees and sick non pregnant women involving the collection of samples and screening of women for malaria and bacterial/mycotic reproductive Tract Infections (RTIs) (at first antenatal visit for pregnant women) during first visit at the selected hospitals within the study period.

Inclusion and exclusion criteria

Inclusion criteria

- Women of all ages, attending ANC for the first time (pregnant participants).
- Gestational age ≤ 32 weeks (for pregnant participants)
- Willing to take part in the study and give consent

Exclusion criteria

- Women reporting to have taken antimalarial and/or antibiotic treatment within the previous 4 weeks

- Women aged below 18 years without a guardian present to give written

Consent

Data and sample collection

One vaginal swab was collected from each participating woman by a trained midwife using a dry cotton swab by placing it in the vaginal cavity for 10 seconds and immediately rolled onto a slide for diagnosis of BV. A Dacron® (Medical Wire & Equipment, Wiltshire, England, United Kingdom) was used to collect a cervico-vaginal sample for the detection of *C. trachomatis*, *N. gonorrhoea* and *T. vaginalis* by PCR. The cervico-vaginal swabs for PCR were placed in cryo vials, labeled with each participant's ID and date of collection. The swabs from each participant was stored at -20° C for a few hours pending extraction which was conducted within 24 hours. Samples collected and investigated from sick non pregnant women served as control.

Malaria diagnosis by microscopy

Thick and thin method was used for malaria diagnosis. Parasite density was determined by assuming 8000 white blood cells (WBCs) per µl and counting the number of parasites per 200 WBCs.

Diagnosis of bacterial vaginosis by microscopy

The vaginal smear samples for the diagnosis of BV was air dried and Gram stained using safranin as a counter stain. Results was classified based on the Nugent criteria and recorded in the appropriate result record form.

Bacterial Inoculation, Isolation and Identification

All the urine specimen were subjected to cultures in order to search for reproductive tract pathogens. Blood agar, Cled agar, MacConkey, Deoxycholate Citrate Agar (DCA) and Nutrient Agar were used for urine specimen. The isolation of bacterial pathogens was done using streak technique to obtain discrete colonies while blood specimen were first inoculated into brain heart infusion broth, incubated for 24 hours before being transferred to solid media. The transfer was repeated every day for seven days before the growth was considered. The plates were incubated at 37 and 30 °C for 24 hours under aerobic conditions. To identify the organisms, the culture plates were examined and recorded for appearance, size, colour and morphology of colonies. Gram stain reaction and biochemical reactions like Catalase, Coagulase and Oxidase, Indole were also carried out on the isolates.

RESULTS

A total of 18 attendees (9.05%), out of the 200 that participated in the study had Bacterial Vaginosis out of which 6 had co-infections with malaria, 8 (4%) had Trichomoniasis out of which 4 had co-infections with malaria, while 8 (4%) and 4 (2%) had Gonorrhoea and Syphilis respectively, all of which had co-infection with malaria. 82(41%) had malaria infection only while 96 (48%) had no malaria infection. A total of 22(11%) of all the pregnant attendees had coinfection of malaria and reproductive tract infections (RTIs). Five

Table 1. Prevalence of concomitant Malaria and RTIs among pregnant ANC attendees and non pregnant ones in the study area.

Infection	Pregnant Attendees			Non pregnant Attendees			P value
	All (N=200) n (%)	Malaria- Uninfected (N= 96) n (%)	Malaria- infected (N= 104) n (%)	All (N=60) n (%)	Malaria- Uninfected (N= 6) n (%)	Malaria- infected (N= 54) n (%)	
Bacterial vaginosis	182 (91)	84(87.5)	98(94.23)	60 (100)	6(100)	54 (100)	0.00
Negative Positive	18 (9.0)	12 (12.50)	6 (5.77)	0 (.0)	0 (0)	0 (0)	
Trichomoniasis	192(96.0)	92 (95.83)	100(96.15)	60 (100)	6 (100)	54 (100)	0.00
Negative Positive	8 (4.0)	4 (4.17)	4 (3.85)	0 (0)	0 (0)	0 (0)	
Chlamydia	199(94.8)	95 (98.96)	104 (100.0)	60 (100)	6 (100)	54(100.0)	0.00
Negative Positive	1 (0.5)	1 (1.04)	0 (0)	0 (0)	0 (0)	0 (0)	
Gonorrhoea	188(94.9)	92 (95.83)	96 (92.31)	58(96.67)	6 (100)	52 (96.30)	
Negative Positive	12 (6.0)	4 (4.17)	8 (7.69)	2 (3.33)	0(0)	2 (3.70)	>.0001
Syphilis	196(98.0)	96 (100.0)	100 (96.15)	59(98.33)	6 (100.0)	53 (98.19)	
Negative Positive	4(2.0)	0 (0.0)	4 (3.85)	1(1.67)	0 (0.0)	1 (1.85)	>0.001

Reproductive Tract Infections were recorded namely Bacterial Vaginosis, Trichomoniasis, Candidiasis, Gonorrhoea and Syphilis.

Only 3 of the 60 non pregnant attendees had Reproductive Tract Infections, all of which had co-infections with malaria. Only two RTIs were recorded among the non-pregnant attendees namely Gonorrhoea 2 (3.33%) and syphilis 1 (1.67%).

There was significant difference in the prevalence of concomitant malaria and RTIs between the pregnant attendees and their non-pregnant counterparts. $P \geq 0$.

DISCUSSION

In this study, 8(4%) of all attendees had Trichomoniasis out of which 4 or 3.85% had coinfections with malaria. Similarly, out of the 18(9.0%) that had Bacterial Vaginosis, 6(5.7%) had coinfection with malaria. This is quite low when compared with the findings by Mullick *et al.*, 1993, that reported up to 40 % prevalence rate of Trichomoniasis and Bacterial Vaginosis in pregnant women in Africa.

Prevalence of Gonorrhoea and syphilis were 12(6%) and 4(2%) respectively while their rates of coinfections with malaria were 7.69% and 3.85% in that order.

Studies conducted by Chico *et al.*, 2012, between 1990 and 2011 reporting point prevalence estimates for malaria and RTIs among pregnant women in West and Central Africa reported the following prevalence: syphilis, 3.5%, gonorrhoeae, 2.7% Chlamydia 6.1%, Trichomoniasis 17.8%, Bacteria Vaginosis, 37.6%

For East and Southern Africa, the estimates were as follows: syphilis 4.5%, gonorrhoea 3.7%, Chlamydia 6.9%, Trichomoniasis 29.1 and BV 50.8%.

Malaria infection could be said to be the main reason for hospitalization of non pregnant women as the prevalence was high (90%), compared to their pregnant counterparts (52%). This could be due to the common practice of taking routine antimalarial drugs by pregnant women in the study area.

However, compared with the non pregnant women, prevalence of Reproductive Tract Infection was higher among the pregnant ones with the following prevalence: BV(9%), Trichomoniasis (4%), Chlamydia (0.5%), Gonorrhoea (6%) and Syphilis (2%).

Only Gonorrhoea (3.33%) and Syphilis (1.67%) where the Reproductive Tract Infections found

among the non pregnant women. The underlying reason for higher prevalence of RTIs among the pregnant women is subject of further research.

The findings of study provide evidence in support of Antimalarial and antibacterial drug combinations that may offer the added benefit of reducing the burden of curable Reproductive Tract Infections during pregnancy, especially in poor resource settings such as the study area where RTIs are prevalent but regular screening, detection and treatment may not be carried out during antenatal visits.

CONCLUSION

The prevalence of malaria, RTI and their coinfection at first ANC in this study population was considerable. There was significant difference in concomitant malaria infections and RTIs between pregnant women and non pregnant ones. However, Pregnancy was found to not affect the prevalence of Reproductive Tract Infections. $P > 0.001$

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Authors' contribution:

All authors made inputs and gave their approvals before submission of this research article. All the authors took active participation in the experiment. Chika Ezenwa played the lead role in writing the article with contributions from other authors.

Declaration of conflicting interest:

The authors declare no potential conflicts of interest concerning this research, authorship, and/or its publication.

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