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***Trichomonas vaginalis* Associated with Adverse Pregnancy Outcomes: Implications for Maternal Health Care Delivery System in South Eastern Nigeria**

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Authors' contributions

This work was carried out in collaboration between all authors. OEC designed and performed the laboratory study. IIR wrote the manuscript, ONC performed the statistical analysis, NO managed the literature review and OAE managed the literature search and typed the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Pregnant women infected with *Trichomonas vaginalis* are often untreated and evidence from the biology and epidemiology of the organism suggests that it may play an important role in causing adverse pregnancy outcomes. Using high vaginal swab from consenting pregnant women, a follow-up hospital based survey of *T. vaginalis* infection and its effect on pregnancy outcome among pregnant women attending antenatal clinics was conducted. Pregnancy outcome was determined on the basis of delivery before 37 weeks of gestation (according to general standards), low birth weight infants and neonatal conjunctivitis. The presence of *T. vaginalis* was re-examined at the point of delivery to exclude treated cases of infection during gestation period. Of the 688 pregnant women who gave birth within the study period, 89(12.9%) were positive for *T. vaginalis* with 31(19.38%) of the positive patients having pre-term delivery. Also, of the total of 89 infants from the infected mothers, 21(24.14%) had low birth weight of below 2.5 Kg, while 31(18.8%) had conjunctivitis. The result shows that the infection of *T. vaginalis* during

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pregnancy is significantly associated with adverse pregnancy outcomes. In view of the public health importance of this common sexually transmitted disease (STD), we advocate routine surveillance of trichomoniasis among pregnant women and prompt treatment of infected individuals and their sexual partners.

Keywords: *Trichomonas vaginalis*; maternal healthcare; neonatal conjunctivitis; birth weight.

1. INTRODUCTION

Trichomoniasis is a sexually transmitted disease with important public health ramifications and impacts upon birth outcomes. The causative agent, *Trichomonas vaginalis*, an ancient eukaryote and protozoan parasite, is known to be responsible for an estimated 180 million infections per year, making it the most prevalent non-viral sexually transmitted pathogen worldwide (WHO, 1995). More women than men are often infected and in both sexes, most infections are asymptomatic, with symptomatic and severe infections more common in women (Kriegar, 2000). The most common symptom among women diagnosed with *T. vaginalis* is vaginal discharge seen in more than 50% of cases (Swygard et al., 2004). Infection is highly associated with the presence of other Sexually Transmitted Diseases (STDs) such as gonorrhea, chlamydia and Human Immunodeficiency Virus (HIV) infections (Spinillo et al., 1997). Trichomoniasis can occur in females if the normal acidity of the vagina is shifted from a healthy, semi-acidic pH (3.8–4.2) to a more basic one (5–6) that is conducive to *T. vaginalis* growth (Schwebke and Hooke, 2003).

The pregnant woman infected with *T. vaginalis* may be at increased risk of an adverse birth outcome such as premature rupture of membranes, premature labour, low birth weight and post abortion or post hysterectomy infection, as well as infertility and enhanced predisposition to neoplastic transformation in cervical tissues (Cotch et al., 1997). *T. vaginalis* has been implicated in playing a critical and unrecognized role in amplifying HIV transmission as well as having a major impact on the epidemic dynamics of HIV in African – American countries (Sorvillo et al., 2005). It is estimated that 747 new HIV cases a year in pregnant women alone are as a result of the facilitative effects of *T. vaginalis* on the transmission of HIV with a significantly higher number of HIV RNA particles in the body fluid of women infected with *T. vaginalis* (Guenther et al., 2005). The treatment of trichomoniasis resulted in a 4.2 fold reduction in the number of HIV particles in vaginal specimens (FMHN, 2004).

Although trichomoniasis is a common worldwide infection, information on the occurrence of the infection in pregnant women and its adverse pregnancy outcome is narrow. In this report, we presented the findings of a hospital based follow-up study on pregnancy outcome of *T. vaginalis* infection. The public health significance of this result is discussed as it affects maternal health care delivery system and the prevention of infection among pregnant women in South Eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at Ebonyi State in South Eastern Nigeria. The inhabitant consists mainly of farmers, civil and public servants, as well as traders. Three different hospitals from three local government area of the state were used for the study namely: Ebonyi State University Teaching Hospital (EBSUTH) in Abakaliki L.G.A, Community Practice Centre Nweazenyi (CPCN) in Izzi L.G.A. and Holy Child Hospital and Maternity (HCHM) in Ebonyi L.G.A. The EBSUTH serves as a major referral centre for maternity clinics while CPCN and HCHM are located in rural areas of the state and majorly patronized by rural dwellers. The period of the study was from November 2006 to August 2008.

2.2 Study Population/Sampling Techniques

A total of 688 pregnant women within the age range of 15-40 years (mean age 26.16 ± 6.73 years) and gestational age of 14 – 36 weeks, with or without complaint of vaginal discharge and who were attending routine antenatal clinic at the hospitals, were enrolled in the present study. The participants were examined for *T. vaginalis* infection at registration and at onset of labour. Obstetric history of the women was used to exclude those with various gynecological problems. Only the women whose pregnancy have been confirmed and registered in the hospital were enrolled for the study. Data for neonatal conjunctivitis was obtained from the doctors after delivery and at 6 weeks post-natal visit. Approval to interact with and collect specimens from the pregnant patients was obtained from the ethical committee of the hospitals. Patient anonymity, good laboratory practice as well as confidentiality of result were assured. All laboratory activities were carried out according to human experimentation in clinical research. Prior to specimen collection, patients were counseled for STDs during pregnancy and assurance of treatment was made for all *T. vaginalis* positive participants. To avoid pregnancy complications or mishandling, the medical doctors collected vaginal swab specimens of pregnant patients in attendance. Specimens were analyzed within one hour of collection and results were made available to the clinics for proper counseling and treatment of infected women.

2.3 Laboratory Analysis

Wet mount preparation was made from vaginal swab samples and viewed with the light microscope to observe motile form of *T. vaginalis*. Positive samples were cultivated in selective medium - Cystein Peptone Liver Maltose Medium (CPLM) which was prepared according to the manufacturer's instruction (Suleyman et al., 2002). Culture was examination for growth at intervals of 24, 48 and 72 hours at a temperature of 37°C. Trichomoniasis was defined as *T. vaginalis* infection confirmed through colour change of medium from deep green to pale green.

2.4 Statistical Analysis

Statistical analyses were carried out and differences in proportion were evaluated using the Chi-square test. Significance for the inferential analytical techniques was set at 0.05 levels ($P < 0.05$).

3. RESULTS

A total of 89(12.9%) of the women were found to be infected out of the 688 that were examined. Majority of the pregnant women (403) were found to register for anti-natal clinics in their third trimester and a higher rate of *T. vaginalis* infection (24.1%) was also found to occur among these women in their third trimester (Table 1).

Of the infected women, 31(19.38%) had pre-term delivery, 44(12.8%) delivered at term, while 14(11.8%) had post-term delivery at the various hospitals used in the study (Table 2). Exactly 21(24.1%) of the 89 infants from the infected mothers had low birth weight of below 2.5 kg, while 31(18.85%) were infected with conjunctivitis. *T. vaginalis* infection was statistically found to be associated with preterm labour birth and low birth weight infants infected with conjunctivitis (Table 3).

4. DISCUSSION

Whereas several studies have been carried out on the prevalence of *T. vaginalis* infection among pregnant women in South Eastern part of Nigeria, this study presents a hospital based epidemiological study on the adverse pregnancy outcomes associated with *T. vaginalis* infection among pregnant women in Ebonyi State in South Eastern Nigeria.

The determination of the effects of *T. vaginalis* on pregnancy outcome showed that infection was found to be associated with low birth weight infants of below 2.5kg, pre-term labour as well as neonatal conjunctivitis. This is in accordance with the findings in a similar study carried out in Maryland USA which showed high level of *T. vaginalis* infection at mid-trimester associated with low birth weight infants, conjunctivitis and premature rupture of membranes (Cotch et al., 1997). In another study the highest level of infection was recorded at third-trimester and opined that it can lead to adverse pregnancy outcomes (Uneke et al., 2006). Several other studies observed that pregnant women infected with *T. vaginalis* were 30% more likely than the uninfected women to have a low birth weight infants infected with conjunctivitis (Afoke et al., 2005; Lynne et al., 2002; Okonkwo et al., 2010).

Table 1. Distribution of *T. vaginalis* with respect to terms of pregnancy at registration

| Terms of pregnancy (weeks) | Number examined at registration | Number positive at registration | Number positive at delivery | Percentage positive at delivery | Percentage positive at registration |
|----------------------------|---------------------------------|---------------------------------|-----------------------------|---------------------------------|-------------------------------------|
| 1-12 (1-3 months) | 50 | 11 | 6 | 12 | 22 |
| 13-24 (4-6 months) | 235 | 36 | 29 | 12.3 | 15.3 |
| 25-36 (7-9 months) | 403 | 97 | 54 | 13.4 | 24.1 |
| Total | 688 | 144 | 89 | 12.9 | 20.9 |

The distribution of *T. vaginalis* with respect to different terms of pregnancy showed that many pregnant women register for antinatal clinics in their third trimester. Health workers view this development as health negligence that could lead to higher incidence of maternal

and perinatal mortality and morbidity, as infections will not be detected earlier for proper treatment (Cotch et al., 1997).

Table 2. Distribution of *T. vaginalis* with respect to terms of delivery

| Terms of Delivery | Number examined | Number Positive at delivery | Percentage Positive |
|-------------------|-----------------|-----------------------------|---------------------|
| Pre-term | 160 | 31 | 19.38 |
| Normal | 378 | 44 | 11.64 |
| Post-term | 119 | 14 | 11.8 |
| Total | 688 | 89 | 12.9 |

Table 3. Distribution of *T. vaginalis* infection with respect to birth weight and neonatal conjunctivitis

| Parameter | Number of infants examined | Number of Infants from Mothers | Percentage Positive | Number of infants from treated mothers | Percentage of infants from treated mothers |
|-------------------------|----------------------------|--------------------------------|---------------------|--|--|
| Birth weight (kg) | | | | | |
| 3.6 above | 160 | 11 | 6.88 | 12 | 7.5 |
| 3.5 – 3.0 | 181 | 15 | 8.29 | 16 | 8.84 |
| 2.9 – 2.5 | 260 | 42 | 16.15 | 24 | 9.21 |
| 2.4 below | 87 | 21 | 24.14 | 3 | 3.43 |
| Total | 688 | 89 | 12.9 | 55 | 7.99 |
| Neonatal Conjunctivitis | | | | | |
| Infected | 165 | 31 | 18.8 | 3 | 1.82 |
| Non infected | 523 | 58 | 11.08 | 52 | 9.94 |
| Total | 688 | 89 | 12.9 | 55 | 7.99 |

The high percentage of pre-term delivery observed in this work among the infected women could be attributed to the fact that *T. vaginalis* is commonly found in the amniotic fluid and has been associated with intra amniotic fluid infection at term (Cudmore et al., 2004). The positive women recommended for treatment and who still tested positive at the point of delivery could be as a result of non compliance with treatment procedures. Treatment of pregnant women and their sexual partners have been advocated to check re-infection (Lynn et al., 2002; Caro-Paton et al., 1997). However, treatment for trichomoniasis which is commonly with metronidazole has been debated because the drug is known to cross the placenta and has been found to be mutagenic in bacteria and carcinogenic in mice, so that the possibility of teratogenicity has been a concern (Cudmore et al., 2004; Car-Paton et al., 1997; Lindmark and Muller, 2003).

Although *T. vaginalis* infection is one of the commonest causes of infection in the newborn and has been implicated as an important contributor to perinatal morbidity and mortality, attention given to the infection is minimal and epidemiological data on neonatal infections due to *T. vaginalis* with its associated risk factors remain inadequately studied (Lynne et al., 2002). Our study shows that a good number of infants were infected with conjunctivitis and

had low birth weights. This is in line with various studies which observed that pregnant women infected with *T. vaginalis* were 30% more likely than the uninfected women to have a low birth weight infants infected with conjunctivitis (Afoke et al., 2004; Lynne et al., 2002; Okonkwo et al., 2010). The infants were likely to have contracted the infection through vulvovaginal contact with the organism at the time of delivery, although some studies have hypothesized that the organism is ingested through meconium or feces (Lynne et al., 2002; Danesh et al., 1995).

Various other infections and conditions of pregnancy have been observed by various authors as causes of adverse pregnancy outcomes. Infections such as HIV, bacterial vaginitis among others have been reported as common causes of pre-term labour, low birth weight infants, neonatal conjunctivitis as well as increased number of stillbirths (Gnaore et al., 1989; Hiller et al., 1995). Also, environmental and nutritional factors as well as obstetric conditions could equally contribute to adverse pregnancy outcomes.

In conclusion, trichomoniasis should be stressed alongside other STDs during antenatal sessions. Also sex education should include the need for barrier protection and proper hygiene during pregnancy.

CONSENT

Consent of all patients used in this study was obtained orally before the study and upon publication they also gave their consent for our finding to be published.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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