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Rationale of Longitudinal Cohort Study on Obstetrical Outcomes of *Trichomonas vaginalis* Infection in Kinshasa, DR Congo

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Abstract

Background: Trichomoniasis is the most common non-viral sexually transmitted infection (STI) in the world, and the occurrence of this infection during pregnancy is responsible for adverse obstetrical outcomes like premature labor, premature rupture of membranes (PROM) and low birth weight (birth weight < 2500 g). The association with a number of factors (maternal age, low level of education, low socio-economic status and multiple sexual partners, etc.) that can be found in our environment suggest its probably high prevalence amongst vaginal infections that are responsible for adverse obstetrical outcomes, but up-to-date estimates are lacking. **Objective:** To assess the obstetrical risk associated with *Trichomonas vaginalis* (*T. vaginalis*) infection in our environment. **Methods:** We designed a protocol for a prospective cohort study which will take place in four medical facilities in the city of Kinshasa, where all pregnant women with a pregnancy of at least 20 weeks and who will give written consent will be included. Vaginal swab specimens will be collected for *T. vaginalis* research by direct microscopy wet mount. Follow-up will consist of recording the process of the pregnancy and obstetrical outcomes. **Conclusion:** Results from this study will allow to enhance management and also bring updated estimates on *T. vaginalis* prevalence and its obstetrical outcomes for infected pregnant woman in our environment.

Keywords

Trichomonas vaginalis Infection, Obstetrical Outcomes, Kinshasa

1. Introduction

Trichomoniasis is the most common non-viral sexually transmitted infection

(STI) in the world, caused by a protozoan called *Trichomonas vaginalis* (*T. vaginalis*) [1] [2] [3].

The extent of *T. vaginalis* infection has been reported in several publications, although some data remain underestimated and, as a result, the burden of *T. vaginalis* infection is not well determined. Recent data from the World Health Organisation (WHO) estimates that there were 156 million cases of *T. vaginalis* infection worldwide (giving a prevalence of 5.3%) in 2016, representing almost half the prevalence of STIs for that year [4].

The global prevalence of *T. vaginalis* infection in pregnant women varies according to geographical region, from 3.9% to 24.6%, with higher prevalence in low- and middle-income countries (*i.e.* Latin America and southern Africa) [5]-[10].

Diagnosis of *T. vaginalis* infection is made by microscopic examination of vaginal swabs to detect the parasite (direct or after staining) [11] [12]. Culture is done in a liquid medium and has the advantage of identifying mobile parasites [11] [12] [13]; it has long been considered the gold standard for detecting *Trichomonas vaginalis* [11] [12] [13] [14]. Treatment is based on imidazoles [3] [14].

T. vaginalis infection is common during pregnancy and is associated with fetal and neonatal morbidity. In fact, *T. vaginalis* infection is associated with obstetric complications such as premature rupture of membranes, premature delivery and low birth weight, as reported in numerous studies [2] [12] [14] [15] [16] [17]. These include the Cotch *et al.* study, which reported that mid-term *T. vaginalis* infection was significantly associated with low birth weight (OR, 1.3 [95% CI, 1.1 - 1.5]), preterm delivery (OR, 1.3 [95% CI, 1.1 - 1.4]) and low birth weight in preterm infants (OR, 1.4 [95% CI, 1.1 - 1.6]) [18]. Another study reported that infection with *T. vaginalis* during the first 8 months of pregnancy was associated with preterm birth (RR, 1.59 [95% CI, 1.18 - 2.14]) [19]. Perinatal transmission of trichomoniasis is rare, but treatment could prevent a respiratory or genital infection in the newborn [20] [21].

The occurrence of *T. vaginalis* infection during pregnancy is associated with a number of factors, including maternal age, low level of education, low socioeconomic status and multiple sexual partners [22]. A history of STIs and poor intimate hygiene have also been found to be associated with vaginal trichomoniasis [14].

The lack of evidence on the actual prevalence of *T. vaginalis* infection during pregnancy, which could be high, and the associated risk factors, could underestimate its extent and also the many obstetric complications associated with it in our environment. The main objective of this study is to take stock of *Trichomonas vaginalis* infection during pregnancy in our community.

2. Objectives

With the aim of assessing the obstetrical risk associated with *Trichomonas vagi-*

nalis infection in our environment, the present study set out to determine the prevalence of *T. vaginalis* infection among pregnant women in the city of Kinshasa in DR Congo, to identify its risk factors and complications, and also to assess the strength of the association between the risk factors identified and the occurrence of *T. vaginalis* infection, and between *T. vaginalis* infection and adverse obstetrical outcomes.

2.1. Rationale for the Study

The occurrence of numerous maternal and perinatal complications during pregnancy suggests the need to identify gravidic and pregravidic risk factors that can be acted upon to reduce the prevalence of these complications. There are, however, complications that arise without any obvious link to a given risk factor. This implies the existence of numerous risk factors whose potential link with these complications has not yet been established. *Trichomonas vaginalis* infection is one of the avenues to be explored, especially as it is known in the literature to be associated with numerous adverse obstetrical outcomes.

2.2. Design and Methods of the Study

2.2.1. Overview of the Study

This prospective cohort study will take place in four medical facilities in the city of Kinshasa, DR Congo, especially the University Clinics of Kinshasa (UCK), Elvic Saint-André Medical Center (ESA), the Centre Hospitalier Mère et Enfant Monkole (CHME) and the Centre Hospitalier de Kingasani (CHK).

Sampling will be convenience non-probability, based on a consecutive series of pregnant women followed up at antenatal care (ANC) in these four health facilities, and will take place over a period of 9 months.

The sample size will be calculated using the following formula [5] [23] [24]:

$$n \geq \frac{2\left(Z_{\alpha} + Z_{1-\beta}\right)^2 \times p(1-p)}{\left(p_0 - p_1\right)^2}$$

- n = minimum sample size.
- Z_{α} = 95% confidence coefficient (its value is 1.96).
- $Z_{1-\beta}$ = corresponds to the power of the test (1 − β); it is the probability of finding a significant difference (1.645).
- p = *T vaginalis* infection prevalence within pregnant women (6.8%).

$$p = \frac{p_0 + p_1}{2}$$

- α = error risk of type I (0.05).
- p_0 = expected proportion of pregnant women not diagnosed with *T. vaginalis* infection who will present adverse obstetrical outcomes.
- p_1 = expected proportion of pregnant women diagnosed with *T. vaginalis* infection. This proportion p_1 is estimated by the investigator on the basis of the estimated value of p_0 and the size of the difference between p_0 and p_1 that he

thinks it is possible and desirable to demonstrate.

- Considering that $p_1 = RR \times p_0$ (with RR as relative risk = 2) and that $p = \frac{p_0 + p_1}{2}$ Then $p_0 = 0.091$ and $p_1 = 0.181$ (on statistic tables).

After incorporating these elements into the formula, the minimum size of our sample is 203, but taking into account those lost to follow-up, abandoned or unsatisfactory data, we have increased it to 250 pregnant women, which will be included in the study. This study will be carried out using our own funds and will not receive any financial support.

2.2.2. Patients' Selection

1) Inclusion criteria

Our study will include.

- Pregnant women at 20 weeks or more, who will attend ANC, and, will give birth in the chosen sites and during the study period;
- Pregnant women who agree to participate freely in the study.

2) Exclusion criteria

Pregnant women recruited who did not give birth at one of the four sites chosen for this study (lost to follow-up), as well as those who will refuse to continue their participation (drop-out), will be excluded.

2.2.3. Variables of the Study and Subjects Follow-Up

- **socio-demographic variables:** age, level of education, marital status, lifetime sexual partners, socio-economic level (defined by the patient's possession score according to the Adjusted Poverty Index or API, which takes in account living standards and goods possession, whose a scoring system allow classification to 4 levels of wealth/poverty), occupation;
- **clinical/paraclinical variables:** parity, gravidity, history of abortion and perinatal/infant death, alcohol and/or tobacco consumption, history of diabetes, HIV and STIs, low birth weight, stillbirth, prematurity, premature rupture of membranes, sexual partner(s)' infection, Body Mass Index (BMI), results of the analysis of vaginal secretions (swab collected before and after treatment if applicable) carried out within 5 minutes of sampling, by direct examination with binocular microscope Olympus XSZ-107BN;
- **pregnancy parameters:** gestational age (at recruitment and delivery), complications during pregnancy;
- **delivery variables:** route of delivery and indication for caesarean section (if applicable), sex, Apgar score, birth weight, height, head circumference, admission in neonatology intensive care unit, neonatal pathologies (neonatal infection, neonatal jaundice, respiratory distress, etc.), other adverse obstetrical outcomes in terms of preterm labor and premature labor (<37 weeks of gestation), premature rupture of membranes (PROM) and intra-uterine growth restriction [IUGR] (based on local charts as a birth weight less than the 10th percentile for the gestational age group) and low birth weight (birth

weight < 2500 g), stillbirth (defined as death of a fetus after 22 weeks of gestation), perinatal mortality (deaths including stillbirths and neonatal deaths before 7 days of life).

2.3. Data Collection Procedure

Pregnant women will be recruited during ANC, following counselling in which they will be told about the purpose and procedure of the study, and will be asked to sign a written consent form when they agree to be part of the study. They will therein, find the opportunity to ask questions and require more detailed information on genital infections and care. All pregnant women with a pregnancy of at least 20 weeks will be included (given that the risk of adverse obstetrical outcomes is significant when the infection occurs in mid-term pregnancy and beyond). Socio-demographic and clinical data will be collected during the consultation, that will end by the vaginal swab collection.

Wet mount smears will be prepared in saline and examined immediately after sampling for identification of *T. vaginalis* under direct microscopy for diagnosis, based on morphological characteristics of trophozoites forms, with their characteristic appearance and motility.

The information gathered will be recorded on pre-established data collection forms. Information relating to the progress and outcome of the pregnancy will then be collected from medical records.

2.4. Expected Study Outcomes

At the end of the present study, the prevalence of *T. vaginalis* infection during pregnancy in our environment will be determined; the sociodemographic and clinical characteristics of pregnant women with *T. vaginalis* infection will be described; the risk factors and complications of *T. vaginalis* infection in this population of pregnant women will be identified and their extent determined; and finally, the strength of the association between, on the one hand, the risk factors identified and the occurrence of *T. vaginalis* infection and, on the other hand, between *T. vaginalis* infection and the adverse obstetrical outcomes, will be established.

2.5. Statistical Considerations

Data will be entered using Microsoft Excel 2013 software and exported to SPSS 24.0 for analysis. For normally distributed parametric data, comparisons of averages are to be made using t-test or ANOVA and comparison of proportions with chi-square test. Correlation between diagnostic of *T. vaginalis* and adverse pregnancy outcomes is planned. Odds ratio will be used to measure strength of association between identified risk factors and *T. vaginalis* infection, and between *T. vaginalis* infection and adverse obstetrical outcomes. Multivariate logistic regression analysis will identify independent determinants of adverse obstetrical outcomes. Testing will be stated significant for p-value ≤ 0.05. All analysis will be generated automatically from a statistical software.

2.6. Ethical Considerations

This research has received the favorable opinion of the staff of the Department of Gynecology and Obstetrics of the University Clinics of Kinshasa, followed by authorization from the Ethics Committee of the School of Public Health of the University of Kinshasa (Approval number: ESP/CE/14/2023). The vaginal swab will be collected at recruitment, and in cases of documented infection, a second control swab will be taken two to four weeks after treatment. Written informed consent [translated into the local language] will be required from each pregnant woman prior to enrolment in the study. The data collected will be treated in strict confidentiality and anonymity.

3. Discussion

T. vaginalis is incriminated as the leading agent of non-viral STI around the world [2] [3]. Besides, trichomoniasis is a neglected parasitic infection demanding more attention to be implemented by health authorities [2] [25] [26]. In our context, we do not know what is the burden of *T. vaginalis* in general population, and only few studies have evaluated its outcomes in pregnancy [27], as several studies for *T. vaginalis* infection has been implemented within specific populations, as sex workers and HIV patients [28] [29]. Trichomoniasis is frequently found in women of reproductive age and also in women suffering from pregnancy-related complications [2] [16] [17]. Nateghi *et al.* found out that the risk of having low birth weight was increased about 43-fold (p = 0.00; OR 43.29; 95% CI 2.79671.98), the risk of PROM about 21-fold (p = 0.00; OR 21.75; 95% CI 4.12 - 136.95) and the risk of abortion about 91-fold (p = 0.00; OR 91.84; 95% CI 15.51 - 544.23) in women with *T. vaginalis* compared to those without *T. vaginalis* infection and the most established association is with preterm delivery [2].

As for Sutton *et al.*, in his study comparing pregnant human immunodeficiency virus-infected and human immunodeficiency virus-uninfected Congolese women, the risk of low birth weight was significantly associated with *T. vaginalis* infection [27] and they wished if further studies could assess this risk before delivery as they took the swab 1 to 2 days after delivery. Our study could be an answer to that, as we will perform vaginal swab from 20 weeks to term pregnancies, and all that before delivery.

The most common method for diagnosing trichomoniasis may be microscopic evaluation of genital secretions ("wet mount"), due to convenience and relatively low cost [1]. Raising concern is about the sensitivity of wet mount method which is estimated to be about 60% to 70% which falls to as low as 35% within half an hour after specimen collection [2] but, as for this study from Nateghi *et al.* [2], when cautiously performed, wet mount can be as detective as culture, and when possible, it is much better to have a combination of the two or even more exams to detect the maximum cases of TV infection.

Discussions raised about screening as a way to tackle negligence towards this infection of significant public health concern, but it was admitted that screening

should be performed for only HIV pregnant women and for other pregnant women, screening may be considered at the discretion of the treating clinician, as the benefit of routine screening [for pregnant women] has not been established [10] [25] [26].

Strengths of the Study

- It will offer an update of the estimates about *T. vaginalis*;
- Genital swab collection is proceed and analysed directly.

4. Limitations

The major weaknesses of our study are:

- It will not be possible to state the true prevalence of *T. vaginalis infection*;
- Some cases may not be diagnosed, due to the fact that we will use only one detection method, the direct microscopy wet mount (due to financial constraints).

5. Conclusion

At the end of this study, knowing the prevalence and risk factors of *T. vaginalis* infection in our environment will allow to enhance management, prevent likely adverse obstetrical outcomes and provide with updated estimates. Further studies can be designed to have a combination of detecting methods and used to a greater sample of pregnant women to reinforce evidence and also, they will help in designing studies addressing vaginal microbiome and infections as they impact obstetrical outcomes and reproductive health.

Authors' Contributions

MMA and LBJ are the principal investigators. MMA generated and designed the study. LBJ participated in the study design and will be actively involved in data collection. MMA, LBJ, LAJ, MNF, NOC, KNB, LMEP, LNN and MFM contributed to the drafting and improvement of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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