Title: The diagnosis and treatment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pregnant women to prevent adverse neonatal consequences.
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Statistical Plan: Evaluating the diagnosis and treatment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pregnant women to prevent adverse neonatal consequences in Gaborone, Botswana

ANALYSIS

Primary outcome measure

- Mother-to-child transmission of CT and/or NG: Neonates of mothers who test positive for CT and/or NG infections will be tested using both ocular and nasopharyngeal specimens.

Secondary outcome measures

- Pregnancy outcome
  - Miscarriage, defined as pregnancy loss before the 20th week of gestation, will be identified through self-report by the participants, and the medical record.
- Perinatal outcome
  - Stillbirth, defined as fetal death at or after 20 weeks gestation and prior to delivery, will be identified through self-report by the participants, and the medical record.
- Neonatal outcomes
  - Preterm birth, defined as delivery prior to 37 weeks' gestation, will be identified through the obstetric record and/or
  - Low birth weight, defined as birth weight of less than 2500g, will be identified through the obstetric record.
  - Conjunctivitis, defined as the presence of swollen eyelids and pus in eyes, will be identified during the care visit by the follow-up nurse/midwife.
  - Pneumonia, defined as cough or difficult breathing plus at least one of the following: Fast breathing, Lower chest wall indrawing. In addition, either crackles or pleural rub may be present on chest auscultation.
  - We will also ask mothers about prior neonatal symptoms and review the obstetric record.
- Maternal outcomes
  - CT and NG infections: The prevalence of maternal CT/NG infections will be assessed among participants in the testing group at baseline and during the third trimester.
  - The incidence of infections between baseline and follow-up in the testing group.
  - Individual cytokines and groups of cytokines associated with mother-to-child transmission of CT/NG, preterm birth, and low birth weight.
- Economic outcomes
  - The total cost of the CT/NG test and treatment intervention for asymptomatic pregnant women.
  - The costs per woman tested, treated, and cured; and the incremental costs per adverse neonatal outcome averted (e.g. neonatal CT/NG infections, preterm birth, and low birth weight) comparing the testing intervention with syndromic management.

Statistical analysis

Prevalence of each STI will be measured as the proportion of individuals testing positive for a given STI divided by the number of individuals tested at each time point. Exact binomial 95% confidence intervals for prevalence will be estimated. Incident infections will be identified when a participant tests negative for an STI during her first ANC visit and positive for an STI during follow-up visits. Time at risk will be the length of time between the first test date and the positive test date. Correlates of CT/NG infections will be identified using both bivariate analyses and multivariate analyses.

Our primary outcome (mother-to-child transmission of CT and NG) and secondary outcomes (composite of preterm birth and/or low birth weight, and prevalence of maternal CT/NG infection at delivery) will be assessed using intention to treat analysis at the clinic level. We will conduct both unadjusted and adjusted analyses, including risk factors for maternal STI and neonatal infection that may be imbalanced between the testing and standard-of-care clinics. Effect measures will be estimated with 95% confidence intervals. All analyses will be carried out using the latest version of Stata statistical software (17.0 or higher; College Station, TX, USA).

To assess immune/inflammatory markers, multiple logistic regression models will be developed in an iterative process to identify individual cytokines and groups of cytokines associated with mother-to-child

transmission of CT/NG, preterm birth, and low birth weight. Additionally, we will identify plasma cytokine signatures that classify study participants with or without preterm birth/low birth weight infants.(1) We will define three equal frequency tertiles or "bins" (low, medium, and high), using all plasma samples. Next, we will use the *VSURF* R package, a random forest method to select the most predictive cytokines to differentiate samples from participants with and without preterm birth/low birth weight infants.(2) From these, we will build a decision tree to classify the samples. To better calibrate the accuracy of the decision tree, we will perform permutation testing.

To estimate the budget impact of CT/NG testing among asymptomatic pregnant women, we will combine the total recurrent costs with capital, training, and start-up costs. Next, we will build a decision analytic model to estimate the cost per pregnant woman tested, tested positive, treated, and cured by time of delivery. If the intervention is effective, we will calculate the incremental costs per adverse neonatal outcome averted (e.g. neonatal CT/NG infections, preterm birth, and low birth weight) comparing the testing intervention with syndromic management. We will also estimate a cost-effectiveness ratio (ICER, difference in costs divided by difference in health outcomes), representing the change in costs per averted DALYs associated with preterm birth/low birth weight infants.

We will also perform a probabilistic uncertainty analysis where parameters are randomly sampled from probabilistic distributions to generate 10,000 parameter sets. For each parameter set, the model will run and produce a distribution of outcomes. We will provide the means and 95% confidence intervals for all cost and health outcomes. If the intervention is effective, we will also calculate mean incremental cost-effectiveness ratios (ICER; $/ DALY averted) compared to syndromic management, assessing cost-effectiveness under a willingness-to-pay threshold informed by the WHO's Gross Domestic Product / capita threshold, which was US$7,961 in Botswana 2019. We will also calculate the probability that the scenarios are cost-effective by assessing the proportion of ICERs that fall below the willingness to pay threshold.(3)

Sensitivity analyses will be conducted through one-way and multi-way methods to identify key cost drivers and assess how changing parameters impacts the overall costs and per patient cost of each strategy.(4)

Sample size

The sample size of 500 participants was determined to have sufficient power to detect a difference in rates of vertical transmission of CT and NG based on 1) the prevalence of CT and/or NG among pregnant women in Botswana (10%),(5) and 2) the risk for vertical transmission of CT/NG to newborn infants during parturition (50%).(49-52). Antenatal CT/NG testing and treatment is expected to reduce vertical transmission by over 85%, from 5% in the control to 0.7% in the intervention group.(49, 50) When accounting for a 7% loss to follow-up rate, 250 women per arm will yield N=232 evaluable subjects. With N=232 evaluable samples per arm, we achieve 80.7% power to detect a 4.3% absolute difference in our primary endpoint, which is the proportion of vertical transmission of CT/NG, between the two groups, using a one-sided Fisher's Exact test, at a 0.05 significance level.

Methods for minimizing bias

Enrollment statistics, including total number of women attending their first antenatal clinic visit, total enrolled, total ineligible, and total who declined to participate, will be recorded in a weekly tally sheet and presented at bimonthly study meetings. Participant characteristics, such as sociodemographic characteristics, gestational age, HIV infection status, syphilis test results, obstetric history (e.g. history of preterm birth), in the testing and standard-of-care clinics will be monitored and compared to assess balance. Attrition will also be monitored and attrition bias will be assessed on a monthly bias.

Data Management

Data will be collected and managed using REDCap electronic data capture tools hosted at the Botswana Harvard AIDS Institute Partnership (BHP). REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Data will be entered offline at study clinics into CRFs on the REDCap mobile application on study tablets. At the end of each working day, data will be checked for any errors, discrepancies and missing data

and will be uploaded to the main server by connecting to the internet. The BHP REDCap server is backed up daily. Backups are sent to a remote server and are also stored on tape. The IT System Administrator is responsible for monitoring backups.

Linking forms that link the participant's study ID with their name and contact details will be recorded on separate hard copy linking forms and will be stored securely in a different location to the electronic data.

References

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