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Evaluating Chlamydia trachomatis and Neisseria gonorrhoeae screening among asymptomatic pregnant women to prevent preterm birth and low birth weight in Gaborone, Botswana: A non-randomized, cluster-controlled trial.

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Abstract

ABSTRACT Objective To evaluate the impact of screening and treating asymptomatic pregnant women for *C. trachomatis* and *N. gonorrhoeae* infections on the frequency of preterm birth or low birth weight infants in Botswana. **Design** Non-randomized, cluster-controlled trial. **Setting** Four antenatal care clinics in Gaborone, Botswana. **Population** Pregnant women aged [?]15 years, attending a first antenatal care visit, [?]27 weeks gestation, and without urogenital symptoms were eligible. **Methods** Participants in the intervention clinics received screening (GeneXpert®, Cepheid) during pregnancy and at the post-natal visit. Participants in the standard-of-care clinics received screening at the postnatal visit only. We used multivariable logistic regression and post-estimation predictive margins analysis. Post-hoc analysis was conducted among subsamples stratified by parity. **Main outcome measures** Preterm birth (<37 weeks gestation) and low birth weight (<2500g). **Results** After controlling for parity, hypertension, antenatal care visits, and clinic site, the predicted prevalence of preterm or low birth weight was lower in the intervention arm (11%) compared to the standard-of-care (16%) (AOR: 0.59; 95% CI: 0.28 to 1.24), but confidence intervals were wide. In post-hoc analysis, the intervention was more effective than the standard-of-care (AOR: 0.20; 95% CI: 0.07-0.64) among nulliparous participants. **Conclusion** A *C. trachomatis* and *N. gonorrhoeae* infection screening and treatment intervention among asymptomatic pregnant women did not significantly reduce preterm or low birth weight outcomes. Post hoc analysis found the intervention reduced adverse outcomes among nulliparous participants.

Evaluating *Chlamydia trachomatis* and *Neisseria gonorrhoeae* screening among asymptomatic pregnant women to prevent preterm birth and low birth weight in Gaborone, Botswana: A non-randomized, cluster-controlled trial.

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Short Title : Asymptomatic STI screening to prevent adverse birth outcomes

Objective

To evaluate the impact of screening and treating asymptomatic pregnant women for *C. trachomatis* and *N. gonorrhoeae* infections on the frequency of preterm birth or low birth weight infants in Botswana.

Design

Non-randomized, cluster-controlled trial.

Setting

Four antenatal care clinics in Gaborone, Botswana.

Population

Pregnant women aged [?]15 years, attending a first antenatal care visit, [?]27 weeks gestation, and without urogenital symptoms were eligible.

Methods

Participants in the intervention clinics received screening (GeneXpert®, Cepheid) during pregnancy and at the post-natal visit. Participants in the standard-of-care clinics received screening at the postnatal visit only. We used multivariable logistic regression and post-estimation predictive margins analysis. Post-hoc analysis was conducted among sub-samples stratified by parity.

Main outcome measures

Preterm birth (<37 weeks gestation) and low birth weight (<2500g).

Results

After controlling for parity, hypertension, antenatal care visits, and clinic site, the predicted prevalence of preterm or low birth weight was lower in the intervention arm (11%) compared to the standard-of-care (16%) (AOR: 0.59; 95% CI: 0.28 to 1.24), but confidence intervals were wide. In post-hoc analysis, the intervention was more effective than the standard-of-care (AOR: 0.20; 95% CI: 0.07-0.64) among nulliparous participants.

Conclusion

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A *C. trachomatis* and *N. gonorrhoeae* infection screening and treatment intervention among asymptomatic pregnant women did not significantly reduce preterm or low birth weight outcomes. Post hoc analysis found the intervention reduced adverse outcomes among nulliparous participants.

Tweetable statement : Screening for curable, asymptomatic STIs during pregnancy reduced the odds of preterm birth and low birth weight among nulliparous women in Botswana.

Keywords: *Chlamydia trachomatis* , *Neisseria gonorrhoeae* , pregnancy, low birthweight, preterm birth, sexually transmitted infections, syndromic management

BACKGROUND

According to a United Nations report from 2021, five million children died before their fifth birthday and 56% of these deaths occurred in Sub-Saharan Africa.(1) The leading cause of death before five years was preterm birth complications(2). Low birth weight, primarily resulting from preterm birth or intrauterine growth restriction, is also associated with increased risk for neonatal death and morbidity. (3) Many causes of preterm birth and low birth weight are preventable.

Common, curable sexually transmitted infections (STIs) such as *Chlamydia (C.) trachomatis* and *Neisseria (N.) gonorrhoeae* are associated with preterm birth or low birth weight.(4, 5) A meta-analysis of 12 studies found that antenatal *C. trachomatis* infection increased the relative risk for preterm labor by 40%. (6) A systematic review and meta-analysis found that *N. gonorrhoeae* infection increased the odds of preterm birth by 55% and low birth weight by 66%. (7)

While antenatal *C. trachomatis* and *N. gonorrhoeae* infections are associated with preterm birth or low birth weight, research is mixed on whether etiologic screening and treatment can reduce adverse outcomes.(8) The majority of low and middle income countries do not screen pregnant women for those infections and continue to adapt the World Health Organization (WHO)-endorsed syndromic approach for managing urogenital symptoms (e.g. vaginal discharge) caused by *C. trachomatis* and *N. gonorrhoeae* infections.(9) Syndromic management allows for same-day treatment of symptoms; however, by only addressing symptomatic infections, asymptomatic infections are untreated.(10) Thus, research is needed to evaluate whether routine screening and treatment of asymptomatic *C. trachomatis* and *N. gonorrhoeae* infections during pregnancy can reduce adverse birth outcomes.

This study evaluated the impact of *C. trachomatis* and *N. gonorrhoeae* screening and treatment on preterm or low birth weight outcomes compared with the standard-of-care, syndromic management. Research was conducted among pregnant women living in Gaborone, Botswana who were asymptomatic for *C. trachomatis* and/or *N. gonorrhoeae* infections at their first antenatal care visit.

METHODS

Study Population

The study population included pregnant women in the Maduo study, which was a cluster-controlled, crossover trial conducted in four antenatal care clinics in Gaborone, Botswana. The primary aim of the main study was to evaluate the impact of asymptomatic screening on post-delivery prevalence and the vertical transmission of *C. trachomatis* or *N. gonorrhoeae* infections. Madua study details have been previously described.(11) In brief, starting in February 2021, consenting pregnant women in both arms were enrolled if they met the following eligibility criteria: aged 15 years or older, attending their first antenatal care visit, 27 weeks gestation or less, not treated for an STI in the past 30 days, and without signs or symptoms of an STI (most commonly vaginal discharge) and therefore not in need of syndromic management (i.e. standard-of-care).

Initially two clinics participated in the study, one intervention and one standard-of-care clinic (February 2021 to mid-September 2021). As previously described,(11) clinics were similar in terms of patient volume and services offered; however, one clinic had space readily available for the screening intervention and was designated the initial intervention clinic. However, COVID-related recruitment challenges resulted in expansion to an additional two clinics, assigned to the intervention arm, in mid-September 2021. In January 2022, the

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standard-of-care clinic crossed-over and received the intervention and two intervention clinics crossed-over and received the standard of care.

Our primary analysis sample included participants with non-missing values for each of our key outcomes (low birth weight, preterm birth), and non-missing values for covariates. We excluded participants who experienced a miscarriage or stillbirth. We also restricted the sample to participants with singleton pregnancies as non-singleton births are more likely to have lower birthweight and shorter gestation.

Intervention

Participants in the intervention clinics received *C. trachomatis*and *N. gonorrhoeae* screening (GeneXpert® CT/NG assay, Cepheid, Sunnyvale, CA, USA) at the first antenatal care visit, the third trimester visit (approximately 36-38 weeks' gestation), and at the postnatal care visit, 6-8 weeks post-delivery. Vaginal samples were self-collected by participants and then transported to a central site for same-day processing according to the manufacturer's instructions. Women were provided with results in-person or by telephone within 24 hours. Participants testing positive for *C. trachomatis* and/or*N. gonorrhoeae* were treated by a study nurse according to US Centers for Disease Control and Prevention 2021 STI Treatment guidelines(12) and were counselled on partner notification. Participants in the standard-of-care arm were tested only at their postnatal visit. If results were positive, they received similar case and partner management.

Data collection

At the first antenatal care visit, participants responded to an interviewer-administered questionnaire that collected data on sociodemographic characteristics, medical history, partner characteristics, condom use, HIV status and treatment, previous STI diagnoses and treatment, and alcohol use and smoking during pregnancy. Data were also abstracted from the participant's hand-held obstetric record, including obstetric and birth history, rapid HIV test results, other laboratory results (e.g. syphilis), weight, and blood pressure. In Botswana, clinicians record obstetric and medical data, laboratory results, treatments, and delivery information in the patient-held record.

At the third trimester visit, participants were asked about new sex partners, STI symptoms, treatment, and diagnoses; as well as alcohol and tobacco use. The patient-held obstetric record was also reviewed to confirm new diagnoses and record blood pressure and laboratory results. At the postnatal care visit, information was collected on the number of antenatal care visits, new STI symptoms and treatment, new sex partners, new test results; and delivery information, including location, delivery type (spontaneous vaginal delivery, breech vaginal delivery, assisted vaginal delivery, elective caesarian section, emergency caesarian section), and singleton vs multiple births. Neonatal information was also collected, including gestational age at delivery, birth weight, length, head circumference, and sex. All study data were entered directly into a Health Insurance Portability and Accountability Act (HIPAA)-compliant REDCap electronic database hosted at the Botswana Harvard AIDS Institute Partnership (Research Electronic Data Capture, Vanderbilt University, USA).**(13)**

Outcomes

The primary outcome of interest was a compositive measure of preterm birth or low birthweight. Preterm birth was defined as <37 weeks, 0 days gestation. Low birthweight was defined as <2500g. Gestational age at delivery and birthweight were abstracted from patient-held obstetric records recorded by midwives. Gestational age was based on the estimated date of delivery that was calculated at the first antenatal care visit using the reported last menstrual period and confirmed by ultrasound when available. If the last menstrual period date was unknown or likely incorrect, symphysis-fundal height measurements were utilized by midwives to determine gestational age. Our study performed ultrasounds on a small sample of 20 women and compared our findings with the recorded gestational age data using a Mann-Whitney test and Fisher's exact test. We found that the estimates were not significantly different.

Primary Exposure and Covariates

Our primary exposure was assignment to either the intervention or standard of-care-arms of the study. Co-

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variates of interest were factors found to be associated with preterm birth(14) or low birth weight(15) in previous research that could be imbalanced between the arms. Initial covariates examined were age ([?]25 years vs above 25 years), relationship status (married/cohabitating vs not), education level (primary level or less vs secondary level or higher), income ([?]2000, >2000 Pula (˜USD 160)/month, or unknown), nationality (Motswana vs other), HIV status (positive vs negative), partner's HIV status (positive, negative, or unknown), parity (prior live birth vs not), recorded history of prior preterm or low birth weight outcome among normal singleton births (ever had a preterm birth or low birth weight infant vs. no history of preterm or low birth weight births among normal singleton births), treatment for STI symptoms between the first and second study visits (STI treatment included azithromycin, erythromycin, ceftriaxone, doxycycline, and metronidazole), proportion of WHO recommended antenatal care visits achieved by gestational age at delivery,(16) delivery mode (spontaneous vaginal delivery vs other), and hypertension during pregnancy (defined as either a systolic pressure of [?]140 mmHg or diastolic pressure of [?]90 mmHg recorded at first antenatal care visit or during the third trimester. We also examined any hypertension vs none, as well as through a three-category variable: "early" hypertension at or before 20 weeks, "late" hypertension after 20 weeks, or no hypertension), alcohol use during pregnancy (using the Alcohol use Disorders Identification Test (AUDIT-C) of any use (AUDIT-C score>0) vs none and harmful use (AUDIT-C score[?]3) vs none and non-harmful),(17) any tobacco use during pregnancy, clinic site where care was received, and enrollment period (Feb 2021-Jan 2022 vs Feb 2022-Dec 2022).

Analysis

All analyses were conducted using STATA 17 (College Station, TX). We assessed whether the proportions of participants who reported miscarriages or stillbirths were similar across study arms using Chi-squared or Fisher's Exact tests. Further, we compared baseline participant characteristics between those missing and not missing birth outcome data using Chi-squared or Fisher's Exact tests for dichotomous variables and Student's t-test or the Wilcoxon-Mann-Whitney tests for continuous variables.

We provided descriptive statistics of the study sample by intervention and standard-of-care arm assignment. We assessed the balance between the arms using Chi-squared or Student's t-tests. To address potential clustering and unmeasured confounding, we assessed results from three statistical models. First, we utilized a multivariable logistic regression model. Next, we estimated a fixed effects model in an attempt to purge some effects of unobserved clinic-level characteristics that may influence birth outcomes.(18) We also estimated a random effects model, with the clinic specified as a random effect. As the intraclass correlation coefficient was 0.0017, suggesting that only 0.17% of the variance in the outcome was due to variations across clinics, we decided not to estimate a generalized estimating equation. For each model, covariates of interest (described above) were entered into a full model and then excluded one at a time, starting with the variable with the largest p-value until all remaining independent variables had p-values <0.20. Then, covariates were readded one at a time in the order they were dropped and retained if they had a p-value of <0.20 or the odds ratio (OR) of the primary exposure was changed by 15%. We also assessed the potential interaction between clinic and intervention assignment. Models were run for the primary outcome (preterm or low birth weight), as well as preterm birth only (includes low birthweight if preterm) and low birth weight only (includes preterm if low birth weight) individually. When the clinic site variable was included, we assessed the models with and without the clinic that did not crossover.

Further, we conducted post-estimation predictive margins analysis to estimate the predicted prevalence of the composite outcome of low birth weight or preterm birth in the intervention and standard of care arms as well as the adjusted risk ratios. In other words, following the logistic regression, all participants were set to both exposure values (standard-of-care and intervention) and the logistic regression coefficients were used to calculate the predicted prevalence for everyone at their observed confounder pattern and assigned exposure. Confidence intervals were calculated using the delta method.(19) Thereafter, the effect of intervention versus the standard-of-care was calculated as an adjusted risk ratio.

In another attempt to balance confounding, we conducted post hoc analysis that stratified the sample by nulliparous and multiparous women.

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ETHICS

The Maduo Study was approved by the Botswana Health Research Development Committee of the Botswana Ministry of Health and Wellness (HRDC #00881) and in accordance with the National Institutes of Health single institutional review board policy, a reliance agreement was approved by the University of Southern California (HS-21-00245) and the University of California, San Diego.

RESULTS

Among 500 participants, 436 (87%) had single, non-missing, live birth outcomes. We excluded 20 participants who experienced miscarriages, 11 who had stillbirths, seven with twin births, and 26 participants who were lost to follow-up (**Figure 1**). The distribution of miscarriages and stillbirths was similar across study arms. Baseline characteristics between the analytical sample and the 26 with missing outcome data were similar in terms of age, relationship status, education, income, HIV status, partner HIV status, primigravida, history of a preterm birth or low birth weight outcome, hypertension, nationality, and alcohol use. However, the standard-of-care group was more likely to have missing birth outcome data (8.7%) compared to the intervention group (2.6%). Our study team was able to contact 11 of the 26 participants missing birth outcome data by phone and determined that they were now living outside of Gaborone.

As seen in **Table 1** , the median age in our analytical sample was 27 years, the median gestational age at first antenatal care visit was 17 weeks, about half of the participants were married or cohabiting, most women were citizens of Botswana, 7% had a recorded measure of hypertension, 17% were living with HIV, and the median number of antenatal care visits was six. Characteristics that differed statistically between intervention and standard-of-care arms included education level, income, citizenship, HIV status, first pregnancy, partner's HIV status, clinic, and study period (p≤0*05 for all).

The overall prevalence was 13.3% for the composite outcome of preterm birth and/or low birthweight, 9.4% for preterm birth, and 8.5% for low birthweight. Without controlling for potential group imbalance, the prevalence of preterm birth or low birthweight was not significantly different between the intervention (14%) and standard-of-care arms (13%).

Results from the multivariable logistic regression can be found in**Table 2** . In all tested models, exposure to the intervention arm was associated with reduced odds of the composite outcome of preterm birth or low birthweight after controlling for primigravida, antenatal care visits, hypertension, and clinic site; however, the confidence intervals crossed one. Further, odds ratios were similar when limiting the sample to participants in cross-over clinics that received the intervention and standard-of-care assignment over time.

Using post-estimation analysis from the multivariable logistic regression model, the predicted prevalence of the composite outcome of preterm or low birth weight was higher in the standard-of-care (15%) group compared to the intervention group (11%) (**Figure 2**); however, the adjusted risk ratio (ARR) had confidence intervals that crossed one (ARR: 0.67; 95% CI: 0.38 to 1.17). Results were similar for preterm birth (ARR: 0.65; 95% CI: 0.33 to 1.28) and low birth weight (ARR: 0.57; 95% CI: 0.28 to 1.16).

In the posthoc analysis, the stratification reduced some of the differences between the intervention and standard of care groups. Among nulliparous participants, the groups were now balanced on nationality, HIV status, and time period; however, the groups continued to differ on education level, income, and partner's HIV status. Although the HIV prevalence was 5% among nulliparous participants, 18% of participants said their partners were living with HIV and 29% did not know their partner's status. In the multiparous group, the intervention and standard of care arms differed on income level, nationality, and partner HIV status. The HIV prevalence among multiparous women was 24%, and 20% reported that their partners were living with HIV and 31% did not know. **Table 2** also provides results from the multivariable logistic regressions stratified by prior live birth. After controlling for hypertension and clinic, the intervention reduced the odds of the composite outcome, preterm and low birth weight, among the nulliparous group. However, no effect was found among multiparous women and the odds ratios switched directions.

DISCUSSION

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Main findings

We evaluated the impact of *C. trachomatis* and *N. gonorrhoeae* screening and treatment on preterm birth or low birth weight among asymptomatic pregnant woman in Gaborone, Botswana. The intervention arm had a lower predicted prevalence of a composite outcome of preterm or low birth weight compared to the standard-of-care, after adjustment for parity, antenatal care visits, hypertension during pregnancy, and clinic site; however, the confidence intervals were wide. In post hoc analysis among nulliparous women, the intervention appeared to reduce the odds of preterm birth or low birth weight outcomes by 80%, necessitating further research into reasons for a differential effect.

Strengths and Limitations

A strength of this study was the prospective design that allowed us to compare STI screening and treatment results with birth outcomes. The majority of previous observational studies that compared *C. trachomatis* or *N. gonorrhoeae* infections during pregnancy with birth outcomes were unable to determine receipt of treatment.(7, 20) Further, this study enrolled a unique cohort of pregnant women that would have been missed by the syndromic approach for management of STIs. While the World Health Organization recommends countries enhance syndromic management by gradually scaling-up laboratory screening;(21) the guidelines for management of asymptomatic *C. trachomatis* or*N. gonorrhoeae* infections are currently being developed and more research is needed. Finally, because low and middle income countries rely on syndromic management during pregnancy(9), there is a lack of data on the prevalence and impact of asymptomatic, curable STIs.

Our study is subject to several limitations. First, neither participants nor clinics were randomized and thus unmeasured confounding between intervention assignment and birth outcomes is likely. We attempted to address threats to internal validity through the cross-over design, controlling for imbalanced participant characteristics, and estimating a fixed effects model that purges clinic-level unobserved variables. Second, birth outcome data were missing not at random. If those missing data in the standard-of-care arm were more likely to have an adverse outcome, then our study results were attenuated. However, we made every effort to contact participants with missing data and determined that almost half were missing because they had moved away from Gaborone. Third, estimates of gestational age and birth weight were subject to mismeasurement. Birth weight measurement procedures and scale accuracy were not validated by the study. Although gestational age was not measured by the study, a sub-sample of women with a study ultrasound received estimates similar to the clinic recorded results, and 70% of participants had access to routine ultrasound prior to their third trimester visit. Finally, the sample size for this study was determined to find a difference in post-delivery prevalence and vertical transmission of *C. trachomatis* and *N. gonorrhoeae* and thus was underpowered to find a difference in preterm birth or low birth weight frequency.

Interpretation

Our finding that STI screening and treatment could reduce adverse birth outcomes compared to syndromic management adds some support to previous systematic reviews and meta-analyses. Adachi et al., found eight studies that evaluated the effect of screening on adverse pregnancy outcomes. (8) Five studies found that treatment with erythromycin reduced adverse birth outcomes.(24-28) Two systematic reviews and meta-analyses assessed the relationship between antenatal infection and adverse birth outcomes. One review found 107 relevant observational studies reporting on 12 pregnancy and fertility-related outcomes, (20) and the meta-analysis results concluded that *C. trachomatis* was associated with preterm labor (unadjusted OR =1*29; 95% CI 1*11–1*50) and low birth weight (unadjusted OR = 1*80; 95% CI 1*20–2*71). However, that review found substantial heterogeneity across studies and only two studies reported whether women had received treatment. Another systematic review and meta-analysis assessing *N. gonorrhoeae* found that women with *N. gonorrhoeae* were more likely to experience preterm birth (OR 1*55, 95% CI 1*21 to 1*99, n=18 studies) and low birth weight (OR 1*66, 95% CI 1*12 to 2*48, n=8). (7) Included studies were all observational, most did not control for confounding, and most women with *N. gonorrhoeae* had received treatment.

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A recent study investigated the relationship between *C. trachomatis, N. gonorrhoeae* , and *Trichomonas (T.) vaginalis* screening and treatment among pregnant women living with HIV in South Africa(29) and found no association between screening and preterm birth or low birth weight. However, this study was limited to women living with HIV, did not exclude symptomatic participants, found the testing group was less likely to be on anti-retroviral therapy, and treated participants for *T. vaginalis* . Further, participants in that study had high levels of persistent infections as 27% were positive for *C. trachomatis* at repeat testing.(30) No participants in the current study were positive at test-of-cure.(31)

Given the equipoise around the impact of STI screening on adverse pregnancy outcomes, future research may consider individual randomization or other methods for addressing confounding, measurement bias, and lack of power. As discussed by Low in a recent commentary,(32) observational studies face major threats to internal validity as many factors are associated with both STIs and pregnancy outcomes. Case-control and cohort studies are subject to measurement bias when receipt of treatment is not recorded because treatment likely reduces the risk of the outcome.(32) Cluster trials, such as the current study and the upcoming WANTAIM Trial(33) seek to reduce confounding and measurement bias; however, confounding can still occur through unmeasured baseline imbalance and clustering has a negative impact on the power to detect an effect and thus cluster designs typically require larger sample sizes.(34)

Conclusion

In conclusion, the screening and treatment intervention among asymptomatic pregnant women reduced the frequency of preterm or low birth weight infants compared to the standard-of-care among nulliparous women; however, the effect size among multiparous women and the entire sample was uncertain. As prematurity and low birth weight are leading causes of preventable death for children under five years, more research designed to detect differences in birth outcomes is needed for evaluating the effectiveness and cost-effectiveness of antenatal *C. trachomatis* and *N. gonorrhoeae* screening interventions.

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Disclosure of interests

Dr Klausner reports personal fees from Cepheid, during the conduct of the study, and personal fees from Danaher, outside the submitted work. None of the other authors declares a conflict of interest. Completed disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

JDK and CM were the principal investigators of this study. JDK, CM, and AW wrote the study protocol with technical expertise from DRM. KR, LT, NN, EH, and RR implemented the study and collected data. AM managed the data collection and quality assurance processes. Study implementation was supervised by JDK, CM, AW, RR, CMB, and AM. AW and MLW performed the data analysis. AW wrote the draft version of the manuscript; all authors contributed to the final version.

Details of ethics approval

Study approval was provided by the Botswana Health Research Development Committee of the Botswana Ministry of Health and Wellness (HRDC #00881) and in accordance with the National Institutes of Health single institutional review board policy, a reliance agreement was approved by the University of Southern California (HS-21-00245) and the University of California, San Diego.

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Clinical Trial Information: ,

1. Date of registration: July 9, 2021
2. Date of initial participant enrollment: February 24, 2021
3. Clinical trial identification number: NCT04955717
4. URL: https://clinicaltrials.gov/ct2/show/NCT04955717
5. Data sharing information: No IPD will be shared with other researchers

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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