Fetal growth and birth weight are independently reduced by malaria infection and curable sexually transmitted and reproductive tract infections in Kenya, Tanzania, and Malawi: A pregnancy cohort study

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ABSTRACT

Objective

Malaria and sexually transmitted and reproductive tract infections (STIs/RTIs) are highly prevalent in sub-Saharan Africa and associated with poor pregnancy outcomes. We investigated the individual and combined effects of malaria and curable STIs/RTIs on fetal growth in Kenya, Tanzania, and Malawi.

Methods

This study was nested within a randomized trial comparing monthly intermittent preventive treatment for malaria in pregnancy with sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine, alone or combined with azithromycin. Fetal weight gain was assessed by serial prenatal ultrasound. Malaria was assessed monthly, and *Treponema pallidum*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Chlamydia trachomatis* and bacterial vaginosis at enrolment and in the third trimester. The effect of malaria and STIs/RTIs on fetal weight/birthweight Z-scores was evaluated using mixed-effects linear regression.

Results

1,435 pregnant women had fetal/birth weight assessed 3,950 times. Compared to women without malaria or STIs/RTIs (n=399), malaria-only (n=267), STIs/RTIs-only (n=410) or both (n=353) were associated with reduced fetal growth (adjusted mean difference in fetal/birth weight Z-score [95% CI]: malaria=-0.18 [-0.31,-0.04], p=0.01]; STIs/RTIs=-0.14 [-0.26,-0.03], p=0.01]; both=-0.20 [-0.33,-0.07], p=0.003). Paucigravidae experienced the greatest impact.

Conclusion

Malaria and STIs/RTIs are associated with poor fetal growth especially among paucigravidae women with dual infections. Integrated antenatal interventions are needed to reduce the burden of both malaria and STIs/RTIs.

Keywords

Malaria in pregnancy, sexually transmitted infection, reproductive tract infection, bacterial vaginosis, fetal growth, birthweight.

INTRODUCTION

Despite efforts to reduce its burden [1], an estimated 46 to 52 million pregnancies were at risk of malaria infection in sub-Saharan Africa in 2020 [2]. Most malaria infections (>80%) during pregnancy remain asymptomatic [3] yet are associated with maternal anemia and impaired fetal growth [4, 5], leading to small-for-gestational-age (SGA), low birthweight (LBW) newborns, and preterm delivery [6].

Curable sexually transmitted and other reproductive tract infections (STIs/RTIs) such as syphilis (*Treponema pallidum*), chlamydia (*Chlamydia trachomatis*), gonorrhoea (*Neisseria gonorrhoeae*), trichomoniasis (*Trichomonas vaginalis*) and bacterial vaginosis are also common in sub-Saharan Africa [7]. Syphilis screening and treatment is part of standard antenatal care throughout sub-Saharan Africa, but other STIs/RTIs are managed via syndromic algorithms [8]. Like malaria, most STIs/RTIs are asymptomatic and often remain undetected and untreated [9]. Exposure to STIs/RTIs during pregnancy is associated with poor birth outcomes such as preterm birth and LBW [7].

Infants born preterm, SGA, or with LBW are at increased risk of neonatal morbidity and mortality [6] and possibly cardio-metabolic diseases in adult life [10].

Despite malaria and STIs/RTIs being highly prevalent in sub-Saharan Africa, few studies have investigated their dual-impact on fetal growth and pregnancy outcomes [11]. Fetal growth evaluation requires accurate gestational age estimation and serial ultrasound to assess fetal weight. Most studies in sub-Saharan Africa relied on LBW and SGA at birth as proxy indicators of intrauterine growth restriction. However, both have limitations in identifying intrauterine growth restriction. Firstly, LBW may result from either intrauterine growth restriction, preterm delivery, or both [12]. Secondly, SGA newborns may be growth-retarded or constitutionally small but healthy [13].

Finally, newborns may have failed to achieve their biological growth potential but still be above the cut-off for LBW or SGA [13].

Only a few and small studies have used ultrasound to assess the effect of malaria on fetal growth [4, 5, 14, 15]. To our knowledge, no study has investigated the effects of STIs/RTIs on fetal growth trajectories or the consequences of both malaria and STIs/RTIs using ultrasound.

METHODS

Study design and population

This cohort study was nested in a randomized partially placebo-controlled trial conducted from March 2018 to August 2019 involving 4,680 pregnant women comparing monthly intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine, alone or combined with a single course of azithromycin at enrolment conducted in Kenya, Tanzania and Malawi [16]. Of these women, one-third were randomly selected into a nested cohort for fetal growth monitoring by serial ultrasound. In order to have a power of 80% to detect an expected proportion of women with STIs/RTIs was 40% in sulfadoxine-pyrimethamine arm compared to 30% in dihydroartemisinin-piperaquine / dihydroartemisinin-piperaquine + azithromycin, with alpa=0.025, 432 women per treatment arm were needed. To allow for 13% loss to follow-up, 500 women were recruited per arm. Women attending antenatal care were enrolled if HIV-negative, had a viable singleton pregnancy between 16 and 28 weeks gestation, no known heart disease, had not received sulfadoxine-pyrimethamine during the current pregnancy, and had no known allergy to the study drugs.

Data collection procedures

Details of data collection have been described elsewhere [16]. In brief, demographic data and medical history were collected at enrolment. Women were screened for urinary tract infection (using urine dipsticks) and hypertensive disorders (blood pressure >140/90 mmHg ± proteinuria), prior medication usage, and maternal anthropometrics were recorded at each antenatal visit. Hemoglobin level was assessed (Hemocue 301 or 201) at enrolment, in the third trimester, and at delivery.

Estimation of gestational and fetal weight

Using ultrasound and standard methodology, gestational age was estimated based on crown-rump length until 13^{+6} weeks [17], and from 14^{+0} weeks by using an algorithm of head circumference and femur length [18], head circumference only [18] or femur length only [19], depending on availability of fetal biometrics. Serial ultrasound was performed at enrolment if gestational age was $\geq$ 22 weeks, at approximately 25-28 weeks gestation, and at approximately 32-35 weeks, and fetal weights were estimated based on head circumference, abdominal circumference and femur length [20].

Detection of malaria

Women were screened for malaria at enrolment using malaria rapid diagnostic tests (mRDTs) (CareStart™ Malaria Pf/PAN (HRP2/pLDH) Ag Combo) as per national policy in Kenya and Tanzania. In all three countries, women with fever (≥ 37.5^0C) or recent history of fever were also screened with mRDTs.

In Kenya and Malawi, regardless of treatment arm, women with positive mRDTs were treated with artemether-lumefantrine, and IPTp dosing was deferred for four weeks. In Tanzania, women with positive mRDTs in the sulfadoxine-pyrimethamine arm were treated with artemether-lumefantrine, and IPTp was deferred for four

weeks. However, women in the dihydroartemisinin-piperaquine and dihydroartemisinin-piperaquine/azithromycin groups who had positive mRDTs at enrolment were given their first course of IPTp but at later visits artemether-lumefantrine was administrated if mRDTs were positive, and IPTp was deferred for four weeks.

Peripheral maternal venous blood was collected at all visits and at delivery, along with cord and placental blood. Thick and thin blood smears were prepared, Giemsa stained, and independently double-read by experienced microscopists; where results were discordant, a third reading was performed to determine the final result [16]. Dried blood spots were also prepared for quantitative real-time polymerase chain reaction (qRT-PCR) [16]. Finally, placental biopsies were taken at delivery for malaria histology [16].

Detection of STIs/RTIs

As part of standard care, pregnant women were pre-screened for HIV. Women living with HIV were provided treatment per national guidelines and excluded from the study. All women were subsequently screened for syphilis with SD-Bioline point of care tests and, if positive, they were treated with 2.4 million units intramascular benzathine penicillin G. Additionally, clinic staff routinely asked women if they had experienced any symptoms associated with STIs/ RTIs. At any visit, if a woman responded in the affirmative, she was treated by the clinic staff according to national syndromic management guidelines recommended by the WHO [8]. Apart from routine care, clinic staff collected vaginal swabs and stored them on site until the end of the trial, at which time the samples were shipped to a regional reference laboratory in East Africa for retrospective batch analysis. Serum and vaginal swab samples were collected at enrolment and between 32-36 weeks. Serum samples

were tested for rapid plasma reagin and confirmatory syphilis testing with *Treponema pallidum* Hemagglutination assays. Vaginal samples were tested for chlamydia and gonorrhoea DNA by RT-PCR (*Artus*® CT/NG QS-RGQ Kit), trichomoniasis with SACASE^{TM} Real-TM Kit, and bacterial vaginosis using the Nugent scoring.

Pregnancy outcome

At delivery, birthweight was measured using digital scales (Seca GmbH & Co. KG., precision 10g or ADE M112600, precision 5g) and head and abdominal circumferences using flexible tape. Birthweights recorded >1 hour post-delivery were adjusted for the physiological weight loss [21].

Statistical analysis

Analyses were conducted using Stata software, v16 (Stata Corp, Texas, USA). Malaria exposure was defined as testing positive at any time-point by any assay: mRDT, microscopy, qRT-PCR, and/or placental histology. STIs/RTIs exposure was defined for individual STIs/RTIs and as a composite variable with positive test for any STIs/RTIs at any time-point. For the longitudinal analyses, women were considered negative until their first malaria and/or STIs/RTIs episode and thereafter considered positive. Four unique exposure groups were generated to assess if malaria and STIs/RTIs co-infection affected growth trajectories; a control group with neither malaria nor STIs/RTIs; malaria-only; STIs/RTIs-only; and malaria plus STIs/RTIs. The primary outcome was Z-scores for fetal weights and birthweight using a sex-specific Tanzanian reference chart [22] based on previous evidence indicating that a local growth curve is more representative than the international growth curve [23]. Our approach aligns with recent recommendations by the International Federation of

Gynecology and Obstetrics (FIGO) on the accuracy of growth curves [24].

Secondary outcomes were birthweight Z-scores alone, growth trajectories based only on fetal weights Z-score, SGA (birthweight <10th percentile) [22], LBW (birthweight <2.5Kg), preterm delivery (gestational age <37 weeks), and newborn abdominal circumference in millimeters and head circumference in millimeters or Z-scores based on INTERGROWTH-21st reference [25].

Women with a non-viable pregnancy outcome (miscarriage, stillbirths), twin pregnancy, severe congenital malformations, or missing data on malaria and STIs/RTIs were excluded. Furthermore, observations with weights measured <14 days apart, gestational age <18 weeks or ≥45 weeks, birthweights <250g or ≥6,500g, or fetal/birthweight Z-score >±5, were excluded.

Linear regression models and linear mixed-effects models were used to assess the effect of malaria and/or STIs/RTIs on birth size and growth trajectories respectively. All crude models were adjusted for study design factors (study arm, site, and gravidity [paucigravidae, i.e. primi- and secundigravidae, and multigravidae]). In mixed models, these same design factors were included as fixed effects, gestational age at visit was included as a time factor, and individual participant as a random effect to account for within-subject clustering. In addition, other potential confounders, selected based on the statistical analysis plan for the main trial, including rainfall patterns, malaria transmission intensity, patterns of parasite resistance to sulfadoxine-pyrimethamine, maternal age, gestational age at enrolment or delivery, socioeconomic status, maternal body-mass index, bednet use, number of IPTp doses received, hemoglobin levels, and sex of the fetus/newborns, were considered if associated with the outcome variable with a $p<0.2$ in the univariate models and retained in final models if p-values were <0.1.

Malaria infection is more detrimental in paucigravidae and undernourished women than in multigravidae and well-nourished counterparts. Thus, we fitted models with interaction terms to investigate possible effect-modification between malaria and gravidity or malaria and maternal body-mass index. The interaction between malaria and STIs/RTIs was also assessed.

To assess if the effect on growth trajectories was due to poor growth close to delivery, models only including Z-scores for fetal weights but not birthweight, were also generated. Finally, as fetal weight gain is mainly in the third trimester, a linear regression model was generated with a single fetal weight Z-score in the third trimester as the outcome, and malaria infections or STIs/RTIs occurring before the fetal weight estimation as exposure.

Additionally, a dose-response relationship was assessed by comparing the impact of number of malaria episodes on birth weight Z-score using the group with one malaria episode as the reference group. Furthermore, the model on STIs/RTIs was repeated after categorizing STIs/RTIs exposure by: 1) composite STIs/RTIs only at enrolment, between weeks 32 and 36, or both at enrolment and between weeks 32 to 36; 2) only one type of STIs/RTIs, or multiple STIs/RTIs. Finally, we assessed the effect of malaria and STIs/RTIs on SGA, LBW and preterm delivery using Poisson regression with robust error variance.

RESULTS

Study population

Of the 1,586 women randomly selected for fetal growth monitoring, 1,435 were eligible for analyses. Of the 1,435 participants, 573 (39.9%) were >22 weeks at

enrolment and had fetal weight assessed,1,007 (70.2%) had fetal weight assessed between approximately 25-28 weeks and 1,045 (72.8%) between approximately 32-35 weeks. Birthweights were available for 1,325 (92.3%) participants. Thus, 3,950 observations of fetal weight/birthweights were included in the longitudinal analysis (Figure S1).

The distribution of the 1,435 women was similar across study arms and countries. The mean age was 24.9 (SD 5.8) years. Only 2.8% were underweight (body-mass index <18.5 Kg/m^2) at enrolment, whereas 33.2% were overweight (25-29.9 Kg/m^2) or obese (≥30 Kg/m^2). Among the newborns, 13.4% were SGA, 4.3% were preterm, and 8.1% were LBW (Table 1). Baseline maternal characteristics were similar between included and excluded mother-newborn dyads, except that; a higher proportion of excluded women were from Malawi and paucigravidae, the proportion of bed net use at enrolment also differed significantly between the two groups and this proportion was lower among excluded women (Table S1).

Malaria infection was common: 43.4% (623/1,435) of women had at least one episode during pregnancy, and 46.3% (364/787) of paucigravidae had malaria (Table 2.a). Malaria prevalence varied across study sites and arms, being highest in Malawi and in the sulfadoxine-pyrimethamine arm (Table S2). Women with malaria had lower socioeconomic status, were younger, had lower body-mass index and hemoglobin levels at enrolment, and more often came from rural areas (Table S2). Similarly, a high prevalence of STIs/RTIs was observed, with over half of the women having STIs/RTIs detected either at enrolment, in the third trimester, or at both timepoints (Table 2.b). Bacterial vaginosis was the most common, with 34.6% (449/1,297) of the women testing positive for bacterial vaginosis at least once during

pregnancy. Only 1.9% (27/1,407) and 4.2% (54/1,298) of the women had syphilis and gonorrhoea, respectively. Among women with STIs/RTIs a higher proportion were from Tanzania. Women with and without STIs/RTIs had similar demographic characteristics across study arms (Table S3). Fetal biometry in second and third trimesters by gestational age and gravidity is described in Table S4.

Effect of malaria and STIs/RTIs on growth trajectories

There was a trend towards lower mean birthweight Z-scores among women with malaria infection and STIs/RTIs compared to women without (adjusted mean difference [aMD] [95% CI] malaria:-0.10 [-0.22,0.02], p=0.09; STIs/RTIs:aMD=-0.09 [-0.21,0.02], p=0.12) (Table 3.a+b). Malaria exposure was also associated with a higher proportion of newborns being SGA (aRR:1.50 [1.14-1.97], p=0.004) (Table 3.a). The effect was more evident among paucigravidae women with malaria or STIs/RTIs (Malaria: aMD for birthweight Z-score= -0.19 [-0.35,-0.03], p=0.02 and SGA aRR=1.84 [1.26-2.69], p=0.002); STIs/RTIs:aMD for birthweight Z-score =-0.17 [-0.33,-0.01], p=0.04) (Table 3.a+b). There was a tendency towards a dose-response relationship between the number of malaria episodes and impact on birthweight Z-score, although this was not statistically significant (1 vs 2 malaria episodes aMD -0.12 [-0.41,0.16], p=0.39; 1 vs 3+ malaria episodes aMD -0.32 [-0.72, 0.09], p=0.13). Infection with both malaria and STIs/RTIs in paucigravid women had an even more pronounced effect on birthweight Z-scores (aMD=-0.34 [-0.57, -0.11], p=0.003) (Table 4a) and SGA (aRR=2.53 [1.37-4.67], p=0.003) (Table 4.b). The same effect on birthweight and risk of SGA was not observed among multigravidae (Tables 3 and 4).

Neither head circumference nor abdominal circumference differed significantly among malaria or STIs/RTIs exposed compared to non-exposed newborns (Tables 3 and 4). No statistically significant effect of the individual STIs/RTIs on birthweight was observed, albeit there was a trend towards lower birthweight Z-score among newborns whose mothers had bacterial vaginosis (crude MD=-0.13 [-0.21, 0.08], p=0.06) (Table S5).

The effects of malaria and STIs/RTIs on growth trajectories were investigated using mixed-effect regression models on fetal weights and birthweight Z-scores (Table 5). Malaria infection was associated with a lower weight Z-score over time (aMD=-0.12 [-0.22, -0.03], p=0.01) (Table 5.a). The effects differed significantly by gravidity strata ($P_{interaction}$=0.01) and were more pronounced among paucigravide (weight Z-score [95% CI] over time aMD=-0.17(-0.31, -0.04), p=0.01) than multigravidae (aMD=-0.07 [-0.21, 0.07], p=0.34) (Table 5.b+c). There were no significant interaction between BMI and malaria ($P_{interaction}$=0.48). STIs/RTIs also reduced weight Z-score over time (aMD=-0.11, -0.20, -0.01, p=0.03), again with paucigravidae being most affected (Table 5.d+e).

The magnitude of the effect on growth trajectories was similar after exposure to malaria-alone (aMD=-0.18 (-0.31, -0.04), p=0.01), STIs/RTIs-alone (aMD=-0.14, -0.26, -0.03, p=0.01) or to malaria plus STIs/RTIs (aMD=-0.20, -0.33, -0.07, p=0.003) (Tables 5.g), and there was a non-significant interaction between malaria and STIs/RTIs ($P_{-interaction}$=0.18). Again, infection with both malaria and STIs/RTIs impacted growth trajectories more in paucigravidae than multigravidae (aMD=-0.30, -0.48, -0.11, p=0.001 vs -0.11, -0.30, 0.09, p=0.28) (Table 5.h+i).

Models containing only fetal weight Z-scores but not birthweight yielded similar results (Tables 3 and 4.a).

Fetal weight in the 3rd trimester, assessed by a single measure, was also lower among paucigravidae after malaria (aMD=-0.25, -0.47, -0.03, p=0.02), but not after STIs/RTIs (Table S6). Fetal weight gain over time was lower among women with STIs/RTIs at enrolment than women with STIs/RTIs both at enrolment and in the third trimester (Table S7). The individual STIs/RTIs were not significantly associated with impaired fetal growth, although there was a trend towards lower fetal/birthweight Z-score for trichomoniasis (aMD=-0.11, -0.23, -0.02, p=0.09) (Table S7). Finally, having multiple STIs/RTIs did not further reduce fetal weight gain compared to having a single STI/RTI (Table S7).

DISCUSSION

There was a high burden of malaria and STIs/RTIs; almost 25% of the women had both conditions during pregnancy. This is consistent with previous studies demonstrating a high prevalence of either malaria [26], STIs/RTIs [27], or both [11]. In the current study, fetal growth trajectories were negatively affected by infection with malaria and STIs/RTIs alone or combined. Malaria in pregnancy is characterized by placental sequestration of malaria-infected erythrocytes resulting in placental inflammation [12], poor vascular development [28] and altered flow in the umbilical and uterine arteries [29]. This may explain the association between malaria and fetal growth restriction. Previous smaller longitudinal studies found reduced fetal biometry and weights in the second [15] and third trimester [4] and an increased risk of fetal SGA [14]. We observed a negative impact on fetal growth trajectories based both on fetal weights and birthweights as well as solely on ultrasound-estimated fetal weights. This suggests that the negative effect occurs continuously *in utero* and not only close to birth. Paucigravidae experienced the greatest negative impact on fetal

growth trajectories, a finding consistent with gravidity-associated epidemiology of malaria in pregnancy [6].

The mechanism by which STIs/RTIs affect fetal growth is not well elucidated. One mechanism may be that ascending genital infections lead to intrauterine infection and inflammation, damaging the trophoblast cells and resulting in placental dysfunction [30]. Previous studies on STIs/RTIs used birthweight as a proxy for intrauterine growth restriction [31]. Our study is the first to conduct serial prenatal ultrasound measurements, demonstrating a significant negative association between STIs/RTIs and fetal growth trajectories. Having infection with both malaria and STIs/RTIs was particularly deleterious to pregnancies of paucigravidae, perhaps due to the dual placental insult occurring in this group. However, the interaction between the dual infection was insignificant. This suggests a non-synergistic effect, although this could also be due to the small sample size and the limited power to detect interactions.

Fetal weight gain was reduced over time among women who tested positive for STIs/RTIs at enrolment but not when considering STIs/RTIs occurring only at week 32-36. This suggests that the negative effect of STIs/RTIs on fetal growth alterations is set early in pregnancy, well before fetal growth peaks in the third trimester. Thus, intervention later in pregnancy may not interrupt the causal pathway to reduced fetal growth. Previous studies found a significant association between bacterial vaginosis and SGA at birth, while others have reported a non-significant association [31].

The effect of STIs/RTIs may also depend on the type and number of infections. Our study indicated that the negative effect of STIs/RTIs on fetal growth might mainly be due to bacterial vaginosis or trichomoniasis. Bacterial vaginosis was the most

common cause of STIs/RTIs, especially among women with only one type of STIs/RTIs, and the high prevalence of bacterial vaginosis provided more statistical power to detect an impact on fetal growth. This might explain why having only one type compared to multiple types of STIs/RTIs appeared to be strongly associated with impaired fetal growth.

Our findings have implications for antenatal care and public health in areas where both malaria and STIs/RTIs are prevalent. The dual burden of malaria and STIs/RTIs is under-appreciated in the antenatal care setting and in the research community. This may partly be explained by both malaria infections and STIs/RTIs being largely asymptomatic among pregnant women [9]. Thus, etiological assays to quantify the true dual burden of infections are needed. A systematic review of malaria and STIs/RTIs among pregnant women attending antenatal care facilities in sub-Saharan Africa identified 171 studies with relevant data points for pooling; none reported the prevalence of dual infection [7].

Current antenatal care includes screening strategies for malaria, HIV, and syphilis. Our study suggests the importance of antenatally targeting other STIs/RTIs as well. Women in this study received IPTp to prevent malaria at each antenatal visit and high-quality care in the clinical trial context with treatment of all detected malaria, syphilis, and symptomatic STIs/RTIs. Nonetheless, a consequential and deleterious effect was still observed – even after adjusting for the type and number of IPTp doses. This emphasizes the need to strengthen community sensitisation and public health awareness about the prevalence, consequences and prevention strategies of these infections. As both malaria and STIs/RTIs are often asymptomatic [27], universal early screening and treatment of both conditions may be warranted [26,

32], especially as point-of-care tests for STIs/RTIs are available, in addition to syphilis and HIV [33]. The importance of early syphilis screening and treatment on pregnancy outcomes has been well demonstrated [32]. A similar emphasis on early intervention is needed for other STIs/RTIs, particularly in low and middle income countries with high disease burdens.

Strength and limitation

This is the largest study to date utilising ultrasound for fetal weight estimation concurrently with in-depth testing for malaria and STIs/RTIs. High-quality obstetric ultrasound was ensured by thorough training of sonographers, review of all ultrasound images at the beginning of the study and thereafter 10% randomly selected scans – all performed by a medical doctor with extensive experience in obstetric ultrasound (CS). All anthropometric measurements were performed twice, with a third reading for discrepancies and the average of the two closest readings was considered definitive. Birthweight measured >1 hour after delivery were also adjusted for physiological weight loss [21].

However, this study also has some limitations. First, fetal weight and birthweight were converted into Z-score using the STOPPAM reference chart, as we have previously demonstrated this reference chart to be more appropriate for the setting [23]. However, a similar reference for head circumference and abdominal circumference is not available, and the INTERGROWTH-21st was therefore used for head circumference [25]. Second, previous studies indicated that malaria in either the first or second trimester might be the most detrimental [4, 5]. However, women were enrolled from the second trimester onward. Thus, malaria infections occurring in the first trimester were not accounted for, and some women may wrongly have

been classified as malaria-negative, resulting in an underestimation of the true burden. Third, miscarriage and stillbirth may be due to malaria and/or STIs/RTIs but were excluded in the analyses. Fourth, the prevalence of STIs/RTIs at enrolment were lower among the excluded women, and may represent some selection bias. Finally, some residual confounders could not be ruled out, including genetic factors. However, these are unlikely to have influenced the results as they would be expected to be relatively infrequent and balanced between study exposure groups.

CONCLUSION

Both malaria and STIs/RTIs were common and associated with poor fetal growth, especially among paucigravidae women with dual infections. Early antenatal intervention is key to reducing the dual burden of malaria and STIs/RTIs. Public health awareness campaigns against these infections are urgently needed, alongside screening for all STIs/RTIs and promoting early antenatal care-seeking, to optimise pregnancy outcomes in low and middle income countries.

Author Contributions

GM, RMC, MM, MA, DTRM, JPAL, FOtK and CS conceived and designed the study. GM, RMC, MM, HB, DTRM, QS, GRG, CM, SG, OAM, VM, KSP, HH, PM, RK, JPAL, SK, FM, JRG, MA, FOtK, and CS contributed to the data acquisition. QS, CM, HH, RK, SK, and MA coordinated the laboratory component. GM conducted the statistical analysis and wrote the first draft of the manuscript. All authors contributed to data interpretation and critical revision for important intellectual content. All authors approved the final version submitted.

Conflict of interest

All authors declare no competing interests.

Disclaimer: The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

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The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Ethical approval statement

This study was approved by independent ethics committees in Tanzania (NIMR/HQ/R.8a/Vol.1X/2533), Malawi (P.02/17/2110) and Kenya (SERU 75-3421). Individual, written informed consent was obtained prior to enrolment or any study procedure. CDC Human Research Protections Office reviewed and approved CDC participation as non-engaged.

Data sharing

Individual participant data is available from the Worldwide Antimalarial Resistance Network (WWARN) data repository.

REFERENCES

[1] WHO. WHO Guidelines for malaria - 25 November 2022. 2022. https://reliefweb.int/report/world/who-guidelines-malaria-25-november-2022

[2] Reddy V, Weiss DJ, Rozier J, Ter Kuile FO, Dellicour S. Global estimates of the number of pregnancies at risk of malaria from 2007 to 2020: a demographic study. *Lancet Glob Health* 2023; 11 (1): e40-e47. https://doi.org/10.1016/S2214-109X(22)00431-4.

[3] Desai M, Gutman J, L'lanziva A, Otieno K, Juma E, Kariuki S, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with

499 sulfadoxine-pyrimethamine for the control of malaria during pregnancy in
500 western Kenya: an open-label, three-group, randomised controlled superiority
501 trial. *Lancet*. 2015; 386 (10012): 2507-19. doi: 10.1016/S0140-6736(15)00310-
502 4.
503 [4] Briand V, Saal J, Ghafari C, Huynh BT, Fievet N, Schmiegelow C, *et al*. Fetal
504 Growth Restriction Is Associated with Malaria in Pregnancy: A Prospective
505 Longitudinal Study in Benin. *J Infect Dis* 2016; 214 (3).
506 https://doi.org/10.1093/infdis/jiw158.
507 [5] Schmiegelow C, Matondo S, Minja DTR, Resende M, Pehrson C, Nielsen BB,
508 *et al*. Plasmodium falciparum Infection Early in Pregnancy has Profound
509 Consequences for Fetal Growth. *J Infect Dis* 2017; 216 (12).
510 https://doi.org/10.1093/infdis/jix530.
511 [6] Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J *et al.* Prevention of
512 malaria in pregnancy. *Lancet Infect Dis* 2018; 18 (4): e119–e132.
513 https://doi.org/10.1016/S1473-3099(18)30064-1.
514 [7] Chico RM, Mayaud P, Ariti C, Mabey D, Ronsmans C, Chandramohan D.
515 Prevalence of malaria and sexually transmitted and reproductive tract
516 infections in pregnancy in sub-Saharan Africa: A systematic review. *JAMA*
517 2012; 307 (19). https://doi.org/10.1001/jama.2012.3428.
518 [8] WHO. Guidelines for the management of symptomatic sexually transmitted
519 infections-15 July 2021. 2021. https://www.who.int/news/item/15-07-2021-
520 launch-who-guidelines-for-the-management-of-symptomatic-sexually-
521 transmitted-infections.
522 [9] Chaponda EB, Bruce J, Michelo C, Chandramohan D, Chico RM. Assessment
523 of syndromic management of curable sexually transmitted and reproductive

tract infections among pregnant women: an observational cross-sectional study. *BMC Pregnancy Childbirth* 2021; 21 (1). https://doi.org/10.1186/s12884-021-03573-3.

[10] Osmond C and Barker DJP. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. *Environ Health Perspect* 2000;108 (3). https://doi.org/10.1289/ehp.00108s3545.

[11] Chaponda EB, Matthew Chico R, Bruce J, Michelo C, Vwalika B, Mharakurwa S, *et al.* Malarial infection and curable sexually transmitted and reproductive tract infections among pregnant women in a rural district of Zambia. *Am J Trop Med Hyg* 2016; 95 (5). https://doi.org/10.4269/ajtmh.16-0370.

[12] Chua CL, Hasang W, Rogerson SJ, and Teo A. Poor Birth Outcomes in Malaria in Pregnancy: Recent Insights Into Mechanisms and Prevention Approaches. *Front. Immunol* 2021;12. https://doi.org/10.3389/fimmu.2021.621382.

[13] Rijken MJ, De Livera AM, Lee SJ, Boel ME, Rungwilailaekhiri S, Wiladphaingern J, *et al*. Quantifying low birth weight, preterm birth and small-for- Gestational-age effects of malaria in pregnancy: A population cohort study. *PLoS One* 2014; 9 (7). https://doi.org/10.1371/journal.pone.0100247.

[14] Landis SH, Lokomba V, Ananth C V., Atibu J, Ryder RW, Hartmann KE, *et al.* Impact of maternal malaria and under-nutrition on intrauterine growth restriction: A prospective ultrasound study in Democratic Republic of Congo. *Epidemiol Infect 2009;137 (2)*. https://doi.org/10.1017/S0950268808000915.

[15] Unger HW, Ome-Kaius M, Karl S, Singirok D, Siba P, Walker *et al.* Factors associated with ultrasound-aided detection of suboptimal fetal growth in a

549 malaria-endemic area in Papua New Guinea. *BMC Pregnancy Childbirth*
550 2015;15 (1). https://doi.org/10.1186/s12884-015-0511-6.
551 [16] Madanitsa M, Barsosio HC, Minja DTR, Mtove G, Kavishe RA, Dodd J, *et al*.
552 Monthly intermittent preventive treatment with dihydroartemisinin-piperaquine
553 with and without azithromycin versus monthly sulfadoxine-pyrimethamine to
554 reduce adverse pregnancy outcomes in Africa: a randomised partially placebo-
555 controlled superiority trial. *The Lancet* 2023; 401 (10381): 1020-1036.
556 https://doi.org/10.1016/S0140-6736(22)02535-1
557 [17] Papageorghiou AT, Kennedy SH, Salomon LJ, Ohuma EO, Ismail LC, Barros
558 FC, *et al.* International standards for early fetal size and pregnancy dating
559 based on ultrasound measurement of crown-rump length in the first trimester
560 of pregnancy. *Ultrasound Obstet Gynecol* 2014; 44 (6).
561 https://doi.org/10.1002/uog.13448.
562 [18] Papageorghiou AT, Kemp B, Stones W, Ohuma EO, Kennedy SH, Purwar M,
563 *et al*. Ultrasound-based gestational-age estimation in late pregnancy.
564 *Ultrasound Obstet Gynecol* 2016; 48 (6): 719–726.
565 https://doi.org/10.1002/uog.15894.
566 [19] Altman DG and Chitty LS. New charts for ultrasound dating of pregnancy
567 *Ultrasound Obstet Gynecol* 1997; 10 (3). https://doi.org/10.1046/j.1469-
568 0705.1997.10030174.x.
569 [20] Hadlock FP, Harrist RB, Sharman RS, Deter RL, and Park SK. Estimation of
570 fetal weight with the use of head, body, and femur measurements-A
571 prospective study. *Am J Obstet Gynecol* 1985; 151 (3),
572 https://doi.org/10.1016/0002-9378(85)90298-4.
573 [21] Mtove G, Abdul O, Kullberg F, Gesase S, Scheike T, Andersen FM, *et al.*

574 Weight change during the first week of life and a new method for retrospective
575 prediction of birthweight among exclusively breastfed newborns. *Acta Obstet*
576 *Gynecol Scand* 2022;101 (3). https://doi.org/10.1111/aogs.14323.
577 [22] Schmiegelow C, Scheike T, Oesterholt M, Minja D, Pehrson C, Magistrado P,
578 *et al.* Development of a Fetal Weight Chart Using Serial Trans-Abdominal
579 Ultrasound in an East African Population: A Longitudinal Observational Study.
580 *PLoS One* 2012; 7 (9). https://doi.org/10.1371/journal.pone.0044773.
581 [23] Mtove G, Minja DTR, Abdul O, Gesase S, Maleta K, Divala TH, *et al.* The
582 choice of reference chart affects the strength of the association between
583 malaria in pregnancy and small for gestational age: an individual participant
584 data meta-analysis comparing the Intergrowth-21 with a Tanzanian birthweight
585 chart. *Malar J* 2022; 21 (1). https://doi.org/10.1186/s12936-022-04307-2.
586 [24] Visser GH, Nicholson WK, Barnea ER, Ramasauskaite D, Nassar AH. and
587 FIGO Safe Motherhood, Newborn Health Committee. FIGO position paper on
588 reference charts for fetal growth and size at birth: Which one to
589 use?. *International Journal of Gynecology & Obstetrics* 2021; *152* (2): 148-151.
590 [25] Villar J, Ismail LC, Victora CG, Ohuma EO, Bertino E, Altman DG, *et al.*
591 International standards for newborn weight, length, and head circumference by
592 gestational age and sex: The Newborn Cross-Sectional Study of the
593 INTERGROWTH-21st Project. *Lancet* 2014; 384 (9946*)*.
594 https://doi.org/10.1016/s0140-6736(14)60932-6.
595 [26] Koladjo BF, Yovo E, Accrombessi M, Agbota G, Atade W, Ladikpo OT, *et al.*
596 Malaria in the First Trimester of Pregnancy and Fetal Growth: Results from a
597 Beninese Preconceptional Cohort. *J Infect Dis* 2022; 225 (10).
598 https://doi.org/10.1093/infdis/jiac012.

[27] Zango SH, Lingani M, Valea I, Samadoulougou OS, Bihoun B, Rouamba T, *et al.* Malaria and curable sexually transmitted infections in pregnant women: A two-years observational study in rural Burkina Faso. *PLoS One* 2020; 15 (11): 1–15. https://doi.org/10.1371/journal.pone.0242368.

[28] Moeller SL, Nyengaard JR, Larsen LG, Nielsen K, Bygbjerg IC, Msemo OA, *et al.* Malaria in Early Pregnancy and the Development of the Placental Vasculature. *J Infect. Dis* 2019; 220 (9): 1425–1434. https://doi.org/10.1093/infdis/jiy735.

[29] Mcclure EM, Meshnick SR, Lazebnik N, Mungai P, King CL, Hudgens M *et al.* A cohort study of Plasmodium falciparum malaria in pregnancy and associations with uteroplacental blood flow and fetal anthropometrics in Kenya. *Int J Gynecol Obstet* 2014;126 (1). https://doi.org/10.1016/j.ijgo.2014.01.016.

[30] Cheah FC, Lai CH, Tan GC, Swaminathan A, Wong KK, Wong YP, *et al.* Intrauterine Gardnerella vaginalis Infection Results in Fetal Growth Restriction and Alveolar Septal Hypertrophy in a Rabbit Model. *Front. Pediatr* 2020; 8. https://doi.org/10.3389/fped.2020.593802.

[31] Vedmedovska V, Rezeberga D, and Donder GG. Is abnormal vaginal microflora a risk factor for intrauterine fetal growth restriction?. *Asian Pacific J Reprod* 2015; 4 (4). https://doi.org/10.1016/j.apjr.2015.07.010.

[32] Hawkes SJ, Gomez GB, and Broutet N. Early Antenatal Care: Does It Make a Difference to Outcomes of Pregnancy Associated with Syphilis? A Systematic Review and Meta-Analysis. *PLoS One* 2013; 8 (2). https://doi.org/10.1371/journal.pone.0056713.

[33] Gao R, Liu B, Yang W, Wu Y, Wang B, Santillan MK, *et al.* Association of Maternal Sexually Transmitted Infections with Risk of Preterm Birth in the

United States. *JAMA Net Open* 2021. https://doi.org/10.1001/jamanetworkopen.2021.33413.

TABLES AND FIGURE LEGENDS

Tables

Supplementary figure and tables