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Active case detection and treatment of malaria in pregnancy using LAMP technology (LAMPREG): a pragmatic randomised diagnostic outcomes trial—study protocol

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ABSTRACT

Introduction Malaria is one of the major public health problems in sub-Saharan Africa. It contributes significantly to maternal and fetal morbidity and mortality in affected countries. This study aims to evaluate the impact of enhanced case detection using molecular testing called loop-mediated isothermal amplification (LAMP) on birth outcomes in a prospective study design.

Methods and analysis A pragmatic randomised diagnostic outcomes trial will be conducted in several health institutes in different Ethiopian regions. Women (n=2583) in their first and second trimesters of pregnancy will be included in the study and individually randomised to the standard of care or enhanced case detection arms, and followed until delivery. Enrolment will encompass the malaria peak transmission seasons. In the standard of care arm, a venous blood sample will be collected for malaria diagnosis only in symptomatic patients. In contrast, in the intervention arm, mothers will be tested by a commercially available Conformité Européene (CE)-approved LAMP malaria test, microscopy and rapid diagnostic test for malaria regardless of their symptoms at each antenatal care visit. The primary outcome of the study is to measure birth weight.

Ethics and dissemination The study was approved by the following ethical research boards: Armauer Hansen Research Institute/ALERT Ethics Review Committee (FORM AF-10-015.1, Protocol number PO/05/20), the Ethiopia Ministry of Science and Higher Education National Research Ethics Review Committee (approval SRA/11.7/7115/20), the Ethiopia Food and Drug Administration (approval 02/25/33/I), UCalgary Conjoint Health Research Ethics Board (REB21-0234). The study results will be shared with the institutions and stakeholders such as the Ethiopia Ministry of Health, the Foundation for Innovative Diagnostics, WHO's Multilateral initiative on Malaria - Tropical Diseases Research (TDR-MIM), Roll Back Malaria and the Malaria in Pregnancy Consortium. The study results will also be published in peer-reviewed journals and presented at international conferences.

Trial registration number NCT03754322.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the largest prospective outcomes-based study of its kind to examine the impact of molecular testing of malaria in pregnancy in the absence of IPT.
⇒ Low malaria positivity rate can minimise the impact of the study, and the timing of the study with the malaria peak in seasonal study sites is paramount. Placental damage occurs early in pregnancy, and the optimal study impact will occur for women included in the first trimester of pregnancy.
⇒ Loop-mediated isothermal amplification (LAMP) technology does not differentiate the sexual and asexual stages of the parasite.
⇒ C

INTRODUCTION

According to the WHO, about 229 million malaria cases were reported and approximately 435 000 deaths resulted from the disease globally in 2019.[1] The sub-Saharan region experienced around 92% of these deaths. Mortality is concentrated around several high-risk groups, including pregnant women and infants. In Africa, 33 million women living in malaria-endemic areas become pregnant each year, among which 35% are exposed to malaria during pregnancy. Malaria is caused by the eukaryotic parasite *Plasmodium*, and is transmitted through *Anopheles* mosquito bite. The parasites undergo a complex development cycle in the human host. The intraerythrocytic asexual replication phase of the cycle originates the symptoms and severity of the disease. Thus, the severity of malaria infection varies according to the *Plasmodium* sp (with *P. falciparum* and *P. vivax* originating the most severe cases), parasite variants expressed during the infection and the host immunity. Indeed, in endemic areas, individuals acquired a protective immunity during childhood, and most of the adults present pauci-symptomatic or asymptomatic

form of the diseases. On the other hand, the most vulnerable populations are children under five, individuals in unstable transmission areas (thus not maintaining protective immunity) and pregnant women.

Malaria in pregnancy (MiP) is primarily caused by *Plasmodium falciparum*. During pregnancy, the development of a new organ, the placenta, favours the emergence of a specific *P. falciparum* variant (VAR2CSA) that adheres to the placental chondroitin sulfate A.[2–4] This sequestration impairs the exchange between the mother and the fetus and protects the parasite from the spleen clearance.[2 5 6] As a result, MiP increases the risk of maternal death, miscarriage, stillbirth and neonatal death.[2 7] In areas of seasonal malaria transmission, pregnant women are three times more likely to suffer from severe malaria than their non-pregnant counterparts.[4 8]

Furthermore, MiP leads to adverse outcomes for the mother and infant, especially anaemia and birth weight.[4 9] In 2019, a total of 822 000 low birthweight (LBW) newborns were linked to MiP.[1] In sub-Saharan Africa, anaemia reportedly accounts for about 20% of all maternal deaths.[10] Regarding LBW outcomes, around 19% of infant LBWs are due to malaria and 6% of infant deaths are due to LBW caused by malaria.[7] LBW is thought to be the single most significant risk factor for neonatal and infant mortality.[7] In addition, LBW and anaemia associated with MiP are predictors of poor outcomes in infancy for the child.[11–15] Therefore, MiP affects the preceding generation and the next, impairing cognitive development[16] and thus economic productivity.

MiP prevention relies either on the systematic administration of intermittent preventive treatment (IPT) during the second and third trimesters of pregnancy to reduce the parasitic burden in pregnant women,[1] or 'screen and treat' strategies (detection and treatment of positive cases in women). IPT has proven its efficacy; however, this strategy comes at a cost since empirical use of antimalarial drugs contributes to drug resistance and may lead to side effects.[17–20] In addition, the WHO estimated that only 62% of women receive at least one dose of IPT and 34% at least three doses during antenatal care (ANC).[1] At this current IPT coverage, an estimate of 408 000 LBWs were averted in 2020, but the impact of IPT event is still suboptimal to reduce MiP burden. Furthermore, IPT administration is not possible in the first trimester of pregnancy since sulfamide drugs are contraindicated due to the risk of neural tube malformation. Hybrid strategies are also in place in some settings (screen and treat in the first trimester coupled with IPTp starting at week 24[21 22]). Additionally, screen and treat strategies are limited by the sensitivity of the diagnostic techniques currently available.

Malaria diagnostics rely on parasite visualisation using stained blood smears observed by light microscopy.[23–26] The microscopist identifies parasitic forms within the red blood cells. In experienced hands, the limit of detection is about 50 parasites/µL (p/µL),[23] and 200 p/µL in most settings. The main alternative to microscopy is rapid diagnostic test (RDT). RDT is an antigen test detecting parasitic proteins in the patient's blood within 10–20 min.

Nevertheless, both microscopy and RDT present a relatively high limit of detection (200 p/µL), and are thus missing low-level infections.[27] Importantly, RDTs face parasite 'escape' to diagnostic with the emergence of *Plasmodium* strains that present a deletion in RDT main target, the protein HRP-2,[28–31] leading to false-negative test results. In the past 10 years, molecular-based techniques for malaria diagnostics have emerged as an alternative for sensitive malaria detection. Molecular-based techniques, or nucleic acid tests, rely on amplifying and detecting specific DNA or RNA sequences using DNA and RNA polymerases. PCR and RT-PCR techniques are highly sensitive (Limit of detection (LOD) <0.1 p/µL[32]), but not practical for diagnostics due to the requirements in equipment, reagents and the delays between sample collection and diagnostics. Therefore, in the current practice, these techniques are reserved for epidemiological surveys and research projects. Isothermal approaches, such as loop-mediated isothermal amplification (LAMP), provide a practical, rapid and cost-efficient alternative for malaria diagnostic using molecular techniques.

LAMP technology brings the sensitivity of molecular methods at the field level. The LAMP test is a nucleic acid amplification method that relies on a strand-displacement DNA polymerase which also retains reverse transcriptase activity. The principle of this method is that no denaturation of the DNA template is required, and thus the LAMP reaction can be conducted under isothermal conditions.[33] It is low cost, requires little electricity, provides rapid results and can be performed by minimally trained health workers.[34] Studies have found that LAMP has comparable sensitivity and specificity to PCR, and is superior to microscopy and RDTs.[35] LAMP is a potential point-of-care test (POCT) that provides an alternative to microscopy and RDTs.[36–38] It is a molecular method, which in comparison to PCR is cheaper, simpler and faster, overcoming three major disadvantages of PCR. The method can detect parasitaemia as few as 1 p/µL of blood, below the detection limit of microscopy or RDTs.[12 13]

In previous cross-sectional studies conducted by our group, LAMP proved to be of higher sensitivity and up to 30% greater detection of malaria in pregnant women who are symptomatic in comparison with RDTs and microscopy.[36 37] In addition, pilot studies have shown the benefits of MiP active detection using LAMP and treatment in terms of birth outcomes.[39]

The LAMP for PREGnancy (LAMPREG) study proposes a pragmatic randomised diagnostic outcomes trial using a highly sensitive LAMP technique to detect more asymptomatic *Plasmodium* infections in pregnant women. This consequently results in the early treatment of pregnant mothers and may avert maternal and fetal morbidity and mortality. The meaningful impact will be measured by determining health outcomes for the mother and child, especially reducing infant anaemia and improving birth weight. This study is of particular importance in Ethiopia,

where IPT is not used for pregnant women, and therefore, accurate screening is paramount.

METHODS AND ANALYSIS

Study area

The study will be conducted at health institutions (health centres and hospitals) across three regions of Ethiopia. Six study sites will be initiated across three regions, namely Amhara, Gambella, and Southern Nations, Nationalities and Peoples' (SNNP) regions.

Study design

The LAMPREG study is a prospective randomised diagnostic outcome trial. The study is an international collaboration from the University of Calgary, Alberta, Canada, and Armauer Hansen Research Institute (AHRI), Jimma University and Amhara Public Health Institute in Ethiopia.

The goal is to determine whether: (1) LAMP provides a clinically measurable benefit compared with the current first-line diagnostic test of Giemsa-stained microscopy, and whether (2) enhanced case detection of asymptomatic mothers with LAMP has added value in terms of outcomes. Symptomatic and asymptomatic first and second-trimester mothers will be included in the study and individually randomised to one of two arms: standard of care (SOC) or enhanced case detection arm using LAMP for malaria. Mothers will be enrolled at the first ANC visit and then followed during the ANC visits until the delivery day, and the newborn will have a follow-up of 28 days after delivery (table 1).

Mothers randomised to the SOC arm will be tested for *Plasmodium* infection by either microscopy or RDT only when they exhibit at least one of the following symptoms of malaria : fever, chills, rigours, sweating, severe headache, generalised body and joint pain, or pallor, as per the Ethiopia Food Medicine and Health Care Administration and Control Authority. In the intervention arm, mothers, whether symptomatic or asymptomatic, will be tested by a commercially available CE-approved LAMP malaria test (Human Diagnostics LoopAMP (Wiesbaden, Germany)) at each ANC visit in addition to RDT and microscopy. The purpose of doing all tests in the intervention arm is to determine how many additional cases LAMP identified. Haemoglobin (Hgb) will be measured at each ANC visit for mothers randomised to both arms. Blood samples in the form of dried blood spots and aliquots will be collected in both groups for storage a table 1.

Source population

All mothers presenting to the study sites (health centres or hospital) in Gambella, SNNP and Amhara regions to start ANC follow-up. Women are compensated for travel cost to improve protocol adherence.

Table 1 Study intervention on pregnant women, following the SPIRIT guideline recommendations

	Study period							
	Enrolment	Randomisation	Postrandomisation					
Time point	Screening	0	ANC1 (0–13 weeks+6 days)	ANC2 (14–27 weeks+6 days)	ANC3 (28–35 weeks+6 days)	ANC4 (36–38 weeks)	Delivery	28-day newborn assessment
Enrolment								
Informed consent	X							
Eligibility screening	X							
Randomisation		X						
Arms								
Standard of care (S)		◆	—	—	—	—	—	◆
Intervention (I)		◆	—	—	—	—	—	◆
Assessments								
Obstetric ultrasound	S, I							
Haemoglobin test			S, I	S, I	S, I	S, I	S, I	
Malaria test with microscopy			I, S*	I, S*	I, S*	I, S*	I, S*	
Malaria test with RDT			I	I	I	I	I	
Malaria test with LAMP			I	I	I	I	I	
Sample storage			S, I	S, I	S, I	S, I	S, I	
Newborn assessment							S, I	S, I
Newborn weight							S, I	S, I
Placenta weight							S, I	
Placenta blood smear							S, I	

*Only women symptomatic in the standard of care arm.
ANC, antenatal care; LAMP, loop-mediated isothermal amplification; RDT, rapid diagnostic test; SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials.

Study period

Inclusions started in July 2021 and encompass the malaria peak season (September to December) in the sites where malaria transmission is seasonal. Inclusion period can be extended in sites where malaria transmission is year round to achieve the target sample size.

Study population

The study population will comprise pregnant women who fulfil the following inclusion criteria.

Eligibility criteria

- First or second-trimester pregnancy.
- Can provide consent to participate in the study.
- Agrees to come back to the clinic for ANC follow-up, delivery and newborn check-up at day 28 postdelivery.
- Ages 18 years and above.

Exclusion criteria

- Antimalarial medication intake (currently taking or history of intake in the past 3 weeks prior to date of screening).
- Signs and symptoms of severe malaria.
- Labelled to have a high-risk pregnancy as per the Ethiopia Ministry of Health guidelines.[40]
- Severe anaemia (Hgb is 70g/L or less).
- Ultrasound shows multiple pregnancy.

Randomisation procedure

Pregnant mothers presenting to the ANC clinics will be randomised to either SOC arm or intervention arm using a sequence implemented in REDCap electronic data capturing system version 8.11.5 (https://www.project-redcap.org/) and managed by AHRI. In addition, randomisation will be stratified by parity since women in their first pregnancy are at higher risk of placental malaria, and that previous exposures to specific parasitic variants during pregnancy can have a protective effect.[3 41 42] Participants are randomised by the data managers, and enrolled by the study nurses and medical officer in each study site.

Variables

Primary outcome

- Proportion of newborns with LBW (<2500 g).

Secondary outcome (dependent variables)

- Actual birth weight (gram).
- Small for gestational age (birth weight below 10th percentile).
- Maternal Hgb during pregnancy and at delivery.
- Fetal Hgb.
- Fetal loss (stillbirths, miscarriage, abortion).
- Premature births (<37 weeks of gestation).
- Neonatal death.
- Signs of *Plasmodium* infection: current (with the presence of asexual parasites in placental blood) or past infection (presence of malaria pigment in the placenta blood).

A composite outcome of these variables will also be analysed.

Other endpoints

Performance of LAMP, RDT and microscopy, compared with qRT-PCR (gold standard) including sensitivity, specificity and positive and negative predictive values, will be evaluated. Signs of *Plasmodium* infection either current (with presence of asexual parasites in placental blood) or past infection (presence of malaria pigment in placental blood) will be evaluated by placental blood smear.

Subgroups to be analysed

Intention to treat (*ITT*): All randomised study subjects. This will be considered the primary population for the analysis.

Per protocol (*PP*): All randomised study subjects completing the whole study period (complete cases). For a specific analysis, study subjects with missing data on any of the variables in the model will be excluded from the analysis. Analyses of this population are seen as a sensitivity analysis to investigate whether conclusions are sensitive to assumptions regarding the pattern of missing data.

The following subgroups (both ITT and PP populations) will be analysed independently:

- Primiparous mother.
- Asymptomatic malaria: women who did not present symptoms at any ANC visit but were detected positive for *Plasmodium* spp infection by any diagnostic method.
- Submicroscopic malaria: any women diagnosed positive for malaria by molecular method only (LAMP or post hoc gold-standard RT-qPCR).
- Malaria species identified: *P. falciparum*, *P. vivax* and mixed infections (three subgroups).
- Women who present a positive malaria testing in placental blood at delivery.
- Women who deliver an LBW newborn.

Sample size

The sample size calculation for this study is based on detecting a difference in the proportion of deliveries with a LBW, which was felt to be the most clinically important outcome variable. The prevalence of deliveries with a LBW in Ethiopia was estimated to be 17.3%. An alpha of 0.05 was selected with an allocation ratio of 3:1 in favour of the enhanced case detection arm to maximise the impact of the intervention. The study will be powered to detect an absolute difference in the proportion of deliveries with an LBW of 5%, so a proportion in the enhanced case detection arm of 12.3% compared with 17.3% in the SOC arm. Using a continuity correction and an attrition rate of 20%, a total sample size of 2583 is required to achieve 80% power.

Data collection and laboratory methods

Sociodemographic and clinical data

Sociodemographic characteristics and clinical data (obtained from medical history and physical examination) will be collected using the uniformised case report forms.

Clinical examination

At inclusion, portable obstetric ultrasound is performed by a trained medical officer to assess gestational age (Clarius C3, Clarius, Canada). Women's height and weight are recorded at each visit using standard scales available in the health centres. Newborn weight is recorded during the head-to-toe assessment, at delivery and before first feeding. Digital scales dedicated to the study were implemented in the health centres to ensure uniform measurement of newborn weight.

Laboratory procedures

Whole blood is collected from all included women at each antenatal visit. Microscopy procedures are performed as per the Ministry of Health recommendations, using 10% Giemsa smears, and the microscopist will examine 8000 white blood cells before calling a slide negative. The RDTs used will be the Core Pan/Pf/Pv that detects malaria at the species level. All the commercial tests are performed according to the respective manufacturer's instructions. Maternal and newborn Hgb are measured directly in the health centres using POCT Hgb device (HumaMeter (HumanDE, Germany) and HemoCue (HemoCue, Sweden)). The LAMP test used in this study is HumaLoop (Pan, Pf, Pv, HumaLoop Eiken, Japan). The 'Pan' test (which detects all *Plasmodium* spp) is first performed, followed by the species-level test if positive. Each test includes positive and negative controls for quality assurance reasons.

At delivery, placenta blood will be collected from the maternal side of the placenta. Microscopy will be performed on placenta blood to assess the presence of malaria pigment. Table 1 summarises the procedure performed at each study visit. Importantly, placenta blood will be preserved for malaria screening using LAMP technology and reference techniques. The preserved blood aliquots will be processed using gold-standard techniques to assess the true burden of malaria in the study population.

Data management

Raw data will be entered in REDCap electronic data capturing system version 8.11.5 (https://www.project-redcap.org/) and managed by AHRI. Data entry and data management procedures were developed, and only authorised users have access to the database. Data entry procedures are subject to validation by the data manager, under the principal investigator's responsibility.

Statistical analysis plan

The sensitivity, specificity, predictive values and kappa coefficient will be determined using SISA online statistical software. We will use STATA V.15, SPSS 28.0.1.1 and the R package hreport for data interpretation. The sensitivity, specificity and predictive values will be calculated for microscopy and LAMP versus the gold standard of PCR. Statistical analysis of epidemiological variables and malaria positivity will be determined using univariate binomial regression. Variables found to be statistically significant with initial analysis will then be characterised through multivariate regression analysis. Risk ratios for primary outcome variables for intervention against SOC arm will be calculated. Subgroup analysis will be done for potential confounders, and to compare positivity by LAMP and microscopy in the intervention arm. Interim analyses are planned at 25% of the inclusion target and 25% of the completion target. The principal investigator makes the final decision to terminate the trial. The complete statistical analysis plan will be uploaded in the trial registry.

Patient and public involvement

No patient was involved.

ETHICS AND DISSEMINATION

Ethics

The study was approved by the following ethical research boards: AHRI/ALERT Ethics Review Committee (FORM AF-10-015.1, Protocol number PO/05/20), the Ethiopia Ministry of Science and Higher Education National Research Ethics Review Committee (approval SRA/11.7/7115/20), the Ethiopia Food and Drug Administration (approval 02/25/33/I), UCalgary Conjoint Health Research Ethics Board (REB21-0234). These approvals are subject to proper study conduct and will be renewed on a yearly basis until the study's completion. Protocol amendment will be submitted to the ethical research boards. Pregnant women's safety was the priority during the study design, including the severe adverse event monitoring after treatment for positive women. The study inclusion procedure was designed to protect women who are illiterate by using a third person, independent of the study staff, as a witness during the procedure. All the study staff will be trained in Good Clinical Practice—ICH6. Personal information collected during the study is stored in locked cabinets under the responsibility of the principal investigator. Only study numbers are used in downstream analysis.

Dissemination

The study results will be shared with all the investigators and their institutions, through local symposiums involving faculty, students and staff. The study results will be shared with stakeholders such as the Ethiopia Ministry of Health, the Foundation for Innovative Diagnostics, WHO's Multilateral initiative on Malaria - Tropical Diseases Research (TDR-MIM), Roll Back Malaria and the MiP Consortium. The study results will also be published in peer-reviewed journals and presented at international conferences.

Notably, study outcomes will be summarised in pamphlets and disseminated in the communities through field extension workers.

DISCUSSION

Thirty-three million women were at risk of MiP in 2019. MiP impacts newborns' health and pregnancy outcomes, including birth weight, miscarriage and stillbirth.[2 43] In addition, there are long-term effects in children born from infected mothers, including increased susceptibility to severe malaria and infections.[2 15 16] Additionally, MiP impairs neurodevelopment and cognitive development.

The emergence of multi-drug resistant *Plasmodium* strains threatens current MiP prevention strategies based on IPT. Importantly, IPT does not cover the first trimester of pregnancy, while infections before and in the first trimester affect birth outcomes.

LAMPREG aims to measure the impact of active case detection of malaria in pregnant women attending ANC in two regions of Ethiopia. In addition, LAMPREG will measure birth outcomes in women offered active detection of *Plasmodium* infection compared with the SOC. Notably, the samples preserved during LAMPREG will be assessed *a posteriori* for the actual burden of *Plasmodium* infection in the study populationand presence of parasitic variants that may affect birth outcomes.

The impact of LAMPREG will be considerable in our estimation. The ability of LAMP technology and other molecular diagnostic tools to provide highly sensitive testing to remote health centres has the potential to be a turning point in the prevention of MiP.

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Contributors RFG and CK drafted the manuscript. RFG, CK, ZMB, MT, BT, DY, AGB and DRP revised the manuscript. AGB and DRP designed the study. RFG, ZMB, BT and DY organised the study in the field. RFG, ZMB, MT, BT, DY and AGB implemented the study in the field. RFG, ZMB, BT and DY are study coinvestigators. DRP, AGB and MT are study investigators. DRP is the principal investigator, conceived the study and obtained the funding. All members of the LAMPREG group have substantially contributed to the conception, design or organisation of the study. All authors approved the final version.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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