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The question of the early diagnosis of asymptomatic and subpatent malaria in pregnancy: Implications for diagnostic tools in a malaria endemic area

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ABSTRACT

Objectives: Malaria in pregnancy (MIP) is a major healthcare challenge in low-income countries with high malaria endemicity. Early but accurate diagnosis and appropriate treatment is the hallmark of preventing disease progression/adverse outcomes in the mother, foetus and neonates. We assessed the comparative diagnostic performance of Malaria Rapid Diagnostic Test (mRDT), microscopy and PCR for malaria diagnosis in pregnant women for early detection of asymptomatic malaria in pregnant women.

Study design: Five hundred and twenty Pregnant women attending study clinics within Ikene and Remo North LGAs with gestational age between 16 and 29 weeks, willing and consented; were enrolled into the study. Blood samples collected via venepuncture were screened for malaria using microscopy, mRDTs kits, and PCR techniques on their first visit (V_1) and at delivery. The parasite positivity rates, sensitivity and specificity were calculated and compared for each technique using PCR as the standard. Data was entered into REDCap® online database and analysis done using Stata and MedCalc®.

Results and conclusions: Average age of enrolled women was 28.8 years and mean gestational age was 21.0 weeks. The parasite positivity rates were 4.3%, 8.8% and 25.0% for microscopy, mRDT and PCR at V_1 and was 2.4%, 3.4% and 43.4% at delivery, respectively. Sensitivity for microscopy and mRDT was 11.2% and 30.3% respectively at V_1, while specificity was 98.2% and 98.5%. At delivery, the sensitivity reduced to 1.6% and 4.9%; while specificity was 96.9% and 97.6% respectively. Only 2.3% cases correlated with all three diagnostic methods. Our data showed a decrease in sensitivity of the diagnostic methods as pregnancy progressed, which may be due to very low parasitaemia, but high specificity. Our study demonstrated a high rate of subpatent parasitaemia amongst pregnant women. This finding therefore raises the question of the effect of subpatent parasitaemia on the health of the mother and foetus.

Introduction

Malaria in pregnancy (MIP) is still a major healthcare challenge in countries where there is high transmission of malaria disease. Nigeria with a large population of women within their reproductive age, has the highest infection, transmission, and at-risk population for malaria globally [1–3]. MIP presents potential health risks and adverse pregnancy outcomes to mother, foetus and neonate [4–7].

Asymptomatic malaria often defined as the detection of asexual or sexual parasites in the blood of patient, with absence of acute clinical

Abbreviations: MIP, Malaria in pregnancy; mRDT, Malaria Rapid Diagnostic Test; PCR, polymerase chain reaction; IPTp-SP, intermittent preventive therapy in pregnancy using sulfadoxine – pyrimethamine; CRF, case report forms; ACT, Artemisinin Combination therapy; LBW, low birth weight; IUGR, intra-uterine growth retardation.

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symptoms of malaria during a specified period [8,9]. Subpatent malarial occurs when the parasite levels in asymptomatic individuals becomes so low, (usually from less than 100 parasites/μL to >10 parasites/μL of blood), making it extremely difficult for the parasites to be detected by microscopy or malaria rapid diagnostic kits (mRDT), but only through PCR [10,11]. This is practically impossible in many Nigeria antenatal care (ANC) settings. This issue needs urgent attention as a recent global review showed that submicroscopic MIP is present in every malaria transmission setting [12].

Malaria diagnosis in poor resource settings is highly dependent on symptomatic presentation of subjects at clinics. Accurate parasitological diagnosis is a big challenge especially in local ANC clinic due to lack of requisite technology and expertise to make diagnosis. Malaria Rapid diagnosis test kits (mRDT) are often used as alternatives but have several limitations and sometimes unable to detect parasites at very low densities in cases of asymptomatic and subpatent malaria [13–15].

To mitigate against the adverse effects of MIP, WHO recommends the use of sulphadoxine-pyrimethamine intermittent preventive treatment of malaria in pregnancy (IPTp-SP) from second trimester of pregnancy [16,17]. The use of IPTp-SP has been shown to significantly reduce the adverse events associated with MIP [4, 5, 16, 18].

The use of IPTp-SP coupled with the reduced immunity occasioned by pregnancy, increases the number of MIP patients with asymptomatic or subpatent parasitaemia especially if the causative organism is *P. falciparum* [10, 19–21]. Asymptomatic infections may also result from residual or recrudescent parasitaemia from earlier clinical episodes and may become full blown malaria [8,22]. This may result in trans-placental transmission of parasites just before or during delivery [23] resulting in possible neonatal malaria. This study therefore assesses the comparative performances of microscopy, mRDT and PCR in diagnosing asymptomatic MIP within an endemic malaria community and investigate the possible correlations in asymptomatic and subpatent malaria.

Materials and methods

Study site and patients' enrolment

The Study was a prospective, observational carried out in five centres within Ikenne and Remo North Local Government areas in Ogun State, South-western. It was part of a larger study on the use of sulphadoxine-pyrimethamine (SP) in the management of MIP in Southwest Nigeria. The study sites were: Babcock University Teaching Hospital, Ilishan Remo (BUTH), the study focal site, Ilishan Community Hospital (ICH), Ikenne Primary Health Care Centre (IKPHC), Christ Apostle Church Spiritual and Healing Maternity Home (CAC) and Isara General Hospital (ISH). The study was conducted from June 2018 to December 2020.

Inclusion and exclusion criteria

Pregnant women in their first trimester but not exceeding 29 weeks at first visit. They should not have taken the SP drug prior to enrolment and have no other symptoms apart from fever, or other common symptoms of malaria such as headache, chills and weakness; absence of other concomitant illnesses, such as complicated malaria, respiratory tract infection, urinary tract infection, HIV or other viral infections.

Collection of samples

All recruited women were clinically examined by qualified medical personnel. Baseline data on socio-demographic, obstetric, medical history and history of last malaria episode were collected. Subjects were screened for malaria parasites on their first visit (at enrolment; V_1) and at delivery. Blood (5mls) were collected via venepuncture into EDTA treated blood collection bottle. From the collected blood, 5 μL was used for mRDT testing, another 15 μL was used to prepare the thick and thin for microscopy. The remainder of the blood was sent for PCR analysis.

Parasitological analysis

Rapid diagnostic testing was done using SD-Bioline PfHRP2 RDT kit (Standard Diagnostics Inc, Geonggi-do, Republic of Korea) according to the manufacturer's instructions. Quality assurance on the mRDT kits were monitored at intervals during the study.

Thick and thin film slides were prepared using the WHO standard protocol for smear preparation of microscopy [24]. Two WHO certified microscopists independently read the stained slides. The parasite densities for malaria positive slides were determined using thick films and calculated using the WHO protocol and assuming a leucocyte count of 8000/uL. Quality assurance for the microscopy was carried out in the Malaria laboratory, Institute for Advanced Medical Research and Training, University of Ibadan, Ibadan, Oyo state, Nigeria by WHO certified malaria microscopists.

Polymerase chain reaction was done after Genomic DNA was extracted using QIAamp DNA Mini kit (Qiagen Hilden, Germany), according to manufacturer's instructions. A nested PCR approach targeting the 18SrRNA gene was adopted to detect the presence of *P. falciparum* as previously described [25,26].

Data collection and analysis

The sample size was determined using the Leslie-Kish formula for single proportions [27]: $N = z^2pq/d^2$. Where N = desired sample size; z = the standard normal deviation corresponding to 95% confidence level i.e., 1.96; p = *P. falciparum* infection prevalence 42.7% [28] q = 1- p and d = degree of accuracy, set at 0.05. Therefore, N = 376, 20% addition for loss to follow-up = 75. Total required sample size is 451.

Data collection and analysis

All data were collected using specifically designed case report forms (CRFs) and entered into REDCap® online database [29,30]. The geometric means of malaria positive slides and percentage positivity were calculated. The sensitivity, specificity, positive predictive and negative predictive values of each of the diagnostic methods was determined using PCR reference standard. Asymptomatic malaria was also assessed for all positive samples. Data was analysed using Stata® 17, MedCalc® and Microsoft Excel® 2019. Statistical significance was set at p-value < 0.05.

Ethical considerations

A community approval was obtained for the study in the town hall meeting without prejudice to written informed consent for each participant or her hubby/guardian for patients less than 18 years. Due to cultural norms, as much as was possible consent was also obtained from spouses either directly or by telephone. The study was approved by Babcock University Research and Health Ethical Board.

Clinical care

Each client was assessed for malaria. If positive, they were treated with artemisinin combination therapy. If negative, they were placed on IPTp-SP and requested to come for follow-up visits monthly until delivery. At childbirth, blood samples were again assessed for malaria parasites. Appropriate treatments were given when necessary. Standard University of Babcock Teaching Hospital safety measures for the care of patients was observed.

Results

Enrolment and baseline characteristics

A total of 520 pregnant were enrolled into the study, however 359 (69%) completed the study. Average age of participants was 28.8 years, BUTH had statistically significant higher age ($p < 0.0000$), while CAC and IKPHC had significantly lower age. Estimated mean gestational age was 21 weeks, BUTH had statistically significant lower gestational age ($p < 0.0000$) while CAC and IKPHC had significantly higher value (Table 1). The use of malaria prevention interventions was very low amongst the pregnant women as only 17.5% reportedly slept under insecticide treated nets (ITN). This was similar across site, except IKPHC having more participants use ITN. More participants (48.8%) used indoor insecticide spray compared to ITN (table1). The baseline demographics was fairly similar across all sites. This was significant because our study was carried different healthcare-level facilities, showing a homogeneity of patients enrolled across sites.

Obstetrics characteristics showed that 32.0% of the enrolees were primigravida, while 68.0% were multigravida. No statistically significant difference was observed in malaria infections between primigravida vs multigravida women at V_1 ($p = 0.681$, Chi2) and at delivery ($p = 0.641$, Chi2) using microscopy, mRDT and PCR. Further analysis showed no difference in the primigravida group ($p = 0.422$, Chi2) of V_1 vs Delivery and multigravida group ($p = 0.631$, Chi2) at V_1 vs Delivery respectively, using the three diagnostic methods (Table 2). At term, 69.0% of the women delivered at the study sites. The median gestational age at delivery was 38.5 weeks. The average number of IPTp-SP taken during pregnancy was 4.0 while 22.0% reported using ACTs at least once during the study.

Clinical malaria outcomes

At enrolment 88 (16.9%) participants reported to having one or more malaria symptoms in the last 14 days. The commonly reported symptoms were headaches [67] and followed by fever [24], although none had axillary body temperature above 38^0 C during the study (Table 2). More symptoms were reported at enrolment (31.5%) than at delivery (3.6%) and this was statistically significant (Table 2). There was a larger percentage of positive asymptomatic clients at delivery (95.0%) than at V_1 (71.1%) using all diagnostic methods, this was also statistically significant ($p < 0.0000$). A higher number of subpatent parasitaemia were recorded at delivery [5] compared to enrolment [2], but this was not statistically significant.

Parasitological assessment

At enrolment, positive parasitological diagnosis of malaria was low for microscopy 22 (4.3%) and mRDT 46 (8.8%), but higher for PCR 129 (25%). This was further reduced at delivery to 7 (2.4%) and 10 (3.4%) for microscopy and mRDT respectively (Table 3). The reverse was the case for PCR, with a greater of percentage positivity at delivery 134 (43.4%) than at enrolment (Table 3). The positive diagnosis correlation amongst the diagnostic was low; especially for microscopy and mRDT at V_1 and delivery. Microscopy and PCR diagnostic correlation was also very low, 3 cases at V_1 and 1 case at delivery (Table 3). The mRDT vs

Table 1
Baseline Maternal Characteristics at Enrolments (Visit 1) and Delivery Details.

Characteristics Visit 1	BUTH n (%) or mean (SD)	CAC n (%) or mean (SD)	ICH n (%) or mean (SD)	IKPHC n (%) or mean (SD)	ISH n (%) or mean (SD)	Total n (%) or mean (SD)
Enrolment per site (%)	232 (44.6)	92 (17.7)	75 (14.4)	70 (13.5)	51 (9.8)	520
Mean Age at enrolment ± SD (years)	30.4 ± 4.40	27.0 ± 5.90	28.2 ± 5.69	26.9 ± 5.58	28.0 ± 6.14	28.8 ± 5.43
p-value	0.0000[a]	0.0037[b]	0.354	0.0052[c]	0.3684	0.7530
Range (years)	20–45	14–42	16–42	16–38	19–41	14–45
Mean estimated Gestation Age at enrolment ± SD (weeks)	19.7 ± 3.08	22.3 ± 3.63	21.2 ± 3.37	21.2 ± 3.89	23.6 ± 3.38	21.0 ± 3.61
p-value	0.0000[d]	0.0008[e]	0.5599	0.6032	0.0000 [f]	0.884
Range of Gestation Age (weeks)	15–28	16–29	16–28	16–29	16–28	15 –29
Gravidity						
Primigravida	62 (27.0)	35 (38.5)	25 (33.8)	30 (42.9)	13 (25.5)	165 (32.0)
Multigravida	168 (73.0)	56 (61.5)	49 (66.2)	40 (57.1)	38 (74.5)	351 (68.0)
p-value	0.169	0.225	0.756	0.070	0.341	0.053
Malaria Preventive Measures						
ITN Usage	34 (14.7)	15 (16.3)	13 (17.3)	21 (30.0)	8 (15.7)	91 (17.5)
Home Insecticide Usage last 1 Month	122 (54.7)	36 (39.1)	34 (45.9)	31 (44.3)	26 (51.0)	249 (48.8)
Delivery Details	**BUTH**	**CAC**	**ICH**	**IKPHC**	**ISH**	**Total**
Number Deliveries (per % enrolled)	181 (78.0)	54 (58.7)	55 (73.3)	37 (52.9)	32 (62.7)	359 (69.0)
p-value	0.311	0.379	0.751	0.212	0.685	0.348
Mean Estimated Gestation Age at Delivery ± SD (weeks)	38.1 ± 2.90	39.1 ± 2.00	38.3 ± 2.31	39.5 ± 1.74	39.0 ± 1.90	38.5 ± 2.56
p-value	0.1570	0.3335	0.2500	0.1347	0.2952	0.3928
Min - Max Estimated Gestation Age at delivery (weeks)	22–41	33–43	32–44	32–42	35–42	22–44
Mean IPT-SP Taken before Delivery ± SD	4.2 ± 1.67	3.7 ± 1.34	4.0 ± 0.86	4.1 ± 1.11	2.8 ± 1.00	4.0 ± 1.20
Number of Patient treated with ACT	39 (21.5)	18 (33.3)	13 (23.6)	2 (5.4)	7 (21.9)	79 (22.0)

BUTH - Babcock University Teaching Hospital Ilishan Remo; CAC - Christ Apostle Church Spiritual and Healing Maternity Home;
ICH - Ilishan Community Hospital, Ilishan Remo; IKHP - Ikenne Primary Health Care Centre; ISH - Ishara General Hospital, Remo North LGA.
ITN – Insecticide treated net.
[a] Statistically significant higher mean age compared to study mean age using ttest ($p < 0.0000$)
[b] Statistically significant lower mean age compared to study mean age using ttest ($p < 0.0037$)
[c] Statistically significant lower mean age compared to study mean age using ttest ($p < 0.0052$)
[d] Statistically significant lower gestation age compared to study mean age using ttest ($p < 0.0000$)
[e] Statistically significant higher gestation age compared to study mean age using ttest ($p < 0.0008$)
[f] Statistically significant lower gestation age compared to study mean age using ttest ($p < 0.0000$)

Table 2
Clinical Details.

Clinical Observations	VISIT 1 n (%)	DELIVERY n (%)	*P* value
Positive symptomatic[d]	41 (28.9)[α]	7 (5.0)[β]	0.0000[ϒ]
Positive Asymptomatic	101 (71.1)[α]	134 (95.0)[β]	0.102
Total parasitaemia positive	142 (27.3)	141 (39.3)	0.008[φ]
Negative Symptomatic	47 (53.4)	Nil	
Subpatent Malaria (defined as parasite densities < 100 parasites per μL of blood)	2 (9.1)	5 (71.4)	0.447
Total subject assessed	520	359	
Symptoms			
Headache	67 (41.9)	1 (7.7)	
Fever	24 (15.0)	3 (23.1)	
Chills	21 (13.1)	2 (15.4)	
Nausea and vomiting	12 (7.5)	2 (15.4)	
Loss of appetite	10 (6.3)	1 (7.7)	
Body pain	7 (4.4)	1 (7.7)	
Joint pain	5 (3.1)	Nil	
Muscle pain	4 (2.5)	2 (15.4)	
Body weakness	4 (2.5)	Nil	
Dizziness	3 (1.9)	Nil	
Abdominal pain	2 (1.3)	Nil	
Catarrh, Cough, Diarrhoea, Fatigue, Bitter taste	1 (0.6)	Nil	
Cold	Nil	1 (7.7)	
Total symptoms experienced	164 (31.5)	13 (3.6)	0.000[Ϗ]
Total subjects with symptoms	88 (16.9)	7 (1.9)	0.000[§]

[α] Statistically significant difference in positive symptomatic cases vs positive asymptomatic cases at V_1 using Pearson chi^2 test ($p = 0.0000$)
[ϒ] Statistically significant difference in positive symptomatic cases at V_1 vs positive symptomatic cases at Delivery using Pearson chi^2 test ($p = 0.0000$)
[β] Statistically significant difference in positive symptomatic cases vs positive asymptomatic cases at DEL using Pearson chi^2 test ($p = 0.0000$).
[φ] Statistically significant increase in parasite positive cases at Delivery compared to V_1 using Pearson chi^2 test ($p = 0.008$).
statistically significant higher number of symptoms at V_1 compared to Delivery using Pearson chi^2 test ($p = 0.000$).
[§] Statistically significant difference in number of individuals with symptoms at V_1 and Delivery using Pearson chi^2 test ($p = 0.000$).
[d] All parasitological diagnosis was done using any or all malaria parasite diagnostic methods (microscopy, RDT & PCR)

PCR, performed better with 39 correlations at V_1 and 5 at delivery. Only in 12 (2.3%) cases did all 3 methods agree at enrolment but no alignment at delivery (Table 3). The negative Diagnosis Correlation was higher for microscopy vs mRDT and much higher for all 3 diagnostic methods.

3.4. Diagnostic test evaluation

Sensitivity, specificity, positive, negative predictive and accuracy (which is overall probability that a patient is correctly classified) values of each of the 3 methods were calculated using the formulas in Fig. 1 [13,31]. The diagnostic capabilities showed much higher sensitivities at enrolment than at delivery, although all were less than 60%. The specificities were high at both enrolment and delivery (Table 4).

Discussion

Our data has demonstrated that asymptomatic malaria in pregnancy is prevalent in our environment [4, 32, 33]. Early diagnosis and treatment is essential to prevent the fulminant diseases [19]. Therefore, effective diagnostic methods are paramount to the management of asymptomatic MIP. This situation is complicated by the fact that sulphadoxine-pyrimethamine does not clear the parasites completely.

Table 3
Malaria Parasitological Assessment.

Parasite Positivity	VISIT 1 n (%)	DELIVERY n (%)	*P* value
MIC	22 (4.3)	7 (2.4)	0.1666
Geometric mean parasite density (parasites/μL)	909	143	
Subjects assessed	515	293	
mRDT	46 (8.8)	10 (3.4)	0.0034[γ]
Subjects assessed	520	292	
PCR	129 (25.0)	134 (43.4)	0.0001[ρ]
Subjects assessed	517	309	
Positive Diagnosis Correlation			
MIC vs mRDT	13 (2.5)	1 (0.34)	0.001 [μ]
MIC vs PCR	15 (2.9)	1 (0.32)	0.001[∞]
mRDT vs PCR	39 (7.5)	5 (1.7)	0.001[Σ]
MIC vs mRDT vs PCR	12 (2.3)	Nil	
Negative Diagnosis Correlation			
MIC vs mRDT	94 (19.8)	106 (38.8)	0.000[a]
MIC vs PCR	5 (1.1)	10 (3.7)	0.017[b]
mRDT vs PCR	9 (1.9)	12 (4.4)	0.054
MIC vs mRDT vs PCR	366 (77.2)	145 (53.1)	0.003[c]
Gravidity[Ω]			
Primigravida	49 (34.5)	40 (28.4)	0.422
Multigravida	93 (65.5)	101 (71.6)	0.631

MIC – microscopy; RDT – rapid diagnostic test, PCR – polymerase chain reaction
[γ] Statistically significant difference mRDT positivity rates at V_1 vs Delivery using Pearson chi^2 test ($p < 0.003$)
[ρ] Statistically significant difference when comparing mRDT positivity rates at Visit 1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[μ] Statistically significant difference is diagnosing capability V_1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[∞] Statistically significant difference is diagnosing capability V_1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[Σ] Statistically significant difference is diagnosing capability V_1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[a] Statistically significant difference is diagnosing capability V_1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[b] Statistically significant difference is diagnosing capability V_1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[c] Statistically significant difference is diagnosing capability V_1 vs Delivery using Pearson chi^2 test ($p < 0.001$)
[Ω] Parasite diagnosis was made using any or all of microscopy, RDT & PCR

$$\text{Sensitivity} = \frac{\text{TP}}{(\text{TP} + \text{FN})} \times 100$$

$$\text{Specificity} = \frac{\text{TN}}{(\text{TN} + \text{FP})} \times 100$$

$$\text{PPV} = \frac{\text{TP}}{(\text{TP} + \text{FP})} \times 100$$

$$\text{NPV} = \frac{\text{TN}}{(\text{TN} + \text{FN})} \times 100,$$

Fig. 1. Diagnostic Test Formulas. (where TP = true positive, FP = false positive, TN = true negative, and FN = false negative, PPV = positive and predictive values, NPV = negative predictive values).

We therefore propose that IPTp-SP may contribute to the phenomenon of asymptomatic malaria. This is evidenced from our data which demonstrated relatively more asymptomatic parasitaemia at delivery than at recruitment (Table 2). Our challenge now is how to detect

Table 4
Parasites Diagnostic Outcomes.

Test Method	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
V_1 PCR vs MIC	11.7%	98.2%	68.2%	76.9%	76.6%
DEL PCR vs MIC	1.6%	96.9%	14.7%	74.7%	73.1%
V_1 PCR vs RDT	30.2%	98.5%	86.7%	80.9%	81.4%
DEL PCR vs RDT	4.9%	97.6%	40.0%	75.5%	74.4%
V_1 MIC vs RDT	59.1%	93.5%	75.2%	87.3%	84.9%
DEL MIC vs RDT	16.7%	97.0%	64.6%	77.7%	76.9%

V1 – Enrolment visit time-point
Del – Delivery visit time-point

asymptomatic parasitaemia quickly and efficiently during pregnancy and immediate post-partum period.

Results from this study clearly shows that the performance of mRDT was comparable to microscopy and could be used to replace microscopy in rural areas where microscopes are not present or difficult to maintain [34]. Apart from the technical challenges and availability, other factors such as drug pressure, strain variation, or approaches to blood collection may complicate the parasitological diagnosis of malaria [10,13].

The incidence of positive parasitological diagnosis using microscopy and mRDT was low both at first visit, with further reduction at delivery. The contrary was the case for PCR diagnosis, where more cases were diagnosed positive at delivery (43.3%) than at enrolment (25%). This was indicative of higher presence of subpatent parasitaemia. This is in agreement with a study in Zambia, where investigators found a low incidence of malaria positivity using these three diagnostic methods, but almost half of the cases had subpatent parasitaemia [11]. Other studies have also reported similar negative effects of low parasitaemia or subpatent malaria [13, 20, 21, 35]. There were clear significant differences in the capabilities of the diagnostic methods to correctly diagnose the malaria infection at V_1 and at delivery. There was poor correlation when comparing the capabilities of 2 individual methods to give the same diagnosis. Only in 12 cases was there a perfect agreement using all three methods of diagnosis with no correlation seen at delivery, similar to other reported studies [13,21].

Calculating the sensitivity and specificity of diagnostic capabilities of malaria parasitological test instruments is important in determining the validity of the test methods to accurately diagnose the disease, especially when there are other confounding factors [36]. Ojurongbe et al. used a composite method in determining the gold standard to use in calculating the diagnostic capabilities. Our study however opted to use both PCR and microscopy for parasitological diagnosis to reflect the true conditions in the field. There were low sensitivity higher specificities of diagnostic methods. A significant finding in this study was reduction in both the sensitivities and specificities between V_1 and delivery.

There was a high incidence of asymptomatic malaria, which could be attributed to the sub-patent malaria parasites, which was more observed at delivery, than at enrolment. A more targeted study is required at this time to test this hypothesis. The observed difference in the diagnostic capabilities of the three test methods at V_1 and delivery is difficult to explain from our data. This phenomenon has been previously well reported [8, 10–13, 19–22, 35]. Although it may be argued that, this could be as a result of the IPTp-SP or ACT taken during the period of pregnancy which may have led to the low numbers of symptomatic cases. Physicians need to go an extra mile in diagnosis asymptomatic/subpatent malaria, and when this is done the patient should be treated immediately [12].

We are therefore left with the challenge of finding a suitable and easy to use diagnostic method for the detection of asymptomatic malaria in our rural areas where the mortality from pregnancies is highest and MIP contributing to this unacceptably high figure. It is evident from our data that the performance of mRDT was only at best equal to that of microscopy.

Conclusion

In conclusion, the WHO/MDG goal of complete malaria eradication and zero adverse events of MIP to both mother, foetus and neonate is on course to being achieved. From our data we can only conclude that while we could use mRDT to make a diagnosis of malaria when symptoms are present, it may not be very reliable for the diagnosis of asymptomatic malaria especially MIP. The good news is that the IPTp-SP is still efficacious in preventing MIP, however the long-term effects of asymptomatic/patent warrant increased consideration by malariologists especially in endemic countries like Nigeria.

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Declaration of Competing Interest

The authors declare no competing or conflict of interest.

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