Anthropometric Estimates and Comparative Evaluation of Diagnostic Methods for Malaria Parasitemia in Pregnant Women and newborn babies in Southwestern Nigerian Communities

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Abstract

Malaria in low-birth-weight newborns affects the prognosis. The anthropometric estimates and comparative evaluation of diagnostic methods for malaria parasitemia in pregnant women and newborn babies in southwest Nigerian communities was investigated in this study. Demography, BMI of mothers and anthropometric data of the newborn children were analysed for malaria infection risk. Venous blood sample from pregnant mothers (n=112) attending routine antenatal clinics and the cord blood during delivery of the newborn (n=112) were analysed Plasmodium infection and comparative evaluation of the diagnostic performance of Rapid Diagnostic Test (RDT), Giemsa Microscopy and PCR assay were evaluated for specificity and sensitivity using the multivariate logistic analysis. Among the pregnant women from various tribes, 90.9% were of the Yoruba tribe, 70.2% had secondary school education, 63.6% belonged to mid-income class, 72.7% had received Intermittent preventive treatment (IRT) and 37.5% slept under ITN ($p<0.05$). A significant decrease in average BMI of the malaria positive mothers from all study locations (28.18 Kg/m^2, 25.05 Kg/m^2 and 26.30 Kg/m^2) was observed compared to the average BMI of the non- infected pregnant mothers ($p= 0.034$). The anthropometric values of infected babies significantly decreased in chest circumference ranging between 20.44 to 21.83cm compared 23.15cm in uninfected babies ($p<0.05$). The average malaria parasite density in infected mothers and babies (18,345 and 6,486 per 200WBC) with higher prevalence of 11.78% and 8.00% respectively of *Plasmodium falciparum* was found in infected mothers and infected babies respectively, compared to other *Plasmodium* species. Higher Plasmodium detection rate by PCR (21.62%) for both mothers and babies compared to microscopy and RDT was recorded. Poor education, poverty and poor use of preventive measures are major risk factors for the high prevalence of malaria among pregnant women. PCR-based methods should be considered as part of diagnosis for early detection of mother-to-child transmission of malaria and reduction of risk of infection for the newborn particularly in endemic areas.

Keywords: Anthropometric, Malaria Parasite, P. falciparum, Pregnant Women, Newborn babies

1.0 Introduction

Malaria infection in pregnancy and among the newborn is a major public health concern in both sub-tropical and tropical regions across the globe (Emmanuel *et al.*, 2018). About 214 million people are infected by malaria parasites accounting for nearly 438,000 deaths worldwide (Sheikhzadeh *et al*., 2016). Each year, up to 3 million people dies of the disease worldwide and majority are young children particularly from Sub-Saharan Africa (NIH, 2010). Malaria is endemic in Nigeria, with 97% of the population at risk (Okorie, *et al.,* 2011). Five ecological zones including mangrove swamps, rain forest, Guinea-savannah, Sudan-savannah, and Sahel-savannah define the intensity and seasonality of transmission and distribution of mosquito vector species.

Plasmodium falciparum is the predominant malaria species with high infectivity rate in Nigeria and other sub-Saharan countries (Malaria Operational Plan, 2016). It is a leading cause of mortality in children under five years of age, and is responsible for an estimated 300,000 total deaths annually. *P. falciparum* infection contributes to an estimated 11% of maternal mortality, 25% of infant mortality, and 20% of under-five mortality (Malaria Operational Plan, 2016). In Nigeria, a very high malaria burden with approximately 51 million cases and 207,000 deaths are reported annually, while 97% of the total

population (approximately 173 million) is at risk of the infection (WHO, 2014). In low-income settings, high malaria in pregnancy is associated with anaemia leading to spontaneous abortion, stillbirth, prematurity and low birth weight (WHO, 2016).

There are several reports of the prevalence of malaria in pregnancy but high rates of cord and maternal blood malaria was majorly reported (Nnaji *et al.,* 2011). In spite of the malaria prevalence rates in southwest Nigeria, microscopy of Giemsa-stained thick blood films has been the standard laboratory method for diagnosis and speciation of malaria parasites (Murray, 2003). This method often provides false result due to the use of poor quality of light microscopy for detection (Faye *et a*l., 2013), and limitation in detection of mixed infections, when *Plasmodium* species is present at low levels (Balaghaleh *et a*l., 2018).

The molecular detection of *Plasmodium* infection using a Polymerase chain reaction (PCR) assay has gained importance due to high diagnostic sensitivity and specificity (Fuehrer *et a*l., 2011). PCR diagnostic method was very useful for detection of mixed infections (Ebrahimzadeh *et al*., 2017) and could be adapted for routine diagnostics (Hanscheid, 2002). Recent reports on congenital malaria suggests that the incidence is increasing but it is difficult to determine whether the clinical disease is due to parasite acquired before delivery or as a result of contamination by maternal blood at birth (Malaria, 2008). Several conflicting reports of cord blood malaria in low-birth-weight newborns affect the prognosis with their anthropometric values. This study investigates the anthropometric estimates and comparative evaluation of diagnostic methods for malaria parasitemia in pregnant women and newborn babies in southwest Nigerian communities.

2.0 Method

Ethical approval

Approval for the study was obtained from the Olabisi Onabanjo University Teaching Hospital Health Research Ethics Committee (OOUTHETH: 2334555). Informed consent from the pregnant mothers was sought from individuals that met the criteria of the study. The identity of the participants in this study remains anonymous and they were assured of confidentiality in view of the intricacy and sensitivity of the study. Participants were informed of the benefit of the study and freedom to withdraw if they wished to do so.

Sampling

Venous blood sample (5ml) was collected in EDTA containers each from pregnant mothers that regularly attend the routine antenatal clinics and the cord blood during delivery of the newborn at major health facilities and Primary Health Care centers in Isara, Iperu, Ikenne, Ogere and Sagamu located in Ogun state, South West Nigeria (NPC, 2007). Each subject demography, BMI of mothers and anthropometric data of the newborn children were collected from their hospital records. Apparently healthy pregnant women and their newborn who were not positive to malaria parasite infection were included as control subjects. From calculated sample size determined from a case-control study according to Jayanthi et al (2013), a total of 112 and 111 blood samples were collected from subjects and control respectively based on previous prevalence (Agomo et al. 2009). The anticoagulated blood samples were refrigerated at about 4-6^{o}C until analysis.

Inclusion Criteria: Pregnant women in third trimester with no history of immunocompromised condition such as HIV, cancer, were included and those that attend antenatal clinics and were willing to participate in the study.

Exclusion Criteria: Pregnant women with history of any other confounding factors, immunocompromised or any chronic disease apart from malaria were excluded as well as non-consenting individuals.

Rapid Diagnostic Test (RDT)

Pre-coated membrane strip with mouse monoclonal antibodies specific to HRP-II of *P. falciparum* with colloid gold that conjugate with the Malaria *Plasmodium falciparum* antigen in the blood sample was used for the RDT assay. The conjugant along the membrane chromatographically to the test region "p.f" formed a visible line as the antibody-antigen-antibody gold particle complex with high degree of sensitivity and specificity indicating a positive test. Both the test line and control line in the result window showed visible red line, after few minutes. The control line is used for procedural control and should always appear if the test procedure is performed properly. The presence of only coloured band (control line"C") within the result window indicates a negative result while non-appearance of any test line suggests invalid result.

Microscopy

Slides were labelled on the frosted end, and with a micropipette, 6µl of blood was placed for the thick film and 2µl of blood sample was used for the preparation of thin film. All the slides were air dried horizontally. For the thick film, approximately 2-3ml

of Giemsa stain was used to cover the slide for 10 minutes and rinsed with phosphate buffer. For thin film slides, all the prepared slide were fixed with methanol and with Giemsa stain for 2 minutes and diluted with phosphate buffer and allowed to stain further for ten minutes. Each slide was gently and allowed to aid dry.

PCR assay

Genomic DNA was extracted from the blood samples using quick-DNA Universal kit according to manufacturer's description. The obtained DNA was estimated using Thermoscientific Nanodrop Spectrophotometer at absorbance of 260nm. The PCR was carried out separately for each gene as described in Table 1. All the preparations were made to a volume of 25µl containing 2X Master Mix with Standard Buffer 12.5µl, 10µM Forward Primer of 0.5ul, 10µM Reverse Primer 0.5µl, template DNA 2.0ρl of each extracted chromosomal DNA. All the tubes were serially arranged in a thermal cycler block (NYX Technik Inc; Model ATC401, USA). The amplification reaction was carried out in 30 repeated temperature cycles at *Initialization* 94°C for 30 seconds; denaturation 94°C for 30 s, annealing (varies for each plasmodium species in Table 1 based on optimal temperature gradient assay), elongation 68°C for 60 seconds. For each of the target gene, their respective primers and different amplification temperatures conditions was used for the amplification**.**

Table 1; Primers used for the detection of Plasmodia genes

Parasite	Target gene	Primer	Sequences (5' – 3')	Annealing temperature for 60s
P. falciparum	***Pfcrt***	***Pfcrt F***	TGGTAAATGTGCTCATGTGTTT	52°C
		Pfcrt R	AGTTTCGGATGTTACAAAACTATAGT	
P. malariae	***rMAL***	***rMAL1***	ATAACATAGTTGTACGTTAAGAATAA	56°C
		rMAL2	AAAATTCCCATGCATAAAAAATTATA	
P. vivax	***rVIV1***	***rVIV1***	CGCTTCTAGCTTAATCCACATAACTG ATAC	52°C
		rVIV2	ACTTCCAAGCCGAAGCAAAGAAAGT CCTTA	
P. ovale	***rOVA1***	***rOVA1***	ATCTCTTTTGCTATTTTTTAGTATTGG AGA	55°C
		rOVA2	GAAAAGGACACATTAATTGTATCCTA GTG	

Data analysis: All data obtained from the study were computed and anlysed with Statistical package for Social Science (SPSS) Version 21.0. Variables of socio-demographic data of pregnant women recruited for the study were analysed into number and percentage of frequency using chi square. Anthropometric values for both control and test subjects were analysed and significance of variables from locations were analysed using ANOVA taking the $p < 0.05$. Comparative level of significance of PCR, microscopy and RDT used for the detection of malaria parasite was determined using ANOVA at $p<0.05$.

3.0 Results

Demographic profile and Malaria infection pattern of the pregnant women and babies

The demographic status of the pregnant women from various study locations were analysed in Table 2. Among the pregnant women from various tribes, 90.9% were Yoruba and less than 10% were from other tribes. Considering the educational status of the recruited pregnant women, 70.2% had secondary school education (Sagamu) compared to other locations. Economic status of the recruited pregnant women showed that 63.6% from Remo north belong to mid-income class while 4.5% from Remo north belong to high income class. In Table 3, the malaria infection pattern among pregnant women from different locations indicated that 46.9%from Ikenne do not have malaria at all compared to others. Based

on intermittent preventive treatment, 50% were given the treatment while 50% were not given. The highest percentage of 72.7% were given Intermittent preventive treatment (IRT) and 49.1% were given treatment while 50.9% were not given. From Remo, 72.7% were given treatment while 27.3% did not receive treatment. From Remo, level of compliance are 4.6%, 63.6%, 4.6%and 27.3% for once, twice, thrice and four times respectively. Considering usage of Insecticide treated nets, 37.5% slept under Insecticide treated nets (ITN) while 62.5% did not sleep under the Insecticide treated nets (ITN) nets.

Table 2, Demographic profile of the pregnant women

Characteristics		**Ikenne**	**Sagamu**	**Remo North**	X^2	**P-value**
		N (%)	N (%)	N (%)		
Age (years)	15-22	6 (18.8)	15 (26.4)	6 (27.3)		
	23-30	15 (46.9)	32 (56.1)	14 (63.6)	27.04	0.004*
	31-50	11 (34.4)	10 (17.5)	2 (9.1)		
Tribe	Yoruba	26(18.8)	42 (73.7)	20 (90.9)		
	Igbo	1 (3.1)	15 (26.3)	0 (0.0)	21.07	0.001*
	Hausa	1 (3.1)	0 (0.0)	0 (0.0)		
	Others	4 (12.4)	0 (0.0)	2 (9.0)		
Educational status	Primary	8 (25.0)	10 (17.5)	2 (9.1)		
	Secondary	20 (62.5)	40 (70.2)	12(54.5)	25.17	0.003*
	Tertiary	4 (12.5)	7 (12.3)	8 (36.4)		
Economic Status (Per month)	Low income	17 (53.1)	33 (57.9)	7 (31.8)		
	Middle income (16,000-30,000)	15(46.9)	24 (42.1)	14 (63.6)	30.41	0.001*
	High income (40,000)	0 (0.0)	0(0.0)	1 (4.5)		

(* significant, p< 0.05)

Table 3, Malaria infection among the pregnant women

Characteristics		**Ikenne**	**Sagamu**	**Remo**	**X2**	**P-value**
		n(%)	n(%)	n(%)		
Frequency of malaria during pregnancy	Not at all	15 (46.9)	29 (50.9)	13 (59.1)		
	Once	10 (31.3)	14 (24.6)	3 (13.6)	23.98	0.001
	Twice	7 (21.9)	10 (17.5)	4 (18.2)		
	More than once	0 (0.00)	4 (7.0)	2 (9.1)		
Intermittent preventive treatment	Yes	16 (50.0)	28 (49.1)	16 (72.7)	31.01	0.002
	No	16 (50.0)	29 (50.9)	6 (27.3)		
Treatment Taken	One	5 (15.6)	11 (19.3)	1 (4.6)		
	Twice	9 (28.1)	23 (40.4)	14 (63.6)	41.25	0.001
	Three times	6 (18.8)	15 (26.3)	1 (4.6)		
	Four times	12 (37.5)	8 (14.0)	6 (27.3)		
Sleep under ITN	Yes	12 (37.5)	22 (38.6)	11 (50.0)	27.64	0.046
	No	20 (62.5)	35 (61.4)	11 (50.0)		

P< 0.05 significant**;** ITN, insecticide-treated nets;

Comparative anthropometric pattern of malaria infected mothers and babies

In Table 4, The comparative anthropometric values of malaria positive mothers recruited from different

locations of study showed a significant decrease in average BMI of the malaria positive mothers (28.18 Kg/m^2, 25.05 Kg/m^2 and 26.30 Kg/m^2 in Ikenne Remo and Sagamu respectively) compared to the average BMI of the non- infected pregnant mothers (p= 0.034) as shown in Table 4. There was a significant weight decrease of infected pregnant women but no significant difference was recorded in the mean height of the infected mothers. The anthropometric values of infected babies significantly decreased in chest circumference ranging between 20.44 to 21.83cm compared 23.15cm in uninfected babies (Table 5).

Table 4, Comparative anthropometric values of malaria positive mothers

Anthropometric values	Control	Ikenne	Remo	Sagamu	F-value	P value
	Mean ±SD					
BMI (Kg/m2)	28.31±4.72	28.18±3.52	25.05±5.68	26.30±2.82	1.542	0.034*
Height (m)	1.48±0.11	1.49±0.13	1.58±0.06	1.50±0.10	2.098	0.643
Weight (Kg)	64.76±3.87	54.84±3.23	63.00±4.79	60.10±4.46	1.754	0.002*

(*p<0.05 is significant; BMI- Body mass index)

Table 5, Comparative anthropometric values of malaria infected babies

Anthropometric values (unit)	Controls	Ikenne	Remo	Sagamu	F-value	P value
	Mean ±SD					
Ch. Cir (cm)	23.15±8.1	21.83±5.02	21.20±5.01	20.44±4.27	2.954	0.036*
Head (cm)	25.13±4.4	23.67±2.75	24.40±4.71	24.33±2.50	3.283	0.051
Length (cm)	35.18±5.5	33.83±3.49	33.20±2.32	34.86±3.61	3.304	0.041*
Mid up. Arm (cm)	9.09±4.49	9.83±3.72	8.70±4.52	8.77±2.64	4.602	0.032*
Weight (kg)	3.19±0.58	3.08±0.39	3.10±0.75	2.98±0.60	2.019	0.021*

(*p<0.05 is significant; Ch. Cir. =Chest Circumference, Mid up. Arm- Mid upper arm.

Malaria parasite infection burden and comparative plasmodium detection with diagnostic methods

Figure 1 showed average malaria parasite density in infected mothers and babies (18,345 and 6,486 per 200WBC). Prevalence of 11.78% and 8.00% of *Plasmodium falciparum* were found in infected mothers and infected babies respectively, and lower prevalence of 4.67% and 5.43% of *Plasmodium malariae* was found in the infected mothers and infected babies respectively. Comparative detection rates indicate higher detection rate was shown by PCR (21.62%) for both mothers and babies compared to microscopy and RDT. Similarly, higher sensitivity (100%) and specificity (98.45%) (95%CI: 0.681-0.928; =0.001) as shown in Table 5.

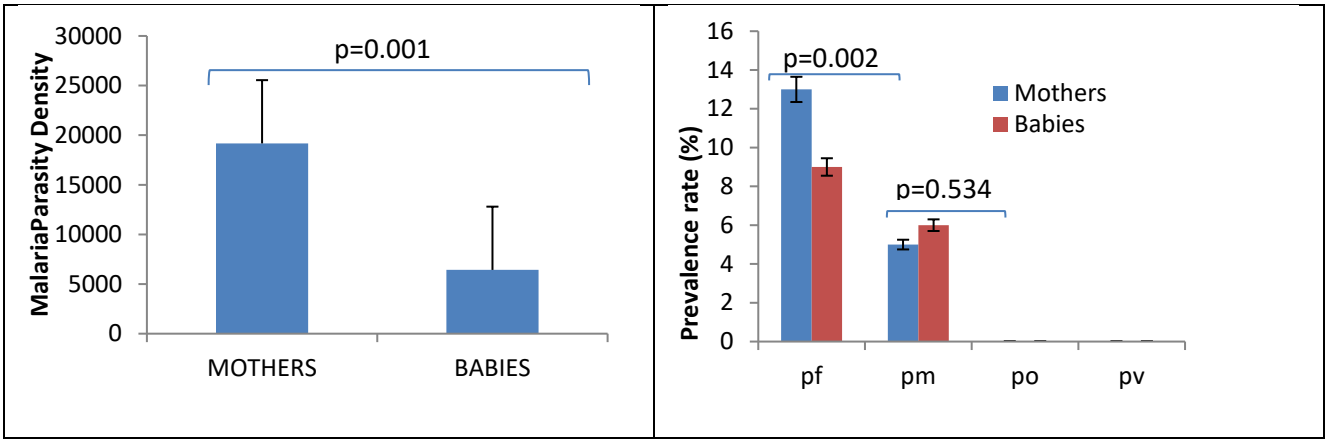

Figure 1; Comparative malaria parasite density and proportion of *Plasmodium* species detected

Table 6, Overall comparative plasmodium detection rate of diagnostic methods

Methods	Detection rate (%)		Sensitivity (%)	Specificity (%)	95%CI	**P-value**
	Mother/Babies					
Microscopy	17.12/5.41	72.56/52.87	81.90/80.02	85.78/83.21	0.344-0.891	0.001
RDT	18.92/14.41	63.33/60.19	70.56/63.21	80.64/65.98	0.452-0.731	0.032
PCR	21.62/21.62	100.00/100.00	100.00/99.65	98.45/98.01	0.681-0.928	0.001

(*Significant, p<0.05, %, Percentage rate)

4.0 Discussion

The impact of malaria among pregnant women was shown by their declined demographic status. The high rate of pregnant women attending ante-natal clinic for treatment were mostly ages 23 to 30 years which was similarly reported by Agomo *et al* in 2009 and Akaba *et al.,*(2013) at Abuja, Nigeria. Tertiary health facility influenced the increased ante-natal care compared to locations with inadequate health facilities. Remo is predominantly a Yoruba settlement with few other tribes living or working in this location. Culture, language and housing set up in this Yoruba community reveal the environmental factors that could influence malaria vector control. There is paucity of report on the housing pattern relating to the tribal culture on the prevalence of malaria among pregnant women. Considering the educational status of the recruited pregnant women, good knowledge of malaria was associated with higher level of educational status of the women. In 2015, Fana *et al* reported similar high rate of poor literacy among pregnant mothers seeking ante-natal care in Northern parts of Nigeria, Aju-Ameh *et al.,*in Benue state in 2016, Oladimeji *et al.,*(2019) in southwest Nigeria. Level of education of pregnant women has been linked with good health awareness and health-seeking behaviour (Oladimeji *et al.,* 2019). This low literacy also affects many mothers in getting treated for malaria. Low economic status observed in these locations suggests high poverty level among the pregnant women suffering from malaria. This has grossly affected their capability to use available goods and services to treat malaria. Many women may be unable to afford transportation costs, to health care centres, pay for consultation with healthcare providers, and pay for drugs and many therefore resort to local herbs which most times adversely affect the outcome of their pregnancy. In addition, economic status based on income level affect the ability of mothers to access good malaria preventive measures such as insecticide treated nets (ITNs) and indoor residual sprays (Diala *et al.,* 2013); Oladimeji *et al.,* 2019).

The comparative anthropometric values of malaria positive mothers recruited from different locations of study showed that there is significant decrease in average BMI of the malaria positive mothers (Tangpukdee 2007). Poor nutrition usually increase pregnant women's risk of severity of malaria infection. Pregnant women with low mid-upper arm circumference and BMI were most likely to have high placental parasite loads (Lovel., *et al.,*2005). There was also a significant decrease in weight of pregnant women infected with malaria compared with the uninfected pregnant mothers. Malaria-related symptoms, such as diarrhea and abdominal pain (Monsalve *et al,* 2011), may lead to malabsorption of nutrients and decreased intake of nutrients, respectively. No significant difference was recorded in the mean height of the malaria positive pregnant mothers and the mean height of the non-infected pregnant mothers, though the average height of the malaria positive mothers were found to be lower than the average height of the non-infected pregnant mothers. A significant decrease in chest circumference of malaria infected babies is consistent with the report that malaria in pregnancy remains a major cause of preterm delivery and symmetric restriction of fetal growth. (Monsalve *et al,* 2011,). Recorded low head circumference, length and the mid upper arm length, and weight of malaria infected babies is associated with proportionate fetal growth retardation (Kalanda, *et al*., 2005). Though, relevant evidence indicated that the relationship between malaria infection and low birth weight may depend upon the mother's nutritional status

(Unger *et al*., 2016). It was previously reported that malaria infection and malnutrition may act along similar physiological pathways affecting the placental development and nutrient transfer (Dellicour*, et al*., 2007; Umbers*, et al*., 2011; Black, *et al*., 2013).

The high overall microscopic and PCR detection rates among mothers and babies further showed increasing prevalence rate of malaria infection in Nigeria. It is important that the healthcare professionals and patients have full confidence in the laboratory result, and the diagnostic results benefit the patient and the public health through compliance to the Malaria Microscopy Quality Assurance (QA) (WHO, 2016). Hospitals and many health centres require expert microscopist for the management of malaria cases which is the gold standard in endemic countries for identifying mixed infections, prevent treatment failures, and quantifying density (Pedro, 2018). *Plasmodium falciparum* infections were higher in mothers and babies compared to other parasites. This is further confirming previous report of *P. falciparum* endemicity in these locations with severe complications associated with metabolic dysfunction which could increase both maternal and neonatal mortality. Comparative detection supports high detection rate by PCR genotyping but Microscopy diagnosis with microscopy should never be neglected as it remains the gold standard in endemic areas. Although, microscopy allows estimation of parasitic densities and identification of all species and is cheaper than the other methods, PCR provide high sensitivity and specificity. However due to its high cost and required expertise, it may not be deployable for routine diagnosis in several LMICs (Kersey, *et al.,* 2018).

5.0 Conclusion

Poor education, poverty and poor use of preventive measures are major risk factors for the high prevalence of malaria among pregnant women and children in this locality. Regular blood smear microscopy with RDTs must be emphasized in low resourced settings in LMICs. PCR-based methods should be considered as part of diagnosis for early detection of mother-to-child transmission of malaria and reduction of risk of infection for the newborn particularly in endemic areas.

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