CENTER OF EXCELLENCE FOR OPEN SCIENCE

Open Access

Email: cpch@ceospublishers.com

CEOS Pediatrics and Child Health

Research Article

Received date: January 15, 2023
Accepted date: February 15, 2023
Published date: February 17, 2023

***Corresponding Author**

Roland Funwei, Department of Pharmacology, Babcock University, Ogun state Nigeria, Tel: 2348036712769, E-mail: okporu2@gmail.com

Citation

Roland Funwei, Precious Umukoro, Gabriel Uyaiabasi, Wasiu Hammed, Chika Okangba et al. (2023) Sensitivity of Rapid Diagnostic Test, Microscopy and Pcr in Asymptomatic Malaria Detection in Rural Community from Southern Nigeria. CEOS Pediatr Child Health 1: 1-8

Sensitivity of Rapid Diagnostic Test, Microscopy and Pcr in Asymptomatic Malaria Detection in Rural Community from Southern Nigeria

Roland Funwei[1,2*], Precious Umukoro[3], Gabriel Uyaiabasi[1,2], Wasiu Hammed[2], Chika Okangba[4], Obaro Micheal[3], Olusola Ojurongbe [5,6], Oladapo Walker[1,2] and Catherine Falade[3,7]

[1]Department of Pharmacology, Babcock University, Ogun state Nigeria
[2]Center for Advanced Medical Research and Biotechnology (CAMRAB), Babcock University. Ogun state, Nigeria
[3]Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria
[4]Department of Medical Microbiology, Babcock University, Ogun state Nigeria
[5]Department of Medical Microbiology, Ladoke Akintola University of Technology, Oyo State, Nigeria
[6]Center for Emerging and Re-emerging Infectious Diseases (CERID), Ladoke Akintola University of Technology, Oyo State, Nigeria
[7]Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan, Nigeria

Abstract

Background: Asymptomatic falciparum malaria is a major diagnostic and public health challenge to global malaria control and elimination programmes. In malaria high transmission settings, passive and active case detection is required to reduce the malaria burden. This study evaluated the performance of CareStartTM rapid diagnostic test, microscopy and polymerase chain reaction (PCR) amongst asymptomatic individuals of southern Nigeria.

Method: A cross-sectional community-based study was conducted from November 2018 to February 2019 to evaluate the performance of CareStartTM, microscopy and PCR amongst asymptomatic individuals.

Results: The sensitivity, specificity, accuracy, and positive and negative predictive values of CareStartTM and microscopy were analysed against PCR as the reference. Malaria prevalence was 55.4%, 51.2% and 68.7% for CareStartTM, microscopy and PCR. The sensitivity and specificity of RDT was 66.1% and 68.6% while accuracy, positive and negative predictive values were 66.9%, 82.6% and 47.3% respectively. Similarly, the sensitivity and specificity of microscopy were 65.2% and 80.4% while accuracy, and positive and negative predictive values were 69.9%, 88.2% and 50.6% respectively. The kappa's value for RDT (0.31) and microscopy (0.39).

Conclusion: The performance of the CareStartTM RDT is comparable with microscopy in asymptomatic malaria parasite detection. An ultra-sensitive nucleic acid-based point-of-care diagnostic devices are required to accurately detect asymptomatic malaria parasite in Nigeria.

Keywords: Asymptomatic malaria, RDTs, Microscopy, PCR and Nigeria

Abbreviations:

COVID-19	Coronavirus Disease-19
DBS	Dried blot spot
FP	False positive;
FP	False negative
mRDT	malaria rapid diagnostic test;
PCR	Polymerase Chain Reaction
pfHRP-2	Plasmodium falciparum Histidine-Rich protein-2
pLDH	Plasmodiun Lactose Dehydrogenase hrp2-histidine-rich protein-2
RDT	Rapid Diagnostic Test
ssrRNA	small subunit ribosomal ribonucleic acid;
TN	True negative;
TP	True positive,
WHO	World Health Organization

Introduction

The public health burden of malaria is devastating, especially in sub-Saharan Africa which contribute the highest global challenge to efforts geared towards malaria elimination [1]. Malaria impacts health, social and economic dimensions either directly or indirectly [2]. Nevetheless, malaria is an eminently preventable and treatable disease caused by *Plasmodium species,* transmitted to the humans during a blood meal of an infected female *Anopheles* mosquito [3]. Children under five and pregnant women are the most vulnerable to malaria morbidity and mortality [4]. In 2020, over 241 million people were infected with malaria and 627,000 deaths occurred with an estimated 95% of global malaria cases and deaths reported in sub-Saharan Africa [3]. These figures are a significant decrease from the estimated fatality of one million estimated in 1998. Despite the significant gains made in malaria mortality reduction, recent data shows that malaria burden surged by 15 million malaria cases and 68,000 deaths in recent years. Approximately, two-thirds of the additional malaria deaths in 2020 were linked to disruption in the provision of malaria prevention, diagnosis and treatment during the COVID-19 pandemic [3,5]. This is in addition to the economic burden of the disease [6].

Quality vector control systems, accurate diagnosis and treatment, drug-efficacy surveillance and compliance to WHO-recommended guidelines are key to reducing the morbidity and mortality of malaria [7]. Malaria symptoms are non-specific, although, fever remains the hallmark symptom of malaria but it is also commonly associated with other tropical diseases like measles, dengue fever and typhoid disease in the tropics [8]. Symptom-based clinical management of malaria lead to inaccurate diagnosis and mistreatment [9]. In appropriate diagnosis and presumptive malaria treatment are one of the major contributors of drug pressure and subsequent emergence of drug resistance [10]. Therefore, WHO recommended all suspected malaria cases should undergo microscopy and/or RDT for confirmatory diagnosis before antimalarial drug therapy [11]. Thus, accurate parasitic diagnosis of malaria is one of the pillars of a successful malaria program as it engenders correct treatment and reduces drug pressure which may lead to the development of parasite resistance.

Parasite based malaria treatment is achieved by the identification of malaria parasites or its circulating antigen in host's blood [12]. Conventional malaria diagnostic methods in clinical settings include microscopy and rapid diagnostic tests (RDTs) while polymerase chain reaction (PCR) is used in reference laboratories [13]. The applicability and choice of each diagnostic method varies widely on cost, expertise, malaria epidemiology and locality of the setting.

For several decades, Microscopy remains the gold standard for clinical malaria diagnosis in most endemic countries [13]. The uniqueness of microscopy as a diagnostic tool lies in its ability to detect all the major *Plasmodium species*, as well as gametocytes of *P. falciparum* in the hands of expert microscopist [14,15]. However, slide preparation, expertise, equipment, quality reagent and deficit infrastructure limits its applicability in resource limited settings [16]. At high parasite densities, microscopy is reported to have excellent diagnostic performance regarding its specificity and sensitivity, however, sub-microscopic levels, sensitivity is low [17]. The limit of detection for microscopy ranges between 50–200 parasites per microliter [11].

On the other hand, RDTs are immuno-chromatographic lateral flow devices developed to detect malaria antigens in blood [18,19]. RDT detects malaria parasites by targeting its antigens, histidine-rich protein- 2 (*hrp2*), which is exclusively expressed by *P. falciparum* and *Plasmodium* lactate dehydrogenase (*p*LDH), expressed by all known human *Plasmodium species* in the host blood [20].

Malaria RDTs are preferred diagnostic tool in low infrastructural setting where equipment, reagents and expertise to handle the microscope is lacking [15]. They are simple to use, requires no skill and has a short turn-around time. However, extreme temperature, humidity and persistent presence of circulating antigens after asexual parasite clearance limits RDT specificity [20–22]. Another striking limitation for RDTs is the occurrence of false negative results arising from parasites with *hrp2* gene deletion [24,25]. Despite these limitations, RDT uptake continues to increase in the last decade in field and clinical malaria diagnosis in resource limited settings to augment microscopy. They are reliable and have high performance when the parasite density and *hrp2* concentrations are high [13,26].

PCR-based methods of malaria diagnosis are highly effective in detecting asymptomatic and sub-microscopic infections [27]. PCR- based methods amplify parasites DNA and thus are highly sensitive. When compared to microscopy, PCR-based diagnosis have higher sensitivity in low parasitaemia infections [12,28]. However, operational cost, complexity and long turn-around time limits its use in clinical settings [12,27].

Asymptomatic parasite carriage in *P. falciparum* infections is well established [27,29]. Contrary to previous assumption that asymptomatic infections are peculiar to high transmission areas due to acquired partial immunity, studies have shown that asymptomatic infections exist in low transmission areas of endemic countries [30]. Parasitaemia in asymptomatic malaria range from microscopic to submicroscopic densities hence, making detection difficult and highly dependent on the sensitivity of the diagnostic tool [31]. Since asymptomatic patients develop no clinical manifestation, they evade treatment and hence, serve as reservoir for malaria transmission [27,32]. Asymptomatic *Plasmodium* carriage is reportedly responsible for 20-50% of all human-to-mosquito malaria transmission in submicroscopic population [33]. There is paucity of data on RDTs performance on asymptomatic malaria diagnosis in line with WHO active case detection in our environment. Therefore, this study is designed to evaluate and compare the performance of CareStart™ RDT, microscopy and PCR amongst asymptomatic population from southern Nigeria. The other consequence of the study is to demonstrate if CareStart™ can be used as a stand-alone diagnostic method in resource poor settings.

Material and Methods

Study location and design

A cross-sectional study focused on diagnosis and treatment of unsuspected asymptomatic malaria was carried out in November 2018 in a riverine community (Akeddei) situated between latitudes 4°55'N and longitudes 6°04'E in the Niger Delta region of southern Nigeria. The study location is comprised of freshwater swamp with heavy flooding which is surrounded with thick forests. The wet season starts with an intense rainfall from March to October while a very short dry season is observed from November to February each year [34]. The primary occupation of the indigenes of Akeddei is peasant fish farming.

Malaria transmission in the study location is holoendemic to hyperendemic thus, transmission is all year round with peak in the wet season. The whole population is at risk for malaria. Malaria control measures in the community is not optimal, therefore, malaria is the commonest reason for hospital visit. Hence, early diagnosis and treatment is key to reducing mortality and morbidity in the population.

Case Definition

As the definition of asymptomatic malaria is not uniform, we defined asymptomatic malaria using the definition proposed by Greenwood [35]. Thus, asymptomatic malaria is defined as individuals who were completely afebrile at the time they were tested. They should also be free of other symptoms like malaise, lethargy, vomiting, diarrhea, body pains and headaches. However on microscopy, they would have significant parasitaemia.

Study Population

The population of the study community was 5 months to 41. Although, ost of the people who reported in the health center for the treatment of malaria were children below of the age of five years. There was no sex discrimination in those afflicted with the illness. The community was sensitized on the purpose of the study and community consent was obtained from the community. The purpose of the study which was mainly diagnostic was well explained to the community before the start of the study. The study population comprised asymptomatic participants from 5 months to 41 years of both sexes. A total of 163 participants were screened for malaria using RDTs and confirmatory screening was carried out in the laboratory with microscopy and PCR.

Laboratory Procedures

Finger prick blood was collected from the index finger to perform RDT following sterile procedures. A thick blood smear was prepared on microscope slides, and blood spots on filter paper (Whatman™ 3 MM) for PCR analysis. The results from Carestart™ (Access Bio, Inc. USA) brand of *Plasmodium falciparum* histidine rich protein -2 (*Pf*HRP-2) based mRDT was considered for decision making. The test preparation and interpretation were done according to the manufacturer's instructions. Reading and interpretation of test results were carried out by community health workers and interpreted within the specified 15–20 min test window. The test was considered

positive, when the test and control lines were visible in their respective test windows, negative when only the control band was visible and invalid when the control band was not visible. Faint test lines were also considered positive. Thick blood films was used to confirm parasitaemia by an expert microscopist who was blind to the mRDT result later in the laboratory. A thick blood film was declared negative after 100 consecutive high-power fields were viewed without parasites being detected. Calculation of Parasite density was carried out by counting the number of asexual parasites against 200 leukocytes, assuming a leukocyte count of 8,000/μL [36,37].

Genomic DNA was isolated from dried blood spots (DBS) using Dried Blood Spot DNA Isolation Kit (Norgen Biotek Corp. Canada), according to the manufacturer's instructions. The extracted DNA was stored at –20°C until PCR amplification. DNA samples were amplified by specie-specific primer pairs to amplify *P. falciparum*, P. *vivax*, *P. malariae* and *P. ovale* genes of the small subunit ribosomal ribonucleic acid (ssrRNA) as previously described [39]. Both primary and nested amplifications were performed in a 20μL reaction volume containing: 5μL 5X Master mix (FIREPol™), 0.5μL each of forward and reverse primers, 12μL nuclease-free PCR grade water and 5μL DNA template on a thermal cycler (Eppendorff AG 22331 Hamburg, Germany). The primary *Plasmodium* genus-specific amplification reaction was followed by nested *P. falciparum, P. vivax, P. malariae,* and *P. ovale* species-specific PCR amplification. Both primary and nested PCR thermal cycling conditions were: Initial denaturation at 94°C for 5 minutes, followed by 30 cycles, respectively, 1 min at 94°C for denaturation, 2 min at 58°C for annealing temperature, and 2 min at 72°C extension, then final extension of 5 min at 72°C. PCR grade water was used as negative control while a previously identified positive sample in the laboratory was used as positive controls and was included in each PCR run. Amplicons were loaded into a 1.5% agarose gel pre-stained with EZ-Vision In-Gel™ dye (AMRESCO.LLC). The sample loaded gel was allowed to electrophorese at 100V for 60 min alongside 100 base pair DNA molecular ladder (Solis BioDyne™, Estonia) for amplicon size separation. The presence or absence of *Plasmodium species* was confirmed with a species-specific representative amplicon base pair size.

Data Analysis

Data was independently entered into Microsoft Excel spreadsheet and IBM SPSS (version 23) for statistical analysis. The number of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) were calculated. The performance characteristics of malaria RDTs and microscopy was further analyzed by calculating the sensitivity [TP/ (TP+FN)], specificity [TN/ (FP+TN)], negative predictive value, positive predictive value, diagnostic accuracy and kappa's value to evaluate the level of agreement. Kappa's value of 0.21 – 0.40 as fair agreement, 0.41 to 0.60 was considered moderate agreement while 0.61 to >0.80 was considered substantial to almost perfect agreement [39]. The level of statistical significance was considered at $\rho \leq 0.05$.

Results

Baseline demographic and clinical characteristics of 166 asymptomatic participants, stratified into three age groups; ≤ 5 (34.3%), 6- 11 years (43.4%) and ≥ 12 (22.3%) respectively, were evaluated. Asymptomatic malaria prevalence was higher in age group 6-11, (73.6%, 63.9% and 61.1%) as detected by PCR, CareStart™ RDT and microscopy respectively, but there was no statistically significant difference in the malaria prevalence amongst the three diagnostic techniques ($\rho = 0.250$). However, within the <5 age group, there was a statistically significant difference in the diagnostic methods as PCR wasmost sensitive ($\rho = 0.023$). A higher proportion of the participants were female (56.6%), accordingly, malaria was more prevalent in female than the male participants but statistically significant difference was only seen in males amongst the three diagnostic methods evaluated ($\rho = 0.007$). A total 160 (96.4%) participants had normal body temperature (≤ 37.5°C) while only 6 (3.6%) were febrile (≥ 37.5°C at baseline. PCR detected significant proportion of asymptomatic malaria compared to RDT and microscopy ($\rho = 0.001$). When body weight (kg) was stratified into ≤ 15kg (27.1%), 16 – 35kg (56.6%) and ≥ 36kg (16.3%), there was no statistically significant difference in malaria positivity rate amongst the three diagnostic methods with regards to body weight (Table 1). Of the 166 participants, 85/166 (51.2%) were positive for *P. falciparum* by microscopy. Of this group, 18/85 (21.2%) has parasite density less than 200 p/μL while 67/85 (78.8%) had parasite density greater than 200 p/μL. Of the ≤ 200 p/μL, RDT detected 44.4% and PCR 77.8% while microscopy, the gold standard for parasite count determination, recorded 100% ($\rho = 0.001$). Similarly, RDT detected 82.1% and PCR 89.6% for parasitaemia ≥ 200 p/μL against microscopy's 100% ($\rho = 0.002$) (Table 1).

Table 1: Baseline Demographic and Clinical Characteristics of Study Participant

Characteristics	PCR n=114 (%)	RDT n = 92 (%)	Microscopy n = 85 (%)	ρ value
Age (Years)				
≤ 5 (n = 57)	39 (68.4)	26 (46.2)	26 (46.2)	0.023*
6 – 11 (n = 72)	53 (73.6)	46 (63.9)	44 (61.1)	0.250
≥ 12 (n = 37)	22 (59.5)	20 (54.1)	15 (40.5)	0.242
Total	**144 (68.7)**	**92 (55.4)**	**85 (51.2)**	**0.028**
Gender:				
Male (n = 72)	54 (75.0)	39 (54.2)	37 (51.4)	0.007*
Female (n = 94)	60 (63.8)	53 (56.4)	48 (51.1)	0.210
Temperature (°C):				
≤ 37.5 (n = 160)	111 (69.4)	87 (54.4)	81(48.8)	0.001*
≥ 37.5 (n = 6)	3 (50)	5 (83.3)	4 (66.7)	0.473
Weight (kg)				
≤ 15 (n = 45)	27 (60.0)	22 (48.9)	17 (37.8)	0.109
16 – 35 (n = 94)	71 (75.5)	57 (60.6)	59 (62.8)	0.066
≥ 36 (n = 27)	16 (59.3)	13 (48.1)	9 (59.3)	0.633
Parasite density (p/µL):				
≤ 200 (n = 18)	14 (77.8)	8 (44.4)	18 (100)	0.001*
≥ 200 (n= 67)	60 (89.6)	55 (82.1)	67 (100)	0.002*

PCR speciation of malaria recorded high prevalence of 68.7% for *P. falciparum* 0.6% for *P. ovale* and for 6.0% *P. malariae* respectively. No *P vivax* was detected in the study population. In all, more female were positive for *P. falciparum* (63.85%) while more male were infected with *P. malariae* (8.3%). However, the difference in male and female infectivity rate was not statistically significant. Only 1/10 (10%) *P. malariae* occurred as mono infection (Table 2).

The asymptomatic malaria detection rate of RDT and microscopy was compared to PCR as the reference. The RDT recorded 76 true positive, 16 false positive, 38 false negative and 36 true negative. While microscopy detected 74 true positive, 11 false positives, 40 false negative and 41 true negative respectively (Table 3).

Table 2: Plasmodium species positive rate by Gender

Gender	*P. falciparum* (%)	*P. ovale* (%)	*P. malariae* (%)	*P. vivax* (%)
Male n = 72	54 (75)	0	6 (8.3)	0
Female n = 94	60 (63.8)	1(1.1)	4 (4.3)	0
Total (n = 166)	114 (68.7)	1 (0.6)	10 (6.0)	
	ρ = 0.084		ρ = 0.228	

Table 3: Performance of Malaria RDT and Microscopy against PCR

Variable	RDT	Microscopy
True Positive	76	74
False Positive	16	11
False Negative	38	40
True Negative	36	41

The overall performance of RDT and microscopy based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy recorded 66.7%, 69.2%, 82.8%, 48.3% and 67.5% for RDT and 64.9%, 78.8%, 87.2%, 50.2% and 69.2% for microscopy. The level of agreement (kappa's value) for RDT and microscopy was similar (Good measure of agreement) when compared with PCR as the reference standard. There was no statistically significant difference ($\rho = 0.201$) in the kappa's value. However, microscopy was superior in specificity ($\rho = 0.030$) than RDT (Table 4).

Table 4: Performance of Microscopy and Malaria RDT against PCR as Gold Standard

Variable	RDT (%, CI)	Microscopy (%, CI)	ρ value
Sensitivity	66.7 [57 – 75]	64.9 [55 - 74]	0.409
Specificity	69.2 [55 – 81]	78.8 [65 – 89]	0.030*
Positive Predictive Value	82.8 [76 – 88]	87.2 [80 – 92]	0.166
Negative Predictive Value	48.3 [40 – 56]	50.2 [43 – 57]	0.406
Accuracy	67.5 [60 – 74]	69.2 [62 – 76]	0.414
κ- statistics	0.32	0.38	0.201

Discussion

To sustain the current decline in the global malaria burden, passive and/or active detection of asymptomatic malaria parasites and treatment is required in high transmission settings to reduce parasite reservoirs. This will further reduce malaria transmission in endemic regions and support strategic planning towards malaria elimination. This study evaluated and compared the performances of malaria RDT, microscopy and nested PCR in asymptomatic malaria detection in a malaria endemic riverine community from southern Nigeria. Nigeria shoulders the highest burden of global malaria with more cases in the rural settings [40]. Malaria diagnosis in these settings, mainly relies on presumptive and clinical symptoms due to lack of microscopy at the peripheral level. Thus, the programmatic deployment of malaria RDT by WHO is aimed at parasite-based malaria case management at the community level to promote appropriate malaria management with cost-effective, sensitive and specific malaria diagnostic tool [41].

Asymptomatic malaria was more prevalent in children aged 6 – 11 years across the three diagnostic procedures. This prevalence may be attributable to acquired immunity resulting from several episodes of malaria attacks during early stage in life [42]. The high prevalence of asymptomatic malaria is worrisome in malaria endemic regions with high transmission [43], as seen in our study, 68.7% of the participants was confirmed parasitaemic with nested PCR amplification. While nested PCR remains the most sensitive diagnostic technique for asymptomatic malaria, the detection rates of RDT and microscopy was comparable. Implying that in settings where microscopy is not readily available, RDT can be deployed for both symptomatic and asymptomatic malaria parasite detection prior to medication.

Asymptomatic malaria prevalence was female biased compared to their male counterpart in our study. While there are no clear explanation to this gender bias malaria infectivity rate, different researchers have attributed it to several factors [44,45]. A survey conducted in Uganda reported female gender was disproportionately associated with higher incidence of malaria across all age groups, especially at childbearing age, and concluded that female make more frequent visits to health facilities independent of malaria with higher risk of seeking care at these facilities [44]. A similar gender based study was conducted in Ghana which reported 62% malaria cases in women, but attributed the prevalence to low educational status and preferential malaria self-medication at home by their male counterparts [45]. Conversely, other authors have reported male biased malaria prevalence [46].

Parasite density is a significant determinant of malaria parasite detection, especially for RDT and microscopy. While the specificity of RDT was lower at parasite density ≤ 200 parasite/μL, microscopy recorded false positive when compared to nested PCR. This is not surprising as the detection limit of RDT is

considered at ≥ 200 parasite/μL [47]. Interestingly, the detection rate was significantly comparable when parasite density was ≥ 200 parasite/μL in all diagnostic methods.

Plasmodium falciparum is the predominant *species* in Nigeria and sub Saharan Africa [48], as our study detected more *falciparum* in all and *malaria* in males while the only *ovale* was detected in a female participant during PCR speciation.

The overall performance of RDT was comparable with microcopy in this study. The sensitivity of RDT was higher than microscopy, although, there was no statistical significance. However, the specificity of microscopy was significantly higher than RDT.

The low specificity in RDT is due to poor detection of parasite at low parasitaemia [49]. Besides specificity, RDT performed comparably with microscopy in the detection of asymptomatic malaria. Thus, RDT is reliable and could be deployed for malaria diagnosis at peripheral health facilities and routine malaria diagnosis at all levels of healthcare delivery.

Conclusion

The performance of CareStart™ mRDT is comparable with microscopy in the detection of asymptomatic malaria and should be encouraged whenever the decision to treat malaria is reached irrespective of fever status to guide appropriate malaria case management in malaria endemic and high transmission settings.

Author Contributions

R.F and C.F conceptualized and designed the study, R.F.; P.U.; GU.; W.H and C.O carried out the methodology, P.U and W.H entered data into SPSS software, R.F and C.F validated the data, R.F., P.U.; G.U.; and C.O.; carried out the formal data analysis, C.F.; O.O.; OW investigation, C.F.; O.O.; and O.W.; gave financial resources, R.F.; P.U.; GU.; W.H and C.O carried out the data curation, R.F.; P.U.; and W.H.; drafted the manuscript, C.F.; O.O; and O.W; reviewed and edited the scientific content of the draft, R.F.; and C.F.; supervised the project. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Ethical Consideration

Ethical approval for this study was obtained from Bayelsa State Ministry of Health.

Informed Consent Statement

As the majority of the population were uneducated, verbal informed consent was obtained from all participants (parents/guardians in the case of minors) before any study related procedures were carried out. The study procedure was carefully explained to each guardian in addition to a general education on the procedures which was done before the study started. This was in addition to community consent mentioned above. Each finger prick was carefully examined to ensure there was no loss of blood after the procedure. Each finger prick was covered by sterile plaster for a few minutes before the children were sent home. Information about participants was concealed with utmost confidentiality while participation was voluntary.

Data Availability Statement

On request

Acknowledgments

We thank the community leaders, parent and guardians for their cooperation.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Badmos AO, Alaran AJ, Adebisi YA, Bouaddi O, Onibon Z et al. (2021). What Sub-Saharan African Countries Can Learn from Malaria Elimination in China. Trop. Med. Health 49: 86.

2. Sachs J, Malaney P (2002) The Economic and Social Burden of Malaria. Nature 2002 415: 680-5.

3. WHO (2022) Fact Sheet about Malaria Available online.

4. Omang J, Ndep AO, Offiong D, Out F, Onyejose K (2020) Malaria in Pregnancy in Nigeria: A Literature Review. Int. Healthc. Res 3: 346-8.

5. Weiss DJ, Bertozzi-Villa A, Rumisha SF, Amratia P, Arambepola R et al. (2021) Indirect Effects of the COVID-19 Pandemic on Malaria Intervention Coverage, Morbidity, and Mortality in Africa: A Geospatial Modelling Analysis. Lancet Infect. Dis 21: 59-69.

6. Orem JN, Kirigia JM, Azairwe R, Kasirye I, Walker O (2012) Impact of Malaria Morbidity on Gross Domestic Product in Uganda. Int. Arch. Med 5-12.

7. Hemingway J, Shretta R, Wells TNC, Bell D, Djimdé AA et al. (2016) Tools and Strategies for Malaria Control and Elimination: What Do We Need to Achieve a Grand Convergence in Malaria? PLoS Biol 14: e1002380.

8. Singhi S, Chaudhary D, Varghese GM, Bhalla A, Karthi N et al. (2014) Tropical Fevers: Management Guidelines. Indian J. Crit. Care Med. Peer-Rev. Off. Publ. Indian Soc. Crit. Care Med 18: 62-9.

9. Um C (2016) Malaria Treatment in Children Based on Presumptive Diagnosis: A Make or Mar? Pediatr. Infect. Dis. Open Access 1.

10. Okoro C, Chukwuocha U, Nwakwuo G, Ukaga C (2015) Presumptive Diagnosis and Treatment of Malaria in Febrile Children in Parts of South Eastern Nigeria. J. Infect. Dis. Ther 3: 2332-0877.1000240.

11. Gimenez AM, Marques RF, Regiart M, Bargieri DY (2021) Diagnostic Methods for Non-Falciparum Malaria. Front. Cell. Infect. Microbiol. 11.

12. WHO (2012) Disease surveillance for malaria control : an operational manual. Published 2012. Accessed 16.

13. Berzosa P, de Lucio A, Romay-Barja M, Herrador Z, González V et al. (2018) Comparison of Three Diagnostic Methods (Microscopy, RDT, and PCR) for the Detection of Malaria Parasites in Representative Samples from Equatorial Guinea. Malar. J 17: 333.

14. Barber BE, William T, Grigg MJ, Yeo TW, Anstey NM (2013) Limitations of Microscopy to Differentiate Plasmodium Species in a Region Co-Endemic for Plasmodium Falciparum, Plasmodium Vivax and Plasmodium Knowlesi. Malar. J 12: 8.

15. Oyegoke OO, Maharaj L, Akoniyon OP, Kwoji I, Roux AT (2022) Malaria Diagnostic Methods with the Elimination Goal in View. Parasitol. Res 121: 1867-85.

16. Azikiwe C, Ifezulike C, Siminialayi I, Amazu L, Enye J (2012) A Comparative Laboratory Diagnosis of Malaria: Microscopy versus Rapid Diagnostic Test Kits. Asian Pac. J. Trop. Biomed 2: 307-10.

17. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S (2009) Malaria Diagnosis: A Brief Review. Korean J. Parasitol 47: 93-102.

18. Cunningham J, Jones S, Gatton ML, Barnwell JW, Cheng Q et al. (2019) A Review of the WHO Malaria Rapid Diagnostic Test Product Testing Programme (2008–2018): Performance, Procurement and Policy. Malar. J 18: 387.

19. Moody A (2002) Rapid Diagnostic Tests for Malaria Parasites. Clin. Microbiol. Rev 15: 66-78.

20. Kavanaugh MJ, Azzam SE, Rockabrand DM (2021) Malaria Rapid Diagnostic Tests: Literary Review and Recommendation for a Quality Assurance, Quality Control Algorithm. Diagnostics 11: 768.

21. Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J (2014) Plasmodium Falciparum Parasites Lacking Histidine-Rich Protein 2 and 3: A Review and Recommendations for Accurate Reporting. Malar. J 13: 283.

22. Poti KE, Sullivan DJ, Dondorp AM, Woodrow CJ (2020) HRP2: Transforming Malaria Diagnosis, but with Caveats. Trends Parasitol 36: 112-26.

23. Yan J, Li N, Wei X, Li P, Zhao Z et al. (2013) Performance of Two Rapid Diagnostic Tests for Malaria Diagnosis at the China-Myanmar Border Area. Malar. J 12: 73.

24. Funwei R, Nderu D, Nguetse CN, Thomas BN, Falade CO (2019) Molecular Surveillance of Pfhrp2 and Pfhrp3 Genes Deletion in Plasmodium Falciparum Isolates and the Implications for Rapid Diagnostic Tests in Nigeria. Acta Trop 196: 121-5.

25. Prosser C, Gresty K, Ellis J, Meyer W, Anderson K et al. (2021) Plasmodium Falciparum Histidine-Rich Protein 2 and 3 Gene Deletions in Strains from Nigeria, Sudan, and South Sudan. Emerg. Infect. Dis 27: 471-9.

26. Slater HC, Ding XC, Knudson S, Bridges DJ, Moonga H et al. (2022) Saad, N.J.; De Smet, M.; Bennett, A.; Dittrich, S.; Slutsker, L.; et al. Performance and Utility of More Highly Sensitive Malaria Rapid Diagnostic Tests. BMC Infect. Dis 22: 121.

27. Okyere B, Owusu-Ofori A, Ansong D, Buxton R, Benson S et al. (2020) Point Prevalence of Asymptomatic Plasmodium Infection and the Comparison of Microscopy, Rapid Diagnostic Test and Nested PCR for the Diagnosis of Asymptomatic Malaria among Children under 5 Years in Ghana. PLoS ONE 15.

28. Wangai LN, Karau MG, Njiruh PN, Sabah O, Kimani FT et al. (2011) Magoma, G.; Kiambo, N. Sensitivity of Microscopy Compared to Molecular Diagnosis of P. Falciparum: Implications on Malaria Treatment in Epidemic Areas in Kenya. Afr. J. Infect. Dis 5: 1-6.

29. Akindeh NM, Ngum LN, Niba PTN, Ali I.M, Ayem OLO et al. (2021) Assessing Asymptomatic Malaria Carriage of Plasmodium Falciparum and Non-Falciparum Species in Children Resident in Nkolbisson, Yaoundé, Cameroon. Children 8: 960.

30. Jiram AI, Ooi CH, Rubio JM, Hisam S, Karnan G et al. (2019) Evidence of Asymptomatic Submicroscopic Malaria in Low Transmission Areas in Belaga District, Kapit Division, Sarawak, Malaysia. Malar. J 18: 156.

31. Agbana HB, Rogier E, Lo A, Abukari Z, Jones S et al. (2022) Gyan B Aidoo, M.; Amoah, L.E. Detecting Asymptomatic Carriage of Plasmodium Falciparum in Southern Ghana: Utility of Molecular and Serological Diagnostic Tools. Malar. J 21: 57.

32. Kimenyi KM, Wamae K, Ochola-Oyier LI (2019) Understanding P. Falciparum Asymptomatic Infections: A Proposition for a Transcriptomic Approach. Front. Immunol 10: 2398.

33. Okell LC, Bousema T, Griffin JT, Ouédraogo AL, Ghani AC et al. (2012) Factors Determining the Occurrence of Submicroscopic Malaria Infections and Their Relevance for Control. Nat. Commun 3: 1237.

34. Funwei R, Akpe AB, Tonkiri A, Akuegbe DE, Gilbert A et al. (2021) Age-Dependent Prevalence of Malaria in Akeddei Community in South-South Nigeria. Pan Afr. J. Life Sci 5: 257-62.

35. Greenwood BM (1987) Asymptomatic Malaria Infections - Do They Matter? Parasitol. Today 3: 206-14.

36. WHO (2016) Available online.

37. WHO (2010) Basic Malaria Microscopy: Tutor's Guide; World Health Organization.

38. Funwei RI, Thomas BN, Falade CO, Ojurongbe O (2018) Extensive Diversity in the Allelic Frequency of Plasmodium Falciparum Merozoite Surface Proteins and Glutamate-Rich Protein in Rural and Urban Settings of Southwestern Nigeria. Malar. J 17: 1.

39. Gatti S, Gramegna M, Bisoffi Z, Raglio A, Gulletta M et al. (2007) Klersy, C.; Bruno, A.; Maserati, R.; Madama, S.; Scaglia, M.; et al. A Comparison of Three Diagnostic Techniques for Malaria: A Rapid Diagnostic Test (NOW Malaria), PCR and Microscopy. Ann. Trop. Med. Parasitol 101: 195-204.

40. Nwele DE, Onyali IO, Iwueze MO, Elom MO, Uguru OES (2022) Malaria Endemicity in the Rural Communities of Ebonyi State, Nigeria. Korean J. Parasitol 60: 173-9.

41. Falade CO, Ajayi IO, Nsungwa-Sabiiti J, Siribié M, Diarra A et al. (2016) Malaria Rapid Diagnostic Tests and Malaria Microscopy for Guiding Malaria Treatment of Uncomplicated Fevers in Nigeria and Prereferral Cases in 3 African Countries. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am 63: S290-7.

42. Doolan DL, Dobaño C, Baird JK (2009) Acquired Immunity to Malaria. Clin. Microbiol 22: 13-36.

43. Heinemann M, Phillips R, Vinnemeier C, Rolling C, Tannich E et al. (2018) High Prevalence of Asymptomatic Malaria Infections in Adults, Ashanti Region, Ghana, 2018. Malar. J 19.

44. Okiring J, Epstein A, Namuganga JF, Kamya EV, Nabende I et al. (2022) et al. Gender Difference in the Incidence of Malaria Diagnosed at Public Health Facilities in Uganda. Malar. J 21: 22.

45. Quaresima V, Agbenyega T, Oppong B, Awunyo JADA, Adu Adomah P et al. (2021) Are Malaria Risk Factors Based on Gender? A Mixed-Methods Survey in an Urban Setting in Ghana. Trop. Med. Infect. Dis 6: 161.

46. Cucunubá ZM, Guerra A, Rivera J, Nicholls R (2012) Comparison of Asymptomatic Plasmodium Spp. Infection in Two Malaria-Endemic Colombian Locations. Trans. R. Soc. Trop. Med. Hyg 107.

47. McMorrow ML, Aidoo M, Kachur SP (2011) Malaria Rapid Diagnostic Tests in Elimination Settings—Can They Find the Last Parasite? Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis 17: 1624-31.

48. Oboh MA, Oyebola KM, Ajibola O, Thomas BN (2022) Nigeria at 62: Quagmire of Malaria and the Urgent Need for Deliberate and Concerted Control Strategy. Front. Trop. Dis 3.

49. Ogunfowokan O, Ogunfowokan BA, Nwajei AI (2020) Sensitivity and Specificity of Malaria Rapid Diagnostic Test (MRDT CareStatTM) Compared with Microscopy amongst under Five Children Attending a Primary Care Clinic in Southern Nigeria. Afr. J. Prim. Health Care Fam. Med 12: 2212.

CEOS
CENTER OF EXCELLENCE FOR OPEN SCIENCE

CEOS is an growing Open Access Publisher with diverse peer reviewed journals supported by Aca demic Editors, Scholars, Physicians, Instructors,

CEOS Publishers follow strict ethical standards for publication to ensure high quality scientific studies, credit for the research participants. Any ethical issues will be scrutinized carefully to maintain the integrity of literature.

CEOS Publishers believes scientific integrity and intellectual honesty are essential in all scholarly work. As an upcoming publisher, our commitment is to protect the integrity of the scholarly publica-tions, for which we take the necessary steps in all aspects of publishing ethics.

All the articles published in CEOS Publisher journals are licensed under Creative CommonsCC BY 4.0 license, means anyone can use, read and dow-nload the article for free. However, the authors reserve the copyright for the published manuscript.