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Diagnosis of Malaria: Comparing Malaria Rapid Diagnostic Test and Blood Film Microscopy among Febrile Children at a Tertiary Health Facility in Lafia Nasarawa State Nigeria

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Abstract **Background:** Malaria is an infectious disease caused by Plasmodium and transmitted by the bite of an infected female Anopheles mosquito. This study determined the knowledge of caregivers about malaria, prevalence of malaria and compares the results of testing for malaria using rapid diagnostic test (mRDT) and microscopy. **Methods:** A prospective study carried out among children < 15 years in Lafia Nigeria. Testing was done using the Histidine Rich Protein-2 RDT kit and blood film microscopy. Bivariate analysis was done. Significant p is < 0.05. **Results:** Mean age of this study population is 15.0 ± 4.6 years. The overall incidence of malaria using RDT was 45.4% while 16.5% was reported positive using microscopy. The positive RDT was highest among children aged 6 month to < 5 years. There was a statistically significant difference (p < 0.0001) between malaria diagnoses by RDT compared with the microscopy which implies that there is difference between the number of positive cases by mRDT and numbers of positive cases by microscopy. Overall 40 (14.1) participants were positive to both mRDT and microscopy in this study. **Conclusions:** There is high incidence of malaria in this study as one out of two febrile children seen had malaria. Rapid Diagnostic Test is a more efficient diagnostic tool for malaria compared with the microscopy. We therefore recommend; more efforts to be directed to halting the rising trend of new cases of malaria and RDT should be deployed at all levels of healthcare in diagnosing all febrile illness and prompt treatment based on the National guidelines.

Keywords: *children, febrile, malaria, microscopy, rapid diagnostic test*

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1. Introduction

Malaria has remained a leading public health problem and cause of deaths in tropical and subtropical countries. [1] It is an infectious disease caused by Plasmodium species and transmitted by the bite of an infected female Anopheles mosquito. [2] These mosquitoes primarily habitat of the tropical and subtropical parts of the world. [3] The four known species of *Plasmodium* genus that cause human malaria are; *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae*.[4] Occasionally, humans become zoonotically infected with Plasmodium specie that normally infect monkeys such as the *Plasmodium knowlesi.* [5]

Malaria is the predominant cause of elevated body temperature in our environment. [6] This avoidable disease has reached epidemic proportions in the the sub-Saharan Africa which accounts for over 90% of the global malaria cases. [7] Malaria cases have reduced from 251 million in 2010 to 228 million in 2019. [8] Globally, malaria affects more than three billion people while deaths from it are ravaging the African countries. [9] In Nigeria, malaria accounted for over 60% of out-patient visit and 20-30% of admission among children. [10,11] It is the commonest cause of death among children aged five years and below. [12]

The malaria National control policy had mandated the conduction of parasitological test to confirm diagnosis before treatment. This is by using either the Rapid Diagnostic Test for malaria [mRDT] or blood film microscopy before commencing treatment of uncomplicated malaria. [13] Microscopy, the gold standard is faced with challenges in its performance such as in emergencies, inadequate power supply and the limitation in the technical expertise required. [14] The mRDT on the other hand is readily available in commercial quantities, easy to perform and interpret without need for power or any elaborate training. [15] The result is read in 10 – 15 minutes. [15] The mRDT can be antigen based like the Histidine-rich protein-2 in the present study but can also be antibody based, the former is preferred. [14]

A recent study by Ahmed *et al* in Ethiopia, reported a 3.9% prevalence of malaria among under five children using the mRDT only. [16] Another study by Garba *et al* in Nigeria among under five children reported 46.6% and 8.5% prevalences of malaria using microscopy and mRDT respectively. [17]

Complications from malaria which may lead to death can be avoided by preventing malaria, promptly diagnosing it if not prevented and giving appropriate treatment. [18] Malaria continues to be a global challenge with about half of the world's population being at risk of the disease and under–5 children being the most vulnerable. [15] Fatally afflicted children often die less than 72 hours after developing symptoms. [14] In those children who survive, malaria drains vital nutrients from them impairing their physical and intellectual development. [7,11] In general, malaria is a curable disease if diagnosed and treated promptly and correctly.

An earlier study in this centre by Bello *et al* reported that malaria accounted for 20% of admissions and 18% of the overall childhood mortality. [19] The above study was among those with complications from malaria (severe malaria). The present study determined the burden of new malaria cases using microscopy and mRDT in a health facility in Lafia, which is the only secondary and tertiary health facility in this locality.

2. Objectives

This study seeks to;

i. Determine the incidence of malaria in febrile children 6 months to 15 years visiting DASH Lafia

ii. Compare the incidence of malaria using microscopy and malaria Rapid Diagnostic Test. (mRDT) among children 6 months to 15 years with febrile malaria visiting DASH Lafia.

3. Materials and Methods

3.1. Study Area

The study was carried out at the Dalhatu Araf Specialist Hospital (DASH), Lafia. This is a referral centre for most private Hospitals, General Hospitals and primary health facilities in the state. Lafia is the capital city of Nasarawa State, one of the six North – Central States.

3.2. Study Population

The study populations are febrile children aged 6 months – 15 years visiting the Dalhatu Araf Specialist Hospital in the Southern senatorial zone of Nasarawa state.

3.3. Study Design

A cross-sectional, hospital-based study was carried out between 1st August to the 31st October 2020 among children visiting DASH at the Paediatric Out-Patients Department (POPD), General Out-Patient Department (GOPD) and the Emergency Paediatrics Unit (EPU).

3.4. Sample Size Determination

The formula for sample size determination of a cross sectional study of febrile children was used.

$$n = \frac{Z^2 PQ}{d^2}$$

Where n= Sample size
Z= Z statistic for a level of confidence or alpha-deviate
P = prevalence or proportion from a previous study. The prevalence rate of 20% reported at Nnewi Nigeria. [15]
d= degree of precision
Therefore
Z= 1.96 at alpha=0.05
P= 0.2
Q=1 – P = 1 – 0.2 = 0.8
d= 0.05

$$Samplesize(n) = \frac{1.96^2 x0.2(1-0.2)}{.05^2} = 246$$

Final sample size, N = n + NRR

Non Response Rate = $\frac{246}{100} X10$ = 24.6 approx= 25.

The study was conducted on a final sample size of a minimum of 271 persons.

3.5. Sampling Method

A convenience sampling was used in the recruitment until the desired number was reached. The parents / care-givers and or the child were approached at presentation. The purpose of the study was explained to them and those that consented where recruited for the study while assent was gotten from children aged seven years and above.

3.6. Eligibility Criteria

3.6.1. Inclusion Criteria

a) All febrile children aged 6 months – 15 years visiting Dalhatu Araf Specialist Hospital Lafia, Nasarawa from 1st August to 31st October 2020 are eligible
b) Those whose parents / care-givers gave informed consent
c) All those children that tested positive to malaria (whether uncomplicated or complicated)
d) Children aged seven years or more who gave assent

3.6.2. Exclusion Criteria

a) Children whose parent declined consent
b) Children who tested positive to malaria or had used anti-malaria in the past two weeks.
c) Children seven years and above that decline assent
d) Children who are very sick
e) Children with co-morbidities such as typhoid, pneumonia

3.7. Ethical Consideration

A formal ethical clearance was obtained from the DASH Research Ethics Committee where issues related to consent, confidentiality and safety of the patient were addressed.

3.8. Data Collection Procedure

A pre-tested questionnaire was administered to the participants after recruitment in order to collect information on their demographic and socioeconomic variables by trained research assistants. Parasitology was done using mRDT at first and subsequently microscopy.

3.9. Blood Sampling and Examination

Approximately 0.5-1.0 ml of venous blood was drawn from each participant into an EDTA tube. All blood samples were transported to DASH parasitology laboratory where the laboratory analysis was carried out. About two drops of blood was taken each for the RDT and for preparing slides for thick and thin blood film. The sampling site is often cleaned with spirit swab for sterilization and allowed to dry. A dry cotton wool swab is made available for arresting the bleeding after taking the desired quantity of blood. A column is created for recording the malaria results based on microscopy and mRDT.

Thick and thin blood films were prepared and rapid testing using the Histidine Rich Protein-2 (HRP-2) CareStart^{TM} (Pf) RDT kit was done concurrently for each blood sample. The blood films were stained with diluted Giemsa and then examined microscopically for the presence of malaria parasites; 100 fields under 100 x magnification were examined from the thick film before the slide was considered negative. All the children had microscopy and mRDT. Most of the care-givers are full time house wives. They normally give paracetamol to a febrile child before coming to the hospital.

3.10. Data Analysis Plan

All data generated from the research was analyzed and presented using SPSS version 23. A descriptive analysis was done showing frequencies among age groups and percentile. At the bivariate level, chi-square test and fisher's exact test was used with some variables to determine the significant association between some variables of interest and presence of malaria in children. Mean and standard deviation of quantitative variables were determined.

3.11. Confidentiality of Data

To maintain privacy and confidentiality, data was collected anonymously without identifiers and stored on a pass worded retrieval system where only the principal investigator had access to it.

3.12. Study Duration

This study lasted for three months, from August 1st and October 31st 2020. During which samples were collected, processed and read.

4. Results

4.1. The Socio-demographic Characteristics of the Study Participants

The average age of participants in this study was 15.0 ± 4.6 years. Most participants (67.3%) were aged 6 months to less than 5 years. There were more males (54.6%) while females were (45.4%) (Table 1).

Table 1. Socio-demographic characteristics of the study participants

Variables	Frequency (%)
Age: mean ± SD	15.0 ± 4.6 years
< 5	191 (67.3)
5 – 10	53 (18.7)
> 10	40 (14.0)
Sex	
Male	156 (54.6)
Female	128 (45.4)

SD = Standard deviation.

4.2. Social Status of Care-givers

Majority of the participants, 66.9% lived in rural locations compared to the urban dwellers. Based on level of education of care-givers of the participants in this study, a quarter of the care-givers (25%) were not educated while only 22.9% had completed tertiary education. Most of the study participants (53.2%) were unemployed as at the time of the study (Table 2).

Table 2. Social status of care-givers

Variables	Frequencies (percentages)
Location	
Rural	190 (66.9)
Urban	94 (33.1)
Education Qualification of care-giver	
Not educated	71 (25.0)
Primary	61 (21.5)
Secondary	87 (30.6)
Tertiary	65 (22.9)
Employment status of care-giver	
Employed	133 (46.8)
Unemployed	151 (53.2)

4.3. Prevalence of Malaria by Age Group and Sex

Of the 191 febrile children who were aged 6 months to < 5 years, 82 (42.9%) were positive for malaria using mRDT and 28 (14.7%) were positive using microscopy. Similarly, out of 53 participants who were between

5 – 10 years old 23 (43.4%) were positive using mRDT and 8 (15.1%) were positive using microscopy and out of 40 participants who were >10 years old, 24 (60.0%) were mRDT positive and 11 (27.5%) were microscopy positive. There were differences noticed between the use of mRDT or microscopy for malaria diagnosis by age of participant but not statistically significant (p = 0.132).

Likewise breakdown of prevalence of malaria by sex revealed 69 (44.2%) male were mRDT positive and 60 (46.9%) female were mRDT positive. Similarly 24(15.4%) males were positive by microscopy and 23 (18.0%) females were positive by microscopy. The observed differences were not significant statistically (p = 0.560).

Of the 129 (45.4%) with positive RDT, it was highest among those aged less than five years 82 (82/129 = 63.6%). While of the 47 (16.5%) with positive microscopy, most 28 (28/47 = 59.6%) were under five years. The overall prevalence of malaria in this study using mRDT was 45.4% and microscopy was 16.5% (Table 3).

4.4. Comparing Results of mRDT and Microscopy

In all, there were 129 (45.4%) positive casesof malaria using mRDT and 47 (16.5%) positive cases using microscopy. This was found to be statistical significance (p < 0.0001) which implies that there is difference between the number of positive cases by mRDT and numbers of positive cases by microscopy.

Overall, 40 (40/284 = 14.1) participants were positive to both mRDT and microscopy (Table 4).

Table 3. Prevalence of malaria by age and sex

Variable		mRDT (%)		Microscopy (%)			
		Neg	Pos	Neg	Pos	χ^2	p value
Age group (years)	6m - < 5 yr	109 (57.1)	82 (42.9)	163 (85.3)	28 (14.7)	4.048	0.132
	5 – 10	30 (56.6)	23 (43.4)	45 (84.9)	8 (15.1)		
	> 10	16 (40.0)	24 (60.0)	29 (72.5)	11 (27.5)		
Sex	Male	87 (55.8)	69 (44.2)	132 (84.6)	24 (15.4)	0.340	0.560
	Female	68 (53.1)	60 (46.9)	105 (82.0)	23 (18.0)		
		155 (54.6)	129 (45.4)	237 (83.5)	47 (16.5)		

Neg = Negative, Pos = Positive, χ^2 = chi square, mRDT = Rapid Diagnostic Test for malaria.

Table 4. Comparing results of mRDT and microscopy

		Microscopy				
		Neg (%)	Pos (%)	Total	χ^2	p value
mRDT	**Neg**	148 (95.5)	7 (4.5)	155 (54.6)	35.778	**< 0.0001**
	Pos	89 (69.0)	40 (31.0)	129 (45.4)		
	Total	237 (83.5)	47 (16.5)	284 (100)		

Neg = Negative, Pos = Positive, χ^2 = chi square, mRDT = Rapid Diagnostic Test for malaria.

5. Discussion

This study showed an overall incidence of 45.4% of malaria among symptomatically diagnosed febrile children at the DASH Lafia using mRDT and 16.5% using microscopy. This may be attributed to variation in sensitivities of the two tests. This high prevalence could be due to the study being conducted in the peak of raining season during malaria high transmission season. The season is usually characterized by collection of stagnant water (due to poor or blocked drainage systems) which is good breeding sites for the vectors, overgrown grasses around the houses and caregivers not using ITN. Risk factors associated with febrile malaria in children include access to and utilization of malaria care which might be a challenge in rural areas. Most of the participants in this study (66.9%) were from the rural area. This creates barriers that might include bad roads and transportation means as a risk factor. Another risk factor could be the caregiver educational qualification. About 25% of the care-givers in the current study are not educated at all while 21.5% had primary school certificate. This low level of education might be a barrier for the participant on clear understanding of healthcare services availability including the free ones. Furthermore, the finding of more than half (53.2%) of the participants in this study being unemployed, might explain the high prevalence found. This hinders the resources for transportation as well as for visiting the hospital for appropriate investigation and treatment.

In addition, this study found a higher proportion (positivity) of malaria was observed using the mRDT compared to the thick film microscopy technique. This is similar to earlier studies both within and outside Nigeria. [14,20,21] This is not surprising as RDT is a sensitive and specific tool for diagnosing malaria, while microscopy could be fraught with errors of high false negatives as it is largely examiner dependent. [22] Similarly, it could also be due to the presence of Histidine Rich Protein -2 antigen (HRP-2) which is known to persist at detectable levels for more than 14 days even after the symptoms of malaria could have disappeared and hence can over – diagnosed malaria. In other word, positive cases detected by Plasmodium falciparum HRP-2 test may be regarded as false positive cases. This study circumvented this limitation by the exclusion from this study of those that had used anti-malaria in the preceding two weeks. This study showed high malaria positivity among children under-five years similar to earlier studies. [23,24] In contrast, a study in Gusau Nigeria reported a higher positivity from microscopy 46.6% than RDT 8.5% respectively. [17] The exact reason for their findings is not clear but the possible explanations are; the RDT storage could have been compromised based on the epileptic power in their store as noted in their article. The laboratory

scientist are probably not WHO (World Health Organization) certified microscopists. Besides, their study was done at the beginning of raining season and it lasted only for a month.

The higher skills (technical know-how) required in performing an ideal microscopy compared with the RDT may also be a reason for the observation in the current study. Hence, making RDT for malaria an important screening tool for diagnosing malaria at all levels of health care in view of its high sensitivity and ease of performance. Overall, 40 (14.1%) were positive to both RDT and microscopy.

6. Conclusions

There is high incidence of malaria in this study as almost one out of two febrile children seen had malaria. Rapid Diagnostic Test is a more effective and efficient diagnostic tool for malaria compared with the microscopy in view of the former's ease of performance and with minimal training.

7. Recommendations

This study recommends that:

1. More efforts are required to stem the rising trend of new cases of malaria.
2. Malaria RDT should be deployed at all levels and be used in diagnosing all febrile illness and prompt treatment based on the National guidelines. There is need for training of laboratory scientists to be certified WHO malaria microscopist.

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Conflict of Interest

The authors declared no conflict of interest.

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