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Reliability of Rapid Diagnostic Tests in the Diagnosis of Malaria in Febrile Under-five Children in North Central Nigeria

Olufemi Mubo Olubiyi[1], Olubunmi Abiola Olubiyi[2], Bilqis Wuraola Alatishe-Muhammad[3], Mayowa Daniel Pemi[4], Fatai Akindele Olaniyan[5], Oladele Feyisola Olagbegi[6], Gloria Omonefe Oladele[4].

1. Department of Family Medicine, Bafrow Medical Centre, Serrekunda, The Gambia.
2. Medical Research Council Unit, The Gambia, at the London School of Hygiene and Tropical Medicine, Atlantic, Boulevard, Banjul, The Gambia.
3. Directorate of Planning, Research and Statistics Kwara State Ministry of Health, Ilorin. Nigeria.
4. Department of Family Medicine, Federal Medical Centre, Bida, Nigeria.
5. Department of Family Medicine, University College Hospital, Ibadan, Nigeria.
6. Department of Paediatrics, Bafrow Medical Centre, Serrekunda, The Gambia.

Corresponding Author: Dr Olubunmi Abiola Olubiyi, Email: abiolasalamiolubiyi@gmail.com

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Abstract

OBJECTIVE: The study is aimed at determining the reliability of rapid diagnostic tests in the diagnosis of malaria. METHODOLOGY: A cross-sectional hospital-based study among 161 febrile under-five children without obvious focus of infection seen at the GOPC of FMC, Bida. An HRP-2 based RDT and thick film microscopy were used for diagnosis. Data was analyzed and summarized by descriptive statistical methods, means, standard deviation and Chi-square test for associations. Level of significance was *p* value of <0.05. RESULTS: There was a male to female ratio of 1.4: 1 in the participants with mean age of 31.75 ± 15.18 months (Mean ± SD). Malaria diagnosis by thick film microscopy (TFM) reported a prevalence of 62.7 %, higher than 53.4% detected using RDT. The most frequently affected age groups were 12-23 months. The outcome of the TFM and RDT in febrile under-five child without obvious focus of infection was not influenced by the age and sex. Illness duration of three or more days (p <0.001*) and absence of cough were associated with positive RDT result (p <0.001*). Logistic regression analysis showed a statistically significant positive relationship between duration of symptoms and positive RDT result. The accuracy of RDT was found to be 63.4% with 74.4% PPV, 50.7% NPV, false positive rate of 36.7% and false negative rate of 36.6%. **CONCLUSION**: The findings in this study demonstrated the association between the rate of RDT positivity and the degree of parasitemia. Under field conditions, the RDT performance was well below the WHO recommended requirements and the performance of this HRP-2 based RDT test was below the manufacturer's preset performance level.

KEY WORDS: malaria diagnosis, febrile under-five, RDT.

Introduction

Malaria has been known to plague man since the beginning of ages and it can be seen mentioned in the ancient Roman, Chinese, Indian as well as Egyptian manuscripts and later in numerous Shakespearean plays, where it was allegedly caused by gods and deities controlling either air, humors (body fluids) or insects (like mosquitoes).[1] Nigeria, the most populous African country (estimated population > 170 million)[2] has the highest people at risk of malaria. Out of the teeming under-5 population, only 29% and 49% are covered by malaria prevention with Insecticide Treated Nets (ITN) and antimalarial coverage respectively; leading to about 3 million reported cases of childhood malaria in 2007 alone.[3] Available records in Nigeria showed that 50% of the population experience at least one episode of malaria per year and further showed that malaria accounts for 45-66% of all out-patient hospital visits and 30% of hospitalization.[4-6]

A global annual under-five mortality of 1 to 2 million is attributed to malaria and as much as 627,000 of such deaths occur in sub-Saharan African children.[7-10] Malaria is the third leading cause of under-five mortality.[3] Nigeria, along with 3 other sub-Saharan countries, constitute 50% of malaria-related mortality worldwide.[11] According to WHO, under-five mortality in Nigeria stands at an alarming 124/1000 live birth, and this is well above the global average of 48/1000 live birth.[3, 9] These deaths are unnecessary and could have been prevented if the WHO- T3 initiative is properly implemented.

A high prevalence of malaria in the under-five children regardless of the test used was reported by Iwuafor and colleagues in a study in Calabar, Nigeria.[12] According to the study, 46 (45.5 %) of the febrile patients had malaria parasitaemia by RDT, while 41 (59.4 %) had malaria parasitaemia by microscopy method.[12] There is an alarming overestimation of the proportion of febrile

illness attributed to malaria in resource-poor endemic regions due to lack of capacity for proper laboratory diagnosis; with majority of the healthcare providers relying on the clinical/ presumptive diagnosis as the basis for malaria chemotherapy.[8]

Generally, malaria RDTs are lateral flow immuno-chromatographic antigen-detection devices that capture dye-labeled antibodies on a strip of nitro-cellulose such as dipstick, card malaria RDT or cassettes/ wells; and they are able to detect malaria antigens like: HRP 2 (histidine-rich protein), pLDH (plasmodium-specific lactate dehydrogenase) enzyme and Aldolase enzyme.[13, 14] Some RDTs detect single specie (either *P. falciparum* or *P. vivax),* some detect multiple species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) and some further distinguish between *P. falciparum* and non-*P. falciparum* infestation, or between specific species.[14]

The use of rapid diagnostic tests (RDTs) have appreciably shortened the waiting time for test results to 15-20 minutes.[8, 13] Other attractive advantages have been elucidated and impressive sensitivity of RDT variously documented: Sensitivity of 100% and a positive predictive value (PPV) of 100% (that is, better than blood smear microscopy under field conditions) have been reported.[15, 16] Findings in Angola and Uganda ascribed sensitivity and specificity higher than the manufacturer's specifications to RDT.[17, 18] In another study, RDTs was found to perform equally well or even better than microscopy under field conditions.[19-21] Batwala et al. in Uganda, regarded RDT to be not only more sensitive but also more feasible than microscopy.[22] Ajumobi et al., investigated diagnostic performance of histidine-rich protein 2 (HRP-2)–based malaria rapid diagnostic test (RDT) in Kaduna, Nigeria (a mesoendemic area for malaria) by comparing RDT results with expert microscopy results

from 295 febrile under-five children.[16] The research revealed that 11.9% (35/295) tested positive with RDT compared with 10.5% (31/295) by microscopy; thus reporting: 100% sensitivity, 98.5% specificity, 88.6% positive predictive value (PPV), and 100% negative predictive value (NPV).[16] The RDT sensitivity was not affected by transmission season, parasite density, and age.[16] However, the use of RDTs is not widely accepted due to debates on their reliability that vary under various conditions. Using real-time polymerase chain reaction (PCR) as the "gold standard" method to assess the "Accuracy and Reliability of Malaria Diagnostic Techniques for Guiding Febrile Outpatient Treatment in Malaria-Endemic Countries", Rakotonirina et al., sampled 313 patients with clinical suspicion of uncomplicated malaria in 2 primary health centers in Madagascar; a very high degree of agreement was observed for conventional microscopy (0.99) and the HRP2-based test (0.93) and high for the pLDH-based test (0.82).[23] This study in north central Nigeria is aimed at determining the reliability of rapid diagnostic test in the diagnosis of malaria in febrile under-five children. The information obtained from this study is expected to fill gaps seen in study done on diagnosis of malaria. The result of the study will also help to inform health workers to consider the use of rapid diagnostic test in the diagnosis of malaria in febrile under-five children.

Methodology

The study was carried out in Bida Local Government Area of Niger State in North-Central Nigeria. The study centre was the General Outpatient Clinic of the Federal Medical Centre Bida. It is located in the ancient city of Bida with an estimated population of about 235,053.[24]

The study population were both male and female patients aged between 6 and 59 months seen at the GOPC of FMCB who

presented with fever without obvious focus of infection in whom presumptive diagnosis of malaria was made. The study was a hospital-based cross-sectional observational study, done over a period of seven consecutive months, from November 2016 to June 2017. Though a minimum sample size of 161 parents/caregivers of under-five children was obtained, 166 were recruited by systematic random technique but two opted out. Febrile children aged 6 to 59 months with no focus of infection whose parents/ guardian consented to participate in the study were included in the study while parents who were visitors were excluded from the study.

The research tool was pre-tested at the out-patient clinic of the Family Medicine Practice Center Outpost, Gawu-Babangida. An interviewer administered questionnaire was administered to evaluate information from 164 parents/ guardians of febrile under-five children three of which offered incomplete information. Therefore, a total of 161 participants proceeded to blood collection. (Figure 1). The collection of blood sample, using the capillary blood method and the RDT was done in the Family Medicine Departmental side laboratory using the Double-G malaria device for in-vitro self/ laboratory testing, batch number MAL13708, marketed by Davinny pharmaceutical limited. Ethical approval was obtained from Hospital Research Ethical Committee (HREC), Federal Medical Centre Bida.

Data analysis was done using IBM statistical software package (SPSS) version 23. A total of 161 questionnaires and samples were analyzed. Results were presented using tables, graphs and charts. Prevalence of malaria in febrile under-five children was determined using descriptive statistics. The Chi-square test was used to test for degree of association between categorical variables. The level of significance was set at P- value of less 0.05. Binary logistic regression was used to determine predictors (child's age,

duration of symptoms, presence of cough at presentation and history of previous hospital admission) of positive RDT test. Based on the results from the 2-by-2 table, the sensitivity, specificity, false negative rate, false positive rate, and the accuracy, as well as the likelihood ratio of both the positive and negative results were defined.

Results

Table 1: Age and sex distribution of the children

Variable	Frequency (N= 161)	Percentage (%)
Age (months)		
6-11	14	8.7
12-23	43	26.7
24-35	32	19.9
36-47	30	18.6
48-59	42	26.1
Sex		
Female	66	41.0
Male	95	59.0
Mean ± SD	31.75 ± 15.18	

The most frequently encountered age group was 12-23 months with a frequency of 43 (26.7%). The mean age of the patients was 31.75 ± 15.18 months. The male to female ratio was 1.44: 1.

Table 2: History of current illness

Variable	Frequency (N = 161)	Percentage (%)
Duration of symptoms before presentation		
< 3 days	73	45.3
≥ 3 days	88	54.7
Fever		
At presentation	42	26.0
In the last 24 hours	64	39.8
In the last 72 hours	55	34.2
Symptoms presented with (history of current illness) *		
Reduced play	150	93.2
Shivering	38	23.6
Sweating	3	1.9
Vomiting	104	64.6
Aches/ body pains	6	3.7
Abdominal pains	1	0.6
Poor appetite/ bitterness of mouth	58	36.0
Convulsions	7	4.3
Cough	59	36.6

*: Presentation with multiple symptoms

Though all the subjects had a history of fever, 55 (34.2%) had fever within the last 72 hours and 64 (39.8%) had fever within the last 24 hours of presentation

Table 3: Comparison of malaria rapid diagnostic test (RDT) positivity with actual parasitemia identified by thick film microscopy (TFM)

	RDT tests				
	Positive	Negative	Total	χ^2	*p* value
Blood film	n (%)	n (%)	N (%)		
Parasite seen	64 (63.4)	37 (36.6)	101 (100.0)	10.783	0.001*
Not seen	22 (36.7)	38 (63.3)	60 (100.0)		
Total	86 (53.4)	75 (46.6)	161 (100.0)		

Evaluation	RDT (%)
Sensitivity	63.4%
Specificity	63.3%
Positive Predictive Value	74.4%
Negative Predictive Value	50.7%
False Positive rate	36.7%
False Negative rate	36.6%
Accuracy	63.4%

Measure of agreement (Kappa): 0.254; χ^2: Chi square; *: p value < 0.05

Table 4: Association between RDT positivity and degree of parasitemia

	RDT tests				
Blood film parasitemia	Positive n(%)	Negative n (%)	Total N (%)	χ^2	*p* value
None	22 (36.7)	38 (63.3)	60 (100.0)	25.262	<0.001*
+ 1	30 (48.4)	32 (51.6)	62 (100.0)		
≥ + 2	34 (87.2)	5 (12.8)	39 (100.0)		

χ^2: Chi square; *: *p* value < 0.05

Less than half, 30 (48.4%), of the samples with +1 TFM results were RDT positive, while 34 (87.2%) of those with ≥+2 TFM report were RDT positive.

Table 5: Predictors of RDT positive results using binary logistic regression

Variable	B	*p* value	OR (95% CI)
Age (months)	-0.068	0.534	0.934 (0.754 – 1.158)
Duration of symptoms (≥ 3 days)	1.328	0.001*	3.774 (1.786 – 7.973)
Cough	-1.681	<0.001*	0.186 (0.084 – 0.414)
Previous hospital admission	-0.705	0.081	0.494 (0.224 – 1.090)

B: Coefficient of logistic regression; OR: Odds ratio; 95% CI: 95% Confidence Interval; Predictive value: 73.1%; R^2: 0.294; χ^2:38.758; *p* value: <0.001

Symptom duration of three or more days (B= 1.328), (p= 0.001), (OR= 3.774) (95% CI= 1.786 – 7.973) and absence of cough (B= -1.681), (*p*= <0.001), (OR=0.186) and (95% CI= 0.084 – 0.414) showed relationship with a positive RDT result in statistically significant manners

Discussion

In this study, more males, 95 (59.0%), in the under-five group participated in the study than females, 66 (41.0%). This translated to a male to female ratio of 1.44: 1. More males were affected across all age groups except in the 6-11 months age group.

Similarly, a study conducted in a rural hospital (South-Eastern Nigeria) by Iloh et al. recruited 116 (59.2%) males and 80(40.8%) females with a male to female ratio of 1.5:1.[25]

Also in this study, the mean age of the patients was 31.75 ± 15.18 months, this is similar to a mean age of 30.4±5.6 months in the population studied by Iloh et al.[25]. However, age was not a predictor of RDT positivity.

The differences between the findings in this study and other cited works could be due to differences in age groups, method used in diagnosis and the geographic regions involved.

The prevalence of uncomplicated malaria in febrile under-five children presenting without obvious focus of infection seen at the GOPC of FMCB; as measured by the two routinely utilized diagnostic methods (TFM, and RDT) were 62.7 % and 53.4% respectively. The outcome of a study done at Aba, Nigeria where a prevalence of 65.0% (using RDT) was reported, and that of the study by Chineke et al.- who reported 71.1% overall prevalence in under-five children from 2012-2014; were both higher than the findings in this study.[26, 27] The proportion of febrile patients reported by Achizie and colleagues, was almost a replica of the findings in this study (61.2 % prevalence by microscopy and 55.4 % by RDT ($p < 0.05$)).[12] This high prevalence is an indication that malaria burden among under-five children is still very high, as non-immune individuals, (under-five children and pregnant women) bear most of the morbidity and mortality due to malaria in sub-Saharan Africa.[26, 28]

The observed prevalence in this study was much higher than the findings of 12% to 50% reported by Ojurongbe *et al*. and other authors such as: Achizie et al., Okeke et al. and Mazigo et al..[8, 12, 29-31] These discrepancies might be attributed to the differences in malaria endemicity in the various study areas, variations in malaria diagnostic methods, and the degrading effects of geographical climatic conditions on RDT kits used.[32-35]

This study revealed a statistically significant (*p* value: 0.001) relationship between RDT positivity and the degree of parasitemia demonstrated by TFM, such that higher level of parasitemia was associated with greater number of positive RDT results. As shown in this study, 30 (48.4%) of the +1 TFM results were RDT positive while 34 (87.2%) of those with ≥+2 TFM report were RDT positive. In a similar study, Kosack et al. observed an increased sensitivity of RDT tests as the level of parasitemia increases from a ++ to ++++ ($p < 0.005$).[36] Sensitivity of RDTs increases with an increase in parasitemia with a limiting value of 100 parasites/µl.[37] Bronner et al. observed that low parasitemia (0.1%) causes lack of reactivity with the pan-malarial antigen aldolase, and according to the works of Wongsrichanalai et al., RDT sensitivity declined at parasite densities of < 500/mcL blood for *P. falciparum* and < 5,000/mcL blood for *P. vivax*.[38, 39] A study by Shakya and colleagues showed that at the low parasite density, the RDT tested was almost insensitive.[40] Another study in the Brazilian Amazon by Andrade et al. published in the year 2010, showed superior performance of RDT (Optimal-IT) over microscopy in symptomatic malaria cases with low parasitemia under field conditions.[41]

These observed discrepancies might be due to [16, 40, 42]: (i) variations in parasite density and insufficient detection of low parasitemia by the rapid diagnostic test (ii) possible genetic heterogeneity of Pf HRP-2 expression,

deletion of HRP-2 gene, presence of blocking antibodies for Pf HRP-2 antigen or immune-complex formation that is: the prozone phenomenon, at high antigenemia or due to unknown causes. (iii) Sequestration of parasites (iv) false-negative reactions (v) differences in the antigen detected by rapid test detects, as only live parasites producing pLDH. and vi) differences in the skills of the microscopist employed.[16, 40, 42]

This study thus showed the disparity between RDT and TFM for the diagnosis of malaria parasitemia in the under-five febrile children without obvious focus of infection. The use of malaria thick film microscopy as gold standard against the RDT under field conditions, showed that the HRP-2 based RDT was able to correctly identify more than half of microscopically diagnosed malaria cases; while about a one-third had negative malaria parasite slides correctly identified as such by the RDT. The overall sensitivity of 63.4% recorded in this study is low implying that sizeable cases of malaria would be missed, while the recorded 63.3% specificity also means that a reasonable number of children would receive un-needed treatment; if RDT result was used for making a diagnosis prior to treatment. The false positive rate and false negative rate were 36.7% and 36.6% respectively.

Studies have shown RDT to be sensitive in the diagnosis of malaria and the sensitivity of RDT reported in this study was comparable to the 69.6%, reported from Lagos.[43] However, it was lower than the 73.7%-100% variously reported across Nigeria, the manufacturer's specifications and the WHO's recommendations for selecting and procuring RDTs.[14, 44-48] Furthermore, findings in Angola and Uganda ascribed sensitivity and specificity that were higher than the manufacturer's specifications to RDT.[17, 18] In another study, RDTs were found to perform equally well or even better than microscopy under field settings.[19-21] Batwala

et al. in Uganda noted that RDT was not only more sensitive but also more feasible than microscopy.[22]

In this study, the accuracy of RDT was found to be 63.4% with 74.4% PPV, 50.7% NPV and a J index (Reliability) of 0.267. The Kappa degree of agreement between the RDT method and TFM recorded in this study was 0.254 (0.469 when compared only with high degree of parasitemia on TFM). The reported accuracy of RDT in this study was similar to the 0.6 (60%) reported by Mbata et al. in Port Harcourt, but much lower than the 76.8% reported in North-West Nigeria and the 88.3% reported in River state, Nigeria.[47-49] The kappa degree of agreement between the tested RDT and malaria microscopy in this study was lower than the 99% (0.99) documented by Isa et al. in North Central Nigeria.[44] Likewise, the J index (0.267) recorded in this study was much lower than the 0.5 reported by Mbata et al.[49]

Variations in sensitivity between the different studies might be due to the differences in the types of RDTs used, test methodology, and the skills of the microscopists.[50] Other reasons for our low positivity rate using RDT could probably be due to low parasite density, as RDT does not detect low parasite density of < 50 mcl.[50] The high number of negative results for both RDT and microscopy noted in this study suggested the possibility of over-diagnosis of malaria if based on clinical diagnosis alone. This is in agreement with previous studies.[8, 22, 42, 51, 52]

Limitations

The mismatch of malaria antigen-antibody quantity in the RDT kits could have contributed to the high negative results leading to the poor performance of the RDT. Also, microscopy, against which the RDT was measured is known to be microscopist-dependent, which might have influenced the outcome of the results since there was no inter-observer validation.

Conclusion

This study was carried out to determine the reliability of RDTs in the diagnosis of malaria in febrile under-five children in North Central Nigeria with the use of TFM and RDT respectively. The RDT performance was well below the WHO recommended standards and even the manufacturer's specifications under field conditions. The degree of parasitemia affected the rate of RDT positivity which was not affected by both age distribution and gender of the under-five children while the pattern of clinical presentation generally did not influence the outcome of parasitological tests.

List of abbreviations

CI: Confidence Interval

FBC: Full Blood Count

FMCB: Federal Medical Centre, Bida

FMPC: Family Medicine Practice Center

GOPC: General Out-patients' Clinic

HIV: Human Immunodeficiency Virus

HRP-2: Histidine Rich Protein 2

HREC: Hospital Research Ethical Committee

IgG: Immunoglobulin G

ITN: Insecticide Treated Net

MCS: Microscopy, Culture and Sensitivity

NMIS: Nigeria Malaria Index Survey

NPV: Negative Predictive Value

OR: Odds Ratio

OTC: Over-the-Counter

PCM: Paracetamol

PCR: Polymerase Chain Reaction

Pf HRP: Plasmodium falciparum Histidine Rich Protein

pLDH : Plasmodium Lactate dehydrogenase

PPV: Positive Predictive Value

RDT: Rapid Diagnostic Test (for Malaria)

SPSS: Statistical Package for Social Sciences

T_3 initiative: Test, Treat and Track initiative

TFM: Thick Film Microscopy

UNICEF: United Nations International Children's Emergency Fund

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Hospital Research and Ethics Committee (HREC) of The Federal Medical Centre, Bida. (See attached additional file). Prior to administration of the questionnaire and sample collection, the study's aim and benefits were explained to each of the parents/guardians of the participants and written informed consent was obtained from them. During the data collection, all methods were performed in accordance with the World Health Organization standard guidelines and regulations.

Conflict of interests

The authors declare that they have no conflict of interests.

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