ORIGINAL ARTICLE

Uncomplicated malaria in children: The place of rapid diagnostic test

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ABSTRACT

Background: Malaria has remained a major cause of morbidity and mortality among the under-five children in Nigeria. Prompt and accurate diagnosis of malaria is necessary in controlling this high burden and preventing unnecessary use of anti-malarial drugs. Malaria rapid diagnostic test (MRDT) offers the hope of achieving this goal. However, the performance of these kits among the most vulnerable age group to malaria is inadequate**. Materials and Methods**: In this cross-sectional study, 433 out-patients, aged <5 years with fever or history of fever were enrolled. Each candidate was tested for malaria parasitaemia using ACON® malaria *pf*. Thick and thin films were also prepared from the same finger prick blood for each candidate**. Result:** Malaria rapid diagnostic test had sensitivity of 8.3%, specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 74%. The sensitivity of MRDT increased with increasing age. This effect of age on sensitivity was statistically significant (P = 0.007). Similarly parasite density had significant effect on the sensitivity of MRDT (P = <0.001). **Conclusion**: Histidine-rich protein-2 based MRDT is not a reliable mean of diagnosing malaria in the under-five age children with acute uncomplicated malaria.

Key words: Febrile, malaria, malaria rapid diagnostic test, out-patient, under-five

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INTRODUCTION

Blood-slide microscopy using thick and thin smears stained with Giemsa has remained the gold standard in the diagnosis of malaria for many years.[1,2] However, microscopy has its draw backs and limitations; it has high-fixed cost, and demands well-trained and experienced laboratory technician and microscopist.[3,4] These limitations have undoubtedly hindered the accurate diagnosis and reporting of malaria infection with all its attendant consequences.[5,6]

Recently, Malaria Rapid Diagnostic Tests (MRDTs) have been developed and are in operation in various settings that lack reliable microscopy.[7,8] Because of low-fixed cost and less demand for skilled training, MRDTs have prospect for wider distribution and use in comparison to microscopy.[9] These tests are being put forward as a potential solution for targeting valuable antimalarial drugs to those who need them. Several studies have shown high performance of MRDTs in the diagnosis of malaria.[10-18] There are fewer data on test performance in children, with varying reports on the effect of age on MRDTs performance.[10,13-15,19,20] Nigeria has adopted MRDTs as a diagnostic tool, where microscopic diagnosis is not feasible.[21] However, only very few studies have been done to assess the performance of these devices in the under-five age group in the country[10,11] and none to the best of my knowledge has been reported from northeastern Nigeria. This may be particularly necessary due to variation in histidine-rich protein-2 produced by *Plasmodium falciparum* from different regions and countries.[22,23] In addition, the hot climatic condition of Maiduguri, with ambient temperature reaching 45°C at the peak of the hot dry season in March and April,[24] which is well above the recommended storage temperature of 2°C to 30°C by manufacturers[25], could adversely affect the performance of these kits if there is disruption in the cold-chain.

This study is thus aimed at determining the performance of MRDTs in the diagnosis of malaria among under-five aged children in an out-patient clinic in semi-arid Maiduguri.

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MATERIALS AND METHODS

This was a hospital based cross-sectional observational study carried out at the paediatric general out-patient (PGOP) unit of University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Borno State of Nigeria. University of Maiduguri Teaching Hospital is a centre of excellence for infectious diseases and immunology. It serves as a referral centre for the six states in the region as well as the neighbouring countries of Cameroun, Chad, and Niger.

Consecutive children aged below five years presenting to the PGOP unit of the UMTH with presumptive malaria defined as fever (axillary temperature >37.5°C), and/or history of fever in the 72 hours prior to presentation,[27] who met the inclusion criteria were studied after an informed consent and approval by the research and ethical committee of the hospital were studied using malaria rapid diagnostic kit, ACON® Malaria *p. f*. (MRDT). The minimum sample size was calculated at 377 using Taylor's equation[28] and '*P*'-value was taken from a study in Jos Nigeria.[29]

Patients were excluded from the study if they were human immune-deficiency virus (HIV) positive, or had rheumatologic disease, or had a history of antimalarial treatment within 4 weeks prior to presentation or malaria prophylaxis, or had treatment with cotrimoxazole, tetracycline and clindamycin.

On the day of inclusion, demographic and clinical information were recorded using a questionnaire. Thick and thin blood smears were prepared, and the MRDT was performed from the same finger or heal prick blood sample. Number was allotted to every participant at the point of entry and was used for identification of MRDT kit, slides and questionnaire from the same patient. Results of the tests were disclosed to the guardians and those with positive malaria parasitaemia, and or positive MRDT were given antimalarial drugs free of charge that were sponsored by the researchers.

All MRDT tests were performed by a trained laboratory scientist and the results were read and interpreted within 15 minutes according to the manufacturer's instructions. For each MRDT the result was classified as negative, positive or invalid. All tests without a control line were considered invalid and were repeated.

Four slides were prepared for each patient; two thin and two thick smears and were air-dried. The thin blood smears were fixed with methanol and the thick smears were left unfixed. Each slide was subsequently stained with 10% Giemsa solution for ten minutes. All blood smears were examined microscopically under ×100 oil immersion. The thick smears were used for diagnosis of *Plasmodium* species and for parasite-density counting. Smears were considered negative if no parasites were seen in 100 oil-immersion fields. For positive smears, the number of parasites was counted against 200 white blood cells (WBC). Parasite density was calculated assuming 8,000 WBC per microlitre using the formula:[30]

$$\text{Parasite density} = \frac{\text{Number of parasites counted} \times 8000}{\text{Number of leukocytes counted}}$$

The thin smears were examined to confirm the parasite species for positive samples. Presence or absence of gametocyte in all negative slides was also recorded, and gametocyte-density counts (number of gametocytes/1,000 WBC) performed. All slides were double-read, blinded, by the first author and the laboratory scientist from the Department of Microbiology UMTH. There was an agreement of >95% between the first author and the laboratory scientist in slide reading.

Analysis of data

Data obtained were entered into a computer to generate a database. Analysis was done using Statistical Package for the Social Sciences (SPSS version 16.0, Chicago, IL, USA). Baseline characteristics (demographical, clinical, and parasitological) were analysed using descriptive statistics; mean, mode, medians, standard deviation, as appropriate. Results were presented in tables and figures wherein required. Frequencies and proportions were compared using Chi-square (χ^2) and strength of association was tested using Contingency Coefficient.

Sensitivity (True positive/True positive + False negative ×100), specificity (True negative/True negative + False positive ×100), PPV (True positive/True positive + False positive ×100) and NPV (True negative/True negative + False negative ×100) were calculated using the method of Galen and Gambino[31]. Further stratification by category of parasitaemia; <100, <1,000 and ≥1,000 parasites/µl for MRDT was done. A 95% confidence interval (95% CI) was given and a *P* -value of <0.05 was considered significant.

RESULTS

A total of 433 children were studied. There were 238 (55%) males and 195 (45%) females (M:F ratio 1.2:1). The mean age of the study population was 19.2 ± 14.3 months. Approximately half of the children studied, 203 (46.9%) were aged 12 months and below. The least frequency was observed among the 49 months and above age category, 18 (4.2%) [Table 1].

In all, 168 (38.8%) of the study population had fever at presentation with axillary temperature ranging between 37.6 and 40.1°C, whereas 264 (61.2%) had history of fever within the preceding 72 hours. The mean, median, and mode of the axillary temperature of the studied population were 37.2°C, 37°C, and 38°C respectively.

In all, 120 children (27.7%) had malaria parasitaemia, which was detected using microscopy. *Plasmodium*

falciparum was the only species detected in all the 120 malarial positive cases. The parasite density was generally low in this study. Fifty eight (48.3%) of the positive patients had parasite densities of <100/µl of blood, whereas only 9 (7.5%) patients had density of 1000/µl and above. Age was the only variable that was significantly associated with parasite density ($x^2 = 15.26$, $P = 0.004$). Using contingency coefficient (c) a significant but weak positive correlation was found between age and parasite density ($c = 0.344$, $P = 0.041$) [Table 2].

Malaria rapid diagnostic test was positive in only 10 (2.3%) of the children studied. The remaining 423 (97.7%) were negative. All the 10 (100%) MRDT positive children were also positive, microscopically. Although, MRDT showed a poor sensitivity of 8.3% (95% CI: 3.4-13.2), the test provided an excellent specificity of 100% with comparable similar positive predictive value of 100% and negative predictive value of 74%.

The MRDT positivity rate was generally low across the age categories, although, it increased progressively as the age advanced. It increased from 1% in the 0-12 months of age group to 11.1% in the 49-59 months of age group. This difference was statistically significant ($x^2 = 10.53$, $P = 0.001$) [Table 3]. Contingency coefficient (c) showed a statistically significant positive correlation between age and positivity of MRDT ($c = 0.178$, $P = 007$). The sensitivity also showed similar pattern of progressive increase with advancing age up to 48 months. This pattern was statistically significant ($x^2 = 8.110$, $P = 0.004$). The specificity and PPV, however, remained unchanged at 100% across various age group categories. There were slight variations in the NPV, but these variations were not statistically significant ($x^2 = 0.080$, $P = 0.777$) [Table 4].

The positivity rate of MRDT was low among both sexes. Males had slightly higher positivity rate 7 (2.9%) compared to the females 3 (1.5%). However, using Fisher's exact test, this difference was not statistically significant ($P = 0.525$) [Table 3]. Similarly, the sensitivity of MRDT was low among the sexes, although, males had slightly higher sensitivity of 9.9% as against 6.12% for females. This difference was, however, not statistically significant ($P = 0.525$). The specificity and PPV were excellent at 100% among both sexes. The NPV was comparable at 72.2% for the male as against 76.04% for the female ($x^2 = 0.765$, $P = 0.436$).

The positivity rate of MRDT was low irrespective of temperature at presentation. It was slightly higher among the afebrile children 2.6% compared to the febrile ones

Table 1: Age and Sex distribution of the study population

Age group (months)	Sex		Total *n* (%)
	Male *n* (%)	Female *n* (%)	
0-12	118 (27.3)	85 (19.6)	203 (46.9)
13-24	66 (15.2)	55 (12.7)	121 (27.9)
25-36	25 (5.8)	31 (7.2)	56 (12.9)
37-48	21 (4.9)	14 (3.2)	35 (8.1)
49-59	8 (1.9)	10 (2.3)	18 (4.2)
Total (%)	238 (55)	195 (45)	433 (100)

Table 3: Malaria rapid diagnostic test outcome by various variables among the study population

Variables	Rapid diagnostic Test		Total *n* (%)	x^2	*P*-value
	Positive *n* (%)	Negative *n* (%)			
Age groups (months)				10.53	0.001*
0-12	2 (1)	201 (99)	203 (100)		
13-24	2 (1.7)	119 (98.3)	121 (100)		
25-36	1 (1.8)	55 (98.2)	56 (100)		
37-48	3 (8.6)	32 (91.4)	35 (100)		
49-59	2(11.1)	16 (88.9)	18 (100)		
Sex					
Male	7 (2.9)	231 (97.1)	238 (100)		0.525**
Female	3 (1.5)	192 (98.5)	195 (100)		
Temperature at Presentation					
Fever	3 (1.8)	165 (98.2)	168 (100)		0.747**
No Fever	7 (2.6)	258 (97.4)	265 (100)		

*Statistically significant P <0.05; **Fisher's exact test

Table 2: Parasite density by various variables among the study population

Variables	Parasite density			x^2	*P*-value
	<100/µl *n*	100-999/µl *n*	≥1000/µl *n*		
Age groups (months)				15.26	0.004*
0-12	31	22	1		
13-36	20	25	3		
37-59	7	6	5		
Sex					
Male	31	33	7	2.286	0.319
Female	27	20	2		
Temperature at presentation					
Fever	22	23	3	0.525	0.769
No fever	36	30	6		

*statistically significant P < 0.05

Table 4: Predictive indices of malaria rapid diagnostic test by age in the study population

Age group (months)	Malaria rapid diagnostic test							
	Sen (%)	x^2	*P*-value	Spe (%)	PPV (%)	NPV (%)	x^2	*P*-value
		8.110	0.004*				0.080	0.777
0-12	3.7			100	100	74.1		
13-24	6.1			100	100	73.9		
25-36	6.6			100	100	74.6		
37-48	30.0			100	100	78.1		
49-59	25.0			100	100	62.5		

Sen – Sensitivity; Spe – Specificity; PPV – Positive predictive value; NPV – Negative predictive value; *Statistically significant P < 0.05.

1.8%. Using Fisher's exact test, this difference was, however, not statistically significant ($P = 0.747$) [Table 3]. Similarly, the sensitivity was higher, 9.7%, among the afebrile children as against 6.3% among the febrile ones, though not statistically significant ($P = 0.738$). Specificity and PPV were excellent at 100% in both groups, while NPV was higher among the afebrile children though not statistically significant ($x^2 = 0.226$, $P = 0.651$).

There was a marked disparity in the sensitivity of MRDT across the parasite density categories. At parasite density of <100/µl, MRDT detected no case of malaria parasitaemia out of 58 patients diagnosed microscopically, while at a density of ≥1000/µl, all the 9 (100%) children with malaria parasitaemia were detected by MRDT [Table 5]. This difference was statistically significant ($x^2 = 47.690$, $P = <0.001$). Using contingency coefficient (c) to test the strength of the association, there was strong positive correlation between the stratified parasite density and sensitivity of MRDT ($c = 0.687$, $P = <0.001$). The predictive values of a positive test was 0% among those with parasite density of <100/µl as there was neither true positive nor false positive detected within the group and excellent at 100% among the remaining two groups [Table 6].

DISCUSSION

This study established a wide disparity between rapid diagnostic test and microscopy in the diagnosis of malaria parasitaemia in the under-five febrile children. The overall sensitivity of 8.3% recorded in this study is very low implying that most cases of malaria would have been missed if treatment decision was based on MRDT result. It is far below the sensitivity of 82% and 69.6% reported from Enugu[11]and Lagos[10] in Nigeria, respectively, and 98.8% from Sierra Leone.[12] Several factors could explain this low sensitivity of the test kits in the patients studied. Several studies have shown that the ability of MRDT to detect presence of malaria parasite is directly proportional to the parasite density.[10,11,15,18] The observed increase in sensitivity of the test kits with increasing parasite density suggests inherent inability of the kit to detect parasite at low densities, thus, this wide disparity could be attributed to the overall low parasite density observed in this study. However, other workers[12,14] found no difference in sensitivity across categories of parasite densities. This may be due to high density parasitaemia recorded by Gestl *et al.*[12] in the Sierra Leone with inter-quartile parasite density range of 2620-79921 parasite/µl and 82.8% having parasite density ≥1000/µl as compared to this study where approximately 50% of the patients had parasite density of <100/µl which is far below the reported detection limit of HRP-2 based MRDT.[10,32] Further, the categorisation of parasite density could have a masking effect, if the upper limit of the lowest category is above the detection limit of the kit and this may be the situation in the finding of Laurent *et al.*[32] from Tanzania where the lowest category of parasite density was 500/µl, which is above the reported detection limit of HRP-2 kits.

Table 5: Malaria rapid diagnostic test outcome by parasite density among the 120 patients with positive malaria parasitaemia

Parasite density (*n*/µl)	Rapid diagnostic test		Total *n* (%)
	Positive *n* (%)	Negative *n* (%)	
<100	0 (0)	58 (100)	58 (100)
100-999	1 (1.9)	52 (98.1)	53 (100)
≥1000	9 (100)	0 (0)	9 (100)
Total *n* (%)	10 (8.3)	110 (91.7)	120 (100)

Table 6: Sensitivities and positive predictive values of malaria rapid diagnostic test by parasite density among the 120 patients with positive malaria parasitaemia

Parasite density (n/µl)	Malaria rapid diagnostic test			
	Sensitivity (%)	x^2	*P*-value	Positive predictive value (%)
		47.690	<0.001*	
<100	0			0
100-999	1.9			100
≥1000	100			100

*Statistically significant $P < 0.05$

The increase in sensitivity with increasing age in this study is similar to the findings from Lagos[10], Nigeria, and Tanzania.[14] This could be attributed to the increase in parasite density with increasing age observed in this study. In malaria holoendemic area, clinical malaria in infancy has been shown to be associated with very low parasite densities due to early loss of passively acquired maternal antibodies and delay in acquiring premunition.[33-36] However, other studies have found no significant change in sensitivity with increasing age.[13,15] This inconsistent findings may be due to variation in size of age grouping among the various studies as well as age group studied.

Sex and temperature at presentation had no significant effect on the sensitivity of MRDT in this study similar to the finding from Burkina Faso[37] with respect to sex. However, other workers[10,15] found a higher sensitivity in those with temperature of >37.5°C at presentation. It is not clear why there is inconsistency in the effect of temperature at presentation on the sensitivity of MRDT, however, history of fever as an inclusion criteria is subjective compared with objective measurement of temperature at presentation.

The high specificity of 100% obtained in this study is similar to findings in other studies.[10,11,13,15,18] This current finding provides confidence that there is no risk of wrongly treating any patient without malaria if diagnosis is done by MRDT. Nonetheless, other workers have reported lower specificities.[12,14-17] The disparity in specificities between

different studies could be due to differences in inclusion criteria. For instance, Kaushik *et al.*[16] from India found 25% of children who were positive for HRP-2 test had history suggestive of use of antimalaria drugs along with other treatments for fever, probably affecting parasitaemia but not the antigenaemia and thus high false positive results. In addition, malaria endemicity seems to inversely affect the specificity of MRDT; this is evident in the study by Abeku *et al.*[15] from Kenya and Uganda where specificity of 99.9% was recorded from hypoendemic site and 65.0% from mesoendemic site in the same study. The specificities in this study remained unchanged irrespective of category of parasite density, temperature at presentation, age and sex. However, some previous studies have found parasite density, age and temperature at presentation to significantly affect the specificity of rapid diagnostic test (RDT).[11,15,38] This disparity in the effect of age may be due to the differences in age of the studied population. While this study was limited to children under five years of age, Abeku *et al.*[15]and Bisoffi *et al.*[38] studied both children and adults. Although this study did not find parasite density and temperature at presentation to significantly affect the specificity of MRDT, other workers have reported a significant association between parasite density[11,15] and temperature at presentation.[15] This difference may be attributable to the generally low parasite densities observed in the present study.

The predictive value of a positive test of 100% implies that all positive MRDT truly have parasitaemia and thus treatment decision could reliably be based on it. This finding is comparable to the reported PPV in earlier studies,[10,15,18] but higher than that reported by others.[11,12,14] The variation in PPV among studies using HRP-2 kit may be due to failure to exclude patients who have had antimalarial drug prior to presentation thereby leading to high rate of false positivity. In addition, differences in malaria endemicity could also account for the observed differences.[15] The PPV in this study was not affected by age, sex, temperature at presentation and parasite density. This is similar to the finding of Abeku *et al.*[15] with regard to effect of temperature at presentation. Laurent *et al.*[14] showed PPV to decrease with increasing age, and this statistics could not be demonstrated in this study probably due to differences in the ages of the study population in the two studies.

The negative predictive values of 74% on the other hand, infers that the probability of a negative result excluding malaria is 0.74 and hence the need to exclude malaria by other means. This is lower than reported NPV in other studies.[10-12,14,15,18] This differences could be explained by the high rate of false negativity in this study, which resulted from the inability of the kit to detect malaria antigen at low parasite density in the face of overall low parasite densities recorded. This is corroborated by the progressive increase in NPV with increase in parasite density. Other workers[11,15] also demonstrated similar relationship between NPV and parasite density. In addition, temperature at presentation had no effect on NPV in this study, which is in agreement with a previous report.[15]

CONCLUSION AND RECOMMENDATION

Malaria rapid diagnostic test is a poor screening tool for malaria infection in the under-five aged children. While a positive rapid diagnostic test result in this age group is diagnostic of malaria; a negative result, on the other hand, is not a sufficient evidence to withhold antimalarial treatment. Hence, routine use of MRDT for the diagnosis of malaria among the under-five children should be done with caution.

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