

Challenges of Malaria Diagnosis In Paediatric Patients At A Nigerian Hospital

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ABSTRACT

Aims: This study compares the performance of routine malaria diagnostic tests, and explores the challenges of malaria diagnosis in paediatric patients in an endemic setting in South West Nigeria.

Study design: Cross sectional study

Place and duration of the study: The study was conducted at the children's outpatient and emergency units of the University College Hospital, Ibadan, Nigeria. Patients seen between May and August, 2013 were enrolled in the study.

Methodology: The records of all 532 children aged six months to 12 years who received treatment for an acute febrile illness at the hospital during the study period were reviewed. The proportion of children classified as having malaria by clinical diagnosis, Rapid Diagnostic Test (RDT) and blood smear microscopy were compared. Factors associated with test positivity were explored using multivariate analysis.

Results: By clinical diagnosis 45.2% of children were diagnosed as having malaria, 37.6% tested positive to malaria parasite on RDT and 19.3% had positive blood smears on microscopy. Logistic regression showed that with RDTs, younger children were less often found to be positive than older children [OR: 0.594 (0.401-0.879)]. A similar lower probability of positivity was found for younger children on microscopy [OR: 0.624 (0.391-0.996)]. Positive smears were however recorded 3.9 times more often for those who gave a history of fever compared to those who did not [OR: 3.882 (1.154-13.057)].

Conclusions: The true malaria morbidity among these paediatric patients remains questionable due to the differences in the results produced by the different diagnostic methods. The clinical implication of RDT-positive but microscopy-negative samples may be grave if microscopy results are erroneous. Quality control systems and surveillance of routine malaria diagnostics are imperative to limit misdiagnosis of malaria in this endemic setting.

Keywords: Malaria; rapid diagnostic tests; microscopy; paediatric; Nigeria)

1. INTRODUCTION

Malaria morbidity and mortality among Nigerian children is one of the highest in the world [1]. Prompt diagnosis and treatment have been identified as critical factors to reducing this burden [2]. A diagnostic challenge in malaria endemic areas has been the non-specific clinical symptoms of malaria which makes laboratory confirmation necessary in any febrile child [2]. Microscopy has long been the standard of malaria diagnosis, but newer diagnostic tests have now been introduced particularly in peripheral health care settings [3]. Many of the new rapid diagnostic tests for malaria are usually compared with microscopy as the gold standard. However, in the absence of expert microscopists, errors in microscopy make it difficult to assess the reliability of these new diagnostic techniques in routine settings [4].

33 Lack of adherence to malaria test results has led to concerns about the capacity of African
34 health systems to implement the policy of laboratory-confirmed malaria in children [5]. In
35 many places endemic areas clinical diagnosis of malaria has continued and this has been
36 attributed to several factors [5]. Nigeria like other malaria endemic countries in sub-Saharan
37 Africa has had a challenge reaching the goal of universal testing in line with the WHO's
38 recommendation [6]. In implementing this policy, it is important to also identify how to do so
39 effectively and efficiently, while avoiding potential problems associated with malaria
40 diagnosis [7]. In the era of evidence based medicine, it is often necessary to provide
41 additional context specific evidence from routine clinical settings to assess the challenges
42 with implementing confirmatory diagnostics in the management of malaria in endemic
43 settings in Nigeria. This study compares the performance of available malaria diagnostic
44 tests, and explores the challenges of malaria diagnosis among paediatric patients in an
45 endemic setting in South West Nigeria.

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48 **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY** 49 **(ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)**

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52 The study was conducted at the children's outpatient and emergency units of the University
53 College Hospital, Ibadan. The hospital serves as a referral hospital for South West Nigeria.
54 The hospital is located in Oyo state, South West Nigeria, a region with the highest malaria
55 parasite prevalence of 50.3% among children under 5 years.[8] In Nigeria, peak malaria
56 transmission occurs during the rainy season from April to October each year. Transmission
57 in South West Nigeria is holoendemic and occurs all year round. Plasmodium falciparum is
58 responsible for 97.3%, of all malaria infections in South West Nigeria.[8]

59 All 532 children aged six months to 12 years who received treatment for an acute febrile
60 illness at the hospital between May and August, 2013 were recruited for the study. Patients
61 were recruited from the children's out-patient department and the Children's Emergency
62 Ward, of the University College Hospital Ibadan over this four month period.

63 Using a cross sectional design, the record of patients seen over the study period were
64 reviewed to assess malaria diagnostic practices. Rapid diagnostic tests had been recently
65 introduced at out-patient departments to augment diagnosis with microscopy. Prior to the
66 study all clinicians were informed to request for RDTs in parallel with microscopy for all
67 febrile children presenting at paediatric entry points of the hospital.

68 The documented clinical diagnosis before laboratory test, malaria rapid diagnostic tests
69 result and microscopy examination of blood smears results was compared. The RDTs and
70 smears were done independently by personnel in different laboratories.

71 The SD Bioline^R kit for the detection of *P. falciparum* specific histidine rich protein-2 (Pf.HRP-
72 2) was used for all tests. SD Bioline RDT is WHO approved with reported sensitivity and
73 specificity of 90.2% and 98.5% for *P. falciparum* under routine conditions.[9] The test uses a
74 rapid antigen capture assay in a lateral flow immunochromatographic test, similar to a
75 pregnancy test, with a visual result in <15 min.

76 The protocol for malaria diagnosis in the routine malaria laboratory is Giemsa stained thick
77 and thin blood smears. Asexual *Plasmodium falciparum* parasites identified were counted
78 against 200 white blood cells. Smear was declared negative if no parasites were found after
79 examining 100 high power fields. Microscopy was performed by the usual laboratory
80 scientists in the hospital.

81 The primary outcome measure was the proportion of children classified as having malaria by
82 clinical diagnosis, RDT and blood smear microscopy. Malaria diagnosis was compared for
83 children under five years and those five years and older. Diagnosis was also compared
84 based on records of axillary temperature readings (<37.5⁰C or ≥37.5⁰C) at presentation
85 using the Chi-square test.

86 The dataset was analyzed using appropriate tests in StatsDirect^R version 2.8.0 software.
87 Binary logistic regression analysis was used to explore factors associated with positive RDT
88 and Microscopy results

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90 3. RESULTS AND DISCUSSION

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92 Patient Characteristics

93 Out of the 532 records of children seen during the study period, only 525 were used as
94 seven had either microscopy or RDT test result missing. There was no difference in the
95 characteristics of children with missing records compared to those used for the study. The
96 median age of children was 2.2 years (IQR: 1.0-5.1 years). The proportion of male children
97 was higher than that of females (57.1% : 42.9%). The common presenting complaints were
98 history of fever (91.4%) and vomiting (42%) [Table1]. Previous drug treatment for malaria
99 was reported by 46.3%.

Demographic and clinical characteristics	N=525(%)
Age group	
<5 years	364 (69.5)
5 years and above	160 (30.5)
Sex	
Male	299 (57.1)

Female	226 (42.9)
*Presenting symptoms	
Fever or history of fever	479 (91.4)
Vomiting	223 (42.0)
Chills	26 (4.9)
Headache	36 (6.8)
Duration of symptoms before presentation	
≤ one day	101 (19.0)
2-3 days	236 (44.4)
≥4 days	178 (33.5)
Drug history	
Artemisinin –based Combination drugs	182 (34.6)
Chloroquine	15 (2.9)
Quinine	10 (2.0)
Sulphadoxine-Pyrimethamine	4 (0.1)
**Others	35 (6.7)
No drug	279(53.7)

100 *multiple response **Amodiaquine, herbal medicine, Paludrine, unknown injections

101 **Table 1: Demographic and clinical characteristics of children**

102

103 **Diagnosis of malaria**

104 The Venn diagram (Figure 1) displays the distribution of positive malaria test results. Clinical
105 diagnosis had 120 suspected malaria cases not confirmed by RDT or Microscopy. RDT had
106 99 positive malaria tests which did not agree with microscopy. Of these, 48 (48.5%) had
107 been on malaria treatment prior to presentation.

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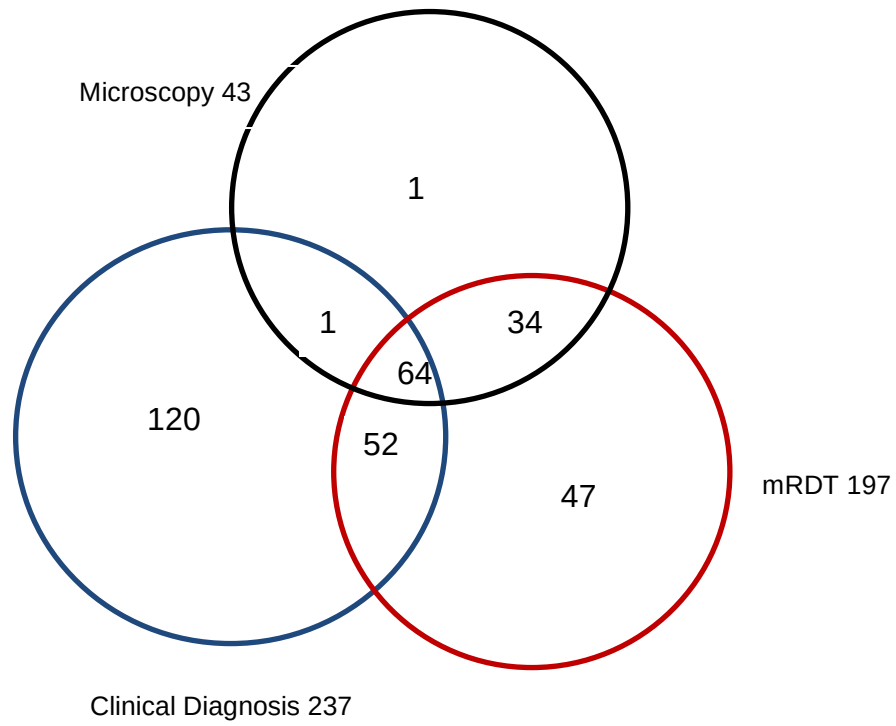


Figure 1: Malaria diagnosis by treatment modality

While the diagnosis of malaria was significantly higher for older children for all diagnostic modalities, there was no significant difference in diagnosis based on the presence of fever among children of different age groups. Clinical diagnosis estimated 7.6% more children as having malaria than did RDT and 26.1% more than microscopy. See Table 2

Diagnosis modality			Age <5 years n(%)	Age ≥ 5 years n(%)	Total N (%)	P value
Clinical diagnosis of malaria			153 (42.0)	84 (52.5)	237(45.2)	0.027
Positive RDT			123 (33.8)	74 (46.3)	197 (37.6)	0.007
Positive blood film on microscopy			60 (16.5)	40 (25.0)	100 (19.3)	0.022
Positive to both RDT and microscopy			59 (16.2)	39 (24.4)	98 (18.7)	0.027
Diagnosis modality			Temperature <37.5	Temperature ≥ 37.5	Total N (%)	P value
Clinical diagnosis of malaria			105(46.5)	127(44.3)	237(45.2)	0.618
Positive RDT			81(35.8)	115(40.1)	197 (37.6)	0.328
Positive blood film on microscopy			39(17.3)	61(20.9)	100 (19.3)	0.298
Positive to both RDT and microscopy			38(16.8)	60(20.6)	98 (18.7)	0.282

Table 2: Proportions of children diagnosed with malaria using different modalities stratified by age and presence of fever

Predictors of test positivity

Tables 3 and 4 indicate the factors associated with RDT positivity and Smear positivity for the patients. With RDTs, younger children were less often found to be positive than older children [OR: 0.594 (0.401-0.879)]. A similar lower probability of positivity was found for younger children on microscopy [OR0.624 (0.391-0.996)]. Positive smears were however recorded 3.9 times more often for those who gave a history of fever compared to those who did not [OR: 3.882 (1.154-13.057)].

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Variable	Odds ratio (95% Confidence Interval)	P value
Sex (male)	0.834 (0.576-1.207)	.34
Sex (female)	referent	
History of fever (yes)	1.598 (0.798-3.201)	.19
History of fever (no)	referent	
Sign/symptom of severe illness (absent)	0.869 (0.5361-0.410)	.57
Sign/symptom of severe illness (present)	referent	
Age group (<5 years)	0.594 (0.401-0.879)	.009
Age group (≥5 years)	referent	
Temperature at presentation (<37.5°C)	0.868 (0.5911-0.274)	.47
Temperature at presentation (≥37.5°C)	referent	
Previous use of antimalarial (No)	0.745 (0.5141-0.080)	.12
Previous use of antimalarial (Yes)	referent	
Illness duration (days)	0.994 (0.9531-0.036)	.77

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Table 3: Predictors of RDT positivity

Variable	Odds ratio (95% confidence interval)	P value
Sex (male)	1.102 (0.6981-0.738)	.68
Sex (female)	referent	
History of fever (yes)	3.882 (1.154-13.057)	.03
History of fever (no)	referent	
Sign/symptom of severe illness (absent)	0.961 (0.531-1.739)	.90
Sign/symptom of severe illness (present)	referent	
Age group (<5 years)	0.624 (0.391-0.996)	.048
Age group (≥5 years)	referent	
Temperature at presentation (<37.5°C)	0.951 (0.597-1.516)	.83
Temperature at presentation (≥37.5°C)	referent	
Previous use of antimalarial (No)	0.863(0.548-1.359)	.53
Previous use of antimalarial (Yes)	referent	
Illness duration (days)	0.966(0.906-1.031)	.30

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151 **Table 4: Predictors of blood smear positivity on microscopy**

152 **Discussion**

153 This article reviews daily clinical practice of malaria diagnosis comparing three frequently
 154 used diagnostic modalities in a Nigerian tertiary hospital setting. The prevalence of malaria
 155 of 19.1% as detected by routine microscopy was lower than the expected 50.3% for a peak
 156 transmission period in a high malaria endemic region of Nigeria [8]. In the absence of other
 157 superior confirmatory tests microscopy is often used as the gold standard in clinical practice
 158 in this setting. Although previous studies show that RDT often overestimate malaria
 159 prevalence relative to microscopy [9], the difference of 18.5% observed in this study was
 160 larger than expected and raises the possibility of false negatives on microscopy. Some bias
 161 in the microscopy result is apparent as a history of fever significantly affected results but not

162 objective clinical evidence of fever using axillary temperature. This implied microscopy error
163 with possible missed malaria diagnosis would have dire consequences if patients are left
164 untreated. This may be responsible for the often documented practice of clinicians
165 continuing to treat patients with negative microscopy results for malaria.[10] Other studies
166 have also reported limitations in microscopy in routine settings [11,12] . Thus, the possibility
167 of a superior performance of RDT to routine microscopy which has been documented in
168 other studies needs to be considered in this setting as well [13]. This diagnostic challenge
169 with microscopy has serious implications for referral centres tasked with the responsibility of
170 making definitive malaria diagnosis since RDT use is often limited to peripheral health care
171 settings [9].

172 Useful as RDTs are, the tendency for false positive result also raises concern about the
173 possibility of over treatment. In the situation where more than 40% of those having
174 diagnostics done had previously received antimalarial treatment, the line between positives
175 and negatives becomes blurred. Persistent antigenicity for as much as 37 days after malaria
176 treatment has been demonstrated for HRP2-based RDTs [15]. As such, for patients
177 returning with symptoms within two to four weeks of treatment, RDTs may not be the best
178 malaria diagnostic test [15]. The evidence of parasitaemia also needs to be interpreted with
179 caution since finding malaria parasites in an ill person in highly endemic regions does not
180 necessarily mean that the illness is caused by the parasites and other tests may be
181 necessary to explore other causes of fever [16]. Patients testing negative on RDT can
182 however be considered less likely to have malaria as such further testing with routine
183 microscopy may not be required [14].

184 Findings from this study show that clinical diagnosis overestimated malaria among patients
185 relative to RDTs and Microscopy and does not seem very useful in making malaria diagnosis
186 with certainty [17]. This buttresses the need for parasite based diagnosis. However the
187 choice of which malaria test to use needs the clinician to take into consideration the patients'
188 circumstances and the available skill for diagnosis.

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190 **4. CONCLUSION**

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192 This study highlights the attendant challenges with decision making that clinicians may face
193 in the use of malaria diagnostic tests in clinical practice in a Nigerian hospital. The low
194 confidence in malaria test results which is often reported may be related to the observed
195 performance of malaria tests in routine settings. The true malaria morbidity among these
196 paediatric patients remains questionable due to the differences in the results produced by
197 the different diagnostic methods. RDTs appear to have the potential to improve routine
198 diagnostics, but the clinical implication of the many RDT-positives, Microscopy-negative
199 samples may be grave if microscopy results are erroneous. For the policy of universal
200 testing to be effective in Nigeria, quality-control systems and surveillance of routine malaria
201 diagnostics are imperative.

202 **ACKNOWLEDGEMENTS**

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207 **COMPETING INTERESTS**

208 The authors declare that they have no competing interests.

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211 **AUTHORS' CONTRIBUTIONS**

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213 RO conceived the study and drafted part of the manuscript, OI analysed the data and drafted
214 the manuscript, BO participated in the design of the study and coordination of data
215 collection, BB helped to draft the manuscript. All authors read and approved the final
216 manuscript.

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218 **CONSENT (WHERE EVER APPLICABLE)**

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221 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

222 The patient data retrieved from records were anonymous and all information were routine
223 information collected in the course of routine clinical practice. All data analysed were
224 collected as part of routine diagnosis and treatment. Approval to use patient data was given
225 by the head of the paediatrics department.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

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Here is the Definitions section. This is an optional section.

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Term: Definition for the term

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280 **APPENDIX**

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