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Original Article

Paediatrics Section

Utility of Rapid Diagnostic Test for the Diagnosis of Malaria in Children: A Cross-sectional Study

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ABSTRACT

Introduction: Malaria is one of the primary causes of paediatric deaths. Rapid and accurate diagnosis, followed by effective treatment, is the main strategy for malaria control. Microscopy has been the gold standard test for diagnosing malaria for over a century. It offers good sensitivity, identifies various species, and measures parasitaemia levels. However, microscopy is time-consuming, labour-intensive, and requires technical expertise. Therefore, there is a need for an easily performed test that is more sensitive and reliable, especially in resource-limited settings. Rapid Diagnostic Tests (RDTs) are a viable alternative due to their simplicity, ease of use, accuracy, and reproducibility.

Aim: To compare RDT with microscopy for diagnosis of malaria in children.

Materials and Methods: This cross-sectional study was conducted in the Paediatric Ward of Government Stanley Medical College and Hospital, Chennai, Tamil Nadu, India, from September 2020 to February 2021. The study included children under 12 years of age with acute febrile illness, hepatosplenomegaly/splenomegaly, or anaemia and thrombocytopenia. Thick and thin blood smears, as well as RDT, were performed using venous samples. The collected data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 23.0. To determine significance in categorical data, the Chi-square test/Fisher's exact test was used. The efficacy was evaluated using the Receiver Operating Characteristic (ROC) curve, which provided sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.

Results: Among the 71 participants, 49 (69%) were males and 22 (31%) were females. Malaria presence, as determined by microscopy and RDT, was observed in 20 (28.2%) and 47 (66.2%) children, respectively, out of the total 71 enrolled in this study. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of RDT compared to microscopy were 100%, 48%, 44.7%, 100%, and 63.4%, respectively. The Area under the ROC (AUC) curve of RDT for diagnosing malaria was 0.740 (95% CI, 0.628 to 0.852; p-value=0.001). There was a statistically significant association between clinical response to antimalarials in microscopy-negative but RDT-positive subjects (p-value=0.0005).

Conclusion: The present study demonstrates that RDT could be used as an alternative to microscopy in the diagnosis of malaria.

Keywords: Microscopy, Parasitaemia, Protozoa, Rapid diagnostic test

INTRODUCTION

Malaria is a disease of global importance and is one of the primary causes of paediatric deaths. According to the World malaria report 2020, India had reported 5.6 million malaria cases in 2019 [1]. Malaria is caused by intracellular plasmodium protozoa transmitted to humans by female Anopheles mosquitoes. There are five species of plasmodium, namely *Plasmodium vivax* (*P. vivax*), *P. falciparum, P. malariae, P. ovale*, and *P. knowlesi* [2]. Among these, *P. vivax* and *P. falciparum* are most commonly seen in Tamil Nadu. The prevalence of *P. vivax* in Tamil Nadu, India is around 90%, and *P. falciparum* is less than 10% [3]. Clinical manifestations of malaria include fever (100%), hepatosplenomegaly (64%), pallor (43.5%), altered sensorium (21.3%), convulsions (18.5%), icterus (10.2%), and circulatory collapse [4]. Efficient management of malaria in children requires rapid and accurate diagnosis. Diagnosis based on clinical features alone has low specificity [5,6]. The World Health Organisation (WHO) recommends prompt parasitological confirmation in all clinically suspected malaria cases, either by microscopy or by using malaria-specific Rapid Diagnostic Tests (RDTs), before starting treatment [7]. In the laboratory, malaria is diagnosed using different techniques, including conventional microscopic diagnosis by thick and thin peripheral blood smears, Quantitative Buffy Coat (QBC) method, RDTs, and molecular diagnostic methods such as Polymerase Chain Reaction (PCR) [8].

Microscopy remains the gold standard test for the diagnosis of malaria for more than a century [9]. It has good sensitivity, allows species and stage identification, and determines the level of parasitaemia. Despite these strengths, microscopy is time-consuming, labour-intensive, and requires technical expertise [10]. The sensitivity and specificity of microscopy vary widely and are influenced by factors such as timing and quality of smear collection, laboratory skills, and the level of parasitaemia. Therefore, it is ill-suited as a diagnostic tool in settings where resources are limited and the caseload is high.

Lateral-flow immunoassay, often called RDT, is an effective alternative as it is simple, easy, accurate, and reproducible. The detection is performed by an immunochromatographic assay with monoclonal antibodies directed against target parasite antigen(s) that are impregnated on a test strip. Malaria antigens targeted by RDTs are Histidine Rich Protein-2 (HRP-2), parasite-specific Lactate Dehydrogenase (pLDH), and aldolase enzymes [11].

Although microscopy remains the gold standard method for the diagnosis of malaria, it requires a high level of technical expertise and additional time to diagnose malaria, which is often not possible in remote rural areas and acute care settings. Therefore, alternative diagnostic methods that are timely and effective are required to identify malaria, particularly in endemic areas. PCR has been used as the standard reference in many studies to compare malaria diagnostic techniques. Due to cost constraints and poor accessibility to PCR-based diagnosis, we compared RDT with microscopy for malaria diagnosis and also correlated the RDT results with clinical response. The primary objective of this study

is to compare RDT with microscopy for the diagnosis of malaria in children, and the secondary objective is to compare their results with clinical outcomes.

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Paediatric Ward of Government Stanley Medical College and Hospital, a tertiary care hospital in Chennai, Tamil Nadu, India, from September 2020 to February 2021. The study received approval from the Institutional Human Ethics Committee (IEC/2020/1010. EC registration number-ECR/131/Inst/TN/2013/RR-22), and informed consent was obtained from the parents.

Inclusion criteria: The inclusion criteria for the study were children under 12 years of age presenting with clinical or haematological features suggestive of malaria, such as fever lasting less than two weeks with hepatosplenomegaly or isolated splenomegaly, and fever lasting less than two weeks with anaemia and thrombocytopenia. Anemia and thrombocytopenia were defined according to WHO classification, with Haemoglobin (Hb) levels below 11 g/dL in children from six months to five years of age, and below 11.5 g/dL in children from 6-12 years of age categorised as anaemia [12]. Thrombocytopenia was defined as a platelet count below 150,000/mm^3.

Exclusion criteria: Subjects who had received antimalarial therapy within two weeks of presentation and those with *P. malariae* and *P. ovale* parasites in the blood smear were excluded from the study. Additional investigations were performed to rule out other tropical infections.

Sample size calculation: The sample size was calculated based on a reference study conducted by Azikiwe CCA et al., [10] using the specificity of RDT antigen test compared to microscopy as the gold standard and a malaria prevalence rate of 17% (as per departmental statistics for the previous year). With an absolute precision of 10% and a 90% confidence interval, the required sample size was 52. A total of 71 subjects were included in the final study.

Study Procedure

Sample collection: Using sterile precautions, 2 mL of venous blood sample was collected in an EDTA container. Thick and thin blood smears were prepared from the EDTA samples within 10 minutes of blood collection. Additionally, an RDT using the SD Bioline malaria antigen kit was performed using the blood samples.

Microscopy: The thick and thin films were made from the EDTA samples and stained with Leishman stain using standard methods by an experienced pathologist. The slides were examined under 100X magnification. Parasites were counted against 200 White Blood Cells (WBCs). A slide was considered positive if at least one parasite was found. A total of 100 fields were examined before determining the slides as positive or negative. The thick blood smears were used to determine the presence or absence of parasites, while the thin smears were used for species identification and quantification. *P. falciparum* was identified by the presence of crescent shaped gametocytes in Red Blood Cells (RBCs) and a high parasitaemia level. *P. vivax* was identified by the presence of ring trophozoites and Schüffner's dots without any change in RBC morphology. Ring trophozoites and Schüffner's dots with distorted RBC morphology were seen in *P.ovale* infection whereas band-shaped trophozoites were seen in *P.malariae* infection All blood smears were examined by the same person to eliminate interobserver variation.

Rapid Diagnostic Test (RDT): The RDT was performed on 5 µL of blood using the SD BIOLINE rapid test following the manufacturer's instructions by the principal investigator. The kit contains monoclonal antibodies specific to HRP-2 of *P. falciparum* and LDH of *P. vivax*. The membrane strip is pre-coated with monoclonal antibodies as three separate lines: a *P. vivax* line indicating infection due to *P. vivax* species, a *P. falciparum* line indicating infection due to *P. falciparum* species, and a control line indicating the validity of the test. 5 µL of blood and two drops of buffer were added to the sample well and diluent well, respectively, and the results were read within 20 minutes. The test was considered positive when the antigen line was visible in the test window along with control, and negative when only the control band was visible. The visualisation of both the *P. vivax* and *P. falciparum* lines along with the control line indicated mixed infection. Faint test lines were also considered positive. The test was considered invalid if the control line failed to appear within the result window. The test was limited to the detection of antigens of *P. falciparum* and *P. vivax*. The recommended storage temperature by the manufacturer is 1°C-40°C, and all the test kits were within the shelf life.

Subjects who tested positive for malaria either by microscopy or RDT were treated with antimalarials according to the National Vector Borne Disease Control Programme (NVBDCP) guidelines for malarial treatment [13]. Subjects were followed-up daily for defervescence. Clinical response was defined as fever clearance for at least four consecutive days after treatment.

STATISTICAL ANALYSIS

The collected data were analysed using IBM SPSS Statistics for Windows, version 23.0 (Armonk, NY: IBM Corp). Descriptive statistical analysis was performed, and the results of continuous measurements were presented as mean and standard deviation (Min-Max). The results of categorical measurements were presented as numbers and percentages. To determine the significance in categorical data, the Chi-square test/Fisher's exact test was used. Fisher's exact test was used when the sample size was small. To assess efficacy, the ROC curve was used, and sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated. In both of the aforementioned statistical tools, a probability p-value of less than 0.05 was considered significant.

RESULTS

A total of 952 subjects with fever were assessed, out of which 71 subjects satisfied the inclusion criteria. Seventeen subjects were excluded due to previous antimalarial treatment. Among the 71 subjects, 49 (69%) were males and 22 (31%) were females. A total of 47 subjects tested positive for malaria either by malaria RDT (n=47, 66.2%) and/or through microscopy (n=20, 28.2%). Among the confirmed cases, 25 (53%) had *P. vivax*, 20 (42%) had *P. falciparum*, and 2 (5%) had mixed infection [Table/Fig-1].

Rapid Diagnostic Test (RDT)	Microscopy		Total
	Positive	Negative	
Positive	20	27	47
Negative	0	24	24
Total	20	51	71

[Table/Fig-1]: Comparison of RDT with microscopy for malaria diagnosis.

Among the malaria-positive subjects, the majority of children belonged to the age group of 9-12 years. However, there was no statistically significant association between age and malaria in the study (p-value=0.066) using the Chi-square test [Table/Fig-2]. The male-to-female ratio was 3.1:1 in *P. vivax* and 2.3:1 in *P. falciparum*, with a male preponderance in both groups. However, there was no statistically

Age in years	*P.vivax*	*P.falciparum*	Mixed	Total
1-4 y	10	3	1	14
5-8 y	7	5	Nil	12
9-12 y	8	12	1	21
Total	25	20	2	47

[Table/Fig-2]: Age distribution of malaria.
p-value calculated using Pearson's Chi-square test was 0.066 which was statistically not significant

significant association between gender and malaria (p-value=0.704) using the Chi-square test in the study.

Fever was associated with chills and rigor in 47 (66%) subjects. Among them, 35 (74%) tested positive for malaria either by RDT/microscopy. There was a statistically significant association between the clinical features of fever with rigor and malaria (Chi-square value=8.029, p-value=0.045). The mean duration of fever at the time of enrolment in the study was 5.6 days. Seizures and altered sensorium were observed in both vivax and falciparum infections. Among the 47 malaria-positive subjects, clinically 45 (96%) had splenomegaly, 34 (72%) had pallor, and 26 (55%) had hepatosplenomegaly [Table/Fig-3]. There was a statistically significant association between pallor and malaria in the study (Chi-square value=8.762, p-value=0.033). Among the laboratory parameters, 39 (83%) had anaemia and 8 (17%) had normal haemoglobin levels. The majority of subjects had moderate anaemia. Thrombocytopenia was observed in 43 (91.4%) subjects.

Clinical features	*P.vivax* (n)	*P.falciparum* (n)	Mixed (n)	Total n (%)
Fever with chills and rigor	19	14	2	35 (74%)
Vomiting	9	11	1	21 (45%)
Cough	5	3	0	8 (17%)
Seizures	1	2	0	3 (6%)
Altered sensorium	1	2	0	3 (6%)
Pallor	18	14	2	34 (72%)
Hepatosplenomegaly	14	11	1	26 (55%)
Splenomegaly	25	18	2	45 (96%)

[Table/Fig-3]: Common clinical features of malaria encountered in the present study (N=47).

Out of the 71 subjects tested, 20 (28%) tested positive for malaria by microscopy. 10 (50%) subjects had *P. vivax*, 9 (45%) had *P. falciparum*, and 1 (5%) had mixed infection. RDT was positive for 47 (66%) subjects, of which 25 (53%) had *P. vivax*, 20 (42%) had *P. falciparum*, and 2 (5%) had mixed infection [Table/Fig-4].

		Rapid diagnostic test				
Species		Negative	*P.vivax*	*P.falciparum*	Mixed	Total
Microscopy	Negative	24	15	11	0	50
	P.vivax	0	10	0	1	11
	P.falciparum	0	0	9	0	9
	Mixed	0	0	0	1	1
Total		24	25	20	2	71

[Table/Fig-4]: Comparison of species identified by RDT with microscopy.
Values are presented as n. p-value calculated using Pearson's Chi square test was 0.0005 which is highly significant

All subjects who tested positive for malaria in microscopy also tested positive in RDT. The species identified by RDT were concordant with microscopy. There was a statistically significant association between microscopy and RDT for the diagnosis of malaria in the present study (Chi-square value=80.946, p-value=0.0005). However, there was no statistically significant association between the day of fever and the diagnosis of malaria by microscopy (p-value=0.132) or RDT (p-value=0.425).

Even though microscopy showed negative results, subjects who tested positive in RDT were started on antimalarial medication according to guidelines after ruling out other tropical infections. All patients treated with antimalarial medication had a significant clinical response in the form of fever clearance for four days [Table/Fig-5]. There was a highly statistically significant association between the clinical response to antimalarial medication in microscopy-negative but RDT-positive patients using Fisher's exact test (p-value=0.0005). Among the 47 subjects with malaria, 22 (46.8%) were treated with artesunate and 25 (53.2%) were treated with chloroquine.

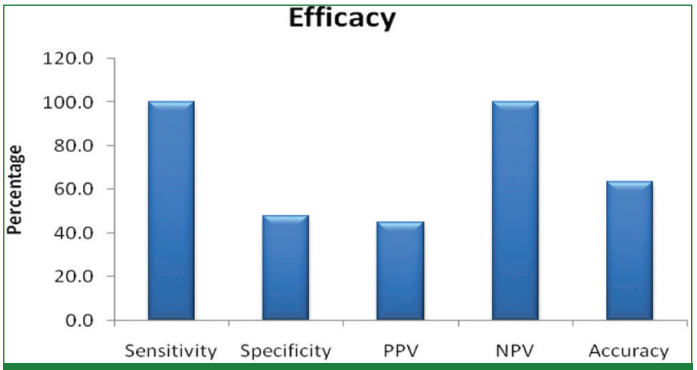
Microscopy negative	RDT positive			Total	Outcome
	P.vivax	*P.falciparum*	Mixed		
Artesunate	0	11	1	12	Afebrile
Chloroquine	15	0	0	15	Afebrile
Total	15	11	1	27	Afebrile

[Table/Fig-5]: Clinical response of microscopy negative but RDT positive subjects treated with antimalarial medications.
Values are presented as n. p-value using Fisher's-exact test was 0.0005 which was statistically significant

The Kruskal-Wallis test showed that there was no statistically significant association between the day of response and different antimalarial medications, Kruskal-Wallis Chi-square value=0.302, p-value=0.860 [Table/Fig-6]. Considering microscopy as the gold standard, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of RDT were 100%, 48%, 44.7%, 100%, and 63.4%, respectively [Table/Fig-7].

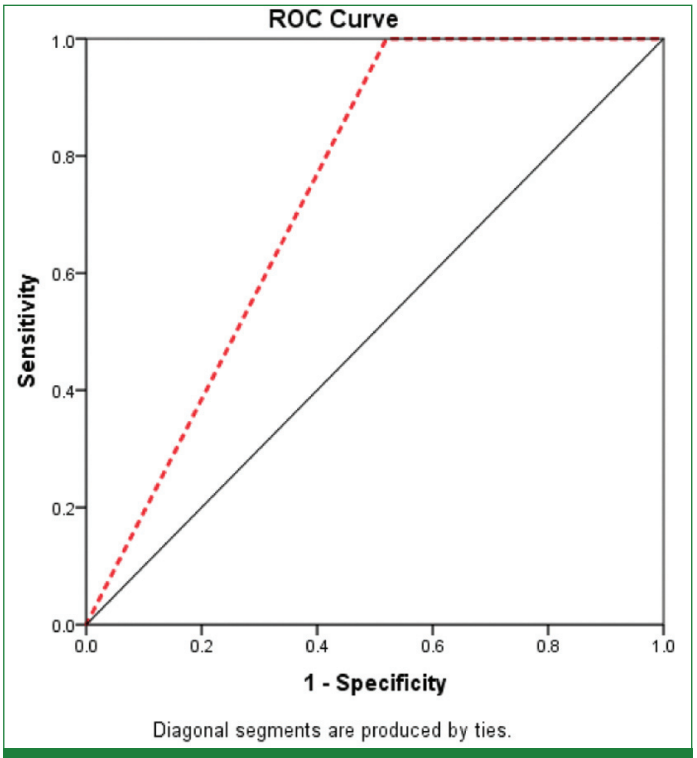
Antimalarial drug	Number of subjects	Mean day of response	SD
Chloroquine	25	1.6	0.64
Artesunate	22	1.7	0.67

[Table/Fig-6]: Comparison between the day of response and antimalarial drug by Kruskal-Wallis test.
p-value using Kruskal-Walli's test was 0.860 which was statistically not significant



[Table/Fig-7]: Sensitivity, specificity, positive and negative predictive value of RDT.

The area under the ROC curve of RDT for diagnosing malaria was 0.740, p-value=0.001, with a 95% confidence interval of 0.628 to 0.852, which was highly statistically significant [Table/Fig-8].



[Table/Fig-8]: Receiver Operating Characteristics (ROC) curve of RDT.

DISCUSSION

Malaria continues to be a challenging problem in the developing world. The definitive diagnosis and confirmation of disease status are crucial for evidence-based medicine. However, the recommended method of three thick and thin blood smears for malaria diagnosis can often cause delays in acute care settings. Therefore, there is a need for an easily performed test that is more sensitive and reliable, especially in resource-limited and acute care settings.

In the present study, among the 47 cases of malaria, 53% were identified as *P. vivax*, 42% as *P. falciparum*, and 5% as mixed infection. *P. vivax* infection was more common than *P. falciparum*, consistent with findings from other studies conducted in Delhi and Uttar Pradesh, India [14,15]. The most common symptom observed was fever associated with chills and rigor in the majority of cases. Thrombocytopenia was observed in 91% of malaria cases, which was consistent with the findings of Yadav et al., where 83.2% of children with malaria had thrombocytopenia [16].

In the present study, two different diagnostic methods (Microscopy and RDT) were used for malaria diagnosis and treatment. RDT identified malaria in 66% of subjects, while microscopy only identified it in 28% of subjects. There was a significant association between clinical response to antimalarial medications in RDT-positive but microscopy-negative patients, indicating that RDT yielded better results compared to microscopy in this study. Similar results were observed by Berzosa et al., who compared microscopy, RDT, and PCR for the detection of malaria parasites. They found that RDT had higher sensitivity and specificity compared to microscopy when using PCR as the reference [17].

The present study highlights that microscopy missed 38% of malaria cases. A meta-analysis of 42 studies concluded that microscopy missed approximately 50% of PCR-positive malaria infections [18].

Several studies in the literature have demonstrated the superior diagnostic performance of RDT compared to microscopy for malaria diagnosis, using PCR as the reference standard [19-23]. Stauffer WM et al., found that RDT had better sensitivity and negative predictive value (97% and 99.6%, respectively) compared to microscopy, which had values of 85% and 98.2%, respectively [19]. A similar study by Diallo MA et al., showed that RDT had better sensitivity and negative predictive value (97.3% each) compared to microscopy, which had values of 93.2% and 87.2%, respectively, when PCR was used as the reference standard [21]. Andrade BB et al., demonstrated in their study that RDT was superior to microscopy in the diagnosis of symptomatic malaria, even with low parasitaemia [20].

Mfuh KO et al., found that RDT had better sensitivity and negative predictive value (78% each) compared to microscopy, which had values of 57% and 66%, respectively [22]. Madkhali AM et al., reported that RDT had better sensitivity than microscopy in diagnosing *P. falciparum* malaria among febrile patients in the Jazan region [23].

The better performance of RDT in the present study could be attributed to two factors. Firstly, the sensitivity of microscopy is limited to 50-100 parasites/mcL of blood, and subjective interpretation as well as observer error could have contributed to the reduction in diagnostic accuracy. Secondly, RDT targets two antigens, HRP2 of *P. falciparum* and pLDH of *P. vivax*, rather than a single antigen. Specificity and positive predictive value were low in the present study since we compared RDT with microscopy, considering microscopy as the gold standard. Mfuh KO et al., found that RDT had a better specificity of 94% when PCR was used as the reference standard [22].

RDTs are simple to use, accurate, less expensive, and provide results within 15-20 minutes. The correct interpretation of RDTs is less subjective than that of microscopy. However, RDTs have certain limitations. False positive results can occur due to persistent antigenemia after treatment, and false negative results can occur due to HRP2 gene deletion and low parasitaemia.

The present study demonstrates that RDTs would definitely be a key tool in diagnosing malaria in remote and acute care settings. RDTs can be used as an alternative to microscopy in situations where reliable microscopic diagnosis is not available. The implementation of RDTs helps in timely diagnosis of malaria and the early initiation of antimalarial treatment. The authors believe that expert microscopy remains an essential tool for the diagnosis of malaria, and it is necessary to reinforce training in endemic areas.

Limitation(s)

This study had several limitations. First, the data relied on suspected cases in patients presenting at the participating hospital, which may introduce selection bias. Second, the evaluation of RDT was conducted using microscopy as the gold standard, despite being aware of its limitations. Comparing RDT with another test, such as the QBC test or PCR, which have higher specificity, could have improved the specificity, positive predictive value, and accuracy of the study.

CONCLUSION(S)

In this study, the authors demonstrated that RDT was more accurate in the diagnosis of malaria. Additionally, the rapidity of the test and absence of any interobserver variation make RDT a better diagnostic tool. Therefore, the present study provides evidence that RDT can be used for timely diagnosis and treatment of malaria in resource-limited settings.

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