Performance Characteristics of Malaria Rapid Diagnostic Test and Its Utilization in Management of Febrile Patients in Korogwe District (Tanga) Tanzania

Kachua Ndewa[1,2,*], Charles M. Kihamia[1], Marycelina Mubi[1], George Semango[3]

[1]Muhimbili University of Health and Allied Sciences, Tanzania
[2]Korogwe District Hospital, Tanzania
[3]Kilimanjaro Christian Medical Centre, Tanzania

Abstract　Background: Malaria diagnosis continues to be the foremost among the challenge in malaria control strategies in Sub-Saharan countries including Tanzania. The World Health Organization changed malaria treatment Guidelines in 2010 to restrict the use of antimalarial drugs to parasitologically confirmed malaria cases in response to the overuse of antimalarial drugs. Malaria rapid diagnostic tests (mRDTs) are presented as a mean to implement the new guidelines for malaria parasitologically confirmed cases prior to initiating antimalarial drug therapy for mRDT positive patients. Methods: A hospital-based cross-sectional study among patients with fever and malaria-like symptoms was done between May and June, 2014 in Korogwe district hospital in a convenient manner. Data were cleaned and analysed in Epi-Info version 3.5.4 computer software, Chi-square test was used to compare proportions between two groups with P-value set at 0.05. Results: Results revealed that sensitivity, specificity, positive predictive value and negative predictive value of mRDT at 95% CI was 97.6%, 97.4%, 91.0% and 99.3% respectively. All patients with positive mRDT were treated with antimalarial drugs, while patients with negative mRDT results were treated with either antimalarial (P-value = 0.001) or antibiotics (P-value = 0.005) at 95% CI. The results of patients with negative mRDT results were less likely to be further investigated at 95% CI (P-value = 1.000). Conclusion: The mRDT had good sensitivity and specificity if compared with blood slide microscopy. Clinicians adhered to the 2010 World Health Organization guidelines adopted by the National Malaria Control Programme for patients with positive mRDT result; however, they did not adhere to malaria diagnosis and management guidelines in case of negative mRDT results.

Keywords　Malaria Diagnosis, mRDT Sensitivity and Specificity, Overuse of Antimalarial Drugs, Clinicians Adherence to 2010 World Health Organization Guidelines

1. Background

Malaria is a major global public health dilemma that exerts a high mortality and morbidity in many countries, particularly in resource-poor countries in Sub-Saharan Africa, South East Asia and Latin America, and probably contributes to keep these countries in poverty [1]. The World Health Organization estimates that 3.4 billion people globally are at risk of malaria and 0.6 million deaths occur due to malaria (90% in Africa) most being children and pregnant women [2]. Diagnosis of malaria is still a challenge mainly based on increasing presumptive malaria treatment, and non-adherence to malaria parasitological confirmed negative results. Malaria over diagnosis, still practiced around the world, results in unnecessary use of antimalarial drugs, exaggeration of the burden of the disease, delay of appropriate treatment and sometimes death due to non-malaria causes of fever [3].

A clinical diagnosis of malaria is a traditional method of management of patients with fever and most practiced in developing world, Tanzania being one of them. This method is least expensive and most widely practiced in peripheral setting. Clinical diagnosis is based on the patient's signs, symptoms and on findings of physical examination. This method is fast but it lacks precision due to other diseases presenting with the same signs and symptoms like malaria such as common viral or bacterial infections and other febrile illnesses [4-7]. When managing febrile patients, it is impossible to know if the condition is due to malaria or other disease on the basis of clinical presentation hence an accurate diagnosis is essential. Laboratory diagnosis is an essential component of malaria control and elimination strategy that promises accurate diagnosis and treatment with effective medication within 24 hours of onset of illness if patients use effective medication. When managing febrile patients, it is impossible to know if the condition is due to malaria or other disease on the basis of clinical presentation

hence an accurate diagnosis is essential. As the incidence of malaria has diminished globally, a better understanding of non-malarial fever is important for effective management of patient's illness and rational use of antimalarial drugs. Globally malaria diagnosis by microscope is gold standard diagnostic method with high sensitivity and specificity provided that the quality of malaria microscopy is good. When good materials and skilled technicians are available, malaria microscopy is an excellent method to diagnose malaria as it can detect malaria parasites as few as 20 malaria parasites/µl. Malaria Rapid Diagnostic tests (mRDTs) are devices that assist in the diagnosis of malaria by providing evidence of the presence of malaria parasites in human blood within a short time (15 to 20 minutes). Malaria rapid diagnostic tests are easy and quick to perform as they can be performed easily by any health professional that has been given a short training. This method is cost-effective especially when Artemether-Lumefantrine (ALu) is used as first line treatment for malaria [8]. Malaria case management could be improved by using mRDTs as this method can be used even in areas where microscopy diagnosis is impractical [9]. Therefore mRDT is a reliable test for diagnosis and patient management in areas where blood slide microscopy is impractical [10-12].

2. Materials and Methods

Study Design

This was a hospital-based cross-sectional study of all patients with fever and malaria-like symptoms. The study was conducted between May and June 2014, to assess performance characteristics of malaria rapid diagnostic test and its utilization in the management of febrile patients.

Study Population

All patients with fever and malaria-like symptoms, attending health care and treatment in outpatient clinic at Korogwe district hospital. In this study fever is defined as body temperature above 37.5 ºC while malaria-like symptoms are signs and symptoms of malaria like headache, muscles aches, tiredness, nausea, vomit and diarrhea associated with fever.

Sampling Technique

The sample was obtained on convenience basis and the patients available at the time of data collection were selected. All outpatients reporting complaints of fever and malaria-like symptoms to the reception were given the opportunity to be included in the study through convenience sampling. Fever was defined as body temperature above 37.5 ºC or raised body temperature within the past 24 hours. The selected individuals were given laboratory identification number and enrolled for the test after they signed the informed consent form.

Data Collection Procedures

A structured questionnaire was administered to obtain the demographic information after patients were seen by the clinician and the clinical diagnosis of malaria was made. In the laboratory, blood sample for mRDT, thin and thick smears for Giemsa staining technique were taken and labeling was done on the frosted part of the slide using a pencil. Labeling included the initials of patient name, identification number, date, month and year in which the specimen was collected. The principal investigator performed all the malaria rapid diagnostic test and the results were used to treat patients. Both field laboratory technicians and the third slide reader at Muhimbili University of Health and Allied Sciences were blinded to mRDT results. Stained slides were handed over to the field technician with specimen tracking form where he/she entered his/her results daily. After the clinician attended to the patients, the questionnaire form was filled by a nurse in order to record the patient's management decision from the prescriber (Exit interview).

Ethical Considerations

Ethical approval for this study was obtained from Muhimbili University of Health and Allied Sciences (MUHAS) through the MUHAS IRB committee. Permission for dissertation field work was obtained from the Korogwe district Executive Director and the Korogwe District Medical Officer.

Diagnostic Tool

The SD BIOLINE Malaria Ag P.f/Pan test cassette contains a membrane strip, which is precoated with mouse monoclonal antibodies specific to HRP-II of *P. falciparum* on test line *Pf* region and with mouse monoclonal antibodies specific to lactate dehydrogenase of Plasmodium species (*P.falciparum, P. vivax, P. malariae, P. ovale*) on test line Pan Region respectively. The mixture of mouse monoclonal antibodies specific to HRP-II of *P.f* and mouse monoclonal antibodies specific to pLDH of Pan. They move along with membrane chromatographically to the test region (*Pf* and Pan) and form a visible line as the antibody-antigen-antibody gold particle complex formed. All the test lines and control line in the result window are not visible before applying any sample. The control line is used for procedural control and should always appear if the test procedure is performed correctly.

Operative Protocol

Sample was obtained on convenience-basis from the patients that happened to be available at the time of data collection were selected. The mRDT test result was reported directly by the field laboratory technician. The blood smears were examined for 7- 10 minutes per slide by a qualified laboratory technician in the field as the first reader, followed by the second reader at Korogwe district hospital. For positive smears, the number of parasites was counted in reference to 200 white blood cells (WBCs). The third reading was done by a competent microscopist who was blinded to

previous results in Dar-es-salaam at Muhimbili University, Parasitology and Entomology department.

Data Processing and Analysis

Data were entered, cleaned and analysed in Epi-Info version 3.5.4 computer software and presented in form of frequency tables, means and standard deviations. Frequencies and cross tabulation was produced to obtain odd ratio, *P*-value and Chi- square tests, for each of the independent variables at 95% confidence interval. A Chi-square test, *P*-value, correlation coefficient and kappa value were done in comparing degree of inter-observer agreement between reader one, two and three blood slides microscopy. Statistical significance for statistical tests was achieved when *P*-value is ≤ 0.05

3. Results

Demographic Characteristics

A total of 2,100 people aged one month to 95 years attended the outpatient department (OPD) at Korogwe district hospital. Out of these, 385 patients were recruited and enrolled in the study based either on malaria-like symptoms or raised body temperature on arrival (body temperature ≥ 37.5).

Table 1 shows that most of participants 245 were female (63.6%), while 140 (36.4%) were male, most of participants (79) were in age group of $0 - 5$ years (20.5%) and 40 years (10.4%). were in the age group $42 - 50$ years, as least group.

Table 1. Demographic characteristics of the study population (N= 385)

Variable	Number	Percentage (%)
Sex		
Male	140	36.4
Female	245	63.6
Age group (years)		
<5	79	20.5
5-14	58	15.1
15+	248	64.4

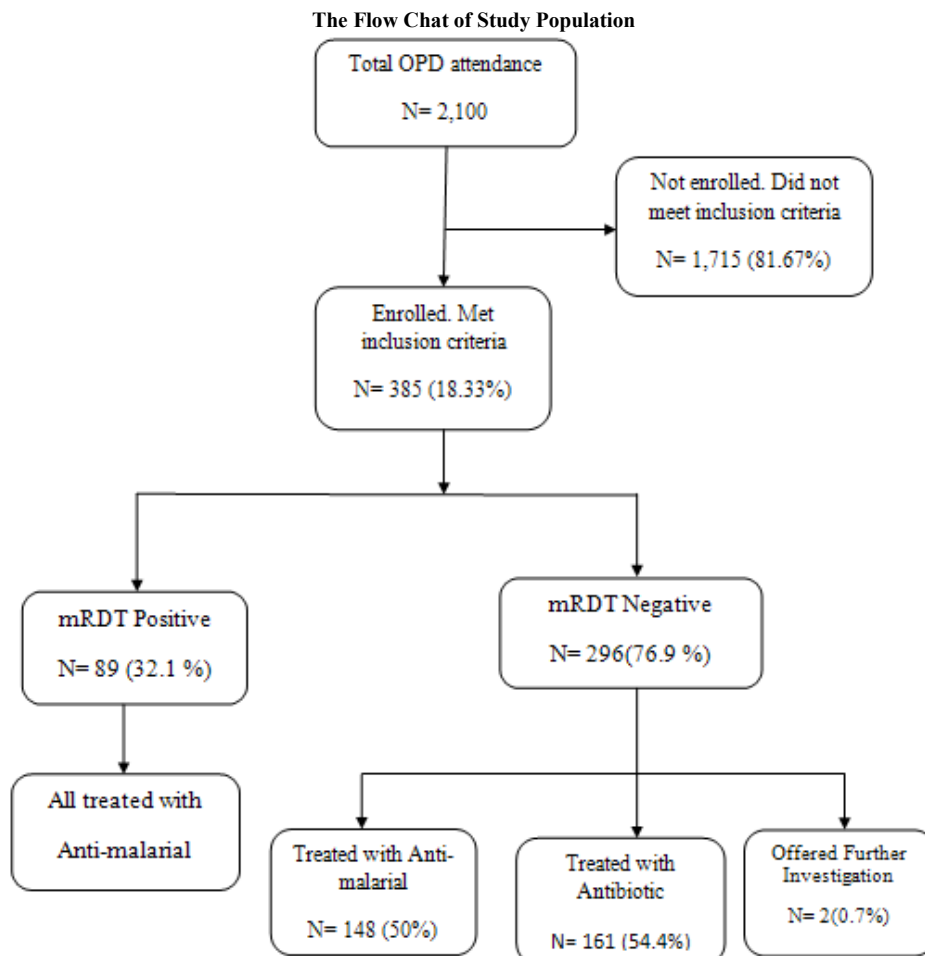**The Flow Chat of Study Population**

Figure 1.   The flow chart for the study population

Table 2. Hospital based malaria prevalence

Age group (years)	Number	Positive	Negative	Prevalence (%)
<5	79	25	54	31.6
5-14	58	25	33	43.1
15+	248	39	209	15.7
Total	385	89	296	23.1

Table 2 shows that overall hospital malaria prevalence at 95% CI was (23.1% and 21.6%) by mRDT and BS respectively.

Table 3. Comparison of Giemsa and mRDT results

mRDT results	Blood slide reader 3			OD	x^2	p-value
	Positive	Negative	Total			
Positive	81	8	89 (23.1%)			
				1.488	3.302	0.001
Negative	2	294	296 (76.9%)			
Total	83 (21.6%)	302 (78.4%)	385 (100%)			

Table 3, shows that the mRDT sensitivity, specificity, positive predictive value and negative predictive value at 95% CI were 91.0%, 99.3%, 97.6% and 97.4% respectively.

Table 4, shows that slides Reader one had 1 discordant results compared to Reader three as reference reader at 95% CI; slide Reader two had 4 discordant results compared to Reader three as reference reader at 95% CI.

Kappa Value

The degree of agreement between Reader one smear microscopy and Reader three gave kappa value of 0.992 The degree of agreement between Reader two smear microscopy and Reader three gave kappa value of 0.969 (Perfect agreement range, 0.81- 1.00). Meaning that there were no significant discordant between Reader one and Reader two in the field compared with Reader three at Parasitology and Entomology MUHAS as reference laboratory.

Table 5, shows that 89(100%) of positive mRDT prescribed antimalarial at 95% CI (x^2=72.289, *P*-value= 0.000 and OD= 2.00). Out of 296 mRDT negative patients, 148 (50%) prescribed antimalarial, while 148 (50%) prescribed antibiotics and 2 (0.7%) offered further investigation.

Table 4. Comparison between reader 1, 2 and 3 blood slides microscopy

Slide Reader 3							
		Positive	Negative	Total	x^2	*P*-value	r
	Positive	82	0	82			
Slides reader 1					3.791	0.001	0.999
	Negative	1	302	303			
Total		83	302	385			
	Positive	79	0	79			
Slides reader 2					3.617	0.001	0.969
	Negative	4	302	306			
Total		83	302	385			

Table 5. Association between mRDT results with further patients management decisions

mRDT results	Antimalarial		Antibiotics		Further investigation	
	Yes	No	Yes	No	Yes	No
Positive (89)	89 (100%)	0	20	69	0	89 (100%)
Negative (296)	148 (50%)	148 (50%)	161 (54.4%)	135 (45.6%)	2 (0.7%)	294 (99.3%)
	x^2= 72.289		x^2= 27.987		x^2= 0.604	
	P-value = 0.000		P-value = 0.005		P-value = 1.0	
	OD = 2.0		OD = 0.243		OD = 0.007	

Table 6. Clinical characteristics by mRDT results

Variables	mRDT Results		Total	OD	x^2	P-value
	Positive	Negative	Total	OD	x^2	P-value
Body Temperature						
Normal (36.9 - 37.4 ºC)	35(39.3%)	177(59.8%)	212	0.436	11.589	0.001
Fever (≥ 37.5 ºC)	54(60.7%)	119(40.2%)	173			
Total	89	296	385			
Intermittent fever						
Yes	61(68.5%)	141(47.6%)	202	2.395	11.99	0.001
No	28(31.5%)	155(52.4%)	183			
Total	89	296	385			
Body malaise						
Yes	89(100%)	285(96.3%)	374	0.762	3.405	0.75
No	0(0%)	11(3.7%)	11			
Total	89	296	385			
Abdominal discomfort						
Yes	62(69.7%)	168(56.8%)	230	1.75	4.739	0.36
No	27(30.3%)	128(43.2%)	155			
Total	89	296	385			
Diarrhea						
Yes	27(30.3%)	69(23.3%)	96	1.433	1.805	0.21
No	62(69.7%)	227(76.7%)	289			
Total	89	296	385			

Table 6 above, 54 (60.7%) patients had fever and were mRDT positive at 95% CI (P-value = 0.001). Patients having intermittent fever and mRDT positive were 61 (68.5%) at 95% CI (P-value = 0.001). While body malaise, abdominal discomfort and diarrhea had none association with mRDT positivity at 95% CI

4. Discussion

The study was conducted during the rainy season; malaria positivity was higher with mRDTs than with microscopy. The study reports the overall hospital malaria prevalence of 23.1% and 21.6% by mRDT and microscopic blood slide respectively (On table 2). Malaria positivity was higher with mRDT than with microscopy and this could either be due to the strict lower cut-off point for microscopy or higher false positivity of mRDT due to persistent antigen in blood [13]. The study found that sensitivity, specificity, positive predictive value, negative predictive value of mRDT (Ag. pLDH/HRP2) were 97.6%, 97.4%, 91.0% and 99.3% respectively in comparison with blood slides microscopy (On table 3) [6]. Out of 385 participants screened for malaria by mRDT, 89 (23.1%) were malaria positive and treated according to malaria diagnosis and treatment guidelines. The remaining portion was mRDT negative, even if they presented signs and symptoms of malaria. Fever has

significant association with malaria case at 95% CI, it is the best symptom of malaria on clinical judgment but it is not specific for malaria case. Intermittent fever has a significant association with a malaria case at 95% CI, is the best symptom to be used in clinical judgment of malaria. Body malaise, abdominal discomfort and diarrhea all at 95% CI had non-association with malaria case, basing on that, it is better not to use such symptoms in clinical judgment, because they are not specific for malaria case (On table 6). In a similar study done in high transmission areas (Benin), patients have acquired a partial immunity to malaria and when infected they present few or no symptoms. Fever, body malaise and intermittent fever were significantly associated with a malaria infection and almost 90% of infected patients presented at least one of these symptoms [14]. This study showed that all 89 (100%) positive patients by mRDT were prescribed antimalarial, while 296 (100%) negative patients by mRDT were prescribed either antimalarial 148 (50%) or antibiotic 148 (50%). There is a significant association of positive mRDT with prescription of antimalarial at 95% CI. Few 20 (22.2%) were prescribed both antimalarial and antibiotics; these are under five years children. For negative mRDT 296 (76.9%) there were significant associations for prescribed antimalarial or antibiotics at 95% CI. There was no association for negative mRDT result to offer further investigation at 95% CI. The World Health Organization guidelines of 2010 adopted by NMCP for diagnosis and

treatment of malaria were full adherent to positive mRDT patients but not adherence to negative mRDT ones (On table 5) [5,15-17]. On the other hand published evidence indicates that in many instances, adherence to mRDT test results is very poor in negative patients compared to positive patients [1]. Other studies showed that antimalarial drugs were prescribed to all patients with positive test results, while in patients with negative results Antibiotics were more likely to be prescribed compared to patients with positive results [18-20]. In a study done in Tanzania (Beyond fever) which included 1005 children with temperature of 38 degrees, a total of 70% had viral disease, 22% had bacteria disease and only 10.9% had parasitic disease [21]. Based on this data high temperature does not confirm malaria case and to offer Anti-malaria to mRDT negative patients is over-use of anti-malaria drugs.

5. Limitations

The data were collected one month after heavy rains, might contribute malaria positivity compared to other season.

6. Conclusions

In rainy season, hospital-based malaria prevalence is still high and children under five years are the most affected age group. The mRDT (Ag. pLDH/HRP-2) manufactured by Premier Medical Corporation Limited (India) and distributed by Ministry of Health Tanzania through Medical Store Department have good sensitivity and specificity as compared with gold standard test (microscopy blood slide). Clinicians adhered to malaria guidelines by prescribing antimalarial drugs to patients with positive mRDT and did not adhere in case of negative mRDT result. The negative mRDT patients were prescribed with either antimalarial or antibiotics, while WHO 2010 guidelines recommend further investigation.

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