Performance of ParaHit and OptiMAL tests in the diagnosis of malaria in Mwanza, north-western Tanzania

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Abstract: Malaria rapid diagnostic tests (RDTs) are non microscopic tests that provide a rapid detection of malaria infections in infected individuals. The objective of this study was to evaluate the performance of ParaHit and OptiMAL tests for detection of malaria infections as compared with routine microscopy. This facility-based study was carried out in Mwanza, north-western Tanzania and involved outpatients attending Igoma Health Centre. Blood samples were tested for malaria infection using the two RDTs and compared with Giemsa stained blood films examined using microscope. A total of 243 individuals (median age= 22 years) were involved in the study. Microscopy had a higher detection rate of 19.7% (48/243) as compared to ParaHit (4.5%) and OptiMAL (3.7%). Low sensitivity of 21.2% and 17%, but high specificity of 99.4% for ParaHit and OptiMAL, respectively was observed. Of all positive blood slides for *Plasmodium falciparum*, 78.7% had low parasite density (80 -720 parasite/μl of blood). These slides were negative for malaria parasite for both RDTs. Over 80% of study participants who reported fever had negative blood slides for malaria parasites by microscopy. On the other hand, 44.7% of those who reported no fever had positive blood slides for *P. falciparum*. Study participants who reported to have fever and high parasite density above 720 parasite/μl were likely to be positive by both RDTs (OR= 6.8; *P*= 0.031529). In conclusion, the overall performance of both RDTs in detecting asexual *P. falciparum* was low as compared to microscopy and their performance were highly affected by parasite density. This calls for further evaluation studies before RDTs are widely used in peripheral health facilities in order to minimize potential severe consequences.

Key words: malaria, rapid diagnostic test, sensitivity, specificity, microscopy, Tanzania

Introduction

Malaria is the major cause of febrile illnesses in tropical countries. It poses a plethora of symptoms that overlap with other febrile illnesses. This overlap of symptoms between malaria and non malarial febrile-illnesses results in significant over-diagnosis of malaria and making decision to treatment difficult (O'Dempsey *et al*., 1993; Greenwood et *al.*, 1997).

Laboratory methods for diagnosis of malaria pose great challenges for most health facilities in developing tropical countries. For many years, diagnosis of malaria has been done by using microscopy, and this technique has remained the gold standard. Microscopic diagnosis involves taking a blood sample, preparing a stained smear, and examining it for parasites (WHO, 2000). The advantage of microscopy is that it can achieve high sensitivity at low cost if done correctly under quality-controlled conditions with high diagnostic throughput. However, despite of being labour intensive and technical expertise demanding, it also requires quality equipment and reagents and the time lag between blood collection and patient consultation often prevents results from being taken into account in the diagnosis (WHO, 2003; Baker *et al.*, 2005). Sensitivity in malaria diagnosis

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by microscopy is often low due to the lack of high quality equipment, the use of low quality stains and other reagents, and lack of supervision and trained staff (Mundy *et al*., 2000; Ishengoma *et al*., 2010). For example, in East Africa re-evaluations of Giemsa-stained slides revealed that standard reading had a sensitivity of 50%-75% and specificity of 59%-96% (Reyburn *et al*., 2004, 2006; Zurovac *et al*., 2006).

The recent introduction of non-microscopic malaria tests (rapid diagnostic tests) to detect individuals infected with malaria has opened a new and important step in an effort to correctly diagnose malaria. Rapid diagnostic tests were developed as an alternative to microscopy for malaria diagnosis. These tests detect specific antigens (proteins) produced by asexual malaria parasites that are present in the blood of infected or recently infected individuals (Kakkilaya, 2003). Several commercially available tests are sensitive, specific and, stable under operational conditions (Mboera *et al*., 2006; Reyburn *et al*., 2007). These have been developed in recent years, and some are approved by WHO to be used in clinical and research facilities (Kakkilaya, 2003).

Improving diagnosis of acute febrile illnesses so that antimalarial drugs are targeted to patients who need them and alternative diagnosis sought in others is therefore a public health priority especially in Sub-Saharan Africa (Bates *et al.,* 2004). Rapid diagnostic tests have advantages over presumptive treatments in their diagnostic precision and potential to help reduce drug costs due to over-prescription. Rapid diagnostic tests have also advantages over microscopy for use in poorly-resourced areas, particularly when diagnostic throughput is low (Goodman *et al*., 1999, 2003). The Ministry of Health and Social Welfare in Tanzania is in the process of introducing rapid diagnostic tests for malaria in its primary health care facilities, where most often microscopic diagnosis is unavailable. This study was therefore, carried out to evaluate the performance of ParaHit and OptiMAL tests for detection of malaria infections as compared with routine microscopy.

Materials and Methods

Study design and population

This was a cross-sectional study conducted at Igoma Health Centre in Mwanza City in north-western Tanzania. Mwanza City lies on the shores of Lake Victoria at 1200-1500m above sea level. Igoma Health Centre has an estimated catchment population of 35,000 people. The study was carried out between November and December 2008. In this study, all attending patients presenting with febrile illnesses were eligible after consenting. Blood samples were collected for preparation of blood films for examination of malaria parasite and RDTs from a finger prick.

The two types of rapid tests used were OptiMAL® and ParaHit®. OptiMAL® test (DiaMed AG 1785 Cressiers, Morat, Switzerland) utilizes the enzyme *p*LDH which is produced by all four species of Plasmodium when present in the blood of infected individuals. ParaHit® test (Span Diagnostics Ltd, India) utilizes HRP-II produced by malaria parasites and released from the infected red blood cells. Malaria rapid tests were performed according to the respective manufacturer's instructions, and were parallel performed on site. Briefly blood from finger prick was drawn into capillary tube and dropped to caste. Buffer solution was added and observed for 15 minutes before reading results. Results were recorded onto a record form.

Thick and thin blood films were prepared and stained with Giemsa stain for microscopy. Microscopy was done by qualified and competent technicians and 10% of all blood slides were read by a second reader for quality control at the National Institute for Medical Research, Mwanza laboratory. Parasitaemia density was determined from thick blood smears by counting the number of parasites per 200 white blood cells. The number of parasites counted in the smear was multiplied by 40 to obtain the parasite density per microlitre (μl) of blood (Dowling & Shute, 1966). A slide was declared negative after the examination of 100 high power fields. Thin blood films were used for identification of Plasmodium species.

Data analysis

Data were entered in MS Excel and analysis was done by using STATA version 10. Proportions, X^2- test and geometric means of parasite densities were calculated. Parasite counts were log-transformed [log (x-l)] and parasite density reported as geometric mean density (GMD). Statistical significance was reported at $P<0.05$. Sensitivity was calculated as TP/TP+ FN), Specificity TN/TN+FP, Positive Predictive Values (PPV) TP/TP+FP, Negative Predictive Values (NPV) TN/TN+FN, and Accuracy of the test TP+TN/N. Where: N= Total number of study participants; TP= Proportion of individuals tested positive by both microscopy and RDTs; TN= Proportion of individuals tested negative by both microscopy and RDTs; FP= Proportion of individuals tested false positive by RDTs; FN = Proportion of individuals tested false negative by RDTs.

Ethical consideration

Ethical clearance was obtained from the Research Committee of The Open University of Tanzania. The permission to conduct the study was obtained from the Mwanza Regional Medical Officer and City Medical Officer of Health. Written consent was obtained from patients and guardians.

Results

A total of 243 patients presenting with febrile illnesses participated in the study with median age of 22 years (range= 0 - 75years). The overall proportion of reported fever was 60.9%. Of all subjects, females accounted for majority (75.7%) of whom 66.2% (98/148) reported fever. The overall prevalence of malaria as detected by microscopy was 19.7% (48/ 243) (Table 1). *Plasmodium falciparum* accounted for the majority (97.3%) of malaria parasites. Only one slide had *P. ovale* (1/48; 2.7%) and the patient was negative for both OptiMAL and ParaHit tests.

Table1: ParaHit and OptiMAL tests compared with microscopy in detection of malaria

Type of RDT		**Microscopy**		
		Positive	Negative	Total
ParaHit	Positive	10	1	11
	Negative	37	195	234 (4.7%
OptiMAL	Positive	8	1	9
	Negative	39	195	234 (3.7%)
Total		47	196	243

The prevalence of malaria by ParaHit and OptiMal was 4.5% and 3.7%, respectively. The geometric mean parasite density ranged from 80–68500 (mean=2294.3 /µl) parasites/µl of blood. Parasitaemia densities were stratified into the strata of 40– 720 (as low); 760–840 (moderate); and >1000/µl (high). Detection thresholds of malaria infections at parasitaemia density were ≥760 and ≥840/µl (GMPD ≥246 and ≥259.2) for ParaHit and OptiMAL tests, respectively. For microscopy, 78.7% of all positive blood slides had parasite density of 80-720/µl of blood (Table 2). Of individuals reported to have fever, >80% had negative blood slides for malaria parasites. However, 44.7% of those who reported no fever had positive blood slides for *P. falciparum*. Those who had fever and high parasitaemia were likely to be tested positive (OR= 6.8), *P*=0.0315 by both rapid malaria tests. However, in contrary fever and high parasitaemia were not associated with blood slides being positive by microscopy (*P*=0.2386).

Table 2: The ability of both RDTs to detect asexual malaria parasites stratified at different levels of parasite density

RDT		Parasite density level			
		40-720 (low)	**760-840 (moderate)**	**>1000 (high)**	**Total**
ParaHit	Positive	0	2	8	10 (21.2%)
	Negative	37	-	-	37
	Total	37	2	8	47
OptiMAL	Positive	0	0	8 (80%)	8 (17%)
	Negative	37	2	-	39
	Total	37	2	8	47

The respective sensitivity was 21.2% and 17.0%. Both tests had a high specificity of 99.4% (Table 3). The accuracy of test for ParaHit and OptiMAL was 0.84 and 0.83 respectively. Of the age groups, *P. falciparum* malaria was detected (33.3%) highest among young children (<10years) and, 2.1% lowest among the elderly (>60years) by microscopy (Figure1).

Table 3: Sensitivity, specificity, positive and, negative predictive values of ParaHit and OptiMAL tests for malaria detection. (CI 95 %)

RDT	**Sensitivity (CI)**	**Specificity (CI)**	**PPV (CI)**	**NPV (CI)**
ParaHit	21.2% (10/47) (CI 7.9, 27.3)	99.4% (10/11) (CI 96.9,100)	90.9% (10/11) (CI 58.7, 99.8)	84.4% (196/232) (CI 71.2, 82.4)
OptiMAL	17% (8/47) (CI 5.6, 23.5)	99.4% (8/9) (CI 96.9,100)	88.9% (8/9) (CI 51.8, 99.7)	83.7% (196/234) (CI 70.5, 81.8)

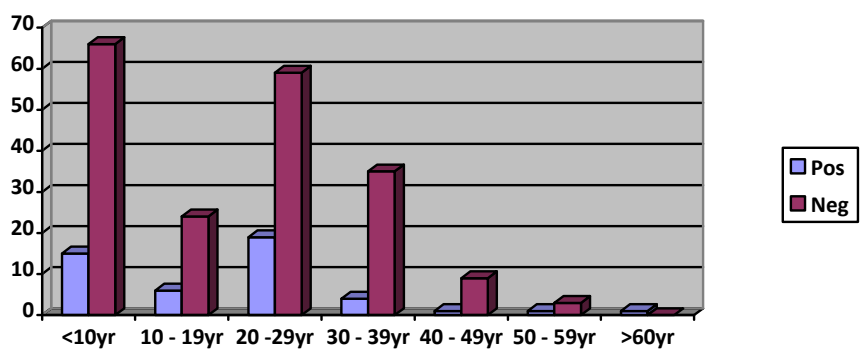

Figure 3: Distribution of malaria infection among age groups as detected by microscopy

Discussion

The overall prevalence of asexual *P. falciparum* malaria detected by both RDTs was found to be low as compared to microscopy. Both the two rapid tests showed a very low sensitivity but very high specificity in our study. Similar low prevalence of asexual *P. falciparum* malaria by microscopy and ParaHit test and low sensitivity were observed in another study in the same region in Tanzania (Kamugisha *et al.*, 2009) and in Philippines (Belizario *et al.*, 2005). In contrary, studies using ParaHit–f test in India showed higher sensitivity (Neeru *et al*., 2005). Using Paracheck-Pf in other areas of Tanzania sensitivities and specificities of >90% have been reported (Mboera *et al*., 2006; Kamugisha *et al*., 2008).

Low parasite levels are more frequently found in low transmission areas. In our study, microscopy detected asexual parasites in patients with parasitaemia density ranged from 40- 68500 parasites/μl, but a high proportion of those with positive blood slides had low asexual parasite densities below detection thresholds of RDTs. Both RDTs could not detect low parasite count of <760 parasites/μl. Low sensitivity of malaria rapid test (PfHRP2) has also been reported elsewhere (Neeru *et al*., 2005; Mboera *et al*., 2006).

Fever and high parasite density were found to have a strong association with positive RDTs in this study. However, microscopy detected high asexual *P. falciparum* malaria infections among individuals who reported no fever. This indicates that asymptomatic infections are high in this area, because during low malaria transmission some individuals might be expected to have a high asymptomatic parasitaemia, a low threshold of parasite density which has been not suggested to reflect clinical malaria better. The sensitivity of the pLDH test in field studies has also been reported to be lower at low parasitaemia in field studies (Baker *et al*., 2005). Generally, RDTs achieve a sensitivity of >90% at high parasite densities >1000 parasites/μl and, the sensitivity decreases markedly below that level of parasite density (Coleman *et al*., 2004; Goodman *et al*., 2006). Further, RDTs have been reported to give false negative results even at higher levels of parasitaemia. Therefore, in cases of suspected severe malaria or complex health emergencies, a positive result may be confirmatory but a negative result may not rule out malaria, and should always be confirmed by microscopy (Reyburn *et al*., 2007).

This study has a number of limitations. This was a cross-sectional study, generalizability of results could not been possible because of study population was at high risk of other non malarial febrile- illnesses, which could affect generalizability of results. Positive results with RDTs do not rule out other possible causes of febrile illnesses such as relapsing fever. False positive results may be due to gametocytemia, persistent viable asexual-stage parasitaemia below the detection limit of microscopy possibly due to drug resistance, persistence of antigens due to sequestration and incomplete treatment, delayed clearance of circulating antigens and cross reaction rheumatoid factor. False negative tests have been observed even in severe malaria with parasitaemia >40000 parasites/μl. This has been attributed to possible genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown causes.

In conclusion, the overall performance of both RDTs in detecting asexual *P. falciparum* was low as compared to microscopy and their performance were highly affected

by parasite density calling for further evaluation studies before its wide use in peripheral health facilities in order to minimize potential severe consequences.

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