Malaria Laboratory Diagnostic Performance: Case studies of two health centers in Zomba, Malawi

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Abstract

The objective of the study was to establish the performance of malaria laboratory diagnostics in routine clinical setting of health facilities in Zomba, Malawi. 246 suspected malaria cases in Matawale and Domasi Health Centers in October-November 2009 and 2010 were tested using two diagnostics methods(rapid diagnostic test (RDT)and Microscopy 144 patients who reported fever in 2009, 52% were referred to the laboratory for malaria test. The positive predictive value for microscopy in this study was 0.86. The study revealed that microscopy missed (30%) and (28%) in 2009 and 2010 respectively and this attributed to challenges with microscopy. RDT missed only 2 of malaria cases results between the two tests performed in the study among the microscopy positives. Microscopy had a lower sensitivity of 70% while Paracheck PfHRP-2 rapid test 91.3%. In conclusion, microscopy produced a low sensitivity when compared to rapid diagnostic tests.

Introduction

Malaria is a major public health problem in Africa especially among under five children and pregnant women. Misdiagnosis of malaria predisposes vulnerable persons to complications. In Mali, Bates and colleagues indicates that effects of malaria misdiagnosis on the most vulnerable in that it leaves other feverish illness such as pneumonia and meningitis untreated which can cause further illness and increase mortality[1]. One challenge in the fight against malaria is proper diagnosis of cases. Reliance on clinical symptoms alone for diagnosis results in high proportions of misdiagnosis. This practice has led to significant overuse of anti-malarial drugs which resulting in drugs being given to people who do not need them and in some cases not given to those who need them3.This forms the basis of national policies in high endemic malaria countries to encourage presumptive treatment for any fever[2]. In Malawi, the Ministry of Health in partnership with the Roll Back Malaria is working on the specific target of at least 80% of those suffering from malaria r to have access and use correct and appropriate treatment within 24 hours. The specific target is under the strategic intervention of case management[7].

Microscopy remains the gold standard for the diagnosis of malaria in resource poor areas where malaria is prevalent4. However, microscopic diagnosis is associated with low accuracy in African setting and this is due to the challenges like defective microscopes, intermittent power and limited time to examine slide due to large number of slides in health facilities and personal competencies which contribute to misdiagnosis of parasites[3].

Use of immunochromatographic rapid diagnostic test (RDT) is said to reduce misdiagnosis of malaria5 because they are more accurate in terms of sensitivity, specificity and stability despite some limitations[3]. Advantages of rapid diagnostic tests when compared with microscopy are simple to perform, fast, low subjectivity i.e. results show little variation between users, no need of electricity. Disadvantages include adversely affected by high humidity and provide no quantification of parasite density (World Health

Organization, 2006). Paracheck Rapid Test Kit for *Plasmodium falciparum* malaria was used and has a recorded sensitivity of 99% and specificity of 100% according to manufacturer's details although in another study by Chinkhumba, also conducted in Malawi, reported sensitivity of 90%[13].

The situation in the study area:

Malaria is the most commonly reported cause of morbidity and mortality in both adults and children. In Malawi, there are more than 8 million cases of malaria annually which ranks the country on 13 among the 19 countries with estimated cases of 90% in African region[6].

Over 85% of malaria infections in Malawi are due to *Plasmodium falciparum* and the main vectors are *Anopheles gambiae and Anopheles arabiensis*[7]. Zomba District Health Office reports not less than 250 000 cases annually. Among the 16 health centers spread throughout the district, only 6 use microscopes for malaria diagnosis. In the health centers microscopy tests are conducted by health surveillance assistants who were trained as Laboratory Assistants due to shortage of qualified laboratory personnel[8].

Study Objectives

The study was conducted to establish the performance of laboratory diagnosis of malaria in local Malawi health facility when microscopy and RDT are used in combination.

Methodology

This was a prospective cross-sectional study conducted in two health centers: Matawale and Domasi in Zomba District in October 2009 and November 2010. The health centers were selected because their laboratories are operational daily, and have high outpatient attendance as compared to the other health centers under the District Health Office. Matawale is estimated to have outpatient attendance of 7, 500 clients in a month, Domasi

Rural Hospital has a monthly outpatient attendance of 5, 290[8]. In the first year was a cross-section study. Inclusion criteria were subjects over 6 months old; all outpatients that the prescribing clinician has indicated require malaria tests. Exclusion criteria was based on subjects less than 6 months of age, all severe cases requiring hospital admission, all cases who had malaria within 1 month prior to the study.

A total of 246 people were recruited in the study. Testing was done using both microscopy and RDT. In November 2009, 144 patients reported fever, Out of the 144 patients 52% were referred to the laboratory for malaria test. This indicates that the presence of fever did not entail reference to the laboratory but based on the discretion of the clinician, 75 individuals were chosen after a clinician had prescribed a malaria test following clinical diagnosis, stratified as follows 48 and 27 subjects from Matawale and Domasi respectively, portioned basing on outpatients monthly attendance.

In October 2010, 102 at Matawale health centre were tested by microscopy, and those that tested negative were the only ones further tested using RDT. 23 tested positive using microscopy and 79 were diagnosed as negative. Out of the 79 that were found to be negative, 40 of were tested again using RDT. Clinical data such as signs and symptoms and treatment before seeking hospital treatment were recorded.

Malaria testing

246 people were finger-pricked for preparation of thick and thin blood smears, and the latter was fixed in absolute methanol for 30 seconds. The slide was then immersed in a freshly prepared

10% pre-filtered Giemsa stain solution for 5-10 minutes, washed in water and left to dry.

Asexual parasites and gametocytes were counted against 200-500 leucocytes, converted to number of parasites per volume assuming 8,000 leucocytes/μL of blood. A negative was registered when no parasites were detected after viewing 100 microscopic fields under 1000x magnifications. The research study used a

single blinding approach in which the laboratory technician performing microscopy test was not aware of the rapid diagnostic test result since the sample collection was done in a separate room.

The RDT kit components were first brought to room temperature. The pouch was opened in order to retrieve the device, sample applicator and dessicant. A sterile blood lancet needle provided was used to prick the patient's finger after cleaning with alcohol. The sample applicator was used to transfer blood to the sample pad on the device in the sample well 'A'. Six drops (300 μL) of the clearing buffer were then dispensed into the well 'B' by holding the bottle vertically. At the end of 15 minutes the results were interpreted in accordance to the test kit used. When only one band appeared in the control window 'C', the result was negative. When, in addition to the control band, a distinct band also appeared in the test window 'T', it was positive. The test was considered invalid when no bands appeared on the device, and a new device was used to carry out the test again.

The clients were then given a questionnaire in which they provided details such as age, gender, their current condition and their medical history with reference to malaria infection. The two laboratory test results were documented on the daily laboratory malaria result record book as well as patient's health pass book.

In this study it was assumed that there were no false positive results for rapid diagnostic tests as well as microscopy. A positive result in the absence of parasites occurs in cases where antimalarials are used by a patient when not required and in persistent antigenemia 28 days after parasite clearance[9]. HRP-2 antigen is known to persist in blood at detectable levels well after symptoms have disappeared and the asexual stage parasites have been cleared from patient's blood[10]. The results of the microscopy and RDT were then compared, and the clinical diagnosis was examined to check whether the symptoms were relative for clinician to prescribe the malaria test. Patients found with malaria were treated and those not with malaria were referred for further testing.

Data Collection and Analysis

Sensitivity and specificity for microscopy results were done by comparing with rapid diagnostic tests results. Rapid Test was used as a reference performance measure for microscopy due to microscopy's inherent problems. This is a different approach from other studies standard that treat microscopy as reference despite introduction of new more sensitive diagnostic tools[11]

Chancellor college ethics and research committee provided the ethical clearance.

Results

Common symptoms

Analysis of the 2009 participants of the most common symptoms and laboratort diagnostic results are shown in table 1 below.

Table 1: Symptoms and Corresponding Laboratory Results

	RAPID TEST		MICROSCOPY		TOTAL WITH SYMPTOM	PERCENT
SYMPTOM	*Negative*	*Positive*	*Negative*	*Positive*		
Cough	3	1	4	0	4	5%
Diarrhea	4	2	4	2	6	8%
Fever	18	21	22	17	39	53%
General malaise	6	1	6	1	7	9%
Headache	12	4	14	2	16	21%
Vomiting	2	1	2	1	3	4%
Total	45	30	52	23	75	100%

Malaria Testing

Among the 2009 participants 69.3% had negative malaria microscopy result. Of these 17.3% were positive to rapid diagnostic test result (while microscopy was negative). Two tested positive for microscopy yet negative for RDT. This could be a result of other types of *Plasmodium*. However these were ignored considering the study was specific for *P. falciparum*.

Table 2: P. *falciparum Microscopy* and Rapid Diagnostic Test results

	Rapid Diagnostic Kit Results		
Microscopy Results	*Positive*	*Negative*	**Total**
Positive	21/30 (91.3%)	2/45 (8.6%)	23
Negative	9/30 (17.3%)	43/45 (82.7%)	52
Total	30	45	74

In 2010 a total of 102 people were tested using microscopy, 23 were diagnosed as positive and

79 were diagnosed as negative, representing 23 %. This time only the negatives were validated by RDT as such, out of the 79 that were found negative using microscopy, 40 were tested again using RDT. 5 out of 40 tested positive with the RDT, representing a 12%, proportion miss by microscopy Table 3.

Table 3: Negative P. falciparum Microscopy validation with Rapid Diagnostic Test results

	Rapid Diagnostic Kit Results		
Microscopy Results	*Positive*	*Negative*	**Total**
Positive	N/A	N/A	N/A
Negative	5	35	40

Using Rapid Test as the standard; microscopy had a sensitivity of 0.7, Specificity 0.96, and otherwise said microscopy missed about 0.3 or 30 percent of malaria cases. The positive predictive value for microscopy in this study was 0.86

Discussion

This study is a known prospective cross-sectional study to look at the performance of laboratory malarial diagnosis in the area/district. This study had sufficient members to make plausible conclusions on performance of laboratory diagnosis. Microscopy missed 30% and 28% (in the two years respectively), cases that were positive. RDT missed only 0.02% of malaria cases results between the two tests performed in the study. In general, microscopy produced a low sensitivity when compared to rapid diagnostic tests. This could be attributed to experience of microscopist, insufficient time to thoroughly examine the slides because of so many specimens to go through or staining failure. Additional challenges noted are that the procedure is labor intensive and variability in stains which affects the quality of the stained slides and in techniques used to collect and process blood affects slide interpretation since challenges faced in this study were also observed in other similar studies3.

These factors contribute to increasing levels of misdiagnosis. Importantly, accurate microscopic diagnosis is a skill still learned with extended training, as such for most facilities to do proper microscopic diagnosis requires experienced microscopists. It should also be considered that an individual microscopist's interpretive expertise may diminish over time10 and this could contribute to alarming misdiagnosis established in the study. Lack of supervision which affects issues of Quality control and assurance of laboratory malarial diagnosis contribute to misdiagnosis. The study reveals that caution be considered with a negative malaria blood smear result in that it does not fully exclude infection particularly at lower parasite levels.

Microscopy is recommended by WHO as the gold standard test for malaria due to the following inherent strengths like detection of malaria parasite densities as low as 5-10 parasites/µl of blood10. However in a hyper endemic country like Malawi with few laboratory personnel to examine a large number of samples in a short time rapid diagnostic test is ideal and microscopy should be used only when determination of parasite density, infecting species as well as stage of circulating parasites is required to aid in patient prognosis.

Fever and headache the common malaria symptoms are non specific which necessitates laboratory confirmation12. Headaches and fevers are symptoms which stand out as being associated with malaria15 yet in this study have a large proportion associated with misdiagnosis where only 54% of the cases with fever and 25% with headaches had malaria. 30 % of positives did not present fever. The increased usage of RDT should take into consideration the limitations of RDT as highlighted by Chinkhumba et al in another study done in Blantyre. In this study sensitivity of RDT was shown to be based on the brand, user training, and previous treatment the candidate had undergone, and low specificity13. The study limitation is that it was done in 2 facilities in one district which can affect generalisability to the country; it was also done in few months of the year. Further studies involving multiple sites in different setting of the country be done to generalize the findings'

Conclusion

The study demonstrated that there is need to introduce/scale up malaria rapid diagnostic tests to all health centers which do not have experienced microscopists. Microscopy should be used only by experienced microscopists when determination of parasite density, infecting species as well as stage of circulating parasites is required to aid in patient prognosis.

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