ORIGINAL ARTICLE

RAPID DIAGNOSTIC TESTS IN MALARIA

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ABSTRACT: OBJECTIVES: Millions of cases of malaria are reported worldwide annually and India belongs to the endemic zone of malaria. The purpose of our study was to diagnose malaria among suspected cases by gold standard microscopy and rapid diagnostic tests (RDTs) and compare the results obtained by the two. **MATERIALS AND METHODS:** Blood samples were collected from 422 patients during three consecutive years. They were subjected to microscopy using Fields stain and rapid diagnostic tests based on the principle of immuno-chromatography for detecting Plasmodium falciparum specific histidine rich protein II and pan malarial species specific enzyme aldolase. **RESULTS:** Out of the 422 samples only 19 samples (4.50%) tested positive for malaria. Of these all the samples were positive by microscopy but only 17 samples were positive by RDTs. Four positive samples were suggestive of co-infection. **CONCLUSION:** RDTs are not able to replace microscopy as the gold standard for malaria diagnosis but are a good replacement in resource poor settings reaching those unable to access good quality microscopy.

KEYWORDS: Malaria, microscopy, rapid diagnostic tests.

INTRODUCTION: WHO estimates that half the world's population is at risk of malaria. In 2012 there were an estimated 207 million cases (with an uncertainty range of 135 million to 287 million) and an estimated 627000 deaths (with an uncertainty range of 473000 to 789000). Approximately 90% of all malaria deaths occur in sub-Saharan Africa and 77% occur in children under 5 years.[1] Malaria is one of the important vector-borne diseases in India with about 1.5 million confirmed cases reported every year.[2]

Plasmodium falciparum (P. falciparum), Plasmodium vivax (P. vivax), Plasmodium ovale (P. ovale) and Plasmodium malariae (P.malariae) are the four main species of malarial parasites that infect humans with the first two species covering the most infections worldwide. P. falciparum malaria is prevalent in Africa, whereas P.vivax is present in greater proportion in parts of Asia and Latin America.

Rapid, accurate and accessible detection of malarial parasites is important in the prevention and treatment of malaria. The commonly accepted gold standard diagnostic method for detecting malaria is microscopic reading of Giemsa stained thick and thin blood films. Microscopy constrains in malaria endemic regions are the need for skilled laboratory technicians, good quality reagents and well maintained microscopes as well as its time consumption.[3]

Rapid diagnostic tests (RDTs) offer the potential to provide accurate and timely diagnosis to everyone at risk reaching those previously unable to access good quality microscopy services. In these regions a considerable proportion of these drugs have been wasted on patients with non-malarial disease due to lack of prompt and accurate laboratory diagnosis.

A malaria RDT sometimes called "dipstick" or "malaria rapid diagnostic device" detects specific antigens (proteins) produced by malaria parasites. These antigens are present in the blood of infected or recently infected people. The RDT works through the lateral flow or immunochromatographic strip (ICS) method and signifies the presence of antigens by a colour change on an absorbing nitrocellulose strip. Three main groups of antigens detected by commercially available RDTs are histidine rich protein 2 (HRP-2), parasite specific plasmodium lactate dehydrogenase (p LDH), aldolase (pan-specific).

The present study was carried out to determine the utility of rapid diagnostic tests for detecting malaria and comparing the results with conventional microscopic diagnosis.

MATERIAL AND METHODS: The study was carried out during three consecutive years from February 2006 to December 2008 in the Dept. of Microbiology in our institute. Clinically suspected cases (>3 years) presenting to our hospital were chosen on the basis of diverse symptoms and signs as fever, headache, chills, myalgia, splenomegaly, nausea, vomiting.

Blood samples approximately 2ml were collected from each patient. Total samples collected during three years were 422. The samples were simultaneously subjected to microscopy and RDTs for detection of malaria. Thin blood smear was prepared from each sample, stained by Fields stain and observed by conventional microscope for presence of parasites. Same sample was also tested by RDTs for presence of P. falciparum and pan malarial species.

The RDTs used were Para HITf (P. falciparum) and Para HIT Total (for pan malarial species) manufactured by Span diagnostics Ltd. Surat, India. These tests are based on the principle of P. falciparum specific histidine rich protein - II (HRPII) and a pan- malarial species specific enzyme aldolase produced by the respective parasites and released into blood. The samples were tested as per manufacturer's protocol and appearance of magenta red coloured bands within 30 minutes was considered a positive reaction. Comparison of results obtained by microscopy and RDTs was then done.

RESULTS: A total of 422 samples were collected during three consecutive years- Table 1. Of these 19 samples (4.50%) were positive for malaria while 403 (95.49%) were negative- Table 2. Distribution of positive samples by microscopy and RDTs is shown in Table 3 & sex wise distribution of positive samples is shown in Table-4. Number of female patients was half as compared to male patients who tested positive for malaria. Majority of the samples were positive by both microscopy and RDTs. But two samples (10.52%) were positive by microscopy only and not by RDTs. Also four samples (21.05%) were positive for RDTs of P. falciparum as well as pan malaria species.

Year	Total
2006	169
2007	141
2008	112
Total	422

Table 1: Total samples

Year	Positive	Negative	Total
2006	5	164	169
2007	5	136	141
2008	9	103	112
Total	**19 (4.50%)**	**403 (95.49%)**	**422**

Table 2: Positive And Negative Cases of malaria

Year	Direct Smear/PBS	RDTs P. falciparum	Pan-malarial antigen
2006	5	4	1
2007	5	2	3
2008	9	7	4
Total	**19**	**13**	**8**

Table 3: Distribution of positive cases

Year	Males	Females	Total
2006	5	-	5
2007	4	1	5
2008	4	5	9
Total	**13**	**6**	**19**

Table 4: Sex wise distribution of positive cases

Year	PBS	RDTs P. falciparum + Pan-malarial species
2006	1 1	- 1
2007	1 1	- 1
2008	2	2
Total	**6**	**4**

Table 5: Smear distribution and RDTs positive cases simultaneously

DISCUSSION: Malaria remains endemic in 104 countries and while parasite based diagnosis is increasing most suspected cases of malaria are still not properly confirmed resulting in over use of antimalarial drugs and poor disease monitoring. Malarial fever continues to be an important public health problem in India. India contributes to about 70% of the total reported cases of malaria in South -East Asia. More than two thirds of the Indian population lives in malaria endemic zone. P. vivax accounts for nearly 50% of total malaria cases.[4]

Malaria diagnosis plays a key role in malaria control and elimination programmes in order to avoid unnecessary anti-malarial therapy to prevent drug resistance and to enhance case finding. It is well documented that treatment based on symptoms results in over treatment of patients who do not suffer from malaria.[5] Although clinical diagnosis is imprecise it remains the basis of therapeutic care for too many febrile patients in malaria endemic areas where laboratory support is often out of reach or negative slide results are disregarded. As a consequence serious non-malarial infections are missed, drugs are wasted, resistance is increasing and the cost effectiveness of the diagnostic tests is reduced.[6-7]

Rapid diagnostic tests (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. This is the case in most of the malaria endemic world. In non-endemic countries RDTs are able to complement microscopy in screening febrile patients.

Target antigens for commercially available RDTs are shown in Table -6.[8] The products come in a number of formats: plastic cassette, card, dipstick, hybrid cassette- dipsticks. RDTs detecting both falciparum specific and non-falciparum (pan specific) target antigens are commonly called combination or combo tests. Pan-specific means the RDTs detect all the four types of plasmodia that infect humans.

Microscopy can detect parasite densities as low as 5-10 parasites per micro-litre of blood. RDTs for P.falciparum generally achieve a sensitivity of more than 90% at parasite densities above 100 parasites per micro liter.[9] In general it is recommended that at least 95% of P.falciparum infections should be detected at 100 parasites per micro liter and at higher parasite densities which is probably similar to good field microscopy.[10]

As per the WHO recommendations for the procurement of RDTs the panel detection score against plasmodium samples should be at least 75% at 200 parasites per micro litre of blood.[11]

In our study maximum positive samples (89.47%) were detected by both microscopy and RDTs indicating a good co-relation between the two at high parasite densities. RDTs failed to detect two positive samples (10.52%) which were diagnosed by conventional microscopy. This indicates that at lower parasite densities microscopy appears to be more sensitive. Also four samples (21.05%) were positive for RDTs of P.falciparum as well as pan-malarial species suggestive of co-infection. Study conducted by Daniel Eibach et al have shown more than 90% sensitivity and specificity by RDTs for the detection of P.falciparum malaria in endemic as well as non- endemic areas.[12]

It is known that a small number of P.falciparum parasites show deletions or mutations of the hrp-2 gene leading to false negative results with RDTs. One of the important factors behind variable sensitivity is genetic variability in diagnostic antigens like PfHRP2 and PfHRP3. It has been reported that the PfHRP2 and PfHRP3 genes vary in size and sequence between different parasite strains [13] and also affect the RDTs sensitivity. [14-15]

Target antigens for commercially available RDTs are shown in Table 6. The strengths and challenges of malaria RDTs have been discussed in Table 7. [9]

Antigen	HRP2	pLDH	Aldolase
P.falciparum specific	✓	✓	✓
Pan specific (all species)		✓	
P.vivax specific		✓	

Table 6: Target antigens for RDTs

Strengths of malaria RDTs	Challenges of malaria RDTs
Relatively easy to use with minimal training required	Costs per test may exceed those of microscopy
Relatively rapid giving timely results	Short shelf life requiring efficient storage, transportation, procurement and distribution systems.
Little or no manipulation of sample required, can be performed in places without laboratories.	Most tests are qualitative (i.e. gives a yes or no answer). Any quantification of parasitemia will require further laboratory based tests.
Most of the RDTs do not require refrigeration, hence tests can be performed where there is no power supply	Intensity of test band varies with amount of antigen present. At low parasite densities this may lead to reader variation in test results.
Uses whole blood (prick or venous blood preferred)	In many cases they are less sensitive (and less specific) than laboratory based tests.

Table 7: Strengths and challenges of malaria RDTs

HRP-2 detecting tests are likely to have greater sensitivity than pLDH and aldolase for detection of current P.falciparum infection in most environments.[9] But it generally takes around two weeks after successful treatment for HRP-2 based tests to turn to negative and may take as long as one month which compromises their value in the detection of active infection.

Our study confirms previous abundant literature showing that RDTs are not able to replace microscopy as the gold standard for malaria diagnosis in most cases. But RDTs offer the potential to provide accurate diagnosis to all, reaching those unable to access good quality microscopy services. The success of RDTs in malaria control will depend on good quality planning and implementation. RDTs have a justified place in laboratories of endemic as well as non-endemic countries. RDTs form a useful adjunct to medical care and reduce anti-malarial drug consumption in resource poor settings.

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