

Research article

An assessment of the usefulness of a rapid immuno-chromatographic test, "Determine™ malaria pf" in evaluation of intervention measures in forest villages of central India

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Abstract

Background: *Plasmodium falciparum* malaria, is a major health problem in forested tribal belt of central India. Rapid and accurate methods are needed for the diagnosis of *P. falciparum*. We performed a blinded evaluation of the recently introduced Determine™ malaria pf test (Abbott, Laboratories, Japan) compared with microscopy and splenomegaly in children in epidemic prone areas of district Mandla to assess the impact of intervention measures.

Methods: Children aged 2–10 yrs with and without fever were examined for spleen enlargement by medical specialist by establishing a mobile field clinic. From these children thick blood smears were prepared from finger prick and read by a technician. Simultaneously, rapid tests were performed by a field lab attendant. The figures for specificity, sensitivity and predictive values were calculated using microscopy as gold standard.

Results: In all 349 children were examined. The sensitivity and specificity for Determine rapid diagnostic test were 91 and 80% respectively. The positive predictive values (PPV), negative predictive values (NPV) and accuracy of the test were respectively 79, 91 and 85%. On the contrary, the sensitivity and specificity of spleen in detecting malaria infection were 57 and 74 % respectively with PPV of 73%, NPV 59 % and an accuracy of 65%.

Conclusions: Determine™ malaria rapid diagnostic test is easier and quicker to perform and has other advantages over microscopy in not requiring prior training of personnel or quality control. Thus, highlighting the usefulness of a rapid antigen test in assessing prevailing malaria situation in remote areas.

Background

Rapid and accurate methods are needed for the diagnosis of *Plasmodium falciparum* malaria [1,2] as malaria is estimated to kill between 1.4 and 2.6 million people each year [1]. WHO South East Asian regional office estimates 19500 deaths due to malaria in India annually [3]. *P. fal-*

ciparum infections in children may become life threatening making rapid diagnosis of extreme importance [4]. No clinical diagnostic criteria are both sensitive and specific for malaria, an undifferentiated febrile illness [5] which may result in the erroneous treatment of millions of non-malaria cases with antimalarial drugs in the ab-

sence of diagnostic malaria microscopy. Excessive anti-malarial drug use is costly, propels the emergence of drug resistant parasites, speeds the obsolescence of affordable drugs and retards malaria control [6]. Prompt diagnosis and effective treatment of malaria improves prognosis [2]. In the absence of diagnostic microscopy in resource poor, remote villages of tribal forested belt of central India, the detection of *P. falciparum* histidine rich protein 2 (Pf HRP-2) in a blood specimen is one method by which expeditious diagnosis of *falciparum* malaria can be made on the spot [7,8]. Recently introduced rapid malaria test, Determine™ malaria pf (Abbott Laboratories, Japan) have been evaluated for its effectiveness in detecting *P. falciparum* malaria in Philippines [9] and in central India [10].

This field study appraised the performance of the rapid diagnostic test in assessing the impact of antimalarial intervention measures in villages of two adjacent Primary Health Centre's of district Mandla, central India. For this study we performed a blinded microscopy and splenomegaly in children as the endemicity of malaria is usually characterized by spleen and parasites rates in children [11].

Material and methods

Study area and study population

This Study was undertaken in two Primary Health Centre's (PHC) of district Mandla. The entire region is undulating and hilly. The villages are generally located on the slopes of the hillocks or on hill tops adjoining perennial streams. The inhabitants are (gond ethnic tribe) illiterate, poorly clothed, health ignorant and work in forest or road construction work etc. An outbreak of *P. falciparum* was recorded in villages of Bizadandi PHC in 1995 (unpublished observation), while in villages of Narayanganj PHC in 1996 [12]. Appropriate intervention measures were undertaken in both the areas by National Anti Malaria Programme (N.A.M.P) which are described previously [13]. Briefly two rounds of focal spraying with DDT (1 g/m²) along with prompt surveillance and treatment to bring the incidence of malaria under control.

For monitoring the impact of intervention measures in these two PHC's, children aged 2–10 yrs with and with-

out fever were examined for spleen enlargement [14] in three villages of each PHC during autumn season (December, 1999), by one medical specialist by establishing a mobile field clinic after obtaining informed consent from their parents. Treatment was given when appropriate as per Indian N.A.M.P. The study is approved by ethic committee of Malaria Research Centre (I.C.M.R), Delhi.

Sampling and data analysis

From these children thick blood smears were prepared from finger prick and read by a technician unaware of the spleen results. Parasites were counted against 200 white blood cells and converted into counts/ μ l assuming the average count is 8000 μ l. Simultaneously, rapid tests were performed by a field lab attendant after only a brief on the spot training according to the manufacturer's instructions taking about 30 minutes to provide one result without reference to the results of the thick smear and spleen. The thick smears were re-examined to discard a false negative results, combined infections or misdiagnosis of species by an experienced technician who was blinded to the previous results of thick smears. The figures for specificity, sensitivity and predictive values were calculated as described previously [10] using microscopy as gold standard. Briefly, sensitivity was calculated as TP / (TP + FN), specificity as TN / (TN + FP), the positive predictive values (PPV) as TP / (TP + FP), and the negative predictive values (NPV) as TN / (FN + TN). The test accuracy, the proportion of all tests that gave correct result, was defined as (TP + TN) / numbers of all tests. The mixed infection of *P. vivax* and *P. falciparum* are treated as falciparum cases for the purpose of analysis. Likewise, the instances of gametocytes only are classified as false positives since gametocytes neither cause illness nor require treatment with blood stage schizonticidal drugs. The Z-test was used for comparison between proportions.

Results

Spleen examination of 199 and 150 children respectively in Narayanganj and Bizadandi Primary Health Centres (PHC) are shown in Table 1. The spleen rate among children who lived in the villages of Narayanganj PHC (78%) was significantly higher ($P < 0.0001$) than that among children of Bizadandi (24%). Fever was found in 16.1 and

Table 1: Results of examination of spleen of children from villages of two PHC, Mandla district.

PHC	Children examined	No. with enlarged spleen	Mean class for enlarged spleen	Spleen rate (%)
Narayanganj	199	155	1.86	78
Bizadandi	150	36	1.16	24

Table 2: Comparison of the results of spleen examination in children with or without fever with microscopic results for malaria.

Symptoms	Microscopy results		Total
	Positive	Negative	
Fever with Spleen	13	60	73
Fever without Spleen	31	6	37
Afebrile with Spleen	96	22	118
Afebrile without Spleen	10	111	121
Total	205	144	349

Table 3: Comparison of the results of Determine malaria Pf and microscopic examination for malaria for 191 children with enlarged spleen.

Microscopy Result	Results of Determine Test		
	<i>P. falciparum</i>	Negative	Totals
<i>P. falciparum</i> , asexual ± sexual ^a	80	8	88
<i>P. vivax</i> , asexual ± sexual	2	5	7
<i>P. falciparum</i> , ± sexual only	8	6	14
Negative	11	71	82
Total	101	90	191

^a asexual ± sexual; asexual stages with or without sexual stage parasites.

Table 4: Performance characteristics of Determine Malaria Pf (rapid test) relative to those of microscopy for patients with enlarged spleen and fever.

Test	Sensitivity % (95 % C.I.)	Specificity % (95 % C.I.)	PPV % (95 % C.I.)	NPV % (95 % C.I.)	Accuracy % (95 % C.I.)
Rapid test	90.91 (85.02–96.98)	79.61 (72.28–93.7)	79.21 (71.06–100)	91.11 (85.08–100)	84.82 (79.73–89.91)
Spleen	57.07 (51.88–62.26)	74.05 (69.45–78.6)	72.67 (67.99–77.3)	58.79 (53.6–63.95)	64.76 (59.75–69.77)
Fever	83.78 (79.91–87.65)	61.86 (56.76–66.96)	79.33 (75.08–83.58)	96.98 (95.18–98.77)	64.18 (61.61–66.75)

3.3 % children in Narayanganj and Bizadandi PHC's respectively without spleen enlargement. The sensitivity and specificity of spleen in detecting malaria infections were 57 and 74 % respectively (Table 2). The positive predictive value (PPV) 73%, negative predictive value (NPV) 59% and accuracy were 65%. On the contrary, fever without spleen had relatively high sensitivity of 84% (P < 0.001) in detecting malaria infections though specificity

is significantly lower (62%). The PPV, NPV and accuracy were 79, 96 and 64% respectively.

The performance of rapid test vs thick smears are shown in Table 3. A good agreement was found indicating high sensitivity of 91% which is significantly higher than that of spleen (P < 0.0001). However, specificity was 79.6%, which is not significant when compared with specificity

of spleen (Table 4). The PPV 79% ($P < 0.05$), NPV 91% ($P < 0.0001$) and accuracy 85% ($P < 0.0001$) were all significantly higher when compared with their respective values of spleen. In all 11 cases showed positive reaction by rapid test for *P. falciparum* while blood smears were negative for malaria parasite. A prolonged re-examination of these slides who were positive by the rapid test, but apparently had negative slides, did not reveal the presence of *P. falciparum* asexual parasites. Further of the eight subjects with *P. falciparum* infection not detected by rapid test, not all had low parasitaemia: five had parasite densities between 60–318/ μl , but the three others had densities between 600–1000/ μl . Out of 14 gametocytes of *P. falciparum*, only 8 (57%) were positive by rapid test.

In addition, 2 subjects with *P. vivax* as detected by microscopy ($2795.81 \pm 3.58/\mu\text{l}$) were found positive for *P. falciparum* by rapid test.

Discussion

Malaria diagnosis, is often made on the basis of presumptive symptoms although this is alarmingly inaccurate [15,16,1]. One of the earliest methods used for estimation of the amount of malaria in a given locality is that of determining the proportion of person with a palpable enlargement of the spleen. This method introduced by Dempster in India in 1848 is commonly used although it is admittedly a rough index for malaria diagnosis and assessment [17]. However, it is still considered a diagnostic aid to every physician as the spleen rate is easier to obtain than the parasite rate [18].

In this study large number of cases with enlarged spleen in Narayananj PHC were asymptomatic, of which fairly large number were having malaria parasites. Thus, in this population a positive correlation between malaria and splenomegaly was observed as recorded elsewhere [18,19]. However, it requires the services of a medical specialist who is not certain whether the spleen is entirely due to malaria. On the contrary, rapid test indicate the presence of *P. falciparum* easily and accurately and since it requires no laboratory or technical equipment, a diagnostic facility can be set up in the most remote areas and in the most rudimentary way which makes it ideal for monitoring large scale control programmes in areas where malaria is a serious problem. This diagnostic test also gives the general practitioner a means of immediate diagnosis, which would overcome the usual delay associated with dependence on medical laboratories. Thus rapid test could be a better diagnostic method in *P. falciparum* malaria than spleen and microscopy.

The fact that a small proportion of children (6%) without *P. falciparum* parasites in thick smears were demon-

strated to have detectable HRP-2 antigen does not reduce the value of the rapid test. Positive results on the rapid test, which can not be verified microscopically have been suggested to be due to circulating *P. falciparum* HRP-2 antigen following treatment [20] or from sequestered parasites [21].

In 4% cases the rapid test gave false negative results compared to the thick smear. Such cases may not have sufficient antigen in the blood for detection by rapid test. We have estimated in our earlier study that the threshold parasitaemia for detection by Determine test to be about 500 parasites μl [10]. However, in this study rapid test failed to detect 3 *P. falciparum* subjects with $\leq 1000 \mu\text{l}$ of parasites. Unexplained false negative results on the rapid test have been reported in many studies [22,23] and rarely even with very high parasitaemia [24,25].

Further, during this study though we did not find the patients with rheumatoid factor, this may need to be investigated further in other malaria endemic areas as a relatively high false positive rate with Determine rapid test was observed in patients with rheumatoid factor in earlier study [9].

Interestingly, in central India the largest state in the country, also contributing highest number of malaria cases (23%) in the country [26], the usual practice under NAMP is to provide radical treatment (Chloroquine and Primaquine) to all fever cases during monsoon and post monsoon season in tribal villages due to inaccessibility. However, as a results of the emergence of drug resistant malaria, when Chloroquine (CQ) resistant *P. falciparum* infections are treated with CQ as CQ is still the first line of treatment in India, these infections may be partially controlled with the persistence of low grade parasitaemia in the peripheral blood [27]. Such patients experience no symptoms or barely symptomatic infections [28,29]. We contend that the asymptomatic infections which were recorded in this study were mainly infections of this type. These asymptomatic carriers in remote villages are putting a strain on malaria control activities. Since the number of febrile patients presenting at most peripheral health facilities in central India far too high to test each patient for the presence of Plasmodium species, hence malaria control programme policy is to provide fever radical treatment. Thus collection and examination of blood smears from asymptomatic patients is not possible. This opens new possibilities for rapid screening of communities at risk particularly pregnant women and children [30,31] in whom correct diagnosis and treatment is especially important [32].

However, a diagnostic test which is to be used in peripheral areas of developing country has to be simple and fast

to perform by unskilled staff. While comparing with other HRP-2, Determine test has some advanced features. The ParaSight F test requires six steps and 50 µl of blood, followed by ICT which required 5 steps and 10 µl of blood while Determine assay require 2 steps and 2 µl of blood. Though final reading time is designed as 30 min, which may be a disadvantage particularly during malaria outbreak, generally a clear red line in patient bar is seen within 10 minutes in mild to moderately parasitaemic cases. In our study the reliability of field worker in reading test results was excellent after only brief on-site training. Finally, the application of the rapid test in the programme will be determined by cost effective analysis. The population and countries at highest risk from malaria are mainly poorest and the most disadvantaged [1]. The price of the test varies with quantity and source of purchase [32]. For example the ParaSight F test sells for US\$ 1.20 / test in Uganda [32], US\$ 2.25 in South Africa [33] and US\$ 10–13 in Europe. The costs of the ICT pf tests ranges from US\$ 1.80 / test in developing countries [32] to US\$ 27 for two test in developed world. These prices appear prohibitive at first sight in a country, which only spends a few dollars/person/year for health care. However the picture may look different when the total costs to disease management are considered for the community and the programme. The cost effectiveness advantage of rapid test in the long term is the reduction of mistreatment in terms of drugs, costs, toxicity and development of resistance [34]. An important advantage of the availability of the rapid test that it does not depend on just one person but can be performed by all staff members of the health clinic.

Conclusions

In a situation like ours where laboratory facilities are poor or non-existent, rapid test is the best option to support malaria diagnosis for case management in malaria control programme. However, persistent positive reactions upto 2 weeks following treatment [21] would not justify the introduction of rapid test as a screening test by village health workers in the region. Since the sensitivity of the rapid tests remains high on treated patients therefore, it will be important to take patients history into consideration during routine usage of the rapid tests. Furthermore, in the study area, people are also subject to other *Plasmodium* infections i.e. *P. vivax*, which are less common and initially indistinguishable. These non-falciparum plasmodial infections have important therapeutic and prognostic implications. Thus a second generation of rapid test's are required which are intended to detect and distinguish *P. vivax* from *P. falciparum*.

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