SHORT REPORT: FIELD EVALUATION OF POSTTREATMENT SENSITIVITY FOR MONITORING PARASITE CLEARANCE OF PLASMODIUM FALCIPARUM MALARIA BY USE OF THE DETERMINE® MALARIA PF TEST IN CENTRAL INDIA

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Abstract. The posttreatment performance of the Plasmodium falciparum histidine-rich protein rapid diagnostic test Determine®Malaria pf (Abbott Laboratories, Tokyo, Japan) was assessed in 70 patients in central India with uncomplicated falciparum malaria who were treated with chloroquine, sulfadoxine-pyrimethamine, and arteether. Data were compared with those of microscopy. Results revealed that the sensitivity for predicting recrudescence by means of the Determine® test after treatment with chloroquine on Day 14 was 75%, with 50% specificity. However, antigenemia was detected in 16% of patients as late as Day 21 in sulfadoxine-pyrimethamine-treated subjects with a drug-sensitive response. Clearance of parasitemia in thick blood smear and clearance of antigenemia appeared to parallel each other only in arteether-treated subjects. The observed diagnostic trends therefore mean that the potential of the Determine® test to detect recrudescence infection is limited.

The recently introduced test that diagnoses Plasmodium falciparum malaria, which uses antigen-detection histidine-rich protein (HRP-2), the Determine®Malaria pf test (Abbott Laboratories, Tokyo, Japan), appears to have good levels of sensitivity and specificity, almost equaling those of microscopy. This diagnostic test is similar to the ParaSight-F test (Becton Dickinson, Sparks Maryland 21152 USA) but has some advanced features: the assay procedure needs only 2 steps and 2 μL of blood, whereas the ParaSight-F test needs 6 steps and 50 μL of blood for testing. However, the Determine® test is relatively slower (30 min) than ParaSight-F test (20 min). Studies in the Philippines indicated that the Determine® test may be used as a diagnostic tool to predict early treatment failures in areas where resistance to currently used antimalarial drugs is an emerging problem.

Therefore, we carried out a field evaluation to assess the performance of the Determine® test in uncomplicated falciparum malaria cases treated with 3 drugs: chloroquine (CQ), the first-line treatment of malaria in the Indian National Anti-Malaria Programme (NAMP), resistance against which is common; sulfadoxine-pyrimethamine (SP), the second-line treatment, resistance against which is uncommon; and arteether (E-Mal; Themis Chemicals, Bombay, India), a highly potent drug recommended for treatment of severe and complicated cases of falciparum malaria.

This study was conducted in 2 villages of Narayanganj Primary Health Centre of the Mandla district (central India). The details of study area have been previously described. Briefly, these villages are thinly populated and are scattered in field and forest, where medical facilities are nonexistent. A mobile field clinic was established during January-February 2000 to screen symptomatic patients for asexual forms of P. falciparum after informed consent was obtained from patients. Blood was obtained from each patient via fingerprick for preparation of thick and thin blood smears for microscopic examination and for analysis via the Determine® test as per the manufacturer’s instructions. Parasitemia was determined from the thick blood smears by counting the number of asexual parasites against 200 leukocytes and assuming that each subject had 8,000 leukocytes/μL.

Patients with uncomplicated falciparum malaria who had no recent history of drug intake, who had parasitemia ≥ 1,000 parasites/μL, and who consented to 28 days of follow-up were enrolled and treated with CQ (1,500 mg for 3 days, administered orally), SP (1,500 mg + 75 mg, single dose, administered orally), or arteether (150 mg for 3 days, administered intramuscularly) by the medical officer. Only adults were enrolled in the study, in accordance with the national drug controller regulation governing the protection of human subjects in medical research.

Initially, patients were assessed every 8 hr to ensure proper clinical management; however, after clearance of asexual parasitemia from peripheral blood, further follow-up by the Determine® test and thick blood smears were performed on Days 2, 7, 14, 21, and 28. All enrolled patients were given insecticide-impregnated bed nets to reduce the risk of reinfection. The reappearance of parasitemia during the course of 28 days’ follow-up was used as reference to assess the accuracy of predicting the outcome of treatment. Epi Info version 6 (Centers for Disease Control and Prevention, Atlanta, GA) was used to calculate diagnostic performance indexes; microscopy was used as the gold standard.

The variables measured were the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Sensitivity was then calculated as TP/(TP + FN), specificity as TN/(TN + FP), and the J index (the overall measure of reliability of a diagnostic test) as [(TP × TN) − (FP × FN)]/[(TP + FN) (TN + FP)]. The study protocol was approved by Institutional Ethics Committee of Malaria Research Centre, New Delhi, India, and conducted in accordance with the Ministry of Health. Statistical significance of the data was determined by t-test.

A total of 70 patients was recruited into this study (38 men, mean age 32.7 ± 11.1, and 32 women, mean age 32.5 ± 13.1). Patients were assigned to the treatment groups as follows: the first patient was provided CQ, the second patient SP, and the third patient arteether, and the subsequent patients were treated with one of these drugs in turn. In all, 30 patients were administered CQ, 30 were administered SP in accordance with NAMP policy for in vivo drug efficacy testing, and 10 were administered arteether (which is kept for emergency treatment). Eight patients did not complete the 28-day follow-
up period (2 from the CQ group, 5 from the SP group, and 1 from the arteether group); we excluded these patients from the calculation of performance indexes. Analysis revealed that after arteether treatment, mean parasite clearance time (MPCT, 20.4 ± 7.0 hr) and mean antigen clearance time (MACT, 12.4 ± 7.4 days) were also significantly shorter (P < 0.05) than they were after SP treatment (MPCT, 26.4 ± 7.6 hr; MACT, 12.4 ± 7.4 days), which were also statistically significant (P < 0.001) when compared with CQ treatment (MPCT, 43.4 ± 11.5 hr; MACT, 25.4 ± 6.9 days).

Further analysis revealed that on admission (Day 0), the sensitivity of the Determine® test was 100% because there were no FN results (Table 1). The specificity could not be calculated at the time of admission because no patient gave either a TN or FP result. The posttreatment parallel readings of Determine® test versus thick blood smears revealed that in the CQ-treated group, 57 and 75% of patients had a positive test against 28.5 and 57% of microscopically detected cases of falciparum malaria, respectively, on Days 14 and 21. All these patients were asymptomatic and were treated with repeated dose of CQ. The sensitivity of the test for CQ therapy after treatment on Days 2, 7, 14, 21, and 28 was, respectively, 100, 75, 94, and 82.4%, with corresponding specificities of 40, 78, 50, and 73% (Table 1).

As expected, posttreatment specificity was poor. The J index values paralleled those of specificity. In the SP-treated group, only 12% patients had detectable asexual parasites by Day 2, though gametocytes alone were seen in 20% of patients at Day 7 of treatment. However, 28% and 16% of patients had detectable antigenemia up to Days 14 and 21, respectively, with a drug-sensitive response. Additionally, our data provide no evidence that gametocytes were responsible for FP results. In the arteether-treated group, only 1 subject showed slower parasite clearance on Day 2 and antigenemia up to Day 7, and none of them experienced recrudescence within 28 days. The sensitivity for predicting recrudescence for SP and arteether could not be calculated from Day 7 until Day 28 because no TP tests were found. Clearance of parasitemia and clearance of antigenemia appear to parallel each other only in arteether-treated subjects.

The reasons for the persistence of the HRP-2 antigen in cured cases are not clear. It may be caused by latent viable parasites (possibly as a result of treatment failure), soluble antigen-antibody complexes, or inherent physiological differences in HRP-2 expression by different parasite clones or strains. Persistence may also depend on the characteristics of the antimalarial drug administered, such as rapidity of action, mode of action, or pharmacokinetics. A highly efficacious drug will eliminate parasites in a shorter time; hence, antigenemia will also be shorter, as recorded in this study with arteether-treated subjects.

Thus, the potential of the Determine® test to detect recrudescence infections within 14 days of treatment, the standard follow-up period during in vivo assessment of malaria response to treatment, are doubtful in this area. Further extended work with larger sample sizes is necessary to elucidate the diagnostic potential of the Determine® test in the late posttreatment follow-up of patients with malaria in areas of different endemicity.

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