Comparison of the OptiMAL Test with PCR for Diagnosis of Malaria in Immigrants

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The OptiMAL test (Flow Inc., Portland, Oreg.), which detects a malaria parasite lactate dehydrogenase (pLDH) antigen, has not been evaluated for its sensitivity in the diagnosis of malaria infection in various epidemiological settings. Using microscopy and a PCR as reference standards, we performed a comparison of these assays with the OptiMAL test for the detection of Plasmodium falciparum and Plasmodium vivax infection in 550 immigrants who had come from areas where malaria is endemic to reside in Kuwait, where malaria is not endemic. As determined by microscopy, 125 (23%) patients had malaria, and of these, 84 (67%) were infected with P. vivax and 36 were infected with P. falciparum; in 5 cases the parasite species could not be determined due to a paucity of the parasites. The PCR detected malaria infection in 145 (26%) patients; 102 (70%) of the patients had P. vivax infection and 43 had P. falciparum infection. Of the five cases undetermined by microscopy, the PCR detected P. falciparum infection in two cases, P. vivax infection in two cases, and mixed (P. falciparum plus P. vivax) infection in one case. Correspondingly, the OptiMAL test detected malaria infection in 95 patients (17%); of these, 70 (74%) had P. vivax infection and 25 were infected with P. falciparum. In this study, 61 (49%) of the 125 malaria cases, as confirmed by microscopy, had a degree of parasitemia of <100 parasites per μl, and 23 (18%) of the cases had a degree of <50 parasites per μl. Our results show that the sensitivity of the OptiMAL test is high (97%) at a high level of parasitemia (≥100 parasites/μl) but drops to 59% when the level is <100 parasites/μl and to 39% when it is <50 parasites/μl. In addition, the OptiMAL test failed to identify four patients whose blood smears contained P. falciparum gametocytes only. We conclude that the sensitivity and specificity of the OptiMAL test are comparable to those of microscopy in detecting malaria infection at a parasitemia level of >100 parasites/μl; however, the test failed to identify half of the patients with a parasitemia level of <50 parasites/μl. Thus, the OptiMAL test should be used with great caution, and it should not replace conventional microscopy in the diagnosis of malaria infection.

Worldwide more than 3 billion people live in areas where malaria infection is endemic. Every year, more than 500 million people are infected with malaria and 2.5 million malaria patients die from the disease (14). A number of factors have contributed to the global resurgence of malaria, including insecticide resistance in the Anopheles mosquito, rapid spread of antimalarial-drug resistance, and increased movement of populations secondary to increased international travel and immigration (2, 5, 10, 11, 13).

The classic method for detection of the malaria parasite is the examination of Giemsa-stained thick and thin blood smears. This method is labor-intensive and time-consuming. It is, however, well documented that microscopy has limitations; for example, its sensitivity decreases in parallel with the density of malarial parasites in the blood (12). When the level of parasitemia is low, diagnosis by using Giemsa-stained blood smears requires long periods of observation and experienced microscopists. Therefore, alternative techniques suitable for the laboratory diagnosis of malaria have been sought for use in both areas where malaria is endemic and areas where it is not.

Two rapid manual tests incorporating a dual-antibody immunooassay against the histidine-rich protein II antigen (HRP-2) of Plasmodium falciparum (ICT Malaria Pf and ParaSight-F test) have been compared and evaluated for their sensitivities in various epidemiological settings (3, 4, 7, 16, 18). Both of these tests detect only P. falciparum parasites. However, the OptiMAL test (Flow Inc., Portland, Oreg.), which was introduced recently, differentiates all four major Plasmodium species associated with human malaria (P. falciparum, P. vivax, P. ovale, and P. malariae). The OptiMAL test detects a species-specific enzyme, parasite lactate dehydrogenase (pLDH), produced by liver malaria parasites (15). We performed an evaluation of the OptiMAL test to assess its sensitivity in detecting malaria infection in immigrant populations in Kuwait using microscopy of Giemsa-stained blood smears and PCR as the reference standards.

MATERIALS AND METHODS

Patients. Blood specimens were collected from 550 individuals who presented with fever at Mubarak Al-Kabeer Teaching Hospital and at the Malaria Screening Laboratory of the Ministry of Health. Most of these individuals had come from countries in the Tropics to reside in Kuwait. This study was approved by the Ethical Review Committee of the Faculty of Medicine, Kuwait University, Kuwait.

Specimen collection. Informed consent was obtained from all patients. Five milliliters of venous blood from each patient and control was drawn into an EDTA-coated syringe for thick and thin blood film preparation, OptiMAL test, and PCR. Thick and thin blood smears were made on-site at the time of specimen collection, and most specimens were evaluated by the OptiMAL test within 1 h of sample collection. All specimens for PCR amplification were frozen at −70°C until used. All microscopy, OptiMAL antigen detection, and PCR assays were performed in a blinded fashion.

Malaria diagnosis with thick and thin blood smears (microscopy). The blood smears were stained with 10% Giemsa stain for 10 min. Smears were considered negative if no parasite was seen in 200 consecutive fields in a thick blood smear. If the blood smear was negative but the OptiMAL test was positive, microscopy was repeated. Parasites in the smear were counted against 200 to 500 leukocytes (WBCs). For the parasite density estimation it was assumed that there were 8,000 WBCs in 1 μl of blood (19, 20).
Malaria diagnosis with OptiMAL. All specimens were tested with the OptiMAL assay (Flow Inc.). This test utilizes a dipstick coated with monoclonal antibodies against the intracellular metabolic enzyme, pLDH, produced by viable malaria parasites. The pLDH is present in, and released from, parasite-infected erythrocytes. Differentiation of malaria species is based on antigenic differences among the pLDH isofoms. The assay was performed following the manufacturer's instructions. Briefly, 1 drop of whole blood was mixed with 2 drops of reagent A, which disrupts the erythrocytes and releases the pLDH, and the specimen was allowed to migrate to the top of the OptiMAL strip. After 8 min, the OptiMAL strip was cleared by adding 2 drops of reagent B. The appearance of a dark band on the strip indicates a positive reaction for any one of the four major malaria-causative species that infect humans. The monoclonal antibody at this site is one against an enzyme common to the four target Plasmodium species. If P. falciparum was present in the test sample, a second band appeared on the strip. The monoclonal antibody at this site is specific for P. falciparum only. A mixed infection with P. falciparum and another Plasmodium species is indicated when both genus- and species-specific bands appear and the genus-specific band is much darker and more intense than the species-specific band. A test control band appears at the top of the strip as an indicator that the test is working correctly. Appearance on the test strip of the genus-specific band only was regarded as indicating P. vivax infection since P. vivax is the predominant species (>75% of the cases) seen in Kuwait (9); however, the species was confirmed by microscopy of a Giemsa-stained blood smear.

Malaria diagnosis with PCR. The PCR and species identification were performed as described by Hang et al. with slight modifications (6). Briefly, parasites were recovered by centrifugation following saponin lysis of 50 µl of blood. DNA was purified after incubation in 20 to 50 µl of boiling buffer (50 mM KCl, 10 mM Tris [pH 8.3], 0.1 mg of gelatin per ml) and boiling for 10 min. All PCRs were carried out with 4 µl of DNA in 25 µl of PCR buffer containing appropriate primers (0.25 mM each) and 1.5 mM MgCl2. Oligonucleotide primers which amplify a 206-bp sequence were used for P. falciparum, and primers which amplify a 131-bp sequence from the gene for the small subunit of rRNA were used for P. vivax (6). These primers were used to detect and distinguish between P. falciparum and P. vivax in a single tube.

The samples were overlaid with mineral oil and amplified for 30 cycles. The DNA amplified by PCR was analyzed on 1.5% agarose gel and visualized under microscopy and PCR assay as the reference standards. Statistical evaluation was done with the unpaired two-tailed Student t test.

RESULTS

A total of 550 individuals were screened for malaria parasites by microscopy of Giemsa-stained blood smears, and 125 (23%) were positive for malaria parasites. Of these, 84 (67%) were infected with P. vivax and 36 were infected with P. falciparum. For five patients the species could not be determined because of a paucity of parasites (40 parasites/µl or fewer) (Table 1). Correspondingly, the OptiMAL test identified malaria infection in 95 patients (17%). Of these, 70 (74%) had P. vivax infection and 25 had P. falciparum infection (Table 1). No mixed infection was detected by the OptiMAL test. The PCR detected malaria infection in 153 (28%) patients: 102 (67%) had P. vivax infection, 43 had P. falciparum infection, and 8 had mixed (P. falciparum plus P. vivax) infection.

The microscopy of blood smears identified 14 cases of P. vivax infection and 11 cases of P. falciparum infection that were not detected by the OptiMAL test. Correspondingly, OptiMAL test detected two P. vivax infections and three P. falciparum infections that were not detected by microscopy. Of the five cases undetermined by microscopy, one was P. falciparum and the other four were not detected by OptiMAL. The OptiMAL test did not identify 25 patients with a level of parasitemia of <100 parasites/µl (14 patients had <50 parasites/µl, and 11 patients had 50 to 100 parasites/µl), irrespective of species or growth stage. Four patients whose blood smears contained only gametocytes were not identified by OptiMAL. Based on microscopic results, the sensitivity of OptiMAL was only 76%. In addition, the OptiMAL test was unable to detect pLDH antigen in two patients with parasitemia levels of 700 and 2,460 parasites/µl (Table 2).

Both the microscopy results and the OptiMAL results were compared with those of the PCR assay. The PCR detected malaria infection in 145 (26%) patients: 102 (70%) of the patients had P. vivax infection, and 43 had P. falciparum infection. When the other methods were compared to PCR, the sensitivity for detection of malaria infection of microscopy was 86% and that of OptiMAL was 66% (Table 1). The species for all five cases undetermined by microscopy were confirmed by PCR; two were P. falciparum, two were P. vivax, and one was mixed (P. falciparum and P. vivax). The OptiMAL and microscopy results and sensitivities at various levels of parasitemia ranging from 20 to 2,750 parasites/µl are shown in Table 2. These results indicate that the OptiMAL test was sensitive (57 of 59 cases, 97%) at a high level of parasitemia (>100 parasites/µl) but that its sensitivity dropped to 59% (36 of 61 cases) for a parasitemia level of <100 parasites/µl. The OptiMAL sensitivity decreased further, to 39% (9 of 23 cases), for a parasitemia level of <50 parasites/µl (P < 0.04 compared to the sensitivity at a parasitemia level of >100 parasites/ml). Furthermore, the OptiMAL test failed to identify four patients with blood smears containing P. falciparum gametocytes only. The OptiMAL test detected P. falciparum infections and P. vivax infections with almost the same sensitivity (Table 2). At least two false-positive cases, which were negative on microscopy as well as by PCR, were observed by OptiMAL (Table 2). Both of these patients had a history of taking antimalarial therapy 3 to 5 days prior to testing. Both of these patients were
negative for malaria infection when they were tested again 10 days later. An attempt was made to determine the correlation between the depth of the color reaction in the OptiMAL test and the level of the parasitemia. Generally, no agreement was found except when the parasitemia level was very low, i.e., <25 parasites/μl, when the reaction was represented by a much fainter test line than when the level was >25 parasites/μl.

**DISCUSSION**

This is the first study to evaluate the sensitivity and specificity of the OptiMAL test using microscopy of Giemsa-stained blood smears and a PCR-based method for the confirmation of malaria infection. The study was done in the immigrant population in Kuwait, a country where there is no malaria transmission. A total of 550 individuals were tested; microscopy of blood smears is a common test for detecting malaria, while the OptiMAL test detected malaria in 17% of the cases. Since more than 70% of the patients in blood smears identified 23% of these as positive for malaria, the presence of blocking antibodies or immune complex formation is a possible explanation. The occurrence of false-negative test results at higher parasitemia levels has been noted by others (1, 7), but the underlying reason is not known. We cannot explain the failure to detect the cases with only gametocytes. Possible explanations include the presence of blocking antibodies or immune complex formation. The two false-positive cases in which the OptiMAL test detected *P. falciparum* but the results were negative by microscopy and by PCR may be explained by the fact that the OptiMAL test is based on the detection of pLDH antigen, which has been shown to remain in the blood at least 7 to 10 days after the initiation of antimalarial therapy. The results were negative when these two patients were tested again after 10 days.

In summary, the sensitivity and specificity in detecting suspected cases of malaria of the OptiMAL test are comparable to those of microscopy at a parasitemia level of >100 parasites/μl, but a major limitation with this test is that approximately half of the patients with a parasitemia level of <50 parasites/μl were not identified by this test. Furthermore, microscopy was required to confirm the presence of malaria-causing species other than *P. falciparum*. In addition, the microscopy of a thick blood film is still needed to make a distinction between trophozoites and gametocytes and for estimation of the parasitemia, which are essential for therapeutic decisions. Thus, in its present form the OptiMAL test should be used with great caution, and it should not replace conventional microscopy in the diagnosis of malaria infection.

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**REFERENCES**