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Evaluation of the OptiMAL Test for Rapid Diagnosis of Malaria

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This study evaluated the ability of a newly developed rapid test for laboratory diagnosis of malaria. OptiMAL is a rapid test that utilizes a dipstick coated with monoclonal antibodies against the intracellular parasite dehydrogenase (PLDH). The differentiation of Plasmodium species is based on antigenic differences between the PLDH forms. Blood samples from 62 of clinically diagnosed patients were examined using the microscopy of Giemsa-stained blood films and the OptiMAL test. The blood films indicated that 27% of the patients were positive for P.falciparum (including one case of mixed infection with P. malariae), while the OptiMAL test recorded 34% for P. falciparum. The OptiMAL test failed to diagnose malaria at concentrations less than 100 per microliter of blood, while those missed by microscopy may be due to sequestration of the parasite coupled with low parasite density. The OptiMAL test was modified by using fingerprick instead of venepuncture and this simplifies the test both in terms of cost and trained personnel. There was no significant difference between the two methods ($x^2 = 1.513; P>0.05$), but the OptiMAL test has the advantages of being faster, requires almost no specialized laboratory experience and extremely sensitive and specific even in field situations. We conclude that the OptiMAL test is an effective tool for the rapid diagnosis of malaria.

INTRODUCTION.

Malaria has had resurgence in many tropical countries. The disease now occurs in more than 90 countries worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria-caused deaths per year (1). Although tremendous efforts and resources have been invested in the study, control and probably eradication of malaria, it continues to be a major problem particularly in the rural areas of tropical Africa. In Nigeria, malaria due to Plasmodium falciparum is holoendemic in many parts of the country (2,3). Multitudes of factors have contributed to the continued persistence of malaria. Some of these are: (i) insecticide resistance in the Anopheles mosquito. (ii) Social instability resulting in movement of unexpected non-immune individuals into the endemic areas and (iii) Failure to develop an effective vaccine (4). Compounding the problem of increasing morbidity and mortality are the emergence and rapid spread of anti malaria drug resistance which necessitate the use of more expensive and sometimes toxic anti malaria and longer treatment course (5).

The ability to diagnosis malaria early and the prompt treatment of infected individuals are very fundamental in the prevention and control of malaria.

Clinical diagnosis of malaria can be very imprecise and should be confirmed with laboratory diagnosis. Definitive diagnosis is currently based on microscopic examination of stained thick and thin blood films and malaria control activities therefore require laboratories. Thus diagnosis of malaria by this method is time consuming and requires considerable expertise for its interpretation.

Recently, a new malaria detection test, OptiMAL (Flow Inc., Portland, Oregon) was introduced. This test is based on detection of parasite lactate dehydrogenace enzymes (PLDH). The aim of this study was to evaluate the diagnostic accuracy of the new optiMAL test as compared to the traditional method.

MATERIALS AND METHODS

Study Area: The location, topography, climate and other geographical indices of Jos where the study was conducted are given somewhere else (6).

Study Population: The population studied consisted of 62 infants and adults resident in Jos with symptoms suggestive of malaria. Informed consent was obtained from the patients or their relations before being recruited in the study and the Helsinki revised declaration principle was strictly adhered to.

PARASITOLOGICAL TECHNIQUES

(a) Collection of Samples:

Two milliliters of venous blood was collected from the patients and thin and thick blood films were made on the same slide. The remaining sample was placed in the EDTA bottle and mixed gently. The anticoagulated samples were refrigerated at 4^0_c if not immediately required for the OptiMAL test. Finger – pricking method was used for the sample collection in a few of the patients, especially infants.

(b) Malaria Diagnosis with thin and Thick Blood Films.

The thin and thick blood films were stained appropriately using the Giemsa's staining method and analysed microscopically at X 1000 magnification for the presence of malaria parasites. The average time spent per slide varied depending on the number of parasites present in the blood sample. The study was blinded, since results of microscopy were not shared with the individual performing the optiMAL test until all samples were processed. Parasitemia levels were calculated with results from stained thick films and parasites were counted in 100 consecutive fields. Parasite densities were calculated by assuming 0.2 microliter of blood per thick film and that each microliter of blood contained 8, 000 leucocytes (7,8).

(c) Malaria Diagnosis with OptiMAL.

All the samples were tested with the OptiMAL assay. Briefly 0.05 millilitre (2 drops) of blood was added to 2 drops of reagent A. and mixed. The sample was then allowed to migrate to the top of the OptiMAL dipstick. After 8 minutes, the optiMAL strip was cleared by adding 4 drops of reagent B. The appearance of 3 dark bands on the strip indicates *P. falciparum* infection while 2 dark bands indicates either P. malariae, P. vivax or P. ovale infections. A positive control band appears at the top of each strip as an indicator that the test is working correctly. Statistical analysis was done using the chi-square at 5 percent level of significance.

RESULTS

Of the 62 patients sampled whose clinical conditions suggested malaria infection, 22 (35.5%) were positive for malaria. The blood film results indicated that 27% (17 of 62) of the patients were infected with malaria based on the morphologies of the various stages of the parasite. Among the patients' P. falciparum was present in all the positive blood films while there was only one mixed infection of P. falciparum and P. malariae. Correspondingly, the optiMAL test results indicated that 34% (21 of 62) of the patients' samples were positive for malaria parasite. The mixed infection of P. falciparum and P. malariae was not differentiated with the optiMAL test and in this particular case showed three bands (see Table 1).

TABLE 1: MALARIA PARASITE DETECTION USING OptiMAL TEST AND GIEMSA – STAINED BLOOD FILMS

	OptiMAL Result		Blood Film Result		Total
Species	Positive No %	Negative No %	Positive No %	Negative No %	
Plasmodium falciparum	21 (34)	41 (66)	17 (27)	45 (73)	62
P. malariae	1 (100)	NA NA	1 (100)	NA NA	1

The blood films indicated one P. falciparum positive sample that was not identified by the optiMAL test. The parasite density in this case was not identified by the optiMAL test. The parasite density in this case was 80 per micro litre of blood. However, optiMAL test detected five cases of *P. falciparum* infection that were not detected with the stained blood films. One of these patients had already been treated with chloroquine phosphate before the optiMAL test was carried out. The parasite density ranges from 80 to 7,120 per micro litre of blood. The trophozoites and gametocytes of *P.falciparum* were present in the positive samples while the trophozoites and schizonts of P.malariae were found in the one case of mixed infection.

DISCUSSION

The study compared the diagnosis of malaria by a new rapid test (OptiMAL) with the traditional microscopy of stained films and found that the two methods yielded comparable results as there was no significant difference between the two methods (X^2 = 1.513; P >0.05).The one case of malaria that was missed by the OptiMAL test may be due to the insensitivity to low parasitemia levels which in this case was 80 per microlitre of blood. It could also be attributed to the fact that the OptiMAL test detects only viable parasites. The one case where the OptiMAL test was negative and the stained blood films detected the parasite was because the patient had already been treated with chloroquine phosphate before the sample was collected. There were 5 cases in which the OptiMAL test was positive while the stained films were negative. This may be due to the sequestration of the malaria parasites. Although the difference is not statistically significant, it shows that even with a very good microscopist, there is the possibility of missing a few of the malaria cases. It was also observed that the OptiMAL test was positive even after the anticoagulated sample had been stored at 4°C for 7 days. In the case of the blood films, the parasitemia levels decreased on storage, until the stained films became negative on the third day. This makes it difficult to diagnose malaria at a more convenient time when the materials would be available.

There are compelling reasons to justify the use of a rapid malaria diagnostic test, especially in developing countries. Definitive diagnosis of infection in malaria control is currently based on microscopy and malaria control activities must therefore require the use of laboratories. The advantages of the OptiMAL test are that (a) it is fast (b) it requires almost no additional laboratory experience, (c) it is extremely sensitive and can be carried out in field situations. This, we believe is an invaluable malaria diagnostic tool of the future. It would do away with the need for slides, their transportation to centralized laboratories and the required infrastructure and trained staff and in situations where the electricity supply is very erratic like Nigeria, the OptiMAL test is a perfectly suitable alternative. This will certainly improve the diagnostic accuracy of malaria at the primary health care level and by community health workers.

The only drawback of the OptiMAL test is that the cost per unit

diagnosis may be currently high and its present use in malaria control programmes may only be cost effective in areas of low transmission where the cost of setting up a microscopic laboratory and equipment exceeds the cost of OptiMAL diagnosis. The OptiMAL test can be modified by using finger-prick samples instead of the venepuncture. This further simplifies the test both in terms of cost and trained personnel and therefore this method of collecting blood sample for the OptiMAL test is advocated.

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