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LABORATORY DIAGNOSIS OF MALARIA IN CHILDREN UNDER FIVE YEARS IN A RURAL COMMUNITY: MICROSCOPY VERSUS MALARIA PF TEST

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ABSTRACT
The morbidity and mortality associated with malaria in children below 5 years is really worrisome especially in the rural communities with little or no laboratory diagnostic facilities. This study was carried out to compare microscopy with Malaria Pf test for the diagnosis of malaria in a rural community in Ideato North Local Government Area of Imo State. Two hundred and fifty blood smears of children below 5 years were stained with Giemsa and examined microscopically for malaria parasites. Also the Malaria Pf rapid diagnostic test was used to test the same blood samples for malaria antigens. Thirty two per cent of the blood samples were positive for malaria parasite. Compared with microscopy, the sensitivity of the Malaria Pf test was 90.0%, the specificity was 98.2%. The positive predictive value was 96.0% and negative predictive value was 95.4%. The Malaria Pf test is reliable in the parasite based diagnosis of malaria in children under 5 years. We recommend the application of this test for parasitological confirmation of malaria in all places where it is not possible to provide facilities for good quality microscopy especially in the rural communities.

KEY WORDS: MALARIA, DIAGNOSIS, CHILDREN, MICROSCOPY, MALARIA PF

INTRODUCTION

Ever since the demonstration by Ronald Ross of the transmission of the parasite causing malaria from humans to mosquito and vice versa (1), malaria remains a scourge the world over especially in the sub-Saharan Africa, parts of Asia and the Americas (1, 2).Each year, 350-500 million cases of malaria occur world-wide (1). The World Health Organization had estimated 881000 deaths resulting from malaria in 2006, 91% of which occurred in Africa (3). Eighty five per cent of these deaths were children under 5 years of age. Another WHO report says 1 out of every 5 children die in Nigeria, 25% of which is caused by malaria (1). The malaria burden is indeed a threat to life and a drain in the economy of the already impoverished people of the sub-Saharan Africa (4, 5).
Early diagnosis, prompt and effective therapy are the pivots of the global malaria control strategy aimed at reducing unnecessary use of antimalarials and also reducing the mortality and morbidity associated with malaria (6). Treatment of malaria based on clinical diagnosis leads to unnecessary use of antimalarials (7, 8, 9, 10) with the attendant economic and health consequences. This is because clinical signs and symptoms associated with malaria are not specific (1, 5, 6). Malaria can be suspected presumptively from the signs and symptoms, but for a definitive diagnosis to be made, laboratory tests must demonstrate the parasite or its components (1). WHO (6) recommends that parasitological confirmation should be part of good clinical practice in order that the quality of care is improved.

Blood film stained with a Romanowsky stain (eg: Giemsa, Leishman and Fields stain) and examined by an experienced laboratory personnel remains the gold standard for laboratory confirmation of malaria diagnosis as this provides more detailed information such as parasite density, the Plasmodium species identification and different stages of the parasite (11). But this method depends on the quality of reagents, of the microscope, and on the experience of the laboratory personnel (1). Furthermore, blood smear may also not yield a reliable result if the slide is scratched and unclean, and if the pH of the buffer is not correct, if the stain contains debris (not filtered) or if the blood has been stored for some time in anticoagulant (12).
There are other methods of testing which include immunological methods like the antigen detection tests (1) as rapid diagnostic tests that produce results within 5-15 minutes, antibody detection tests example: enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT). The last 2 are less sensitive and unsuitable for routine diagnosis of malaria (1, 2, 12). Molecular method employing polymerase chain reaction (PCR) is highly sensitive but expensive, requiring greater sophistication in materials and labour and therefore not suitable for routine diagnosis (12).

The World Health Organisation (6) had stated that rapid diagnostic tests make it possible to provide accurate parasite based diagnosis for remote populations, reaching those who lack access to good quality microscopy services.
Furthermore, it has been reported that Plasmodium falciparum is the most common cause of severe and potentially fatal malaria, causing an estimated 700000 to 2.7 million deaths annually, most of them in young African children (1). Because of this it has been recommended that rapid diagnostic tests in Africa need high sensitivity for *Plasmodium falciparum*, and specificity to avoid over estimation of the malaria burden, false perception of therapeutic failure when fever is due to other illnesses, and unnecessary drug pressure (5).
This study was therefore carried out to assess the effectiveness of the malaria Pf rapid diagnostic test in detecting active malaria infection in children under 5 years of age in a rural community.

MATERIALS AND METHODS

Subjects
The subjects comprise children under 5 years who attend Osina Community Hospital, Osina, Ideato North, and Chika Medical Centre, Osina, Ideato North, both in Imo State from May to August, 2009

Procedures for testing
Consent of the parents/ guardians of the children were obtained prior to sample taking for the tests, and venous blood of the patients were collected.

Microscopy
Thick and thin blood smears were prepared according to standard techniques (13), allowed to air-dry and stained with 10% Giemsa solution for 30 minutes. The thin blood smear was fixed in absolute methanol for about 2 minutes before staining. Afterwards, the stain was washed in running tap water, allowed to air-dry in a slanting position and examined under oil immersion for malaria parasites by experienced Medical Laboratory personnel blinded to the result of the rapid diagnostic test. At least 200 high power fields were examined before a patient test is recorded as negative. Both thick and thin blood films were examined for each patient.

Malaria Pf Test
The kit comprises:

- Cassette contained in a sealed foil (pouch).
- Assay diluent (or buffer) in a dropper plastic bottle.
- Disposable pipettes.

The test was performed strictly according to manufacturer's instructions. The test kit was allowed to equilibrate at room temperature before testing. The cassette was removed from the pouch and placed on a clean and level surface. Using the dropper provided, the blood sample was drawn up to the fill line (about 10 µl) and transferred to the sample well (S) on the test cassette.

Three full drops (about 120 µl) of the sample diluent or buffer were added on the sample well.
The cassette was then examined for the appearance of coloured lines on the result window within (but not beyond) 20 minutes.

Interpretation of results
Positive test is indicated by the appearance of 2 coloured lines, 1 on the control (C) region and the other on the test (T) region. A negative test is indicated by the appearance of only 1 coloured line on the control (C) region and none on the test region. An invalid test is indicated by the non-appearance of coloured line on the control region with or without a coloured line on the test region.

RESULTS
A total of 250 children under the age of 5 were tested with both Giemsa stained blood smears and Malaria Pf rapid diagnostic test for malaria parasite. Eighty patients (32%) were positive by microscopy and 75 patients (30%) were positive by Malaria Pf test. Ten per cent of those positive by microscopy (n=8) were negative by the rapid diagnostic test (RDT) while 4% of those positive by RDT (n=3) were negative by microscopy (table 1). Using the microscopy as gold standard, the sensitivity (14) of the Malaria Pf test was 90.0% and the specificity was 98.2%. The positive predictive value (15) was 96.0% and negative predictive value was 95.4%.

TABLE 1 STATISTICAL VALUES OF MALARIA PF TEST

SENS	SPEC	PPV	NPV	FPR	FNR	LR+	LR-	F-MEASURE
90.0%	98.2%	96.0%	95.4%	1.8%	10.0%	5.0	0.1	93.9%

KEY: SENS=Sensitivity, SPEC= Specificity, PPV= Positive predictive value, NPV= Negative predictive value, FPR=False positive rate, FNR= False negative rate, LR+ = Likelihood ratio for positive tests, LR- = Likelihood ratio for negative tests.

DISCUSSION
The prevalence of malaria parasite among children under 5 years in this community is 32%. The sensitivity of the Malaria Pf test is 90.0% and the specificity is 98.2%. This means that this kit is capable of detecting 90 out of every 100 children with malaria and is also capable of giving a clean bill of health to 98 out of every

100 children who do not have malaria. In other words the kit is good at confirming the presence of malaria with a precision (positive predictive value) of 96.0%, thus enabling prompt and accurate treatment of a child with malaria. It also shows that the kit is good enough for ruling out the presence of malaria (negative predictive value = 95.4%) thus prompting search for other possible causes of febrile conditions in a child. Other studies comparing microscopy with RDTs especially in pregnant women have given equally good results (2, 16). The false positive rate of 1.8% is low and acceptable. Although the false negative rate of 10% seems to be on the high side, other factors that may give rise to false negative and false positive malaria tests need to be considered. Factors such as low parasite density (17, 18), sequestration of parasite in tissue capillaries (5, 19), Mutation of parasites (20), cross-reactivity with rheumatoid factor (5) etc. have variously been reported. Nonetheless, the result of this study gives a reasonable confidence in the diagnosis of malaria in small children. This is considered when weighed with the risk of unnecessarily exposing every child with fever to antimalarials, given the fact that children in the sub-Saharan Africa are also prone to other conditions such as respiratory tract infections (1), septicaemia etc. that usually give rise to febrile conditions. Furthermore, the use of this RDT in the diagnosis of malaria will go a long way to reduce over diagnosis and miss-diagnosis of malaria which give false impression of therapeutic failure and antimalarial drug resistance. This view is re-enforced by the discovery during the course of this study that laboratories that claim to diagnose malaria by microscopy use very low quality microscopes and some lack electricity and use reflected light from the sun using a mirror. This will obviously produce miss-leading results. Therefore, in any situation where it is not possible to provide good quality microscope and well experienced laboratory personnel, then the rapid diagnostic test is highly recommended. Moreover, the need to insist on parasitological confirmation of malaria before treatment has variously been emphasised (6). However, there is this suggestion that parasitological confirmation of diagnosis of malaria was recommended in all cases except for children under 5 years of age residing in areas of high prevalence of *Plasmodium falciparum* (5). This suggestion was in consideration of the risk of not treating false negative children. This argument sounds plausible but however, we are of the opinion that parasitological confirmation of diagnosis is necessary even in children under 5 years given the fact that should it turn out that the child does not have malaria after all, it will take days of worsening condition for
that to become obvious and a wasted opportunity for earlier search and adequate treatment for the real cause of the ailment. Worse still, some of such cases could unfortunately be misconstrued as antimalarial drug resistance with further administration of more expensive and complex antimalarials posing a further risk to the child and financial loss to the parents. This will also increase drug pressure on malaria parasite due to sub therapeutic dose encountered by newly acquired parasites, thus helping the parasite to develop resistance to the drug (2, 4, 5).

CONCLUSION AND RECOMMENDATION

We conclude that the Malaria Pf rapid diagnostic test is comparatively good for the parasite based diagnosis of malaria in children under 5 years. It is therefore recommended that the kit be provided in all places where facilities for microscopic diagnosis of malaria could not be provided and where laboratory personnel were under trained for recognition of malaria parasites in stained blood films. This will go a long way to reduce the miss-diagnosis and over diagnosis of malaria in our environment. Selection of drug resistant malaria due to drug pressure will be reduced if the policy of parasite based diagnosis prior to treatment is adopted even in children under 5 years of age. We also recommend that strict quality assurance measures be adopted in the use of the rapid diagnostic tests, a well trained and regularly retrained medical laboratory personnel designated to monitor the use within a given area and the results regularly compared with microscopy. Finally, it is necessary that laboratory personnel in both public and private health institutions be retrained in microscopic identification of malaria parasite if the war against malaria is to be won.

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