Research Paper

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Comparative Study of Rapid Malaria Antigen Test with Giemsa Stained Peripheral Smear for Diagnosis of Malaria

Medical Science

KEYWORDS : Malaria, Blood smear, ACCUCARE test

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ABSTRACT

Malaria is thought to kill between 1.5 to 2.7 million people worldwide each year. The most common test for malaria diagnosis remains the microscopic examination of giemsa or the fields – stained blood smears. The serological test is based on the detection of Plasmodium falciparum specific histidine rich protein II (HRP II) and a pan malarial species specific LDH. Method: This study included 220 patients. For each sample thin and thick smear blood films were made and examined for malaria parasite by light microscopy and also by Accucare test. Results: The blood films results indicated that 21 (9.5%) patients were infected with malaria and the rest 199 (90.5%) were malaria negative. Among positive patients Plasmodium vivax was detected in 5 cases (23.8%) and Plasmodium falciparum in 10 cases (47.6%) and 6 cases mixed infection (28.6%) correspondingly, the ACCUCARE test results indicated that 20 (9.1%) of the patient sample were positive for malaria parasites and 200 (90.9%) were malaria negative.Out of 20 patients cases, infection with Plasmodium vivax accounted for 5 (25%) while infection with Plasmodium falciparum accounted for 9 (45%) and 6 (30%) with mixed infection.

Introduction :

Malaria continues to be one of the main public health problem in the world. Malaria is more serious in the poorest countries and among population living under most difficult and impoverished conditions.

Estimates of mortality may vary from 1.5-2.7 million malaria death world wide each year. Malaria is endemic in India.. Vector resistance and parasite resistance are the problems in malaria control in India. Microscopic examination of stained blood films has been the most widely used technique for detecting malarial parasites, but the correct interpretation of blood films requires considerable expertise, which may not be available at the periphery of the health care system1. Moreover, the microscopic method is not suitable for malaria surveillance and control programmes, where large number of blood films have to be examined. Antigen detection methods have been developed for situations in which reliable microscopy may not be available. In view of this problems, considerable emphasis has been given to malaria immunodiagnosis with the aim of developing reliable and sensitive procedures, which can be used for specific diagnosis for malaria specially in developing countries.

There are some recently developed antigen tests available, which are based on detection of histidine rich protein- 2 and specific to falciparum malaria and pan malaria specific LDH for other plasmodium species. These tests are simple, rapid, sensitive and suitable for on the spot diagnosis of malaria in the world.

Present study was conducted to find out sensitivity and specificity of serodiagnostic test, that is commercially available, to correlate Giemsa based microscopy, pf HRP II antigen assay test, pan malaria LDH and find out which is best applicable in rural conditions.

Plasmodium falciparum accounts for the majority of lethal infections. While the three other species (P.vivax,P.ovale, P.malaria) causes less severe malaria. The infection is characterized by intermittent fever with chills and anemia2-4.

In many developing- world , a presumptive diagnosis of malaria is based upon the presence of fever alone. While statistically justifiable in some regions, such an approach inevitably leads to the overuse of antimalarial drugs. Under ideal circumstances, the clinical suspicion of Malaria would be confirmed by a laboratory test that is simple to perform, rapid, sensitive, specific and less expensive.

The most common test for malaria diagnosis remains the microscopic examination blood smears. However, the examination of blood films requires technical expertise and the availability of a good – quality microscope. The microscopy is also time consuming and has limited sensitivity when parasitemia is low44-6.

Besides these majorities of Malaria cases occur in rural areas where there is a little or no access to reference laboratories and in many areas microscopy is not available. Keeping all these in, study was done to compare microscopic examination of blood films with newly develop Immuno Chromatography Test. The test is based on the detection of Plasmodium Falciparum specific HRP II and a Pan Malarial Species specific LDH produced by the respective parasites and released into the blood.

MATERIALS AND METHODS

Study included 220 patients. 60% of patients had history suggestive of malaria i.e. rigor, chill, rise of high temperature with profuse sweating. Another 40% gave the history of irregular fever;

For each patient peripheral blood sample was collected into a sterile tube containing potassium EDTA. Thin and thick smear blood films were made immediately after blood collection, stained with Giemsa stain and examined for malaria parasite by light microscopy. All the blood samples were tested with ACCUCARE (one step Malaria Pf/Pv antigen test) and results were compared to those obtained from examination of thin and thick blood smears. The test is based on the detection of P. falciparum species specific HRP II and a pan malarial species specific LDH produced by the parasites and released into the blood.

RESULTS

Positive for P.falciparum: Appearance of two magenta red colored bands, one each in the anti falciparum antibody region, and control region.

Positive for P.vivax or other Plasmodium species: Appearance of two magenta red colored bands, one each in the pan malarial species specific LDH region, and control region. (Plasmodium vivax is most commonly encountered in India).

Negative: Appearance of only one magenta red colored band in the control region.

Error: No band observed in control or test region indicates improper test procedure or deterioration of reagents.

The magenta red colored test bands indicate reactive result representing the binding of antigen antibody complex to a monoclonal antibody that has been pre-immobilized on the test strip. In nonreactive sample no magenta red colored band is seen in test region. A reactive procedural control band is also built into validate the results as well as proper test performance.

Table 1.

	Peripheral Smear		ACCUCARE	
	Positive	%	Positive	%
Pf	10	47.6	9	45
Pv	5	23.8	5	25
Mix	6	28.6	6	30
Total	21	100	20	100

A total of 220 blood samples were tested by the Accucare method and the results were compared to those obtained from examination of thin and thick smear blood films. The blood films results indicated that 21(9.5%) patients were infected with malaria and the rest 199 (90.5%) were malaria negative. Among positive patients Plasmodium vivax was detected in 5 cases (23.8%), Plasmodium falciparum in 10 cases (47.6%) and mix infection in 6 cases (28.6%) and correspondingly, the Accucare Test detected 20 (9.1%) Patients were infected with malaria and the rest 200 (90.9%) were malaria negative. Among positive patients Plasmodium vivax was detected in 5 cases (25%), Plasmodium falciparum in 9 cases (45%) and mix infection in 6 cases (30%).

The blood film examination identified 1 Plasmodium vivax positive samples that was not detected by the Accucare Test and 1 Plasmodium falciparum case identified by blood film examination and not detected by the accucare test. ACCUCARE test detected 1 Plasmodium vivax positive sample that was not detected by blood film.

DISCUSSION

The resurgence of malaria has renewed interest in developing not only preventive measures, but also rapid diagnostic techniques. A multitude of factors has contributed to the reemergence of malaria, like Insecticide resistance in the Anopheles Mosquito, Social instability resulting in movements of unexposed non immune individuals in areas

where malaria is endemic, and
The failure to develop an effective malaria vaccine.

These necessitate the use of more expensive and sometimes toxic antimalarial drugs and longer treatment courses. In addition, the cyclic recurrence of malaria epidemics has a tremendous impact on the health infrastructure in developing countries and adversely affects local economics. One of the most pronounced problems in controlling the morbidity and mortality caused by malaria is limited access to effective diagnosis and treatment in areas where malaria is endemic. Clinical diagnosis of infection with the malaria parasite requires microscopic observation of parasites on a Giemsa – stained blood smear.

Microscopic examination of blood smears requires highly skilled people to perform or interpret results. Several methods have been developed to supplement and replace the conventional microscopic method. The most promising new malaria diagnostics are the serological Antigen detection tests. Accucare is one amongst them. We employed this test and compared it with a conventional smear examination for diagnosis of P. falciparum and P. vivax infection7-9.

The antigen detection test identified (9.1%) as malaria positive while the blood film identified (9.5%) to be malaria positive. Some malaria infections detected by blood film were not detected by the accucare test. This may be explained by the fact that increased awareness of malaria among the general public has led to a rampant misuse of antimalarial drugs in inadequate doses empirically for any fever. Since Accucare detects PLDH which is produced only by living parasites, the blood samples judged negative by Accucare may have been dead parasites and not yet cleared from the host. Two cases of Plasmodium vivax detected by blood film were not detected by Accucare. This may be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentrations below the Accucare tests detection level. One sample in which Accucare detected P.vivax was found to be negative in the blood smear examination. This may be explained by the fact that Plasmodium can sometimes sequester and may not be present in circulating blood10-11.

This test has the added advantage that it can detect all four Plasmodium species and can be used to follow the efficiency of drug therapy since it detects on enzyme produced only by living parasites. Although it has got a number of advantages one needs to keep in mind the cost of the test which may not be affordable by many. The high cost of the test may prevent its regular and routine we in many of the laboratories. However, it was a valuable adjunct at the time of emergency for rapid diagnosis, although microscopy remains the mainstay for the diagnosis of malaria for routine use in countries like India.

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