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Original research article

DETECTION OF MALARIAL PARASITE BY BLOOD SMEAR EXAMINATION AND ANTIGEN DETECTION: A COMPARATIVE STUDY

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ABSTRACT

At present about 100 countries in the world are considered malarious, is thought to kill between 1.1 and 2.7 million people worldwide each year, of which about 1 million are children under the age of 5 years in these areas. Under ideal circumstances, the clinical suspicion of malaria would be confirmed by a laboratory test that is simple to perform, rapid, sensitive, specific and expensive. At the present time, no such test exists. The most common test for malaria diagnosis remains the microscopic examination of giemsa or the fields – stained blood smears. The test is based on the detection of Plasmodium falciparum specific histidine rich protein ii (hrp) and a pan malarial species specific enzyme aldolose, produced by the respective parasites and released into the blood and the test is based on immune chromatography, the test is highly sensitive. **Method**: In this study included 100 patients, 60% of patients had history suggestive of malaria, another 40% gave the history of irregular fever; For each patient peripheral blood sample was collected, thin and thick smear blood films were made immediately after blood collection, stained with Leishman stain and examined for malaria parasite by light microscopy. **Results:** The blood films results indicated that 40 (20%) patients were infected with malaria and the rest 171 (85.5%) were malaria negative. Among positive patients Plasmodium vivax was detected in 24 cases (60%) and Plasmodium falciparum in 10 cases (31%) and 6 cases mixed infection (PV + PF) (15%) correspondingly, the Para HIT Test results indicated that 29 (14.5%) of the patient sample were positive for malaria parasites and 171 (85.5) were malaria negative out 29 patients cases. Infection with Plasmodium vivax accounted for 17 (58.6%) while infection with Plasmodium falciparum accounted for 9 (25%) and 3 (1.3%) with mixed infection of Plasmodium vivax and Plasmodium falciparum.

Keywords: Malaria, Blood smear, Para Hit test.

INTRODUCTION

Malaria is a parasitic infection of global importance and it remains to be one of the most significant cause of morbidity and mortality of humans, worldwide. The disease is a major health

problem in the tropics and subtropics regions. Annually, approximately 500 million people in the world suffer from malaria and about 1 million deaths occur due to this infection. Current efforts to control malaria focus on reducing attributable morbidity and mortality by prompt diagnosis of suspected malarial infection with rapid and accurate diagnosis for effective therapeutic intervention.

The protozoan parasite belongs to the genus Plasmodium. Four species of malaria parasite that are known to infect humans are Plasmodium falciparum, Plasmodium vivax, and Plasmodium ovale and Plasmodium malariae. Plasmodium falciparum accounts for the majority of infections that term out to be lethal. While the three other species cause a less severe form of malaria. The infection is characterized by intermittent fever with chills and anemia[1-3].

At present about 100 countries in the world are considered malaria, about half of which are in sub-Saharan Africa. Although this number is considerably less than it was in the mid 1950s, more than 2.4 billion of the world's population is still at risk.

Malaria is thought to kill between 1.1 and 2.7 million people worldwide each year, of which about 1 million are children under the age of 5 years in this areas[2-5].

In many developing- world settings, a presumptive diagnosis of malaria is based upon the presence of fever alone. While statistically justifiable in sole 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 expensive. At the present time, no such test exists. The most common test for malaria diagnosis remain the microscopic examination of Giemsa or Fields – Stained blood smears. However, the examination of blood films requires technical expertise and the availability of a good – quality microscope. The microscope is also time-consuming and has limited sensitivity when parasitemia is low[3-5].

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 a study was done to compare microscopic examination of blood films with newly develop Immuno Chromatography dipstic Test.

The test is based on the detection of Plasmodium Falciparum specific Histidine Rich protein II (HRP II) and a Pan Malarial Species specific enzyme Aldolose, produced by the respective parasites and released into the blood and the test is based on Immuno Chromatography. The test is highly sensitive and specific for the diagnosis of Plasmodium Falciparum, Plasmodium Vivax, Plasmodium Ovale and Plasmodium Malarial Infection.

MATERIALS AND METHODS

After approval of the Institutional Ethics Committee and inform consent form the patient in this study included 100 patients attending Mamata General Hospital 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; Patients that have been treated for malaria in the previous four weeks were excluded from this study. 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 Leishman stain and examined for malaria parasite by light microscopy. According to standard practice a thin blood smear was examined for 15 minutes and for a thick blood film 200 fields were visualized. All the blood sample was tested with Para HIT total dipstick test according to manufacturers instruction and results were compared to those obtained from examination of thin and thick blood smears.

The test is based on the detection ofPlasmodiumm falciparum specieshistidinee rick protein II (HRP

II) and a pan malarial species specific enzyme Aldolase, produced by the respective parasites and released into the blood.

RESULTS

Positive: Appearance of three magenta red colored bands, one each in the anti falciparum antibody region, anti malarial antibody region and control region indicates that the sample is reactive for Plasmodium falciparum and mixed infection with another malarial species. (Plasmodium vivax is most commonly encountered in India).

Negative: Appearance of only one magenta red colored band in the control region indicates that the sample is non-reactive for Plasmodium species.

Error: No band observed in control or test region indicates improper test procedure or deterioration of reagents. Repeat the test using a fresh test strip.

The magenta red coloured 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 non-reactive sample no magenta red coloured band is seen in test region. A reactive procedural control band is also built into validate the results as well as proper test performance.

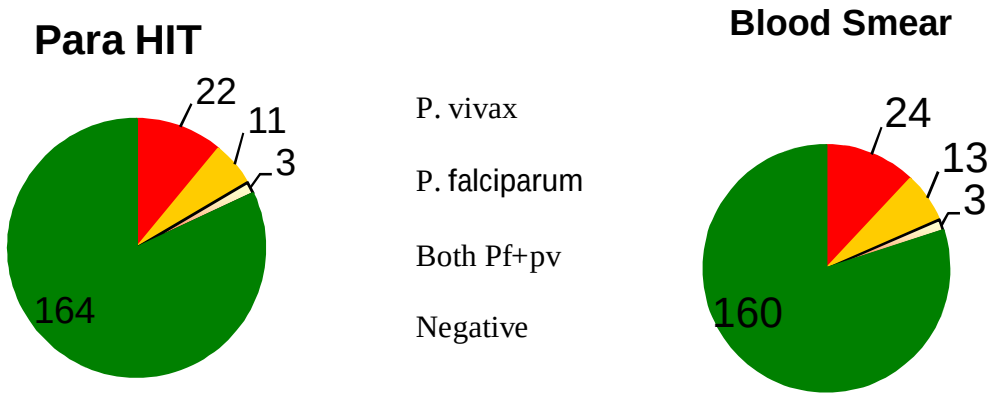

Fig.1: Examined Blood smear report & Para hit report

Table:1. Comparision of blood smear examination and antigen detection

Parameters	Blood Smear		Para HIT	
	Positive	%	Positive	%
PF	10	(25%)	9	(31%)
PV	24	(60%)	17	(58.6%)
Mixed	6	(15%)	3	(10.3%)
Total	40	(20%)	29	(14.5%)

A total of 200 blood samples was tested from the month of March 2007 to December 2007 for malaria parasites by the Para HIT method and the results were compared to those obtained from examination of thin and thick smear blood films. The blood films results indicated that 40 (20%) patients were infected with malaria and the rest 171 (85.5%) were malaria negative. Among positive patients Plasmodium vivax was detected in 24 cases (60%) and Plasmodium falciparum in 10 cases (31%) and 6 cases mixed infection (PV + PF) (15%) correspondingly, the Para HIT Test

results indicated that 29 (14.5%) of the patient sample were positive for malaria parasites and 171 (85.5) were malaria negative out 29 patients cases. Infection with Plasmodium vivax accounted for 17 (58.6%) while infection with Plasmodium falciparum accounted for 9 (25%) and 3 (1.3%) with mixed infection of Plasmodium vivax and Plasmodium falciparum.

The blood film examination identified 7 Plasmodium vivax positive samples that were not detected by the Para HIT Test and 1 Plasmodium falciparum case identified by blood film examination and not detected by the para HIT test. However there was 100% agreement between blood film results and Para HIT results for the other 29 cases.

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, including

(i) Insecticide resistance in the Anopheles Mosquito.
(ii) Social instability resulting in movements of unexposed non immune individuals in areas where malaria is endemic, and
(iii)The failure to develop an effective malaria vaccine.

Compounding the problems of malaria's geographical expansion and of increasing morbidity and mortality are the emergence and rapid spread of antimalarial drug resistance. Which 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, since infected individuals are often too debilitated to work.

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. Para HIT is one amongst them. We employed this test and compared it with a conventional smear examination for diagnosis of Plasmodium falciparum and Plasmodium vivax infection[6-8].

The antigen detection test identified (14%) as malaria positive while the blood film identified (20) to be malaria positive. Some malaria infections detected by blood film were not detected by the Para HIT 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 Para HIT detects PLDH which is produced only by living parasites, the blood samples judged negative by Para HIT 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 Para HIT. 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 Para HIT tests detection level eight blood samples in which Para HIT detected Plasmodium falciparum band were found to be negative in the blood smear examination. This may be explained by the fact that Plasmodium falciparum can sometimes sequester and may not be present in circulating blood. [9,10]

This test has the added advantage that it can detect all fouPlasmodiumum 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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