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Original Article

Role of ICT Malaria Immunochromatographic Test for Rapid diagnosis of Malaria

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Abstract

Objective: To evaluate the sensitivity and specificity of immunochromatographic test (ICT) malaria p.f/p.v using microscopy as the gold standard diagnosis.

Methods: Five hundred and sixty patients of both sexes and all age groups with clinical suspicion of malaria were studied. Venous blood was collected for microscopy and ICT. Thick and thin films prepared and stained with Leishman's stain were examined. ICT malaria test was performed and interpreted according to manufacturer's instructions. Data was analyzed using Epi-6.

Results: A total of 560 cases were studied, 339 males and 221 females with age ranges between 2 to 73 years. Seventy two (12.85%) cases had parasitaemia (with or without sexual forms). On microscopy 65 (11.6%) cases had asexual-stage parasitaemia and 7 (1.25%) cases had P. falciparum gametocytes only. Thirty two cases were infected with P. falciparum, 29 with P. vivax and 4 had mixed infection. For P. falciparum the ICT was 97.0% sensitive, 98.3% specific, with positive predictive value (PPV) of 78.0% and a negative predictive value (NPV) of 99.8%. For P. vivax the sensitivity was only 89.7%, specificity 97.9%, PPV was 70.3% and NPV 99.4%.

Conclusion: Our results are in concordance with previous studies. Rapid tests though expensive are simple to perform and effective diagnostic tools of malaria. They can be used selectively, though microscopy remains the gold standard diagnosis, economical and accurate if performed by skilled technologists (JPMA 56:167;2006).

Introduction

Malaria is one of the most common parasitic diseases and a major health problem world wide infecting 200 million and killing about 2 million people each year.[1] Rapid diagnosis and early treatment of clinical cases is central to the reduction of malarial morbidity and mortality.[2] The two diagnostic approaches currently used are clinical and microscopic examination. Clinical diagnosis of malaria alone is unreliable and should be confirmed by laboratory tests.[3] Microscopic examination of stained thick and thin blood film is currently the standard method of malaria diagnosis.[3,4] This technique remains cost effective however; correct interpretation of the blood films requires considerable expertise and adequate quality control. Secondly, its reliability is questionable particularly

at low levels of parasitemia and in the interpretation of mixed infection.[5,6]

Recently, rapid antigen detection methods have been developed for situations in which reliable microscopy may not be available. These tests are based on the detection of antigen(s) released from parasitized red cells. Malaria antigens currently targeted by rapid diagnostic tests (RDT) are histidine-rich proteins 2 (HRP-2), Plasmodium lactate dehydrogenase (PLDH) and Plasmodium aldolase.[7]

Rapid diagnostic tests such as dipstick format kits for the detection of malaria antigen are now commercially available. In recent years multiple studies have found that rapid dipsticks have excellent sensitivity and specificity when compared with conventional microscopy.[8-10] Recently a new immunochromatographic test kit capable of detecting antigen of Plasdmodium (P.) falciparum and P. vivax (P.f/P.v) has been introduced. This is the immunochromatographic test (ICT) malaria P.f/P.v (Binax Inc., Portland, Maine, USA).

The test is based on detection of the P. falciparum specific antigen and panmalarial antigen. The test uses two colloid gold labelled antibodies that have been immobilized across the test strip. One antibody is specific for the histidine-rich protein 2 (HRP-2) antigen, a water soluble protein produced by trophozoites and young gametocytes of P. falciparum. The other antibody is specific for an antigen that is common to all Plasmodium species.

The purpose of this study was to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ICT malaria P.f/P.v immunochromatographic test using microscopy as a gold standard for the diagnosis of malaria.

Patients and Methods

This was a prospective study carried out at the Department of Pathology and Microbiology, Aga Khan University, Karachi from August 2004 to February 2005. Five hundred sixty patients from both sexes and all age groups with clinical suspicion of malaria having history of fever (temperature >37.5^{o}C) at the time presentation associated with shivering in some cases and with other non specific symptoms like body ache, headache, fatigue and abdominal discomfort were included in the study. Informed consent was obtained from patients or parents as appropriate. All patients who had been treated for malaria in the previous 4 weeks or with other known causes of fever were excluded from study.

Venous blood was collected by the standard venipuncture procedure into EDTA tubes for microscopy and immunochromatographic testing. Thick and thin films were prepared and stained with Leishman's stain and examined at X 1000 magnification by a senior technologist and principal author. The microscopists were unaware of the immunochromatographic test results. Minimum of 200 consecutive fields were counted in thick blood film before classifying result as negative. The sensitivity of thick blood film is about 50 parasites/µl of blood.[7] Immunochromatographic test was performed in microbiology section according to manufacturer's instructions using Now ICT malaria P.f/P.v rapid diagnostic device (Binax, Inc., Portland, Maine, USA). The sensitivity of rapid diagnostic test is more than 100 parasites/µl of blood.[7] These results were read by the second author who was blinded to the microscopy results. The test uses two antibodies that have been immobilized across the test strip. One antibody (test area 1) is specific for the histidine rich protein 2 antigen of P. falciparum (Pf HRP-2). The other antibody (test area 2) is specific for a malarial antigen which is common to both P. falciparum and P. vivax species. Fifteen microlitre whole blood is applied to a sample pad impregnated with colloidal gold labeled antibodies directed against the two malarial antigens. The test was considered valid if the control line was visible and positive if the HRP-2 and/or panmalarial antigen lines were visible. An immunochromatographic test diagnosis of P. vivax malaria was made if panmalarial antigen line was visible. A diagnosis of P. falciparum malaria was made if the HRP-2 was visible, with or without panmalarial antigen.

Data was collected and analyzed using Epi - Info (version 6). Performance indices were calculated for malaria as a whole (diagnosis of either species), P. falciparum malaria (including mixed infection) and P. vivax malaria. For sensitivity and specificity, the ICT malaria test results were compared with microscopy using as a gold standard. Variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP), and number of false negatives (FN). Sensitivity was calculated as TP/(TP+FN), specificity was calculated as TN/(TN+FP), the positive predictive value (PPV) was calculated as TP/(TP+FP) and negative predictive value (NPV) was calculated as TN/(FN+TN) as suggested by Tjitra et al (1999).[10] Test efficiency, the proportion of all tests that gave a correct result, was defined as (TP+TN)/ number of all tests. When the data was analyzed for malaria as a whole, results were considered false positive if P. falcipaurm were detected in thick smear and ICT P.f/P.v detected P. vivax and vice versa. Usually most laboratories read and report mixed infections as P. falciparum alone due to its clinical importance. When analyzing test performance for the detection of P. vivax, mixed infection detected by microscopy were considered true negative (technically ICT showing positive reaction for P. falciparum, and a mixed infection on microscopy would confirm P. falciparum alone, thus P. vivax would be excluded and regarded as true negative and vice versa). As sexual stages do not cause

disease, samples that were HRP-2 or panmalarial antigen positive by immunochromatographic tests but that were asexual parasite negative and gametocyte positive on microscopy were considered false positive.[10,11]

Results

Of 560 patients who met the case definition for clinical malaria 339 were males and 221 were females. The age ranges were between 2 to 73 years. Seventy two (12.8%) cases were found to have parasitemia (with or without sexual forms), 65 (11.6%) had asexual-stage parasitemia (with or with out sexual forms) and 7(1.25%) had P. falciparum gametocytes only. Of the 65 people with asexual-stage parasitemia, 32 (49.23%) were infected with P. falciparum as detected by microscopy, 29 (44.61%) were infected with P .vivax and 4 (6.15%) were infected by both P. falciparum and P. vivax (mixed infection) characterized by presence of asexual forms of P. falciparum and P. vivax with or without sexual forms.

The results of parasite detection by microscopy and immunochromatographic testing are compared in Table 1. The test was sensitive (97.0%) and specific (98.3%) for the diagnosis of P. falciparum malaria, with a PPV and an NPV of 78.0% and 99.8% respectively. The corresponding sensitivity and specificity for the diagnosis of P. vivax malaria 89.7% and 97.9%, respectively with PPV and NPV of 70.3% and 99.4% respectively (Table 2). Sensitivity of P. vivax was significantly different from P. falciparum (P<0.0001) however specificity showed no significant difference between both species. Three cases of P. falciparum and 7 cases of P. vivax were false positive by rapid test, probably due to intake of antimalarial drugs in preceeding weeks, as they have been treated by general practitioners. Two of 29 subjects with P. vivax infection were not detected by panmalarial antigen and 3 of 32 subjects with P. falciparum infection were not detected by HRP-2.

Table 1. Comparison of ICT PF / PV and microscopic examination results (n=560).

Microscopic Result	No. of Samples	Breakup of ICT P.f / P.v results		
		P.falciparum	P.vivax	Negative
P.falciparum a sexual (+ sexual)[a]	32	29	2	1
P.vivax a sexual (+ Sexual)	29	1	26	2
P.falciparum + P.vivax (+ sexual) mixed	04	3	1	0
P.falciparum sexual only	7	5	1	1
Negative	488	3	7	478
Total	560	41	37	482

a + sexual, with or without sexual-stage parasites

Table 2. Performance characteristics of ICT P.F / P.V relative to those of microscopy in patients with a presumptive clinical diagnosis of Malaria (n = 560).

	Sensitivity % (CIa 95%)	Specificity % (CI 95%)	PPV % (CI 95%)	NPV% (CI 95%)	Efficiency %
Total	94.91 (84.9 - 98.7)	96.21 (94.0 -97.6)	74.7 (63.1 - 83.7)	99.4% (98.0 - 99.8)	96.07 %
P.falciparum	97.0 (82.5 - 99.8)	98.3 (96.7 - 99.2)	78.0 (62.0 - 88.9)	99.8 (98.8 - 100)	98.21%
P.vivax	89.7 (71.5 - 97.3)	97.9 (96.2 - 98.9)	70.3 (52.8 - 83.6)	99.4 (98.2 - 99.9)	97.5%

aCI, Confidence interval

Discussion

The recommended method and current gold standard used for the routine laboratory diagnosis of malaria is the microscopic examination of stained thick and thin blood films. In capable hands, this method can be expected to detect 50 parasites/ul (0.001%) parasitemia and to identify to the species level 98% of all parasites seen. However, this procedure is difficult and time consuming, and requires skilled staff.[7] In the past few years efforts have been made to replace the reading of blood film by other techniques. Fluorescence microscopy and PCR has proven to be sensitive but difficult to perform in a routine laboratory practice.

Therefore, the development of easy, rapid and accurate tests for the detection of plasmodia infection is highly desirable.[3] Immunochromatographic rapid tests offer the possibility of more rapid nonmicroscopic methods for rapid diagnosis. In this study we investigated the performance of Now^{R} ICT malaria P.f/P.v test with traditional light microscopy. The study showed 97.0% sensitivity and 98.3% specificity for P. falciparum with a PPV and an NPV of 78.0% and 99.8% respectively. Various studies using monoclonal antibody to HRP-2, same as in present study revealed comparable results in term of sensitivity (range 92-100%) and specificity (range 84-99%) done at national[12] and international levels.[10,13,14] Similar results were reported by Gasser et al[15] using the same kit. However a study from Indonesia reported a low sensitivity for HRP-2 antigen detection tests.[16] For the detection of P. falciparum, the sensitivity and specificity of ICT P.f/P.v (current study), ICT Malaria Pf[13,14,16,17] and Parasight F[18,19] for HRP-2 are, overall, at least equal to those of microscopy performed in a well-organized malaria diagnostic laboratory and much better than those routinely achieved by microscopy in remote primary health centers.[11]

In contrast to the excellent sensitivity for the detection of P. falciparum, the overall sensitivity of ICT P.f/P.v for the detection of P. vivax was less than the desirable level. This is most likely due low parasitemia levels. The pyrogenic threshold, which is the density of plasmodia required to involve a febrile reaction in a given individual is on an average, lower for vivax malaria than for falciparum malaria.[10] Same results for P. vivax malaria were reported by other investigators.[10,12,14] Two subjects with P. vivax infection were not detected by panmalarial antigen and 3 subjects with P. falciparum infection were not detected by HRP-2 antigen. This may be due to (i) low level of parasitemias (ii) very high levels of parasitemias or (iii) gene deletion for the production of HRP-2 antigen. These findings have also been reported in previous studies.[7,10,11,20] Why high falciparum or vivax parasitemias give false negative results, is not known.[11] One microscopy detected P.vivax shown as P. falciparum by ICT P.f/P.v could have been due to mixed infection with P. falciparum, because low-level of P. falciparum have been obscured or over looked in mixed infection dominated by P. vivax. Alternatively P. falciparum parasites can be sequestered in peripheral blood.[14]

An alternative rapid dipstick method (OptiMAL) for the diagnosis of both P. falciparum and P. vivax malaria is also in use. This test uses a monoclonal antibody to the intra cellular antigen parasite lactate dehydrogenase (PLDH). Like ICT P.f/P.v, this test also differentiates species by the use of a P. falciparum specific antibody and a genus - specific antibody. Compared with microscopy and ICT malaria P.f/P.v, this test showed high sensitivity.[3,9,21] However the sensitivity of all rapid immuno-chromatographic tests is low when compared with PCR.[22]

Some kits like ParaSight-F immunoglobulin G (Ig G) monoclonal antibody to HRP-2, cross-react with rheumatoid factor, causing false-positive results.[23] However, there is a little cross-reactivity with infection or rheumatoid factor with ICT P.f/P.v test using IgM monoclonal antibody to HRP2 antigen.[11]

Our results are in concordance with the results reported in previous studies. They add to the evidence that these nonmicroscopical rapid tests for the detection of Plasmodial antigen should develop into essential life saving diagnostic tools which prove to be a valuable adjuncts to clinical assessment of the patients and blood film microscopy under selective circumstances. These tests are rapid and simple to perform and to interpret but not cost effective when compared to microscopy. In our laboratory, at present, the cost of ICT is 2.5 times higher than microscopy (Rs. 430 v/s Rs. 150), though the rates vary from laboratory to laboratory and with type of kit used for the test. The price in US $ is 1.20 per test. It seems to be too high to enable wide spread use in developing countries.[10] They can not replace microscopy which is still the gold standard for diagnosing malaria. Although time consuming, it is economical and accurate if performed by a skilled technologist.

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