Journal of Islamabad Medical & Dental College (JIMDC); 2016:5(1):21-25

Original Article

Evaluation of Immunochromatographic Assay and Microscopy of Peripheral Blood Film for Malaria Diagnosis

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Abstract

Objective: This study aims to evaluate the sensitivity, specificity, positive predictive value, negative predictive value and efficiency of Immunochromatographic (ICT) method for diagnosis of malaria using conventional microscopy of peripheral blood film, as gold standard.

Materials & Methods: This cross sectional study was conducted at private laboratory in Wah Cantt, from February 2014 to January 2015. Four hundred patients from both sexes and all age groups with clinical suspicion of malaria were included in the study. Venous blood was collected by the standard venepuncture practice. Thick and thin films were prepared and stained with Giemsa and Leishman stain for microscopy respectively. ICT assay was performed according to manufacturer's instructions. Results were entered on SPSS version 16.

Results: The overall sensitivity of ICT was 99%, while specificity was 98%, with a PPV of 96% and NPV of 99%. Test efficiency was calculated as 98%.

Conclusions: ICT can be used as effective diagnostic tool for malaria diagnosis by relatively inexperienced persons.

Keywords: Immunochromatographic assay, Malaria, Rapid diagnostic test.

Introduction

Malaria being the number one killer of all the parasitic diseases is considered a serious human health issue. It is estimated that each year at least 200 million people worldwide have malaria, and more than 1 million die of it each year, making it the most common lethal infectious disease.[1] It occurs primarily in tropical and subtropical areas, especially in Asia, Africa, and Central and South America. Certain regions of Southeast Asia, South America, and East Africa are mostly affected by *Plasmodium falciparum* strains resistant to chloroquine. Untreated malaria caused by *P. falciparum* is lethal and life-threatening because of its complication such as cerebral malaria, haemolytic anaemia and acute renal failure. Malaria caused by the other three plasmodia (*P.vivax*, *P.ovale*, *P.malariae*) is usually associated with a low mortality rate.[2] There are increasing reports of a fifth human-infecting species, *Plasmodium knowles*i, which has been described in southeast Asian countries.[3,4]

In Pakistan malaria imposes enormous burden of disease because tropical climatic condition with a poor irrigation system overlaps monsoon season.[5] In Pakistan, malaria burden has increased due to internally displaced persons in Khyber Pakhtunkhwa and natural catastrophes like floods in Punjab and Sindh. The disease prevails throughout the year along the coastal areas and Western border areas. Malaria has a tendency for epidemic outbreaks particularly in Baluchistan, Khyber Pakhtunkhwa and Sindh province. However, the disease is now emerging as a significant health problem in Federally Administered Tribal Areas (FATA) particularly along the international border with Afghanistan and Iran. Malarial epidemics in Pakistan occur at 8-10 years' intervals.[6] In 2013 report WHO declared that 0.6 million malaria cases annually has been reported in Pakistan and only 2% of Pakistani population lives in malaria free region.[7]

Rapid and accurate laboratory diagnosis is imperative for efficient treatment of malaria. The lack of proper diagnosis of malaria is a waste of already meager resources and impacts negatively on the prompt treatment.[8] Although highly debatable, practice of prescribing antimalarials based on clinical diagnosis was acceptable in the past when well tolerated and inexpensive antimalarial drugs like chloroquine was effective. The resistance of plasmodium to

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Received: Dec. 19th 2015; Accepted: April 22nd 2016

traditionally used antimalarial drugs has developed globally and artemisnin-based combination therapy (ACT) is required.[6] There is a need of targeted treatment to the patients with parasitologically confirmed malaria in order to improve quality of patient care, reduce drug pressure and in turn delay in development and spread of drug resistance.

Malarial parasites are detected and identified in the laboratory by examination of Giemsa stained thick and Leishman stained thin blood films.[9] Aptly used microscopy of peripheral blood smears is rapid, inexpensive and reasonably sensitive method which is in use for more than 100 years. For the diagnosis of malaria, peripheral blood smear microscopy for malarial parasite is considered as the 'gold standard' technique.[10] But non-availability of skillful and experienced microscopists in far off medical facilities result in poor interpretation of smears. The peripheral blood smear microscopy sometimes fail to detect sequester *P. falciparum* parasites, especially when the parasitemia is low [≤50 parasites (ml blood)].[11] The other factors which may lead to poor quality of results include unavailability of good microscopes, lack of electricity, shortage or poor quality of available reagents /stains, shortage of staff and high workload.[12] To deal with these confines of microscopy, Malarial rapid diagnostic tests (RDTs) were developed in the mid-1990s.[13] RDTs are used for antigen detection by lateral flow immunochromatography, whereby the dye labeled antibody binds to lysed parasite antigen and passes along the nitrocellulose strip, the resultant complex is arrested on the strip by a band of bound antibody, forming a visible test line. RDTs are easy to use, simple and rapid method which requires minimal training for performance and interpretation of test result.

RDTs are presented in different formats i.e. plastic cassette, card, dipstick or hybrid cassette dipstick which can be easily transported. RDTs neither need laboratory equipment /facilities nor electric power supply; thus, they are appropriate for use by medical facilities with unskilled staff or meager facilities and field tests. WHO recommends that RDTs when compared with microscopy should have at least 95% sensitivity and 90% specificity for all malarial species.[14] These RDTs must be able to reliably detect cases of low parasitemia ~100 parasites / ml (0.002% parasitemia).[14]

RDTs are used in most of the laboratories of private and public setups where technicians are overworked, without supervision and training and mat not be specialized in this field. This study was conducted to evaluate the sensitivity, specificity, positive predictive value, negative predictive value and efficiency of RDT using conventional microscopy as gold standard

Materials and Methods

This cross sectional study was conducted at private laboratory in Wah Cantt, **f**rom February 2014 to January 2015. Four hundred patients from both sexes and all age groups with clinical suspicion of malaria were included in the study. Venous blood sample was collected by the standard venipuncture procedure for microscopy and immunochromatographic (ICT) testing. Both thick and thin films were prepared and stained with Giemsa and Leishman stains respectively and examined at 10x100 magnification. The microscopist was unaware of the ICT test results. Before classifying result as negative, 200 consecutive fields were counted in the thick blood film.[9,10] ICT test was performed by using rapid diagnostic device SD Bioline Malaria Ag P.f/Pan (Standard diagnostics, Inc, Republic of Korea). The test uses strip precoated with monoclonal antibodies specific for the histidine rich protein 2 antigen of P. falciparum (Pf HRP-2) and with other monoclonal antibodies specific to lactate dehydrogenase (LDH) which is common to Plasmodium species (*P.falciparum, P. vivax, P.malariae, P.ovale*).

The manufacturer's instructions were followed while performing ICT test for malarial parasite detection. The ICT test was considered valid if the control line was visible and positive if the HRP-2 and/or panmalarial antigen lines were visible. The diagnosis of *P. vivax* malaria was made on ICT based test if panmalarial antigen line was visible whereas the diagnosis of *P. falciparum* malaria was made if the HRP-2 line was visible, with or without panmalarial antigen line i.e. when two lines are visible, the test interpretation was *P. falciparum*. ICT test cannot distinguish coinfection with both *P. falciparum* and *P. vivax* from infection with *P. falciparum* alone. The samples that were HRP-2 or panmalarial antigen positive by ICT but were negative for asexual parasite and positive for gametocyte on microscopy were considered false positive (FP) because sexual stages do not cause disease.[15, 16, 17]

True positive (TP) results were considered when asexual stages of P. falciparum and any stage of *P.vivax* were detected by standard method of microscopy as well as ICT yielded positive result.

True negative (TN) results were yielded from those samples in which parasite were not detected by microscopy as well as parasite antigen was not detected by ICT method. False negative (FN) results were considered when ICT was interpreted as negative either for *P. falciparum, P. falciparum /P.vivax* or panmalaria and parasite was detected by microscopy. Sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated as was calculated as TP/(TP+FN), TN/(TN+FP), TP/(TP+FP) and TN/(FN+TN) respectively as suggested by Tjitra et al.[15] Test efficiency was defined as (TP+TN)/ number of all tests, the proportion of all tests that gave a correct result.

Table 1: True positive, false positive, true negative and false negative cases.

Gold standard (MP microscopy)			
	Diseased	Non diseased	
Screening test ICT-MP Diseased	128 TP	05 FP	133
Non Diseased	01 FN	TN 266	267
Total	129	271	400

Results

Among total of 400 samples, 125 cases were true positive for malarial parasite by both of above mentioned methods. Out of 125, 68% (85) were male who suffered from malarial infestation while 32% (40) were female. Mean age of the patients was 28.6 ± 14.3 SD years. Age distribution of different age groups which were infected with Plasmodium is shown in Figure 1.

The results of ICT yielding different species of Plasmodium are illustrated in Figure 2. Not a single case of *P. malariae* or *P. ovale* was found during our study. Out of 400 samples, 05 cases were HRP-2 positive by ICT but the gametocytes of *P.falciparum* were not seen during microscopy. These 05 cases were considered false positive.

A single case was recorded in which parasite was detected by microscopy but ICT results was interpreted as negative for *P. falciparum*. This case was recorded as false negative. There were 266 samples in which parasite were not detected by microscopy as well as by ICT method. These cases were considered to be true negative. The overall sensitivity of ICT was 99%, while specificity was 98%, with a PPV of 96% and NPV of 99%. Test efficiency was calculated as 98%.

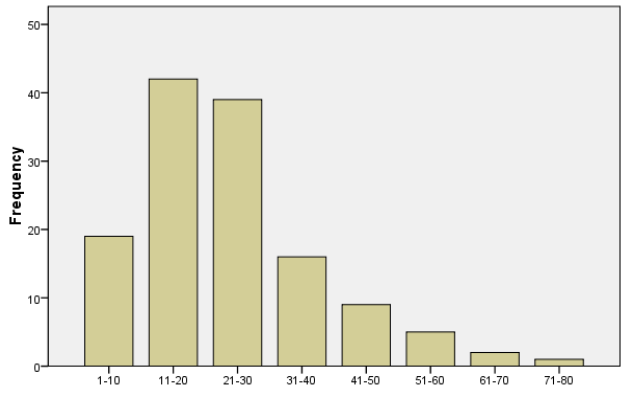

Figure 1: Plasmodial infestation among different age groups (n=125)

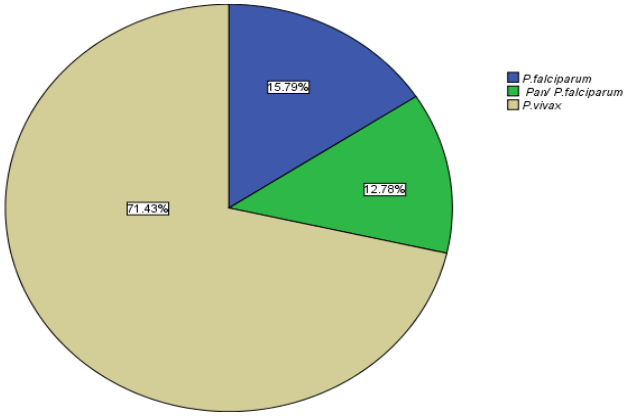

Figure 2: Results of Immunochromatographic technique

Discussion

Diagnosis of malaria based on clinical judgement is the least expensive and the most common practice to treat malaria in our clinical settings. However, the overlapping of malaria symptoms with other diseases impairs its specificity and promotes the indiscriminate use of antimalarial drugs for managing febrile conditions. Moreover, over diagnosis of malaria implies inappropriate treatment of self-limiting viral infections and potentially fatal acute respiratory infections and meningitis. The progress in developing new simple, speedy and accurate tests for *Plasmodiasis* detection is of utmost importance.[18] The rapid tests are also of paramount value in the diagnosis of severe, complicated falciparum malaria and in those cases who have taken antimalarials. Rapid diagnostic tests for malaria have the potential to extend accurate malaria diagnosis in areas where reliable microscopy services cannot be readily provided. Even a non-clinical staff can easily learn to perform and interpret the result of this test. Provision of RDT kits to the health care units for early diagnosis and treatment has resulted in a 2-fold reduction in antimalarial prescription.[19] Despite of increased per test cost of the ICT device as compare to the cost of microscopy, it serves as a reliable diagnostic tool for early detection of disease. About 200 devices are currently in market for use and international organization have tested the efficacies of some devices.[20] Various national and international studies revealed comparable results of our study in terms of sensitivity (range 92-100%) and specificity (range 90-99%).[17,21,22]

In our study, malarial infestation was reported more in male and young age group. Winskill et al. showed an equal preponderance of gender and older age children being more at risk of malaria.[23] The study concluded that ICT had high NPV higher PPV and FP result which was in range between 1% and 2% is in agreement with other studies.[24] Circulating rheumatoid factors and previous recent infections with malaria had been associated with FP results of ICT.[25,26] The persistence of PfHRP2 in the blood of

patients for long periods after clearance of parasites leads to FP results, as determined by microscopy of blood smears.[27,28] In addition, if patients have undertaken self medication prior to presentation, then FN results can occur by even microscopy. This practice of self-medication by patients is very common in our setups and is usually under reported.[29] Reyburn H et al have reported FN results in Tanzanian children with high parasitemia and this could be due to 'flooding' of RDT capture sites.[30]

Our results are in concordance with the results of the study conducted in Karachi by Mahadev S. Harani et al.[17] However a study conducted in Equatorial Guinea by Portero et al reported a sensitivity and specificity of 81.5% and 81.9% respectively for the detection of plasmodia species as compared to microscopy.[31] Certain factors such as target population, local epidemiology and variation in ICT kits influence the reliability parameters of RDT. Furthermore, degradation of these kits due to adverse storage and transport conditions also results in inaccurate interpretations. A lot of effort has been made in recent past to find substitute for the conventional microscopy. Methods such as fluorescent microscopy and flow cytometry are expensive and not suitable for routine clinical laboratory use. Polymerase chain reaction and loop mediated isothermal amplification are expensive and require specialized techniques and equipment. Our findings are parallel with previous reports suggesting reliability of RDTs for malaria parasite detection. However, RDTs have limitations as they are costly as compared to microscopy, have limitation in species identification and give positive result of HRP2 test even after effective treatment.

Conclusion

Blood smear microscopy for parasitological confirmation of suspected malaria is still a 'gold standard' technique but is cumbersome and requires a well-trained and skilled microscopist, good microscope and quality reagents. RDT is quick and easy to perform and can be carried out by relatively unskilled staff with minimal training. RDTs can reduce the time required for the result to initiate appropriate therapy especially for P.falciparum infection. The sensitivity and specificity of RDTs is around 90% relative to standard microscopy and thus can be used as useful diagnostic tool for malaria diagnosis.

Conflict of Interest

This study has no conflict of interest to declare by any author.

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