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Comparison of traditional and modern methods for identification of *Plasmodium* species

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Abstract

If we think about one of the dreaded endemic in the last century, undoubtedly Malaria name comes at the top. In case of infectious disorders, it is very well established fact that earlier the diagnosis of infectious disease, lesser are the chance to spread of such a disease in public domain and subsequently lesser the cascading effect. Still due to the limitations of testing a few no of cases by a clinical laboratory or research entity is also a big hinderance to give accurate results. There are many traditional methods for Plasmodium vivax identification and common in use are Giemsa staining (thick and thin) and Fluoroscence microscopic malarial diagnostic test. In spite of the fact that ibid mentioned methods are effective in diagnosis of Plasmodium vivax yet it was observed that both the methods in one or other way are time consuming and labour intensive. Therefore, one more method came into existence which has the potential to rapidly diagnose the parasite is known as Non microscopic Rapid Diagnostic test (RDT). RDT uses single step immunochromatography assay to identify the Plasmodium vivax. RDT is simple to execute, more convenient, provide fast diagnosis and less labour intensive as compared to the former two. Aim of the current work is to find out the authenticity, precision level of various techniques to identify the malarial parasite and find out which one is more economical, easy to operate and accurate. A total of 104 patients were taken in study who were suffering from malaria caused by Plasmodium vivax. Out of the them, 85 patients were positive by using technique of Flouroscence Microscopic test while a total of 94 patients were positive by using technique of Giemsa staining, On the other hand Non Microscopic Rapid Diagnostic test shown a total of 101 patients positive for malaria.

Keywords: Malaria, Plasmodium vivax, Giemsa stain, Fluorescence Microscopy (QBC), Rapid Diagnostic Test (RDT).

Introduction

Malaria is a life threatening mosquito born disease and is one of the major contributor for increasing health hazards problem in India and the world wide. Plasmodium parasite is one of the most common protozoan transmitted by anopheles mosquito and the causative agent for malaria. As per WHO statistics more than 3.8 billion population of the world is at risk from malaria. As per the World malaria report of 2018[1], in 2017 more than 219.4 million cases were reported in 91 countries worldwide. Deaths due to malaria were reported to an estimation of about half a million. India contributes 89% malarial cases in South East Asia.

A person suffering from malaria always gets fever but vice versa of it is not always true. Malaria is very much curable and preventable by implementing certain preventive measures, timely diagnosis and step by step full treatment. On one hand it is somehow appropriate to accept that in one or two institute it is difficult to test more than hundered cases. Moreover nonproficient staff on microscopy and other diagnostic technique as well as unability to detect the low level parasitemia are few important reason which contributes to the inaccurate diagnosis of malaria which later on having cascade effect and on subsequent diagnosis of such inaccurate cases also contribute to increased no. of mortality, morbidity and huge economical impact which is highly preventable. Therefore to avoid such cases, Rapid Diagnostic Tests (RDTs) proves to be one of the more accurate, time as well as man power saving, economical and easy to operate technique which screen infectious diseases even in resource limited settings or remote locations where traditional techniques are very few or not exist.

The diagnosis of malaria is usually based on a history or presence of fever and dependent upon the demonstration of malarial parasite in the peripheral blood films. The presumptive treatment of fever result in our administration of antimalarial drugs thus reliable diagnosis of malaria is necessary. The aim of this endeavour is to find out the accuracy and authenticity of current method of detecting the presence and identification of the malaria parasite. The present study is carried out on 104 patients suffering from malaria caused by *Plasmodium vivax.* Meerut region is dominated by *vivax* species of *Plasmodium*. While studying the changing pattern in the symptoms of *vivax* malaria it was found that some patients (who were recommended by doctors) were not found positive in above mentioned three methods which was used for the diagnosis of malarial parasite. The current work is focused on the specificity and sensitivity of the various techniques used for the identification of malarial parasite.

In the current work, professional and trained pathologist as recommended by doctors took out the blood sample from the patient whose verbal details were taken at multispecialty hospitals of Meerut. A questionnaire was prepared for the symptoms and the pathological confirmation of the malaria parasite was also done in our research lab.

Materials and methods

RDT (Rapid diagnostic test): The suspected malaria patients were diagnosed by Rapid diagnostic test[2,3] for malaria. It is a rapid coloured immunochromatography assay for qualitative determination of antibodies of malarial parasites (p. f. /p. v.) in human blood. Nitro cellulose membrane of the testing device is immobilized with very specified recombinant antigens (Merozoite Surface Protein: MSP) of Pf at test line zone 1 and recombinant antigen (MSP) of Pv at test line zone 2. Human blood containing malarial antibodies is allowed to react with recombinant Pf/ Pv antigens coupled gold conjugate followed by reaction with recombinant antigens immobilized at test line zones. Appearance of pink /purple clear line in result window at test line zone (1&2) in addition to pink /Purple line "C" at control line zone, indicates positive test result. Presence of pink/ purple line at control line "C" validates the procedure. Non presence of a band in the testing zone recommend not a positive result. Visibility of pink/ purple line at "C" and 1 indicates *Plasmodium falciparum* infection and appearance of purple/pink line at "C" and 2 demonstrates *Plasmodium vivax* infection while the presence of pink/ purple line at "C" 1 and 2 indicates mixed infection with *Plasmodium vivax* and *Plasmodium falciparum*[4].

Giemsa stain: This traditional technique is applied to demarcate cytoplasmic and nuclear morphology of RBCs, WBCs, platelets and parasites. One of the most reliable stain for blood parasites especially in thick films is Giemsa stain having azure B. Concentrated azure B stain must undergo dilution process for use with water buffered to an acceptable level of pH which ranges from 6.8 or 7.0–7.2. Sample or specimen generally comprise of fresh blood which is taken by prick in the finger or the other option in which whole fresh blood with EDTA and has to be taken by method of venipuncture and it is less than one hour old. Specimen slide may be a thick blood film that has kept to dry thoroughly and is not to be fixed and a thin blood film that has to be fixed in absolute methanol and allowed to dry[5].

Fluorescent microscopy: This technique is also been used in patients with antigen positivity. Fingertip or venipuncture is used to take the whole blood in the collection tube which already having EDTA anticoagulant. The blood is filled into acridine orange coated microcapillary. Fluorescent dye acridine orange has an attraction for the nucleic acid in nucleus of the parasite. The malaria parasite picks up fluorescent stain into their nucleus and cytoplasm, so that examination of its morphological characteristics can be done via fluorescent microscopy on given stimulus by UV light at suitable wavelength. Centrifugal quantitative buffy coat or QBC combines an acridine orange coated capillary tube resulting into the formation of an internal float. The float occupies the area in middle between red cells and plasma and settle at various levels in the capillaries[6].

Results and discussion

Species of malarial parasite were identified by using these above mentioned methods. A total of 104 patients were the base of the present study who were hospitalized in multispecialty hospitals of Meerut city from October 2013 to September 2015. (Table-1)

The present work was carried out with the aim of suitably comparing the various rapid methods for their efficiency in terms of accuracy, economical or ease for operation. Accuracy of clinical diagnosis varies with the level of malarial season, age group and with the level of endemicity. A single clinical algorithm cannot be a universal predictor[7,8].

One undisputed fact in case of sound treatment and bring down the level of mortality and morbidity in malaria is rapid diagnosis of the disease. The era of rapid diagnostic techniques has given tremendous advantages to public health which is not only limited to the ambit of better patient management where infections symptoms are not specific to a particular disease (asymptomatic disease), but also it is effective in terms of sound monitoring of outbreaks in high-risk endemic areas as well as widespread health care delivery by minimally trained technicians.

Gustav Giemsa[9] introduced a mixture of methylene blue and eosin stains which later on became the standard norms of malaria diagnosis by investigating Giemsa stained blood smears. In the current work, 94 out of 104 patients diagnosed positive for Giemsa stain in which low level of parasitemia may be one factor. Several researches indicates that the level of diagnosis of malaria by blood film investigation is as high as 75% [10,11]. Other probable reason for not showing positive results might be due to slide washing during staining which results in very few parasite of malaria for analysis and deduction.

In laboratory diagnosis of malaria, Giemsa-stained thick and thin films considered to be one of the most successful pillar in field of microscopy. Due to its ability to be less expensive to deliver, ability to demarcate the species of malaria and ability to even quantify parasites make Giemsa microscopy as the reliable diagnostic equipment for control of this dreaded diseases worldwide yet this technique has many cons which includes the requirement of highly trained professional staff, consistent maintenance of associated machinery as well as very high standard of quality assurance and quality control and later are the reasons that in spite of being mainstay in the diagnostic world this technique made it less attractive in majority of clinical institutions or laboratories.

Poor microscopy has long been recognized in practice and is a function of multiple factors, including training and skills maintenance, slide preparation techniques, workload, condition of the microscope, and quality of essential laboratory supplies. Even among local laboratories with similar equipment and equal training and among reputed experts, abilities vary significantly[12,13]. This variability combined with the risk of untreated malaria in the face of safe, inexpensive therapy in the past led clinicians to treat febrile patients without regard to the laboratory results[14,15]. A professional and well learnt staff should be able to identify different species of Plasmodium with precision in thick blood films at relatively low density of parasites. Many a times it may be required to investigate the thin film for various diagnostic parameters viz. colour, pigment structure, shape and size of erythrocyte, crenation, characteristic dots in the erythrocyte stroma, as well as schizonts and morphological features as well.

During the current research work, it was found that density of malaria parasite infected RBCs by centrifugation coupled with staining of dye acridine orange and Quantitative Buffy Coat method was found positive in 85 out of 104 malaria patients. Problem noticed while using these techniques is that few fluorescence structures looks similar as that of parasites[16].

One more important technique which came as a revolution to the detection system of malaria is non microscopic Rapid diagnostic test. RDT is a device that recognise malaria antigen in a miniscule quantity of blood, ranging from 5–15μL, with the help of immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. Within a short span of time of 5 to 15 minutes this technique provide results in form of a coloured test line. Out of many significant merit of this technique few include viz. does not need capital investment or electricity, smooth in execution and easy to analyse and interpret. Accuracy of rapid diagnostic test is considered to be a useful diagnostic; Rapid Diagnostic Test must achieve more than even 95% sensitivity[17]. Rapid diagnostic test seems to be most reliable and easy technique for the presence and identification of the malaria parasite. In the present study, 101 out of 104 patients showed positive results by this technique. It can even be done by non trained personal without even any laboratory. Notwithstanding the above, in case of suspected severe and multi resistance cases of malaria, laboratory confirmation might be useful (WHO). Many of the malaria smears are being performed in areas of low to moderate transmission like in India and Brazil and this technique is being used in several countries in various special situations. Contrary to the fact that inspite of having qualified and trained staff in reputed clinics and laboratories both in Government or semi government institutes (for e.g. in country like India where on an average, 100 million blood films are being examined on yearly basis, as per WHO report), research from endemic countries like South Africa and India have shown that errors or incorrect clinical diagnosis is quite common[18]. Probable credit for the same might goes to imbalance between huge burden of work and limited trained professionals.

Table-1: List of patients observed.

Age/Yrs	Sex	Giemsa stain	RDT	Fluorescence Microscopy
18	M	Positive	Positive	Positive
47	M	Positive	Positive	Positive
60	F	Positive	Positive	Negative
34	M	Positive	Positive	Positive
26	F	Positive	Positive	Positive
15	M	Positive	Positive	Positive
54	M	Positive	Positive	Positive
24	M	Positive	Positive	Positive
56	F	Positive	Positive	Positive
16	M	Positive	Positive	Negative
19	M	Positive	Positive	Positive
34	M	Positive	Positive	Positive
65	M	Positive	Positive	Positive
20	F	Positive	Positive	Negative

56	M	Positive	Positive	Positive
21	F	Negative	Positive	Positive
67	M	Positive	Positive	Positive
40	M	Positive	Negative	Positive
48	M	Positive	Positive	Positive
60	M	Positive	Positive	Negative
28	F	Positive	Positive	Positive
20	M	Positive	Positive	Positive
25	M	Positive	Positive	Positive
34	F	Positive	Positive	Positive
36	F	Negative	Positive	Negative
35	M	Positive	Negative	Positive
54	M	Positive	Positive	Positive
23	F	Negative	Positive	Negative
22	M	Positive	Positive	Positive
75	F	Positive	Positive	positive
13	F	Positive	Positive	Positive
45	M	Negative	Positive	Positive
25	M	Positive	Positive	Positive
32	M	Positive	Positive	Positive
40	M	Positive	Positive	Negative
21	M	Negative	Positive	Positive
38	F	Positive	Positive	Positive
48	M	Positive	Positive	positive
58	M	Positive	Positive	Positive
45	F	Positive	Positive	Positive
25	F	Positive	Positive	Positive
34	F	Positive	Positive	Negative
30	M	Positive	Positive	Negative
49	M	Positive	Positive	Positive
58	F	positive	Positive	Positive
22	M	Positive	Positive	Positive
34	M	Positive	Positive	Positive

35	M	Positive	Positive	Positive
32	F	Positive	Positive	Negative
20	M	Negative	Positive	Positive
24	M	Positive	Positive	Positive
26	F	Positive	Positive	Positive
41	M	Positive	Positive	positive
26	F	Positive	Positive	Positive
30	M	Positive	Positive	Positive
48	M	positive	Positive	Negative
18	M	Positive	Positive	Positive
43	F	Positive	Positive	Positive
15	M	positive	Positive	Positive
44	F	Positive	Positive	Positive
55	M	Positive	Positive	Negative
46	F	Positive	Positive	Positive
32	F	Positive	Positive	Positive
43	M	Positive	Positive	Positive
21	M	Positive	Positive	Negative
30	M	Positive	Positive	Positive
35	M	Positive	Positive	Positive
41	F	Positive	Positive	Negative
12	M	Negative	Positive	Positive
42	F	Positive	Positive	Positive
28	M	Positive	Positive	positive
37	F	Positive	Positive	Positive
43	M	Positive	Positive	Positive
39	F	Positive	Positive	Positive
18	M	Positive	Positive	Positive
40	F	Positive	Positive	Positive
62	F	Positive	Positive	Negative
50	F	Positive	Positive	Positive
42	M	Positive	Positive	Positive
48	M	Positive	Positive	Positive

36	M	Negative	Positive	Positive
22	M	Positive	Negative	positive
26	M	Positive	Positive	Negative
10	F	Positive	Positive	Positive
45	M	Positive	Positive	Positive
45	M	Positive	Positive	Negative
27	M	Positive	Positive	Positive
22	M	Positive	Positive	Positive
16	F	Negative	Positive	Positive
23	M	Positive	Positive	Negative
29	M	Positive	Positive	Positive
16	M	Positive	Positive	Positive
25	M	Positive	Positive	positive
36	M	Positive	Positive	Positive
25	F	Positive	Positive	Positive
20	M	Negative	Positive	Positive
15	F	Positive	Positive	Positive
34	F	Positive	Positive	positive
37	M	Positive	Positive	Positive
62	M	Positive	Positive	Positive
30	M	Positive	Positive	Positive
48	F	Positive	Positive	Positive
70	M	Positive	Positive	Positive
30	M	Positive	Positive	Negative

RDT-Rapid Diagnostic Test, M - Male ,F-Female

Conclusion

If we see in developed nations, RDTs might be profitable in quarantine febrile travelers/returnees from endemic areas[19-21]. In case of auto use by travelers, however, produces different results[20,21]. RDT is also highly advisable in conditions where other traditional methods viz. Giemsa staining or Fluorescent microscopy are not suitably available, such as in an outbreak circumstances or in occupationally exposed groups. Current RDTs are not intended to replace microscopy. Moreover a detailed and comprehensive planning is required at grass root level for successful implementation of Rapid Diagnostic Techniques. Application of Rapid diagnostic techniques at peripheral levels such as by health workers, in informal health sectors and for self-diagnosis/self-treatment is a challenge[24]. Implementation requires new local-level algorithms for actions to be taken based on RDT results. Quality RDT is a valuable complement to microscopy because it helps to expand the coverage of parasite-based diagnosis to the periphery and minimize exclusively clinical diagnosis.

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