Journal of Infection and Public Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

LED fluorescence microscopy: Novel method for malaria diagnosis compared with routine methods

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ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form
16 November 2016
Accepted 7 January 2017

Keywords:
Malaria
Diagnosis
Light microscopy
LED fluorescence microscopy
Rapid diagnostic test

ABSTRACT

Rapid and accurate diagnosis of malaria is the need of hour for effective management and controlling drug resistance. The conventional and gold-standard method, Light microscopy (LM), is time-consuming, requires trained staff and well-maintained equipments. The newly developed, rapid diagnostic tests (RDT) are fast and reliable, but give only qualitative results, are expensive and have short shelf life. Light Emission Diode fluorescence microscopy (LED FM) may provide a reliable alternative which can be used for routine diagnosis. In order to assess the effectiveness of LED fluorescence microscopy in malaria diagnosis, a cross-sectional study was conducted at a tertiary care teaching hospital in Mumbai. 2–3 ml of blood of 300 patients, who were clinically suspected of having malaria but were not on anti-malarial treatment, was collected in EDTA vials. These specimens were processed to diagnose malaria by three methods, namely—Peripheral smear examination with LM, Peripheral smear examination with LED FM and RDT. The results of all the 3 tests were compared, taking Light Microscopy as the gold standard method. Of the 300 specimens, LM, LED FM and RDT reported 111 (37%), 86 (28.67%) and 107 (35.67%), respectively, as positive. The sensitivity and specificity were respectively 71.2% and 96.3% for LED FM and 91% and 96.8% for RDT. Of the LM positive cases, 53 (47.75%) had parasitic index (PI) <1% and 58 (52.25%) had PI ≥1%. LED FM was found to be only moderately sensitive but highly specific in comparison to Light microscopy. In order to improve the performance of this technique, more precise training in fluorescence staining and reading of the slides, will be required.

Introduction

Malaria is an overwhelming problem in developing countries in tropical region. About 3.2 billion people are at risk of malaria, especially in sub-Saharan Africa, South East Asia and Latin America regions. In 2015 alone, there were about 214 million new cases of malaria and 438,000 deaths. India is amongst the worst affected countries where malaria is one of the most common parasitic infections [1,2].

The global increase in cases of malaria in recent times can be attributed to factors like increasing transmission risk in areas where malaria control has declined, increasing prevalence of drug resistant strains of parasites and insecticide resistant strains of mosquitoes and in a relatively few cases, massive increase in international travel and migration [3]. Presentation with atypical manifestations and complications is intensified [1]. The impact of malaria can be seen globally on both extremes of health-care system; in developing nations, scarce resources lead to inadequate diagnostic procedures, while in affluent countries, poor familiarity with malaria may cause clinical and laboratory misdiagnosis [4,5]. Dearth of rapid and accurate diagnostic method leads to presumptive treatment, which is a factor in development of drug resistance [6]. Hence, there is an increasing need for effective and practical diagnostics for control of malaria globally, since effective diagnosis reduces both complications and mortality from malaria [7]. A key feature of the new malaria control strategy of WHO is rapid diagnosis of malaria, which is available to all those who need it, especially at village level [8].

The clinical presumptive diagnosis cannot be fully relied upon as presentation of malaria is extremely non-specific and mimics a variety of other clinical conditions [9–11]. The gold standard, conventional peripheral blood smear examination is inexpensive, but requires trained staff, well-maintained equipments, a regular supply of reliable reagents, clean water and electricity and a well-executed quality management system; also it is time consuming and may give poor results in cases with low parasitaemia [12,13].

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http://dx.doi.org/10.1016/j.jiph.2017.01.001

The rapid diagnostic tests for antigen detection give only qualitative results, are comparatively expensive and have a short shelf life [14]. They cannot be used to monitor therapeutic response [1]. Moreover, they are unable to detect various species and stages of malarial parasite [15,16]. They also have low sensitivity at low parasitaemia [11].

Considering the problems of atypical clinical presentations, rapid emergence of resistance and expensive drugs; a rapid, easy and reliable tool for malaria diagnosis is required. A fluorescent microscope based on LED of one wavelength can be used as an alternative. Such microscopes have been recently approved by WHO for rapid diagnosis of Tuberculosis [17]. It is a very useful tool in field-settings as the LED microscope consumes less energy, is long-lasting and has brighter view. Additionally, it offers battery operation when no electricity is available. The potential benefits of LED microscope in malaria diagnosis are not yet revealed completely. A little work done in this field reveals its sensitivity and specificity to be 99% and 92%, respectively [14]. A fluorescent dye, Acridine Orange, which stains DNA and RNA instantly, is used for staining in this method [14].

Keeping all the above facts in mind, the present study was carried out in the Department of Microbiology of a tertiary care teaching hospital, to compare the sensitivity, specificity and predictive values of Light microscopy, LED fluorescence microscopy and Antigen detection test for malaria diagnosis.

Methods

After obtaining approval from Institutional Ethics Committee (Permission number-EC/153/2011, Dated-3rd January, 2012), a cross-sectional study was carried out in monsoon season at a tertiary care teaching hospital in Mumbai—an area of perennial malaria transmission. Blood specimen was collected from 300 clinically suspected cases of malaria who attended the fever out-patient department (OPD) of the hospital. Only patients who were not on anti-malarial treatment and were ready to give informed written consent were included in the study. 2–3 ml of blood was collected in an Ethylene Diamine Tetra Acetic acid (EDTA) vial, which was processed for the three tests:-

i) Peripheral smear examination with Light microscopy (LM)
ii) Peripheral smear examination with LED fluorescence microscopy (LED FM)
iii) Rapid diagnostic test for malaria antigen detection (RDT)

For Light microscopy, the thick and thin smears were stained using Leishman's stain solution and were examined under 1000× magnification and identified according to WHO guidelines [18,19]. For LED fluorescence microscopy, the procedure was carried out as described by Gay et al. [20]. The methanol-fixed smears were stained with Acridine orange solution (20 μl per ml of solution) for 1–2 min and covered with cover slip for examination under 1000× magnification of Olympus CX21*i* microscope attached with

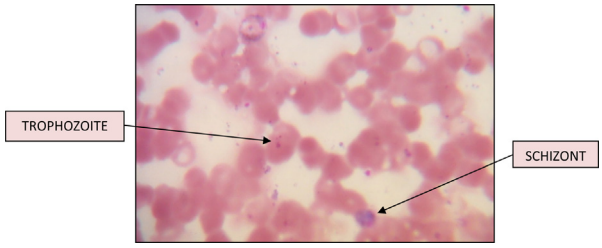

Fig. 1. Leishman stained Blood-smear as seen under 1000× oil immersion lens of Light microscope showing trophozoite and schizont of *Plasmodium vivax*.

Magnus MicroLED fluorescence illuminator. Malaria was confirmed by viewing the fluorescent green staining of DNA and red-orange staining of cytoplasmic RNA of *Plasmodium* within non-nucleated RBCs [20]. At least 100 oil immersion fields were examined before reporting a slide as negative by both the microscopy methods. Each slide was examined by two independent observers and the mean of their results was recorded as the final result. In case of a discrepancy in the reading, the reading was repeated by a third independent observer, where the mean of the two closest of the three results was considered final for that smear. Detection by LED FM was standardized and competency assessed with known LM smear positive and negative specimens. Also 20 samples (10 each of positive and negatives) were run in triplicate and results read by three independent observers before being put to use. All observers were trained prior to reporting LED microscopy. The observers were blinded from the results of other readers. Average time required by the observers was recorded. Turnaround time (TAT) was defined as time elapsed between specimen check-in and reporting of results.

For both the methods, Parasitaemia (Parasitic Index) (PI) was assessed by counting the number of parasite-infected RBCs for every 100 RBCs seen in the thin blood film. If occasional parasites were seen while scanning the thick smear, but none were identified during the process of counting 300–500 RBCs in thin smear, a parasitic index of less than 1% of RBCs was assigned.

Rapid Malarial Antigen detection test was done using SD BIOLINE Malaria Antigen *P.f*/*P.v* test (Standard Diagnostics Pvt. Ltd., Gurgaon, Haryana, India). It is an immuno-chromatographic test, based on detection of monoclonal antibodies specific to HRP-II (histidine rich protein II) of *Plasmodium falciparum* and to pLDH (*plasmodium* lactate dehydrogenase) of *Plasmodium vivax*. A positive result was interpreted in form of coloured band/s, seen after about 15–20 min but not later than 20 min (Figs. 1 and 2).

The results of Leishman's stained Peripheral smear examination were considered as the gold standard. Data was entered into Statistical Package for Social Sciences (SPSS) software version 16. Sensitivity, specificity, positive predictive value and negative predictive value were calculated as per standard formulae. Chi-square test was used to compare results among different categorical variables. A p-(predictive) value of <0.05 was considered as statistically significant.

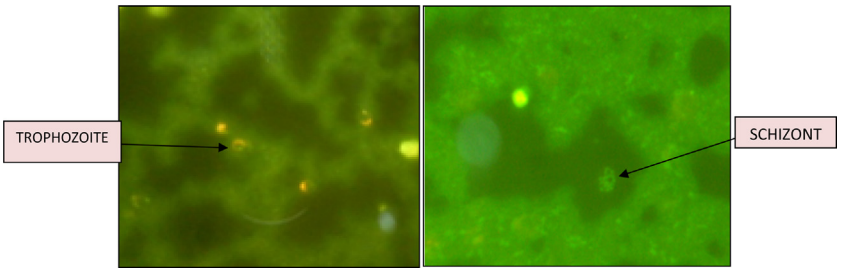

Fig. 2. Acridine Orange stained Blood-smear as seen under 1000× oil immersion lens of LED fluorescent microscope showing trophozoite and schizont of *Plasmodium vivax*.

Please cite this article in press as: Hathiwala R, et al. LED fluorescence microscopy: Novel method for malaria diagnosis compared with routine methods. J Infect Public Health (2017), http://dx.doi.org/10.1016/j.jiph.2017.01.001