Original Article

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Evaluation of Different Diagnostic Techniques for Malaria in a Tertiary Health Care Centre, Karnataka

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Abstract

Context: Malaria is endemic in tropics and subtropics with India contributing to 75% of the cases in South East Region. The early diagnosis of malaria is essential to treat patients and to prevent complications especially in cerebral malaria. *Aims:* To evaluate the diagnostic accuracy of different techniques available like Peripheral smear, Quantitative Buffy coat (QBC), and Rapid Diagnostic tests (RDT) in the diagnosis of malaria. *Settings and Design:* Prospective study for duration of one year. *Methods and Material:* Blood samples from all clinically suspected cases of malaria were routinely subjected to peripheral smear examination, QBC & RDT for the presence of malaria parasite. *Statistical analysis used:* Sensitivity, Specificity, Positive predictive value and Negative predictive value were analyzed using standard formulae. *Results:* Sensitivity, specificity, Positive Predictive value and Negative predictive values were 100%, 99.6%, 97% and 100% for QBC and 100%, 99.2%, 95% and 100% for RDT respectively. *Conclusions:* RDTs are equally or more sensitive and specific than peripheral smear and QBC. Newer Pf /Pv specific antigen card can distinguish mixed and PF infections unlike old Pf/ Pan RDTs. However further studies are required to assess cost effectiveness and efficiency of different RDTs.

Keywords: Malaria Diagnosis; Rapid Diagnostic Test; QBC.

Introduction

Malaria is a major health problem globally with 106 malaria endemic countries and more than 50% of world's population is at risk [1]. India, Bangladesh, Indonesia and Myanmar countries contributes 97% of all malaria cases in the South East Asia region. In India it was known as 'king of diseases' from the ancient times and approximately affects 75 million people annually [2].

India launched National Malaria control programme in 1953 and malaria eradication programme in 1958, despite varies efforts, malaria is still not been eradicated from India. One of the reasons could be presumptive diagnosis and empirical treatment leading to drug resistance. Accurate Diagnosis of malaria is a key strategy in controlling and eradicating malaria. There are various methods for diagnosis of malaria each with its own disadvantages and advantages. In a recent policy WHO recommends parasite diagnosis is a must before antimalarial chemotherapy and to avoid presumptive treatment as for as possible [3,4]. Knowing the significance and benefits of accurate diagnosis of malaria, this study was conducted to evaluate the efficiency of different techniques in diagnosis of malaria.

Materials and Methods

This study was conducted in a tertiary health care centre in Karnataka for duration of one year. All blood samples from clinically suspected case of malaria were tested by routine peripheral smear examination for malarial parasite, Quantitative buffy coat (QBC)examination under fluorescence microscope and Rapid diagnostic tests (RDT) that is Pf /Pv

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specific antigen card test.

Blood samples were collected in EDTA vacutainers tube. Peripheral smears were made and stained with Leishman stain. Smears were thoroughly examined under oil immersion for 10 – 15 minutes, nearly 100 oil fields before labeling as malaria negative.

For QBC, about 75‡l of blood was aspirated in capillary tube coated with acridine orange, a small float was inserted, capped and centrifuged for 10 minutes at 10000 rpm. Buffy coat was examined under customized fluorescent microscope. 20 -25 fields were examined for 2-5 minutes before labeling as malaria negative (Figure 1a).

For RDT, Antigen based Pf (HRP-II) and PV (pLDH) specific card were used. About 5 ‡l of blood was put in sample well with the help of disposable loop provided with the kit. 4drops of assay diluent provided with the kit was added. Result was interpreted after 15 -20 minutes. When only control band appeared with two negative test bands, test was considered negative , when control band and Pf bands appeared with negative Pv band, test was positive for Plasmodium falciparum and vice versa. When all three bands appeared, test was considered as mixed infection with P Falciparum and Vivax. (Figure 1b & Figure 1c) Sensitivity, specificity, Positive Predictive value and Negative predictive value were calculated considering Peripheral smear diagnosis as gold standard.

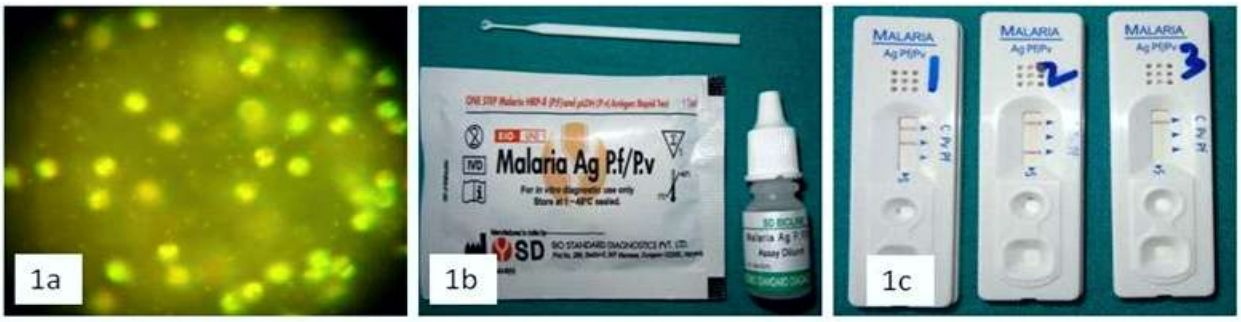

Fig. 1: a. QBC showing P.Vivax, **1b.** RDT kit with Diluent and loop, **1c.** RDT interpretation

Results

During the study period a total of 1485 samples were received in the pathology laboratory with a clinical suspicion of malaria. 185 samples were positive for malarial parasite(MP) on peripheral smear examination of which 180 were positive for Plasmodium Vivax, 4 were positive for plasmodium Falciparum and 1 was mixed infection. 189 samples were positive with QBC technique, of which 187 were Plasmodium vivax and two were P Falciparum. With RDTs 195 samples were positive including 170 Plasmodium vivax, 10 Falciparum and 15 mixed infections (Table 1).

Sensitivity, specificity, Positive Predictive value and Negative predictive values were 100%, 99.6%, 97% and 100% for QBC and 100%, 99.2%, 95% and 100% for RDT respectively.

Table 1: Comparison of Peripheral smear, QBC and RDT tests

Result	Peripheral smear	QBC	RDT
Positive PV	180	187	170
PF	04	02	10
Mixed	01	00	15
Negative	1300	1296	1290
Total	1485	1485	1485

QBC Quantitative Buffy Coat, RDT Rapid Diagnostic Test, PV Plasmodium Vivax, PF Plasmodium Falciparum

Discussion

Accurate diagnosis of malaria is imperative for rational therapy and to reduce drug resistance. Malaria is diagnosed on clinical symptoms and laboratory tests for malarial parasite. Laboratory tests commonly used are peripheral smear examination, QBC and RDT antigen tests [5].

Most of the time malaria is treated empirically without confirmation by laboratory tests, however this is not advisable as other diseases causing fever can be missed and the possibility of treating false positive cases with antimalarials becomes high. In the present

study clinical accuracy was found to be 12-13%, out of 1450 clinically suspected cases, only 195 cases were positive for malaria on laboratory tests. In a study conducted by Gandhi AM [6] 67.86% of cases showed negative peripheral smear who were treated empirically with choloquine.

Previous studies have shown sensitivity and specificity ranging from 84 to 100% for QBC and RDT [7,8,9,]. In the present study we found 100% and 99% respectively. The increased specificity of RDT may be because PF/PV specific antigen card is used instead of Pf/Pan card. Compared to Peripheral smear and QBC, RDTs are more sensitive and specific for diagnosis of P Falciparum and mixed infections. This is important because Falciparum causes severe disease and has high mortality and P. Vivax needs to be treated with primaquine to prevent relapses of malaria.

RDT is simple to perform, any non technical person can be trained, does not require electricity, microscope, however may be more expensive than peripheral smears. Another drawback of RDTs is that it can be positive for 7-14 days after treatment and hence cannot be used to assess response to treatment [10].

QBC is also equally sensitive and specific, but has disadvantages of poor performance in identifying the species, requirement of costly instruments and skilled worker to report. Peripheral smear though inexpensive of the two is laborious to perform, less sensitive, requires electricity, instruments and skilled technician to interpret. Results depend on quality of the smears [11].

Conclusion

Peripheral smears are considered to be gold standard for diagnosis of malaria. RDTs are equally or more sensitive than peripheral smear and QBC. Newer Pf /Pv specific antigen card can distinguish mixed and PF infections unlike old Pf/Pan RDTs. However further studies are required to assess cost effectiveness and efficiency of different RDTs.

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