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Comparison of different diagnostic techniques in *Plasmodium falciparum* cerebral malaria

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Abstract

Background & objectives: Plasmodium falciparum cerebral malaria remains a major health problem in India. The efficacy of treatment of cerebral malaria lies in its early diagnosis through rapid diagnostic methods. ParaSights-F test detects HRP-2 antigen secreted by parasitised red blood cells and quantitative buffy coat assay (QBC) is examination of buffy coat for the presence of malarial parasite stained with acridine orange. This study was performed to evaluate the effectiveness of ParaSight-F test and QBC assay as diagnostic methods in the patients of cerebral malaria.

Methods: Fifty clinically diagnosed patients of cerebral malaria were included in the study. ParaSight-F test, QBC and conventional blood smear examination was done. Patients who were in coma and there were no obvious features of bacterial or viral etiology were investigated for cerebral malaria by these diagnostic methods.

Results: ParaSight-F test, QBC and peripheral blood smears were examined. Patients were followed-up for signs of clinical recovery. ParaSight-F test was positive in 47 patients, QBC in 46 while blood smear examination was positive in 28 cases.

Interpretation & conclusion: Sensitivity and specificity of ParaSight-F test were found to be 96.6 and 94% while QBC showed 97.8 and 100% respectively. ParaSight-F test and QBC were found to be novel methods for diagnosis of cerebral malaria especially in the cases where diagnosis can not be made by conventional blood smear examination due to low parasitaemia. These rapid diagnostic methods help in early therapeutic intervention.

Key words Cerebral malaria – ParaSight-F test – QBC

Introduction

Cerebral malaria is an important cause of mortality and morbidity associated with malaria. Early diagnosis and treatment is vital. Microscopic examination of blood smear is the standard method for malaria diagnosis. The method is easily available and has low cost but its reliability is questionable at low level of parasitaemia[1]. Devices for rapid diagnosis of malaria emerged to meet the need as reliable diagnostic adjunct to microscopy in clinical settings. ParaSight-F test and QBC assay are such diagnostic methods. QBC assay involves direct observation of centrifuged blood in capillary tube, stained by acridine orange. ParaSight-F test is antigen detection in dipstick format in which a monoclonal antibody captures a specific antigen of *Plasmodium falciparum* (PfHRP-2, present in the parasite throughout the erythrocytic cycle) if antigen is present, a positive result is indicated by a second anti-HRP-2 antibody labeled with

coloured marker, which produces visible line on dipstick[2,3].

The present study is done to compare QBC, ParaSight-F test with conventional peripheral blood smear examination for diagnosis of cerebral malaria.

Material & Methods

The study was conducted on patients admitted in the paediatric and medicine wards of JN Medical College Hospital, Aligarh, India who were clinically diagnosed as cases of cerebral malaria from July 2003 to July 2004. A detailed clinical history regarding the duration of fever, its nature and associated symptoms was taken from each patient. All these patients were of acute febrile illness and had no obvious focus of bacterial, viral or fungal infection. All 32 patients included in the study had a history of intake of antimalarial drugs and were followed-up for signs of clinical recovery by monitoring the level of consciousness, disappearance of signs of meningeal irritation.

Study group: Comprised of 50 patients clinically diagnosed as cases of cerebral malaria, who were in coma, and associated symptoms and signs were highly suggestive of *P. falciparum* infection and their CSF cultures were negative for any bacterial infection.

Control group 1: Comprised of 10 patients whose blood smears were positive for *P. vivax* to rule out cross-reactivity in ParaSight-F test.

Control group 2: Comprised of 10 healthy individuals with no history of fever or intake of antimalarial drugs for last three months.

Thick and thin blood films were made on clean glass slides by pricking the finger. Leishman staining was performed[4,5]. For ParaSight-F test a finger prick blood sample was collected in a standardised 50 µl heparinised blood capillary tube and transferred to a dispensing tube containing haemolysing agent. One drop of haemolysed blood was transferred from dispensing tube into a well on plastic plate provided with a kit. The dipstick was placed vertically into the haemolysed blood sample and the sample was rapidly taken up by dipstick through capillary action. One drop of detection reagent containing a rabbit polyclonal antibody raised against Pf HRP-II labeled with sulforhodamine B, was added. Once absorption was complete, one or two drops of washing reagent was added to clear the haemolysed blood. If blood sample was positive for *P. falciparum* a pink line developed almost simultaneously at monoclonal deposit site and a pink broken line above it as reagent control. In negative cases, only the pink broken line appeared. A single test took about 7 min to perform.

QBC was performed by pricking a finger and filling QBC tube with 55 µl blood stopper and float was placed at either end of the tube and then centrifused at 12,000 rpm for 5 min. The QBC tube after centrifugation is placed in paraviewer and examined using a standard microscope equipped with the Paralens ultraviolet microscope adaptor and a 60 x objective connected to fibre optics ultraviolet light module. Parasites were observed in buffy coat and a interface at red blood cells and white blood cells. Cytoplasm of parasite appears red and nuclear chromatin appeared green.

To compare leishman stained blood smear examination with ParaSight-F test and QBC, Z-test was applied for statistical analysis. Different diagnostic tests performed for the diagnosis of *P. falciparum* infections, we have applied Z-test for statistical analysis:

$$Z = \frac{p_1 - p_2}{\sqrt{pq\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Results

In study group Leishman stained blood smear exam-

ination showed different stages of *P. falciparum* in 28 patients. ParaSight-F test was positive in 46 patients and QBC in 47 patients (Table 1).

On comparing ParaSight-F test with Leishman stained blood smear examination ($Z=3.9$, $p < 0.001$) which is statistically significant. Comparison of QBC with peripheral blood smear examination ($Z=4$, $p<0.001$) which is also statistically significant.

Sensitivity, specificity, positive and negative predictive values of ParaSight-F test were 96.6, 94, 100 and 98%, respectively whereas that of QBC were 97.8, 100, 100 and 75%, respectively.

Table 1. Showing comparison of ParaSight-F test and QBC with blood smear examination

Diagnostic test	Cases positive for *P. falciparum* study group n = 50
Leishman stained blood smear examination	28 (56%)
QBC	47 (94%)
ParaSight-F test	46 (92%)

Table 2. Comparative evaluation of various tests for diagnosis of cerebral malaria

Test	Cost (Rs.)	Technical experience	Equipment and facilities needed	Time
ParaSight-F test	150.00	None	Kit based no equipment required	10 min
QBC	45.00	Needs lot of practice	Laboratory QBC kit fluorescent objective centrifuge electricity	10 min
Leishman stained blood smear	1–2/test	Moderate amount of practice and training is required	Laboratory electricity light microscope	35–40 min

Table 2 shows comparison of different diagnostic techniques used in relation to cost technical expertise, equipment, facilities and time required for the diagnosis.

Discussion

The majority of cases of malaria worldwide are treated on the basis of clinical diagnosis and microscopy. Although microscopic examination of blood smear continues to be the gold standard, it has a drawback that it is time consuming and requires an expert microscopist and results are poor in cases of low parasitaemia. Several studies have shown that the ability to diagnose malaria by blood film examination alone is about 75% for *P. falciparum*[6,7].

In this study we have used ParaSight-F test and QBC along with Leishman stained blood smear examination. Out of 50 patients who were clinically diagnosed as cases of cerebral malaria only 28 patients were found positive for *P. falciparum* by microscopy, blood smear may be reported negative due to low parasitaemia, especially in partially or improperly treated cases. Out of these 50 patients, 28 patients (56%) were positive by Leishman stained blood smear examination for various stages of *P. falciparum*. This shows 44% of patients could not be diagnosed by this conventional method.

Our results are in agreement with results reported by Lema *et al*[13], who compared Para Sight-F test and conventional microscopy. Other workers in their respective studies showed high sensitivity, specificity, positive and negative predictive values of ParaSight-F test [3,9–12].

The sensitivity and specificity of ParaSight-F test were found to be 95.74 and 100% respectively. QBC was positive in 47 patients out of 50 patients show-

ing high sensitivity and specificity. Parzy *et al*[13] found QBC to be more sensitive than blood smear examination and advocated its use for urgent diagnosis[13].

On comparing ParaSight-F test and QBC with conventional blood smear examination Z-values were found to be statistically significant. This indicates that these tests may be better options in case of negative blood smear. The sensitivity and specificity of QBC and ParaSight-F test were found to be higher in the present study than reported by other workers[14] due to our selection criteria. We have not included patients of malaria in general and have carried out the tests only where there was strong suspicion due to various associated clinical presentations of *P. falciparum* infection. This is a baseline study comprising small group of patients.

Conclusion

ParaSight-F test and QBC showed high sensitivity and specificity. Although no single test can replace the conventional method of peripheral blood smear examination, these newer diagnostic tests can be used as supplement to microscopic examination of peripheral blood smear where the diagnosis cannot be made on microscopy. These tests can be used at times where there is urgent need of diagnosis to prevent mortality and morbidity associated with *P. falciparum* infection[2,15,16]. ParaSight-F test is very helpful for diagnosis of malaria in areas where facilities of microscopy are not available especially in the night when services of routine laboratories and experienced microscopists are not available[11]. Early diagnosis can lead to timely therapeutic intervention, which can prevent mortality in cerebral malaria patients.

References

1. Molyneux, Mand R, Diagnosis and treatment of malaria in Britain. *Br Med J* 1993; *306:* 1175–80.

2. Iqbal J, Sher A, Rab A. *Plasmodium falciparum* histidine rich protein 2 based immunocapture diagnostic assay for malaria. Crossreactivity with rheumatoid factor. *J Clin Microbiol* 2000; *38*(3): 184–6.

3. Popov AF, Chirkor VP, Nikiforov ND, Sarin BI. The rapid diagnosis of tropical malaria using the Parasight-F test. *LRM Lab Diagn* 1999; 2(5): 56–7.

4. Mendiratta DK, Dhutada K. Narang R, Narang P. Evaluation of different for diagnosis of *P. falciparum* malaria. *Indian J Med Microbiol* 2006; *24*(1): 49–51.

5. Krishna BV, Deshpande AR. Comparison between conventional and QBC methods for diagnosis of malaria. *Indian J Pathol Microbiol* 2003; *46*(3): 517–20

6. Maline LM, Kyi MS, Chiodine PL. Accuracy of routine laboratory diagnosis of malaria in United Kingdom. *J Clin Pathol* 1998; *47:* 740–2.

7. Lee MA, Awl T, Singh M. A comparison of antigen-dipstick assays with polymerase chain reaction and blood film examination in diagnosis of malaria. *Am Acad Med Singapore* 1999; *28*(4): 498–501.

8. Lema OE, Carter JY. Comparison of five methods of malaria detection in outpatient setting. *Am J Trop Med Hyg* 1999; *60*(2): 177–82.

9. Mengesha T, Bresclassic GE. ParaSight-F dipstick antigen test in diagnosis of falciparum malaria in Ethiopia in East. *Afr Med J* 1999; *76*(11): 626–9.

10. Humar A, Oht C Harrington MA, Pillai D, Kaink C. ParaSight-F test compared with polymerase chain reactio and microscopy for diagnosis of *P. falciparum* malaria in travelers. *Am J Trop Med Hyg* 1997; *56*(91): 48–9.

11. Preeji Z. Laboratory diagnosis of malaria by village health workers using rapid manual ParaSight-F test. *Trans R Soc Trop Med Hyg* 1994; *88:* 418.

12. Arora Sandeep, Gaiha Manorma, Arora Anju. Role of ParaSight-F test in the rapid diagnosis of complicated *Plasmodium falciparum* malarial infection. *Braz J Infect Dis* 2003; *7*(5): 1–9.

13. Parzy D, Raphenom G, Martet G, Nicolas P, Touzi JE, Baudon D, Lacamus JC. The quantitative buffy coat test monofluokit. *Med Trop (Mars) France* 1990; 50(1): 97–102.

14. Serougi AO, Amin AM. The quantitative buffy coat capil-

lary tubes versus thin and thick blood films in diagnosis of malaria in Saudi Arabia. *J Egypt Soc Parasitol* 1996; *28*(1): 17–22.

15. New perspective: malaria in Britain. Report of Joint WHO/USAID informal consultation. Geneva, Switzerland: World Health Organization 2000.

16. Bellagra, Ajana F, Caillax M. ParaSight-F in diagnosis of *P. falciparum* malaria. *Pathol Biol* (Paris) 1998; *46*(5): 301–6.

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